SHIMADZU
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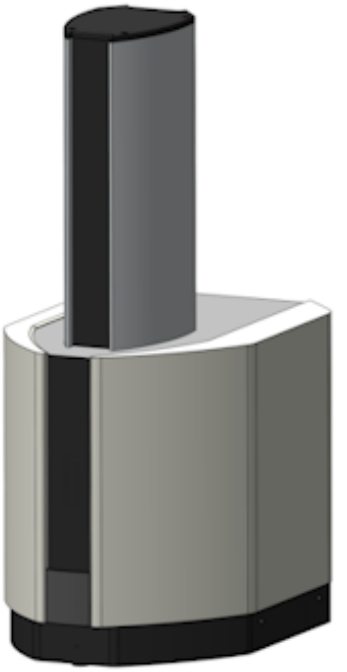
224-20010-80

MALDI-8020 Mass Spectrometer

User Guide

Research use only



Change history

Issue A (May 2017)

Topic	Changes
All	First issue

Issue B (June 2017)

Topic	Changes
Trouble shooting	New section added.

Issue C (July 2017)

Topic	Changes
Search (protein identification using Mascot)	New section added.
Inserting a Target plate	Function buttons moved from Target tab to Acquire tab.

Issue D (July 2017)

Topic	Changes
Cleaning the fans	New section added.

Issue E (July 2017)

Topic	Changes
Mass range & Specifications	Mass range clarified for using high masses.

Contents

CHAPTER 1 Introduction

This chapter provides the following information:

Health and safety precautions

For your safety and the safe operation of the instrument, 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 instrument.

WARNING	High voltage
	Do not remove any panels from the instrument as it can produce lethal voltages. Do not modify the instrument. Keep liquids and flammable vapours away from the instrument.

WARNING	Electric shock
	The power supply must be suitable for that on the rating plate (on the rear mains panel). The power supply to the instrument must be provided from an earthed socket outlet. Do not stretch, twist or coil the mains power cable.

CAUTION	Laser radiation
	The instrument is a Class 1 Laser product which complies with the requirements of 21CFR1040.10 and 1040.11 except for deviations pursuant to Laser Notice No. 50, dated June 24, 2007, and IEC 60825-1 Ed.3 (2014). Do not remove any panels from the instrument - this may result in laser light exposure greater than class 1. Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

信息提供清单

This information is applicable for People's Republic of China only.

部件名称	有害物质					
	铅(Pb)	汞(Hg)	镉(Cd)	六价铬(Cr(VI))	多溴联苯(PBB)	多溴二苯醚(PBDE)
金属框架，机壳	×	○	○	×	○	○
电路板	×	×	×	×	×	×
线束・联接电缆	×	×	×	×	×	×
树脂框架与树脂盖之类	×	○	×	×	×	×
电源组件	×	×	×	×	×	×
电气部件	×	×	×	×	×	×
机械驱动机构	×	○	×	×	○	○
电容器	×	○	○	×	○	○
高压发生部	×	×	×	×	×	×
探测器	×	×	×	×	×	×
监视器	×	×	×	×	×	×

本表格依据 SJ/T11364 的规定编制。

○：表示该有害物质在该部件所有均质材料中的含量均在 GB/T26572 规定的限量要求以下。

×：表示该有害物质至少在该部件的某一均质材料中的含量超出 GB/T26572 规定的限量要求。

环保使用期限标识（标记）是根据《电器电子产品有害物质限制使用管理办法》以及《电子电气产品有害物质限制使用标识要求》制定的，适用于中国境内销售的电器电子产品的标识。标记中央处的数字即为环保使用期限的年数。只要遵守相关产品安全与使用方面的注意事项，则从生产日期算起，在此期限内产品不会对环境造成污染或对人身、财产造成影响。另外，此标识中的期限不同于产品质量与功能的保证期，望周知。

About this guide

This guide contains information and tasks related to the MALDI-8020 instrument. Please discard any previous copies of this guide. Before using the instrument, read this guide.

The images and figures shown are intended as illustrations only and must not be interpreted as actual representation of data, results or equipment. Images and equipment are not shown to scale.

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*.

Using the instrument

Intended use and users

The MALDI-8020 is a MALDI TOF mass spectrometer that operates in conjunction with *MALDI Solutions* software and associated applications. It is typically used for research, quality control and analysis of proteins/peptides, other biological and organic samples.

The instrument and this guide are intended for laboratory use by professional users who are trained to use the instrument and good laboratory practices.

If the instrument is not used for the purpose which it was intended for, any protection will be impaired and we do not accept any liability for the consequences.

During normal use, the instrument is not a biohazard.

Skills

This guide assumes that operators are familiar with:

- standard laboratory procedures and precautions;
- sample preparation;
- using PCs and the Windows operating system.

Training courses are available including sample preparation and using the instrument. Contact your local Shimadzu office, or distributor, for further information.

Using the mouse

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

Typical effects with MALDI-Solutions software	
Left mouse button	Selects buttons, checkboxes, etc.
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.

Limitations of use

The following has an effect on the instrument's ability to operate to its full potential:

- Quality of samples:
    - Purity of samples - impure samples may result in unwanted peaks.
    - Concentrations - too low, or too high, concentrations produce no or unusable spectra.
    - Samples must be dry (wet samples result in longer pump-down times).
    - Type of matrix used.
- Laser power - too low, or too high, will produce no or unusable spectra.

21 CFR 11

The MALDI Solutions software and associated applications provide functions that are in accordance with 21 CFR 11. Therefore, you will be required to log in to access the software. An inactivity timer will log you out automatically.

Target plates and samples

Samples are spotted onto target plates, also known as sample plates, which are then placed into the instrument for data acquisition and analysis. Only use approved target plates in the instrument otherwise you may damage the instrument.

The following are available from your local Shimadzu office, or distributor.

- Target plates, see "Target plates" on page 14.
- Startup calibration kit, order number TO-724R00.

Always keep a record of all samples used in the instrument.

Backup database

Regularly backup the database to secure media. We do not accept any liability for loss of data. See "Database administration" on page 211.

Enter one-byte code alphanumeric characters

For Far Eastern countries. The instrument 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 pump (used to maintain the vacuum in the instrument) may be damaged.

USB/Network precautions

To avoid computer viruses, never download any software or files to the instrument PC. It is your responsibility to secure your network and ensure this protection is appropriate and maintained. It is recommended to use all appropriate means (including anti-virus software, security patches, firewall, etc.) to protect your network from virus intrusion, unauthorised use, alteration, manipulation and disclosure.

Manufacturer

The MALDI-8020 is manufactured by:

Kratos Analytical Limited,
Wharfside,
Trafford Wharf Road,
Manchester,
M17 1GP,
United Kingdom

Tel: +44 (0) 161 888 4400
Fax: +44 (0) 161 888 4401

Web site: www.shimadzu.com/an

Kratos Analytical Limited is part of the Shimadzu Corporation, Japan.

Contact details

For servicing and repairs, contact your local Shimadzu office, or distributor.

Disclaimer and copyright

Trademarks and acknowledgements

MALDI Solutions and MALDI-8020 are trademarks of Kratos Analytical Limited and Shimadzu Corporation, Japan.

Windows is a trademark of Microsoft, Inc.

Summary of data acquisition

1. Wait till the samples on the *Target Slide* are dry.
2. Open the MALDI-8020 instrument door.
3. Load the *Target Slide* into the instrument.
4. Acquire data; the software operates the MALDI-8020 instrument to acquire spectral data.
5. Export the data.

Switching on/off

Switching on

To switch the instrument on, press the switch at the back of the instrument to the ON position; the instrument will power up and pump down the vacuum system.

Switch on the instrument PC and monitor. (If you wish, you can switch on the PC/monitor first and then switch on the instrument.)

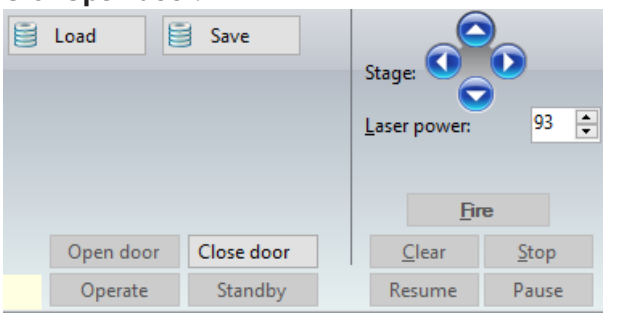
When the front panel LED becomes green and blinking, the instrument is ready to use.

Switching off

We recommend that the instrument is left switched ON. If it is switched off for an extended period and then switched on, it may take several hours for the instrument to reach its operating vacuum.

The sequence is important to ensure that the *Plate carrier* within the instrument is parked correctly.

1. Click **Open door**:



   The stage moves the plate carrier to the front of the instrument, see "Inserting/removing a Target Plate" on page 39.

2. If there is a Target plate in the instrument, you can now remove it.
3. When the front panel LED becomes green and blinking, it is safe to switch off the instrument. Press the switch at the back of the instrument to the OFF position; the instrument will power down.
4. If required, close down and switch off the PC and monitor.

Mains power failure

If the power supply to the instrument fails, or is switched off, the vacuum system will automatically start when the power is restored.

Description of the front panel LEDs

Colour	Status	Function
Pink	Blinking	Instrument is starting up
Blue	Steady	Instrument is in Operate mode
Blue	Blinking	Instrument is acquiring data from the sample
Cyan	Steady	Instrument is in Standby mode
Cyan	Blinking	High voltage ramping in progress
Orange	Blinking	Instrument is venting or pumping down
Green	Steady	Target plate bar code recognised
Green	Blinking	Instrument is ready to load Target plate
Red	Blinking	Instrument fault detected, see "Servicing and fault reporting" on page 206
Yellow	Blinking	Laser cleaning in progress

CHAPTER 2 Target slides & samples

This chapter describes topics associated with target plates, including sample preparation, etc.

Target plates

The following accessories are available for use with the instrument.

FlexiMass-DS disposable targets

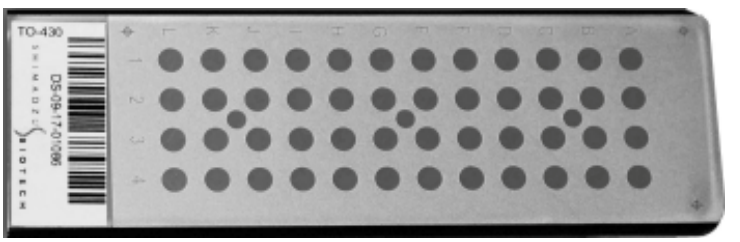
These polymeric targets are disposable (single use) alternatives to the FlexiMass steel targets. The plates can be used as supplied and do not require cleaning prior to use.

Each plate has a unique bar code.

It is strongly recommended that the plates should not be cleaned after use in an attempt to reuse them. Doing so will compromise the surface characteristics and performance of the product and possibly damage the instrument.

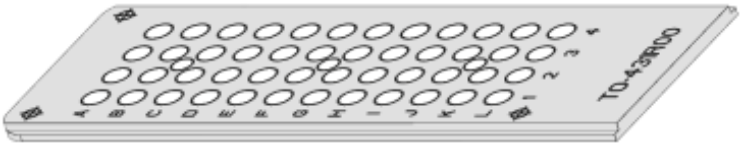
Please dispose of used plates in accordance with local, state and federal regulations.

Number of wells:	Sample = 48 Calibrant = 3
Internal diameter of wells:	Sample = 2.8mm Calibrant = 1.5mm
Order number:	TO-430R00 (four packs of four targets)



FlexiMass-SR48 steel targets

Number of wells:	Sample = 48 Calibrant = 3
Internal diameter of wells:	Sample = 2.8 mm Calibrant = 2.2 mm
Order number:	TO-431R00 (pack of four targets)



Cleaning the target plates (basic)

These instructions are for cleaning target plates (not FlexiMass-DS disposable targets). Either:

- Wipe off any fibres, etc., use a lint-free cloth.
- Remove any finger marks, etc., wipe using HPLC grade acetone.

Cleaning the target plates (chemical)

These instructions are for cleaning target plates (not FlexiMass-DS disposable targets).

The following instructions can also be found at:
http://shimadzu.com/an/lifescience/maldi/support.html

Important notes:

- For targets intended for lipid or low molecular weight (<800Da) analyses omit all steps that include Micro 90 solution.
- Do not attempt to clean the single-use polymeric FlexiMass-DS targets. Doing so will compromise the characteristics and performance of the product and may ultimately lead to damage of the instrument.
- Shimadzu accepts no liability for any damage caused by improper handling of FlexiMass-DS targets.

Items required

When handling solvents, wear suitable personal protective equipment (e.g. safety glasses, lab coat and gloves).

Refer to manufacturer's MSDS (Material Safety Data Sheet) for instructions on safe handling and disposal.

- Ultrasonic bath for target cleaning.
- Cleaning solution concentrate: Micro 90 (available from Sigma Aldrich).
- Hotplate or oven capable of heating a 5% Micro 90 solution to approximately 60°C (140°F).
- HPLC grade methanol and acetone.
- Deionised or HPLC grade water.
- Small oven for drying targets, up to 50°C (122°F).

Cleaning procedure

1. Prepare 100 mL of a 5% (v/v) solution of Micro 90 in deionised or HPLC grade water (5% (v/v) = 5 mL of Micro 90 concentrate + 95 mL water).
2. Heat the 5% Micro 90 solution to approximately 60°C (140°F).
3. Using a tissue and a suitable solvent (e.g. methanol), remove any visible signs of samples from the surface of the target.
4. Place the target face up into a small container and immerse (cover) with acetone.
5. Cover the container and place into an ultrasonic bath.
6. Sonicate the target in acetone for 15 minutes at room temperature.
7. Discard the used acetone. Rinse the target (x2) with 100 mL deionised or HPLC grade water. Discard rinsing solutions.
8. Place the target face up into a small container and immerse (cover) with the heated 5% (v/v) Micro 90 solution.
9. Cover the container and place into an ultrasonic bath.
10. Sonicate the target in the 5% Micro 90 cleaning solution for 15 minutes at room temperature.

11. Discard the used 5% Micro 90 solution. Rinse the target (x2) with 100 mL deionised or HPLC grade water. Discard rinsing solutions.
12. Cover the target with approximately 100 mL of deionised or HPLC grade water. Sonicate target for 5 minutes at room temperature.
13. Cover the target with approximately 100 mL of methanol. Sonicate target for 15 minutes at room temperature. Discard the rinsing solution.
14. Cover the target with approximately 100 mL of deionised or HPLC grade water. Sonicate target for 15 minutes at room temperature. Discard the rinsing solution.
15. Shake the target to remove excess water from the rinsing step.
16. Flood the target with methanol and allow excess to evaporate.
17. Place the target in an oven at 50°C (122°F) for at least 6 hours (e.g. overnight). This oven drying step is necessary to minimise unwanted sample spreading during use.
18. Prior to use, allow the target to adjust to room temperature.

Sample preparation

The procedures in this section are for guidance only. Adapt them to suit your requirements.

Use standard laboratory precautions when preparing samples, for example, in a wet laboratory. Ensure that samples are not prepared within the vicinity of the instrument.

Serial dilutions of samples

The procedures below assume that you have low concentration starting samples. However, if you have a high concentration starting sample, you may wish to make 100 fold or greater dilutions, especially if you require fmol/µL concentrations. Adapt the procedures below to suit.

Make 10-fold dilutions from stock concentrations (10-3 M = 1,000 pmol/µL) :

1. Add 90 µL of 0.1% trifluoroacetic acid to a 0.5 ml micro-centrifuge tube, or similar, using a pipette.
2. Take 10 µL of the stock solution (1,000 pmol/µL) using a pipette. Equilibrate pipette tip by filling and emptying 3 times. Add to the micro-centrifugetube, mix up and down 5 times. The result is a concentration of 100 pmol/µL.
3. Vortex the mix.
4. Repeat this procedure until you reach the required concentration, using a new pipette tip at each stage. Typical concentrations for peptides/proteins are 10 and 1 pmol/µl.

Where a 5 pmol/µL or 500 fmol/µL concentration is required, make 20-fold dilutions. For example, take 95 µL of 0.1% trifluoroacetic acid and to this add 5 µL of sample (e.g. 100 pmol/µL) to give a concentration of 5 pmol/µL.

Calibration samples

A calibration mix must cover the mass range of interest. Typically, a calibration would contain evenly spaced calibrants across the mass range of interest, using a minimum of three calibrants.

Calibrants (amino acid sequence)	Calculated monoisotopic (M+H)+ mass
Bradykinin fragment 1-7 (RPPGFSP)	757.40 Da
Angiotensin 2 human (DRVYIHPF)	1,046.54 Da
Angiotensin 1 human (DRVYIHPFHL)	1,296.69 Da
ProteoMass™ P14R MALDI-MS standard (PPPPPPPPPPPPPR)	1,533.86 Da
[Glu1]-Fibrinopeptide B human (EGVNDNEEGFFSAR)	1,570.68 Da
N-Acetyl-Renin Substrate Tetradecapeptide porcine (C2H2O-DRVYIHPFLLVYS)	1,800.94 Da
ACTH fragment 1-17 human (SYSMEHFRWGKPVGKKR)	2,093.09 Da
ACTH fragment 18-39 human (RPVKVYPNGAEDESAEAFPLEF)	2,465.20 Da
Adrenocorticotropic Hormone Fragment 7-38 human (FRWGKPVGKKRRPVKVYPNGAEDESAEAFPLE)	3,657.93 Da
Cytochrome c equine heart	12,361.96 Da
Bovine serum albumin	66,431 Da

Peptide/protein samples

Preparation

1. Use 10 pmol/µL concentrations.
2. Take 100 µL of each peptide/protein into a micro-centrifuge tube, or similar.
3. Vortex the mix.

For calibrants, you may need to adjust the concentrations in the mix to produce a spectrum of similar intensity peaks, i.e. the monoisotopic peaks are of similar height.

Spotting peptides - general

Add matrix and sample to the wells using a pipette.

1. Spot 0.5 µL of sample.
2. Add 0.5 µL matrix of a-cyano 4-hydroxycinnamic acid 5 mg/mL in acetonitrile/0.1% trifluoroacetic acid (1:1).
3. Leave the spotted target plate for several minutes at room temperature for the samples to dry.
4. Check that the samples are visibly dry before inserting the target plate into the instrument.

Spotting peptides - low concentrations

For very low concentrations, pre-coat the required wells.

1. Spot 0.5 µL of matrix, leave for few seconds, remove the surplus using a pipette and leave to dry.
2. Spot 0.5 µL of sample.
3. Add 0.5 µL matrix of a-cyano 4-hydroxycinnamic acid 5 mg/mL in acetonitrile/0.1% trifluoroacetic acid (1:1).
4. Leave the spotted target plate for several minutes at room temperature for the samples to dry.
5. Check that the samples are visibly dry before inserting the target plate into the instrument.

Spotting proteins

1. Spot 0.5 µL matrix of sinapinic acid, saturated solution (20 mg/mL) in acetonitrile/0.1% trifluoroacetic acid (1:1).

2. Add 0.5 µL of sample.
3. Allow to dry.

Glycan samples

The procedures in this section are for guidance only. Adapt to suit your requirements.

1. Spot 0.5 µL matrix of 2,5-dihydroxybenzoic acid (DHB) at 10 mg/mL in acetonitrile/0.1% trifluoroacetic acid (1:1).
2. Add 0.5 µL of sample.
3. Allow to dry.
4. Optionally, pipette 0.3 µL of chilled ethanol onto the dried spot and allowing to dry.

Sample records

Keep records of all substances which have been used in this instrument.

Service engineers are not allowed to service instruments if it is contaminated with any substance which is radioactive or biologically active; it is dangerous, unless decontaminated.

CHAPTER 3 MALDI Solutions Data Acquisition

This chapter describes the workflow and instructional procedures to produce data for analysis.

Introduction

MALDI Solutions Data Acquisition is an application that enables you to:

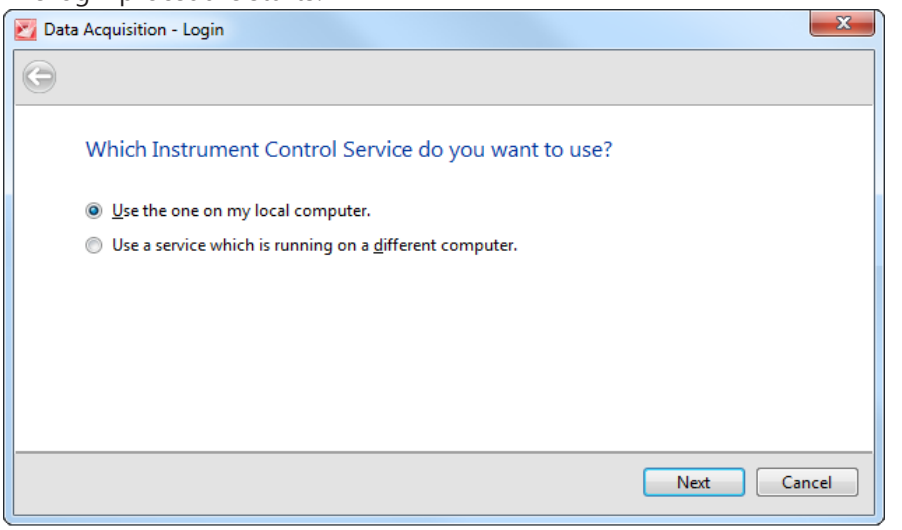
- acquire data from the sample in the form of a spectrum (intensity versus mass/charge (m/z)).
- process the data to smooth the peaks.
- calibrate the data to provide actual masses of the peaks.

The application operates in conjunction with a database and background services to control the MALDI-8020 instrument and feedback data.

Starting Data Acquisition

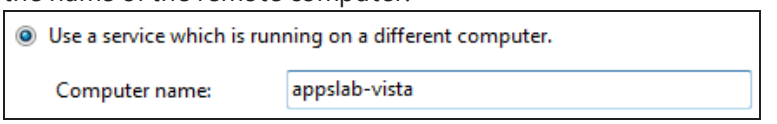
1. On the desktop, click the *Data Acquisition* short cut:


2. The login procedure starts:

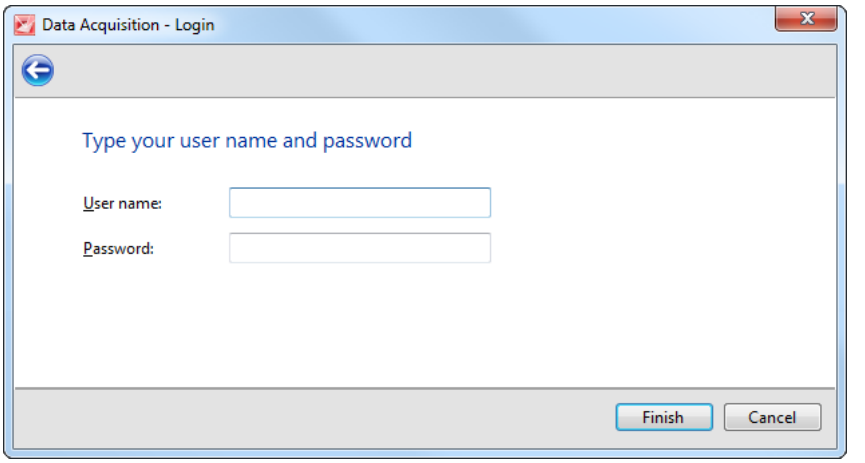


Instrument Control Service is a background service which MALDI Solutions uses. This service is always on the instrument PC.

- If you are using the instrument PC, select the **Use the one on my local computer** radio button.
- If you are logging on from a remote PC/laptop select the **Use a service which is running on a different computer** radio button; you then need to specify the name of the remote computer:



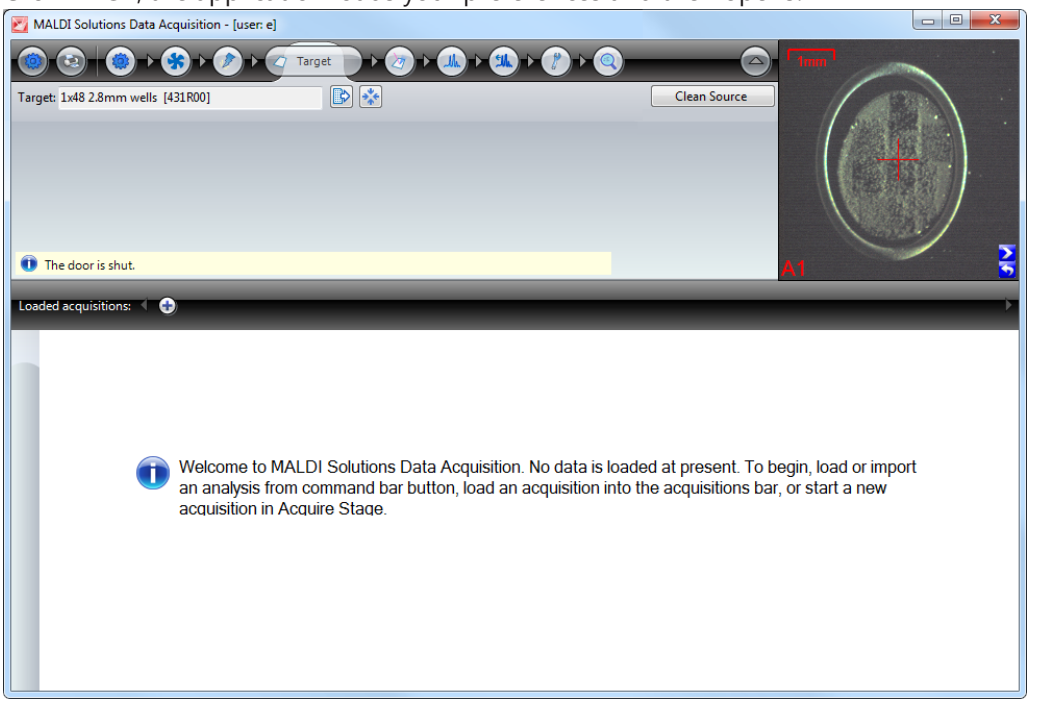
3. Click **Next**:



4. Enter your user name and password.

   User names and passwords are set by your System Administrator using the *Administration Console*, see "Administration Console" on page 175.

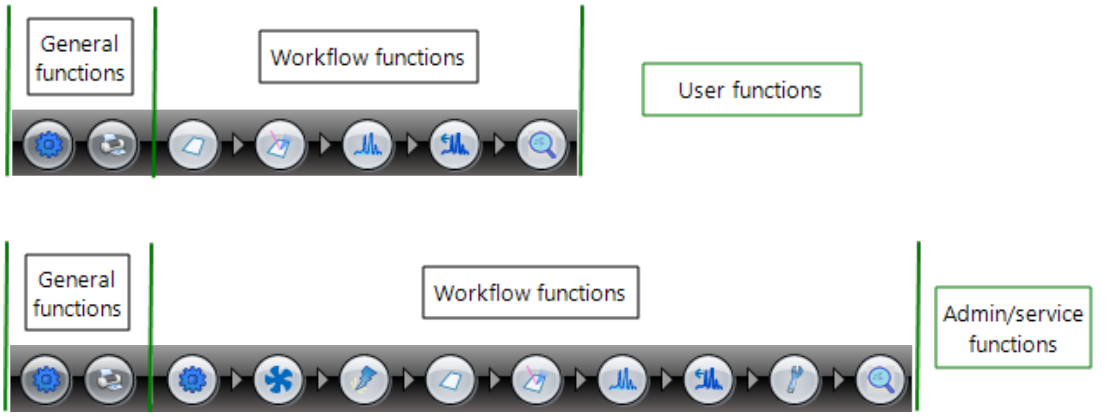
5. Click **Finish**; the application loads your preferences and then opens:



Description of the display

Workflow

The software is designed to guide you through an acquisition. As you work through the application, you only see fields, or features, that are relevant to you at that time.



General functions

Tab	Function
	Settings - use to change your personal settings
	Print Analysis - use to print the display of a spectrum, see "Printing & settings" on page 150.

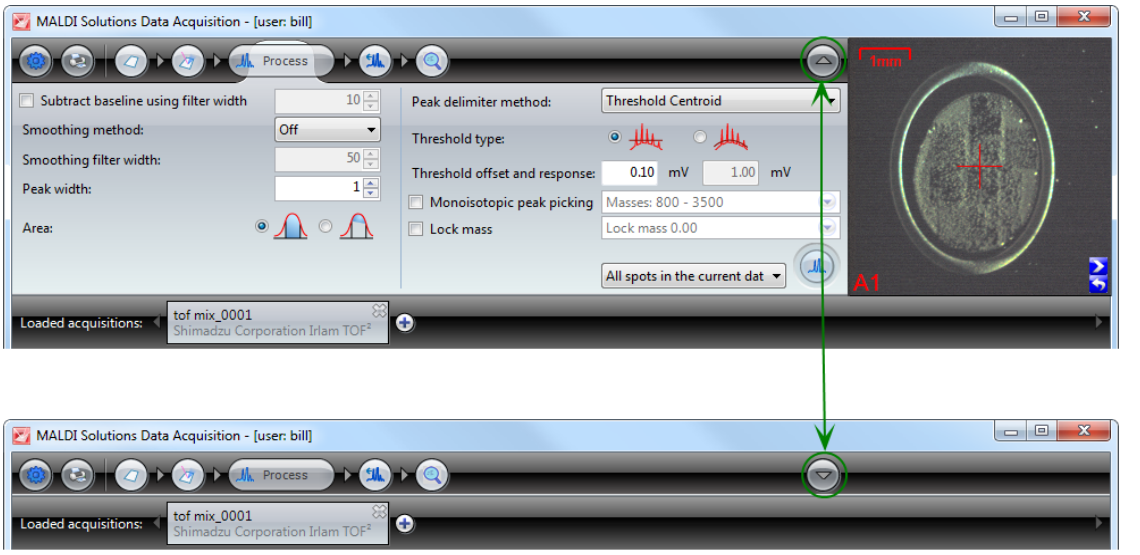
Workflow functions

Tab	Function
	Setup - for service engineers only
	Vacuum - for service engineers only
	Voltages - for service engineers only

Tab	Function
	Target - use to load and unload a Target plate, see "Inserting/removing a Target Plate" on page 39 This tab also includes Source cleaning, see "Cleaning the source plate " on page 194
	Acquire - use to acquire MALDI data, see "Setting acquisition parameters" on page 45
	Process - use to process the MALDI data, see "Processing a spectrum" on page 76
	Calibrate - use to calibrate the MALDI data, see "Calibration" on page 128
	Service - for service engineers only
	Search - not available for this release

Toolbar functions - show/hide

You can hide the toolbar functions to increase the space for displaying the spectrum:



Attributes

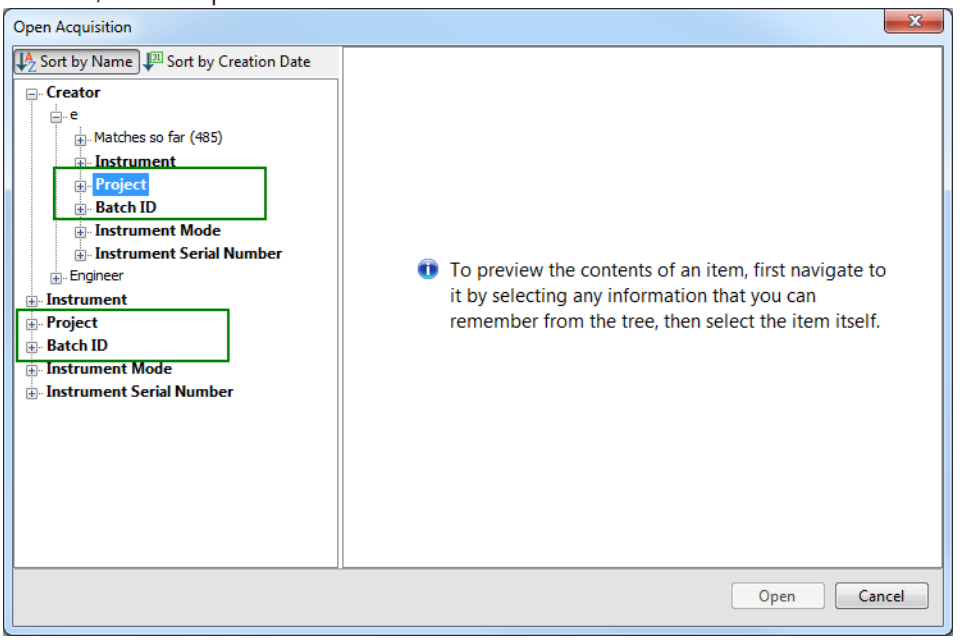
MALDI Solutions Data Acquisition opens and saves information to a database; there are no files.

Attributes are used to describe your data. Whenever you load data, or save data, you use attributes. Examples of attributes include "acquisition name", "customer", "project", "batch ID", "part no", etc. Examples of data requiring attributes include:

- acquired spectral data;
- calibrations.

Attributes may be mandatory, optional, or are provided automatically. The administrator can define attributes using the *Administration Console* application see "Administration Console" on page 175.

When you access the database to retrieve/open data, the attributes are displayed in a browser window; for example:



You can sort the attributes by name, or creation date, by clicking the appropriate button. Click on an attribute to reveal the available choices. As you drill down through the tree structure, the number of entries reduces, narrowing the number of choices, to leave you with the required entry.

Increasing the number of attributes makes finding data easier. Therefore, if you are saving data, provide as many attributes as possible; try not to leave optional fields blank.

Camera viewer

The camera viewer allows you to observe the sample. The cross-hairs show you where the laser strikes the sample.

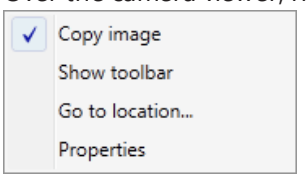
You can:

- copy the image;
- show the toolbar (to change the brightness and contrast of the camera view);
- go to location (move the Target plate to a specific spot);
- un-dock the camera viewer;
- minimise the camera viewer;

Copying the image

You can copy the image of the camera view to the clipboard and subsequently paste it into, for example, a Word document.

1. Over the camera viewer, right-mouse click; a menu box is displayed:

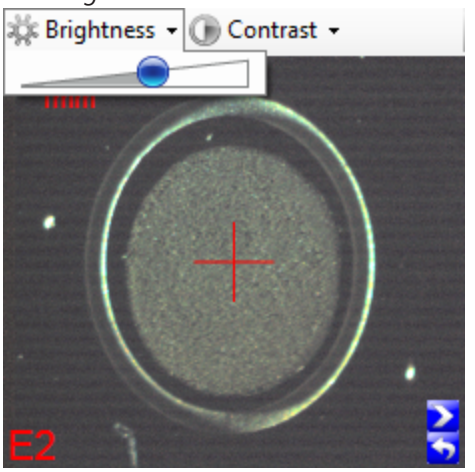


2. Click **Copy image**.

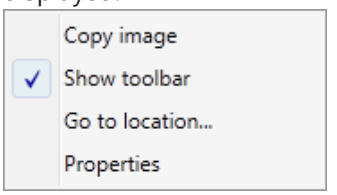
To import the image, use your application's paste feature; you may be able to use the keyboard shortcut Ctrl+V to paste.

Changing the brightness and contrast

The *Brightness* and *Contrast* controls are located in a toolbar, which you can display/hide:



1. If the toolbar is not displayed, over the camera viewer, right-mouse click; a menu box is displayed:

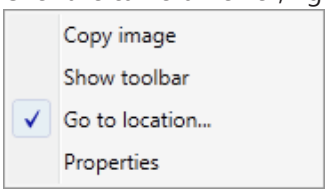


2. Click **Show toolbar**; the *Brightness* and *Contrast* controls are displayed.
3. Click the required toolbar item.
4. Using the mouse, drag the slider to the required level.

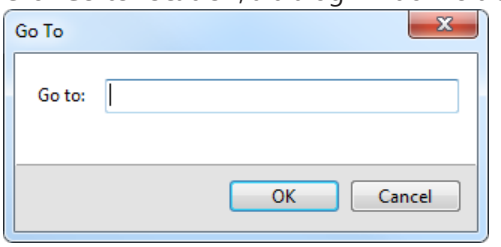
Go to location

If the Target plate is aligned, you can use this feature to move to a specific well position on the plate.

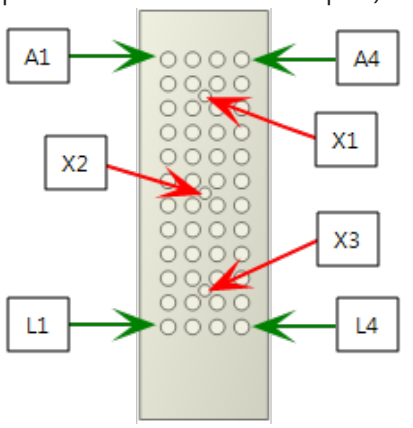
1. Over the camera viewer, right-mouse click; a menu box is displayed:



2. Click **Go to location**; a dialog window is displayed:



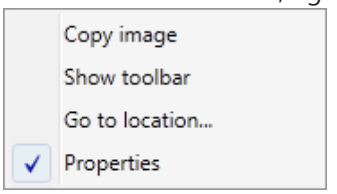
3. Enter the required spot location; diagram below shows the location of the spots (X spots are for calibration samples).



4. Click **OK**; the instrument moves the stage to locate the spot in the centre of the camera.

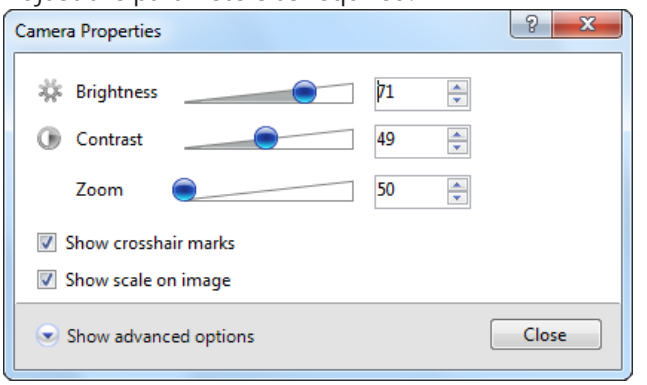
Displaying camera properties

1. Over the camera viewer, right-mouse click; a menu box is displayed:



2. Select **Properties**; a dialog box is displayed.

3. Adjust the parameters as required.



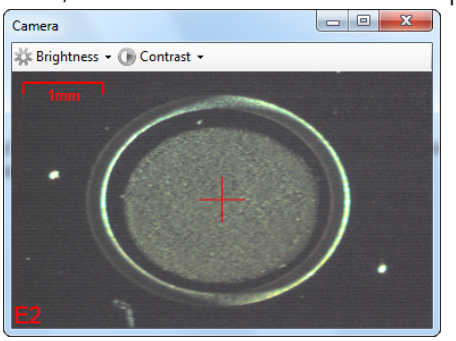
If displayed, the **Show advanced options** are for service engineers; do not adjust the settings.

Un-docking the camera viewer

You can un-dock the viewer, which provides you with a larger image.

1. Click the Undock icon:


; the viewer is un-docked and displayed in a separate window:



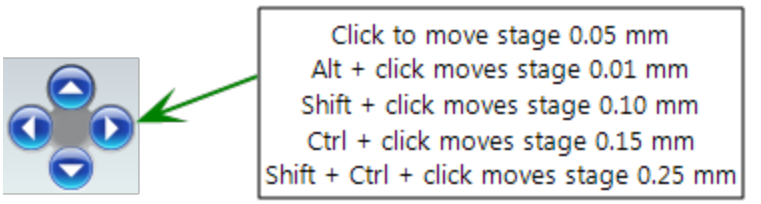
2. To close the window, click the button:


; the viewer is redisplayed within the main window.

Manually moving the stage

The laser fires at the sample where the crosshairs meet. If you are using manual positioning, see "Manual positioning" on page 46, you can move the stage, which is useful when the laser is firing to find a "sweet spot", by either:

- moving the mouse pointer to where you want the laser to fire and click the mouse left-button; the stage moves to that point;
- using the stage control buttons:



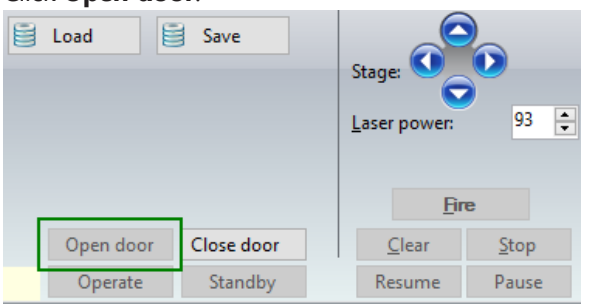
Inserting/removing a Target Plate

All laboratory specimens must be handled according to standard precautions. Use powder-free gloves while handling a *Target Plate*. Avoid touching the spotted areas of the *Target Plate*.

CAUTION	Damage to the instrument
	Do not put wet samples or any other element such as fibre into the MALDI-8020 instrument, moisture and particles within the vacuum system may damage the instrument.

You use MALDI Solutions Data Acquisition to load and unload a *Target Plate*.

1. Ensure that the samples on the *Target Plate* are dry.
2. Click the **Acquire** tab.
3. Click **Open door**:

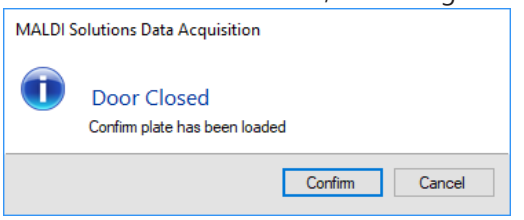


The vacuum system is vented and the stage moves the plate carrier to the front of the instrument.

4. When the front panel LED blinks green, open the front door and insert the *Target Plate* (if required, remove any loaded plate). When inserting the plate, ensure that the orientation of the plate is correct:
FlexiMass-DS disposable targets (TO-430R00) - barcode label at the rear of the plate.

FlexiMass-SR48 steel targets (TO-431R00) - part number at rear of plate.

5. Check that there is no dust, etc. on the O-ring. If there is dust, etc., wipe with a clean lint-free cloth - DO NOT use solvents.
6. Close the front door closed; the dialog box is displayed:

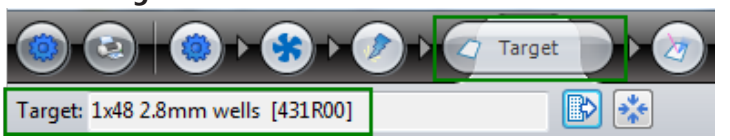


Note: The front door comprises several integral parts which are designed to hold the front door seal in place when the door is closed. However, when the door is closed, the outer part of the door can move by several millimetres; this is normal and does not affect the performance of the instrument.

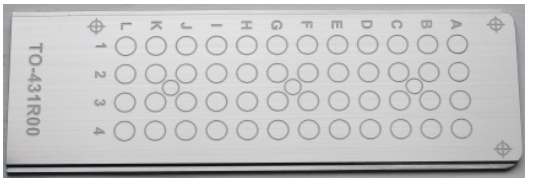
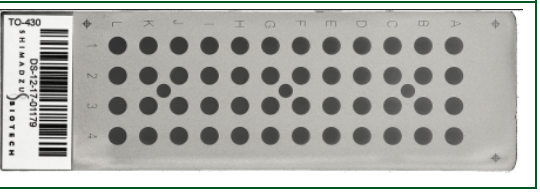
7. Click **Confirm**.
(If you click **Cancel**, you can still continue to load a *Target plate* by clicking **Close door**.)

Loading a target definition

Click the **Target** tab:



There are two types of *Target plates* that can be used in the instrument:

Part No.	Image
TO-431R00 (stainless steel targets)	
TO-430R00 (disposable targets)	

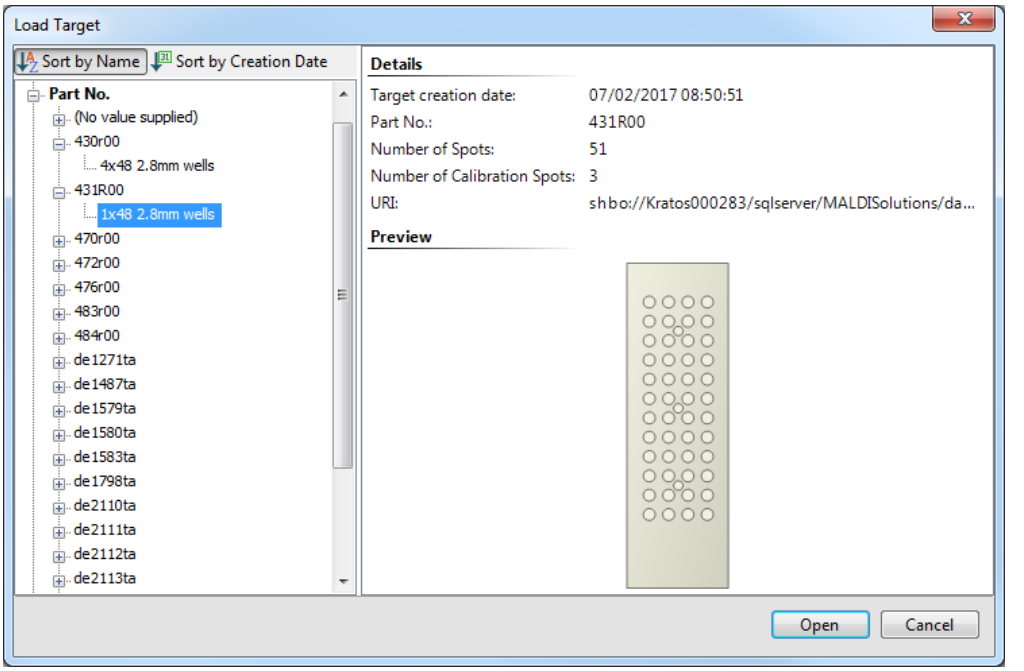
Use the Target definition **1x48 2.8mm wells [431R00]** for BOTH Target plates.

If the Target definition is not shown:

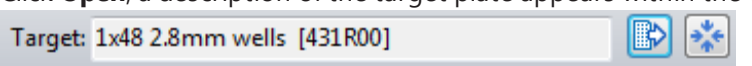
1. Click the **Target** icon:

   The *Load Target* browser displays the available targets from the database:



2. Select 431R00 -> 1x48 2.8 wells; an image of the plate is displayed.
3. Click **Open**; a description of the target plate appears within the *Target* field:



Aligning the target plate

This process allows for possible variations from one target plate to another, etc. You align the target plate using the camera and three wells on the Target plate.

Usually, you perform this procedure once and then only if the alignment strays.

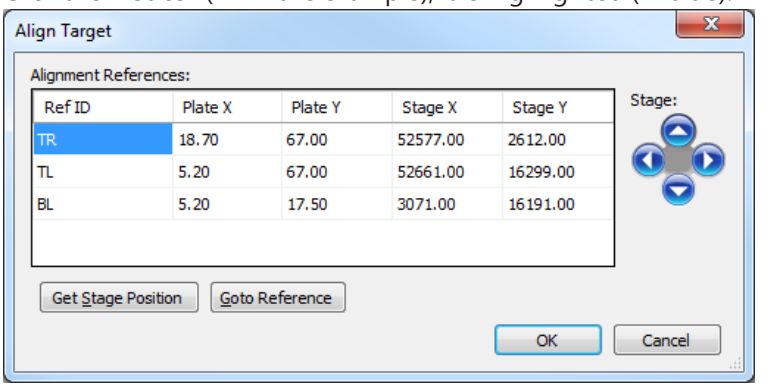
1. Click the **Align Stage** icon:

   The *Align Target* window displays the approximate positions for the target plate based on the target you selected earlier. The reference wells for both types of Target plates is TR, TL and BL, where:

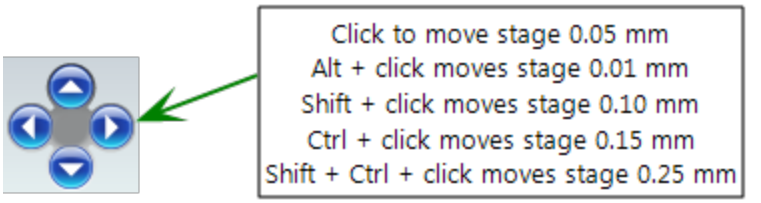
   - TR = Top Right (actual well position is A4)
   - TL = Top Left (actual well position is A1)
   - BL - Bottom Left (actual well position is L1)

2. Click the first cell (TR in the example); it is highlighted (in blue).

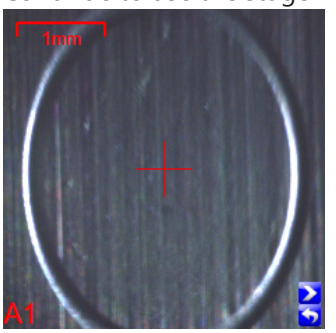


3. Click **Go to Reference**; the stage moves to that location, you can view the progress in the camera viewer.

4. Use the stage movement buttons to check that the stage has moved to the correct spot (A4 is the top right-hand spot).



5. Continue to use the stage movement buttons to centre the crosshairs within the spot:

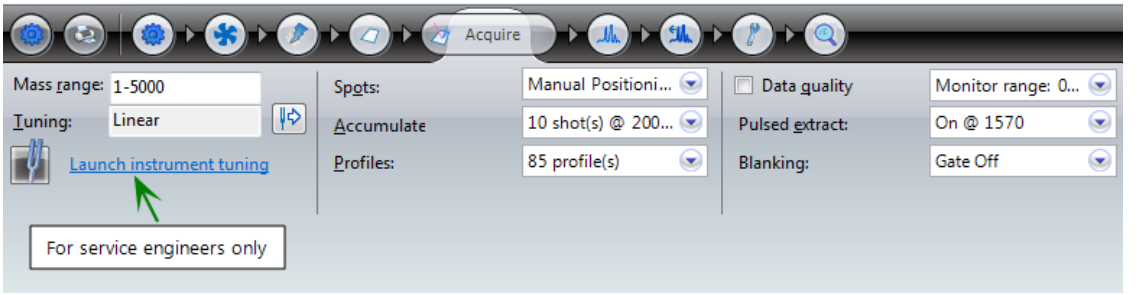


6. Click **Get Stage Position**; the co-ordinates within the first row adjust accordingly.
7. Repeat these instructions for TL and then BL.
8. Click **OK**, the target plate is now aligned.

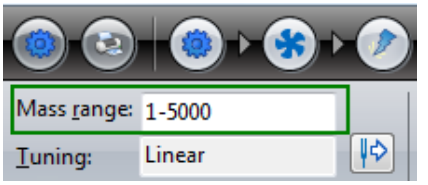
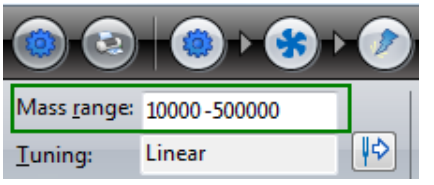
Setting acquisition parameters

The following parameters determine which acquisition data to collect and how the resultant spectrum is produced.

Click the **Acquire** tab to display the acquisition parameters:

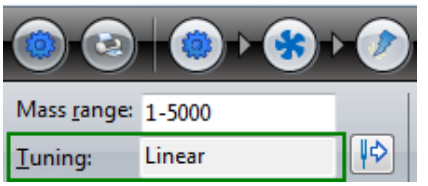


Mass range

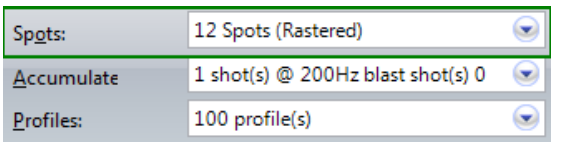
<350 kDa	>350 kDa
For masses up to 350 kDa, enter the mass range (in Daltons) of the data that you wish to acquire. 	For masses above 350 kDa, set a lower limit of 10 kDa. For example, for an upper mass range of 500 kDa, enter "10000-500000". 

Tuning

Use linear tuning modes only.



Spots



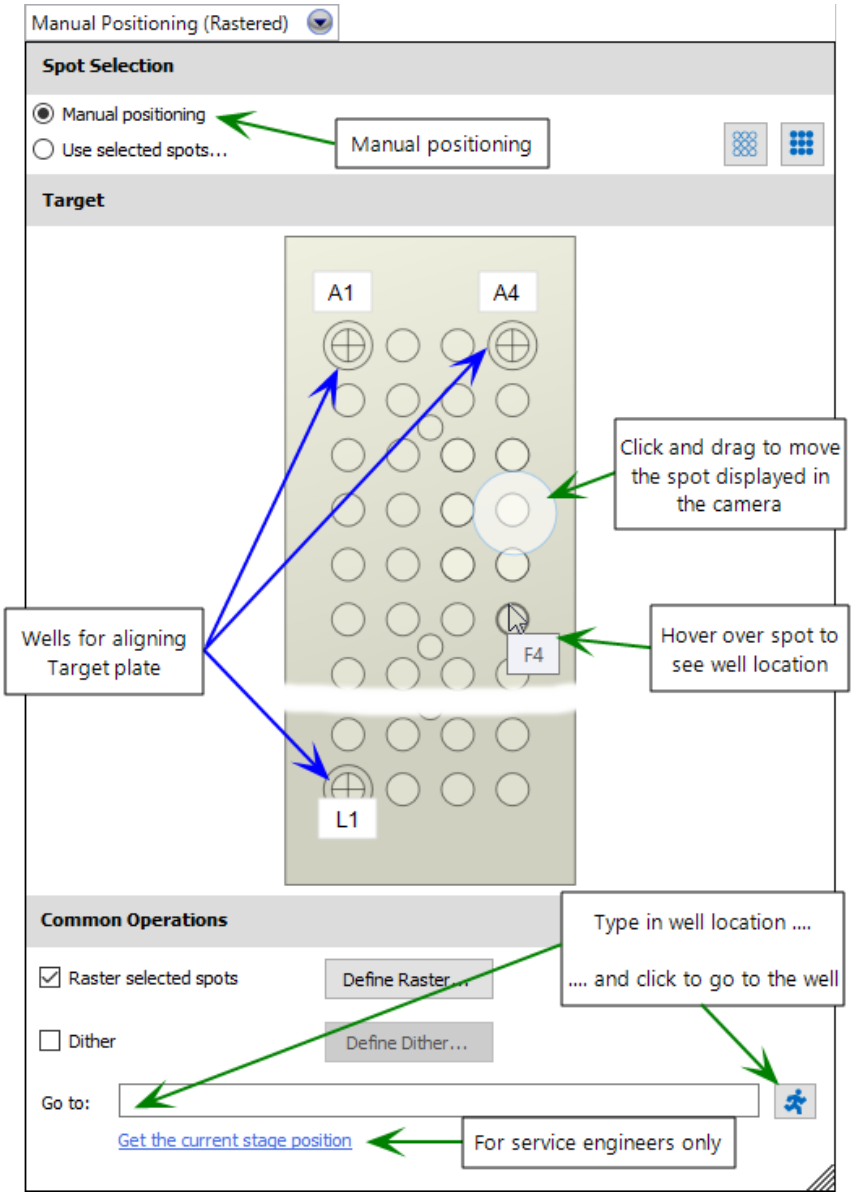
You can choose to:

- manually position the required spot for data acquisition, or
- automatically use selected spots.

Manual positioning

You can use the following procedure to select the required spot to acquire data.

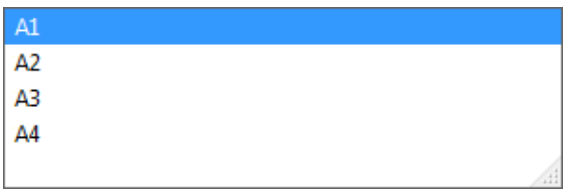
1. Use the drop-down arrow  to open the *Spot selection* window.


Manual Positioning (Rastered)
Spot Selection
Manual positioning
Use selected spots...
Manual positioning
Target
A1
A4
Click and drag to move the spot displayed in the camera
Wells for aligning Target plate
F4
Hover over spot to see well location
L1
Common Operations
Type in well location ....
.... and click to go to the well
Raster selected spots
Define Raster...
Dither
Define Dither...
Go to:
Get the current stage position
For service engineers only

2. If required, select the **Manual positioning** radio button.
3. Go to the required spot:
    a. Resize the window so that you can clearly see the spots (click and drag the bottom right-hand side of the window).
    b. Click and drag the "O" on the target to the required spot to display in the camera.
    c. Hover the mouse over the required spot to display its location.

    Alternatively:

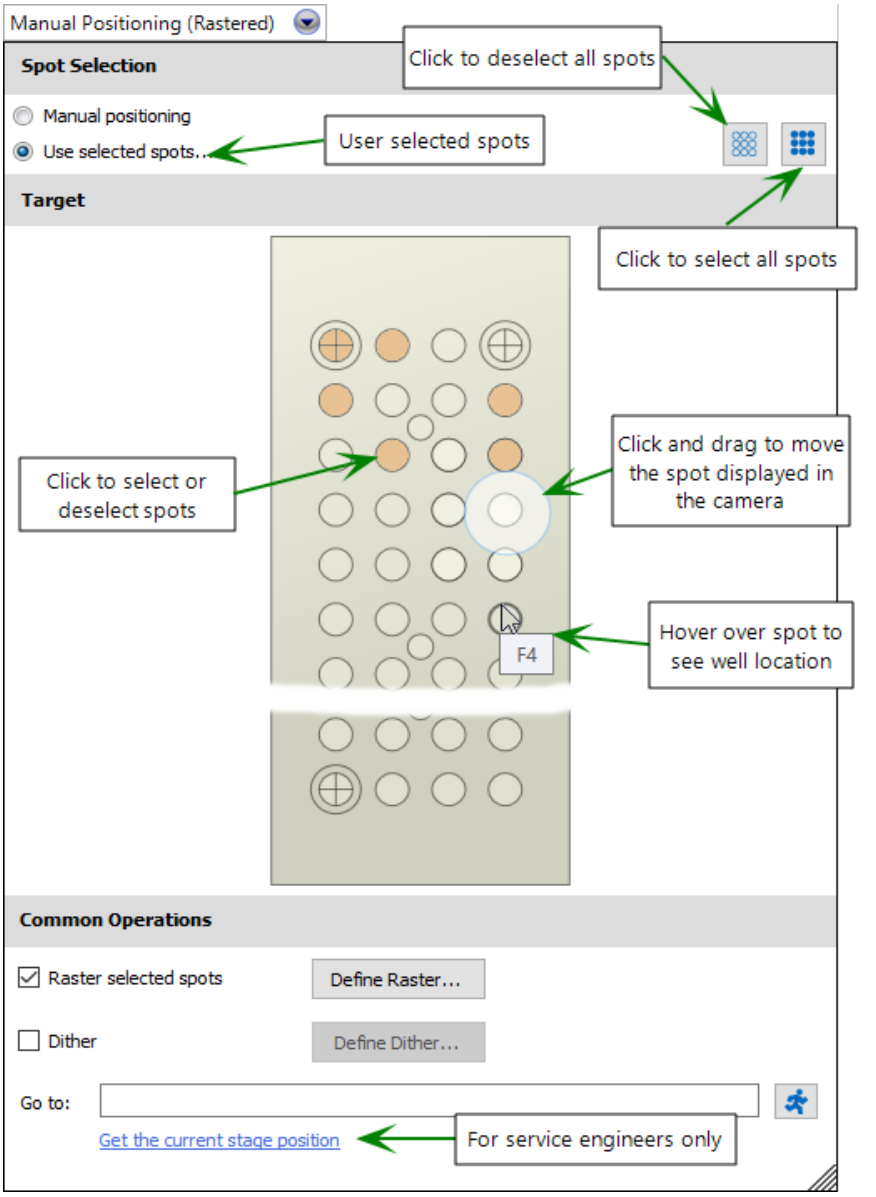
    a. In the **Go to:** field, type in the location of the required spot. As you type, a drop-down list is displayed from which you can select the required spot location:



    b. Click the Go to icon:
     ; the spot moves to the centre of the camera viewer.

Use selected spots

1. Use the drop-down arrow
 to open the *Spot selection* window.


Manual Positioning (Rastered)
Spot Selection
Click to deselect all spots
Manual positioning
Use selected spots...
User selected spots
Target
Click to select all spots
Click to select or deselect spots
Click and drag to move the spot displayed in the camera
F4
Hover over spot to see well location
Common Operations
Raster selected spots
Define Raster...
Dither
Define Dither...
Go to:
Get the current stage position
For service engineers only

Defining rastering

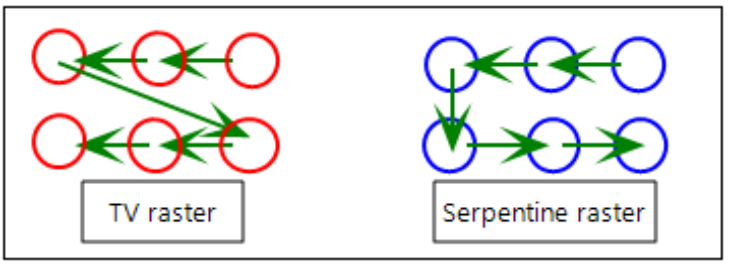
Rastering automatically scans the laser over the spot, rather than remaining static at one specific point.

You have the following choices of raster:

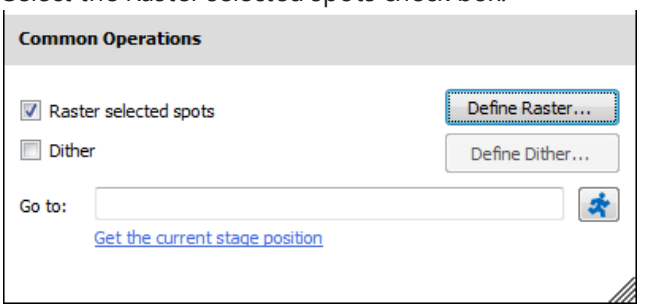
- Regular circle;
- Regular annular;
- Regular square.

You can also define the raster style (the direction of laser shots):

- TV raster;
- Serpentine raster.

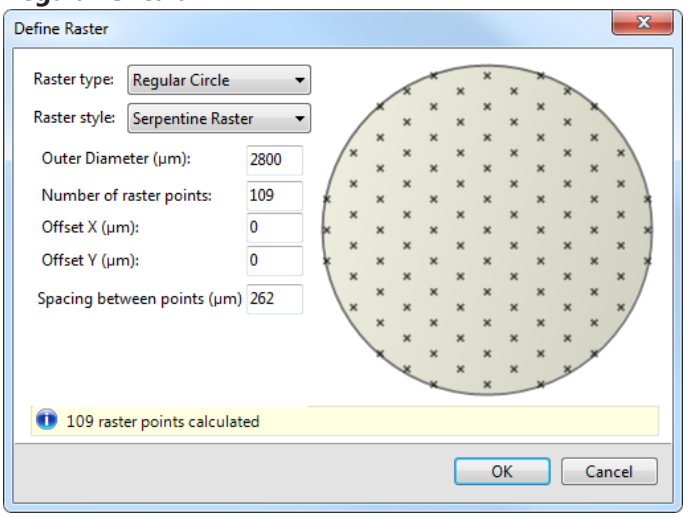


1. Select the Raster selected spots check box:

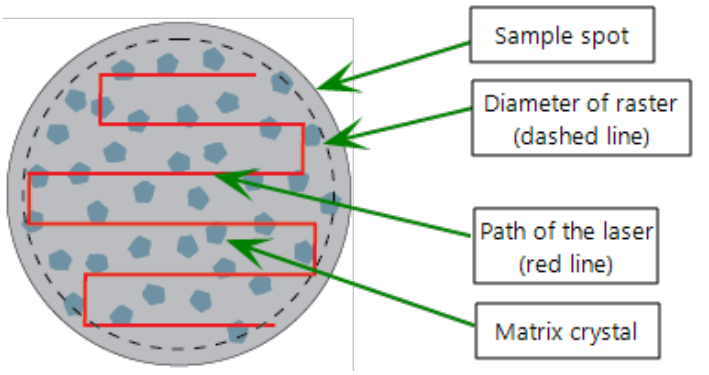


2. Click the **Define Raster** button.
3. Select the required Raster type.

Regular Circular



The laser scans the spot within the diameter of the raster. This is useful if you are using CHCA matrix which typically forms small, evenly distributed crystals over the spot.



a. Select the required **Raster style** from the drop-down list.

b. Type in the required **Outer diameter** of the raster (for the Target plates, the diameter of the well is 2800 µm).

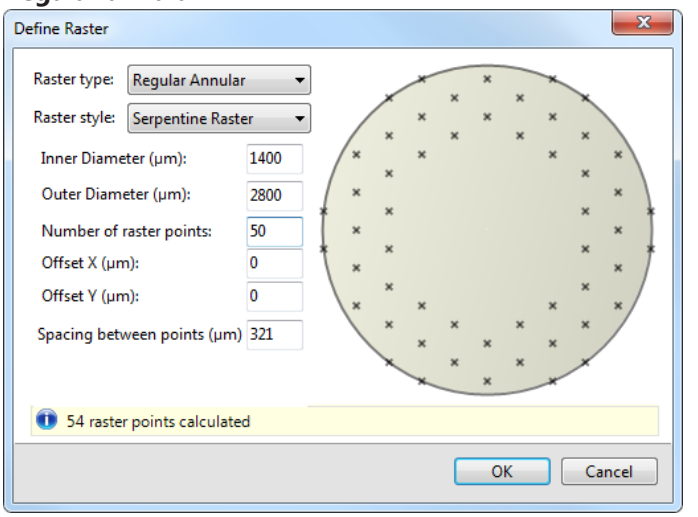
c. Enter the required **Number of raster points**.
A raster point is effectively the position where the laser fires at the sample. You can specify any number of raster points up to 200. The software will use this number, and the available raster area, to calculate the effective number of raster points. Therefore, the number of raster points that you specify may be different from the actual number of points calculated - as displayed in the information bar.

d. The raster is based around the centre of the spot. If you wish to define an offset from the centre, enter a value in the **Offset X** and/or **Offset** Y fields.

e. The **Spacing between points** field is for information only.

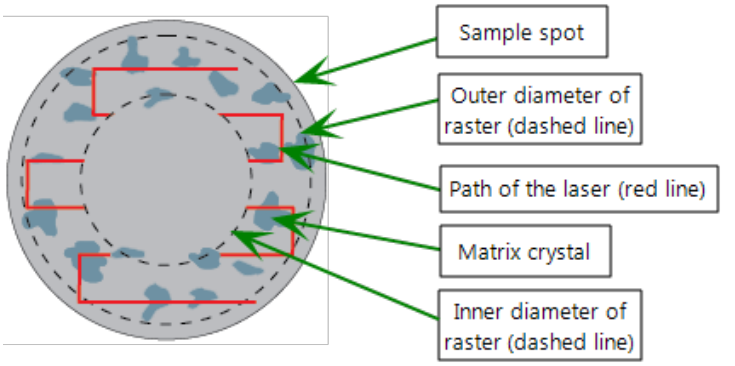
f. Click **OK**.

Regular annular



The laser scans the spot between the inner diameter and the outer diameter rings. This

is useful if you are using DHB matrix which typically forms larger crystals at the edge of the spot.



a. Select the required **Raster style** from the drop-down list.

b. Type in the required **Inner diameter** of the raster.

c. Type in the required **Outer diameter** of the raster (for the Target plates, the diameter of the well is 2800 µm).

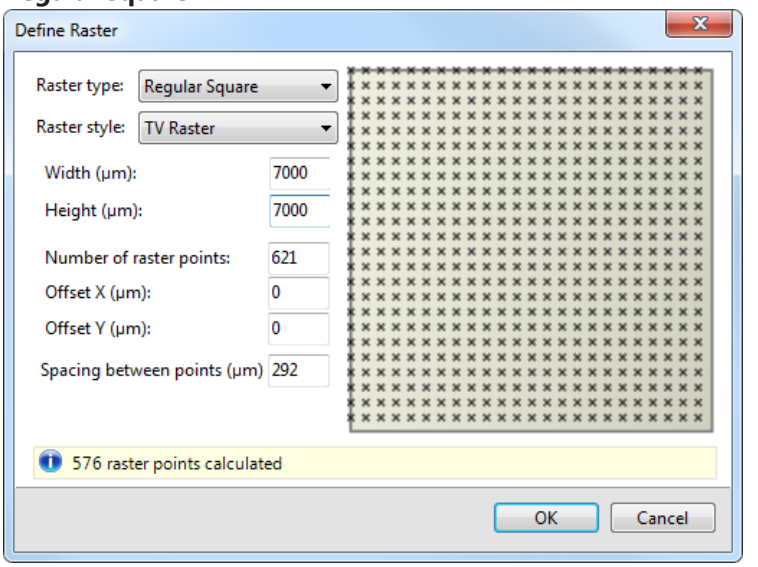
d. Enter the required **Number of raster points**.
A raster point is effectively the position where the laser fires at the sample. You can specify any number of raster points up to 200. The software will use this number, and the available raster area, to calculate the effective number of raster points. Therefore, the number of raster points that you specify may be different from the actual number of points calculated - as displayed in the information bar.

e. The raster is based around the centre of the spot. If you wish to define an offset from the centre, enter a value in the **Offset X** and/or **Offset Y** fields.

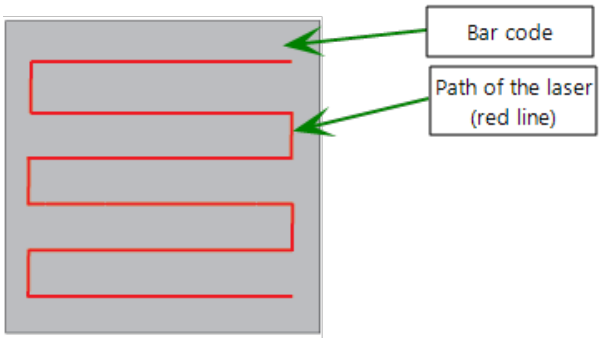
f. The **Spacing between points** field is for information only.

g. Click **OK**.

Regular square



This feature is for use with auto tuning (not available in this release) where the laser will scan part of the barcode. (Auto tune uses the spectrum of the ink in the bar code as a reference.)



a. Select the required **Raster style** from the drop-down list.

b. In the **Width** and **Height** fields, enter the required size of the area.

c. Enter the required **Number of raster points**.
A raster point is effectively the position where the laser fires (up to 1,000 times) at the sample. The software will use this number, and the available raster area, to calculate the effective number of raster points. Therefore, the number of raster points that you specify may be different from the actual number of points calculated.

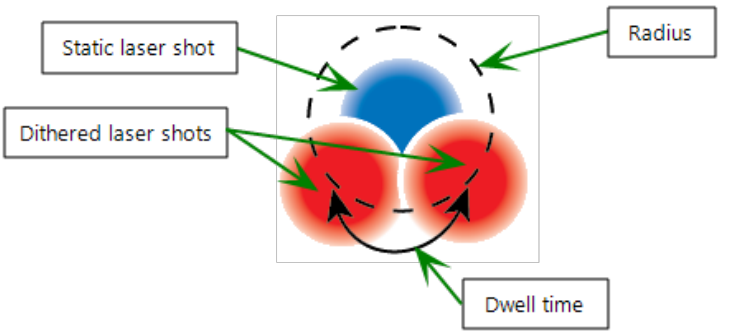
d. The raster is based around the centre of the square. If you wish to define an offset from the centre, enter a value in the **Offset X** and/or **Offset Y** fields.

e. The **Spacing between points** field is for information only.

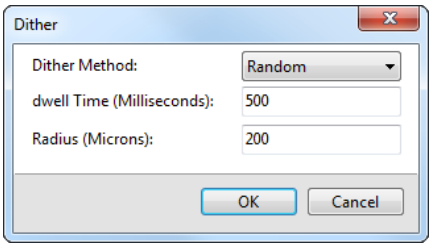
f. Click **OK**.

Defining dithering

Dithering allows the laser to move around a spot, rather than remaining static at one point.



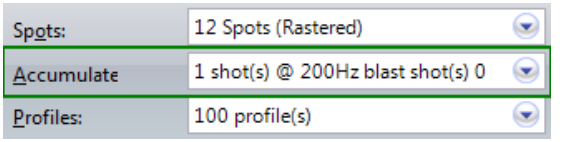
1. Select the **Dither** check box.
2. Click the **Define Dither** button:



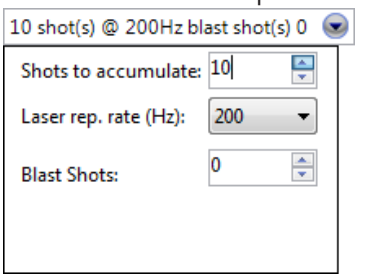
   Note: Only the random dither method is available.

3. Set the **Dwell time** (this is the time from one dither position to the next dither position).
4. Set the **Radius** (defines the distance of the laser spot from the static position).
5. Click **OK**.

Accumulate

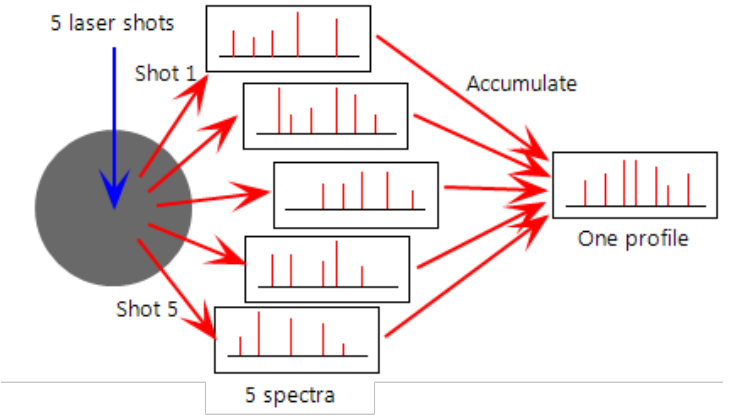


Set the number of required accumulations from the drop-down list:



Shots to accumulate

Each laser shot produces a spectrum. If you set to accumulate 5 shots, the software will collect 5 spectra and then accumulate them into a single composite spectrum called a profile. Maximum number of "shots to accumulate" is 128.



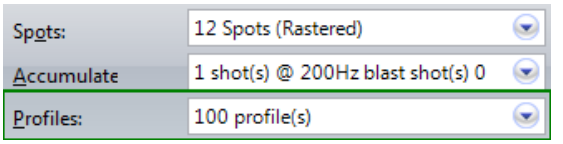
Laser rep rate

The laser repetition rate is the number of times the laser fires in a second.

Blast shots

Blast shots discard the data from the first few laser shots at each position (specified as the number of blast shots). This may help to improve the quality of the spectra acquired in cases where contaminants accumulate in the top layer of the sample/matrix spot.

Profiles



Set number of profiles

Set the number of profiles (spectra) to acquire from each sample spot:

Maximum number of profiles you can set is 30,000.

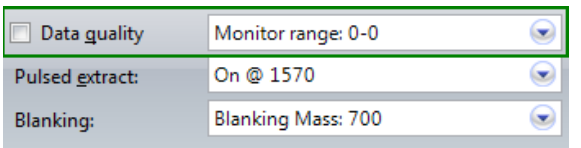
Continuous firing

When you click the **Fire** button, the laser will fire continuously until you click the **Stop** button.

Store individual profiles

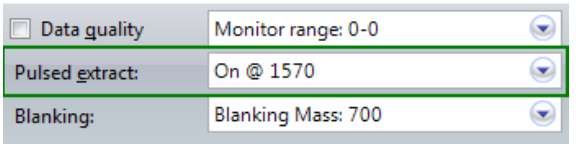
Each profile is stored and you can subsequently view each profile, see "Spectra features" on page 91. Otherwise, do not select this check box unless needed as the database will soon fill up.

Data quality

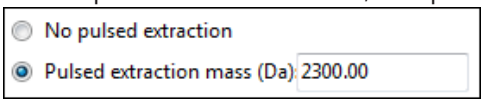


This feature is described in a separate section, see "Data quality" on page 65.

Pulsed extract



Set the pulsed extraction mass, if required, from the drop-down list:



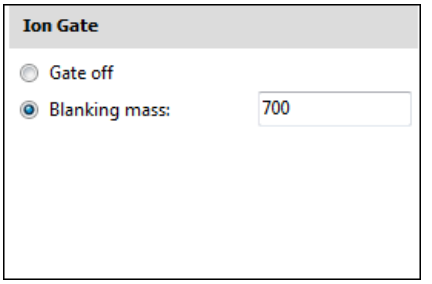
What it does

The instrument generates ions from each laser shot and after a short delay (nanoseconds) extracts them into the flight tube (for subsequent detection). This feature is used to improve the resolution of the mass of interest.

Guidance

Pulsed extraction guidance	
Application	**Settings**
Single-mass samples	Set to the mass of interest.
Broad-range mass samples	Set to approximately ⅔ of the largest expected mass.

Blanking



Gate off

All ions enter the flight tube for detection.

Blanking mass

This feature filters out most ions up to the mass set in the adjacent box. For example, blanking mass set to 700 Da filters out ions between 1 to 700 Da, including most matrix ions.

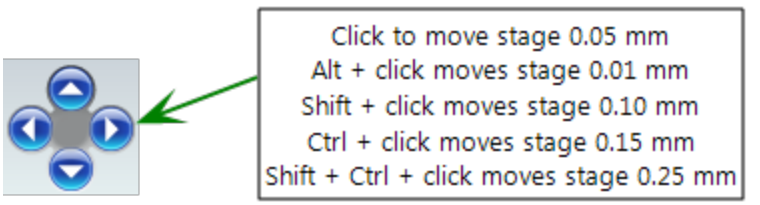
Acquiring a spectrum

Manual positioning

If the required spot is NOT displayed in the Camera window:

Either:

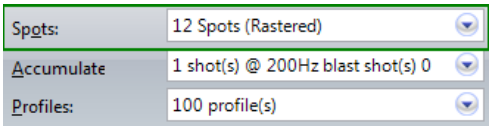
Position the crosshairs in the camera viewer to where you want the laser to fire. You can use the pointer in the camera viewer or the stage movement controls, see "Manually moving the stage" on page 38.



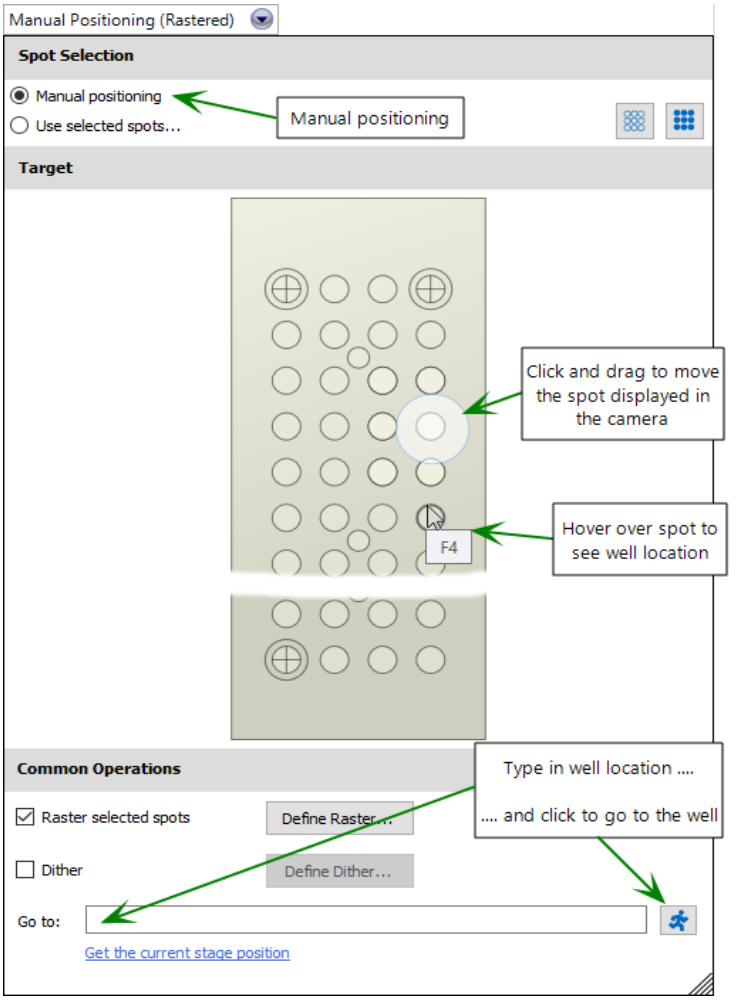
Or:

You can use the following procedure to select the required spot to acquire data.

1. At the **Spots** field, use the drop-down arrow  to open the *Spot selection* window.



2. In the *Spot selection* window, either:
   - Click and drag the circle to the required spot, or
   - In the Go to field, type in the required spot number:



Firing on selected spots

If you have selected spots to fire on (see "Acquiring a spectrum" on the previous page) the instrument will automatically go to the first spot:

Note: If you attempt to use the stage movement controls, the experiment will stop.

Spectrum acquisition

1. Click **Operate**; the instrument switches on the high-voltage components and waits approximately two minutes for the components to stabilise.
2. Click **Fire**, the laser fires.
3. If required, raise or lower the laser power to optimise the spectrum.

During an acquisition

While acquiring, you can:

- adjust the laser power;
- for manually selected spots, move the point where the laser hits;
- clear the acquisition;
- pause the acquisition;
- stop the acquisition (and change the processing parameters);
- resume the acquisition.

Adjusting the laser power

The arbitrary laser power range is 0 to 180.

Select the required laser power:

- too low - sample will not ionise resulting in no or low intensity peaks;
- too high - peaks will be "off the scale" where the detector becomes saturated resulting in poor resolution.

To set the power you can:

- Type in the required power.
- Use the spinner to raise/lower the power.

During the acquisition, you can raise/lower the power as required.

Moving the laser point

You can use the pointer in the camera viewer, or the stage movement controls, to move the laser point, see "Manually moving the stage" on page 38".

Managing an acquisition

At the start of an acquisition, you may raise/lower the laser power to find the optimal setting. In this scenario, accumulated spectra are therefore not required; click **Clear** to remove these spectra.

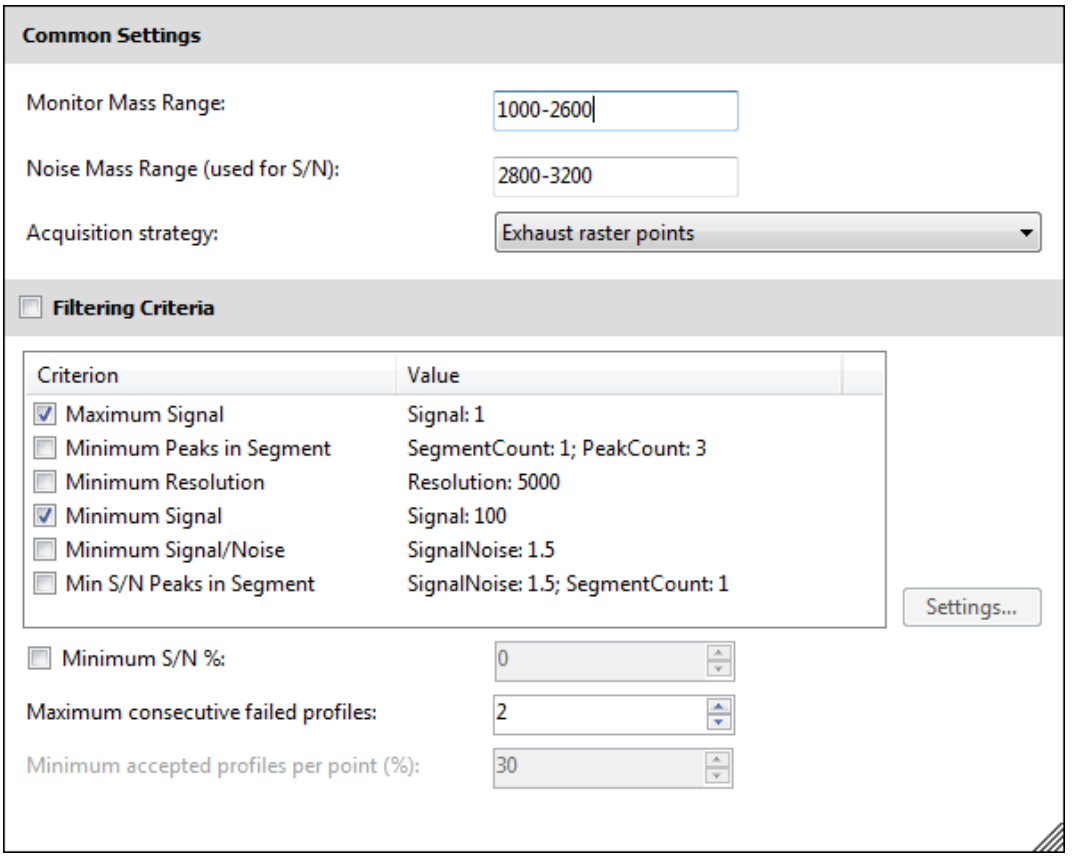
You can also **Pause** an acquisition, adjust the peak processing parameters, and **Resume** the acquisition.

The **Stop** button stops the acquisition, you cannot then resume the acquisition.

The **Standby** button switches off the laser and the high-voltage supplies.

Data quality

Introduction



Data quality is used in conjunction with rastering/dithering, see "Defining rastering" on page 50. It allows you to define the criteria to filter which profiles are kept and which are discarded.

To use this feature, you need to have prior knowledge of your data, usually from previous experiments where this feature was not used. You need to be able to anticipate what peaks you can expect and what criteria needs to be set to achieve your goals.

Common settings

Monitor mass range

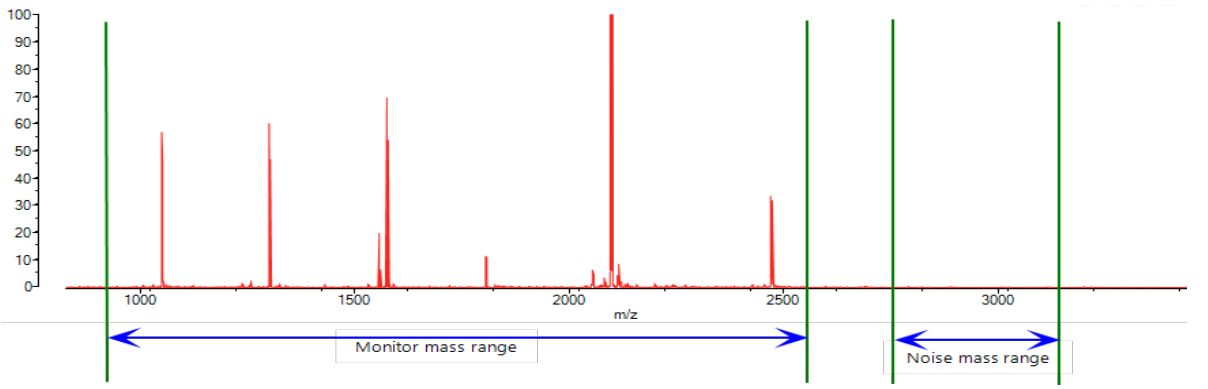
Enter the mass range (Daltons) - only peaks detected in this range will be compared with the filtering criteria, see diagram below.

Ensure that this range covers all the peaks of interest.

Noise mass range

Enter the mass range (Daltons) the criteria are to cover, see diagram below.

Usually, the noise mass range is an area outside of the monitor mass range. Ideally, the range should include only "chemical" noise.

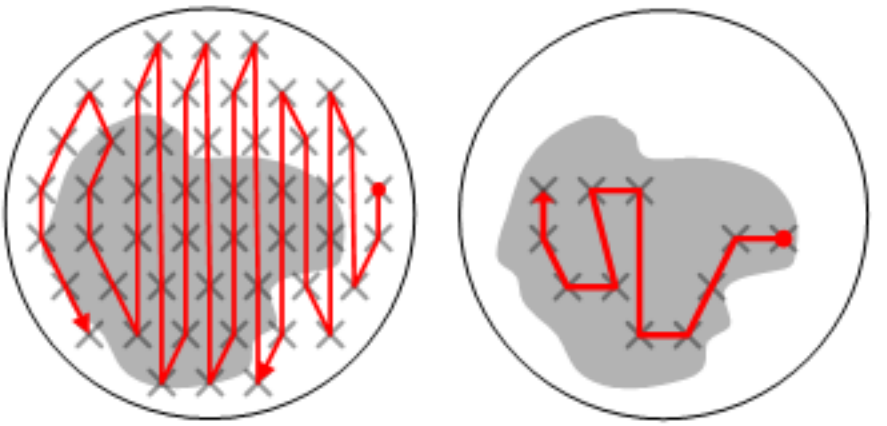


Acquisition strategy

There are two options:

- *Find sweet spots* (typically used to increase acquisition speed or to ensure even coverage of the sample spot);
- *Exhaust raster spots* (typically used to improve data quality where the sample contains matrix or salt crystals on the surface).

Find sweet spots



How the *Find sweet spots* strategy works:

1. The required number of profiles (as set in the **Profiles** field, see "Data quality" on page 65) are distributed between the available raster points. Software attempts to acquire the required number of profiles (pass 1).
2. Each profile is assessed against the **Filtering criteria**. Only those which meet the criteria are accepted.
3. Raster positions which failed to produce acceptable profiles are discarded; the remaining/outstanding number of profiles required to complete the acquisition are distributed amongst the remaining raster positions (that gave acceptable spectra in the

first pass) and a second pass of the sample is performed, with the laser only firing on the remaining raster positions (pass 2).

4. The profiles from the second pass are assessed against the filtering criteria. Raster positions are further filtered depending on whether they produced acceptable profiles.

This process continues until:

- there are no raster points remaining;
- the software has acquired the required number of profiles;
- the software has reached the maximum number of consecutive failures;
- the value in the field **Minimum accepted profiles per point (%)** is reached, see "Minimum accepted profiles per point (%)" on page 75.

Exhaust raster spots

Note: Using this method, throughput may be reduced.

How the *Exhaust raster spots* strategy works:

1. The laser fires on the raster point.
2. Each profile is analysed against the Filtering criteria. However, if the sample contains matrix or salt crystals on the surface, the profiles will fail the criteria and will be discarded, and the acquisition moves to the next available spot.
3. As the surface of the spot is ionised, profiles of the sample are obtained.

The process continues until:

- The software has acquired the required number of profiles;
- The software has reached the maximum number of consecutive failures and all available raster points have been exhausted.

Filtering criteria

To use this feature, select the adjacent check box.

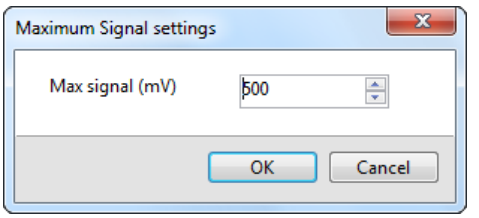
You may need to experiment to determine the optimal value(s).

For each criterion:

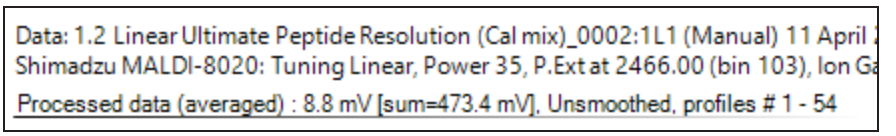
1. Click to highlight the criterion and click **Settings**; a dialog box is displayed.
2. Enter the required value(s).
3. Click **OK**; the dialog box closes.
4. Select the required check box to enable/disable the criterion.

A description of the criteria follows.

Maximum signal



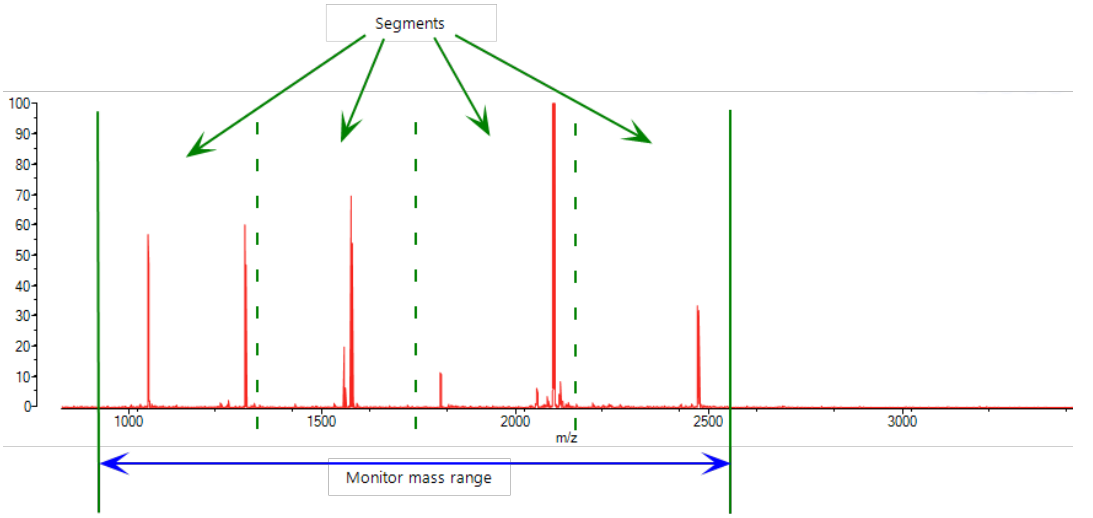
The signal of a displayed peak is shown in the spectrum:



If any part of the processed signal (within the **Monitor mass range** field) exceeds the setting, the profile is rejected.

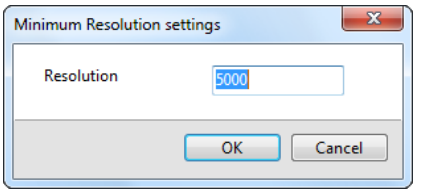
Minimum peaks in segment

The software divides the mass range (within the **Monitor mass range** field) into the specified number of segments.

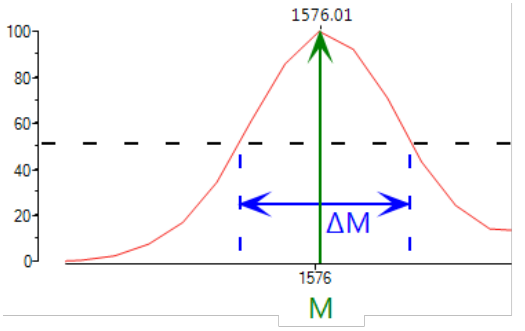


If the minimum number of labelled peaks within each segment is not achieved, the profile is rejected.

Minimum resolution



The resolution is a mathematical description of the shape of the peak:



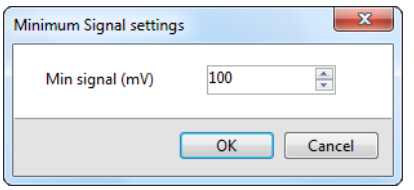
The formula is:

Resolution = M/ΔM

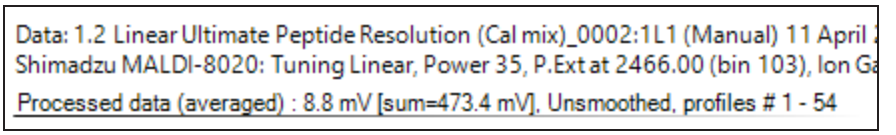
where M = mass and ΔM (dMass) = mass range at 50% intensity of the peak.

If a peak (within the **Monitor mass range** field) does not exceed the setting, the profile is rejected.

Minimum signal



The signal of a displayed peak is shown in the spectrum:

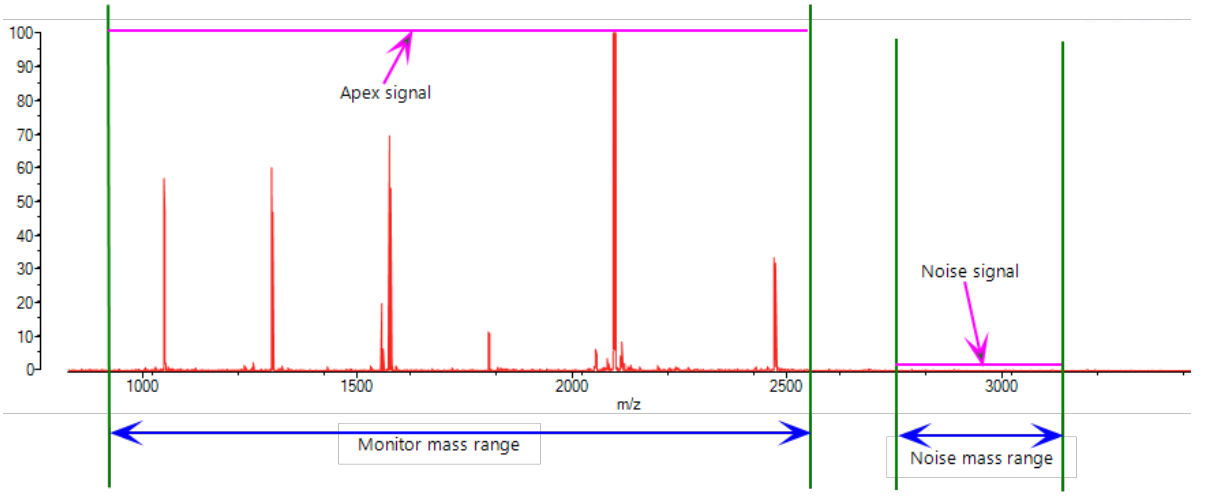


If any part of the processed signal (within the **Monitor mass range** field) does not exceed the setting, the profile is rejected.

Minimum signal/noise

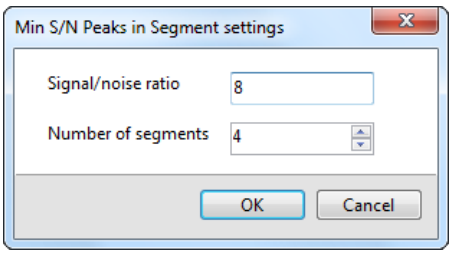
The signal-to-noise (S/N) is a ratio =

$$\frac{\text{Apex signal (within the } \textbf{Monitor mass range} \text{ field)}}{\text{Noise signal (within the } \textbf{Noise mass range} \text{ field)}}$$

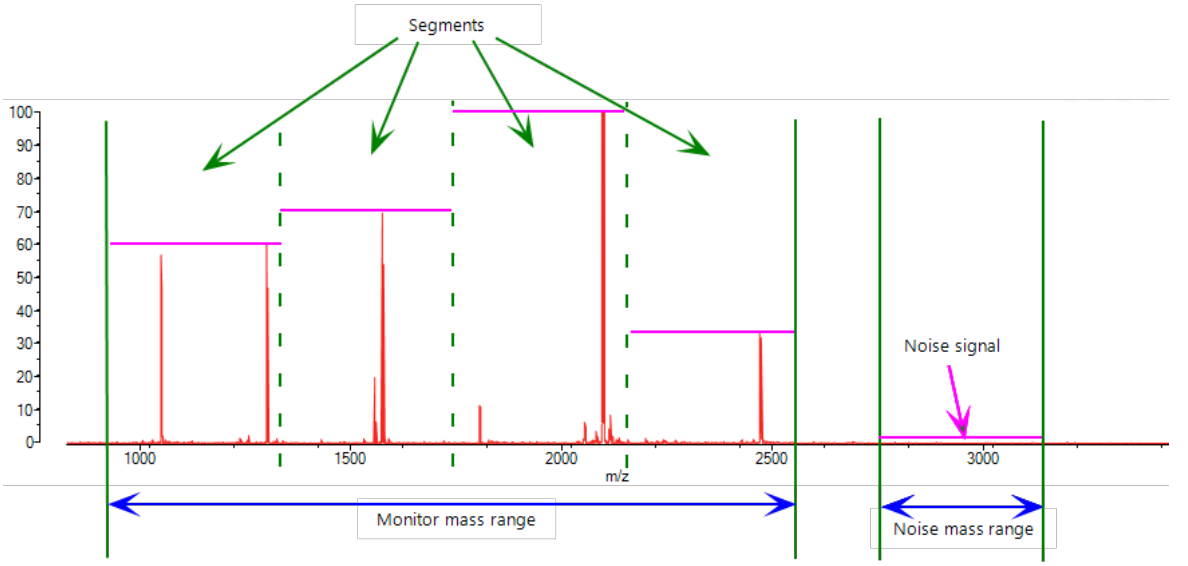


If the minimum ratio is not achieved, the profile is rejected.

Min S/N peaks in segment



The software divides the mass range (within the Monitor mass range field) into the specified number of segments.



Within each segment, the highest intensity peak (Apex signal) are identified. The profile is accepted if the highest intensity peak in each segment has a signal/noise (S/N) ratio equal to, or greater than, the specified value.

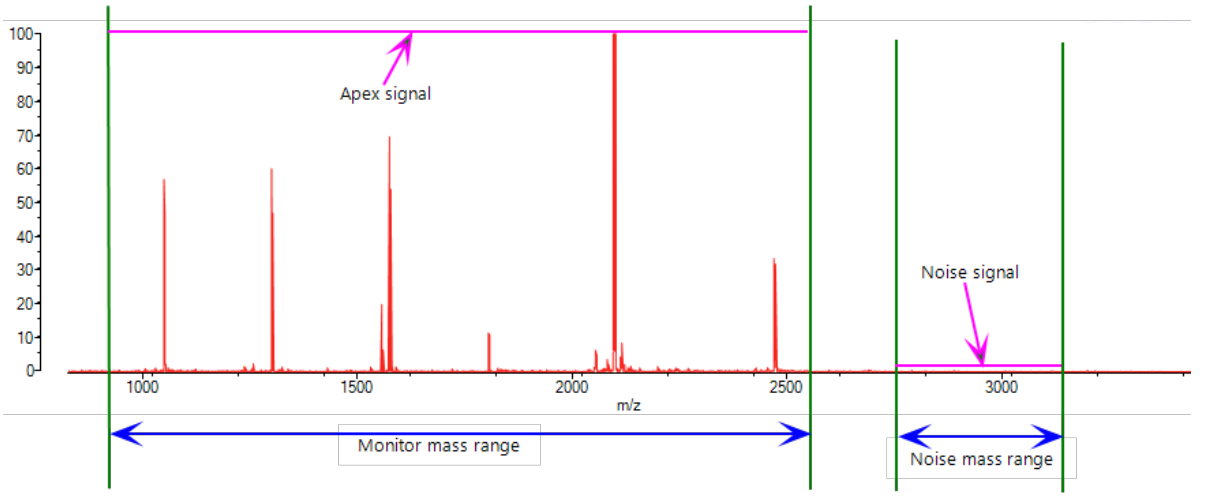
The signal-to-noise (S/N) is a ratio=

$$\frac{\text{Apex signal (within the segment)}}{\text{Noise signal (within the } \textbf{Noise mass range} \text{ field)}}$$

Minimum S/N %

The signal-to-noise (S/N) is a ratio=

$$\frac{\text{Apex signal (within the } \textbf{Monitor mass range} \text{ field)}}{\text{Noise signal (within the } \textbf{Noise mass range} \text{ field)}}$$



The software takes the first profile and calculates the "spectrum quality" where:

$$\text{"spectrum quality"} = \frac{\text{S/N x user setting}}{100}$$

The S/N values of subsequent spectra are compared to the "spectrum quality" value.

Example:

For a user setting of 20% and the first profile having a S/N value of 10, "spectrum quality" = (10 x 20)/100 = 2

- Subsequent spectra that have S/N values of 2 or <2 are rejected.
- Subsequent spectra that have S/N values >2 are accepted.

The initial **Minimum S/N %** value is updated only after consecutive failures.

Maximum consecutive failed profiles

The value specified here determines when the software abandons a poor spot. If the software rejects the specified number of profiles in a row, it moves onto the next spot.

Minimum accepted profiles per point (%)

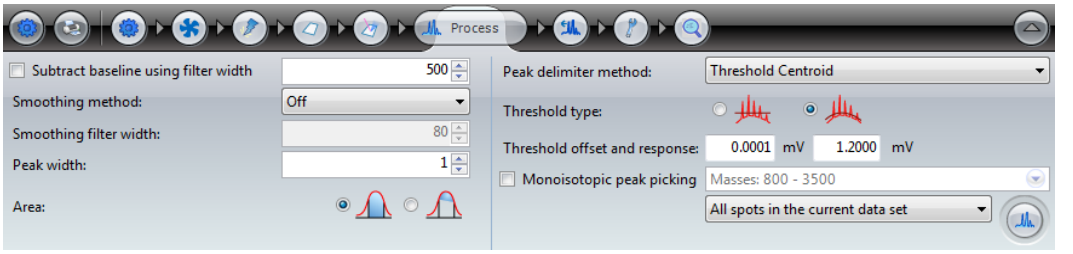
Only applicable if the **Acquisition strategy** is set to **Find sweet spots**.

The software rejects raster points which have failed to provide a certain percentage of good profiles. The percentage can be customised using this setting.

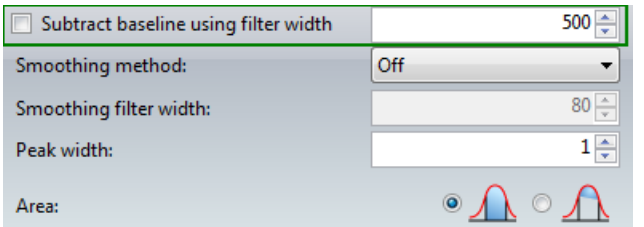
Processing a spectrum

After you have acquired a spectrum, you can apply processing parameters to label peaks, subtract baseline, smooth and/or apply monoisotopic peak picking.

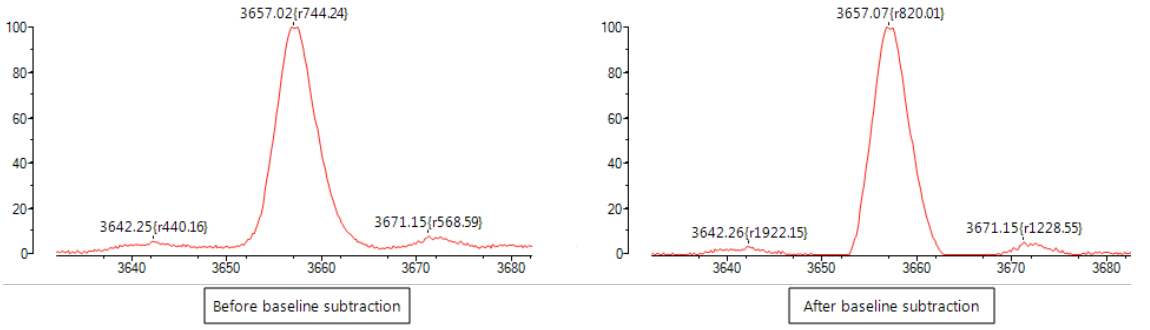
Click the **Process** tab to display the process parameters:



Subtract baseline using filter width



Using a high laser power and/or "chemical" noise (from the sample/matrix) can result in the spectrum not being resolved to the baseline. The **Subtract baseline using filter width** feature allows you to move the baseline so that the spectrum rests on the x-axis, and improves the signal-to-noise ratio and resolution of the peaks.

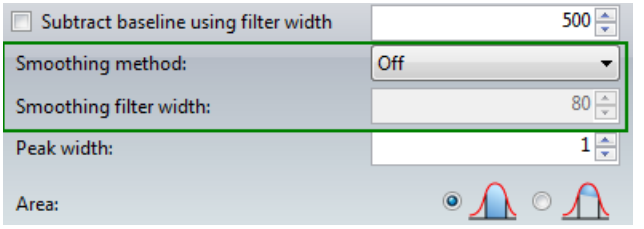


This field defines how high the new baseline is above the x-axis; use the following table for guidance:

Application	Suggested values
Proteins (>20 kDa)	Not selected
Proteins (<20 kDa)	30 to 300
Peptides	3 to 15
Small molecule	3 to 15
Polymers	30 to 500

Select the checkbox to turn the feature on and enter a baseline filter width.

Smoothing method & Smoothing filter width

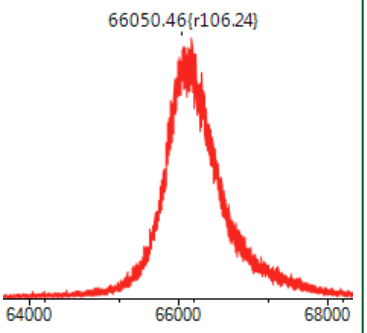
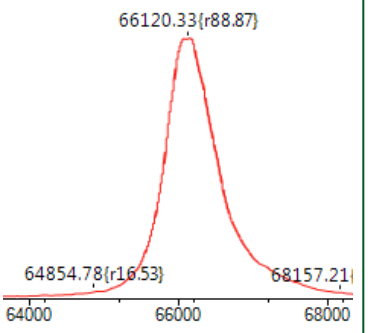
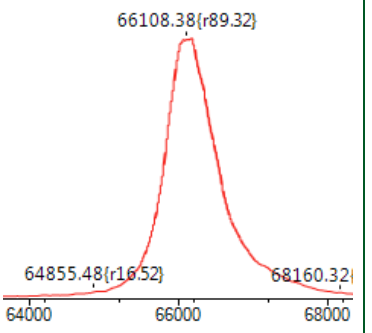


The two fields are used together.

Smoothing reduces the "spikiness" of data caused by background electronic noise. Two mathematical models are available to smooth the peaks:

- Gaussian
- Savitsky-Golay

The effect of these smoothing methods is shown below:

Off - raw data	Gaussian	Savitsky-Golay
		

Gaussian

Gaussian smoothing produces a characteristic symmetric "bell curve" shape that quickly falls off towards plus/minus infinity.

Savitsky-Golay

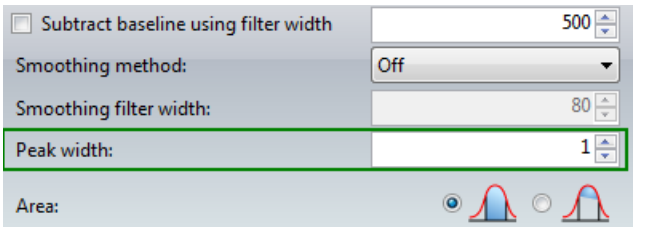
Savitsky-Golay smoothing tends to preserve features of the distribution such as relative maxima, minima and width, which are usually "flattened" by other models.

Smoothing filter width

Use the **Smoothing filter width** field to increase/decrease the amount of smoothing. Generally, the higher the number, the greater the amount of smoothing. Use the following table for guidance:

Application	Suggested values
Proteins (>20 kDa)	10 to 200
Proteins (<20 kDa)	3 to 30
Peptides	1 to 5
Small molecule	1 to 5
Polymers	3 to 50

Peak width

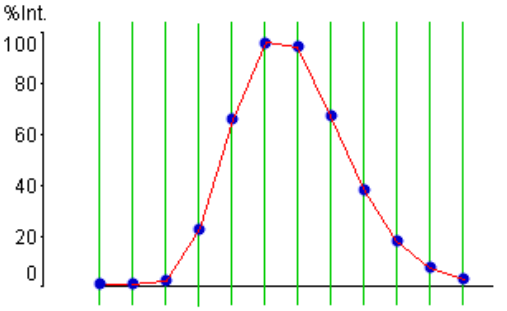


The **Peak width** field allows you to specify the number of channels that are used in the peak calculation. A value of 1 means that all peaks will be labelled, even spikes caused by noise. Therefore, the higher the number of channels you set, the fewer (but significant) number of peaks labelled.

As a guide, a peak width between 5 to 10 channels will result in only significant peaks being labelled.

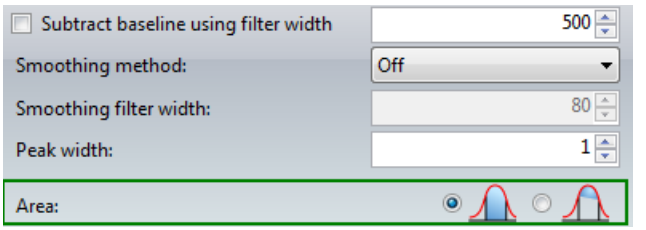
Channel (bin)

Although you see the spectrum as an analogue trace, it is actually made up of thousands of points joined together by straight lines:



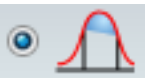
Each point (blue) is a value on a channel (green). Channels are also known as "bins".

Area



The **Area** feature defines the peak area used to calculate the position of the peak.

 Calculates the peak area down to the baseline.

 Calculates the area using the limits of the peak.

You may need to experiment to identify which gives the best results for your samples.

Peak delimiter method

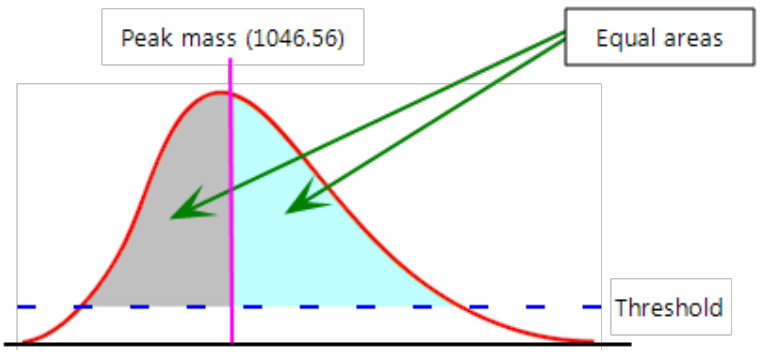
The software supports the following methods for determining the peak mass:

- Threshold Centroid;
- Threshold Centroid 25%;
- Threshold Apex;
- Gradient Centroid;
- Gradient Centroid 25%.

Threshold Centroid

The start and end points of a peak are defined by a constant threshold or an adaptive threshold,, see "Threshold type & Threshold offset and response" on page 85.

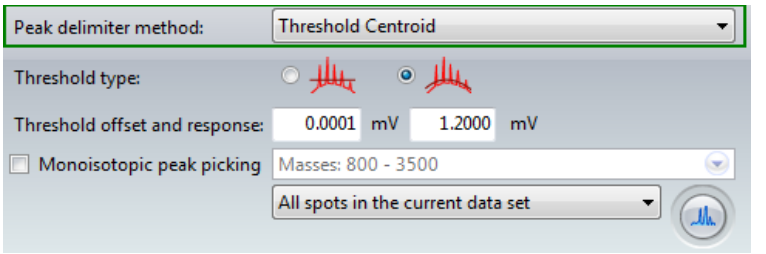
The peak mass is calculated using the area above the threshold. The area is then vertically divided into two equal areas; the peak mass is where the vertical line crosses the curve.

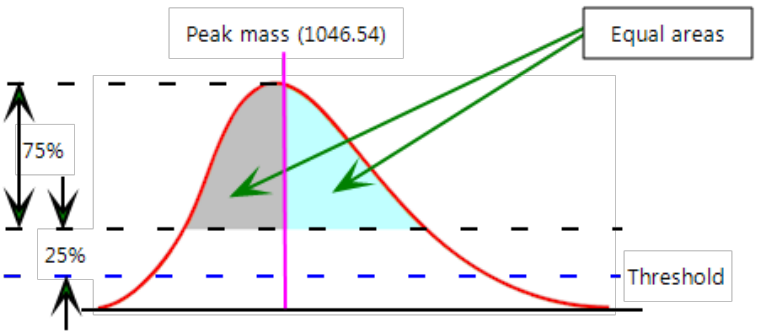


Threshold Centroid 25%

The start and end points of a peak are defined by a constant threshold or an adaptive threshold, see "Threshold type & Threshold offset and response" on page 85.

The peak mass is calculated using the area above the threshold; only the top 75% is used. The area is then vertically divided into two equal areas; the peak mass is where the vertical line crosses the curve.

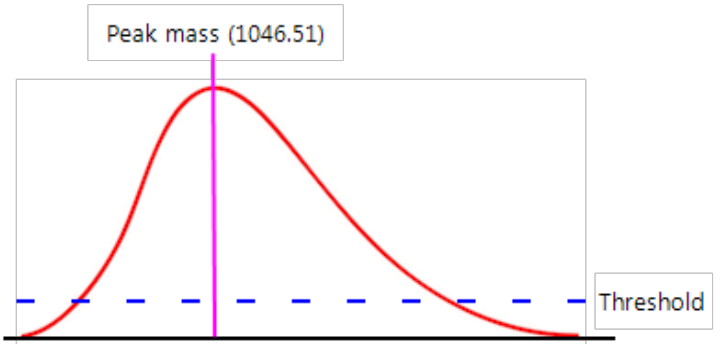




Threshold Apex

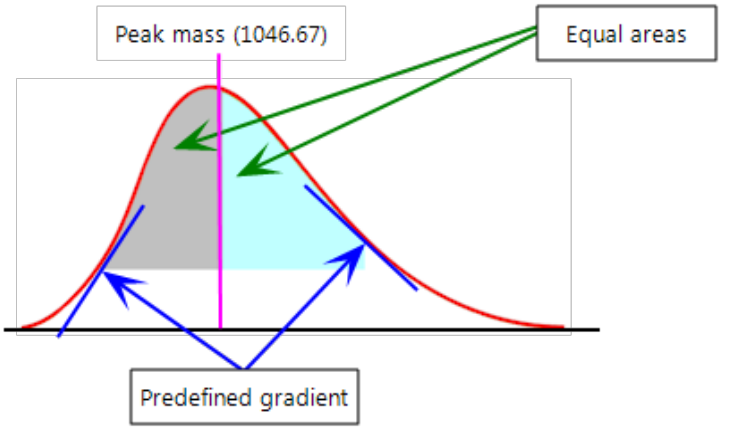
The start and end points of a peak are defined by a constant threshold or an adaptive threshold, see "Threshold type & Threshold offset and response" on page 85.

The peak mass is calculated as the highest point above the threshold.



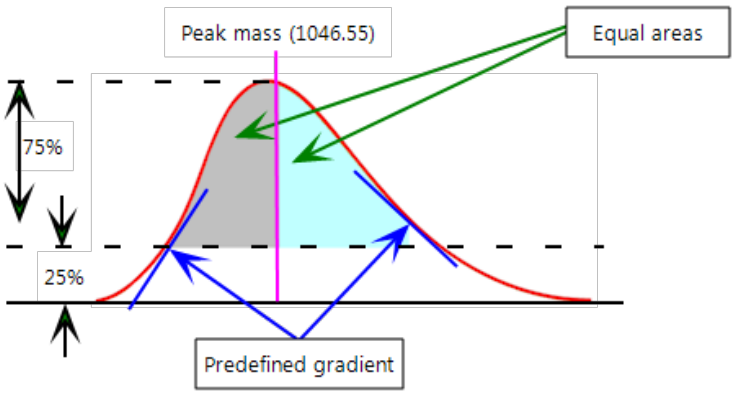
Gradient Centroid

The start and end points of the peak are determined by a predefined gradient. The peak mass is calculated using the area of the curve above the baseline and between the gradient start/end points. The area is then vertically divided into two equal areas; the peak mass is where the vertical line crosses the curve.



Gradient Centroid 25%

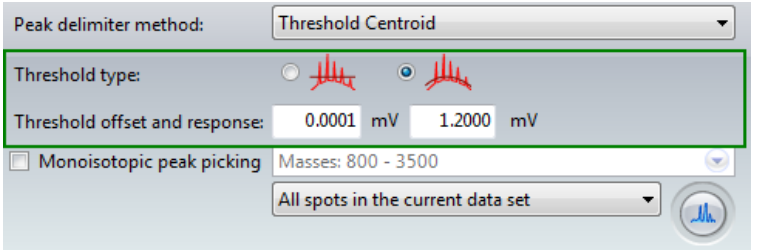
The peak start and end points are determined by a predefined gradient. The peak mass is calculated using the area of the curve above the baseline and between the gradient start/end points; only the top 75% is used. The area is then vertically divided into two equal areas; the peak mass is where the vertical line crosses the curve.



Guidance

- **Threshold apex**
  Use for symmetrical peaks, or highly smoothed data.
  Also used for high mass samples, >10,000 Da.
- **Threshold Centroid 25%**
  Gradient Centroid 25%
  Use for asymmetrical peaks; caused by adducts, modifications, etc. Typically used for peptides, low mass sample <10,000 Da.

Threshold type & Threshold offset and response

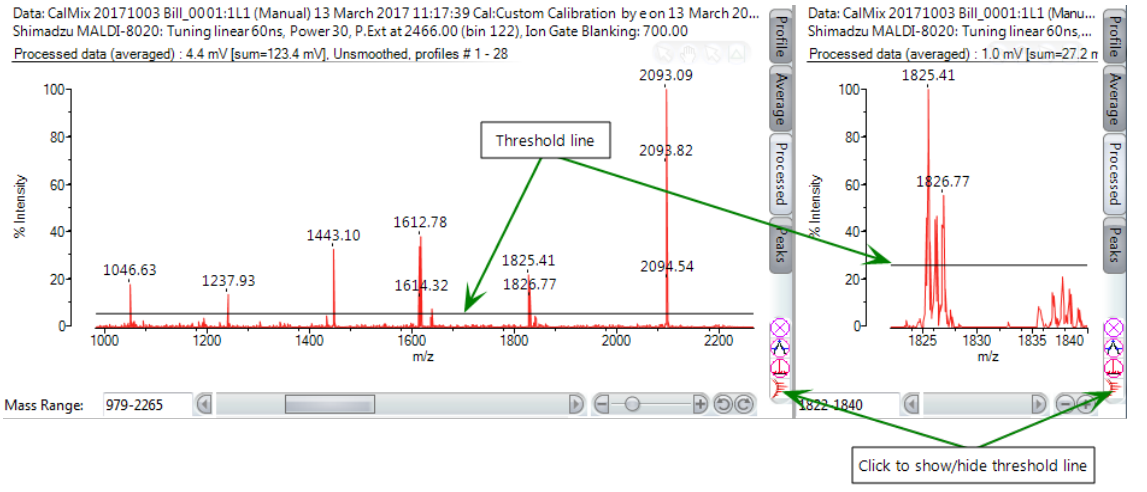


The two fields are used together.

Only available for the threshold peak delimiter methods.

Constant threshold

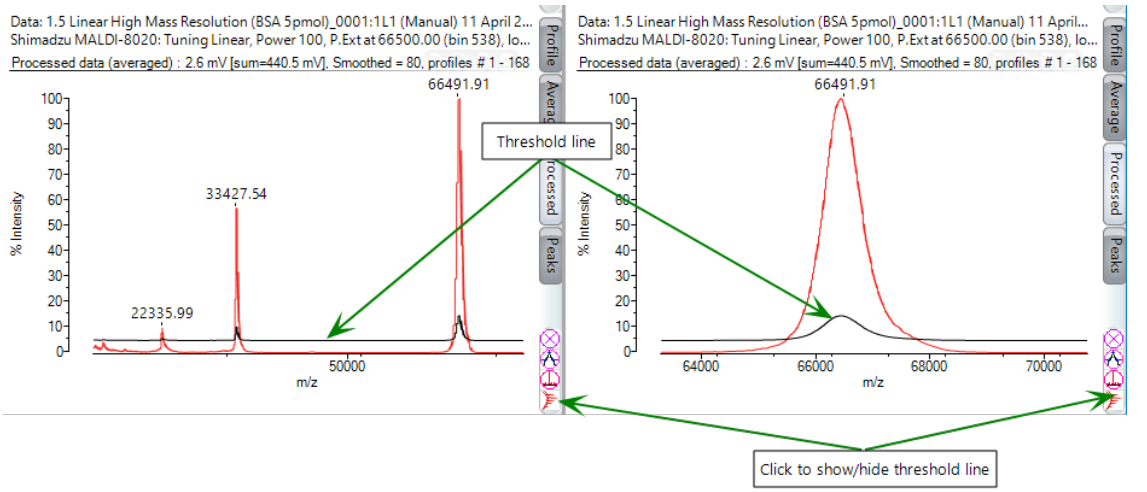
Constant threshold provides a horizontal line; peak masses above are labelled, peaks below are ignored.



Use the **Threshold offset** field to define the height of the threshold. You need to determine the required offset on a spectrum-to-spectrum basis.

Adaptive threshold

Adaptive threshold follows the signal noise; peak masses above are labelled, peaks below are ignored.



In the example above, the signal noise at the lower masses of the spectrum is very large and tapers off towards the higher masses. The threshold line is displayed in a darker colour.

Use the **Threshold offset** and **Response** fields to define the height and shape of the threshold. You will need to experiment with these values to find those that best fit your experiment.

Threshold offset and response

The **Threshold offset** field defines the height of the threshold above the baseline. Peaks above the threshold are included when the software determines the peaks, and peaks below are

ignored.

If you to set the offset to 0.0mV, the threshold would track the baseline of the spectrum. Set this field so that it is at least above the signal noise.

NOTE - If you enter a low threshold value, you may cause the PC to operate slowly as the software attempts to label most of the detected peaks, including noise.

The **Response** field is only available for the Adaptive threshold peak method. This field allows the threshold to track the spectrum. If you set the Threshold offset to 0 mV and the Response to 1, the threshold tracks the baseline of the spectrum.

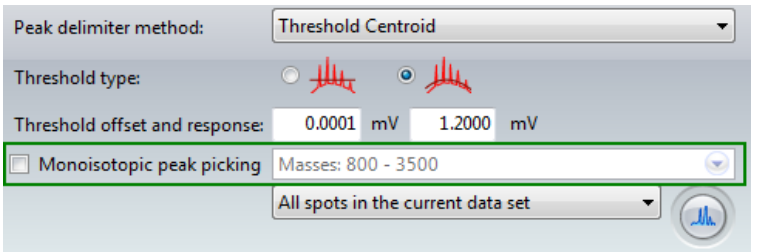
Increasing the **Threshold offset** and the **Response** values changes the shape of the adaptive threshold curve. You can set the noise to fall just below the curve, allowing the algorithm to identify peaks in the spectrum.

Guidance

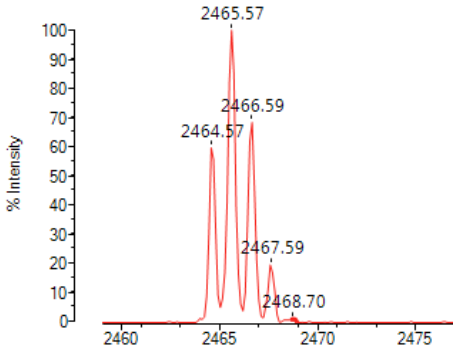
As a starting point, set:

- offset to 0.0 mV
- response between 1 mV and 2.5 mV.

Monoisotopic peak picking



Only applicable if the isotopes are discernible.



Generally, use this feature for experiments using peptides. Experiments with proteins do not usually use this feature.

The monoisotopic mass is the only peak used in this process. It uses the lowest mass containing only the most abundant isotopes, for example, ^{12}C, ^{14}N, ^{16}O.

Click the button

to display the options:

Minimum mass & Maximum mass

The default values (800 and 3,500 Da) are usually sufficient for most peptide experiments.

Minimum isotopes

Specifies the number of **additional** isotopes, n, that must contribute to a peak before it is to be considered as a candidate for Poisson modelling to determine a monoisotopic peak mass. As this field is "additional" the actual total number of isotopes is n+1.

Maximum intensity variation

This is a tolerance parameter that allows the isotope intensity to differ from its theoretical value by a specified percentage. Candidate isotope which are outside this parameter are

discarded.

Overlapping distributions

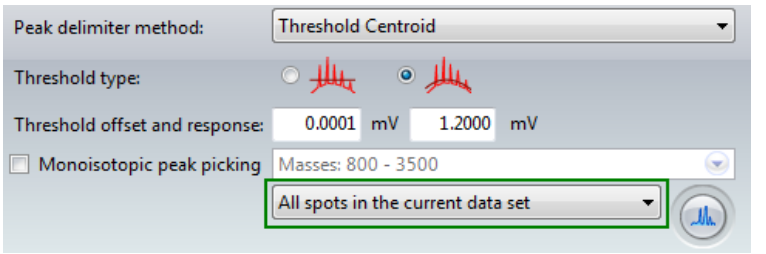
This is a check box, which if selected, permits the Poisson modelling to attempt to distinguish two overlapping isotopic clusters.

Reference: Breen E J, et al. Automatic Poisson peak harvesting for high throughput protein identification. Electrophoresis 2000 June 21 (11):2243–2251.

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.

All spots in data sets



Choose to apply the processing parameters to the current acquisition, or all the loaded acquisitions.

Applying the process parameters

Click the **Process** button

 to apply the parameters.

You can experiment with the parameters and select the button at any time to see the effect on the spectrum.

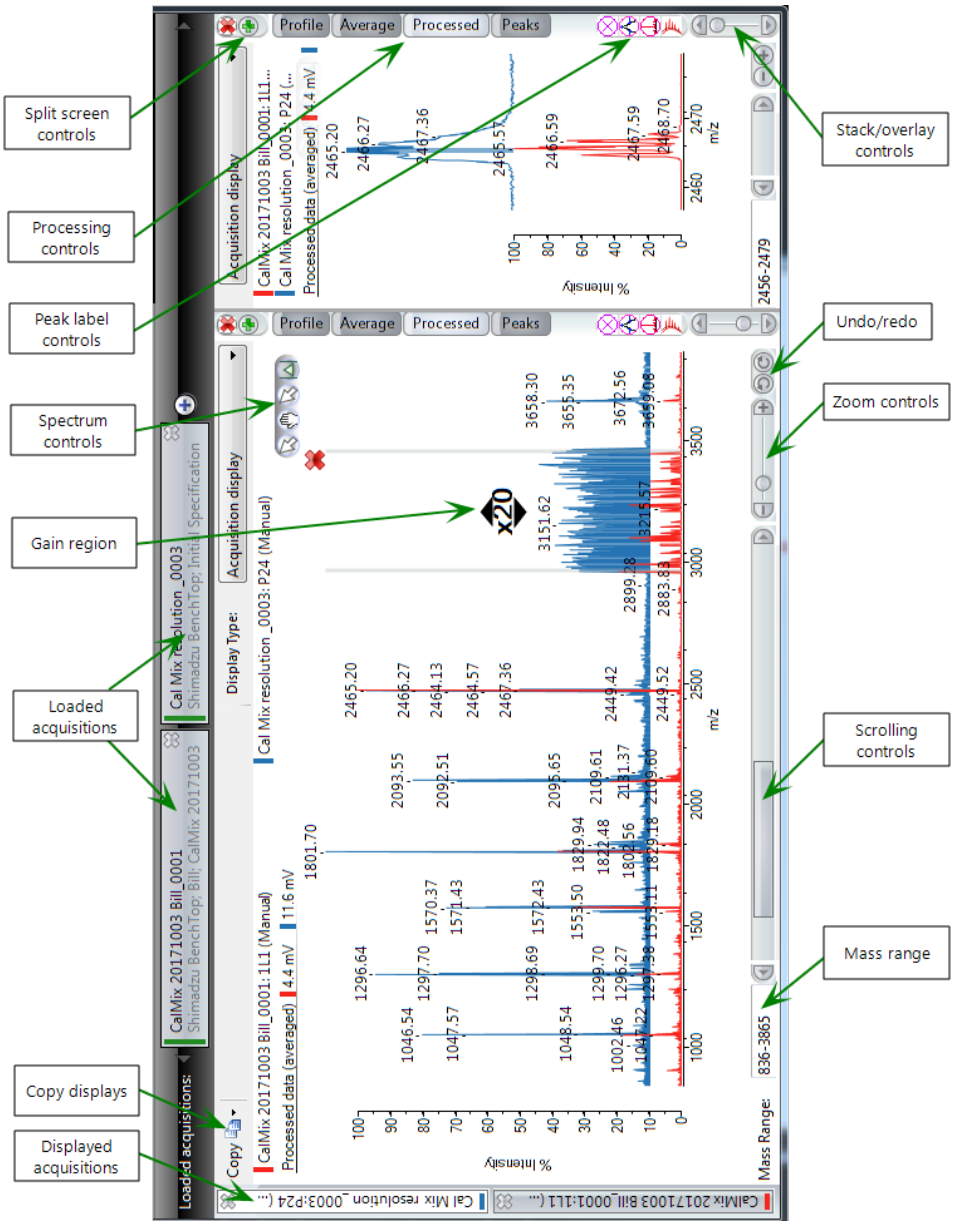
Spectra features

This section describes:

- Display features
- Loading, viewing and exporting acquisitions
- Labelling peaks
- Scrolling and zooming
- Showing baseline threshold data
- Analysing the spectrum
- Processing views
- Comparing spectra
- Split screen controls
- Profile controls
- Changing spectrum colours
- Copying displays
- Displaying mass lists

Display features

The feature shown in the diagram (on the next page) are described in the following sections.


Split screen controls
Processing controls
Peak label controls
Spectrum controls
Gain region
Loaded acquisitions
Copy displays
Displayed acquisitions
Stack/overlay controls
Undo/redo
Zoom controls
Scrolling controls
Mass range
Profile
Average
Processed
Peaks
Acquisition display
Display Type:
Cal Mix resolution _0003: P24 (Manual)
CalMix 20171003 Bill_0001: 1L1 (Manual)
Processed data (averaged)
Loaded acquisitions:
Copy
Mass Range:
836-3865
2456-2479
% Intensity
m/z
x20

Features - top

Features highlighted on the top of the image on the previous page are:

- Displayed acquisitions
- Copy displays
- Loaded acquisitions
- Gain region
- Spectrum controls
- Peak label controls
- Processing controls
- Split screen controls

Features - bottom

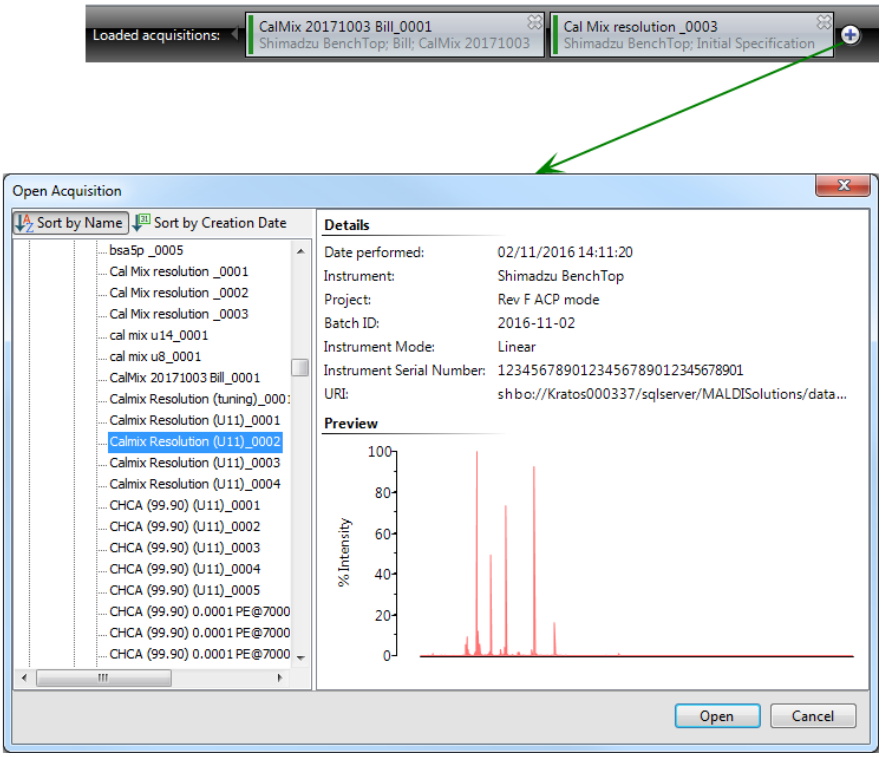
Features highlighted on the bottom of the image on the previous page are:

- Mass range
- Scrolling controls
- Zoom controls
- Undo/redo
- Stack/overlay controls

Loading, viewing and exporting acquisitions

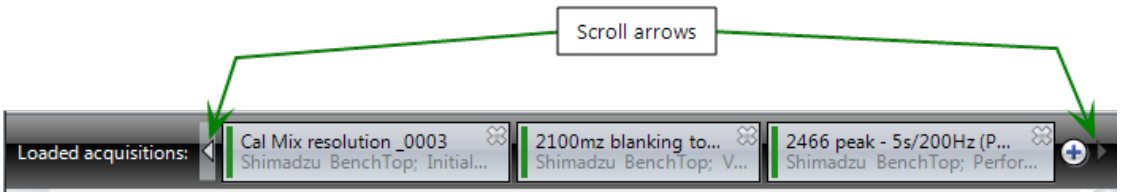
Loading an acquisition

You can load a saved spectrum (for example, from a previous experiment) to compare with current spectra.

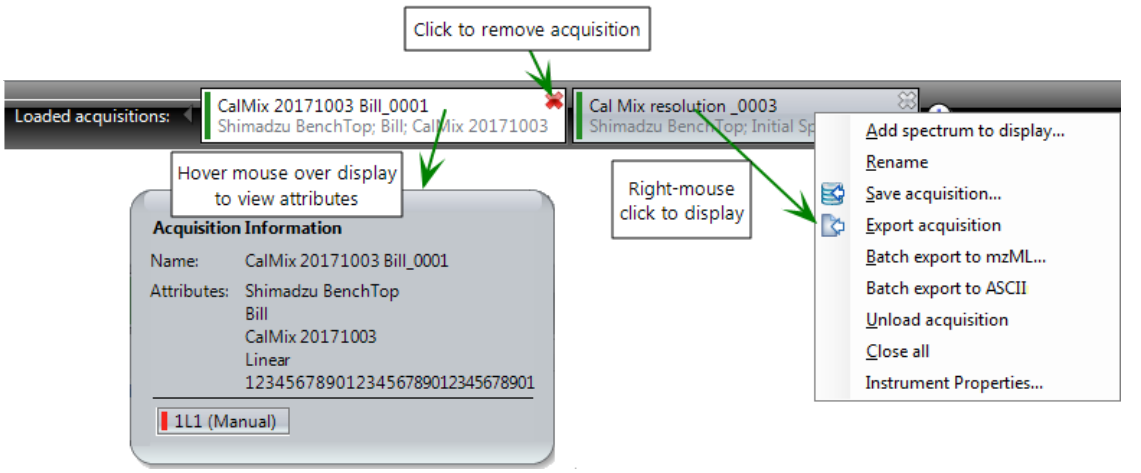


1. Click  .
2. From the **Open acquisition** window, navigate and select the required spectrum.

   If the number of loaded acquisitions exceeds the display, click the scroll arrows to view hidden acquisitions.



When a spectrum is loaded, you have many choices.



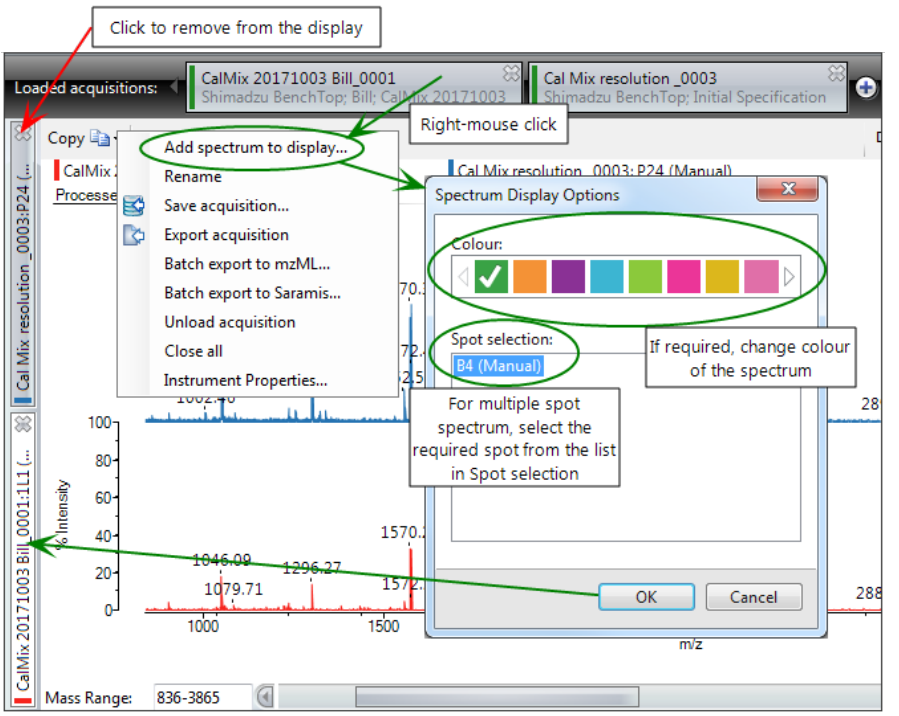
The right-mouse button displays a dialog window which allows you to:

- Add a spectrum to the display
- Rename the spectrum
- Save the acquisition
- Export the acquisition
- Batch export to mzML
- Batch export to ASCII

- Unload an acquisition
- Close all acquisitions
- View instrument properties

Displaying and removing displayed acquisitions

The following features allow you add and remove acquisitions from the display panel.



mV label

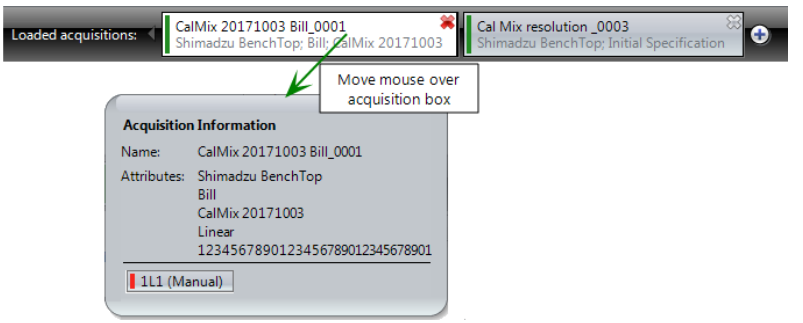
The mV (millivolt) label is primarily used by test and service engineers. This value is for the displayed spectrum; if you zoom in or out, this value will change.

If you are comparing two similar spectra, generally, the spectrum with the highest mV value is the better of the two. When comparing individual peaks, only use this value in conjunction with resolution and signal-to-noise values to determine the best peaks. "Labelling peaks" on page 105

Viewing and changing acquisition information

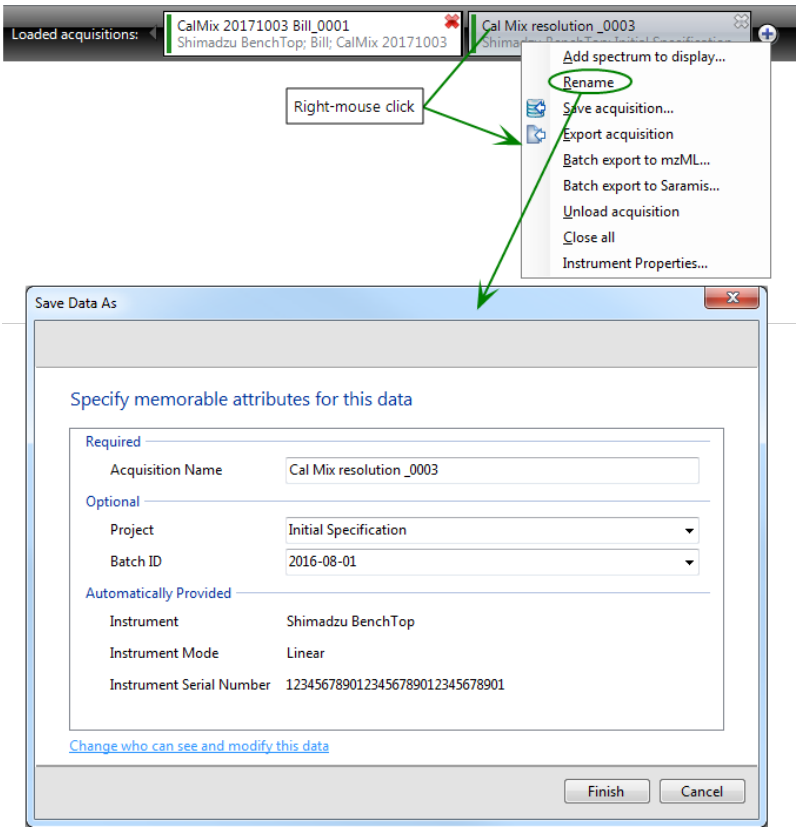
Viewing information

Acquisition information is displayed in a pop-up box.



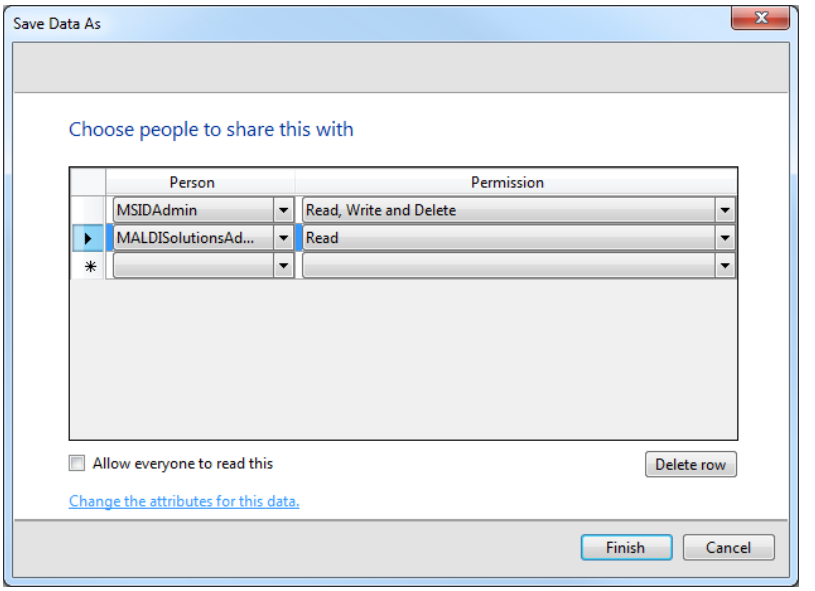
Changing information

You can rename and change optional attributes of the acquisition.



Amend the acquisition name and optional attributes as required.

To allow other users to see and optionally to modify your data, click the **Change who can see and modify this data link**:



 Indicates that the row is selected

 Indicates that the row is empty

To allow everyone to read your preferences, select the **Allow everyone to read this** check box.

To select a colleague, in the **Person** drop-down list, select the required name.

In the **Permissions** drop-down list, select the required permissions.

To delete a row, select it and click **Delete row**.

When done, click **Finish**.

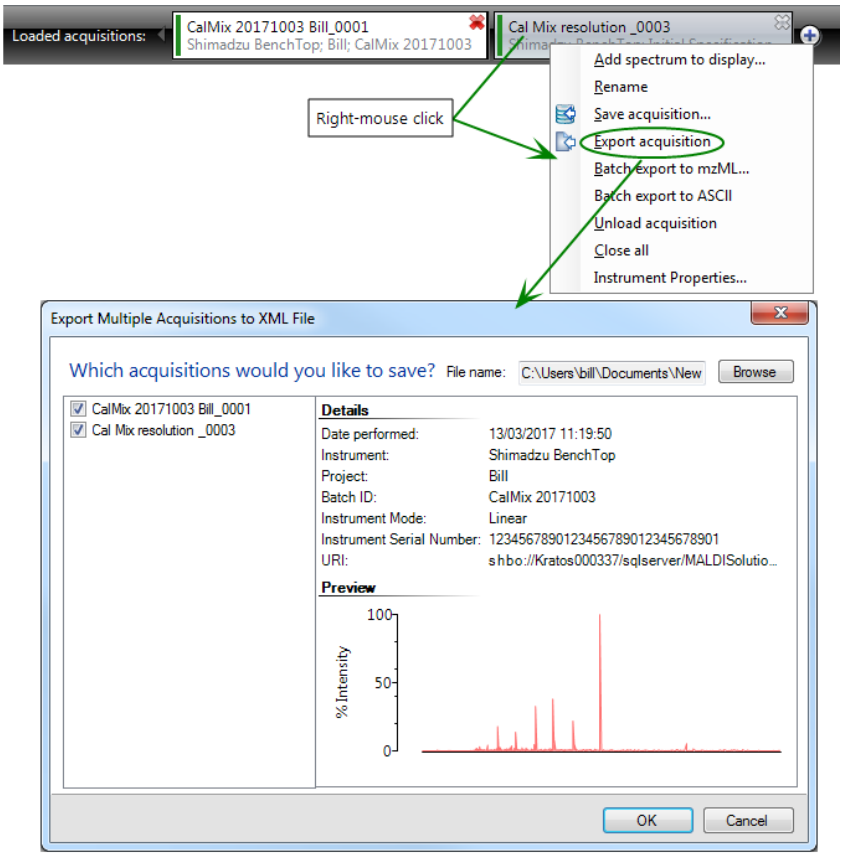
Saving an acquisition

The procedure is very similar to the previous procedure, see "Changing information" on page 100.

Exporting

Export acquisition

This feature allows you to export all, or specific displayed acquisitions to an XML file. You can only export an acquisition that has been saved.



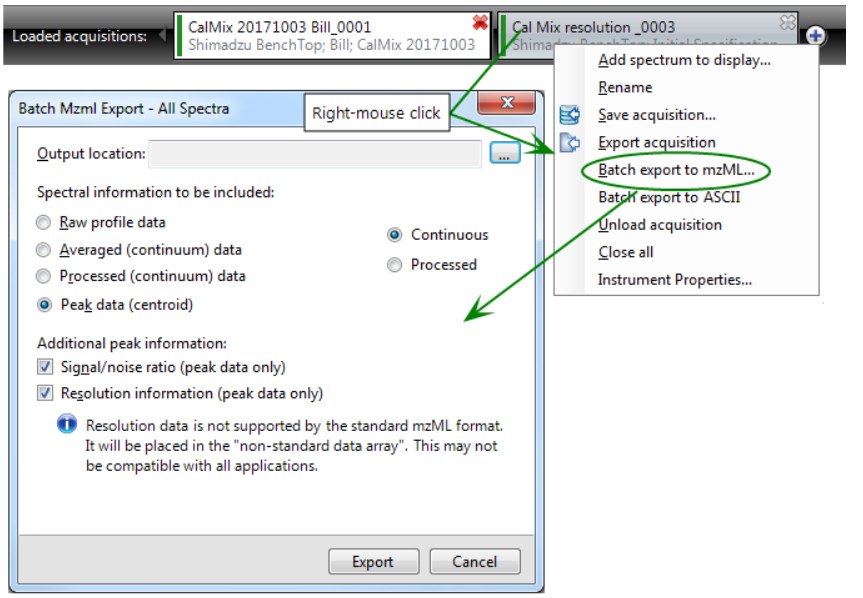
1. Click **Browse** and select the path and file name for the export.
2. Select, or clear, the check boxes for the required acquisitions.
3. Click **OK** to create a file.

```
<?xml version="1.0"?>
- <persistence schema-version="1.0.0.0">
  - <entity-type-dictionary>
    - <entity id="Foundation_IndexableType">
        <element data-type="String" name="BaseObjectIdentifier"/>
        <element data-type="String" name="DateDisplayValue"/>
        <element data-type="String" name="NameDisplayValue"/>
        <element data-type="String" name="TypeName"/>
        <element data-type="String" name="UserDisplayValue"/>
      </entity>
    - <entity id="Foundation_AllowedAttribute">
        <element data-type="String" name="AttributeName"/>
        <element data-type="Bool" name="AttributeRequired"/>
        <element data-type="Int" name="AttributeType"/>
        <element data-type="Bool" name="Deprecated"/>
        <element data-type="String" name="DisplayName"/>
        <element data-type="Guid" name="IndexableTypeID"/>
        <element data-type="Bool" name="UserAttribute"/>
      </entity>
      <entity id="Foundation_AlignmentRefs"/>
    - <entity id="Foundation_AlignmentRef">
        <element data-type="Guid" name="AlignmentRefsID"/>
        <element data-type="String" name="Name"/>
        <element data-type="Double" name="XStageAlignment"/>
        <element data-type="Double" name="XTargetAlignment"/>
        <element data-type="Double" name="YStageAlignment"/>
        <element data-type="Double" name="YTargetAlignment"/>
```

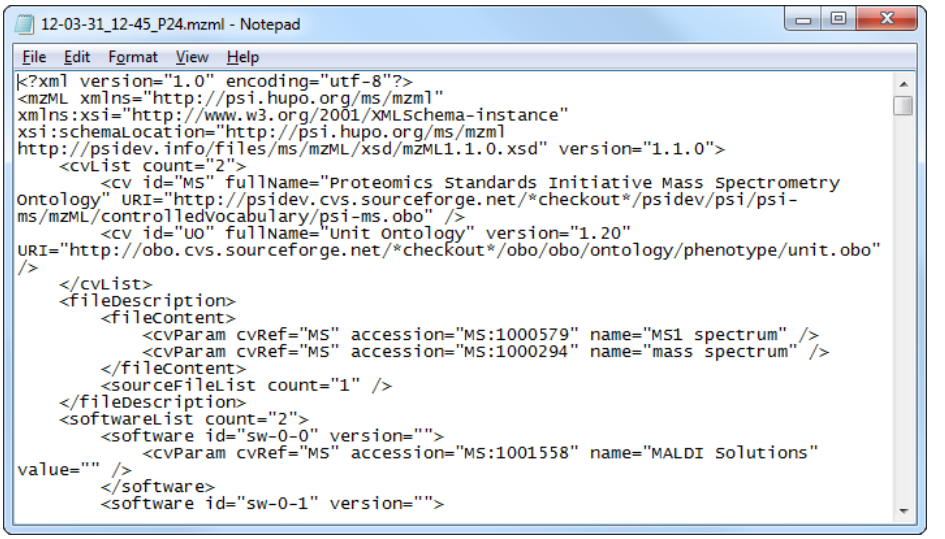
Example XML file

Batch export to mzML

This feature allows you to export the acquisition to mzML format.



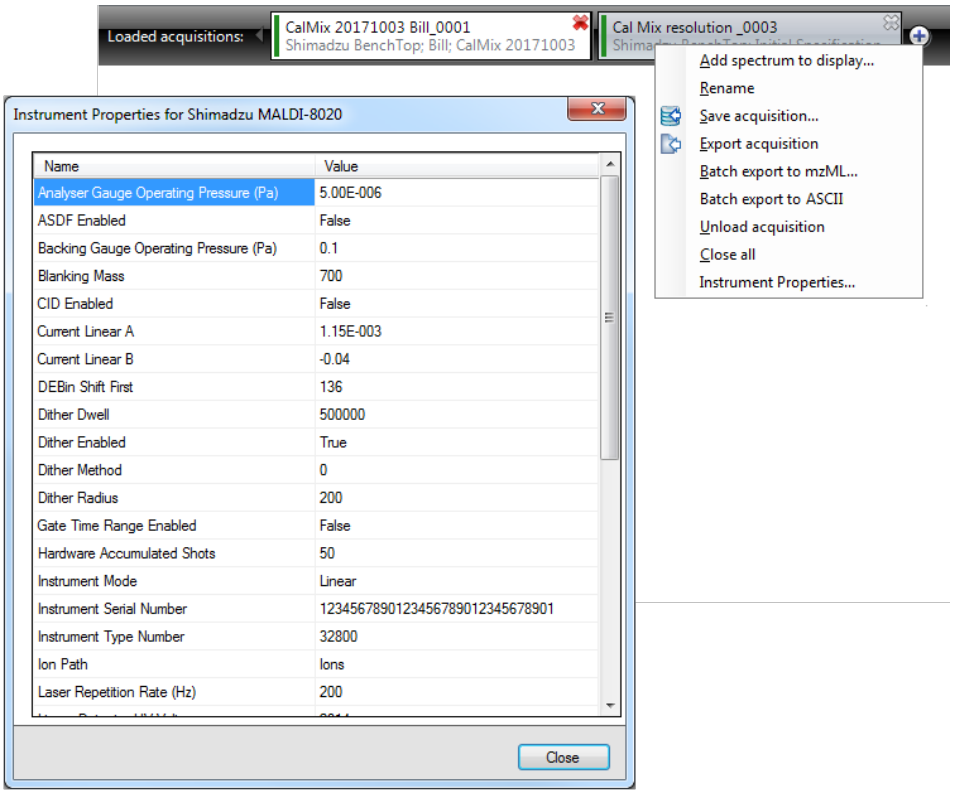
1. Click the browse icon  and define the folder for the export.
2. Select the required spectral information (radio button).
3. Select any additional required peak information (checkboxes).
4. Click **Export** to create a new file within the defined folder.



Example mzML file within Notepad

Instrument properties

The *Instrument Properties* window displays the parameters used by the instrument to acquire the spectrum.

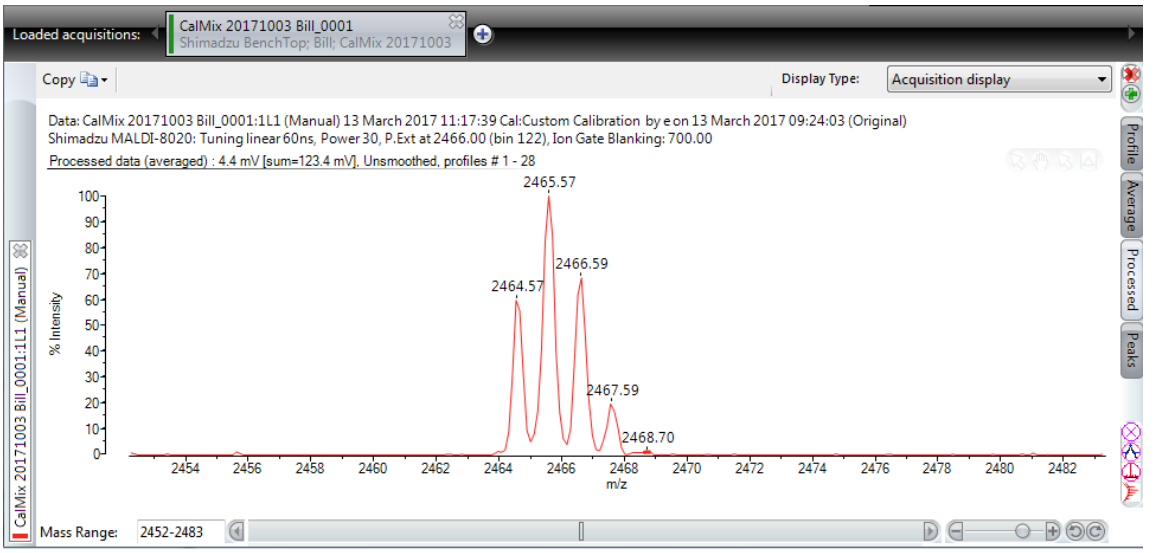


Labelling peaks

Assuming that the peak processing parameters are set correctly, in the processed spectrum, the peaks are automatically labelled.

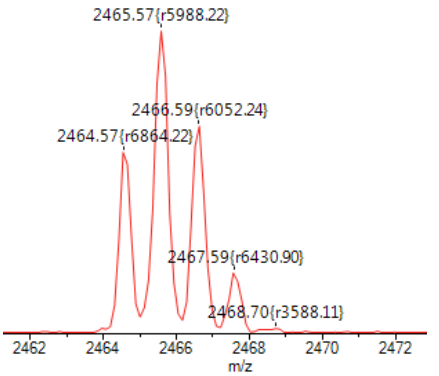
You can also include:

- Resolution, or
- Signal-to-noise ratio.



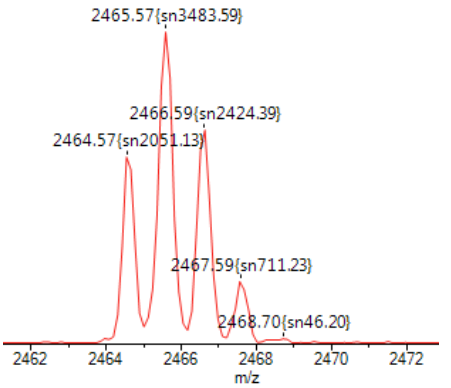
Resolution

Click the **Resolution** icon to display the resolution values alongside the peak values.



Signal-to-noise ratio

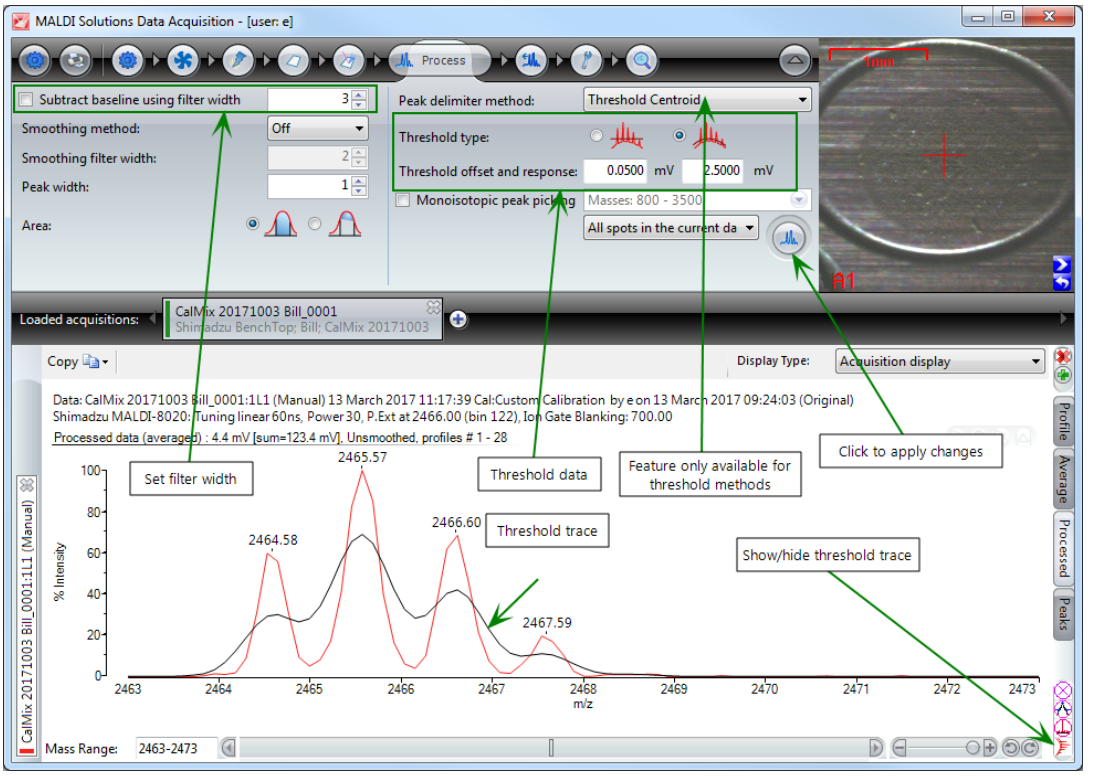
Click the **Signal-to-noise** icon to display the signal-to-noise ratio alongside the peak values.



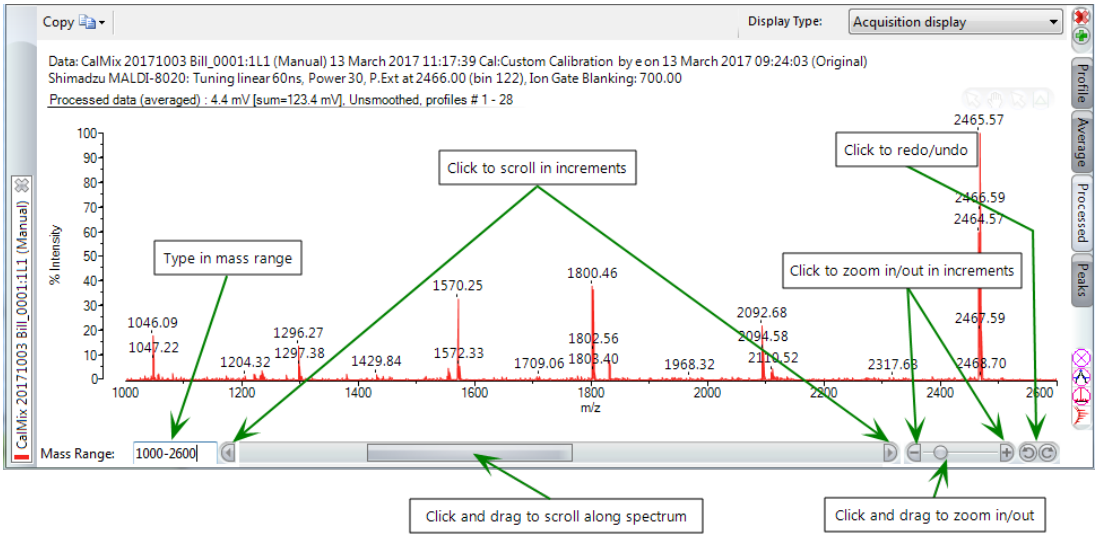
Showing baseline threshold data

If you have selected one of the **Threshold type** radio buttons in the processed spectrum, you can show the threshold trace.

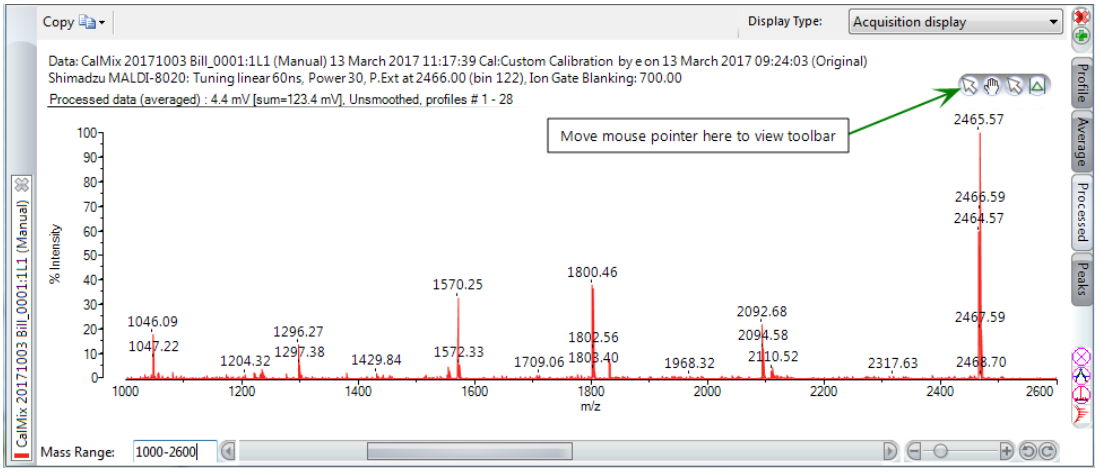
Click the threshold icon to show the threshold data, click again to remove it from the display.


MALDI Solutions Data Acquisition - [user: e]
Process
Subtract baseline using filter width
Smoothing method:
Smoothing filter width:
Peak width:
Area:
Peak delimiter method:
Threshold Centroid
Threshold type:
Threshold offset and response:
0.0500 mV
2.5000 mV
Monoisotopic peak picking
Masses: 800 - 3500
All spots in the current da
Loaded acquisitions:
CalMix 20171003 Bill_0001
Shimadzu BenchTop; Bill; CalMix 20171003
Copy
Display Type:
Acquisition display
Data: CalMix 20171003 Bill_0001:1L1 (Manual) 13 March 2017 11:17:39 Cal:Custom Calibration by e on 13 March 2017 09:24:03 (Original)
Shimadzu MALDI-8020: Tuning linear 60ns, Power 30, P.Ext at 2466.00 (bin 122), Ion Gate Blanking: 700.00
Processed data (averaged) : 4.4 mV [sum=123.4 mV], Unsmoothed, profiles # 1 - 28
Set filter width
Threshold data
Feature only available for threshold methods
Click to apply changes
Threshold trace
Show/hide threshold trace
2465.57
2464.58
2466.60
2467.59
% Intensity
m/z
Mass Range:
2463-2473
CalMix 20171003 Bill_0001:1L1 (Manual)
Profile
Average
Processed
Peaks

Scrolling and zooming



Analysing the spectrum



Spectrum controls toolbar

The display has a toolbar in the top right-hand corner that becomes visible when you move the pointer closer to it:

Icon	Description
	Peak tool - click and drag over a spectrum to zoom, for example, to examine peaks.
	Drag tool - click and drag to scroll the spectrum.
	Move peak label tool - click a peak mass label and drag it to the required location.

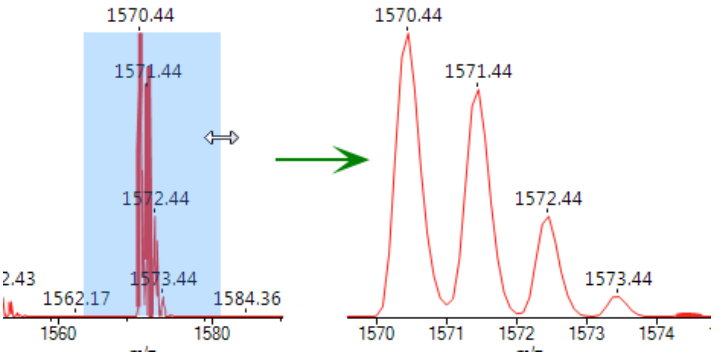
Icon	Description
	Define gain regions tool - click and drag the required region and set the required magnification.

Zooming on peaks/peak information

Zooming on peaks

Click the **Peak** tool on the toolbar:

1. Move the pointer to the start of the zoom.
2. Click and hold the left mouse button and drag the pointer to the end of the zoom:

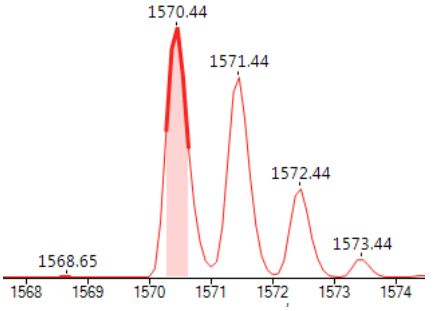


3. Release the mouse button; the spectrum zooms on the selected area.
4. Repeat the zoom to get the required display.

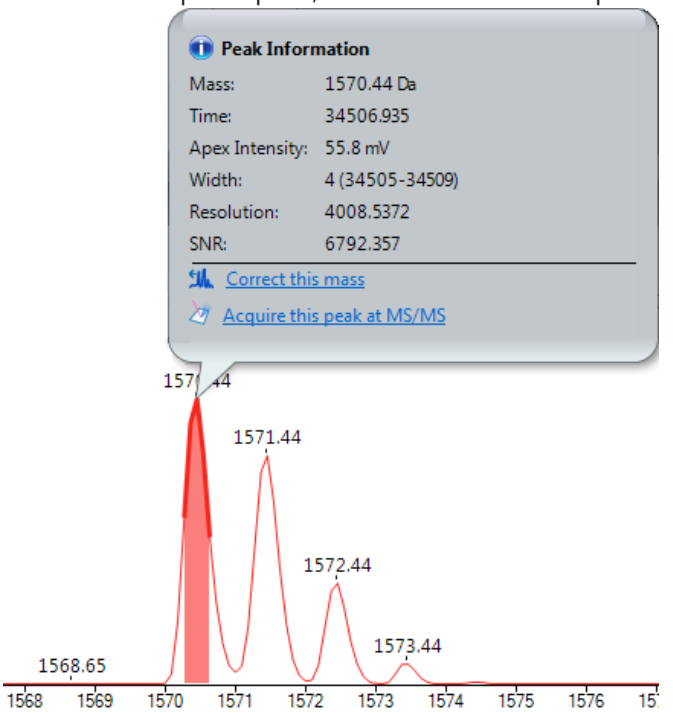
Displaying peak information

Click the **Peak** tool on the toolbar:

1. To highlight the area of a peak, move the pointer over the peak:



2. Click on the required peak; information about the peak is displayed:



Peak information:

Peak information	Description
Mass	Peak mass, in Daltons.
Time	Channel associated with the peak.
Apex intensity	Intensity in mV, see "mV label" on page 96.
Width	Width of the peak, in channels.
Resolution	Resolution of the peak.
SNR	Signal-to-noise ratio.

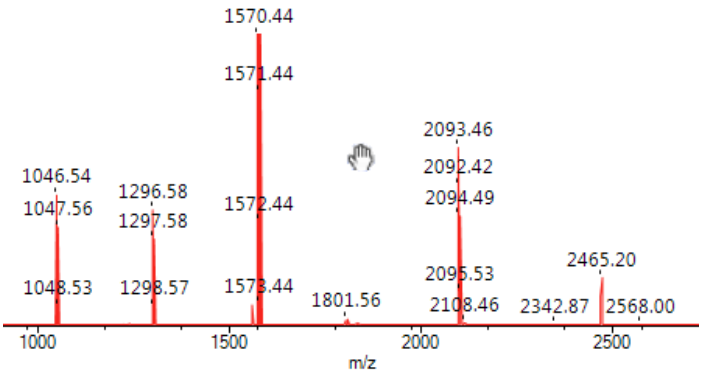
For details about correcting the peak mass, see "Calibration" on page 128.

Dragging the spectrum

1. Click the **Drag** tool on the toolbar:

   The mouse pointer changes to a hand:

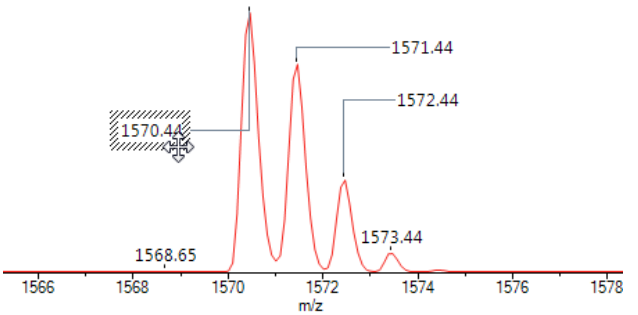
2. Press and hold the left mouse button.
3. Drag the spectrum to the required location and release.

4. To switch off the drag, click the **Peak** tool on the toolbar:


Moving peak labels

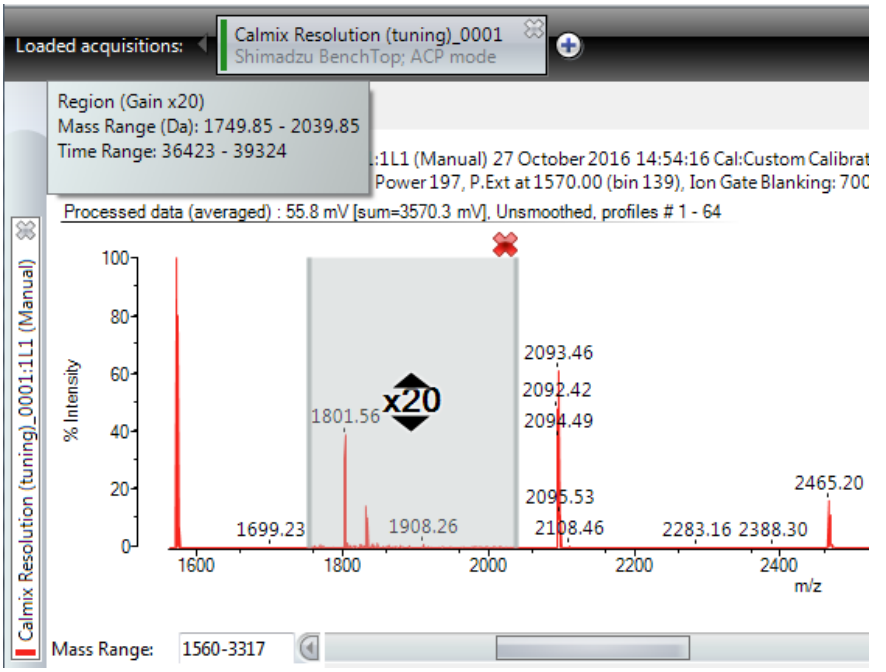
If, for example, a peak label is obscuring the spectrum, you can move the label; a dotted line references the peak.

1. Click the **Move** peak label tool on the toolbar:

2. Click the required spectrum label; it is bounded by a striped box.
3. Click and drag the label (pointer changes to an arrow-tipped cross) to the required location:


4. Repeat for any other labels.
5. To switch off, click the **Peak** tool on the toolbar:


Define gain regions

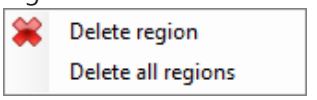


This feature allows you to select a mass range and increase its signal amplitude. You can specify several non-overlapping gain regions for a spectrum.

1. Select the Define gain regions tool from the toolbar:

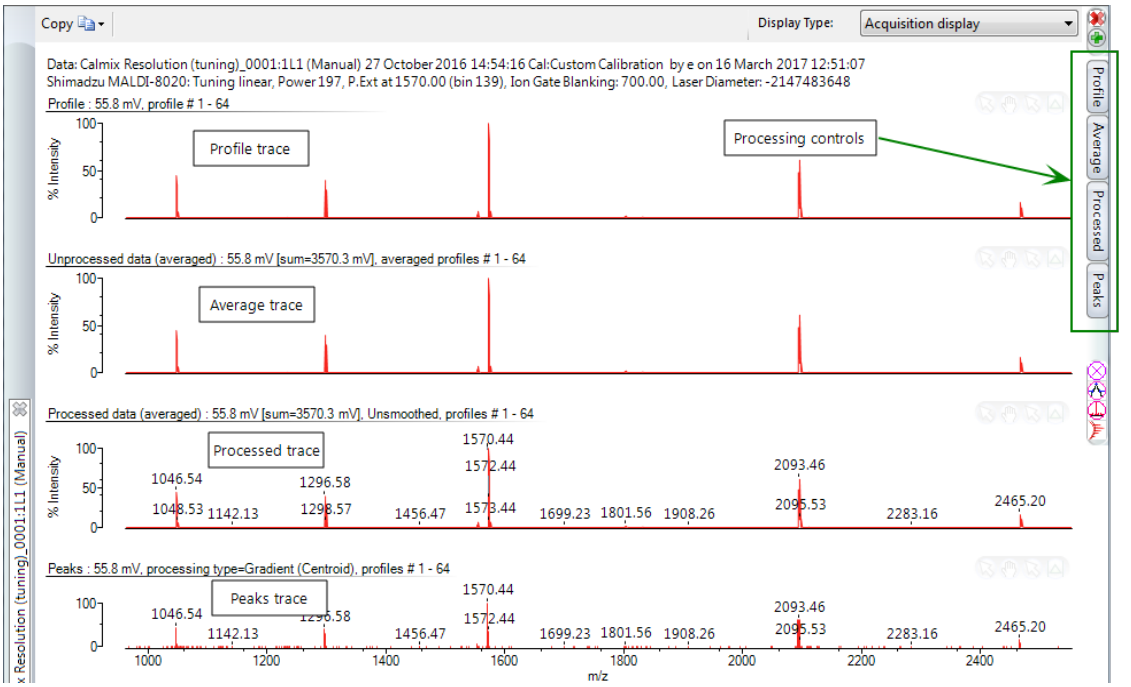
2. Click and drag the mouse pointer over the required region; the display will mark the selected region with a vertical bar at each end and a fill-colour between the bars.

3. Click the up and down arrows to alter the gain of the region. You can set: ×1, ×2, ×5, ×10, ×20, ×50, ×100 ... ×5,000.
4. Hover the mouse pointer over the region, the tooltip contains information about the range including start and end points in mass (Da) or time range (bins).
5. Adjust the width of the region by dragging the sides.
6. To remove the gain region either:
   - Click the remove icon  .
   - Right-mouse click and select either **Delete region** or **Delete all regions**.



Processing views

This feature allows you to view multiple viewpoints on the loaded data. You can also view several different mass ranges simultaneously.



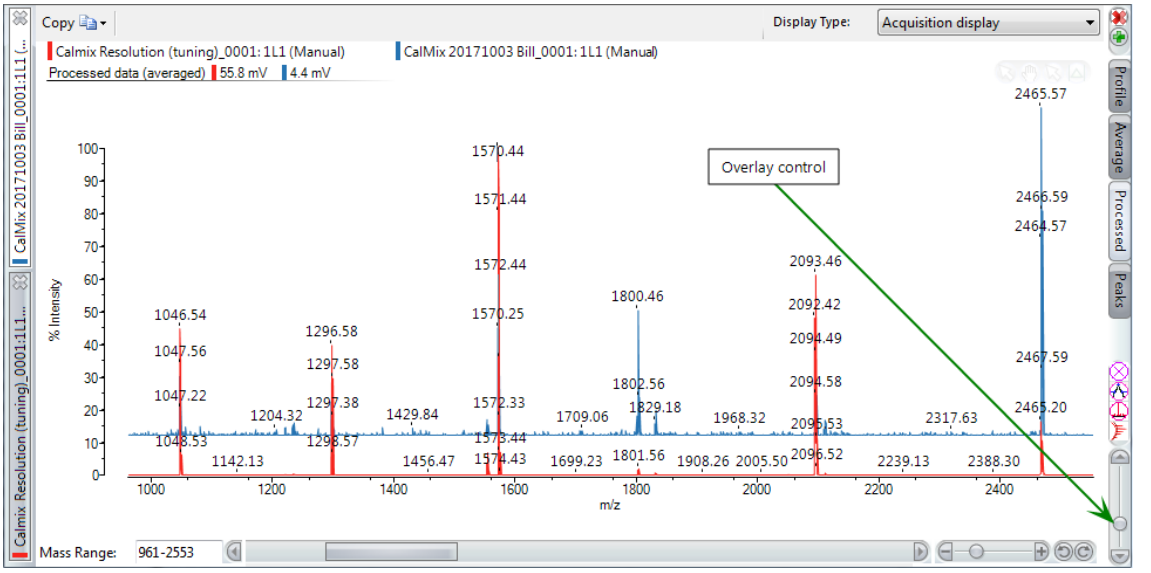
Select the required process buttons (usually the **Profile** and **Processed** profiles are used). The table below describes the buttons.

Feature	Function
Profile	Displays data collected from the sample for the last profile.
Average	Displays the average of all the profiles collected from the sample.
Processed	Displays the averaged data after the application of any smoothing, baseline subtraction, and peak detection to the data.
Peaks	Displays the centroid/apex mass peaks found in the processed data as sticks.

Comparing spectra

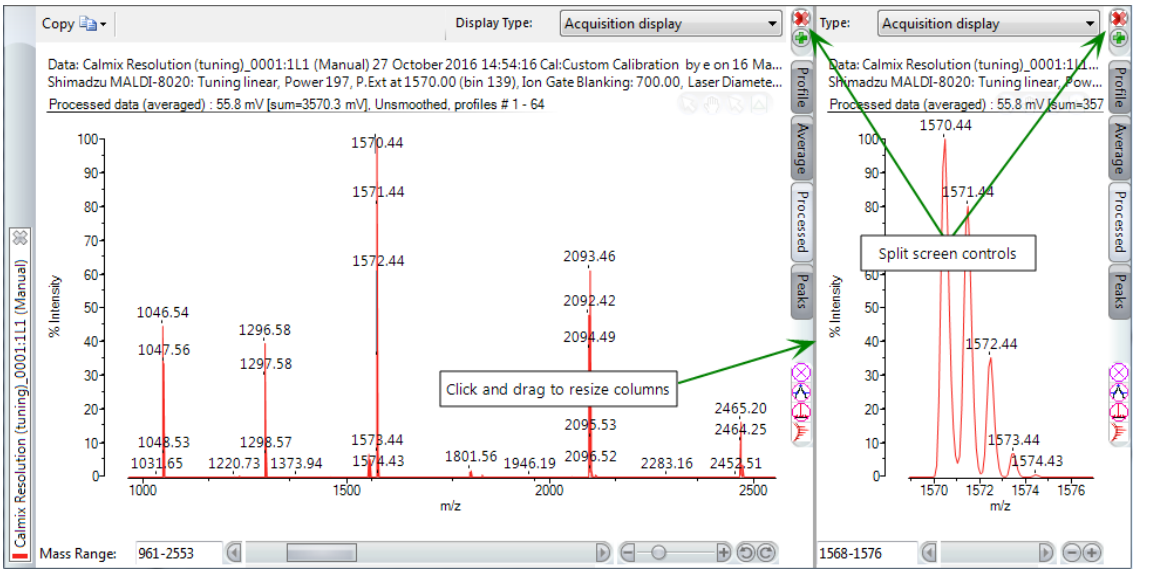
This feature allows you to display several spectra and overlay them so that you can compare them.

1. Display the required spectra, see "Loading an acquisition" on page 93.
2. On the right-hand side is the overlay control; move the slider up and down to change the views:



Split screen controls

Split screen controls allows you to, for example, focus on specific peaks while keeping the main spectrum visible.



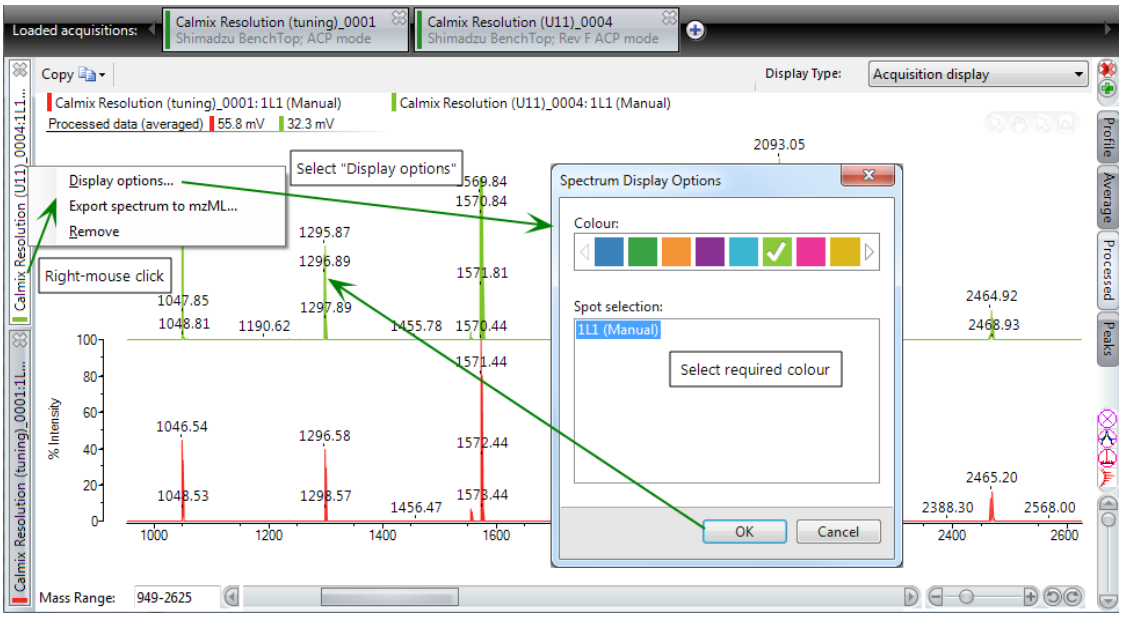
To add a column, click the split screen icon ; the display divides into two columns (realistically you can display up to 3 columns).

To remove a column, click the close screen icon .

To resize each column, drag the splitter bar between each column.

Changing spectrum colours

You can change the colours of the displayed spectra.

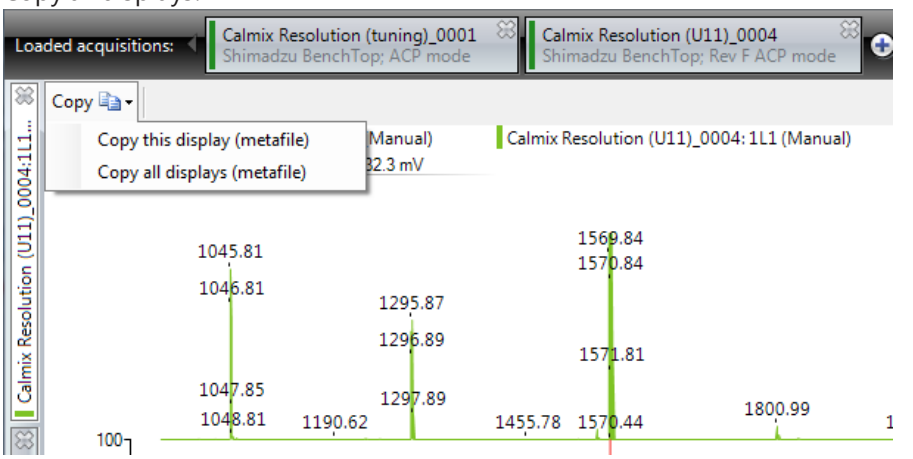


1. Right-mouse click in the required displayed acquisition box.
2. Select **Display options**.
3. Select the required colour of the spectrum.
4. For multiple spot spectrum, select the required spot.
5. Click **OK**.

Copying displays

You can copy an image of the spectra/spectrum to the clipboard for pasting into, for example, Word.

1. Display the spectra/spectrum for copying.
2. Click the icon, which displays two options:
   - Copy this display.
   - Copy all displays.



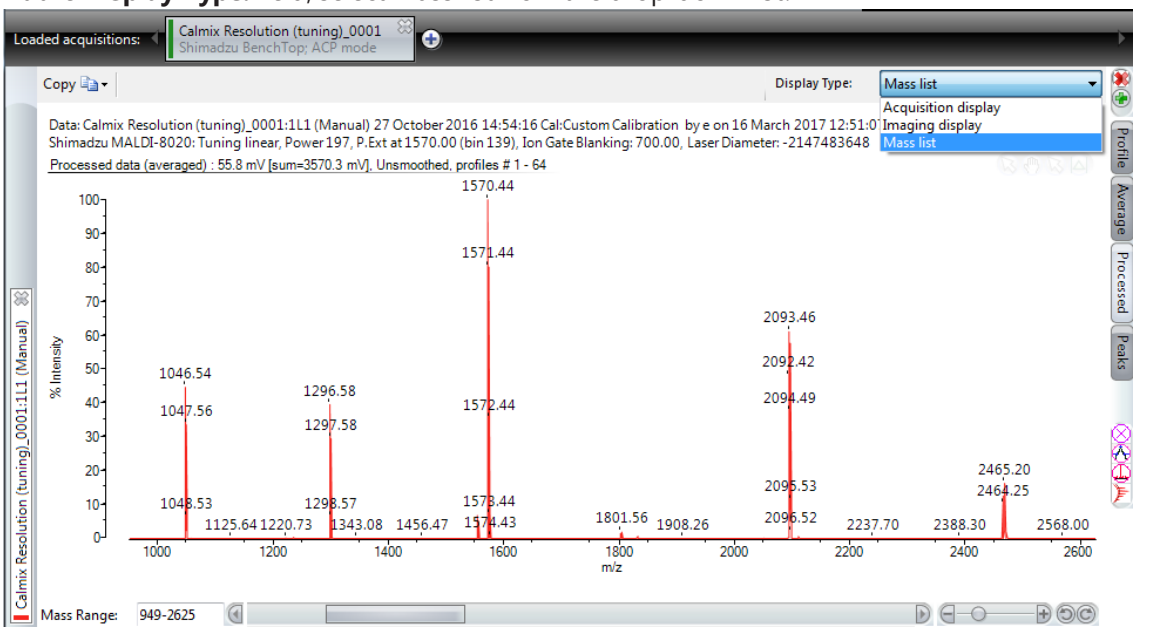
   The image is copied to the clipboard as a meta file.
3. In the target application, paste the image from the clipboard, typically, press Ctrl + V.

Note: **Copy all displays** only works if the display is split, otherwise it copies the displayed spectra/spectrum.

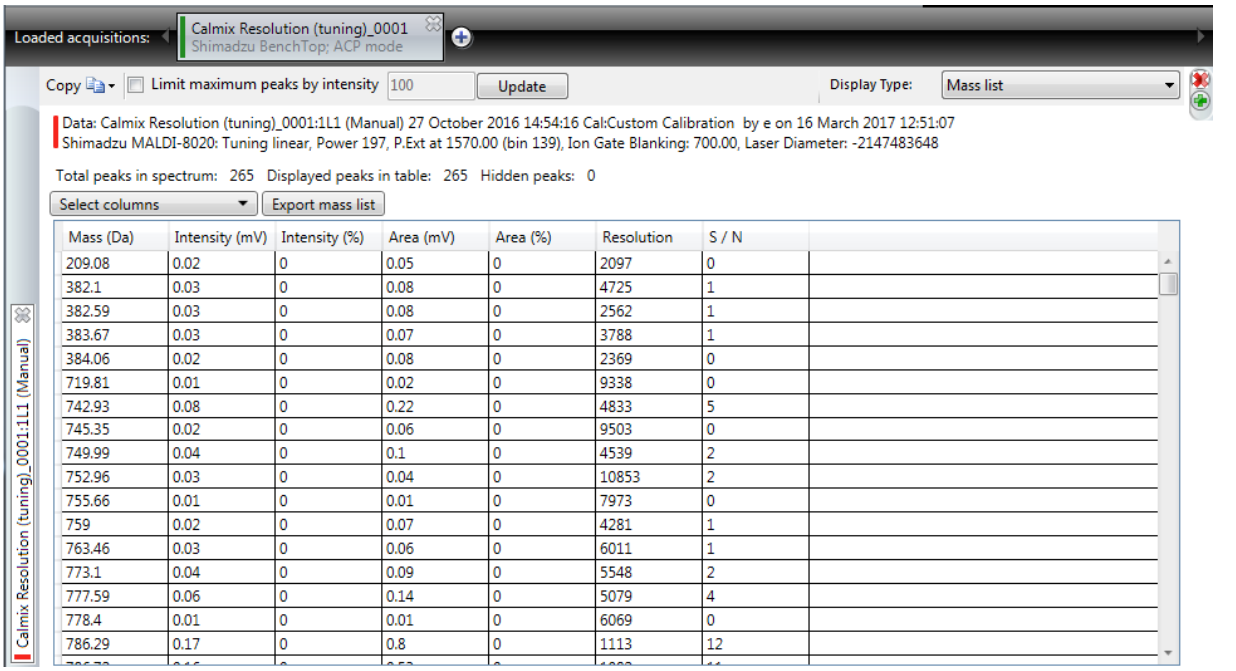
Displaying mass lists

This feature allows you to display a mass list of the acquisition being displayed.

At the **Display Type:** field, select Mass list from the drop-down list:



The mass list is displayed:



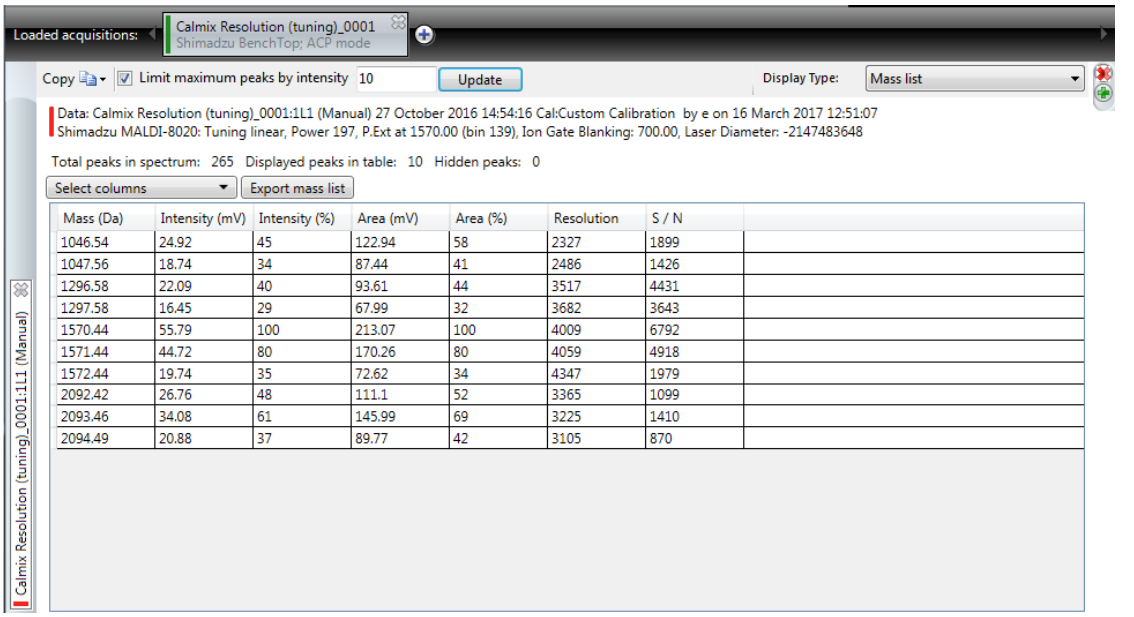
Mass (Da)	Intensity (mV)	Intensity (%)	Area (mV)	Area (%)	Resolution	S / N
209.08	0.02	0	0.05	0	2097	0
382.1	0.03	0	0.08	0	4725	1
382.59	0.03	0	0.08	0	2562	1
383.67	0.03	0	0.07	0	3788	1
384.06	0.02	0	0.08	0	2369	0
719.81	0.01	0	0.02	0	9338	0
742.93	0.08	0	0.22	0	4833	5
745.35	0.02	0	0.06	0	9503	0
749.99	0.04	0	0.1	0	4539	2
752.96	0.03	0	0.04	0	10853	2
755.66	0.01	0	0.01	0	7973	0
759	0.02	0	0.07	0	4281	1
763.46	0.03	0	0.06	0	6011	1
773.1	0.04	0	0.09	0	5548	2
777.59	0.06	0	0.14	0	5079	4
778.4	0.01	0	0.01	0	6069	0
786.29	0.17	0	0.8	0	1113	12

You can limit what is displayed:

- Limit the number of rows.
- Select which columns are displayed.
- Hide peaks.

Limit the number of rows

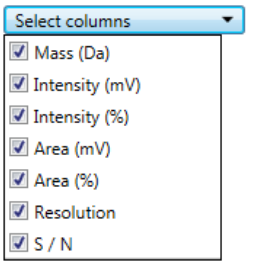
Tick the **Limit maximum peaks by intensity** check box, enter the required value, and click **Update**.



Mass (Da)	Intensity (mV)	Intensity (%)	Area (mV)	Area (%)	Resolution	S / N
1046.54	24.92	45	122.94	58	2327	1899
1047.56	18.74	34	87.44	41	2486	1426
1296.58	22.09	40	93.61	44	3517	4431
1297.58	16.45	29	67.99	32	3682	3643
1570.44	55.79	100	213.07	100	4009	6792
1571.44	44.72	80	170.26	80	4059	4918
1572.44	19.74	35	72.62	34	4347	1979
2092.42	26.76	48	111.1	52	3365	1099
2093.46	34.08	61	145.99	69	3225	1410
2094.49	20.88	37	89.77	42	3105	870

Select which columns are displayed

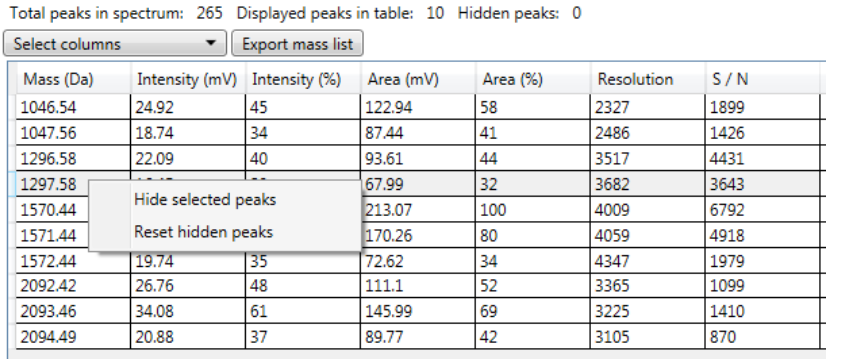
Select the required columns from the **Select columns** drop-down list:



Hide peaks

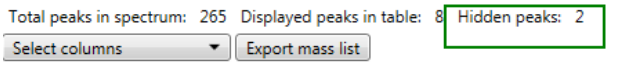
Select the required rows; for selecting several rows you can use Shift (to select a range) or Ctrl (to select individual peaks) in conjunction with clicking the mouse.

1. Right mouse click:



Mass (Da)	Intensity (mV)	Intensity (%)	Area (mV)	Area (%)	Resolution	S / N
1046.54	24.92	45	122.94	58	2327	1899
1047.56	18.74	34	87.44	41	2486	1426
1296.58	22.09	40	93.61	44	3517	4431
1297.58			67.99	32	3682	3643
1570.44			213.07	100	4009	6792
1571.44			170.26	80	4059	4918
1572.44	19.74	35	72.62	34	4347	1979
2092.42	26.76	48	111.1	52	3365	1099
2093.46	34.08	61	145.99	69	3225	1410
2094.49	20.88	37	89.77	42	3105	870

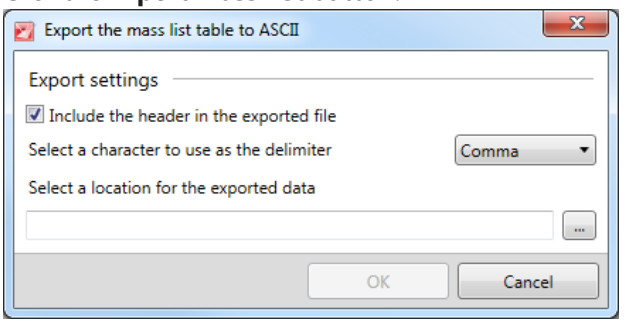
2. Select **Hide selected peaks**; peaks are hidden and the number hidden is displayed.



Mass (Da)	Intensity (mV)	Intensity (%)	Area (mV)	Area (%)	Resolution	S / N
1046.54	24.92	45	122.94	58	2327	1899
1047.56	18.74	34	87.44	41	2486	1426
1296.58	22.09	40	93.61	44	3517	4431
1570.44	55.79	100	213.07	100	4009	6792
1571.44	44.72	80	170.26	80	4059	4918
2092.42	26.76	48	111.1	52	3365	1099
2093.46	34.08	61	145.99	69	3225	1410
2094.49	20.88	37	89.77	42	3105	870

Export mass list

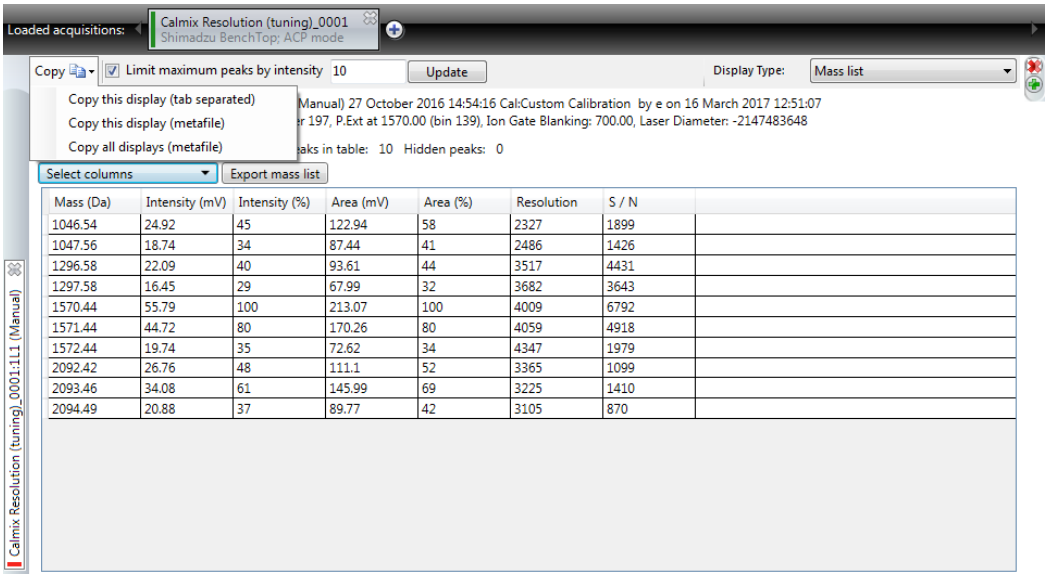
1. Click the **Export mass list** button:



2. Select the required parameters and click **OK**.

Copy mass list

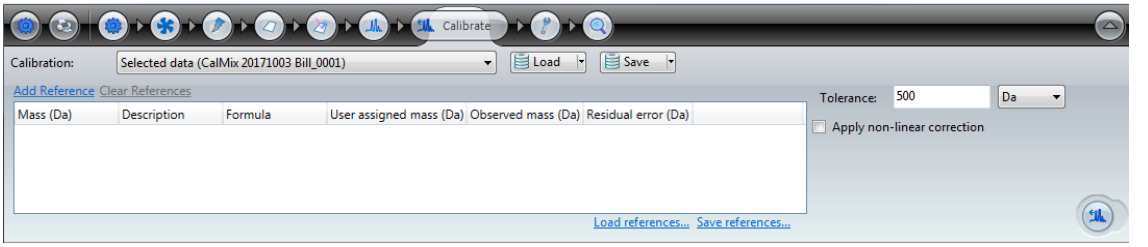
You can copy the mass list as either a tab-separated file (for spreadsheets, etc.) or a meta file for pasting as an image (for word processors, etc.).



Mass (Da)	Intensity (mV)	Intensity (%)	Area (mV)	Area (%)	Resolution	S / N
1046.54	24.92	45	122.94	58	2327	1899
1047.56	18.74	34	87.44	41	2486	1426
1296.58	22.09	40	93.61	44	3517	4431
1297.58	16.45	29	67.99	32	3682	3643
1570.44	55.79	100	213.07	100	4009	6792
1571.44	44.72	80	170.26	80	4059	4918
1572.44	19.74	35	72.62	34	4347	1979
2092.42	26.76	48	111.1	52	3365	1099
2093.46	34.08	61	145.99	69	3225	1410
2094.49	20.88	37	89.77	42	3105	870

Calibration

Introduction

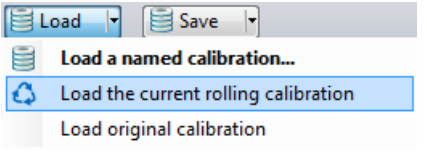


The calibration feature allows you to calibrate the currently loaded spectrum and set which calibration the instrument is to apply to the next MS acquisition.

There are three types of calibration:

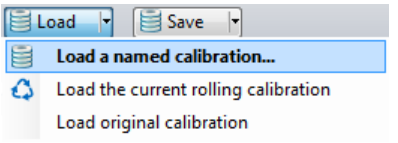
- Rolling calibration;
- Named calibration;
- Preferences calibration.

Rolling calibrations



The instrument is supplied with a default calibration, called a rolling calibration.

Named calibrations



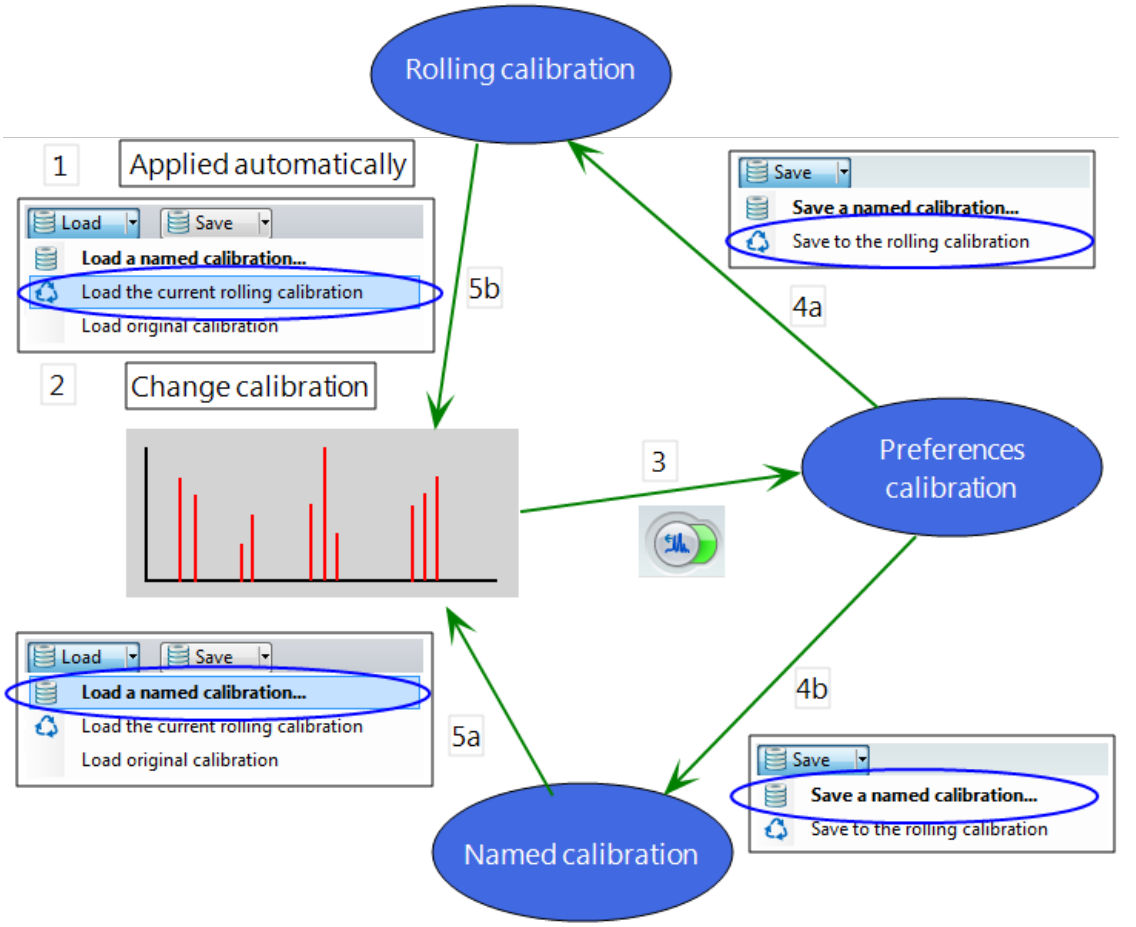
These are calibrations that users have created and saved to the database. All users can load and use these calibrations.

Preferences calibration

If you change a calibration, it is automatically saved as the preferences calibration and stored with your preferences. (Preferences are typically all your settings, for example, acquisition/processing parameters, etc. in use when you log off. When you next log on, these settings are loaded.)

All subsequent acquisitions will use this calibration.

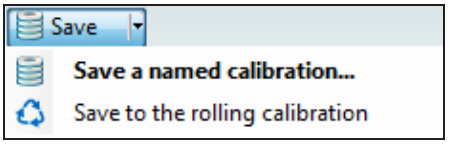
How calibrations are applied



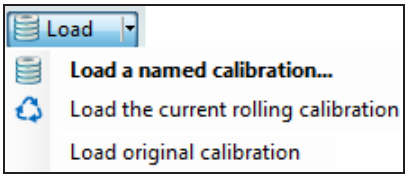
1. When you select the required tuning parameter set in the **Acquire** tab; the associated rolling calibration is applied automatically.

2. Acquire a spectrum and change the calibration in the **Calibrate** tab to suit your acquisition.
3. When you click the **Calibrate** button, the calibration becomes the preferences calibration.

4. From the preferences calibration you can:
    a. Save the calibration as a "named" calibration;



    b. Save the calibration as the rolling calibration (only if you are authorised).
5. The preferences calibration will be used on all subsequent acquisitions unless you:
    a. Load a different "named" calibration;

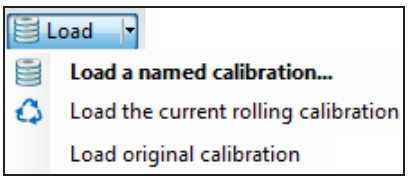


    b. Load the rolling calibration (as used in step 1);
    c. Change the tuning parameter set, then the associated rolling calibration will be used.

If you load a named or a rolling calibration, you can make changes and save it as either a named calibration or a rolling calibration.

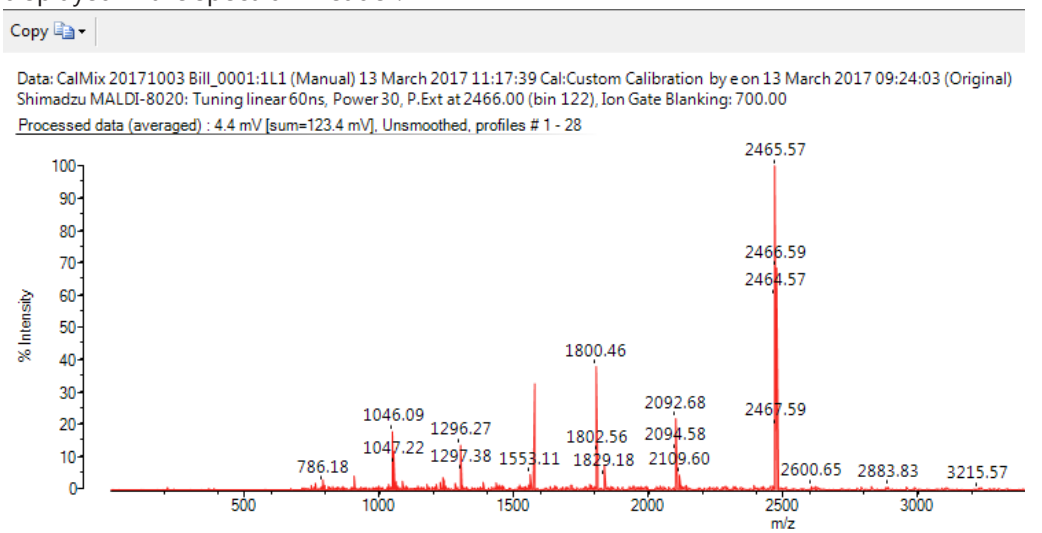
Loading the original calibration

If you wish to reapply the original calibration to the data, click **Load original calibration**.



Spectrum and calibrations

When you save an acquisition, the calibration is saved with the data. Calibration details are displayed in the spectrum header:

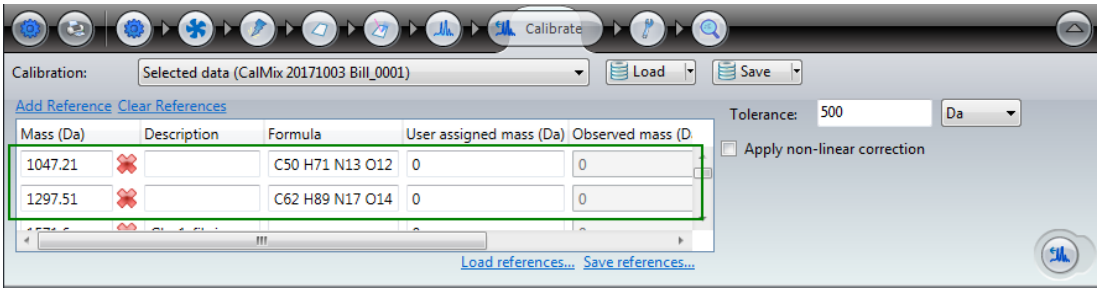


You can subsequently re-load the acquisition and change the calibration.

If a named or rolling calibration is changed, saved acquisitions using that calibration are not updated automatically. However, you can open the acquisition and load the revised calibration.

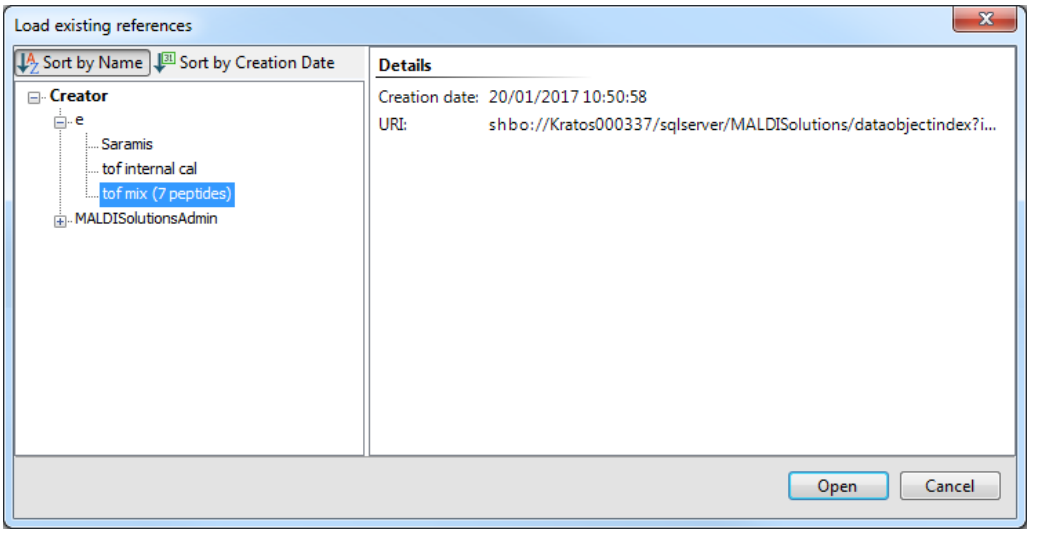
Calibration references

A calibration reference is simply a list of calibrant masses with optional fields for a description and formula. You can save a calibration reference for further use. You can also delete, amend and add references.



Loading calibration references

1. Click the **Load references** link:



2. Navigate to the required reference.
3. Click **Open**; the references are loaded.

Editing calibration references

Deleting a reference

Click the required  button.

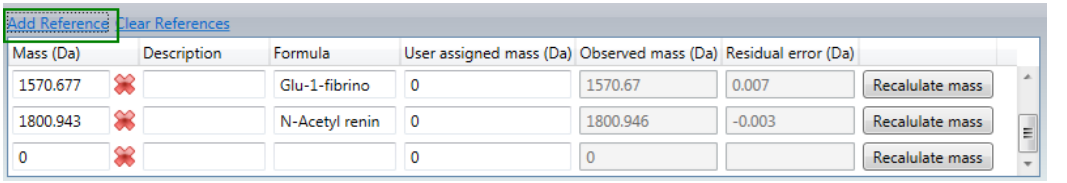
To delete all references, click the **Clear references** link.

Amending a reference

Amend the required fields.

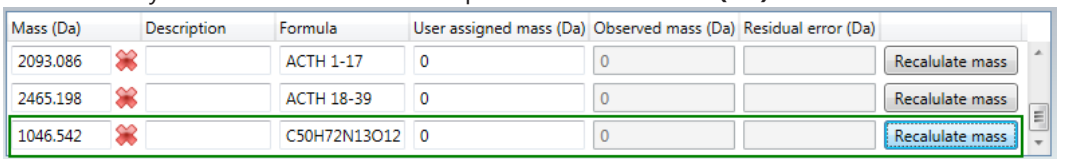
Adding a reference

1. Click the **Add Reference** link, which adds a new line to the bottom of the list.



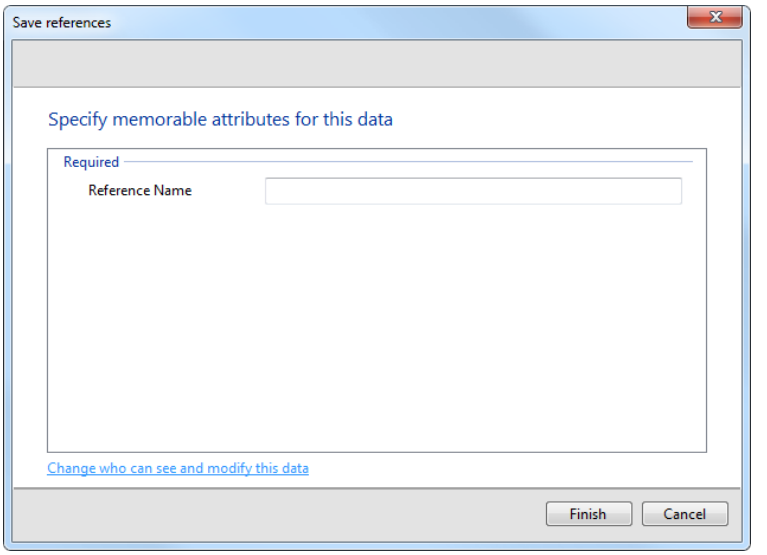
2. Fill in the **Mass (Da)** and optional **Description** and **Formula** fields.
(For the **Mass (Da)** field and the **Formula** field, enter the expected mass. For example, for peptides, formula should include an additional H.)

   If you entered a formula in the **Formula** field, clicking the **Recalculate mass** button automatically calculates the monoisotopic mass in the **Mass (Da)** field.

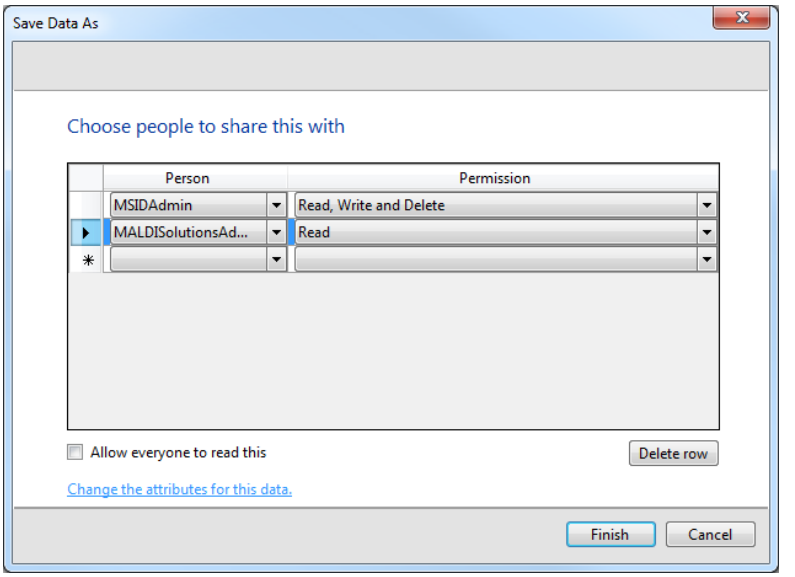


Saving calibration references

1. Click the Save references link:



2. Enter a name that best describes the references.
3. To allow other users to see and optionally to modify your references, click the **Change who can see and modify this data** link:



A selected row is indicated by  . An empty row is indicated by  .

a. To allow everyone to read your preferences, select the **Allow everyone to read this** check box.

b. In the **Person** drop-down list, select the required colleague.

c. In the **Permissions** drop-down list, select the required permissions.

d. To delete a row, select it and click **Delete**.

4. Click **Finish**.

Creating a calibration

Ideally, the calibrants should:

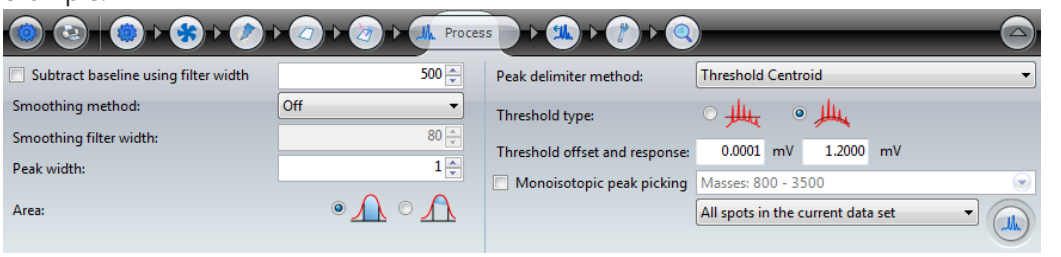
- cover the whole range of masses you expect from your experiment;
- use the same matrix that you will use for the experiment;
- use the same pulsed extraction value as used in the experiment, "Pulsed extract" on page 59.

The process includes:

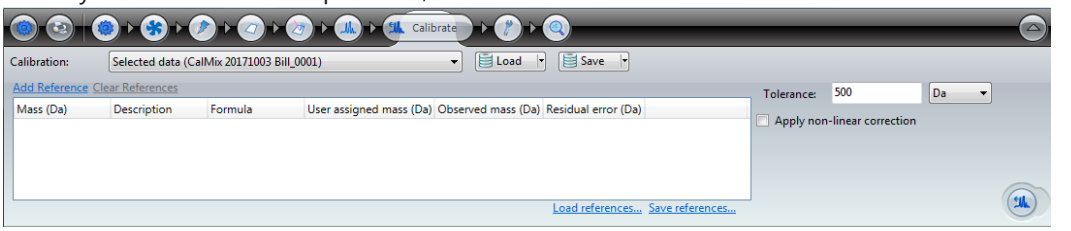
1. Creating a spectrum of the calibrants.
2. Loading/creating calibration references.
3. Setting the calibration tolerance.
4. Linking the peak masses in the spectrum with the references.
5. Calibrating the spectrum.
6. Saving the calibration.

Creating a spectrum of the calibrants

1. Acquire a spectrum of your calibrants.
2. Apply the required processing parameters to label the peaks with their masses. For example:



3. When you have a suitable spectrum, click the **Calibrate** tab:

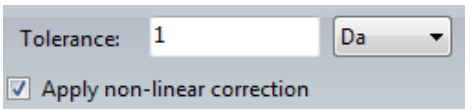


Loading/creating calibration references

Either load, or amend, the list of calibration references. "Calibration references" on page 133.

Setting the calibration tolerance

Tolerance

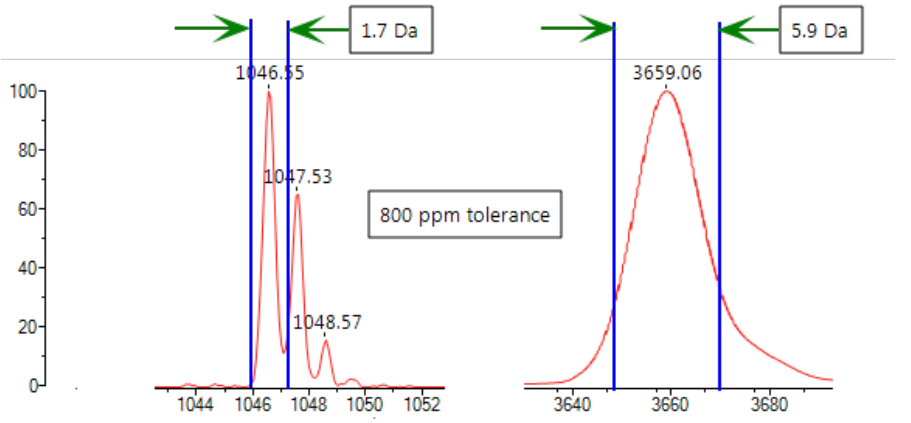


You can specify the tolerance as:

- Absolute mass accuracy using:
  - Da;
  - mDa.
- Relative mass accuracy using:
  - ppt (parts per thousand);
  - ppm (parts per million).

An example of relative mass accuracy follows. Specifying 800 ppm results in a tolerance at 1,000 Da of ±0.8 Da and at 7,000 Da of ±5.6 Da. This is useful as peaks at low masses are sharp and well defined whereas peaks at high mass are usually less well defined and a higher tolerance is

more applicable:



Apply non-linear correction

The instrument operates on the principle that ions are accelerated into a field-free region, and the final velocity of the ions is dependent on their mass. It is the flight times of the ions, i.e. the time from applying the acceleration voltages to the detection of the ions, that are actually measured. The flight times (t) are converted to mass-to-charge ratios (m/z) via a calibration that uses the theoretical principle that m/z is proportional to t^2.

However, the observed relationship between t^2 and m/z is non linear. In the mass range up to approximately 2,500 Da, this can produce errors of around 50 ppm.

Therefore, to improve the mass accuracy, with internal mass calibration, you can apply a "non linear correction factor" to correct for deviations and reduce the errors to typically less than 10 ppm.

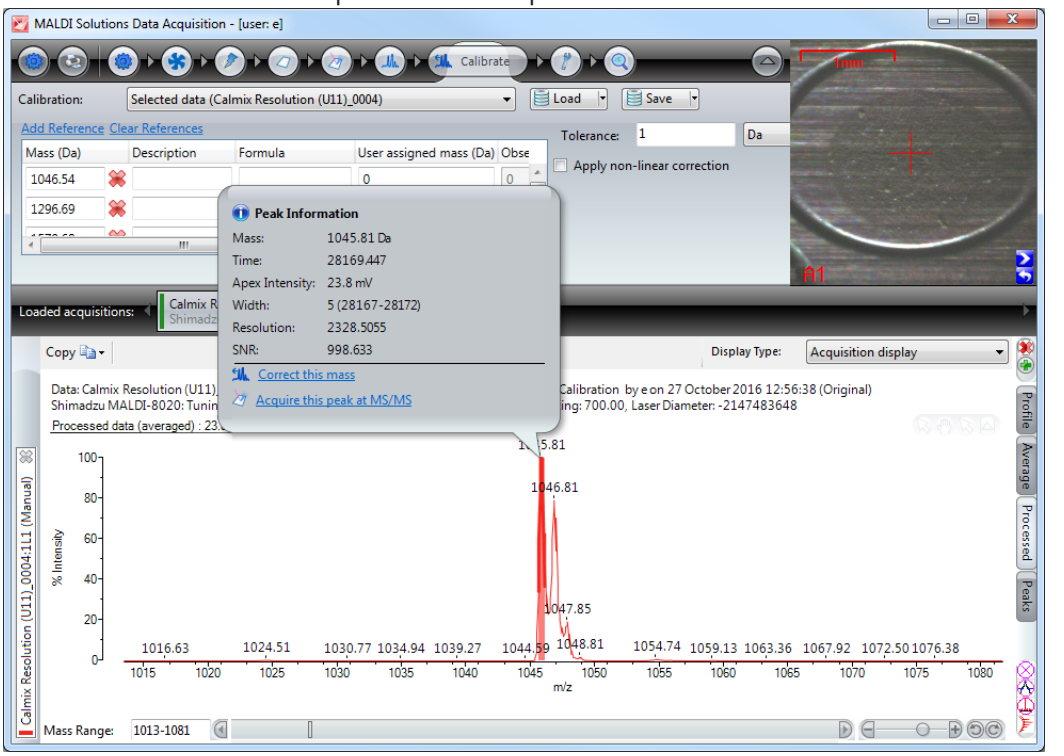
For the correction factor to work, you must have three or more calibrants.

To switch the feature on select the **Apply non-linear correction** check box.

Linking the peak masses with the references

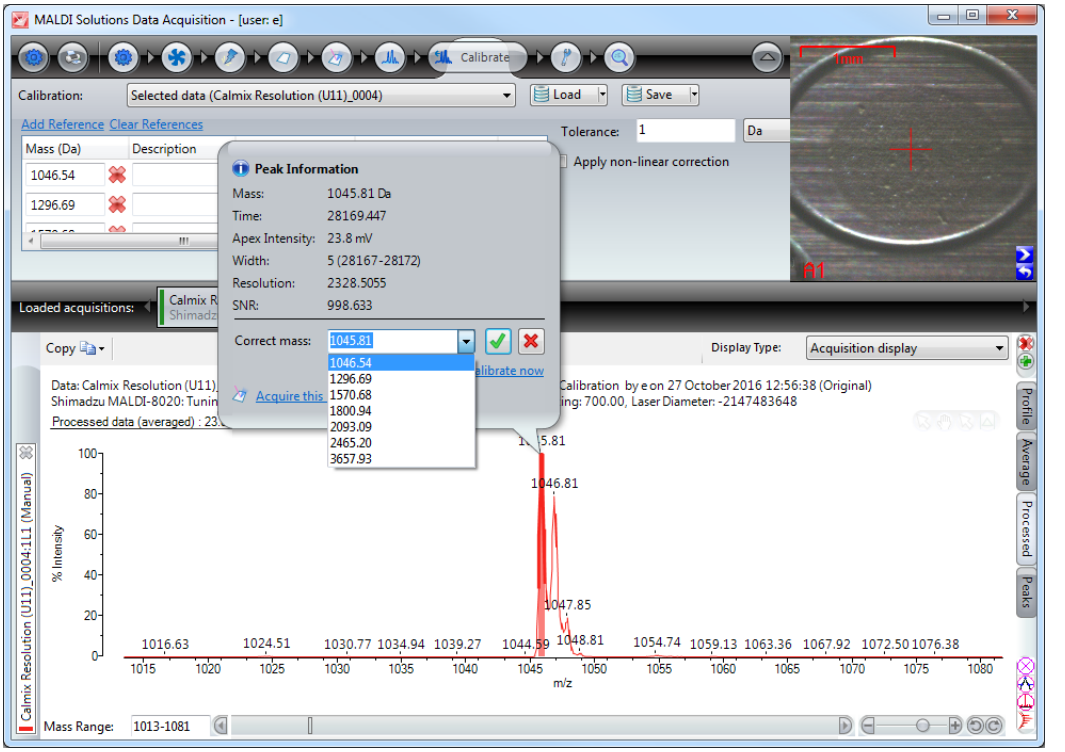
For each peak:

1. Left-mouse click on the first peak that corresponds to the calibration reference:

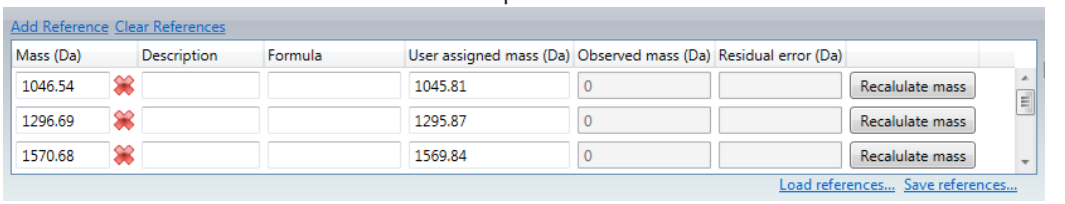


2. In the **Peak information** window, click the **Correct this mass** link.

3. Select the required mass from the drop-down list which displays all the calibration references:



4. Click the tick button  . Within the calibration references, the **User assigned mass** field shows the masses selected from the spectrum.



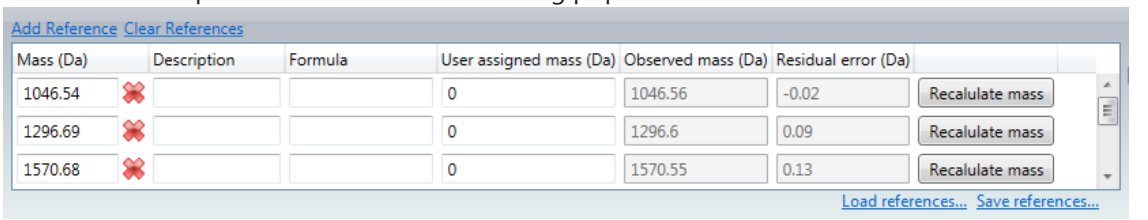
5. Continue to select the required masses.

Calibrating the spectrum

1. When the calibration references are complete, either click the **Calibrate now** link in the *Peak Information* window or click the **Calibrate** button  ; the spectrum masses will adjust as required.

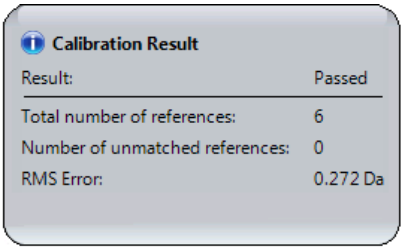
   If the calibration is successful, the button shows green:
   

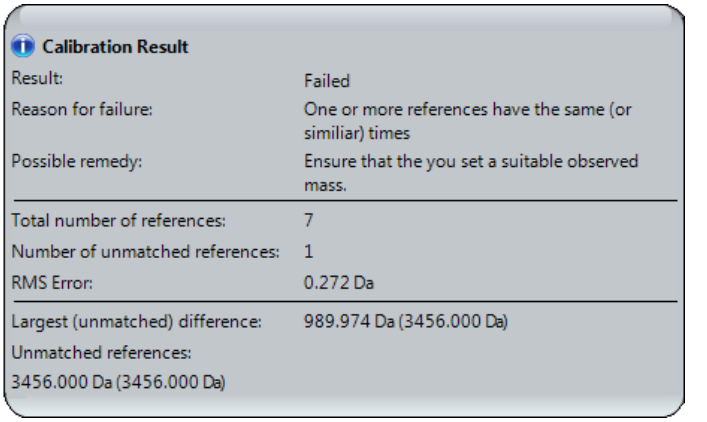
   A successful calibration results in the **User assigned mass** fields clearing and the **Observed mass** plus **Residual error** fields being populated:



   If the calibration is not successful, the button shows as either amber (passed with residuals outside tolerance) or red (fail) colour:
   

To view details about the calibration result, click the green/amber/red area of the button:



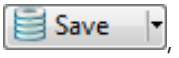


2. If the calibration fails, try increasing the **Tolerance** field so that all peaks would be matched to the corresponding reference values. If the calibration succeeds, reduce the tolerance down to the required value in steps (click the **Calibrate** button between step changes). For example, set the field to 5 Da, then 1 Da, etc. For isotopically resolved peaks, set the tolerance to 500 mDa.

Saving the calibration

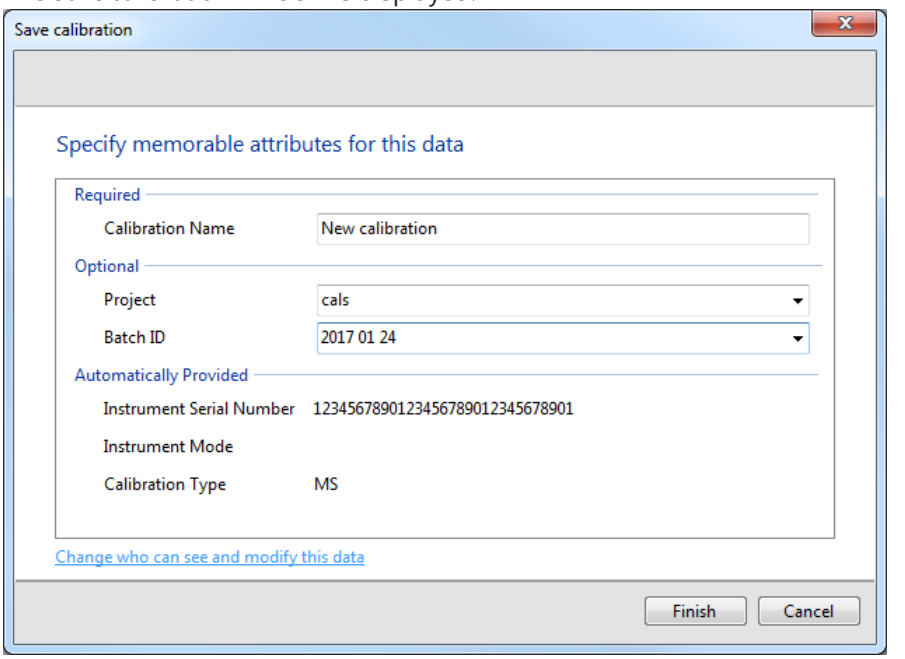
If you have changed the calibration references, you can save the calibration for future use.

Save a named calibration

1. Either click the **Save** button 

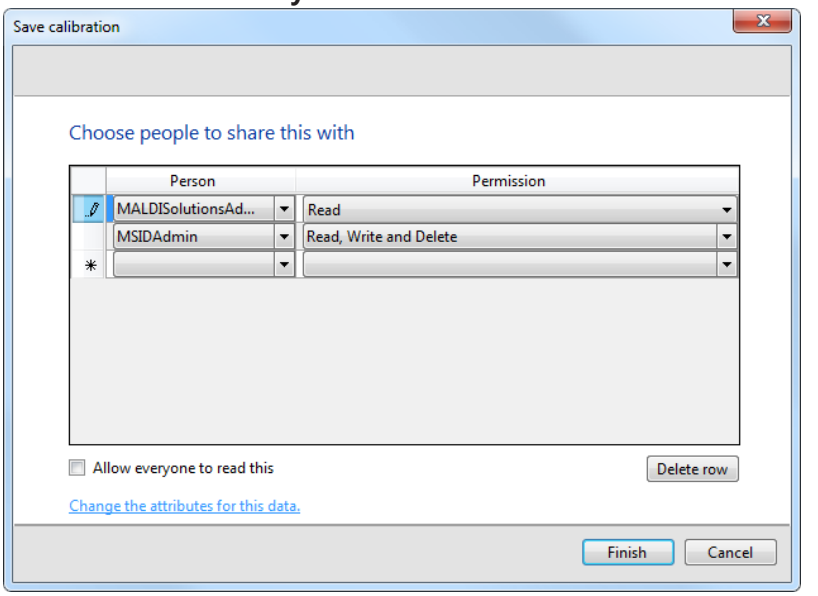
, or select **Save a named calibration** from the drop-down list:

The *Save calibration* window is displayed:



2. Enter a name to identify this calibration.
3. Enter any optional fields as required.

4. To allow other users to see and optionally to modify your calibration, click the **Change who can see and modify this data** link:



A selected row is indicated by  . An empty row is indicated by  .

   a. To allow everyone to read your preferences, select the **Allow everyone to read this** check box.

   b. In the **Person** drop-down list, select the required colleague.

   c. In the **Permissions** drop-down list, select the required permissions.

   d. To delete a row, select it and click **Delete**.

5. Click **Finish**.

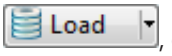
Save to the rolling calibration

The rolling calibration is assigned to the tuning mode. In a multi-user set up, all users who subsequently use the rolling calibration will be using this new calibration. Select **Save to the rolling calibration** from the Save drop-down list.

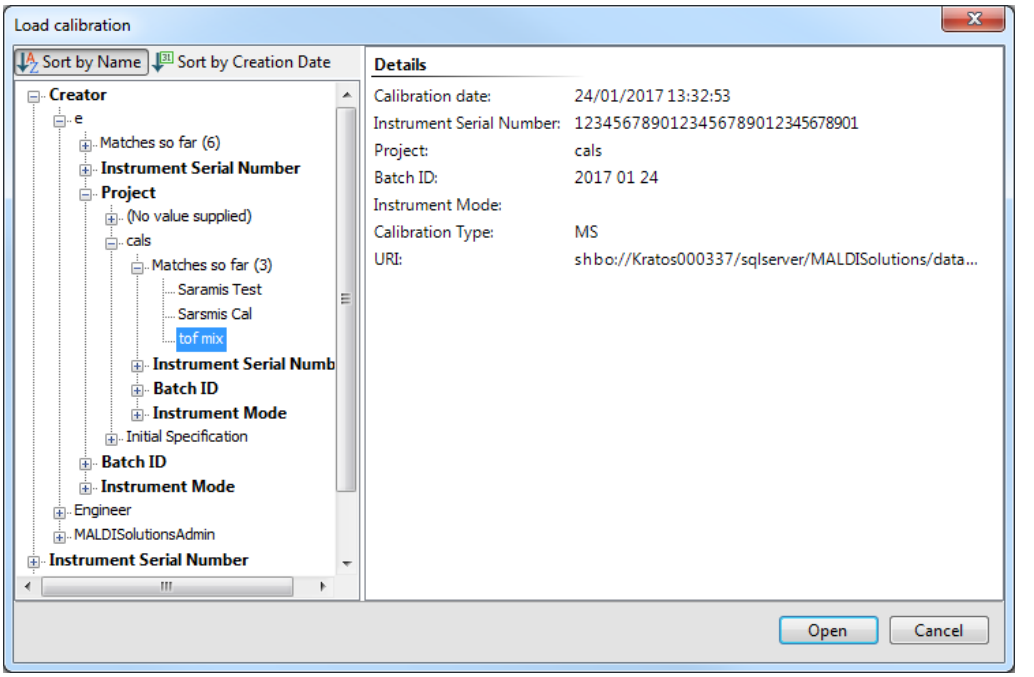
Calibrating a spectrum

Click the **Calibrate** tab to display the calibration features:

Load a named calibration

1. Either click the **Load** button 

, or select **Load a named calibration** from the drop-down list; the *Load a named calibration* window is displayed:



2. Navigate to the required calibration and click **Open**; the calibration is applied to the spectrum and the references used to create the calibration are displayed.

Load the current rolling calibration

This is the calibration assigned to the tuning mode. Select **Load the current rolling calibration** from the drop-down list; the calibration is applied to the spectrum and the references used to create the calibration are displayed.

Load original calibration

Select **Load original calibration** from the drop-down list; the calibration is applied to the spectrum and the references used to create the calibration are displayed.

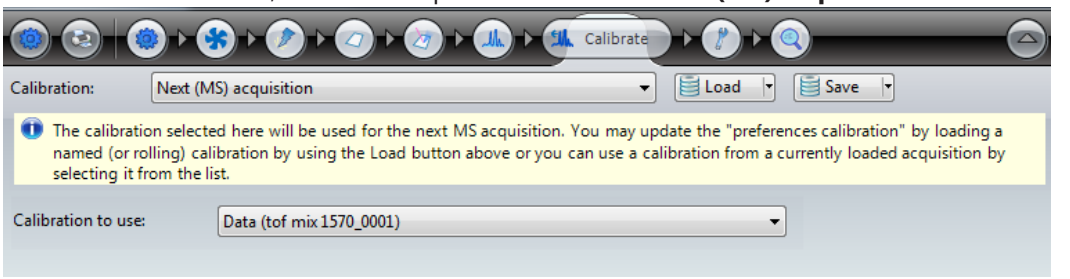
Predefining the next calibration

You can predefine which calibration is applied to the next data acquisition.

You can select a calibration from the currently loaded spectra, or load a calibration from the database. When you next acquire a spectrum, it will use the selected calibration.

Calibration from the currently loaded spectra

1. In the **Calibration** field, from the drop-down list select **Next (MS) acquisition name**:



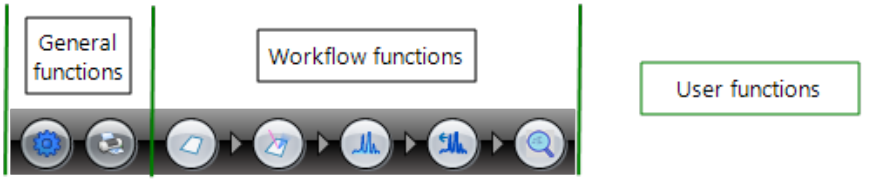
2. In the **Calibration to use** field, from the drop-down list select the required calibration from currently displayed spectra.

Using a predefined calibration

Choose either to load a named calibration or the current rolling calibration.

Printing & settings

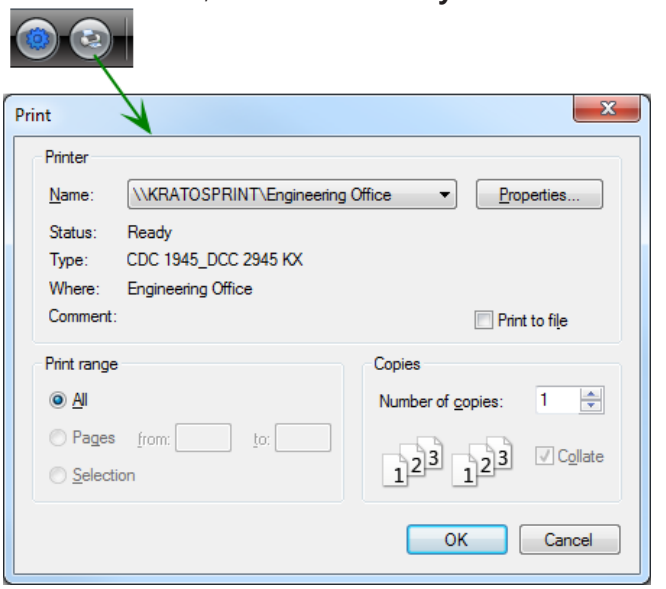
This chapter describes how to use the general functions on the toolbar.



Printing

Use this feature to print the display of a spectrum.

1. From the toolbar, click the **Print analysis** icon:

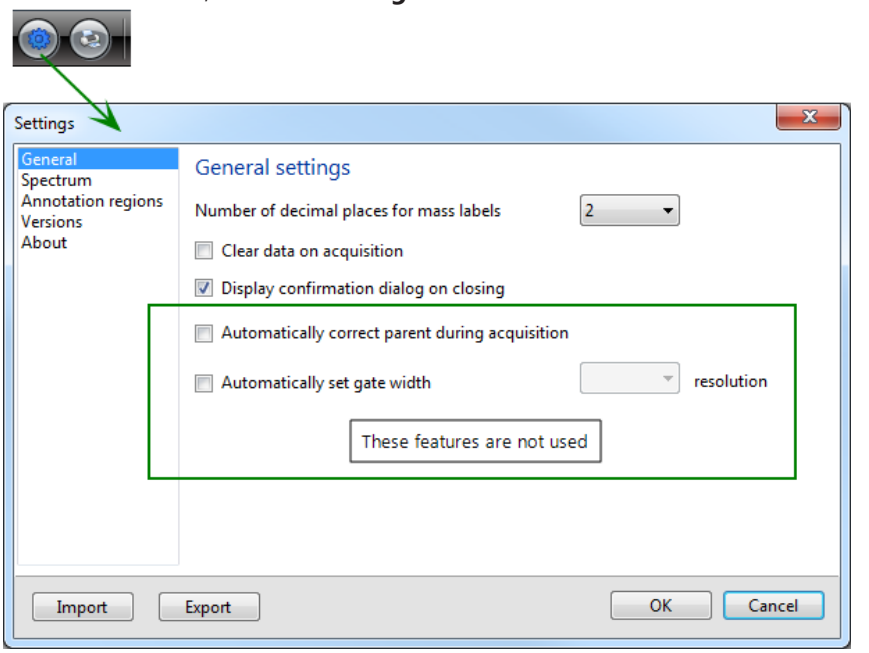


2. If required, adjust the default settings.
3. Click **OK**:

Settings

Use this feature to change your personal settings and view information about the software.

From the toolbar, click the **Settings** icon:



General

Number of decimal places for mass labels

Set the number of decimal places for the mass label you wish to display on the spectrum.

Clear data on acquisition

During an experiment, when you click the **Fire** button you will acquire a set of acquisition data. If you click **Fire** again, you will acquire a second set of acquisition data. If this field is not ticked, the first set of acquisition data remains displayed with the new acquisition. However, if this field is ticked, the first set of acquisition data is overridden by the data of the second acquisition.

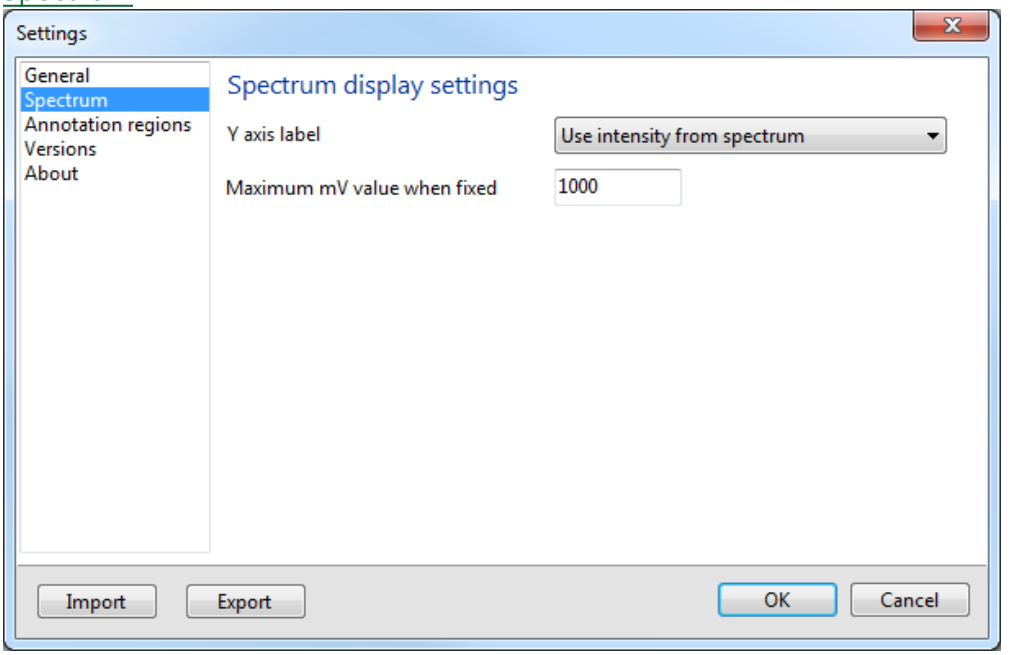
Automatically correct parent during acquisition

Not used on this instrument.

Automatically set gate width

Not used on this instrument.

Spectrum



Y axis label

This field determines how the spectra are displayed:

- **Use the largest intensity from all spectra** - If you have more than one spectrum displayed, the Y axis % intensity is adjusted to use the largest intensity from all displayed spectra.
- **Use intensity from spectrum** - The Y axis is set to % intensity.
- **Use fixed millivolt value** - This field operates in conjunction with the millivolt value set in the next field (Maximum mV value when fixed). The Y axis is set to mV.

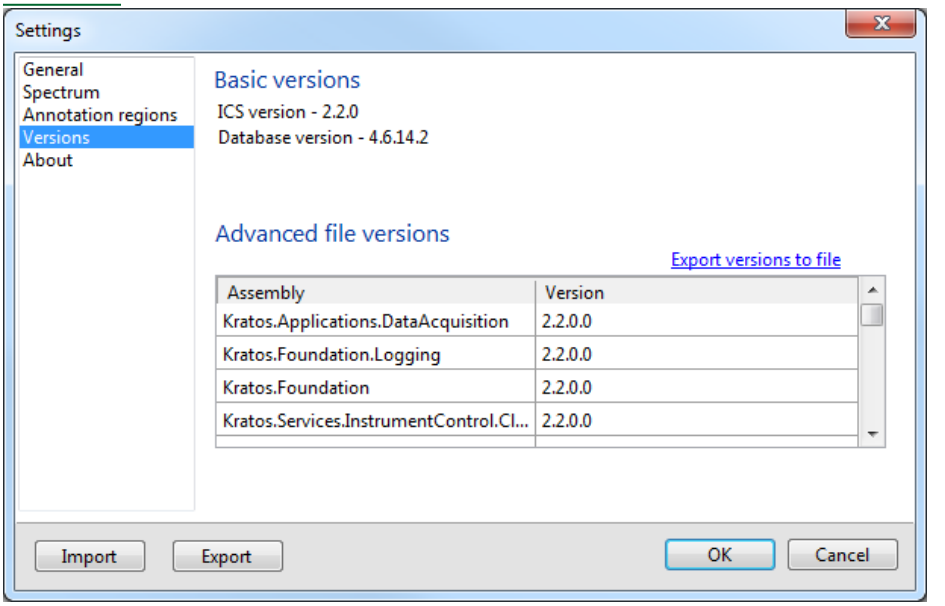
Maximum mV value when fixed

This field operates in conjunction with the **Use fixed millivolt value** field above. Type in the required value.

Annotation regions

Not used on this instrument.

Versions



This feature lists all the version numbers of the software modules. You may be asked to export this list to a file; click the **Export** versions to file link.

About

This feature defines the copyright owner.

Import/export

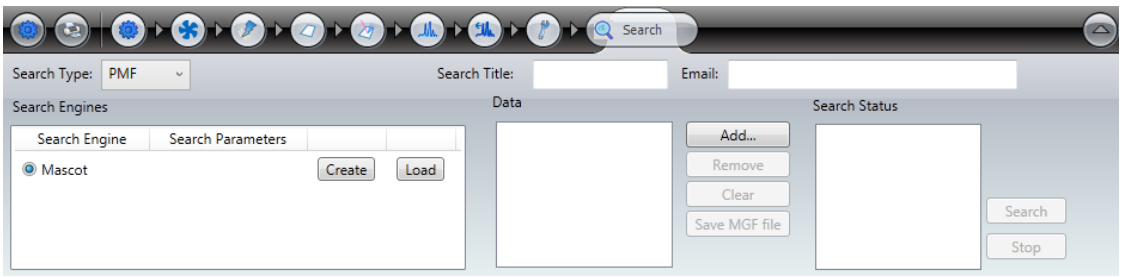
This feature allows you to export your settings to a file. Conversely, you can import the settings of a colleague.

Search (protein identification using Mascot)

You can search a database of proteins using the Mascot search engine to aid your analysis of PMF (Peptide Mass Fingerprint) or ISD (Ion Source Decay) spectrum. However, the results for PMF are for crude confirmation of a protein identification and are not suitable for publication.

To use this feature, you must have access to a Mascot server, or internet access to the Mascot web site.

Click the **Search** tab to display the search parameters:



Mascot parameter sets

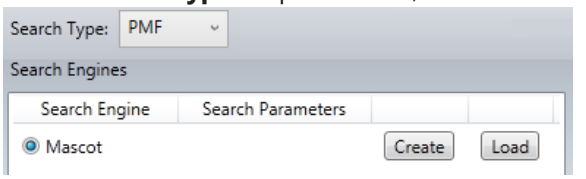
The Search feature requires you to create Mascot search parameter sets, which you subsequently use when setting up a search.

You can:

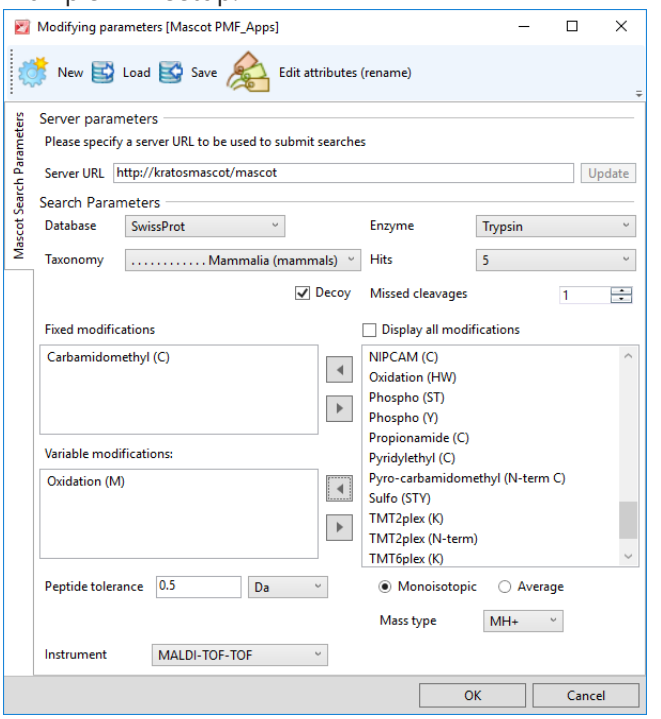
- Create a parameter set; you can subsequently change the values.
- Load a previously saved parameter set, and either:
  - edit the fields and save the changes;
  - save it as a new parameter set.

Creating a Mascot parameter set

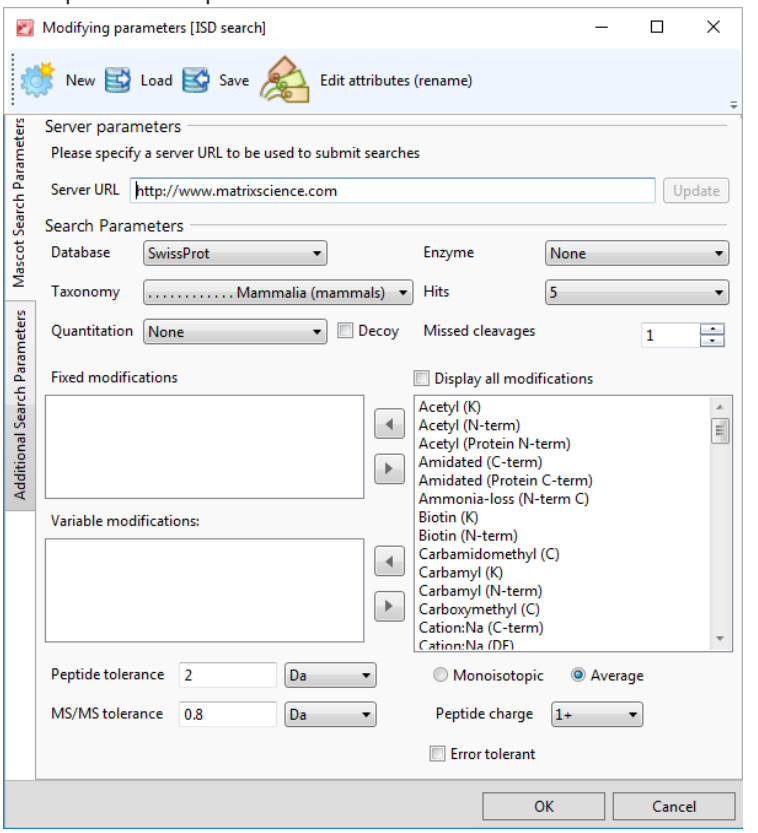
1. At the **Search Type** drop-down list, select either ISD or PMF.



2. Click the **Create** button:
   Example PMF setup:



Example ISD setup:

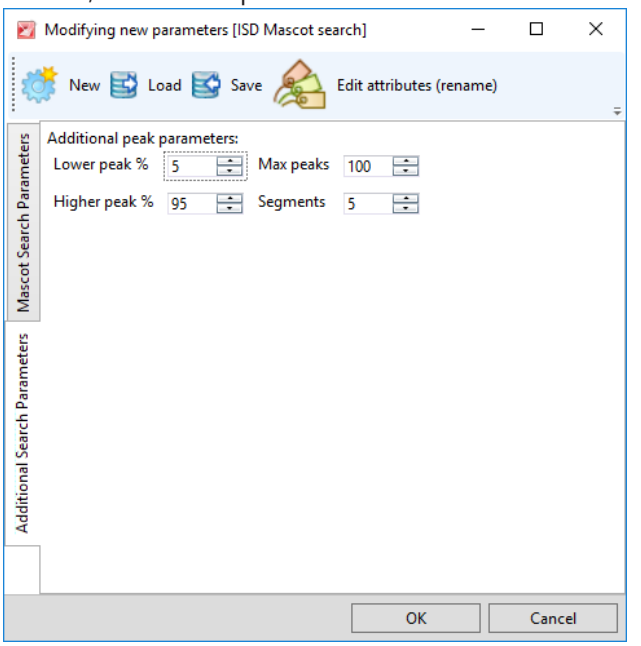


3. Fill in the required **Mascot Search Parameters** fields and click **OK**, refer to the table below.

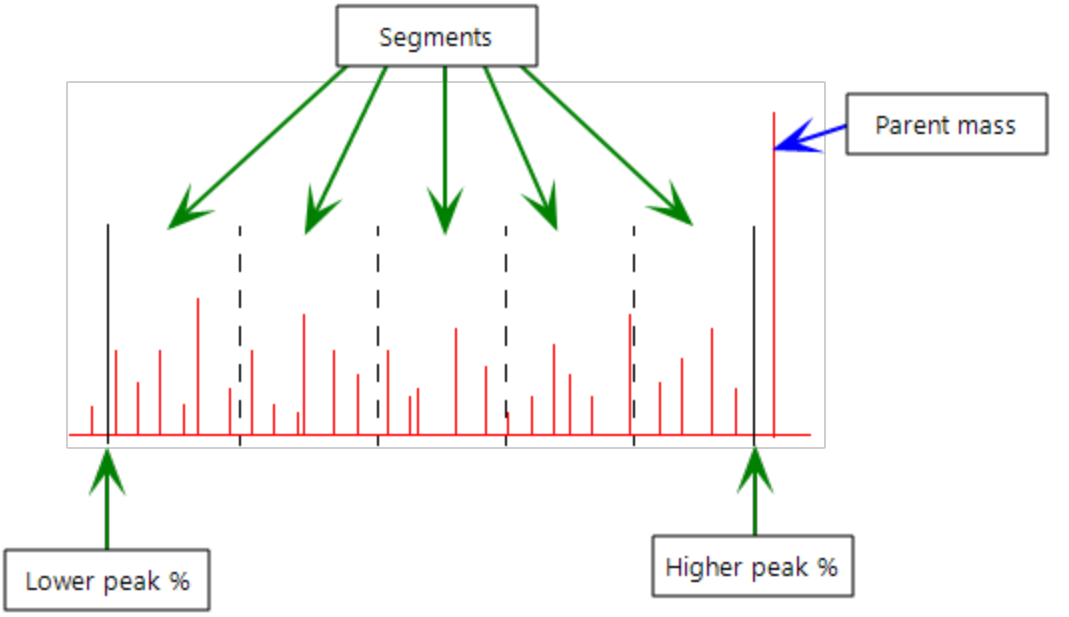
Field	Description
Server URL	The Matrix Science database URL is: http://www.matrixscience.com If you are using a local Mascot server, the URL is typically: http://<mascot server name>/mascot
Database	Select the required database from the drop-down list (usually NCBInr or SwissProt).
Enzyme	For ISD, select None. For PMF, select the required enzyme from the drop-down list.
Taxonomy	Select the required source of the protein.
Hits	Maximum number of hits displayed in a search results report.
Quantitation	Quantitation analysis only.
Decoy	Select to search the decoy database.
Missed cleavages	Generally, select 1.
Display all modifications	Expands the following modification fields to show all available modifications.
Fixed modifications and Variable modifications	The fields allow you to specify any modifications applied to the sample during preparation. Select the appropriate modification(s).
Peptide tolerance	Set the required tolerance and units.
Monoisotopic and Average	Select whether the spectral data is of monoisotopic (PMF) or average masses (ISD).
MSMS tolerance	Set the required tolerance and units.
Peptide charge	Generally, select +1.
Instrument	Select MALDI-TOF-TOF

Field	Description
Mass type	For a PMF search, select MH+.
Error tolerant	Not normally selected.

4. For ISD, fill in the required **Additional Search Parameters** fields and click **OK**:



The Additional peak parameters define which fragment peaks are selected.



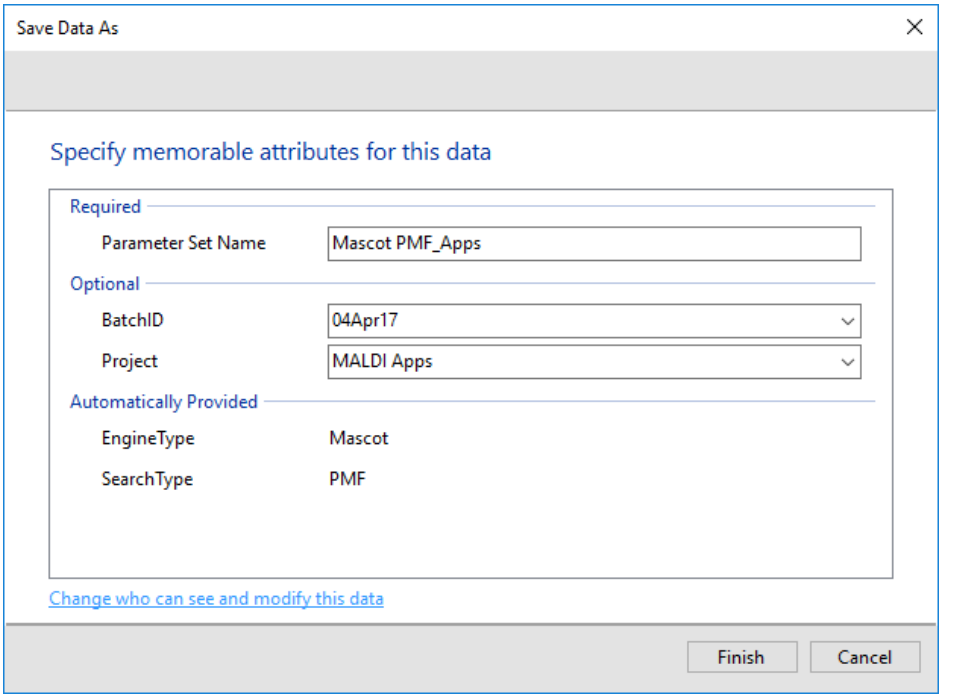
The **Lower peak %** and **Higher peak %** fields set the boundaries for selecting peaks for the fragment mass list. The limits are set as a percentage of the parent mass. Peaks outside the boundaries are ignored.

The **Max 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:

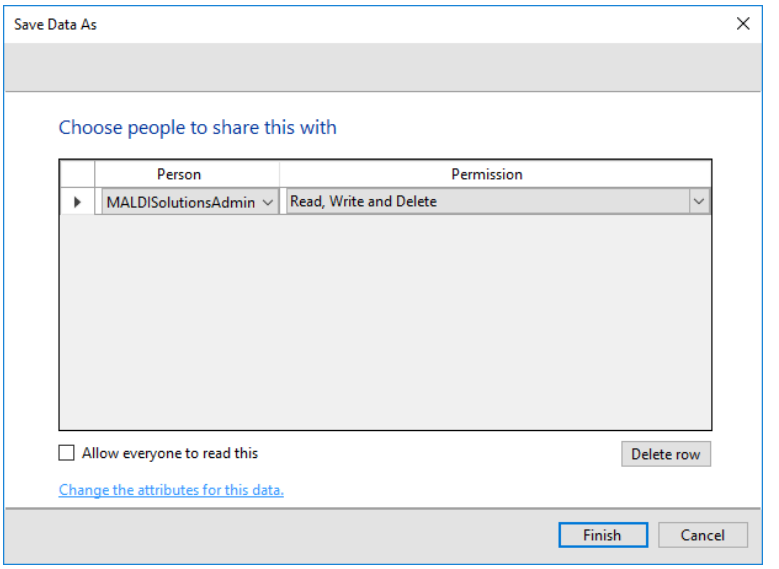
- divides the spectrum into 5 equal segments;
- from each segment picks the 8 (40 divided by 5) most intense peaks;
- uses the 40 peaks for the fragment mass list.

Saving a Mascot parameter set

Following from the previous section, the *Save Data As* dialog is displayed:



1. Enter the required data. For optional fields, enter text or use the drop-down lists.
2. To allow other users to see and optionally to modify your parameter set, click the **Change who can see and modify this data** link.



A selected row is indicated by  . An empty row is indicated by  .

a. To allow everyone to read your preferences, select the **Allow everyone to read this** check box.

b. In the **Person** drop-down list, select the required colleague.

c. In the **Permissions** drop-down list, select the required permissions.

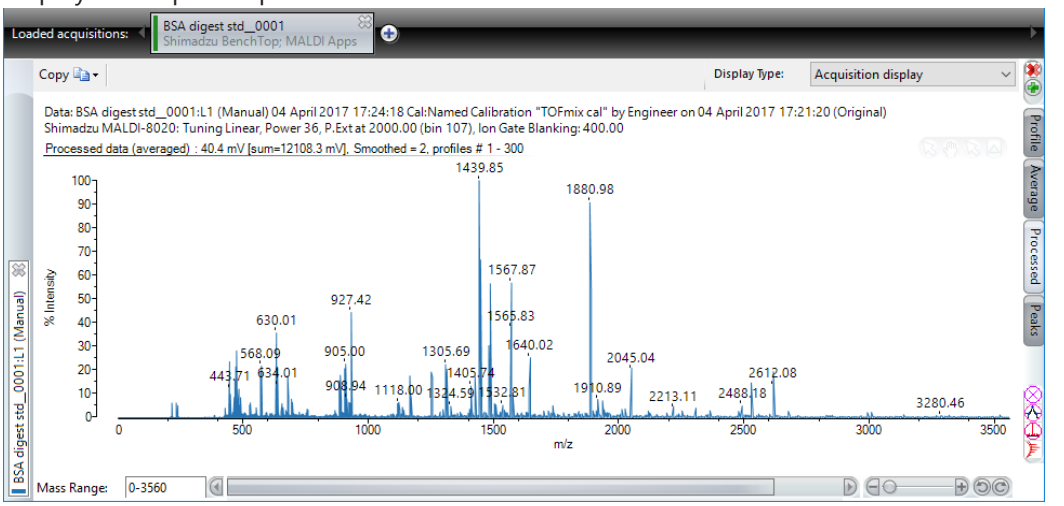
d. To delete a row, select it and click **Delete**.

3. Click **Finish**; the dataset is created; you are returned to the parameter set display.
4. Click **Close**.

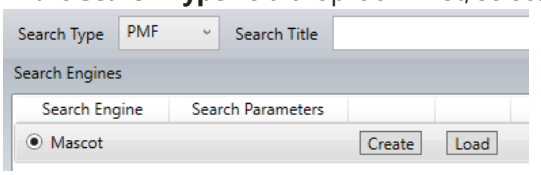
Mascot PMF searching

This procedure describes how to search Mascot using search parameters sets.

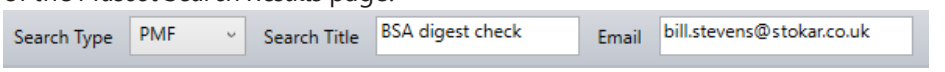
1. Display the required spectrum.



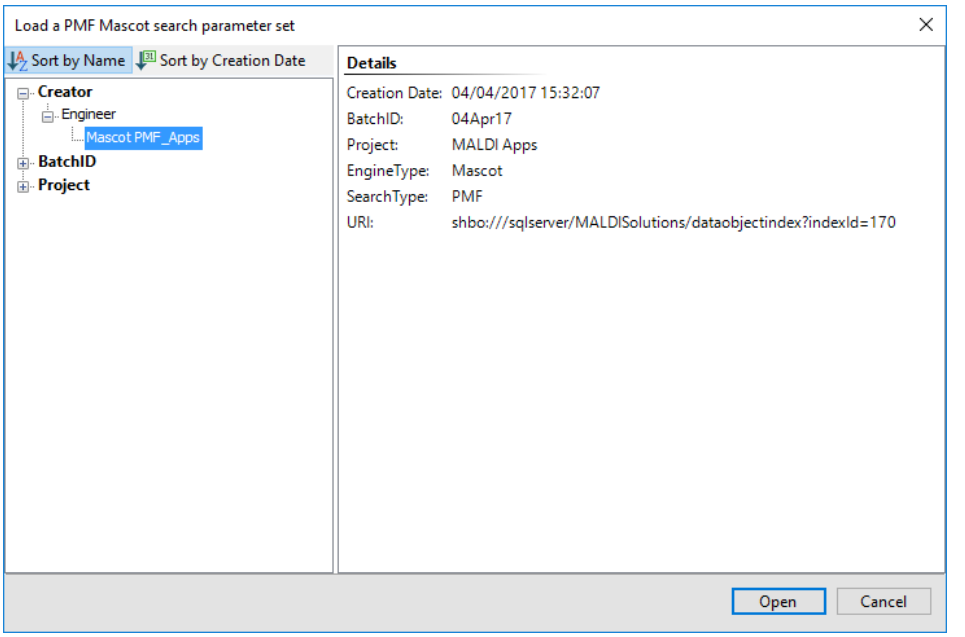
2. Click the **Search** tab to display the search features.
3. In the **Search Type** field drop-down list, select PMF.



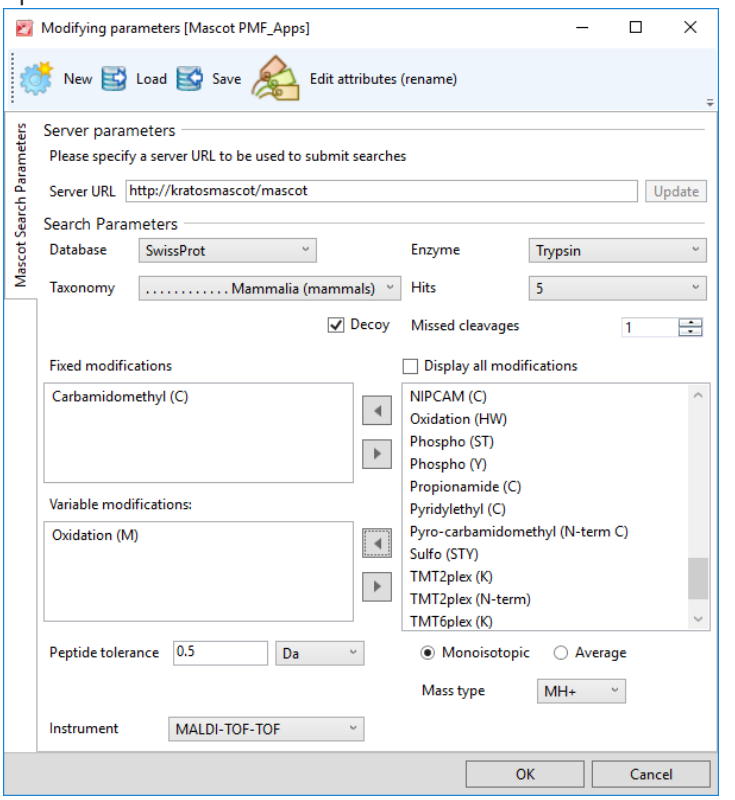
4. In the **Search Title** and **Email** fields, enter the information you wish to appear at the top of the *Mascot Search Results* page.



5. Click the **Load** button associated with Mascot.

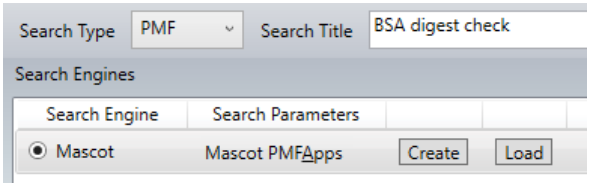


6. Navigate to the required search parameter set and click the **Open** button; the dataset is opened:

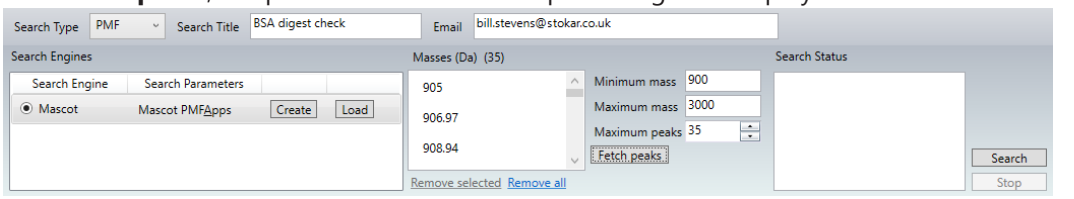


7. If required, make changes to the parameter set and click **Save** to update the parameter dataset, or **Close** to exit without saving.
   or
   If no changes are required, click **OK**.

   In both cases, the name of the parameter set is displayed (in this example, Mascot PMFApps).



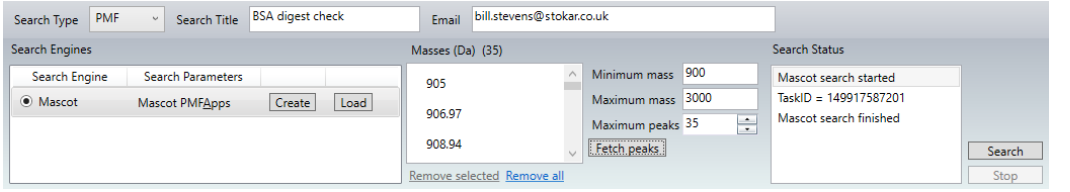
8. Enter required peak range (Da) fields, **Minimum mass** and **Maximum mass**.
9. Set the **Maximum peaks** field.
10. Click **Fetch peaks**; the peak masses within the peak range are displayed:



You can:

- Remove selected data; highlight the required peaks and click **Remove selected** link. Use the Ctrl key to select/deselect individual peak masses.
- Clear all the data; click **Remove all** link.

11. To start a search, click **Search**. The peak masses are sent to Mascot; the progress is shown in the **Search Status** box.



12. When Mascot has finished processing the data, it displays the results within your browser.

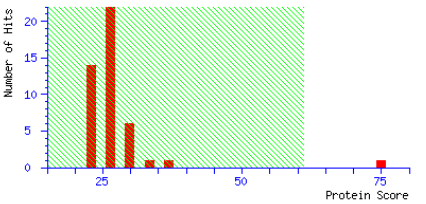
MATRIX SCIENCE **Mascot Search Results**

```
User            :
Email           : bill.stevens@stokar.co.uk
Search title    : BSA digest check
MS data file    : Automatically uploaded data
Database        : SwissProt 2017_06 (554860 sequences; 198649153 residues)
Taxonomy        : Mammalia (mammals) (66684 sequences)
Timestamp       : 4 Jul 2017 at 13:31:51 GMT
Top Score       : 75 for ALBU_BOVIN, Serum albumin OS=Bos taurus GN=ALB PE=1 SV=4
```

	SwissProt	Decoy
Protein hits above identity threshold	1	0
Highest scoring protein hit	75	48

Mascot Score Histogram

Protein score is -10*Log(P), where P is the probability that the observed match is a random event.
Protein scores greater than 61 are significant (p<0.05).



Concise Protein Summary Report

Format As: Concise Protein Summary — Help
Significance threshold p< 0.05 — Max. number of hits 5
Preferred taxonomy: All entries

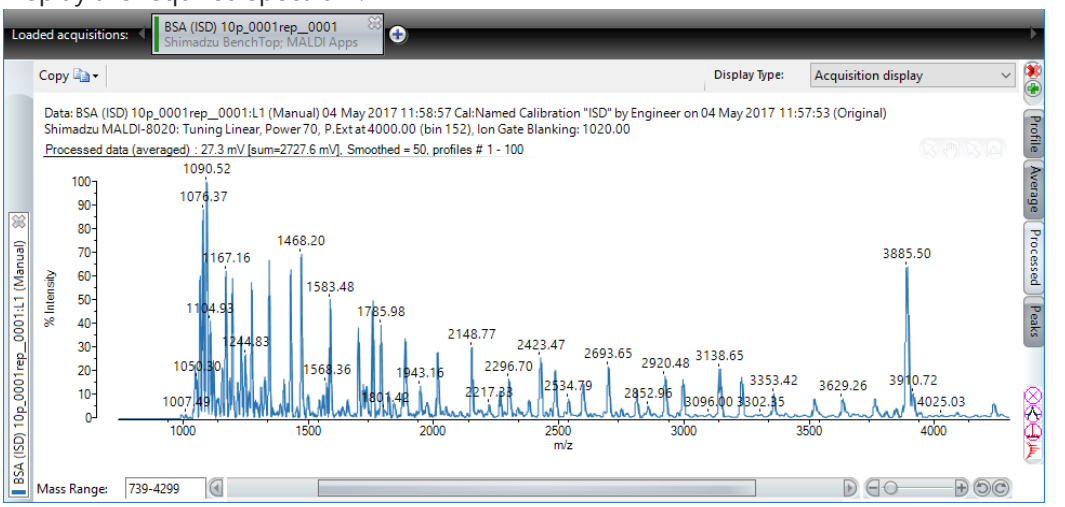
Re-Search All | Search Unmatched

```
1.    ALBU_BOVIN    Mass: 71244    Score: 75    Expect: 0.0021  Matches: 14
      Serum albumin OS=Bos taurus GN=ALB PE=1 SV=4
```

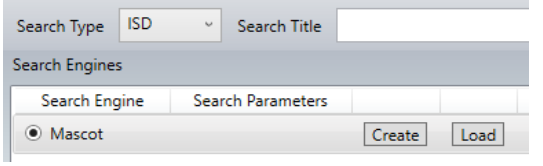
Mascot ISD searching

This procedure describes how to search Mascot using search parameters sets. Also, this section describes how to generate an MGF (Mascot Generic Format - a plain text (ASCII) file containing peak mass information).

1. Display the required spectrum.

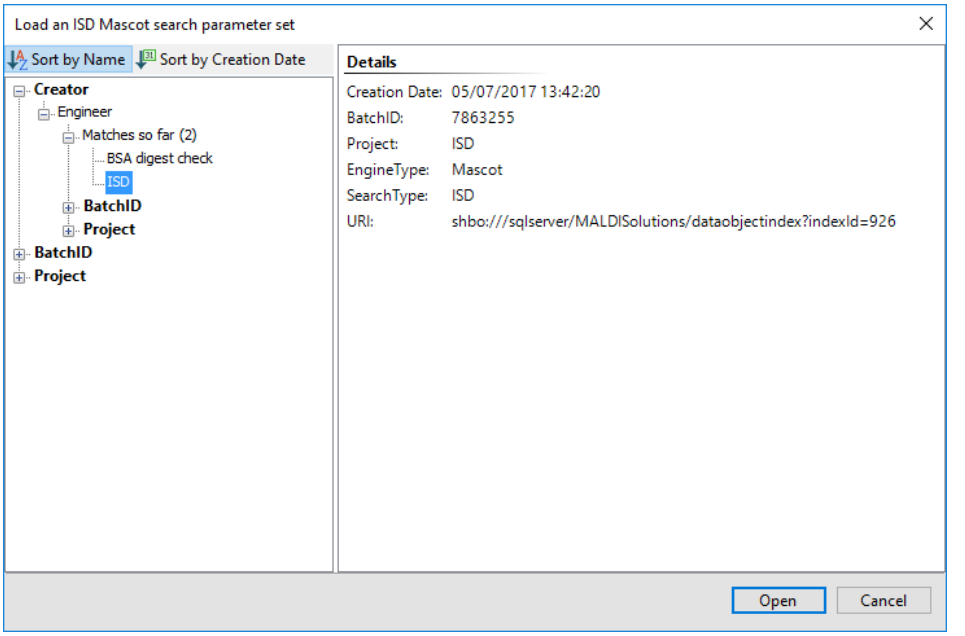


2. Click the **Search** tab to display the search features.
3. In the **Search Type** field drop-down list, select ISD.

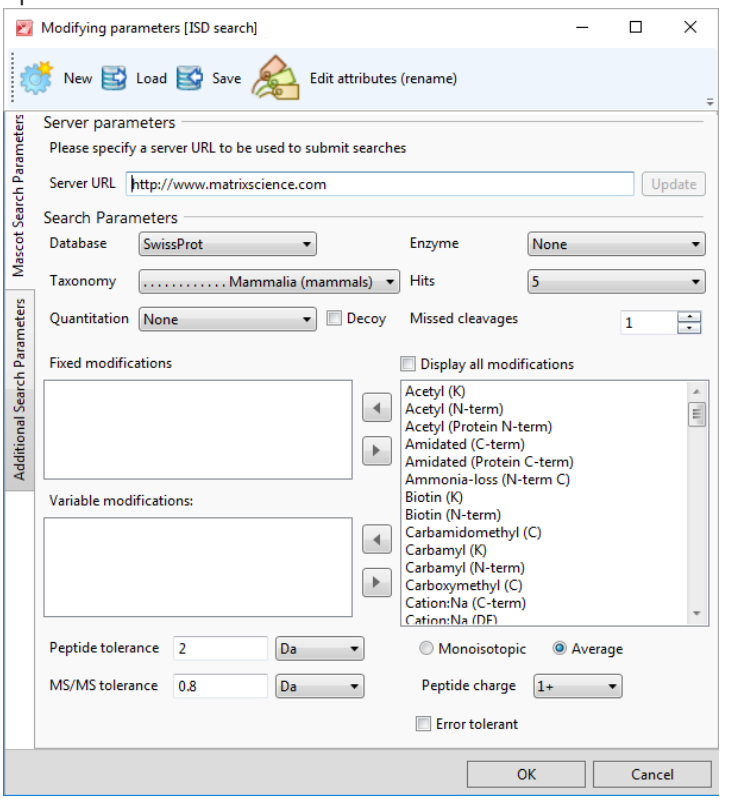


4. In the **Search Title** and **Email** fields, enter the information you wish to appear at the top of the *Mascot Search Results* page.

5. Click the **Load** button associated with Mascot.

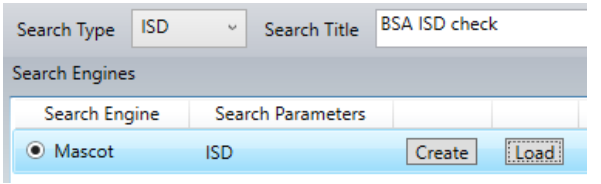


6. Navigate to the required search parameter set and click the **Open** button; the dataset is opened:

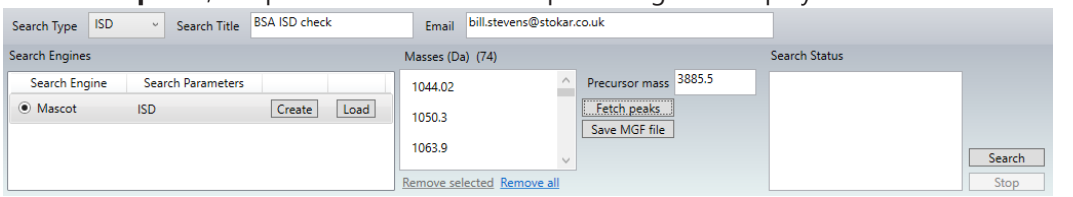


7. If required, make changes to the parameter set and click **Save** to update the parameter dataset, or **Close** to exit without saving.
   or
   If no changes are required, click **OK**.

   In both cases, the name of the parameter set is displayed (in this example, ISD).



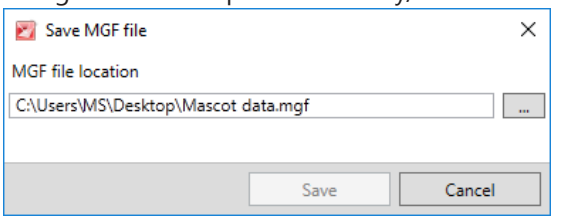
8. Enter **Precursor mass**.
9. Click **Fetch peaks**; the peak masses within the peak range are displayed:



You can:

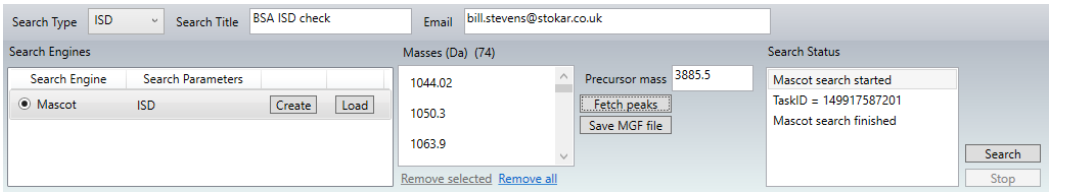
- Remove selected data; highlight the required peaks and click **Remove selected** link. Use the Ctrl key to select/deselect individual peak masses.
- Clear all the data; click **Remove all** link.

10. To generate an MGF (Mascot Generic Format - a plain text (ASCII) file containing peak mass information):
    a. Click **Save MGF file**.
    b. Navigate to the required directory/file and click **Save**.

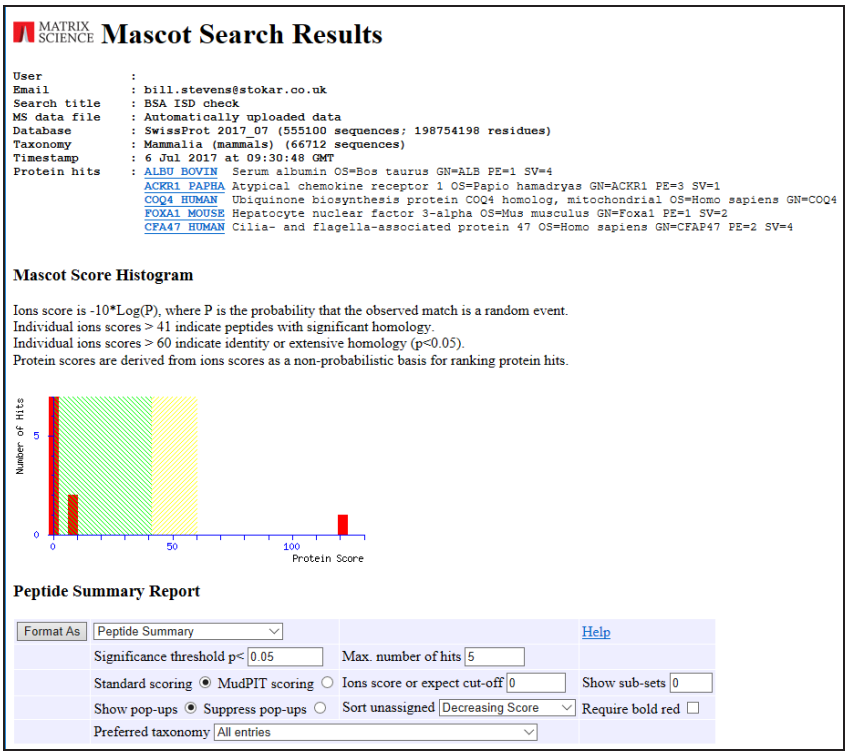


    c. Click **Save**.

11. To start a search, click **Search**. The peak masses are sent to Mascot; the progress is shown in the **Search Status** box.



12. When Mascot has finished processing the data, it displays the results within your browser.



MATRIX SCIENCE **Mascot Search Results**

```
User           :
Email          : bill.stevens@stokar.co.uk
Search title   : BSA ISD check
MS data file   : Automatically uploaded data
Database       : SwissProt 2017_07 (555100 sequences; 198754198 residues)
Taxonomy       : Mammalia (mammals) (66712 sequences)
Timestamp      : 6 Jul 2017 at 09:30:48 GMT
Protein hits   : ALBU_BOVIN  Serum albumin OS=Bos taurus GN=ALB PE=1 SV=4
                 ACKR1_PAPHA Atypical chemokine receptor 1 OS=Papio hamadryas GN=ACKR1 PE=3 SV=1
                 COQ4_HUMAN  Ubiquinone biosynthesis protein COQ4 homolog, mitochondrial OS=Homo sapiens GN=COQ4
                 FOXA1_MOUSE Hepatocyte nuclear factor 3-alpha OS=Mus musculus GN=Foxa1 PE=1 SV=2
                 CFA47_HUMAN Cilia- and flagella-associated protein 47 OS=Homo sapiens GN=CFAP47 PE=2 SV=4
```

Mascot Score Histogram

Ions score is -10*Log(P), where P is the probability that the observed match is a random event.
Individual ions scores > 41 indicate peptides with significant homology.
Individual ions scores > 60 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

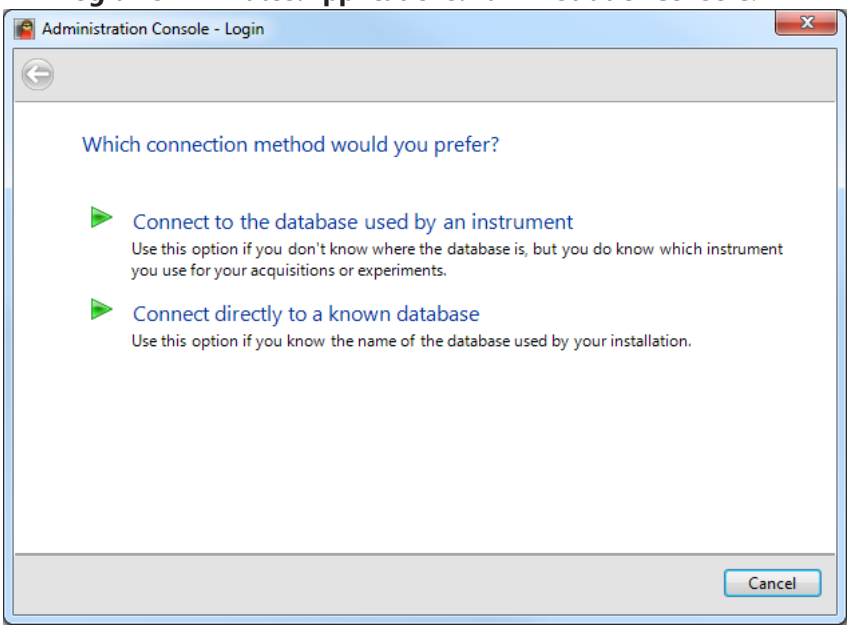
Format As: Peptide Summary — Help
Significance threshold p< 0.05 — Max. number of hits 5
Standard scoring / MudPIT scoring — Ions score or expect cut-off 0 — Show sub-sets 0
Show pop-ups / Suppress pop-ups — Sort unassigned: Decreasing Score — Require bold red
Preferred taxonomy: All entries

CHAPTER 4 Administration Console

The Administration Console application allows users with the appropriate privileges to manage users, attributes and saved data.

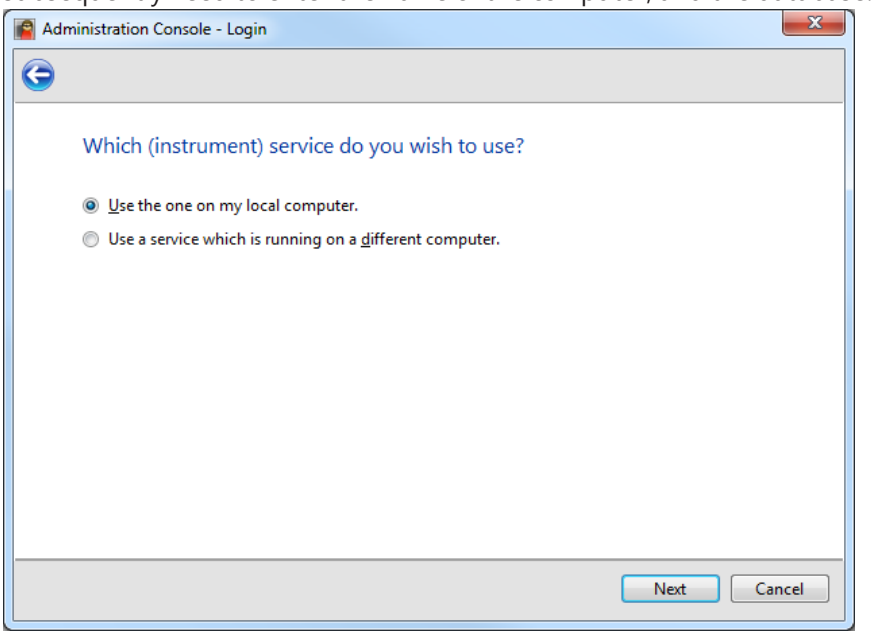
Starting the application

1. Open Windows File Explorer.
2. Navigate to and open **Programs Files => Shimadzu Corporation => MALDI Solutions => Programs => Kratos.Applications.AdministrationConsole**.

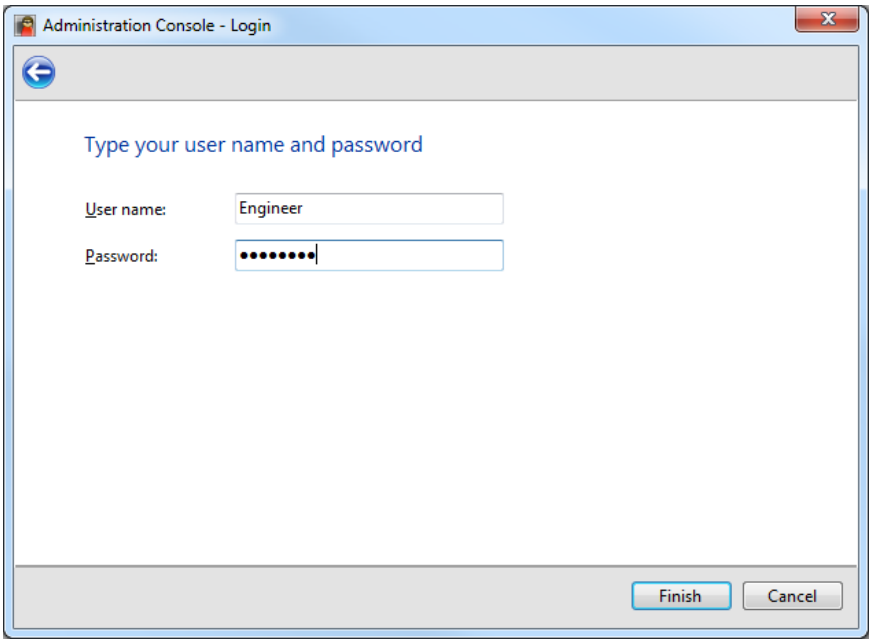


3. Click **Connect to the database used by an instrument**.
   For a remote connection, you will need to know the name of the computer. If you know the name of the database, click the Connect directly to a known database. You will

subsequently need to enter the name of the computer, and the database.

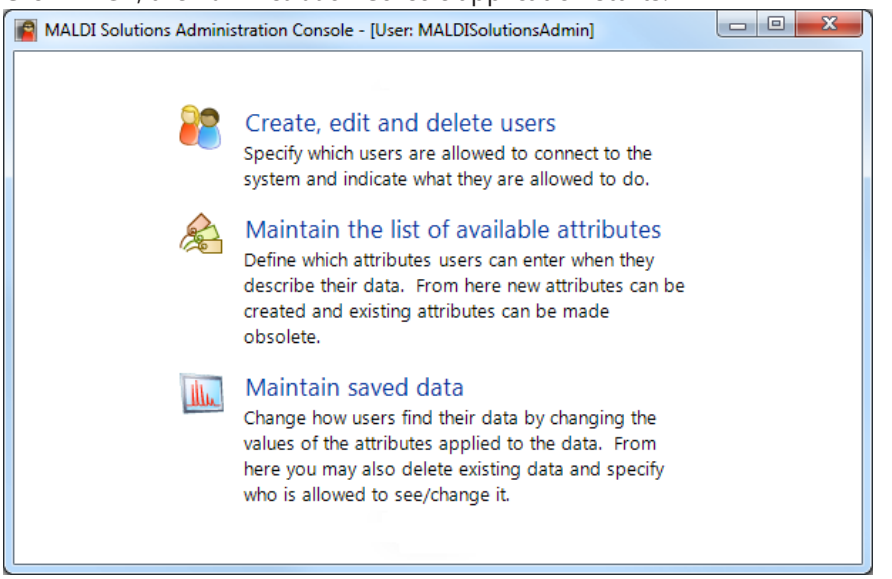


4. Click **Next**:



5. Enter your user name and password.

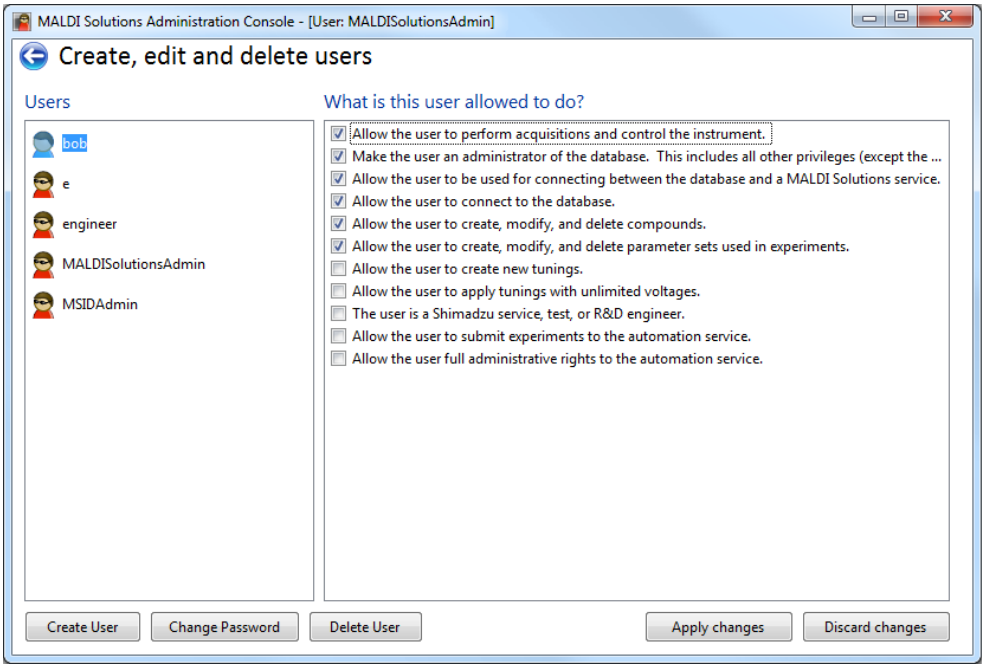
6. Click **Finish**; the *Administration Console* application starts:



Depending on your access privileges, you may not see all the options shown above.

Create, edit and delete users

Click the **Create, edit and delete users** link:



The icons of the users represent:

 Users

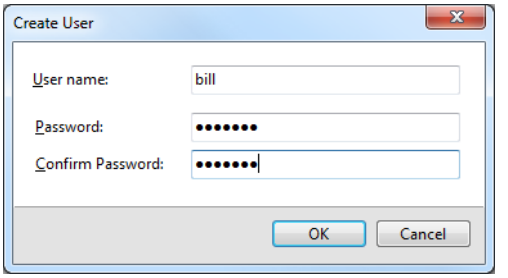
 System administrators

To go back to the Administration Console main screen, click:

 .

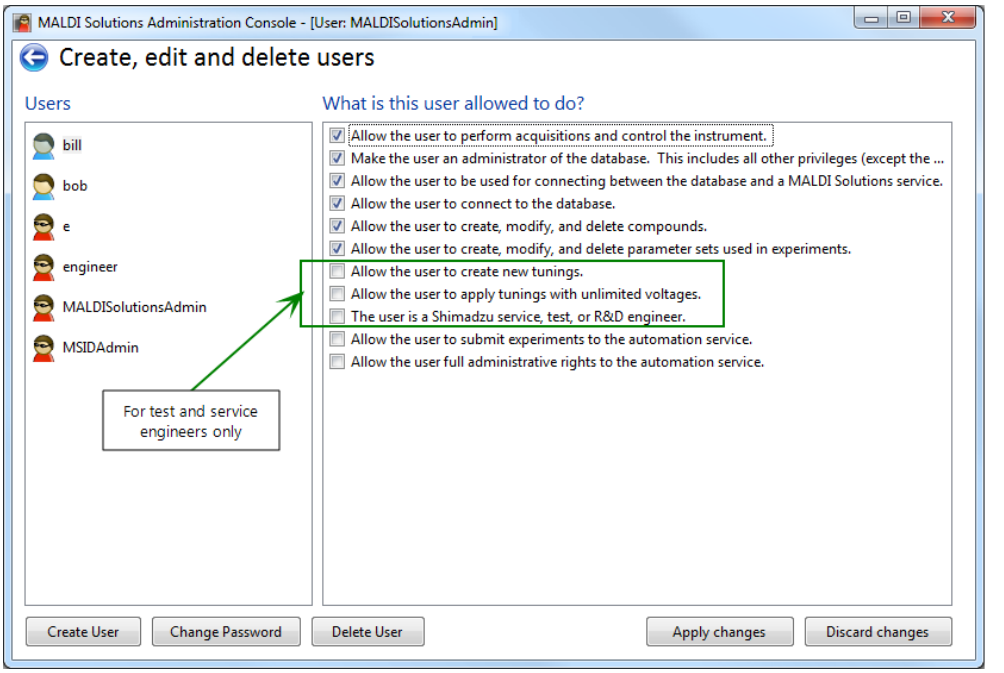
Creating users

1. Click the **Create User** button:



2. Enter the user's name.
3. Enter the user's password and confirm the password.
4. Click **OK**; the new user is added to the list:
5. Select/clear the required check boxes for the new user.
   CAUTION - The highlighted fields below are for test and service engineers. Do not use

these fields for users.



6. Click **Apply changes**.

Editing users

1. Select the required user.
2. Select/clear the required check boxes for the appropriate features for that user.
   CAUTION - The highlighted fields below are for test and service engineers. Do not use these fields for users.
3. Click **Apply changes**.
4. Repeat for any other users.

Deleting users

The system requires at least one system administrator; therefore, you cannot delete the last one.

1. Select the required user.
2. Click **Delete User**.
3. At the confirmatory message, click **Yes**; the user is deleted.

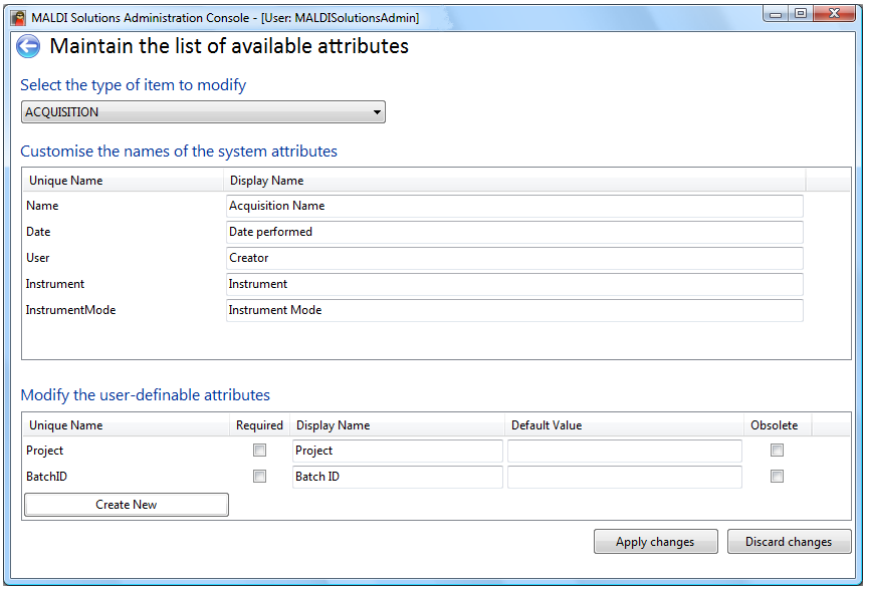
Managing attributes

You can define attributes (fields) that users enter when they save data. This feature allows you to:

- modify existing attributes;
- create new attributes;
- make an attribute obsolete.

Once an attribute has been created, you cannot delete it.

1. Click the **Maintain the list of available attributes** link:

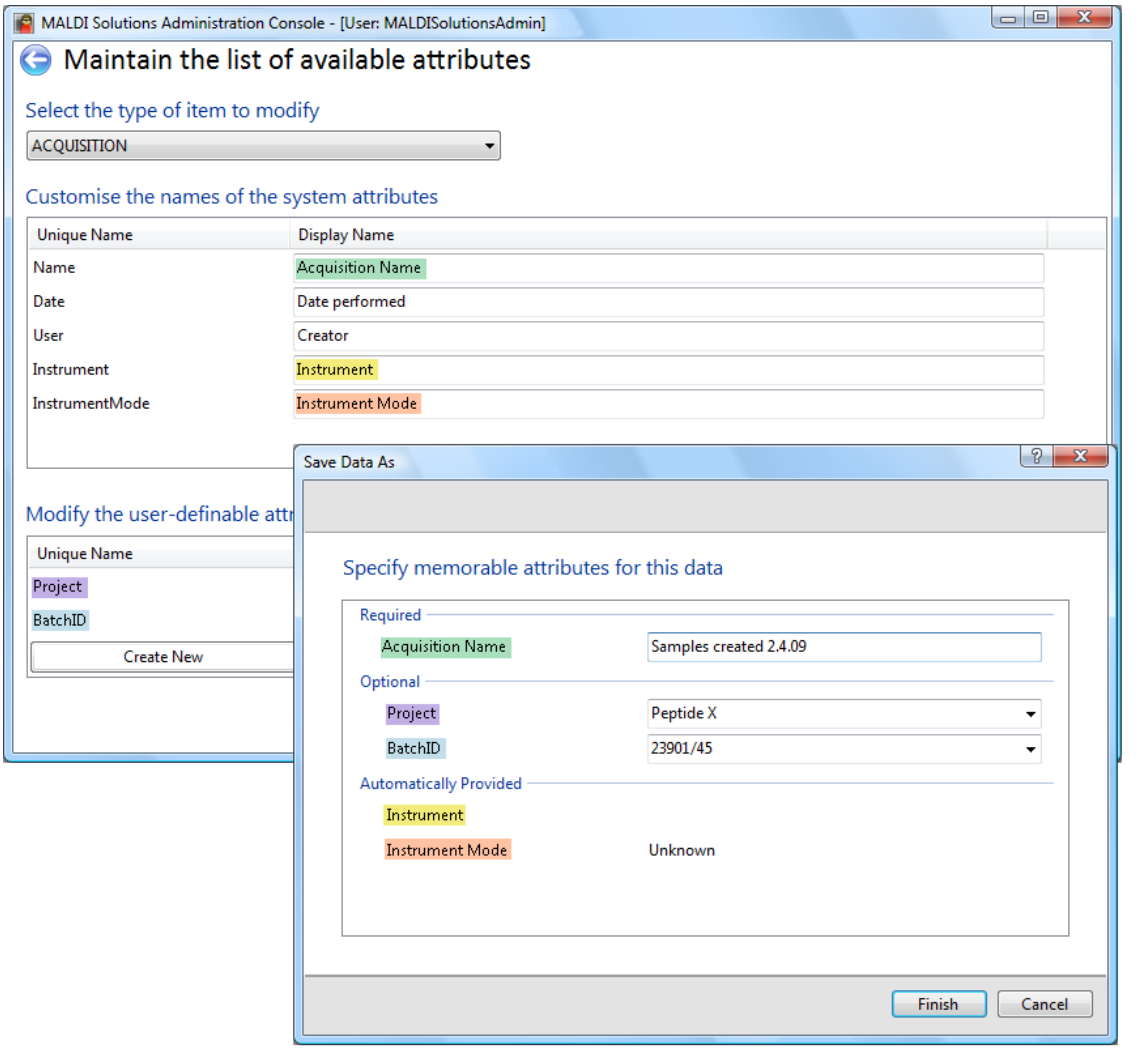


2. To go back to the *Administration Console* main screen, click:  .

About attributes

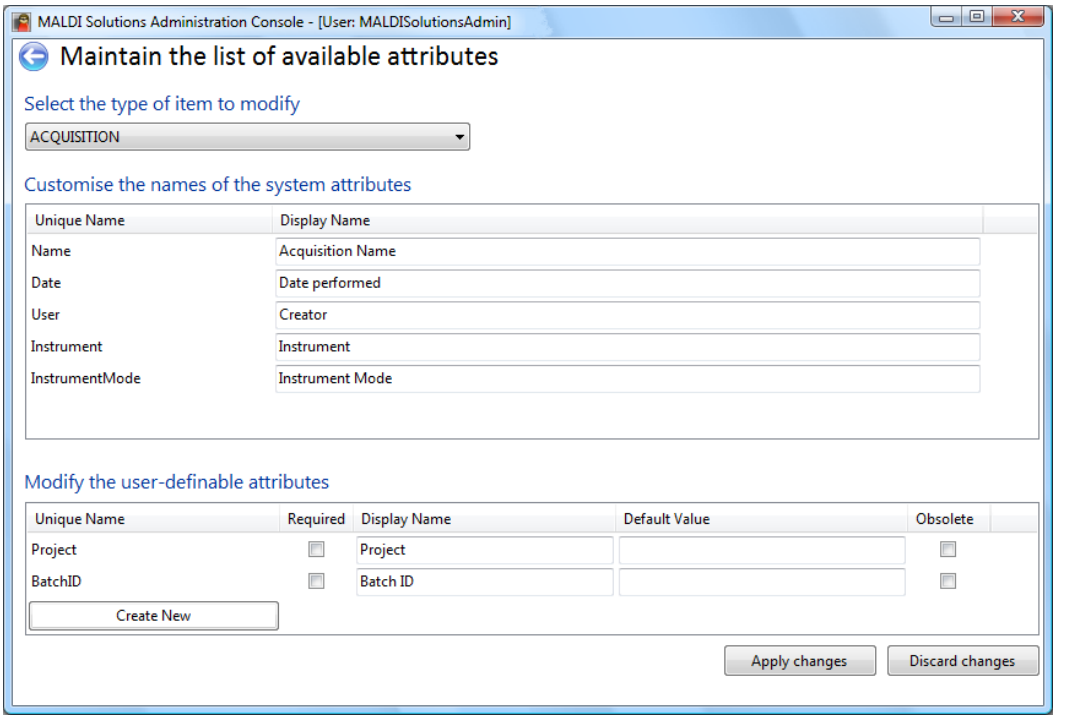
An attribute is a "field" associated with a data set. They are used to allow you to identify the data set within the database, especially if you wish to view the data set at a later date. Increasing the number of attributes makes finding data easier.

In the example below, the *Maintain the list of available attributes* window shows the attributes associated with **ACQUISITION** data sets. The inset window shows you how these attributes are presented to the user when they save the data from an acquisition. The images below are coloured show the relationship between the two.


MALDI Solutions Administration Console - [User: MALDISolutionsAdmin]
Maintain the list of available attributes
Select the type of item to modify
ACQUISITION
Customise the names of the system attributes
Unique Name
Display Name
Name
Acquisition Name
Date
Date performed
User
Creator
Instrument
Instrument
InstrumentMode
Instrument Mode
Modify the user-definable attr
Unique Name
Project
BatchID
Create New
Save Data As
Specify memorable attributes for this data
Required
Acquisition Name
Samples created 2.4.09
Optional
Project
Peptide X
BatchID
23901/45
Automatically Provided
Instrument
Instrument Mode
Unknown
Finish
Cancel

Modifying existing attributes

The fields accept Unicode type, therefore, you can use a local language, for example, Japanese. Limit the number of characters to <100.



Customise the names of the system attributes

Default names are provided. If you wish to change a field, type in the required name.

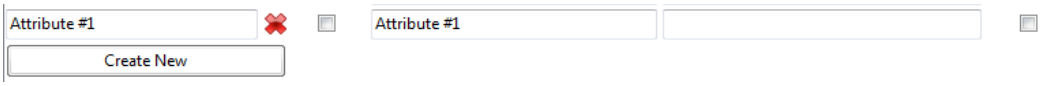
Modify the user-definable attributes

Where appropriate several default attributes are provided. You can modify the default attributes, or subsequent new attributes.

Click **Apply changes**, or **Discard changes**.

Creating new attributes

1. Click **Create New**:



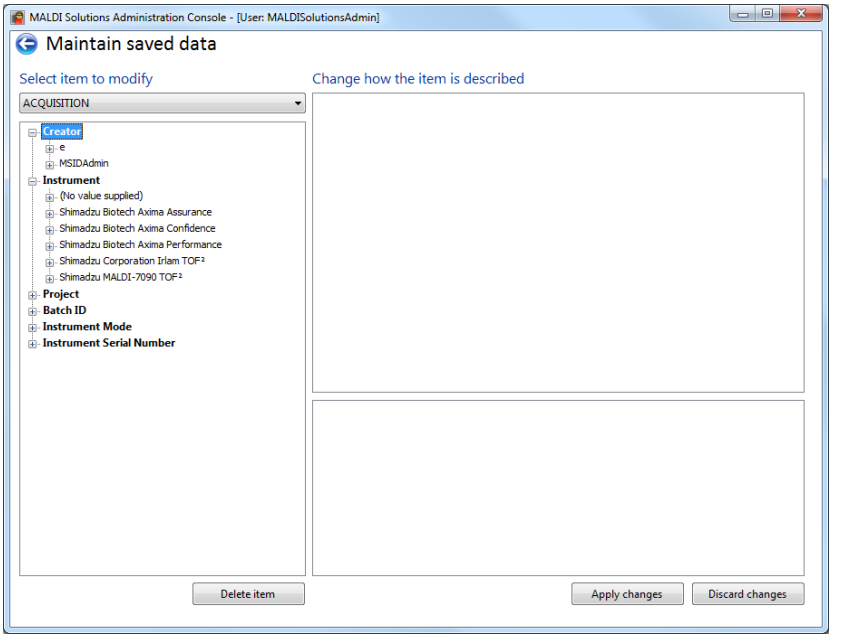
2. Enter the details for the new attribute.
3. To abandon the new attribute, click  .
4. Click **Apply changes**, or **Discard changes**.

Managing saved data

You can manage the data that is saved within the database. This feature allows you to:

- modify the attributes of entries;
- delete entries.

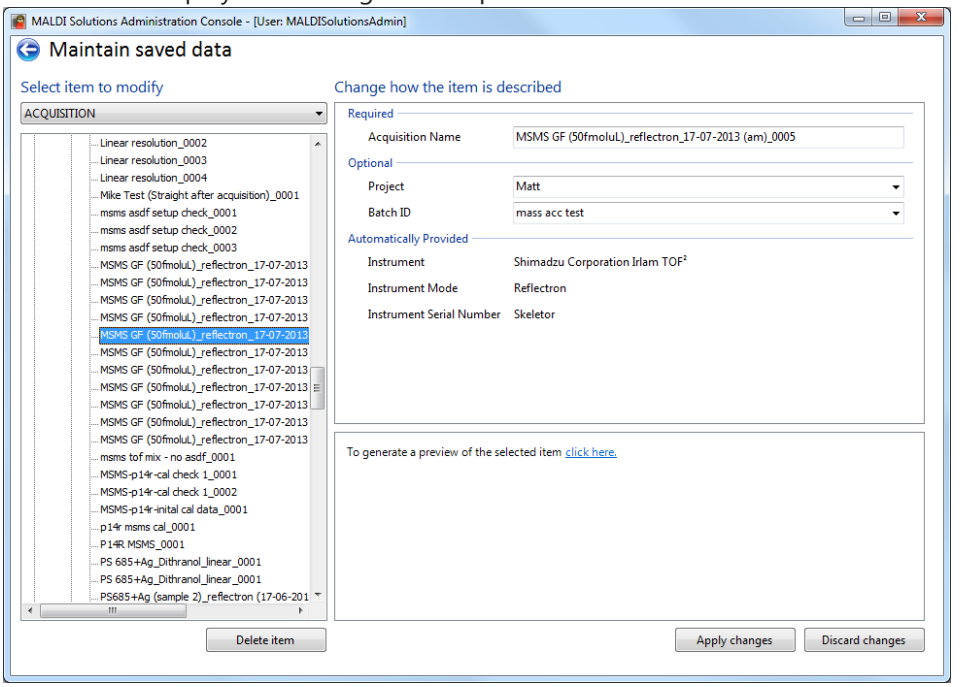
1. Start the "Starting the application" on page 176.
2. Click the **Maintain saved data** link:



3. To go back to the Administration Console main screen, click:  .

Modifying attributes

1. Select the item to modify:
    a. From the **Select the item to modify** drop-down list, select the required category data, for example, **ACQUISITION**.
    b. Navigate through the data structure to the required item and select it; details of the item are displayed in the right-hand pane.



2. If a preview of the data is available, you can use the click here link to display it.
3. Change the fields as required.
4. Click **Apply changes**, or **Discard changes**.

Deleting an item

1. Select the item to delete:
    a. From the drop-down list select the required category data, for example, **ACQUISITION**.
    b. Navigate through the data structure to the required item and select it; details of the item are displayed in the right-hand pane.
2. If a preview of the data is available, you can use the **click here** link to display it.
3. Click **Delete item**; a confirmatory message is displayed.
4. Click **Yes**; the item is deleted from the database.

CHAPTER 5 Maintenance information

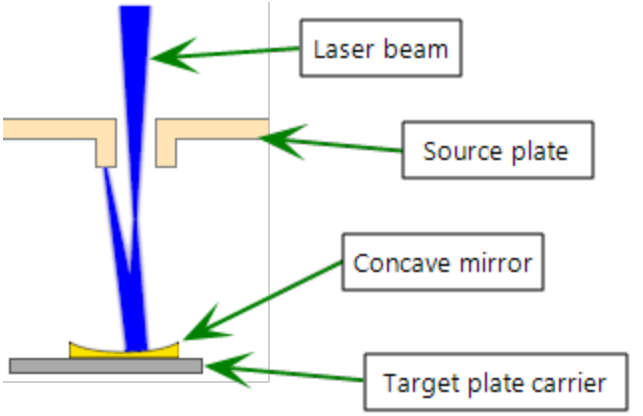
This chapter describes topics associated with maintaining the instrument, what to do if a fault occurs, etc.

Cleaning the source plate

Introduction

Over time, contamination from your samples and matrix can build up on the source plate forming an insulating layer that will adversely affect the operation of the instrument. Therefore, the extraction plate requires periodic cleaning.

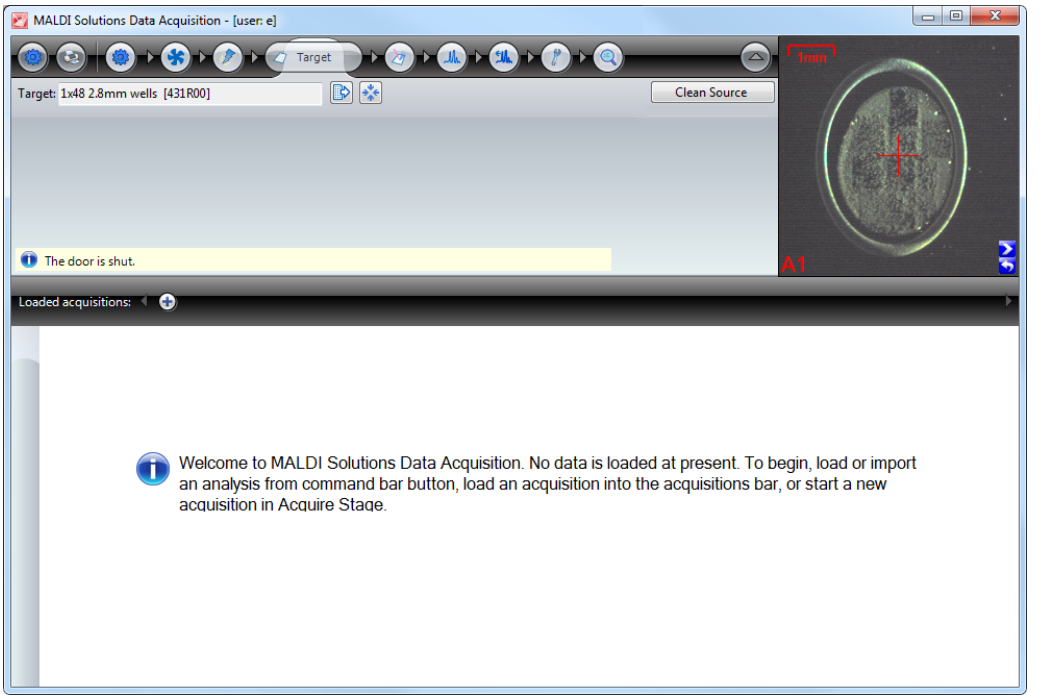
The target plate carrier is fitted with a concave mirror. The instrument uses this mirror to direct and refocus the laser beam (used to desorb/ionise your samples) onto the contamination. The laser then scans the extraction plate, removing the contamination.



Starting source cleaning

This feature is available to users with the appropriate privileges.

1. Click the **Target** tab.
2. Click **Clean Source**:



3. At the confirmation window, click **OK**.

   The software moves the concave mirror in to position. When the instrument is at the operating vacuum, the laser fires. The X/Y stage moves the mirror so that the laser beam rasters the source electrode and cleans it.

   In the Camera window, you can see the laser firing at the source. Also, a message is displayed informing you that source cleaning is in progress.

   The laser cleaning process takes approximately 10 minutes and a confirmation window informs you when the process has finished.

4. Click **OK**.

Cleaning the instrument

Covers

Use a soft damp, or antistatic, cloth to clean the covers of the instrument. Alternatively, you can use diluted washing-up liquid.

- DO NOT use cleaners containing alcohol (such as screen wipes), which corrode plastic.
- DO NOT use acid or alkaline solvents, or abrasive creams.

Monitor

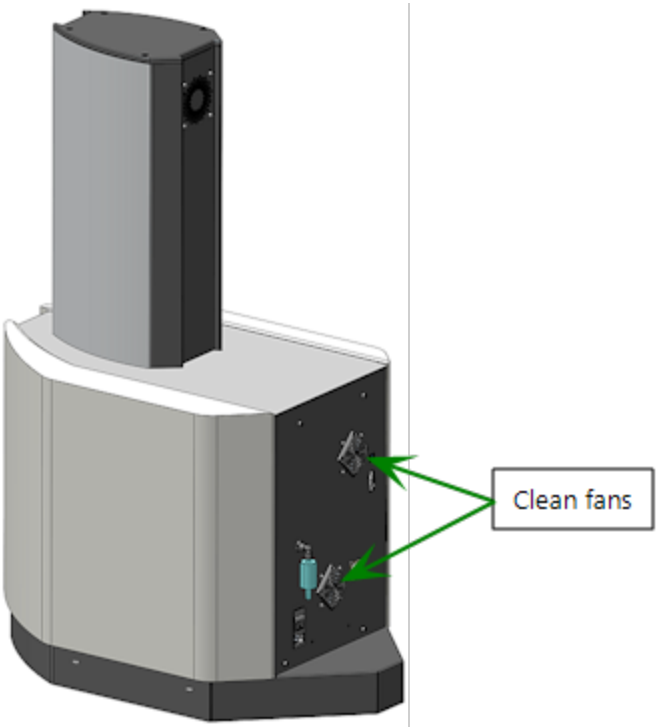
Periodically clean the screen with a soft, dry cloth; DO NOT use tissue paper, etc. as these will damage the screen.

The frame of the monitor can be cleaned with a cloth lightly moistened with diluted washing-up liquid followed by a wiping with a soft, dry cloth.

- DO NOT use cleaners containing alcohol (such as screen wipes), which corrode plastic.
- DO NOT use acid or alkaline solvents, or abrasive creams.

Cleaning the fans

1. Switch off the instrument, see "Switching off" on page 11
2. Remove dust from the two fans using, for example, a vacuum cleaner.
3. Switch on the instrument, see "Switching on" on page 10.



Consumables

Description	Part number	Frequency
FlexiMass-DS disposable targets, pack of 16 polymeric slides	TO-430R00	As and when stocks run low
O-ring door seal	224-00127	If damaged
MALDI TOF Mix Testing kit	TO-724R00	When required

Fitting the O-ring door seal

If the O-ring door seal is damaged, for example by physical knocks, replace it. The replacement O-ring door seal is supplied clean. DO NOT attempt to clean the seal with any solvent as this will change the properties of the seal.

1. Open the Target plate door, see "Inserting/removing a Target Plate" on page 39
2. Using your fingers, lift the seal out of its groove. DO NOT use metal tools as you may scratch the surface. Discard the old seal.
3. If required, carefully clean the groove with a lint-free cloth and HPLC grade acetone - allow to dry before fitting the new seal.
4. Fit the new seal into the groove.

Customer QC tests

The Customer QC (Quality Control) tests identify any deterioration in performance of your instrument. Only personnel who have had basic user training should attempt these tests.

Run these tests monthly.

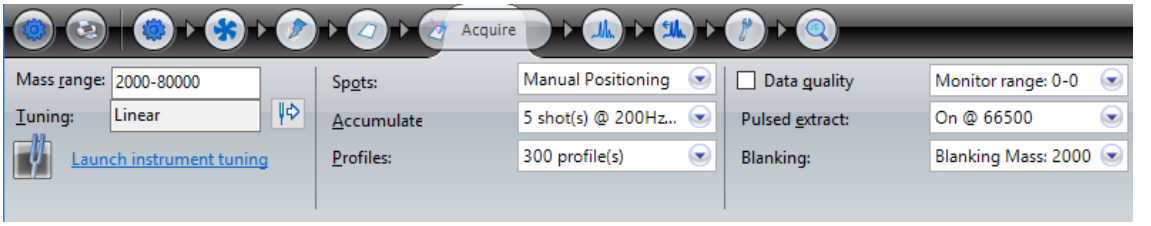
The samples below are available in the Startup calibration kit, order number TO-724R00.

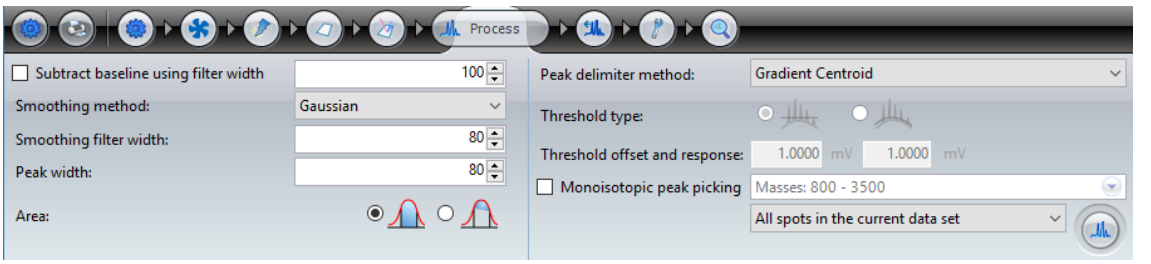
High mass test

Sample

- Test sample: 500 fmol/µL BSA in 70:30 0.1% TFA/acetonitrile
- Matrix: sinapinic acid, 20 mg/mL in 50:50 acetonitrile/0.1% TFA
- Spotting: 0.5 µL sample/0.5 µL matrix

MALDI Solutions setup parameters





Experiment notes

Collect between 30 and 300 profiles.

Pre-test: Identify the laser power that starts producing the matrix peak 225.08 Da (switch Blanking off).

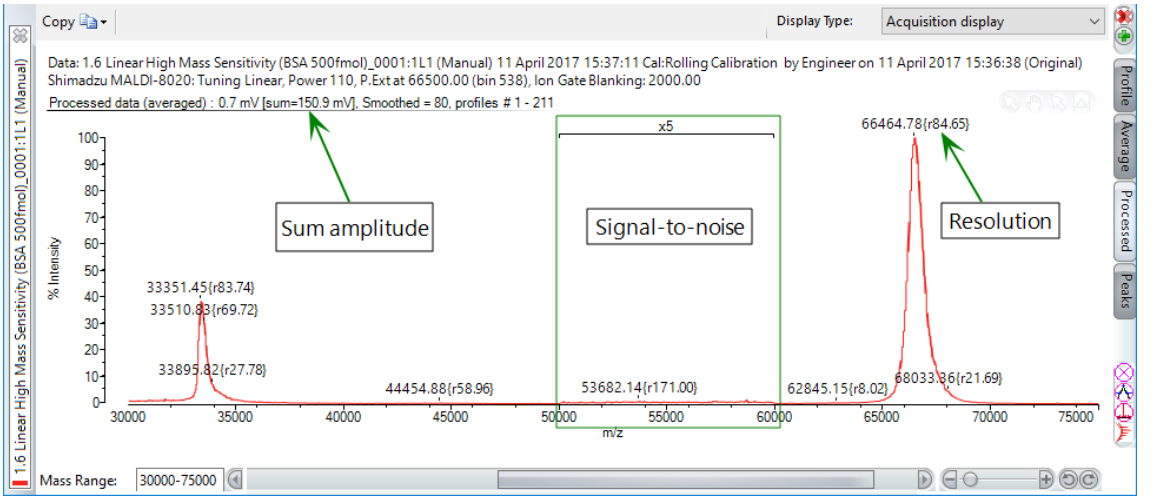
Test: Increase the laser power of the pre-test by 30 to 50. Switch the blanking on at 2000 Da. Move the laser position continuously around the sample, looking for 'sweet spots'. Each 'sweet spots' will only yield data for 2 to 10 profiles.

Results

Test	Specification
Sum amplitude	>500 mV
Resolution	>50
Signal-to-noise	>5:1 (50,000 to 60,000 Da)

Keep a record of the results, see "Results" on page 205.

Typical spectrum

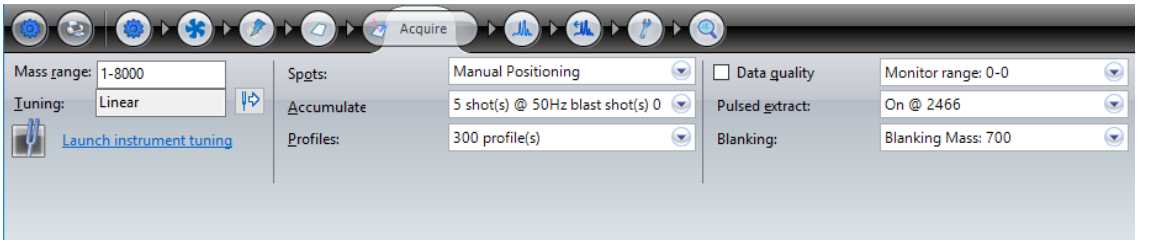


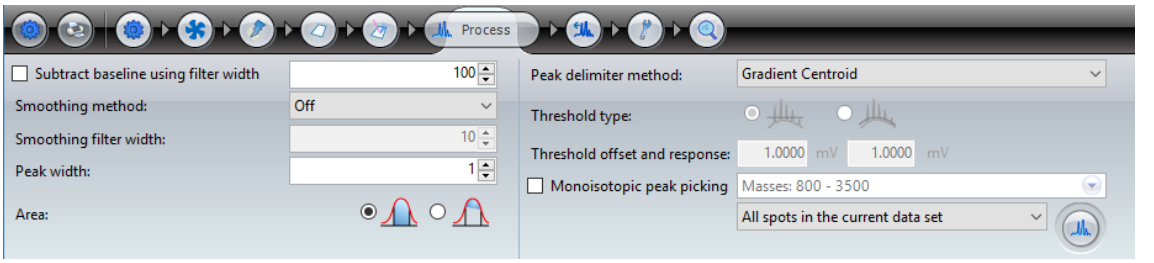
Mass resolution test

Sample

- Test sample: 1 pmol/µL ACTH 18-39 in 70:30 0.1% TFA/acetonitrile
- Matrix: alpha-cyano-hydroxy-4-cinnamic acid, 10 mg/mL in 50:50 acetonitrile/0.1% TFA
- Spotting: 0.5 µL sample/0.5 µL matrix

MALDI Solutions setup parameters





Experiment notes

Collect between 30 and 300 profiles.

Pre-test: Identify the laser power that starts producing the matrix peak 190.05 Da.

Test: Increase the laser power identified in the pre-test. Switch the blanking on at 700 Da. Move the laser position continuously around the sample, to find a position that yields a continuous signal. You can clear the accumulated profiles and start to acquire the data for the test.

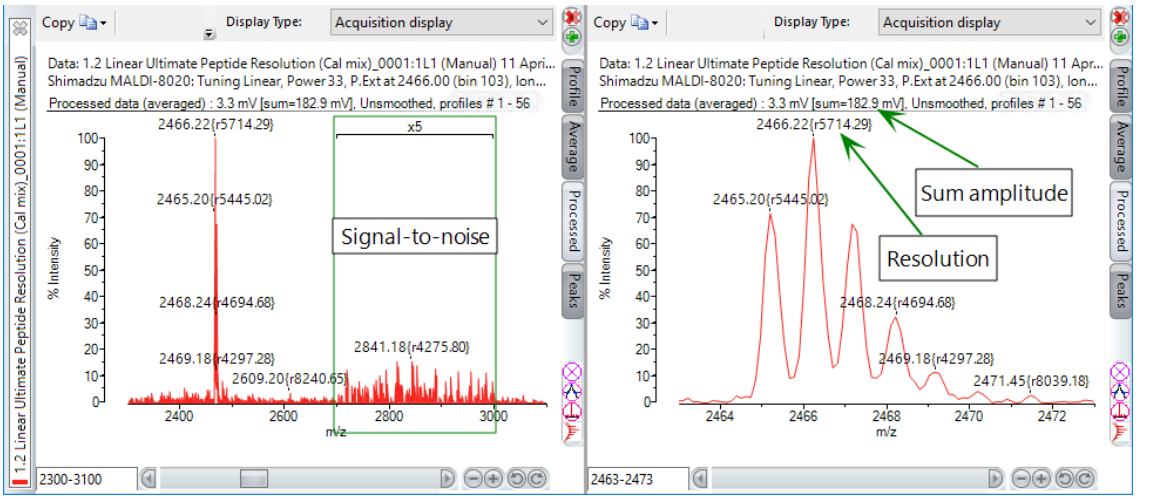
Results

Test	Specification
Sum amplitude	>100 mV
Resolution	>4000

Test	Specification
Signal-to-noise	>5:1 (2700-3000 Da)

Keep a record of the results, see "Results" on the facing page.

Typical spectrum



Results

Photocopy this page and use the copy to record your results.

Test	Specification	Period 1	Period 2	Period 3	Peiod 4
High mass test	Sum amplitude >500 mV				
	Resolution >50				
	Signal-to-noise >5:1				
Mass resolution test	Sum amplitude >500 mV				
	Resolution >4,000				
	Signal-to-noise >5:1				

Test	Specification	Period 5	Period 6	Period 7	Peiod 8
High mass test	Sum amplitude >500 mV				
	Resolution >50				
	Signal-to-noise >5:1				
Mass resolution test	Sum amplitude >500 mV				
	Resolution >4,000				
	Signal-to-noise >5:1				

Servicing and fault reporting

Due to hazardous voltages and a Class 3B laser, only trained service engineers are permitted to carry out preventative maintenance and servicing.

Preventative maintenance

Your instrument is designed for reliability and long life. Preventative maintenance servicing ensures that the instrument continues to provide trouble-free operation. We recommend that the instrument is serviced at least once a year.

After the one-year warranty period, service contracts are available to provide the appropriate level of support. Contact your local service centre for details.

Service engineers are not allowed to service instruments if it is contaminated with any substance which is radioactive or biologically active; it is dangerous, unless decontaminated.

Fault reporting

You are notified of a fault by:

- Front panel red light - flashing;
- Monitor - displaying an information or error message.

Contact your local Shimadzu, or distributor. They will require the following information:

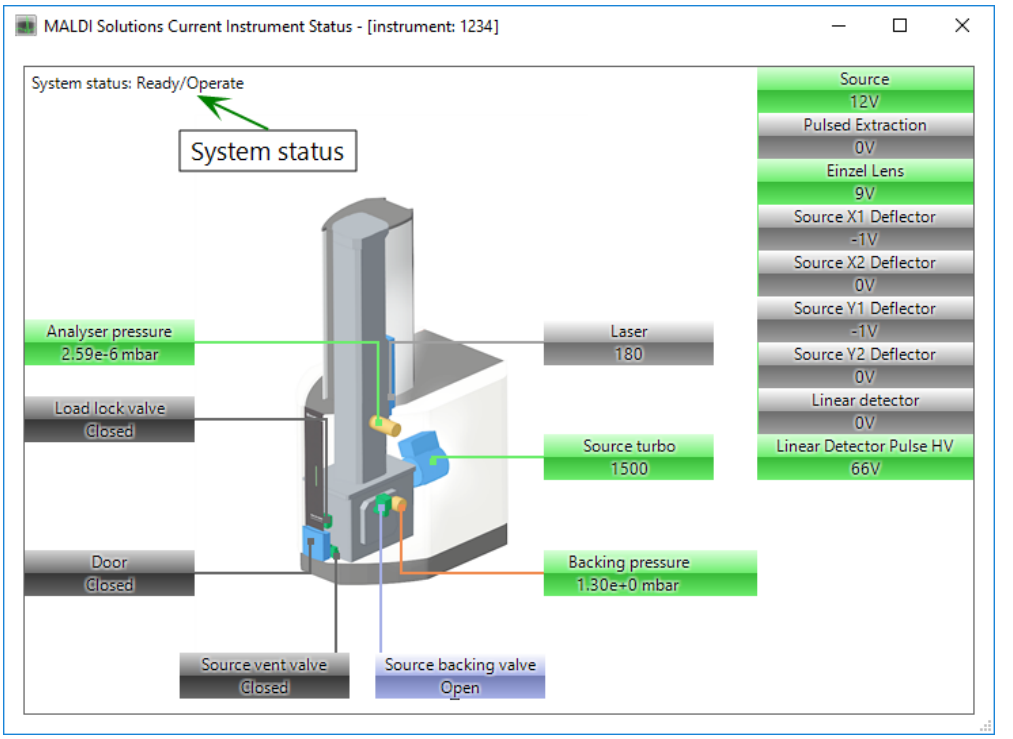
- Type of instrument: MALDI-8020;
- Serial number (shown on the documentation supplied with the instrument, or on the back of the instrument);
- Details of any error messages;
- Symptoms of the fault.

Trouble shooting

This section describes the causes of problems and the corrective action to take when they occur.

Current instrument status viewer

If you suspect that the instrument is faulty, open the status viewer, see "Current instrument status viewer" on page 232.



Instrument problems

If you cannot resolve the problem, contact your local Shimadzu office, or distributor, see "Fault reporting " on page 206.

Problem	Cause/corrective action
The front panel LEDs do not light when the power is turned ON.	Check if the power cable is correctly connected and if 100 to 240 V power is being supplied to the instrument. See "Layout - front " on page 239.
PC cannot communicate with the instrument.	Check that the USB 3.0 cable from the instrument is connected to a USB 3.0 port on the PC. See "Connection diagram" on page 242.
In MALDI Solutions Data Acquisition, the **Operate** button is not active.	Open the *Current instrument status viewer* and monitor the "Analyser pressure". • If pressure is greater than 5.0e-6 mbar, the instrument will not switch to operate mode and the high voltage supply will not switch on, contact your local Shimadzu office, or distributor. • If pressure is less than 5.0e-6 mbar, high voltage supplies may be faulty, contact your local Shimadzu office, or distributor.
In MALDI Solutions Data Acquisition, the **FIRE** button is not active.	Open the *Current instrument status viewer*. • If the "System status" is *Standby*, the laser will not fire; in MALDI Solutions Data Acquisition, click the **Operate** button. • If the "System status" is *Operate*, the laser may be faulty, contact your local Shimadzu office, or distributor.

Problem	Cause/corrective action
After loading a Target plate, instrument takes more than 5 minutes before the **Operate** button is active.	If your samples are wet, the instrument will take longer to achieve operating vacuum. Ensure samples are dry before loading the Target plate.
	Target plates handled without the use of clean powder-free latex gloves. Use gloves when handling target plates.
	Vacuum leak on the front door sealing surface, check that: • Front door is fully closed. • There is no dust on the O-ring door seal. If there is dust, wipe with a clean lint-free cloth. • The O-ring door is damaged, replace it, see "Fitting the O-ring door seal" on page 199.
	Instrument has been switched off for several weeks, wait for vacuum to be achieved - may take several hours.
Source turbo does not reach full speed (1500 Hz).	Instrument has a vacuum leak, contact your local Shimadzu office, or distributor.
	Diaphragm pump is failing, contact your local Shimadzu office, or distributor.
	Inlet filter is clogged, contact your local Shimadzu office, or distributor.
	Source turbo is running too hot, contact your local Shimadzu office, or distributor.
The front door cannot be closed.	Ensure the Target plate is properly inserted into the sample holder, see "Inserting/removing a Target Plate" on page 39.
	The O-ring door seal has come out of its groove. To refit it, see "Fitting the O-ring door seal" on page 199.

Data acquisition problems

If you cannot resolve the problem, contact your local Shimadzu office, or distributor, see "Fault reporting " on page 206.

Problem	Cause	Corrective action
The ion signal is unstable or low.	The laser power is too low for the samples.	Increase the laser power to get a good signal.
	The laser power is degraded.	Increase the laser power to get the same ion signal as before.
	The sample quality is poor.	Use freshly prepared samples.
	The Source plate is contaminated.	See "Cleaning the source plate " on page 194.
	The detector is degraded.	Contact your local Shimadzu office, or distributor.
	High-voltage supply is degraded.	Contact your local Shimadzu office, or distributor.
The base line is too high.	Detector signal cable is damaged.	Contact your local Shimadzu office, or distributor.
	The detector is degraded.	Contact your local Shimadzu office, or distributor.
	High-voltage supply is degraded.	Contact your local Shimadzu office, or distributor.

Database administration

Security implications

This section is for any database administrator who may wish to manually configure the database security.

The Administration Console is designed to simplify the configuration and maintenance of the MALDI Solutions database. To ensure correct operation of the system third-party user management tools (such as Microsoft SQL Server Management Studio) must not be used.

The standard administrative user (which is created during the installation of the product) is assigned the Kratos_Basic and Kratos_Advanced roles. These roles allow the user to perform any operation within the MALDI Solutions product suite. The user also holds the "securityadmin" and "db_owner" roles which allow the user to create users and grant roles. The "processadmin" role is required to ensure that administrators can terminate any database activity for any users that they delete.

When new users are created in the Administration Console, they are given a basic level of access to the database (in particular the Kratos_Basic role). If the Administration Console is then used to promote a user to an administrator, the user will also be assigned the "securityadmin", "db_owner" and "processadmin" roles. This user can then promote other users in the same manner. Newly created users will have the CHECK_POLICY disabled. This means that the passwords entered through the Administration Console will not have to conform to any corporate password policy. Additionally, password aging will be disabled by the console and is not supported by any of the MALDI Solutions products.

Performance implications

During installation of the database, the installation script changes the maximum memory that SQL server is allowed to half the total system memory.

Backing up the database

As backing up the database is probably beyond the scope of most users, we recommend that you arrange for your IT/IS department to arrange this for you. The process, by default, will backup:

- DBBbackup (nightly), and
- LogBackup (every 8 hours).

We recommend that the server/PC that contains the MALDI Solutions database is configured to automatically back up the data. For added security the data should ideally be backed up to a remote location, or to a tape drive which is stored off-site.

The installation media contains a sample back up script (**Database Scripts => Backup.bat**). This is to be used as a guide only to provide a starting point.

Backup and restore

This section describes how to use the *Backup and Restore* application. This application allows you to:

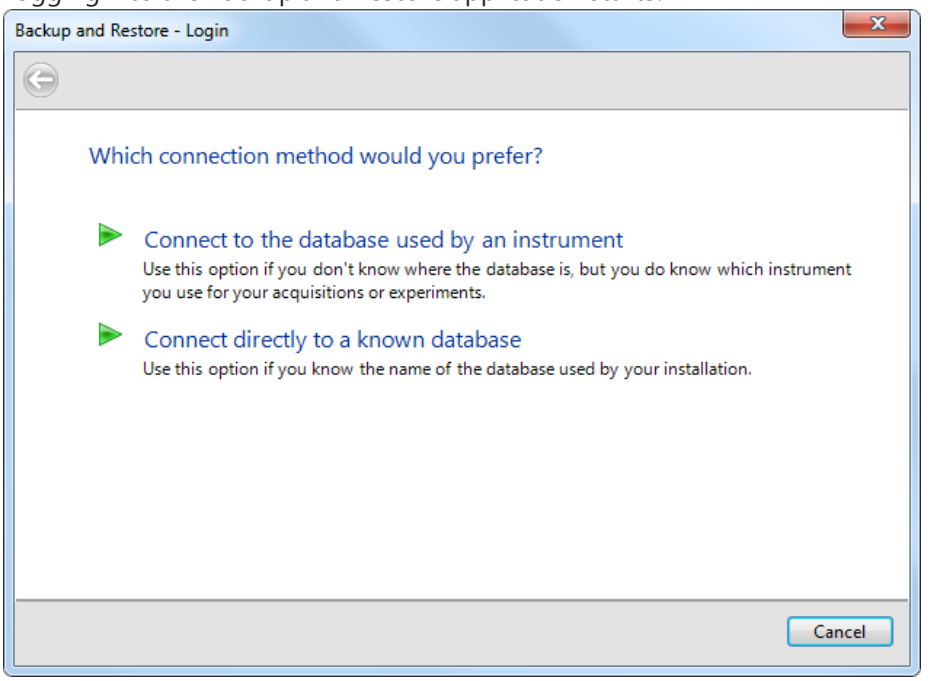
- Store selected data into a backup file. You can send this file to a database held on another computer or to an offline location for restoration at a later date.
- Re-import data from the backup file into the MALDI Solutions database.

Starting the application

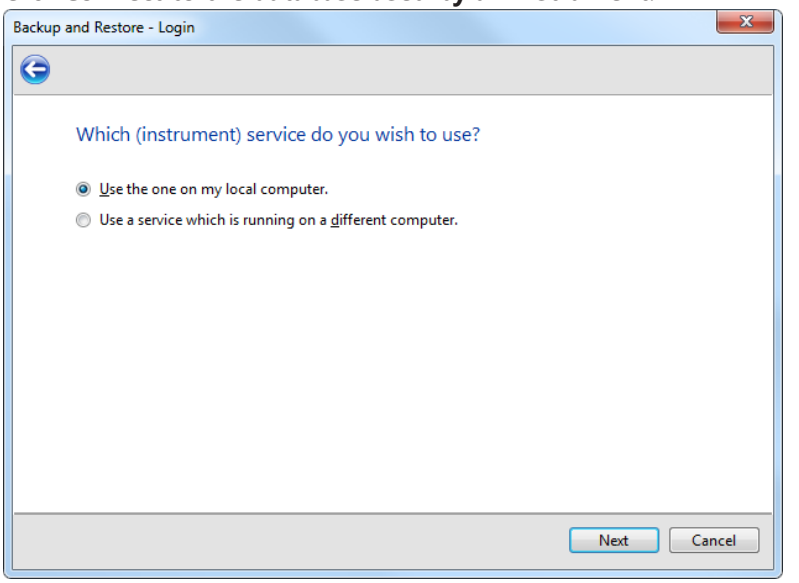
This section assumes that you know how to login and, if required, that you know how to fill in the fields that are presented to you.

1. Open Windows File Explorer.
2. Navigate to and open **Programs Files => Shimadzu Corporation => MALDI Solutions => Programs => Kratos.Applications.BackupRestore**.

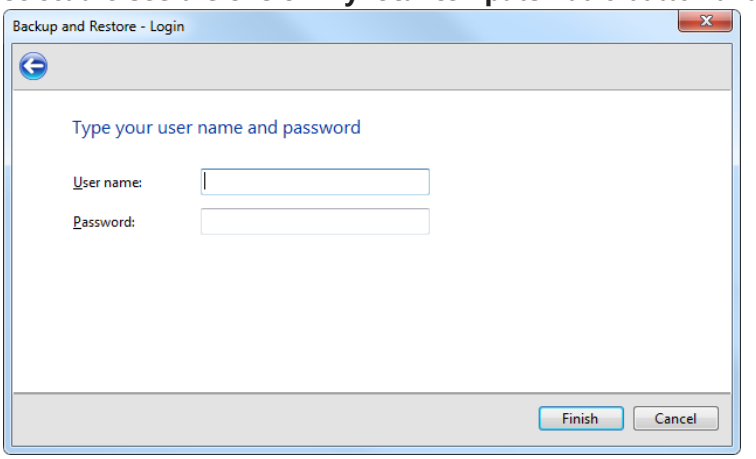
Logging into the Backup and Restore application starts.



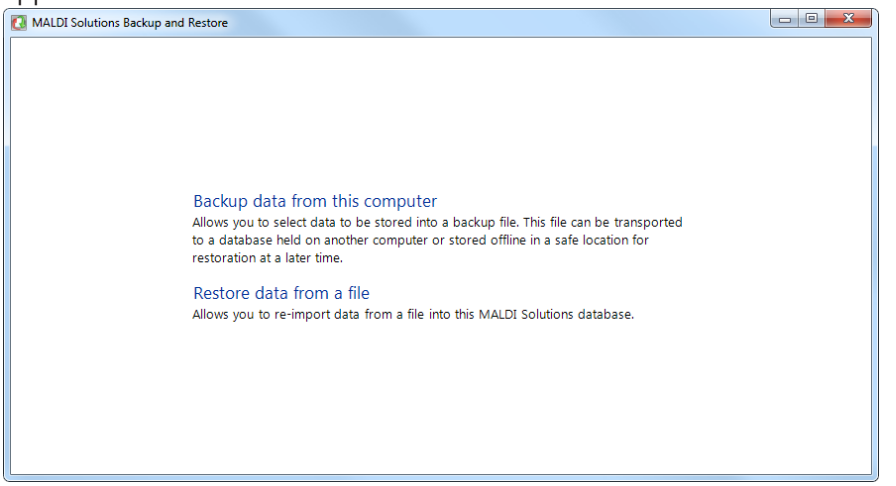
3. Click **Connect to the database used by an instrument**.



4. Select the **Use the one on my local computer** radio button and click **Next**:



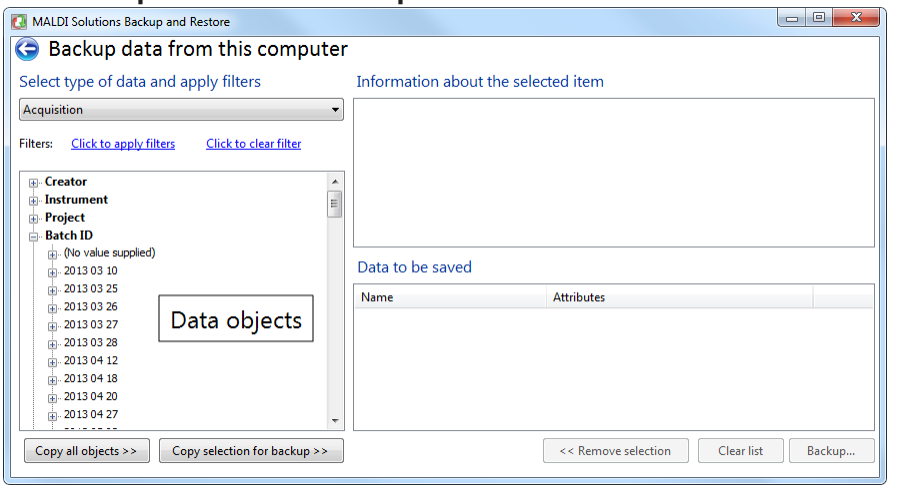
5. Enter your user name and password, and click **Finish** the Backup and Restore application starts:



Selecting data for backup

You can select various data objects for backing up.

Click **Backup data from this computer**:



You can:

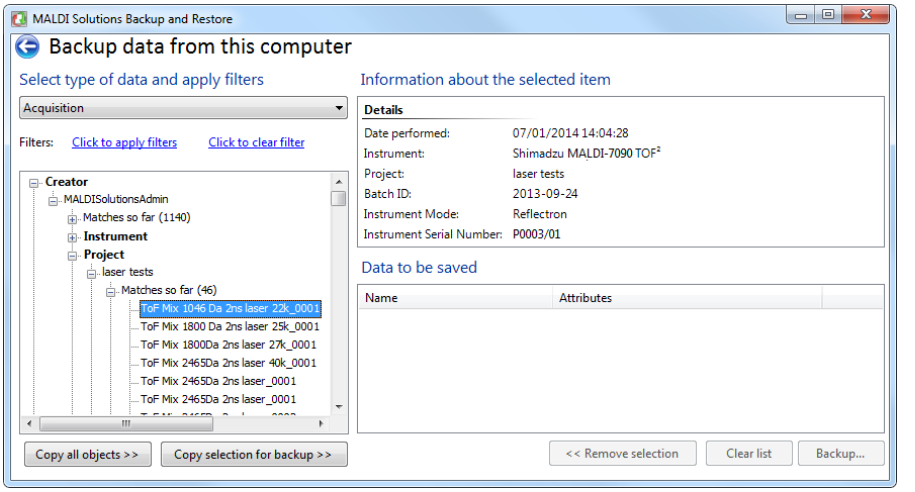
- View information about a data object;
- Select data objects for copying;
- Filter data objects according to attributes.

To go back to the *Backup and Restore* main screen, click:

Viewing information about a data object

1. In the **Select type of data and apply filters** drop-down list, select the required type of data for backing up.

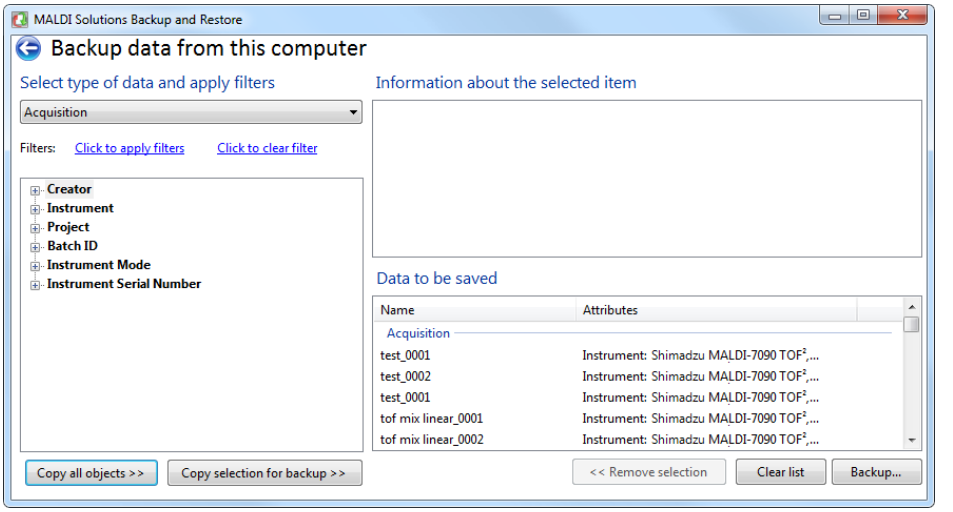
2. Navigate to the required data object and select it; details are displayed in the *Information about the selected item* window.



Copying all data objects

1. In the **Select type of data and apply filters** drop-down list, select the required type of data for backing up.

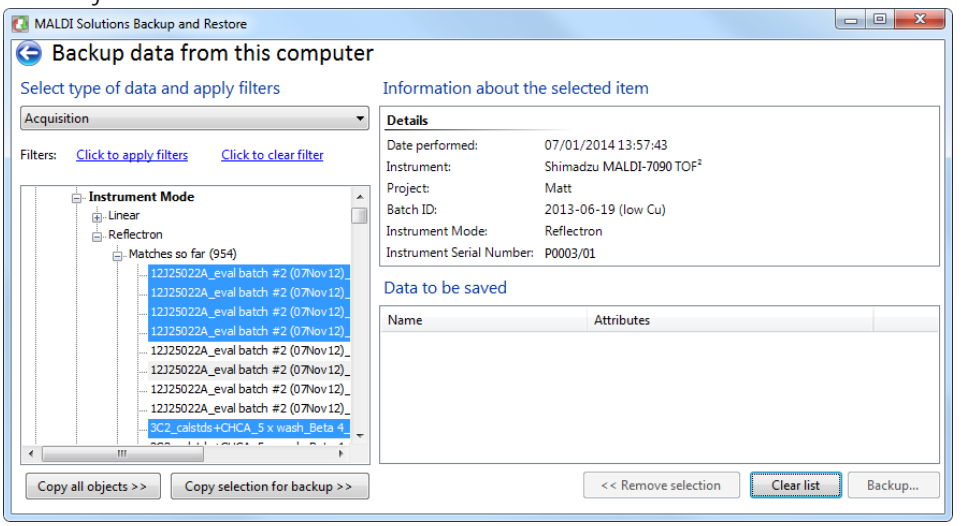
2. Click the **Copy all objects** button; the data objects for backing up are displayed in the *Data to be saved* area.



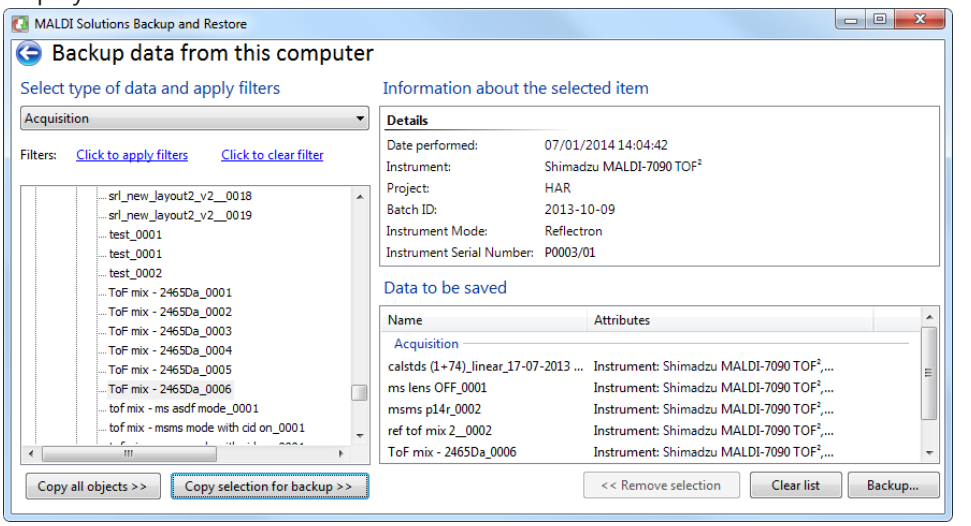
Selecting specific data objects for copying

1. Navigate to the required data objects.

2. Select the required data objects. Use the Shift or Ctrl keys to select a range or multiple data objects.



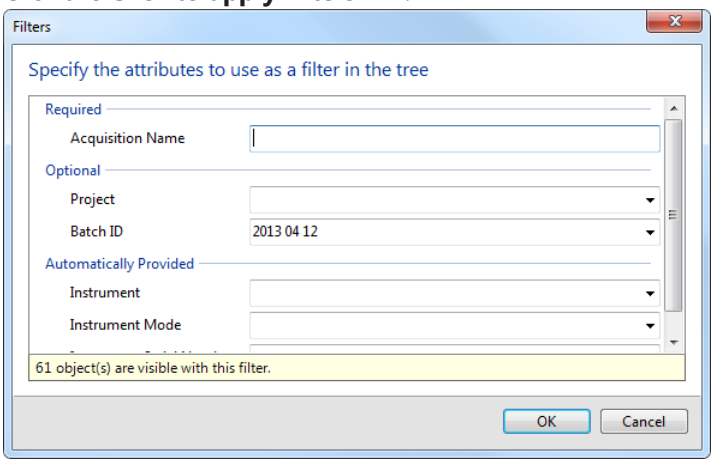
3. Click the **Copy selection for backup** button; the data objects for backing up are displayed in the *Data to be saved area*.



Filtering data objects according to attributes

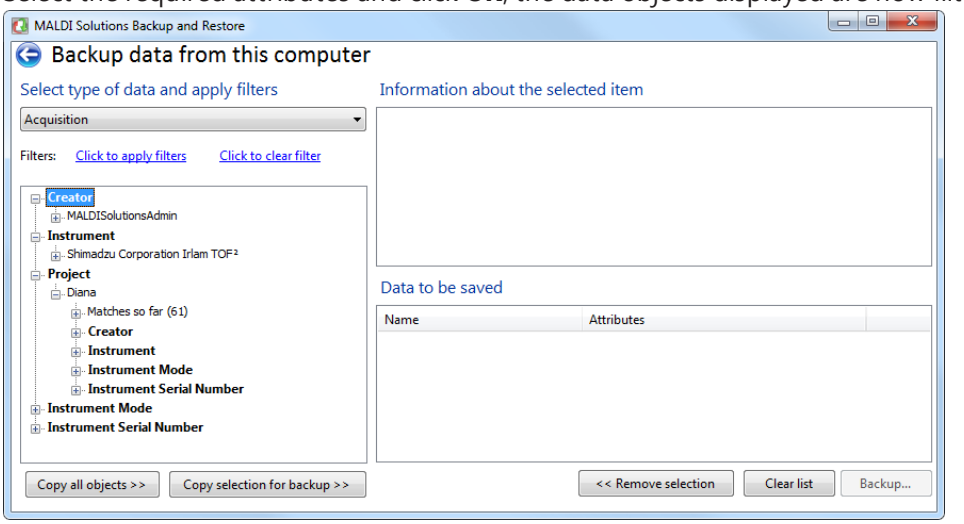
1. In the **Select type of data and apply filters** drop-down list, select the required type of data for backing up.

2. Click the **Click to apply filters** link:



The number of visible data objects is displayed within the window.

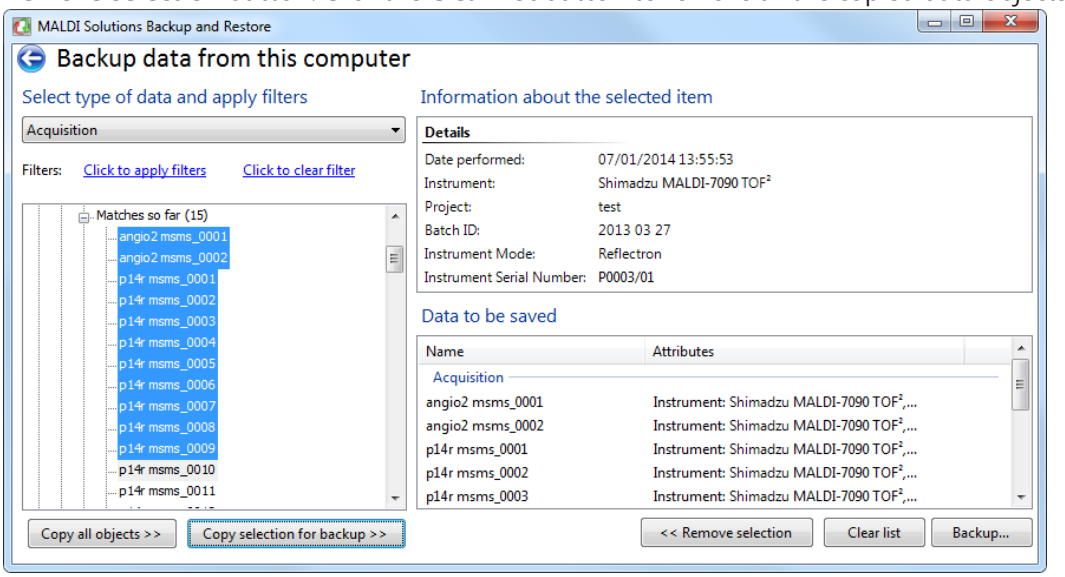
3. Select the required attributes and click **OK**; the data objects displayed are now filtered:



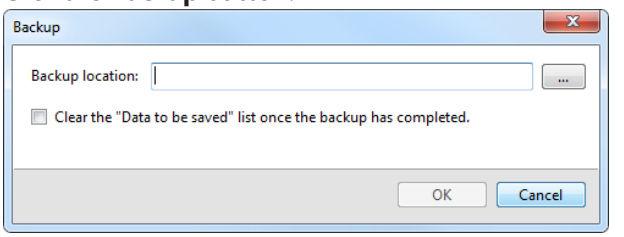
4. To clear the filter, click the **Click to clear filter** link.

Backing up data

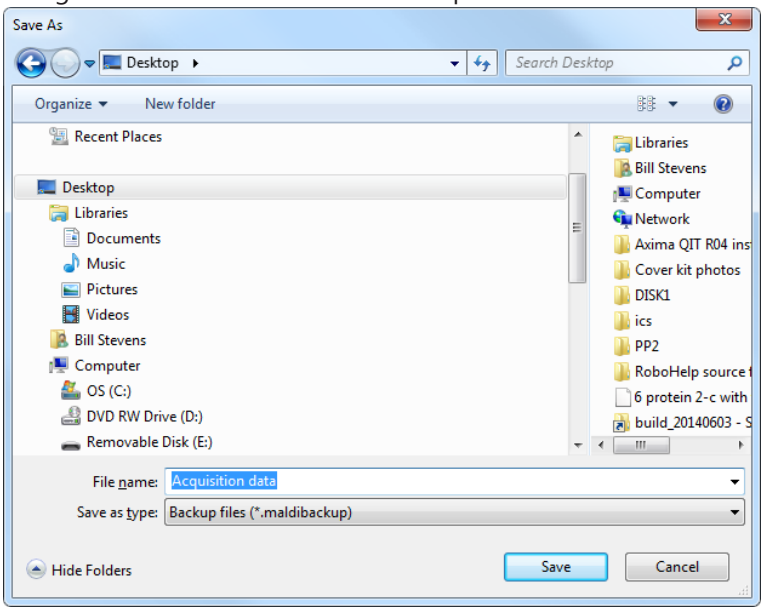
Prior to backing up the copied data objects, you can remove selected objects using the **Remove selection** button. Click the **Clear list** button to remove all the copied data objects.



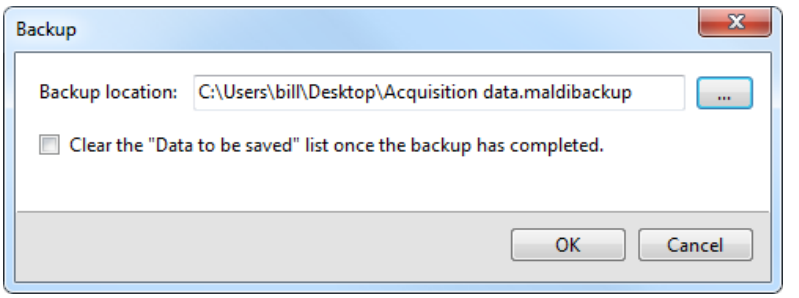
1. Click the **Backup** button:



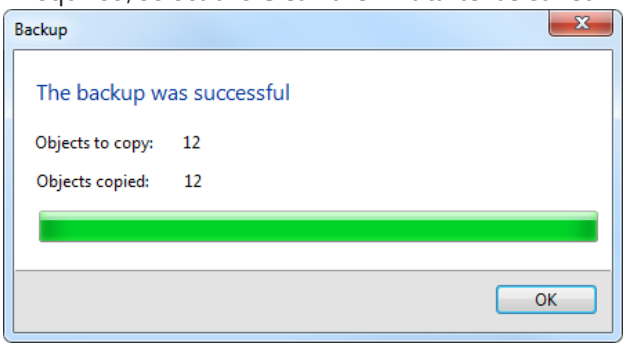
2. Navigate to the location for the backup file:



3. Click **Save**:



4. If required, select the **Clear the "Data to be saved" list ...** check box; back up starts.



5. When the backup has finished, click **OK**.

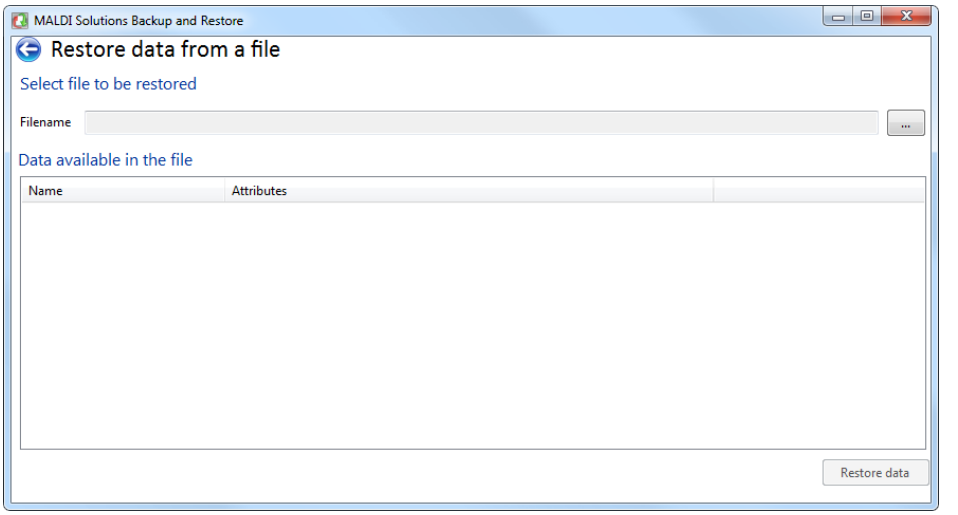
If the backup was not successful, the dialog box informs you.

Restore data

This feature allows you to import backup data into the MALDI Solutions database.

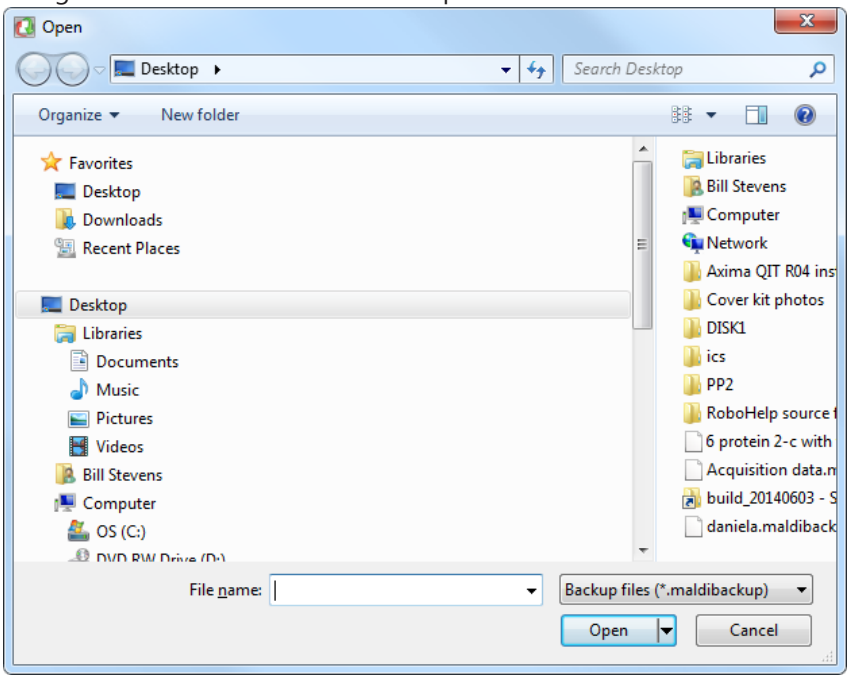
1. If the MALDI Solutions Data Acquisition application is open, close it.
2. Start the *Backup and Restore* application, see "Starting the application" on page 213

3. Click **Restore data from a file**:

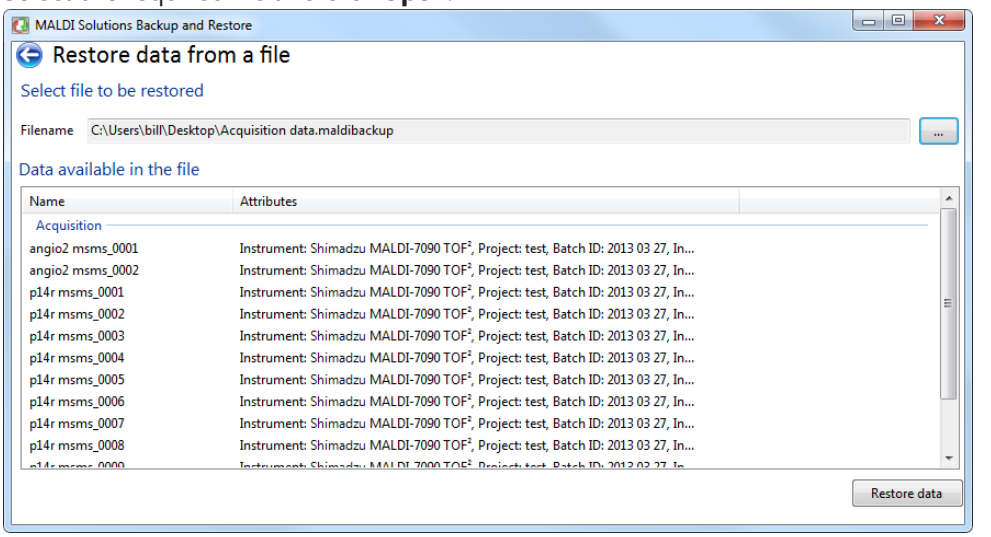


To go back to the *Backup and Restore* main screen, click:

4. Navigate to the location of the backup file:



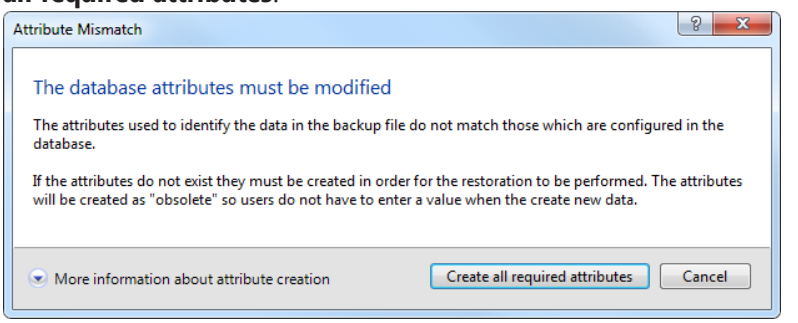
5. Select the required file and click **Open**:



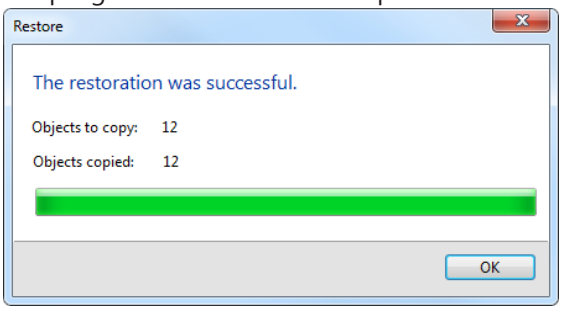
6. Click **Restore data**.

   If the MALDI Solutions database contains copies of the data objects you are attempting to restore, the following window is displayed. Click **Replace data**.

   If there is mismatch regarding attributes, a window is displayed to alert you. Click **Create all required attributes**.



7. When the data is copied to the database, the *Restore* window is displayed showing you the progress of the restoration process.



8. When the restoration has finished, click **OK**.

CHAPTER 6 MALDI-8020 instrument

This chapter describes the MALDI-8020 instrument including:

Current instrument status viewer

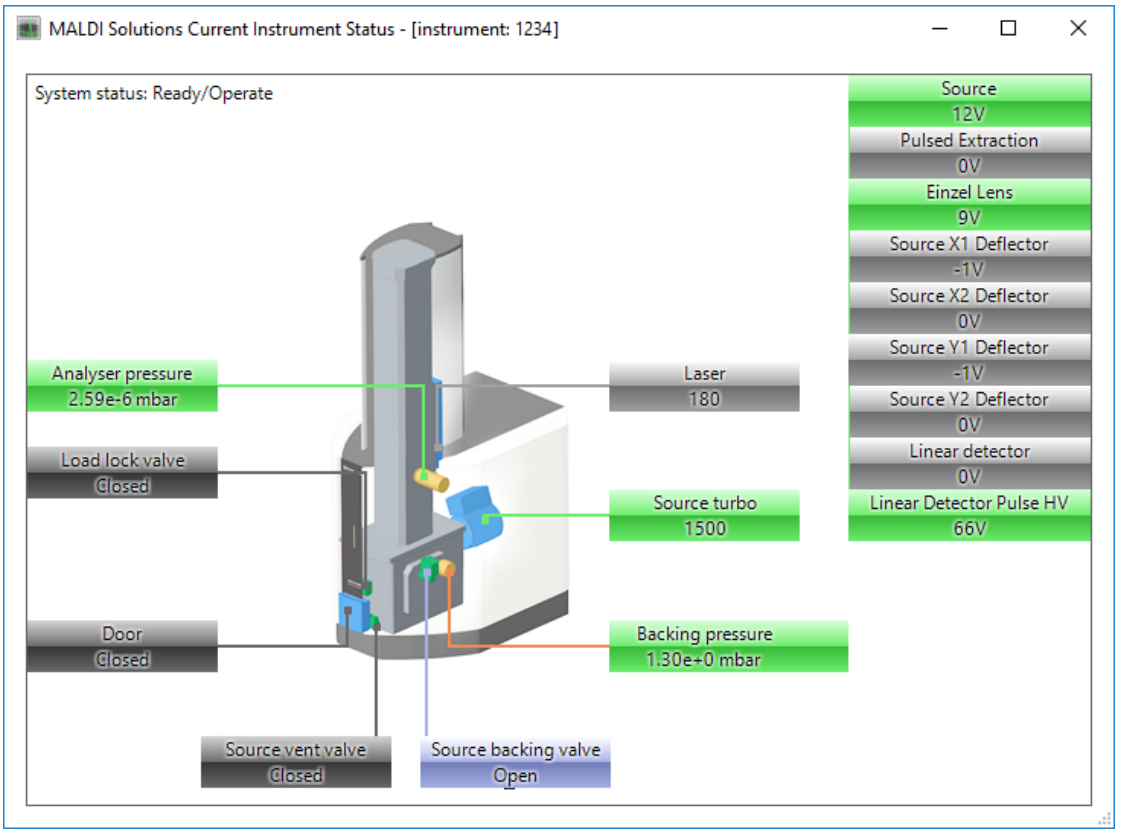
The *Current Instrument Status* viewer shows you a graphical representation of the instrument. Colour-coded annotations give you a visual representation of the status of the components.

The viewer is also used by service engineers, and contains information specifically for their use. For relevant components, their pressures or voltages are shown.

As you use the instrument, the viewer reflects those changes, for example, if you open the door.

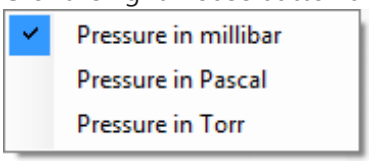
Starting the application

1. Open Windows File Explorer.
2. Navigate to and open **Programs Files => Shimadzu Corporation => MALDI Solutions => Programs =>Kratos.Applications.CurrentInstrumentStatus**.



Changing the units of pressure

1. Click the right mouse button anywhere in the viewer:



2. Select the required unit.

Status

Pressure readings

Colour	Description
Green	At required pressure
Amber	Not at required pressure - progress bar indicates progress to required pressure

Valves/door

Colour	Description
Blue	Open
Black	Closed

Laser

The laser power is displayed; the colour does not change.

Source turbo

Colour	Description
Grey	Turbo is off
Yellow	Turbo is accelerating
Green	Turbo is at full speed
Red	Turbo has failed

Components

For example, source deflectors.

Colour	Description
Grey	Component is off
Green	Component is on
Red	Component has failed

Life cycle of the instrument

Storage

The storage requirements for the instrument are:

- Minimum temperature: -20°C (-4°F).
- Maximum temperature: +60°C (140°F).
- Maximum humidity: 70% relative humidity (non condensing).

Delivery and installation

The instrument is delivered in suitable packaging that includes installation instruction and lifting straps. Shimadzu or Kratos accredited engineers normally install the instrument.

Site requirements

CAUTION	Heavy equipment
	The instrument weighs approximately 86 kg (190 lbs); use suitable mechanical handling equipment to lift the instrument. Check that the new location can support the weight of the instrument.

CAUTION	Access to the mains switch
	Always ensure that there is clear access to either the rear panel mains switch or to the mains plug (where it is plugged into the wall socket) to allow the instrument power to be easily disconnected at any time.

CAUTION	Cooling fans
	Two inlet fans and an outlet fan cool the instrument. Do not obstruct the fans.

Servicing

CAUTION	Servicing
	Only Shimadzu or Kratos accredited service engineers are permitted to service components within the instrument.

We recommend that the instrument is serviced at least once a year, depending on usage and samples used. After the one-year warranty period, service contracts are available to provide the appropriate level of support. Contact your local Shimadzu office, or distributor, for details.

Fault reporting

For further information, see "Servicing and fault reporting" on page 206

Moving the instrument

CAUTION	Damage to the instrument
	Do not move the instrument while the turbo pump is running. Switch off and allow the pump to slow down for at least 30 minutes.

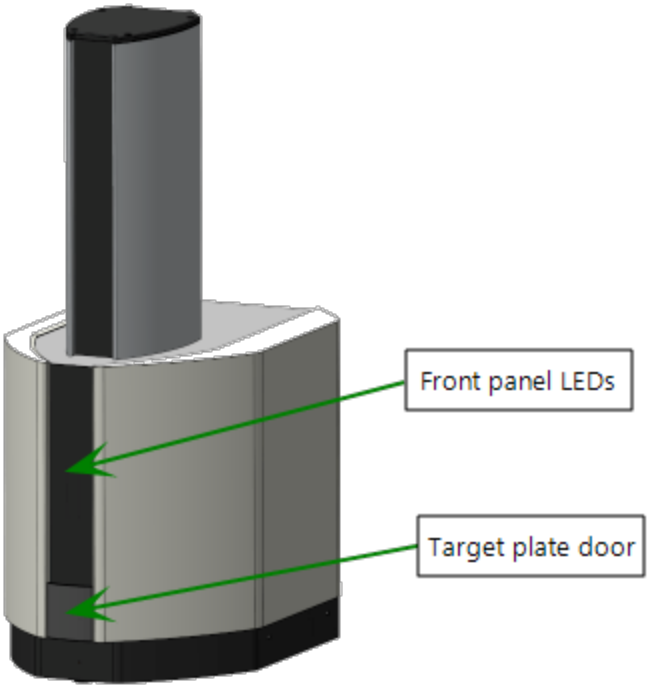
Refer to the Site requirements above.

Disposal

	The instrument is 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. When the instrument has reached the end of its life, contact your local Shimadzu office, or distributor, for details of its disposal.

Instrument layout & connections

Layout - front

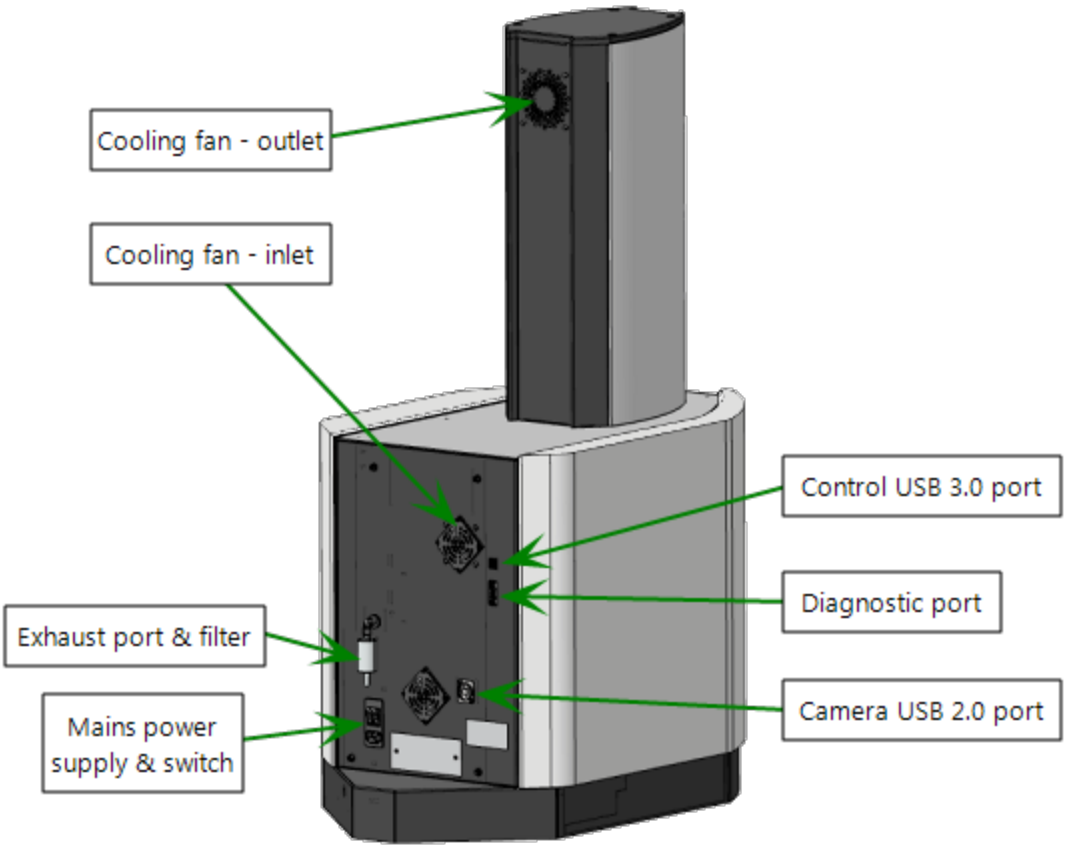


The Target plate door allows you to load and unload target plates from the instrument, see "Inserting/removing a Target Plate" on page 39.

The function of the front panel LEDs is described in the following table (a pulsing LED is 0.5s on, 0.5s off):

Colour	Status	Function
Pink	Blinking	Instrument is starting up
Blue	Steady	Instrument is in Operate mode
Blue	Blinking	Instrument is acquiring data from the sample
Cyan	Steady	Instrument is in Standby mode
Cyan	Blinking	High voltage ramping in progress
Orange	Blinking	Instrument is venting or pumping down
Green	Steady	Target plate bar code recognised
Green	Blinking	Instrument is ready to load Target plate
Red	Blinking	Instrument fault detected, see "Servicing and fault reporting" on page 206
Yellow	Blinking	Laser cleaning in progress

Layout - rear

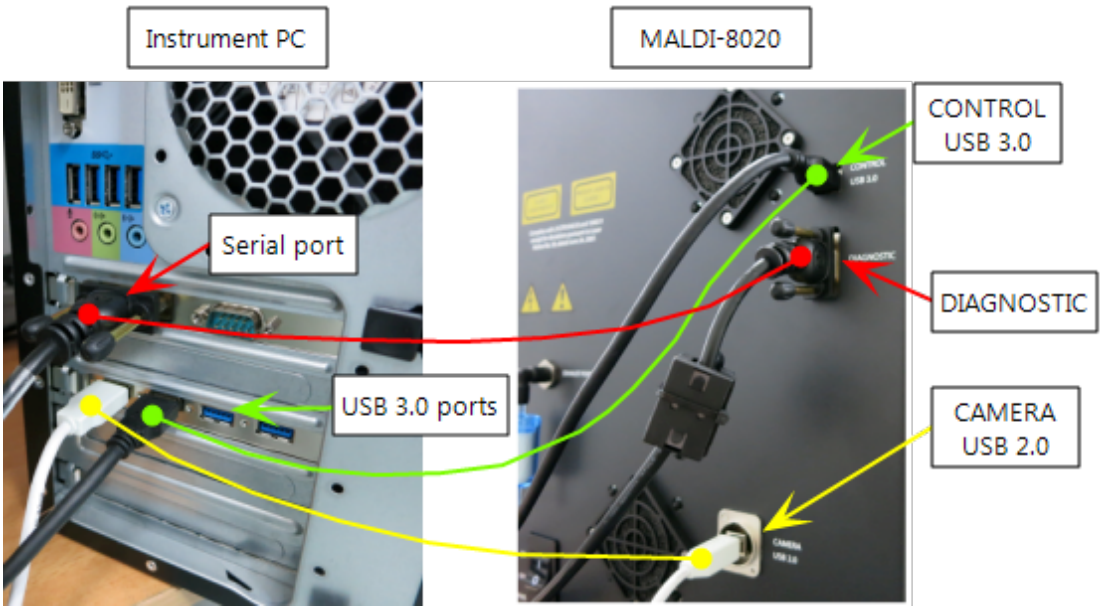


Always ensure that there is clear access to either the rear panel mains switch or to the mains plug (where it is plugged into the wall socket), to allow the instrument power to be easily disconnected from the mains power at any time.

Mains power leads

The instrument is supplied with power leads for the instrument, PC and monitor. If alternative leads are used, they must be suitable for the power rating of the equipment.

Connection diagram

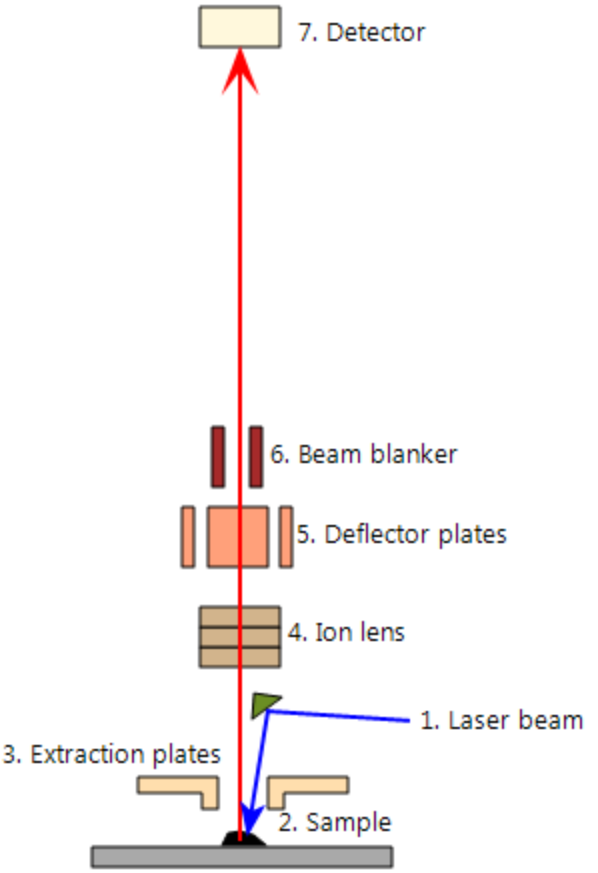


The *Control USB* port is used for communications between the instrument and the PC; the PC controls the instrument and receives data back.

The *Diagnostic* port is used by service engineers to assist servicing and fault diagnosis.

The *Camera USB* port is used to feed the image of the sample for analysis back to the PC. Note that this cable can also be connected to USB 3.0 port on the PC.

How it works



Matrix assisted laser desorption ionisation (MALDI) is the process used to ionise a sample into the gas phase. The following diagram represents the time-of-flight (TOF) vacuum tube and the components used in the mass spectrometer. The numbered points in the diagram correspond to the steps described below.

1. A pulsed UV laser beam is directed onto the sample.
2. Energy from the laser beam desorbs and ionises the sample.
3. Extraction plate provides a high-voltage electrical field to accelerate the ionised particles through the flight tube.
4. An ion lens focuses the ions.
5. Deflector plates steer the ions on a path towards the linear detector at the end of the flight tube.
6. A beam blanker deflects low mass ions (for example, derived from the matrix) away from the detector.
7. The detector detects ions moving in a direct line from the sample (lower-molecular weight ions followed by higher-molecular weight ions).

Ions hitting the detector cause an electrical signal which is recorded by a very high-speed digitiser. The recorded signal is processed by the MALDI Solutions software and presented as a spectrum of intensity versus mass/charge ratio (m/z).

Specifications

Instrument specifications

Dimensions (including stabiliser	600 mm (width) x 745 mm (depth) x 1,055 mm (height). (23.6" (width) x 29.5" (depth) x 41.5" (height))
Weight	Approximately 86 kg (190 pounds).
Power supply	A clean, stable and continuous mains supply is required; 100 to 240 Vac, 50/60 Hz, 1,000 VA single phase.
Electrical insulation	The instrument is classed as an Electrical Installation Category II, pollution degree 2, according to BS EN 61010-1:2012.
Operating temperature	15°C (59°F) to 32°C (90°F).
Performance temperature	18°C (64°F) to 28°C (82°F).
Relative humidity	Less than 70% non condensing at 22°C (72°F).
Sound level	<55 dB(A)
Maximum altitude	2,000 m (6,562 feet).
Location	Indoor use only.

PC specification

The PC used to control the instrument must comply with IEC 60950 IT equipment (the instrument's camera is powered via the USB cable from the PC). This is a regulatory requirement to meet UL (Underwriters Laboratories) approval.

Laser specifications

Wavelength	355 nm
Beam divergence	20 mrad (full angle)

Pulse width	1.5 ns
Repetition rate	50, 100, 200 Hz
Nominal power	80 µJ/pulse

Performance specifications

The performance of the instrument may vary slightly depending on the type of target plate you are using. The following specifications were obtained using FlexiMass-SR48 steel targets.

Mass range	1 to 350,000 Da and 10,000 to 500,000 Da
Mass resolution	>5,000 FWHM (full width half-height maximum) on ACTH 18-39 (2,465 Da)
Accuracy	<20 ppm with internal calibration <150 ppm with external calibration (nearest neighbour external calibration on Fleximass-SR48 steel target, within 30 minutes)
Sensitivity	250 fmol on bovine serum albumin (loaded) 250 amol on Glu-1-Fibrinopeptide B (loaded)

Compliance

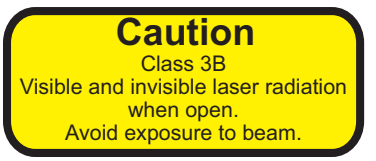
The MALDI-8020 conforms to the relevant European regulation for electrical safety and electromagnetic compatibility (EMC).

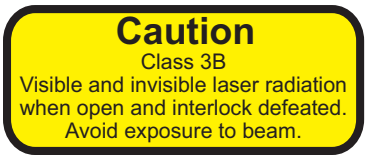
The MALDI-8020 complies with the emissions and immunity requirements of IEC 61326.

Labels

Internal labels

The following label symbols are used within the instrument. (As there is a potential personal injury hazard, access to the inside of the instrument is only allowed by authorised service engineers.)

Label	Meaning
	Safety alert symbol - indicates a general potential personal injury hazard.
	Electric shock symbol - indicates a specific potential personal injury hazard.
	Laser hazard symbol - indicates a specific potential personal injury hazard.
	Class 3B invisible and visible radiation - indicates classification of the laser which is enclosed by a protective housing within the instrument. Text on the label reads "Caution: Class 3B invisible and visible laser radiation when open. Avoid exposure to beam."

Label	Meaning
	Class 3B invisible and visible radiation - indicates classification of the laser which is enclosed by a protective housing within the instrument. Text on the label reads "Caution: Class 3B invisible and visible laser radiation when open and interlock defeated. Avoid exposure to beam."
	Caution moving parts - indicates hazard from exposed lead-screws and moving motors.

Rear mains panel labels

The following label symbols are used on the rear mains panel:

Label	Meaning
	Safety alert symbol - indicates a general potential personal injury hazard.
	Electric shock symbol - indicates a specific potential personal injury hazard.
	Class 1 laser product - indicates that the instrument is safe under all conditions of normal use.

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