Waters Micromass MALDI Q-Tof Premier

Operator's Guide

71500106402 / Revision A

Copyright notice

The information in this document is subject to change without notice and should not be construed as a commitment by Waters Corporation. Waters Corporation assumes no responsibility for any errors that may appear in this document. This document is believed to be complete and accurate at the time of publication. In no event shall Waters Corporation be liable for incidental or consequential damages in connection with, or arising from, its use.

Waters Corporation
34 Maple Street
Milford, MA 01757
USA

Trademarks

Millennium and Waters are registered trademarks of Waters Corporation. MassLynx and Q-Tof Premier are trademarks of Waters Corporation.

Other trademarks or registered trademarks are the sole property of their respective owners.

Customer comments

Please contact us if you have questions, suggestions for improvements, or find errors in this document. Your comments will help us improve the quality, accuracy, and organization of our documentation.

You can reach us at tech_comm@waters.com.

Safety information

General

The Waters® Micromass® MALDI Q-Tof Premier™ Mass Spectrometer is designed solely for use as a mass spectrometer; any attempt to use it for any other purpose is liable to damage the instrument and will invalidate its warranty.

The Waters Micromass MALDI Q-Tof Premier Mass Spectrometer conforms to European standard EN61010-1:2001, Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 1: General requirements.

The instrument has been designed and tested in accordance with recognized safety standards. If the instrument is used in a manner not specified by the manufacturer, the protection provided by the instrument may be impaired.

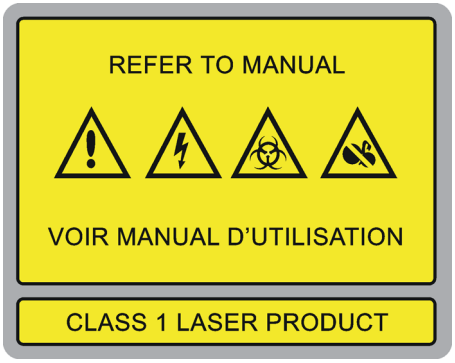
Whenever the safety protection of the instrument has been compromised, disconnect the instrument from all power sources and secure the instrument against unintended operation.

The instrument must be installed in such a manner that the user can easily access and isolate the power source from the instrument.

Laser radiation hazard

Warning: The use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

The MALDI Q-Tof Premier uses a nitrogen laser, producing a concentrated beam of invisible UV radiation. The instrument is a Class 1 Laser product, as indicated by the following label on the top the instrument.



If the operating procedures are followed as described in this manual, the laser beam will be contained within the instrument, and there will be no risk of exposure to laser radiation.

The MALDI Q-Tof Premier must be operated only with all exterior panels fitted. If any panels are removed and the laser safety interlocks are defeated there is a risk of exposure to invisible radiation exceeding Class 1.

The laser safety cover must only be opened by Waters service personnel qualified to service this instrument. When this cover is open and the interlocks are defeated, the instrument is a Class 3B laser hazard, indicated by the following warning label, fixed on the panel.

Output specification of enclosed laser

Wavelength	337.1 nm
Average Power	3 mW @ 10 Hz
Repetition Rate	up to 20 Hz
Pulse Width	4 ns
Pulse Energy	300 µJ @ 10Hz
Peak Power	75 kW
Beam Divergence, Full Angle	0.5 mrad typical

High voltage hazard

The MALDI Q-Tof Premier produces high voltages, presenting risk of electric shock if the instrument is operated without the exterior panels fitted.

The MALDI Q-Tof Premier must only be installed or relocated by qualified Waters field service engineers.

Biological hazard

When you analyze physiological fluids, take all necessary precautions, and treat all specimens as potentially infectious. Precautions are outlined in "CDC Guidelines on Specimen Handling", *CDC – NIH Manual*, 1984.

Suitable protection against biohazards must be taken during maintenance procedures and cleaning, as parts of the instrument are exposed to potentially infectious samples.

Chemical hazard

Good Laboratory Practice should be followed when you are using potentially toxic, caustic, or flammable solvents and analytes.

Pinch Point hazard

The MALDI Q-Tof Premier has moveable parts that may constitute a pinch point. When the MALDI source is moving keep away from the regions that are marked with yellow and gray labels.

Safety symbols

Warnings in this Operator's Guide, or on the instrument, must be observed during all phases of service, repair, installation and operation of the instrument. Failure to comply with these precautions violates the safety standards of the design and intended use of the instrument. Waters Corporation assumes no liability for the user's failure to comply with these requirements.

The following safety symbols may be used in the Operator's Guide, or on the instrument. A **Warning** is an instruction that draws the user's attention to the risk of injury or death; a **Caution** is an instruction that draws attention to the risk of damage to the instrument.

Warning: This is a general warning symbol, indicating that there is a potential health or safety hazard; the user should refer to this Operator's Guide for instructions.

Warning: This symbol indicates that hazardous voltages may be present

Warning: This symbol indicates that hot surfaces may be present.

Warning: This symbol indicates that there is danger from corrosive substances.

Warning: This symbol indicates that there is danger from toxic substances.

Warning: This symbol indicates danger of contamination by a biological agent that constitutes a threat to humans.

Warning: This symbol indicates that there is danger from flammable substances.

Warning: This symbol indicates that there is danger from laser radiation.

Warning: This symbol indicates that there is danger from moving machinery.

MALDI Q-Tof Premier Spectrometer Information

Intended Use

The Waters Micromass MALDI Q-Tof Premier Mass Spectrometer can be used as a research tool to deliver authenticated exact mass in both MS and MS-MS mode. It is not for use in diagnostic procedures.

Calibration

Follow acceptable methods of calibration with pure standards to calibrate methods. Use a minimum of five reference standards to generate a standard curve. The concentration range should cover the entire range of quality control samples, typical specimens, and atypical specimens.

Quality Control

Routinely run three quality-control samples. Quality control samples should represent subnormal, normal, and above-normal levels of a compound. Ensure that quality-control sample results are within an acceptable range, and evaluate precision from day to day and run to run. Data collected when quality-control samples are out of range may not be valid. Do not report this data until you ensure that system performance is acceptable.

Table of Contents

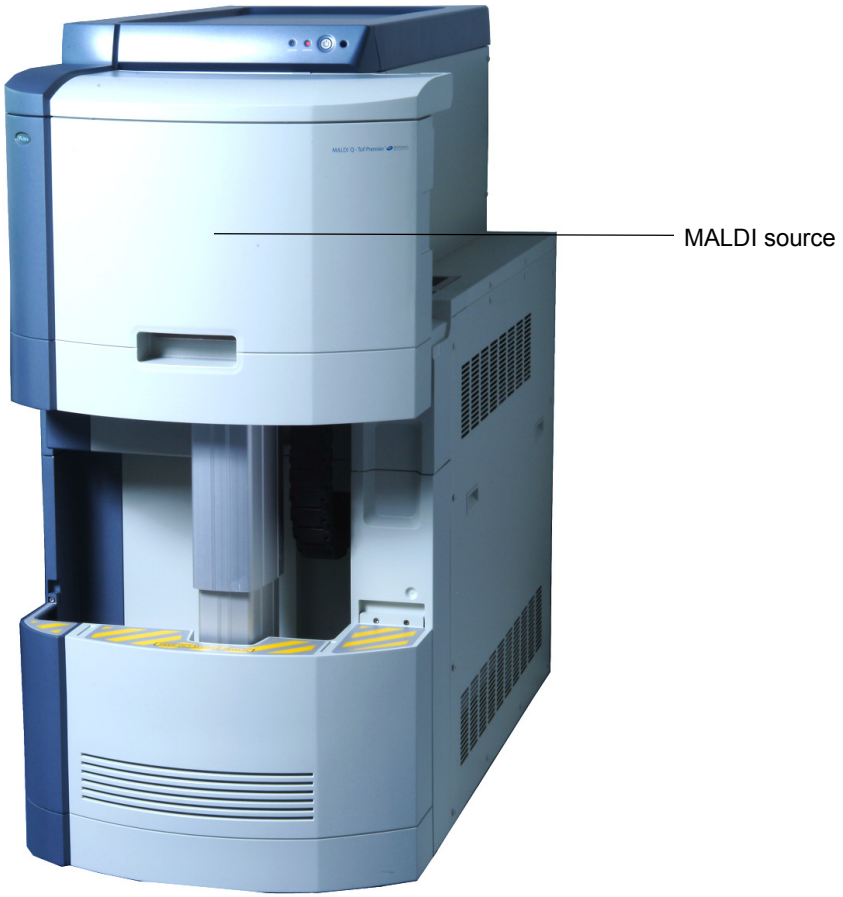
1 Basic Principles

Contents:

Overview

The MALDI Q-Tof Premier provides an efficient switch between API and MALDI modes to deliver the degree of flexibility required in today's research environment. By simply removing the API source, the MALDI source unit is transferred via a motorized stage to the source region, and is fixed in position.

MALDI Q-Tof Premier:



Installing the MALDI source

Before installing the MALDI source the instrument needs to be vented and the standard API or NanoLockSpray source housing has to be removed. These procedures are described in detail in the *Q-Tof Premier Operator's Guide*.

Warning: Risk of pinch point injury while the assembly is being moved into place. Take care to keep fingers away from the moving assembly.

Warning: The ESI probe and source are liable to be hot. To avoid burns take care when removing the source housing and ion block.

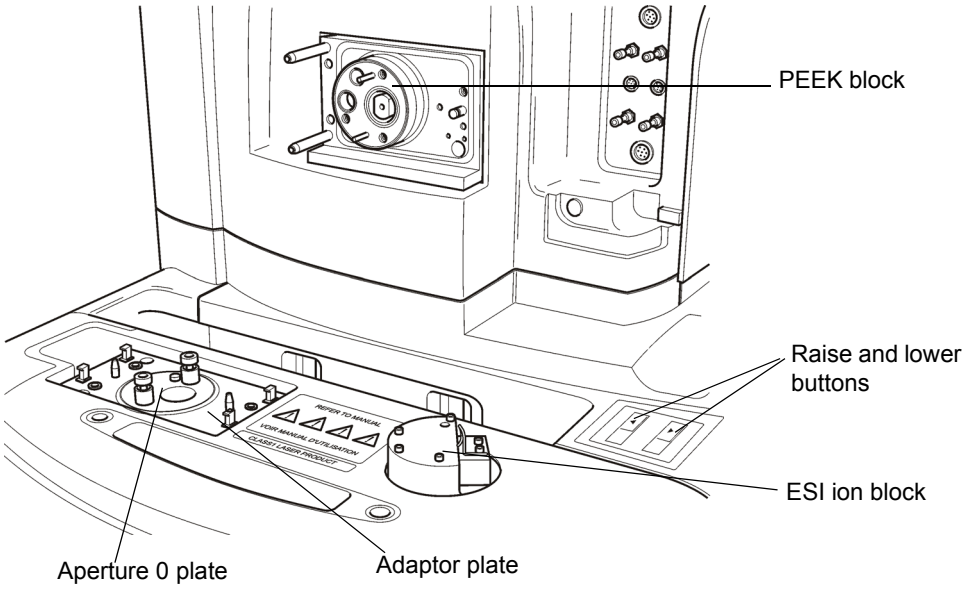
To prepare for installation:

1. Click Vacuum > Vent to vent the instrument.
2. Remove the ESI probe, source housing, source drain block and ion block.

   Important: Check that the 3 O-rings on the PEEK block remain in place.

To install the MALDI source:

1. Remove the top cover of the MALDI unit. This will reveal a repository of the components required to fit the source.



2. Fit the adaptor plate and tighten the three 5-mm captive screws with the appropriate allen key. Ensure that it is square and tight.

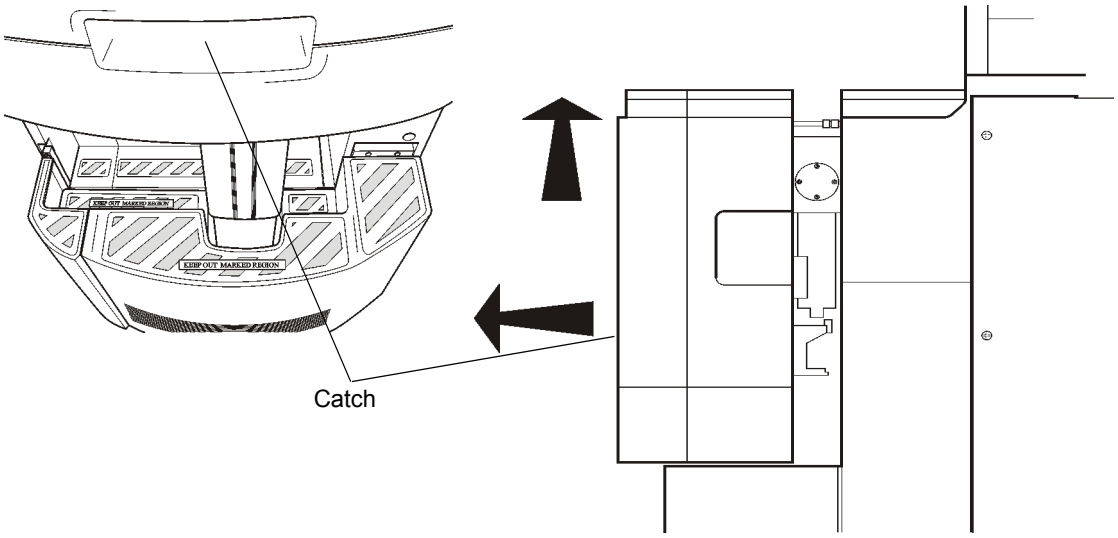
   Tip: On the back, ensure that the O-ring is fitted evenly in the groove.

3. Locate the Aperture 0 plate on the two pillars and tighten the two 6-mm screws with the appropriate allen key.

   Tip: Do not fit the washers used with the ESI ion block. Fitting these will cause a vacuum leak.

   Caution: Take care not to over-tighten the screws.

4. Release the catch on the source (by pulling towards you) and gently withdraw the assembly until it locks into position.



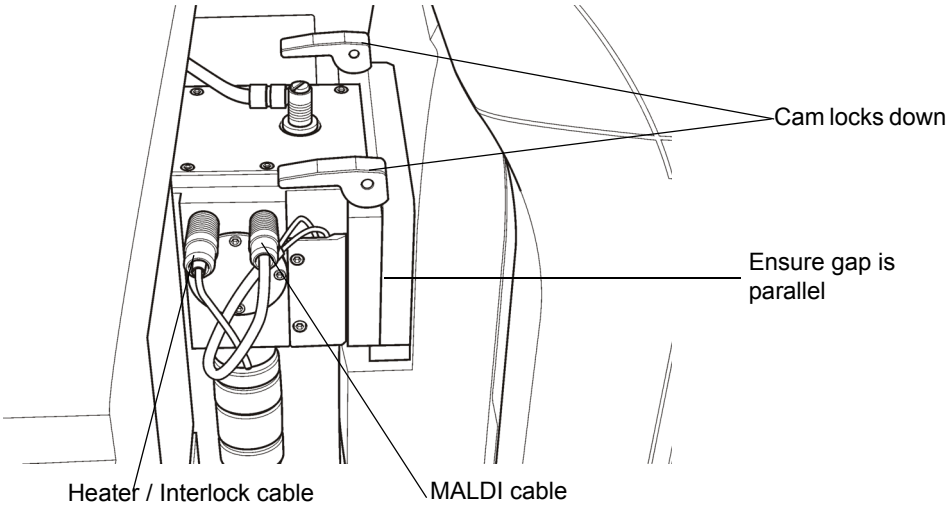
5. Press the Raise button and hold it until the MALDI source rises to its required position.

   Important: There is an audible indication that the source is moving.

6. With the cam locks raised, pull back the catch and advance the source onto the locating pillars.

7. Secure with the cam locks.

   Important: Ensure that the gap, between the source and instrument, is parallel.



8. Plug MALDI cable into the MALDI socket on the front panel, and the Heater/Interlock cable into the DESOLVATION socket.
9. Blank off the nebuliser gas.
10. Select Vacuum > Pump to pump down the instrument. It should be possible to switch the instrument into Operate after approximately one hour.
11. Recondition the detector.

Result: The instrument is now ready to use.

To remove the MALDI source:

1. Unload the MALDI sample plate and select Vacuum > Vent.
2. When the instrument has vented, remove the cables and fit them into their parking positions.
3. Release the cams and pull the source backwards until it locks in its 'out' position.
4. Press the Lower button and hold until the source is at its predefined low position.
5. Release the catch and push forward.

6. Remove the Adaptor plate and Aperture 0 plate and store them, with the allen keys, in the component repository.

Result: The instrument is now ready for the fitting of other sources.

Troubleshooting:

You a cannot switch the instrument into Operate unless 'Vacuum OK' is displayed in the Status bar.

If after requesting Operate the vacuum status is 'Pumped - Tof vacuum out of range', the instrument vacuum has not reached a suitable operating pressure. This may be caused by an extended period between venting and pumping down, while changing sources.

If the vacuum status has not reached Pumped then this may be due to vacuum leaks. If there is clearly a gap or an audible hiss then the source is not fitted correctly.

Either of these can caused by:

- The source not being parallel with the instrument.
- Fitting the washers from the ESI ion block.
- The Aperture 0 plate. Ensure this fitted tightly to the ion block.
- O-rings having fallen out or not being seated correctly. Vent the instrument and check the O-rings before refitting the source.

  Check that the gap, between the source and instrument, is parallel.
- Inappropropriate height of the MALDI Source. If this is the case, contact Waters (see Contacting Waters on page 4-6).

The Tune window

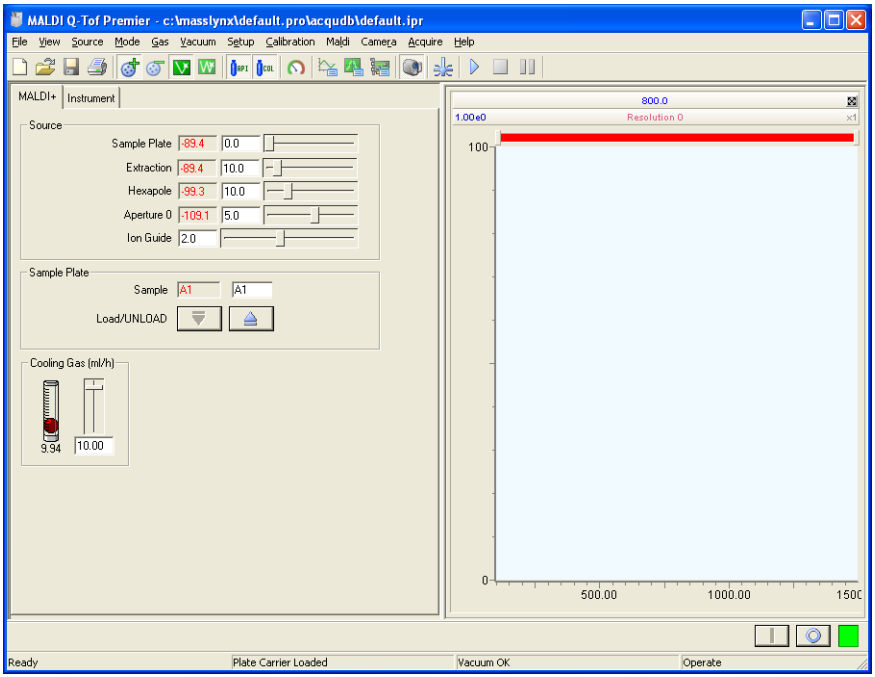
After the MALDI source is installed, the MALDI specific parameters can be viewed.

To view the MALDI Tune window:

On the Tune window select Source > MALDI.

Note: The Source page is not automatically selected, the Heater/Interlock cable must be connected to allow you to view the MALDI source page.

Tune window:



In addition to the tasks that are described in the Q-Tof Premier Operator's Guide you can:

- Load sample plates and select sample wells.
- Display a camera image of the sample.
- Fire the laser and optimize the spectrum.
- Save and recall settings for use with different samples.

- Set parameters on the MALDI source page.

Sample plates

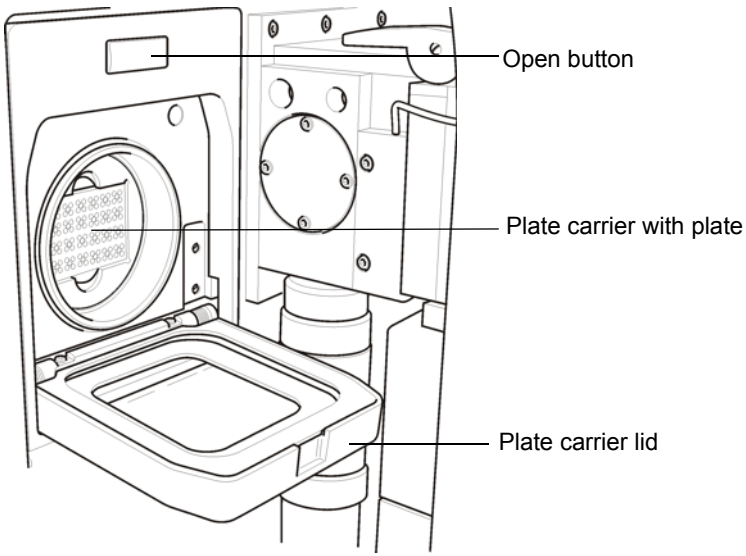
Operating the plate carrier

Important: It is not necessary to select Standby mode when loading and unloading plates.

To load plates:

1. Press the Open button.

   Plate carrier:



2. Take the sample plate by the sides, aligning it so the corner indent is to the left, and place it level with the plate carrier.
3. Close the plate carrier lid.
4. From the Tune window click  .

   Result: The system pumps the plate carrier (this will take approximately 3 minutes) and the sample plate is transported into the source.

5. Enter the sample well number in the sample box.

   The selected sample well is aligned with the laser beam.

   After the sample plate is loaded, the Unload button is highlighted blue to show that Unload is available.

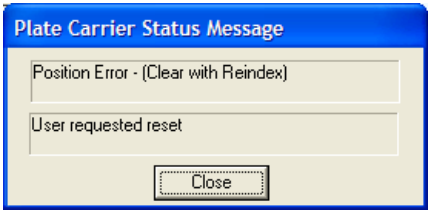
   Neither button is highlighted when the instrument is acquiring data.

To unload a plate:

1. From the Tune window click  .
2. When the carrier has stopped moving open the plate carrier lid and remove the plate, taking care not to touch the surface.

Plate carrier errors

The current status of the plate carrier is displayed in the Tune window Status bar. Error messages are displayed in the Plate Carrier Status Message dialog box. If you connect the Heater/Interlock cable when a plate is already loaded, it is normal to receive the Status Message as shown.



This message box indicates that the sample plate control is restarting. If the plate carrier is in the Unload position this message will not appear.

Other errors give possible solutions in the dialog box and are indicated by the status of the Load/Unload buttons (see Plate carrier errors on page 4-4).

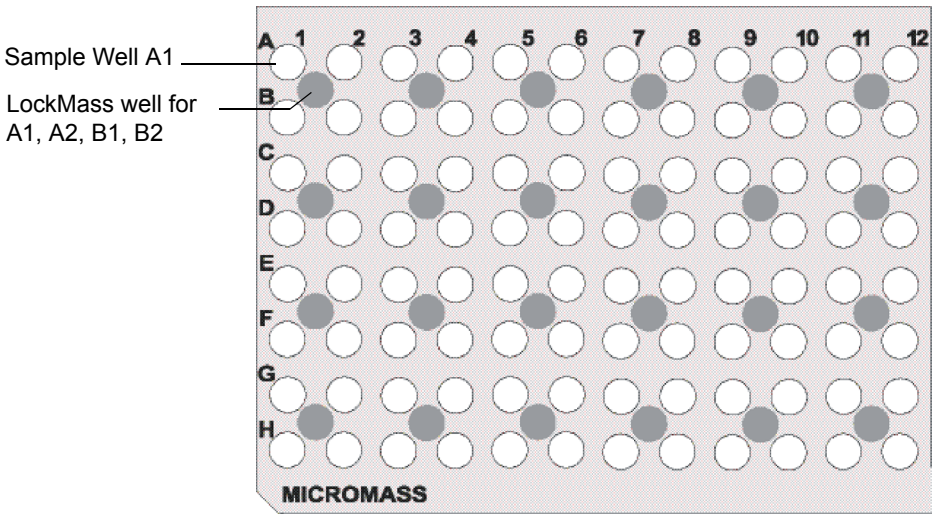
Load/Unload button states:

State	Meaning
Blue	Normal operation - Load/Unload
Red 	Recoverable error. The plate is out of position. The error is cleared by clicking the buttons and the plate reindexes. If it does not recover contact Waters.
Both disabled	This can be one of three things: • Acquiring data • Vented error • Plate carrier error (see page 4-4)

Sample plate formats

The MALDI Q-Tof Premier comes with several plate formats already defined. These are the standard 96 well MALDI sample plate (shown below), 384 well, MassPrep™, DIOS and BigSpot.

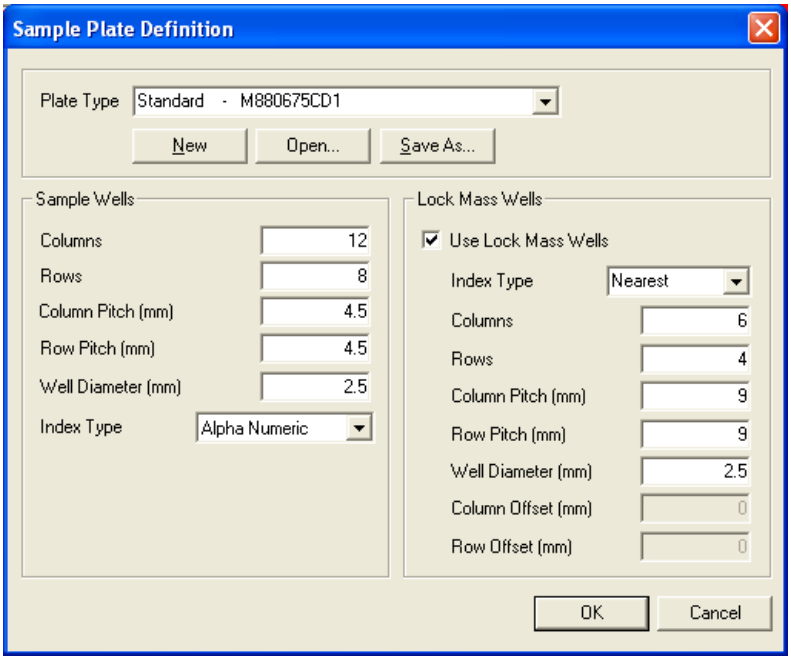
Standard 96 well sample plate:



Viewing and setting plate formats

From the Tune window click Maldi > Sample Plate Definition to open the Sample Plate Definition dialog box.

Sample Plate Definition dialog box:



To select a predefined plate:

1. Select from the drop down list in Plate Type.
2. Click OK.

To create a user defined plate:

1. Click New.
2. Enter relevant parameters for the plate type you are using.
3. Select the Index Type drop down list to select Numerical or Alpha Numeric addressing of the sample wells.
4. Select Use Lock Mass Wells for the system to recognize the near point correction wells used to improve and validate external mass measurement accuracy.
5. Click Save As and save with an appropriate name.

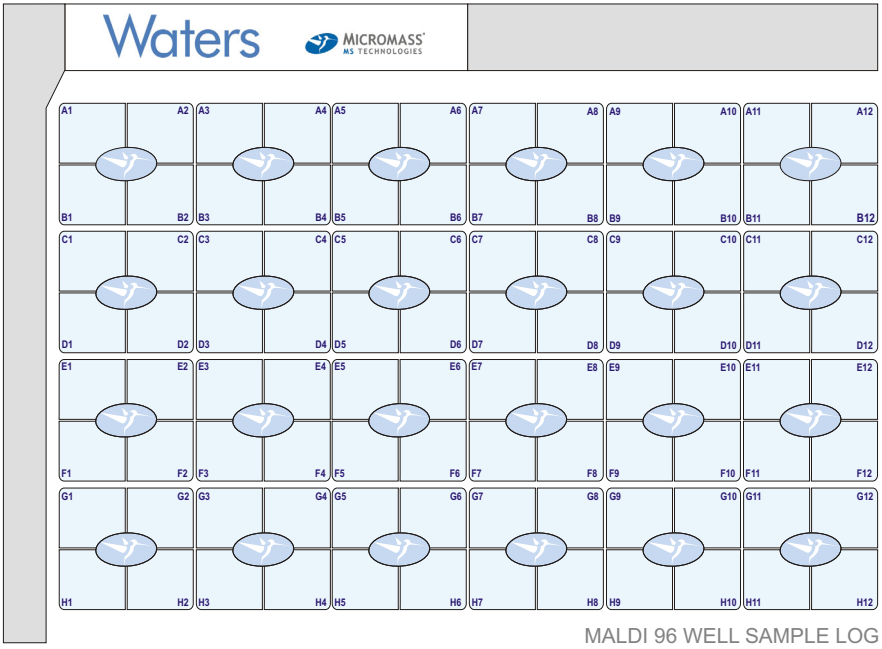
To open a user defined plate definition:

1. Click Open.
2. From the Open dialog box select the required *.mtp file.
3. Confirm the settings are correct, and click OK.

Sample log sheet

A sample log sheet, 'MALDI_SAMPLE_LOG.pdf', is included with your MassLynx installation in the MassLynx folder.

Sample log sheet:



With Adobe Acrobat Reader installed on your PC, you can print log sheets and record sample details.

Obtaining an ion beam

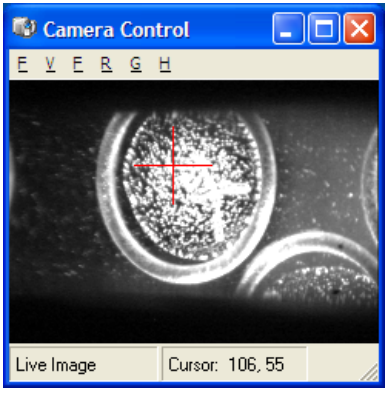
In this section you will learn how to control the laser so that the peak display displays an ion beam.

Controlling the camera

The camera is used to view the sample plate in position. The camera image of the selected sample well has crosshairs indicating the firing point. The point at which the laser has fired on the sample can be seen as a bare patch where the sample has been consumed.

To operate the camera:

1. Click  to open the camera.



2. Click in the status bar to toggle between Live and Static images.

   In Static Image crosshairs can be moved around by clicking in the image. In Live Image the crosshairs cannot be moved.

Aligning the camera image

The laser may fire but may not be perfectly in line with the crosshairs. This can be corrected from the Camera window.

To align the laser position to the camera image:

1. Fire the laser, at a prepared sample well, without moving its position until enough material has been removed from the sample plate to allow you to see where the laser is actually firing.
2. Click the status bar to toggle to Static Image.
3. Click on the camera image at the position of the laser firing mark. This moves the camera crosshairs to position selected with the mouse.
4. When the laser and crosshair positions are satisfactorily aligned with each other, click in the status bar to toggle back to a Live Image.

   The camera software stores the crosshair position.

Aligning the laser and the sample well:

After the camera crosshairs have been set to indicate the laser fire position the center of the sample well may not be aligned with the camera crosshairs.

The sample well position relative to the camera image is set from the Tune window.

To realign the sample well position:

1. Click Maldi > Source Settings > Password and type ‘access’.
2. In the Source Settings dialog box click Sample Plate and select Nudge Using Crosshairs.

   Result: The plate moves to sample well A1.
3. Adjust the sample plate crosshairs until the center of the sample well is aligned with the camera crosshairs.
4. Click  to store the current alignment.
5. Repeat steps 3 and 4 until the sample well center and the laser firing mark are satisfactorily aligned.
6. Click  to accept and use the current alignment.

   Result: The plate reindexes and moves to sample A1 using the latest alignment settings.

Controlling the sample plate

During an acquisition the sample plate is controlled either using a crosshair pointer or by following a predefined pattern.

Acquisitions from the Sample List only use pattern control.

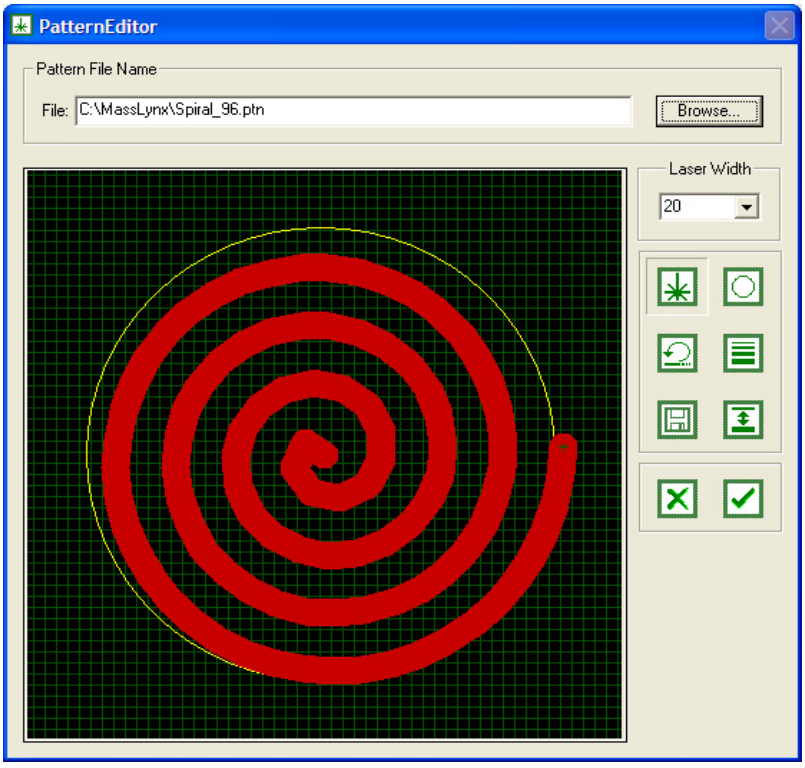
Pattern editor

A pattern is constructed from a series of nodes (up to a maximum of 100). These nodes define a series of points between which the plate carrier moves. When the end of the pattern is reached the movement stops. If an acquisition is being run from the Sample List and the end of pattern is reached the acquisition also stops.

Each pattern is scaled to fit the sample wells, so that there are same number of nodes in a 2.5 mm well as there are in a 2 mm well.

There are 13 predefined patterns, either spiral or straight line, that can be loaded from the Pattern Editor (see Figure titled “Spiral pattern in Pattern Editor:” on page 1-17). Further patterns can also be defined in the Pattern Editor if required. The *.ptn files are comma delimited text files and can be opened and modified in any spreadsheet application.

Spiral pattern in Pattern Editor:



Starting an acquisition

An acquisition can be performed in one of three ways:

- Obtaining an ion beam displays the data on the Tune window peak display, but does not save any data to disk.
- Acquiring data from the Tune window creates a data file and saves data to disk.
- Acquiring data from the Sample List allows you to create multiple data files in a batch format.

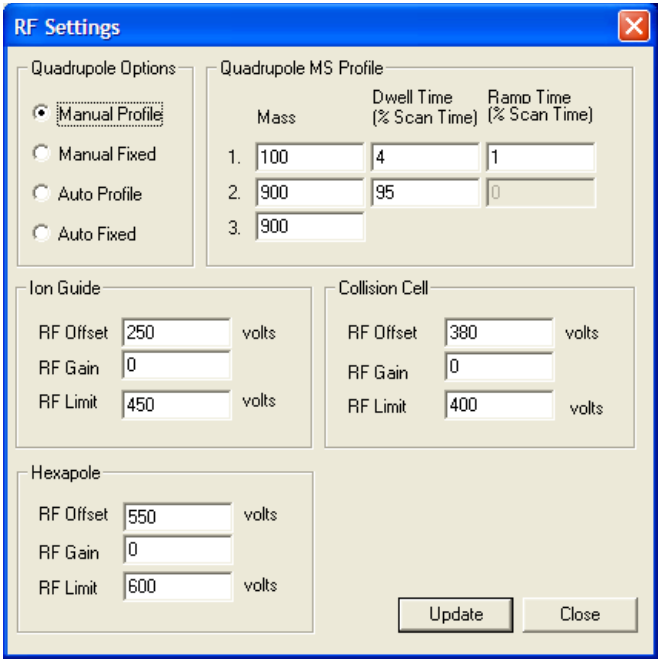
Initial instrument settings

Ensure the following items have been set:

- From the RF Settings dialog box, ensure the values and quadrupole options are set as shown below.

- On the Instrument page, set the detector voltage 100 V higher than for ESI (this is a typical example, actual voltages may vary. Contact Waters for further information).
- The Collision and API gasses are on.
- The cooling gas is set to 10.
- The mass range in the Tuning Settings dialog box is appropriate to your sample.
- The laser firing rate is set to maximum.
- The ion mode and polarity appropriate to your sample.

RF Settings dialog box:



To obtain an ion beam:

1. Click  to switch the camera on and view the sample plate.
2. Select a sample well containing your sample.
3. Click  from the Tune window tool bar.

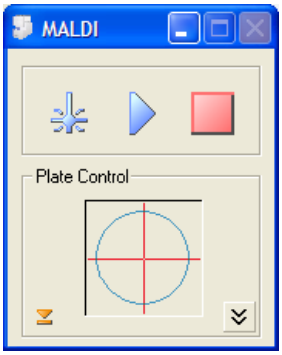
Result: The MALDI control window opens. The appearance of the window will depend on your settings in the Source Settings dialog box.

4. Click  , from the MALDI control window, to fire the laser.

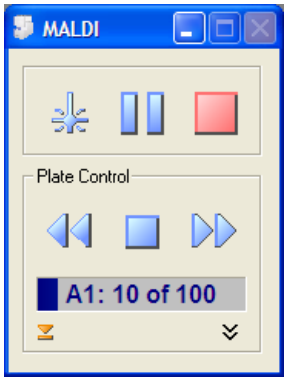
   As appropriate use either the crosshairs or the pattern to move within the sample well.

5. The data produced by the instrument is shown on the Tune window peak display.
6. Click  in the MALDI control window to finish.

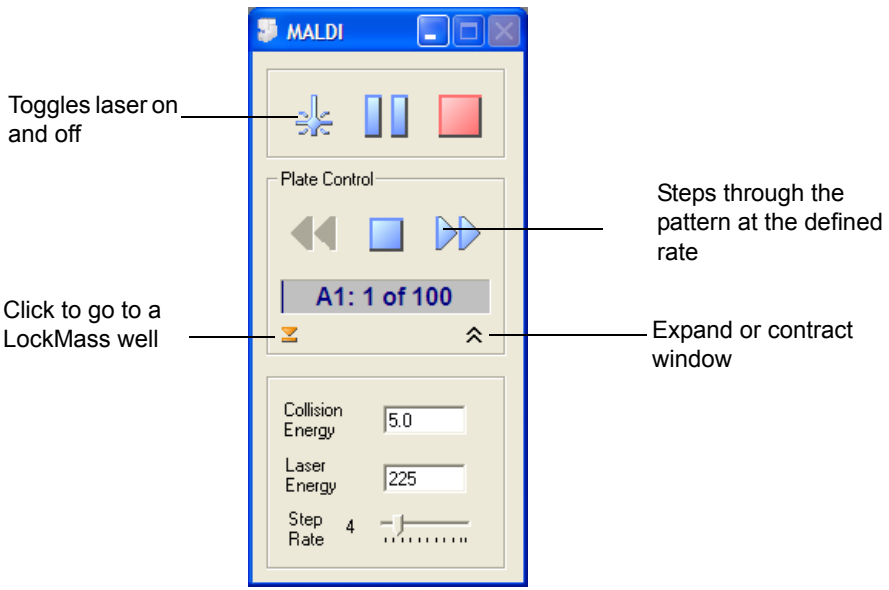
MALDI control windows:



Crosshairs selected



Pattern file selected



Expanded dialog

Acquiring data from the Tune window

Before acquiring data from the Tune window it is necessary to determine:

- What the data file name and comments will be.

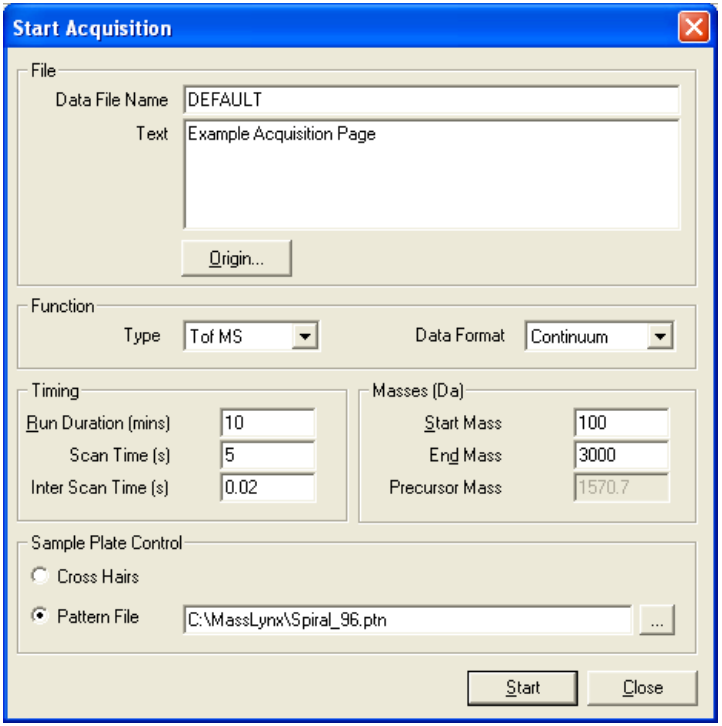
- What type of acquisition will be performed; MS or MS/MS.
- What overall duration and scan times will be used.
- What mass range and, if an MS/MS acquisition is selected, what precursor mass is required.
- What Sample Plate Control type will be used.

To start an acquisition:

1. Click  to switch the camera on and view the sample plate.
2. Select a sample well containing your sample.
3. Click  from the Tune window tool bar.

   Result: The Start Acquisition dialog box opens. The acquisition type and settings are selected from this window.

Start Acquisition dialog box:



4. Enter settings appropriate to your acquisition.

5. Click Start to begin acquiring data.

   Result: The MALDI control window opens. The appearance of the window will depend on your settings in the Sample Plate Control section of the Start Acquisition dialog box.

6. Click  , from the MALDI control window, to fire the laser. As appropriate, use either the crosshairs or the pattern to move within the sample well.

   The data can be observed in Spectrum or Chromatogram, and the peak display.

7. Based on the quality of the data being acquired, adjust the laser energy, collision energy (for MSMS) and pattern step rate until a satisfactory combination of peak intensity and sample consumption is achieved.

8. Click  in the MALDI control window to finish acquiring.

   Tip: Acquire data from the Tune window and arrange the MALDI control dialog so that you can also see the Chromatogram and Spectrum windows. View the chromatogram and spectrum in real-time as you fire the laser.

To acquire data from the Sample List:

Before acquiring data from the Sample List it is necessary to:

- Select a MALDI specific sample list format.
- Complete a sample list entry with the following information:
  - Filename
  - Method (see Method Editor on page 3-2)
  - Sample Well
- Optionally the following information can be specified:
  - File comment text
  - Instrument parameter file

To start a sample list acquisition:

1. Create a sample list with the information as described above.
2. Save the sample list.
3. Click  from the MassLynx Toolbar.

Result: The Sample List begins acquiring; creating the data filename requested, using the selected method and sample well.

2 Calibration

Calibration for MALDI is very similar as for that described for ESI in the *Q-Tof Premier Operator's Guide*. Create calibrations that encompass the mass range that the instrument will acquire over. Acquiring beyond the calibrated range will limit the mass accuracy returned by the instrument.

Contents:

Processing MALDI data

Processing MALDI data involves combining, smoothing, background subtraction and centering spectra. Where this data is used for creating a calibration the processed spectrum is also saved into the file history.

To process data:

1. Right-click and drag beneath the chromatogram, to select and combine spectra.
2. Select the spectrum as the active window by clicking anywhere within it.
3. Select Process > Smooth.
4. In the Smooth dialog box, enter the settings shown in the following table and click OK.

Smooth Settings

Parameter	V and W mode
Smooth window (channels)	3
Number of smooths	2
Smoothing method	Savitzky Golay

5. Select Process > Subtract.
6. In the Background Subtract dialog box, enter the settings shown in the following table and click OK.

Subtract Settings

Parameter	V and W mode
Polynomial order	15
Below curve (%)	10
Tolerance	0.01

7. Select Process > Center.

8. In the Spectrum Center dialog box, enter the settings shown below and click OK.

Center Settings

Parameter	V mode	W mode
Min peak width at half height (channels)	4	6
Centroid top (%)	80	80

9. From the Spectrum window, select File > Save Spectrum to open the Spectrum save dialog box, and click OK.

Note: Saving processed spectra into the file History, allows this processed information to be recalled directly without repeating the individual steps.

Calibrating

To return mass data of consistent accuracy, calibrate the MALDI Q-Tof Premier regularly against reference compounds appropriate to your project:

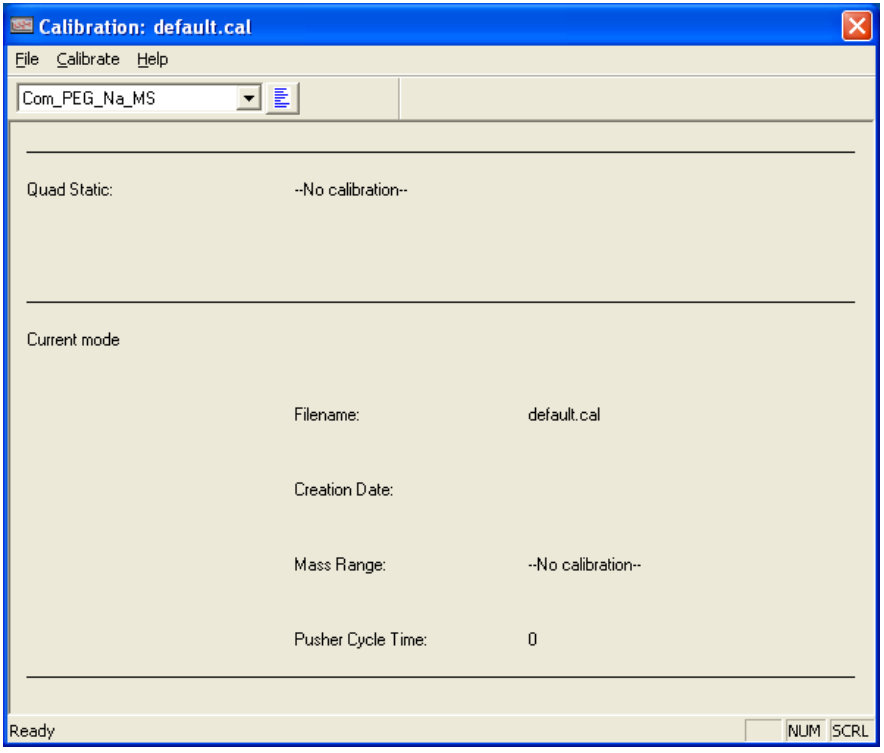
- Daily, or
- Before long automated data acquisitions, or
- For each sample plate.

Calibrations created from MS acquisitions are valid for MS, MSMS and Survey experiments.

Using the Calibration window

From the Tune window, select Calibration > Calibrate TOF to open the Calibration dialog box.

Calibration dialog box:



With this box you can:

- Choose an appropriate reference file from a drop-down list.
- See when the instrument was last calibrated.
- See the instrument settings from that calibration.
- Save your calibration as a file and recall previous calibrations.

Calibrating from previously processed data

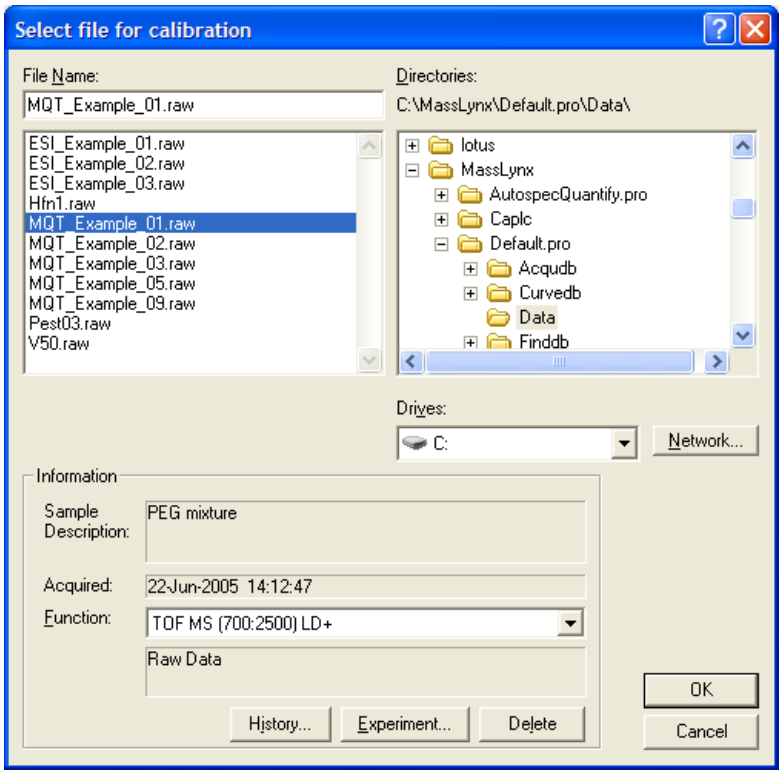
The data acquired, as shown in the previous section or from the Sample List, can be used for calibrating the instrument. In addition, a calibration reference file relevant to the data is required.

In the following section, the example file MQT_Example_01.raw is used to illustrate the calibration steps.

To calibrate form previously processed data:

Tip: Waters recommends you calibrate using PEG 600, 1000, 1500, and 2000, and that you use the reference file Com_PEG_Na_MS, which is appropriate for this sample.

1. From the Tune window, select Calibration > Calibrate TOF to open the Calibration window.
2. Select the relevant reference file for your calibrant.
3. Select Calibrate > Create Calibration.
4. From the Select file for Calibration dialog box, navigate to your raw data file.



5. Click History to display the History Selector dialog box

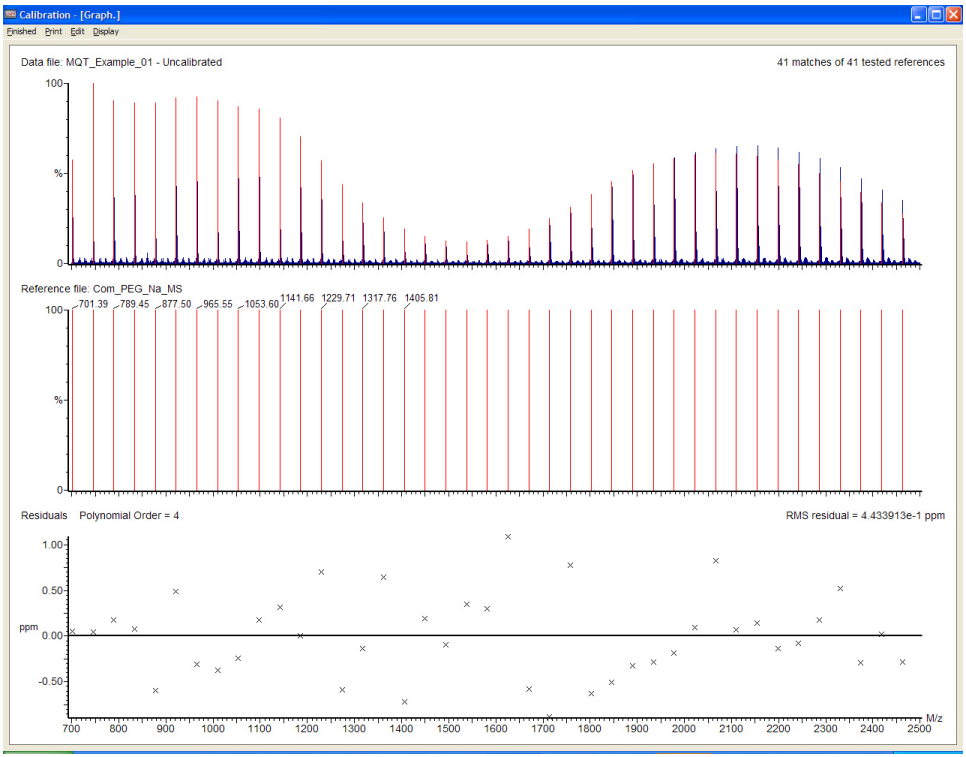
   The box shows the processing steps previously applied to the raw data spectrum.

6. Click the saved version (highlighted black).

   Note: Calibration can only be performed on centered data.

7. Click OK.

   Result: MassLynx performs the calibration calculations and displays the resulting calibration graph.



8. Click File > Save As to save the calibration to accept the calibration. Enter a file name appropriate to your project.

   Caution: Always save the calibration with a new name. Do not overwrite Uncal.cal.

   A polynomial order value of five should be used. If the acquisition mass range is beyond the calibration mass range, create a new calibration using polynomial order one; to ensure the mass measurement accuracy is maintained in the extrapolated region.

   Tip: It is good practice when saving a calibration to use the date as the filename.

   If the calibration is not acceptable, outliers can be removed and peaks reassigned see the *Q-Tof Premier Operator's Guide* for further details.

Using LockMass for Greater Mass Accuracy

You can improve the mass measurement accuracy of acquired data by applying a uniform mass adjustment taken from a simultaneously acquired reference peak. This allows you to compensate for small, local variations in the calibration arising from effects such as drift in laboratory temperature, and is referred to as LockMass.

MassLynx extracts a mass from centered data, compares it to the exact mass that you specify (the LockMass) and applies the adjustment necessary to align these two numbers. This adjustment is automatically applied to all the spectra in a data file, but only to the function that the LockMass is generated from.

- Internal LockMass correction means that the mass of one peak within the sample data is used to correct the calibration.
- External LockMass correction means that one peak from within the data acquired from a LockMass sample well is used to correct the calibration.
- An acquisition may contain data which can be used for both internal and external correction, however only one type is used at any one time.
- Where LockMass is acquired from the Sample List it is of the External type and is saved in a separate function to the sample data within the MassLynx raw file.
- Where LockMass is acquired from the Tune window it is of the External type and is saved in the same function as the sample data within the MassLynx raw file.
- For external LockMass use a sample of a known mass at a concentration that produces approximately 100 counts/second. Stronger concentrations produce distorted mass measurement; weaker concentrations require long acquisition times to produce data that does not contain statistical errors.
- Acquisitions from the LockMass sample well should be short in duration to maximize the acquisition time on the principle sample. A total intensity of approximately 1000 counts is sufficient.
- When acquiring from a sample list if the end of pattern on the LockMass sample well is reached the acquisition stops. With the standard sample plate configuration one LockMass sample well is shared between four sample wells.

Creating LockMass Correction

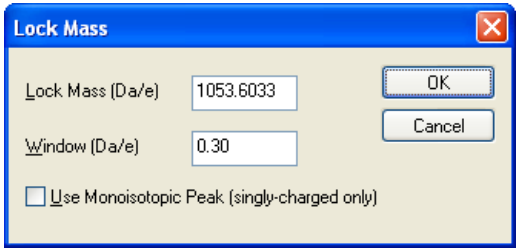
To create a LockMass correction the LockMass data must be processed to centered masses and the specific LockMass peak extracted.

Note: The figures in this section refer to the example file MQT_Example_01.raw (PEG MS - Using an internal LockMass).

To create a LockMass correction:

1. Combine together the spectra that contain the LockMass peaks.
2. Smooth, subtract and center this spectrum as described in Processing MALDI data on page 2-2.
3. Select Tools > Lockmass to open the Lock Mass dialog box.
4. In the box Lock Mass (Da/e), enter the correct mass for your Lock Mass compound.

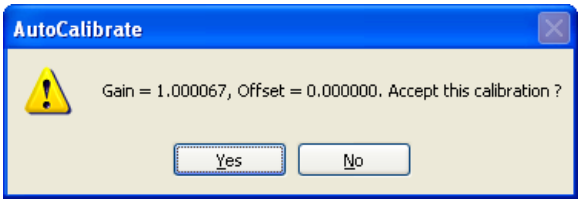
   ### Lock Mass dialog box:

5. In the box Window (Da/e), enter a value to enable MassLynx to identify the peak associated with this mass.
6. Click OK to display the AutoCalibrate window.

   Result: The Gain value displayed in the AutoCalibrate window is the multiplier correction that will be applied to all the spectra in the data file for the selected function.

AutoCalibrate dialog box:



A gain of 1.0 applies no correction to the data.

A gain of >1.0 e.g. 1.000067 increases the mass values.

A gain of <1.0 e.g. 0.999933 decreases the mass values.

7. To accept the LockMass correction, click Yes.

Applying LockMass Correction

Internal LockMass

If using an internal LockMass the steps above have applied the LockMass gain correction to the sample data as well as to the LockMass data. This is because the sample and LockMass data are saved in the same function.

External LockMass from the Tune window

If using an external LockMass acquired from the Tune window the steps above have applied the LockMass gain correction to the sample data as well as to the LockMass data. This is because the sample and LockMass data are saved in the same function.

External LockMass from the Sample List

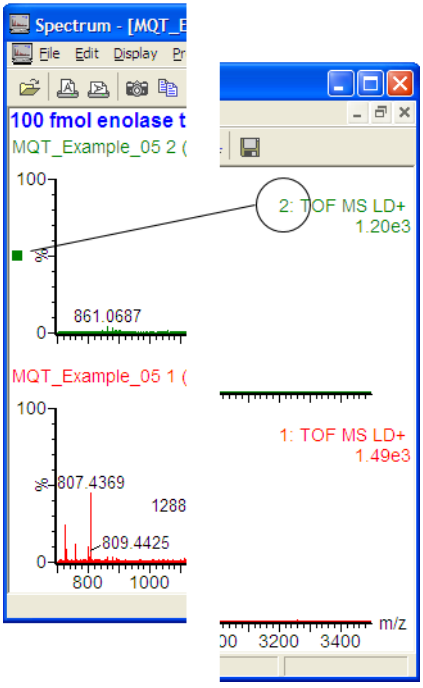
If using an external LockMass acquired from the Sample List the steps above have *not* applied the LockMass correction to the sample data. This is because the sample and LockMass data are saved in separate functions.

To apply the LockMass gain correction to the sample data copy the gain correction value generated above into each data function using the clipboard.

The figures in this section refer to the example file MQT_Example_05.raw (Enolase MS - Using an external LockMass) and assumes that the LockMass correction has already been created.

To apply a LockMass correction:

1. Display a spectrum from all the functions within the data file to which the gain value will be applied.
2. Select the LockMass data as the active function as indicated by the marker in the spectrum display (see below).



3. Select Spectrum > Tools > Modify Calibration
4. In the Gain field, highlight the entire number by double clicking within the field.
5. Either right-click or press Ctrl C to copy this selected value into the clipboard.
6. Close the Modify Calibration window.
7. Select another function.
8. Paste the gain value into the Gain field as in steps 3 and 4.
9. Repeat steps 7 and 8 for all functions as appropriate.

Result: The gain correction created from the LockMass data has been copied to the other data functions. The other data functions are now LockMass corrected.

Notes:

- The gain correction may also be typed into the Gain field.
- Gain values are stored with the data file and are preserved when the data file is closed.

3 MALDI Acquisition

The principles of acquisition for MALDI are similar to those described for API in the *Q-Tof Premier Operator's Guide* and many of the parameters are described there.

Contents:

Method Editor

In addition to the 6 acquisition types described in the Q-Tof Premier Operator's Guide, the MALDI Q-Tof Premier has a further three:

- MALDI MS
- MALDI MSMS
- MALDI Survey

Each type has several pages of parameters. The Scan Conditions page and the LockMass page are common to all three types of acquisition.

Sample List Formats

To use the full functionality of the MALDI methods the MALDI specific sample list format should be selected. This displays the column configuration applicable to the MALDI MS, MSMS and Survey methods.

To change a sample list format:

1. MassLynx Sample List > Samples > Format > Load
2. Select MALDI Q-Tof Premier.

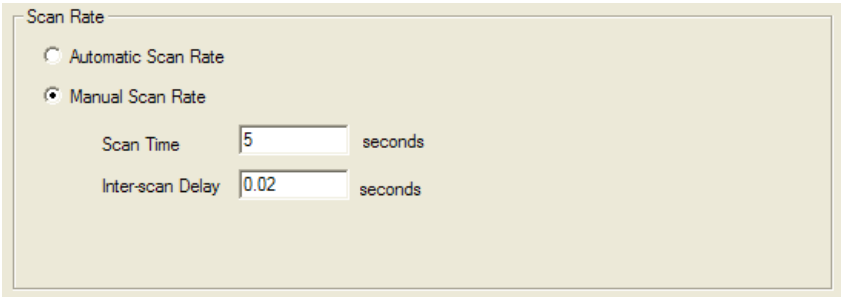
   This is applicable to the MALDI source only.

Scan Conditions page

The Scan Conditions page is used for setting the instrument conditions for the acquisition.

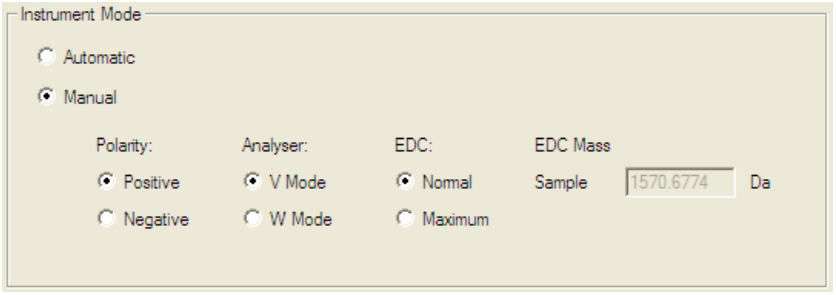
Scan Rate frame

As the samples analyzed by MALDI do not change within one sample well it is not necessary to scan at a high rate.



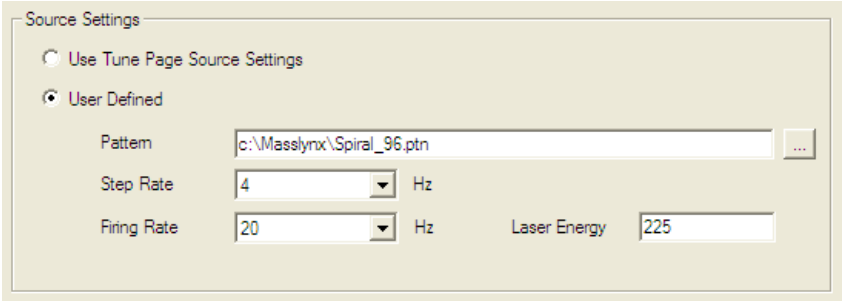
The automatic scan rate is 5 seconds with an Inter-scan delay of 0.02 seconds. To select values different from this select Manual Scan Rate.

Instrument Mode frame



This frame is used to set the ion mode, V or W mode, and whether EDC is used. If you select Automatic the acquisition will be in V positive mode with no EDC.

Source Settings frame



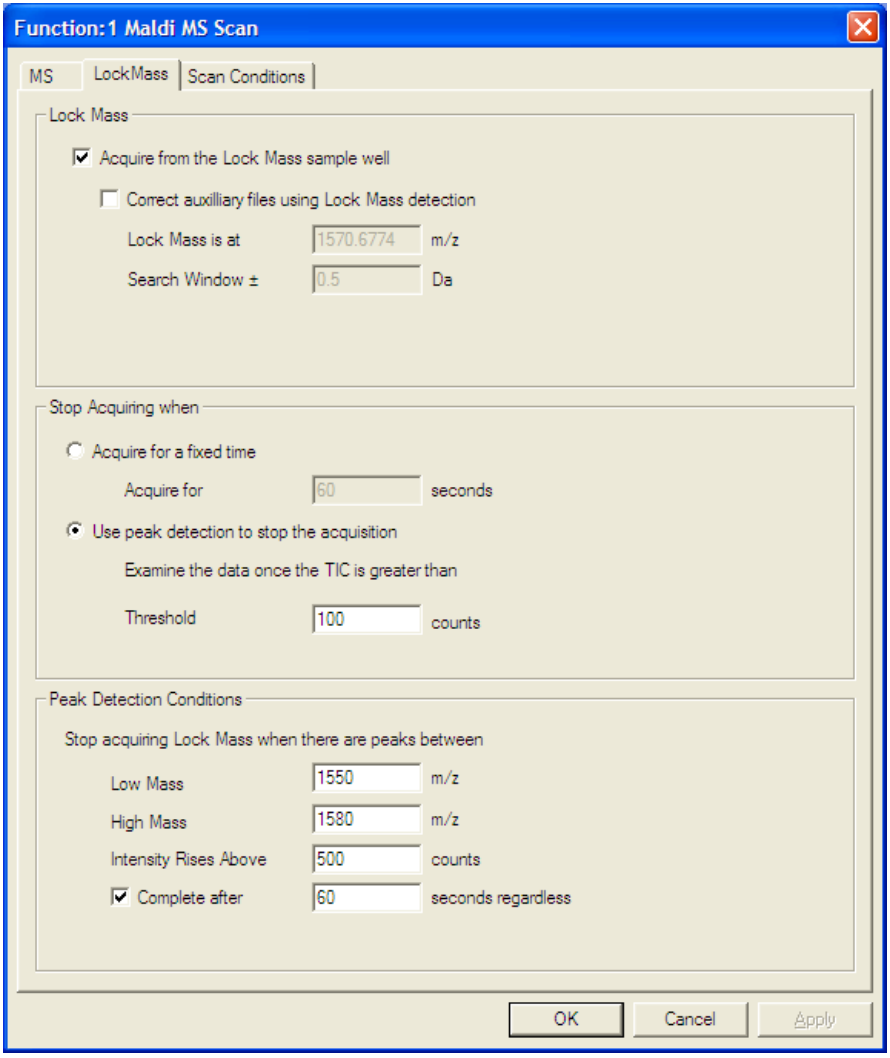
This frame allows you to set the conditions for the laser. Either the settings from the Tune window are used or a user defined pattern file, Step Rate, Firing Rate and Laser Energy can be entered.

When a sample list acquisition is taking place the motion starts at the beginning of the pattern and moves at a constant speed towards the end. The history of the plate movement is used during the experiment. So if the sample spot has been used before, then the plate begins moving from the last used point.

Important: Only one pattern history is held in MassLynx. The pattern specified has to be the same as that last used in the Tune window so the pattern history is consistent.

Tip: The Step and Firing rates should be set to suit the samples. Waters recommends that various settings are tried using manual acquisitions.

LockMass page



When 'Acquire from the LockMass sample well' is checked, the sample plate moves to the LockMass sample well. Data is acquired for the time defined in the set-up as shown above.

The LockMass data is acquired over the same mass range as the MS scan. In addition the LockMass uses the same scan conditions and pattern as the MS acquisition. The data from the LockMass acquisition is always written into the last function in the data file.

Peak detection

In addition to acquiring data for a fixed amount of time, the MALDI Q-Tof Premier offers the option of acquiring until a user-defined quality threshold is reached. Within the MALDI method editor this is referred to as peak detection.

MS Scan and MS Survey

As each spectrum is acquired, it is saved to disk and then combined with all the previous scans into a 'rolling-average'. (This average is actually a sum as no division takes place.) This rolling-average is continued until there is a peak in this combined spectrum with an intensity greater than a user defined threshold. In this way if two samples gave different intensity MS scans, where the first was strong and the second weak, the MS spectrum would be acquired for different lengths of time, but would contain peaks of the same intensity. In this way the data is normalized for the data quality rather than duration.

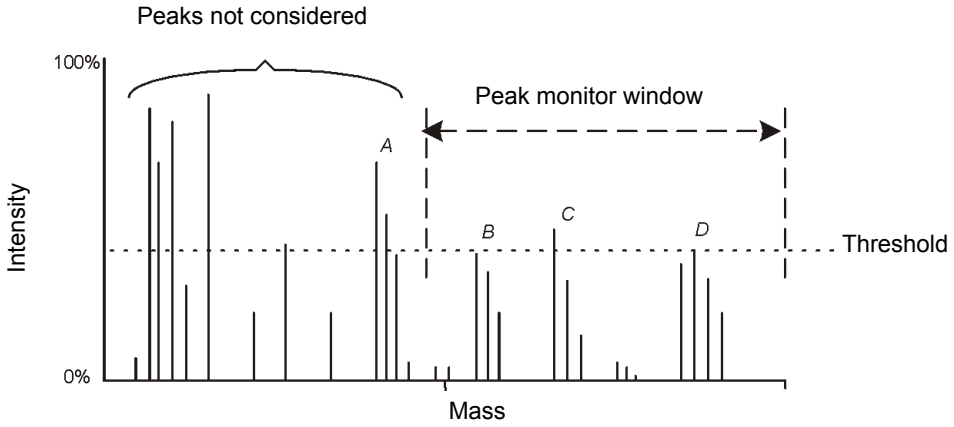
Although every spectrum is recorded, the rolling-average is only started once the chromatogram TIC has risen above a user defined value.

In a MALDI Survey scan only one MS scan is performed. Consequently all the information used to extract peak information, which is used to create the MSMS peak list, must be generated from this one combined spectrum. By combining scans the signal to noise can be enhanced so that weak peaks as well as the strong peaks are recorded.

Within the mass range of the MS scan, a Peak Monitor Window is defined. Only peaks which fall within this window are considered when the rolling-average is being interrogated to determine if there is, or is not, a peak present. If the Peak Monitor Window is set to include all the mass range then the MS scan is stopped when the largest peak in the spectrum reaches the threshold.

If the mass range of the scan were extended down to low mass the matrix ions would be recorded, but they can be 'screened out' so that their intensities, although recorded, do not contribute to the conclusion of the MS acquisition. In this way their relative intensities may be used as a rough guide to the intensity of the signals of interest. Alternatively, if clusters of interference peaks are known to be present, then the peak monitor window could be set to exclude where these lie - remembering that the (accurate mass) exclude list is only applied after the MS scan is complete. Note that there is only one peak monitor window defined within each experiment set-up.

Peak monitor window



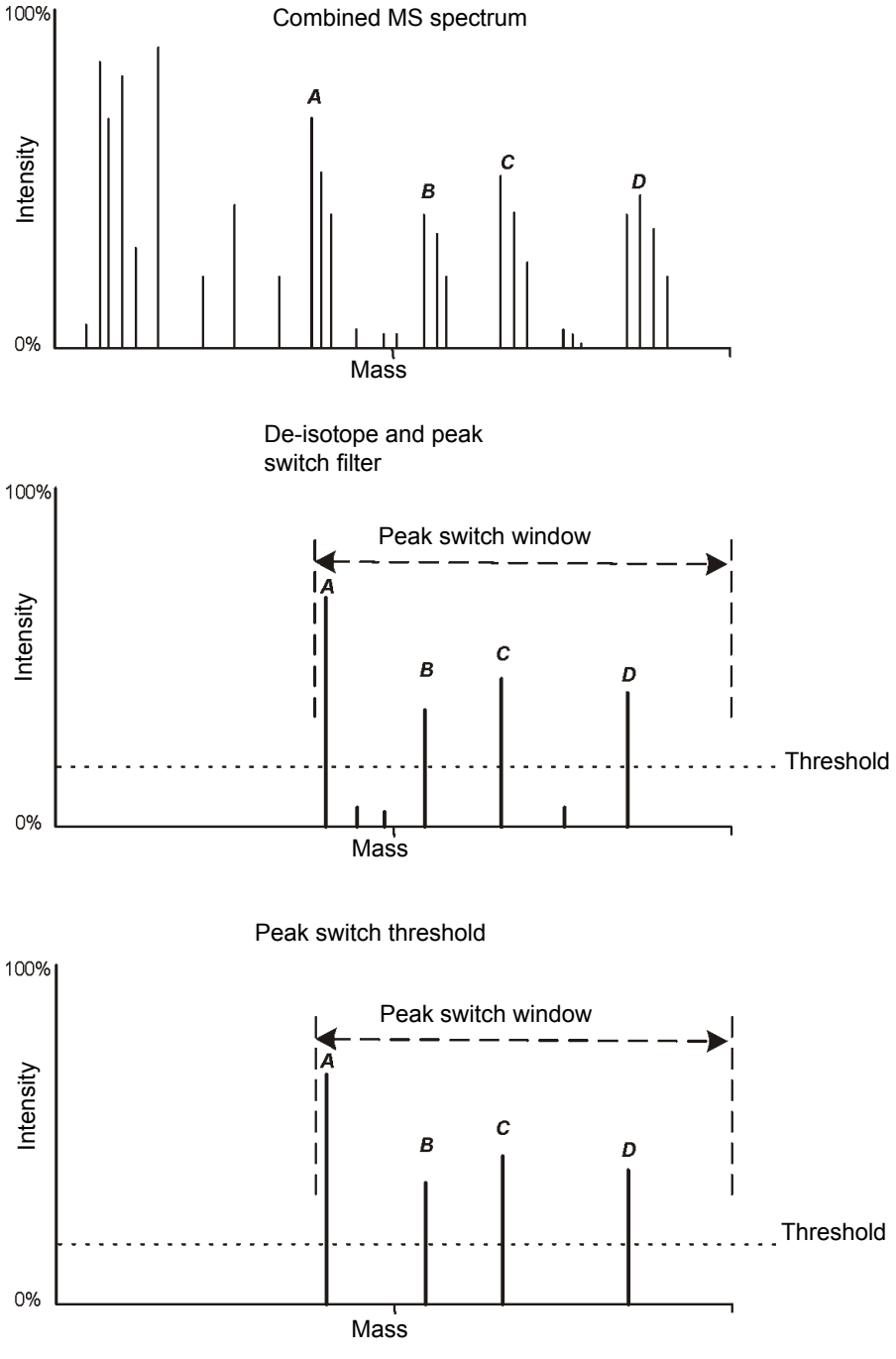
In the example of a completed MS spectrum, a Peak Monitor Window has been defined which extends from ~45% to ~100% of the mass range. Although there are intense peaks, such as A and those at the low mass (left hand) end of the spectrum, these are not considered when the spectrum is interrogated for 'peaks present'. Instead the peak C has exceeded the threshold and this, because it falls within the Peak Monitor Window, has triggered the end of the MS scan. The rolling-average spectrum is not directly visible to the user, it is a virtual spectrum that exists only in the instrument control system.

If the MS scan is part of a Survey experiment then, once the MS scan has completed, the data is processed to determine a list of precursor masses that MSMS will be performed on. It is the rolling-average spectrum that is considered in the Peak Detection phase. If, however, the MS scan is part of an MS experiment, the acquisition completes at this point.

The combined spectrum is peak detected (converted to centroid data) and a Peak Switch Window applied to the result. Any masses outside the Peak Switch Window will not be considered for further processing. Typically the Peak Switch Window will cover a larger portion of the mass range than the Peak Monitor Window. If the peak switch window is set to be the same as the mass range then every peak will be considered. Within this window a Peak Switch Threshold is defined. Once the data has been processed, only peaks above this threshold will be considered as MSMS precursor masses.

The peak-detected spectrum is submitted to a de-isotoping routine. This produces a list of mono-isotopic masses that correspond to the peaks present in the combined MS spectrum. This list is then filtered to remove peaks that have intensities below the peak switch threshold.

Peak windows:



This reduced list is then ranked in intensity order (where the most intense peaks are at the beginning) and, finally, it is compared to the contents of the Include and Exclude settings (see Q-Tof Premier Operator's Guide). This final list then provides the precursor mass information from which automated MSMS will be performed.

The Survey method will then run in MSMS mode. Each mass is acquired in turn, rather than simultaneously.

The experiment continues until either the list is complete or the end of the (MALDI sample plate) pattern is reached. The sequence described above is carried out automatically. The results of the individual steps are not available to the user. In the example shown, the combined MS scan is first filtered by the Peak Switch Window which, in this case, rejects all the low mass peaks. This reduced spectrum is de-isotoped and then all the peaks that fall below the Peak Switch Threshold are rejected, leaving four candidates for MSMS. These are then ranked in intensity order summarized in the following table.

Final MSMS order:

MSMS order	Peak
First	A
Second	C
Third	D
Fourth	B

MSMS Acquisition

The MSMS acquisitions follow a similar method to that of the MS i.e. a spectrum is recorded; that spectrum is combined with all the previous spectra; the combined spectrum is then interrogated for a peak intensity; if the spectrum is below the threshold the acquisition is continued, if the spectrum is above the threshold then the acquisition completes. If there are more MSMS masses available then the cycle is repeated with the next mass until the end of the list is reached.

In a Survey acquisition the mass range of the MSMS acquisition is set independently from that of the MS acquisition. A separate start mass is defined and the end mass is set dynamically as an offset above the MSMS precursor mass. For example, if an offset of 75 were used (and the start mass was 50), then, if multiple set masses were defined of it peaks were detected at

1000 and 1500 amu, the first acquisition would be from 50 to 1075 and the second from 50 to 1575.

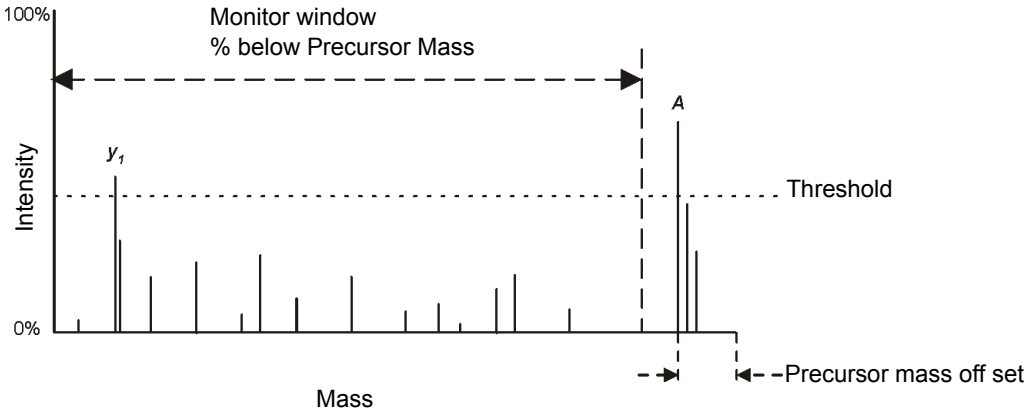
In order to produce fragmentation, the collision energy needs to be defined. This is either done in the same way as it is for the single MSMS scan or by using Collision Energy Profiling. This Collision Energy Profile is a look-up table, that the user can create, which defines the relationship between collision energies and mass. When the acquisition begins, the collision energy is taken from this table and used until that acquisition is completed. The example collision energy profile, C:\Masslynx\QTofPremier\MQT_CE_Profile.txt, is included as part of the software installation.

As each spectrum is acquired, it is combined into a rolling-average (as is done with the MS scan) and this combined spectrum is monitored for 'peaks present'. To this rolling-average a Monitor Window is applied. Only peaks that fall within this window and are above a user defined intensity threshold will be considered. This Monitor Window is defined from the Start Mass of the MSMS mass range up to a (user defined) percentage below the precursor mass.

e.g. If the precursor mass was detected as 1000 amu (the Start Mass set to 50), and the Monitor Window was defined as 10% below the precursor mass, then only when there was a peak in the mass range 50 to 900 amu - which was above a threshold - would this MSMS acquisition stop. This is shown in the following figure (Figure titled "MSMS Monitor window:" on page 3-11) where the peak at y_1 has triggered the end of the acquisition.

This Monitor Window can then be set so that the intensity of the precursor mass is not considered when the spectrum is interrogated. In this way only fragment peaks contribute to the intensity. For example, where one precursor is intense and the second is weak, (then by only considering the fragment ions), the two acquisitions will run for different lengths of time but will have the same fragment intensity when they have finished. In this way the data is normalized for fragmentation quality, not for the time spent on that mass.

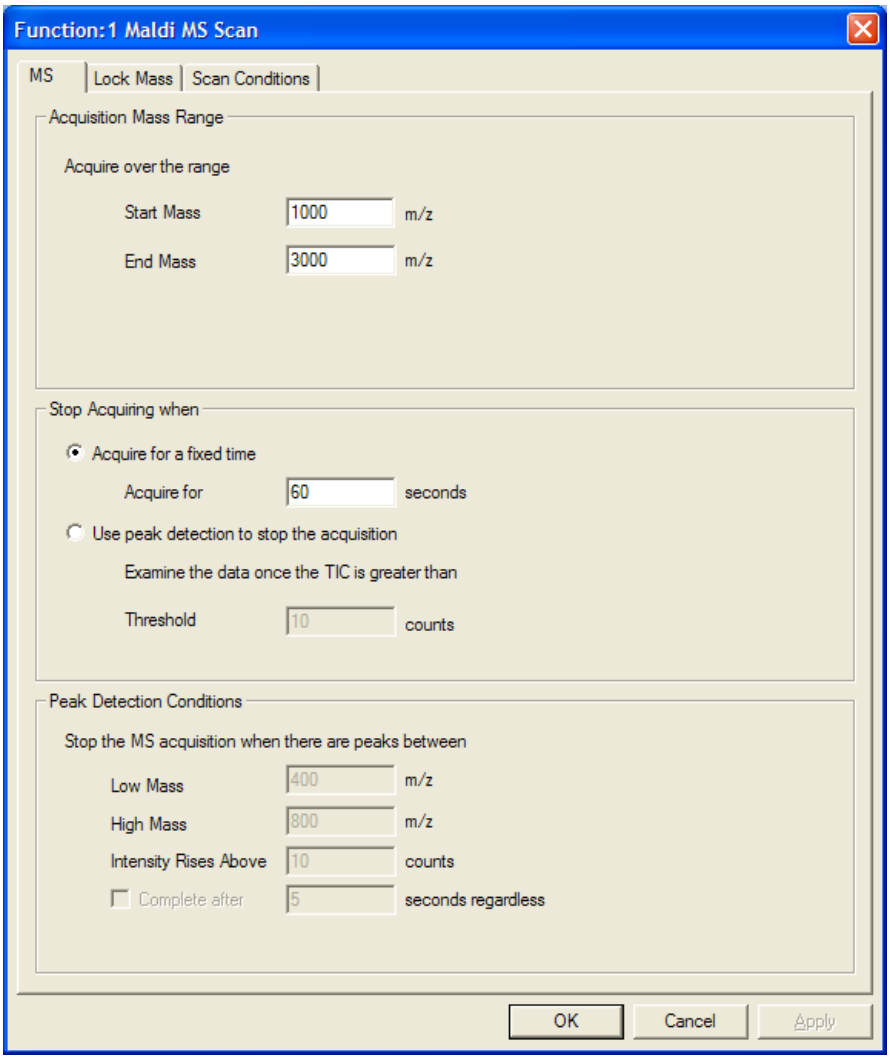
MSMS Monitor window:



Software Setup

MALDI MS method

For a MALDI MS method there are three pages of parameters that need to be considered. The Scan Conditions page and LockMass page are described in the Method Editor on page 3-2. The MS page controls the conditions specific for the MALDI MS scan.



When performing an MS scan there are three things to consider:

- The acquisition mass range

  Enter the Start Mass and End Mass to define the acquisition mass range.

- When to Stop Acquiring data

  This can be done for a fixed time or peak detection can be used to determine when to stop the acquisition.

- The peak detection conditions

These are the same as those described in Peak detection on page 3-6. The low and high mass define the peak monitor window.

MALDI MSMS method

For a MALDI MSMS method there are five pages of parameters that need to be considered. The Scan Conditions page and LockMass page are described in Method Editor on page 3-2. The Collision Energy page options are described in the *Q-Tof Premier Operator's Guide*.

The two remaining pages are:

- The MS/MS page which sets the acquisition parameters.
- The Precursor Mass page which sets the precursor masses of interest.

MS/MS page

When performing an MSMS scan there are two things to consider:

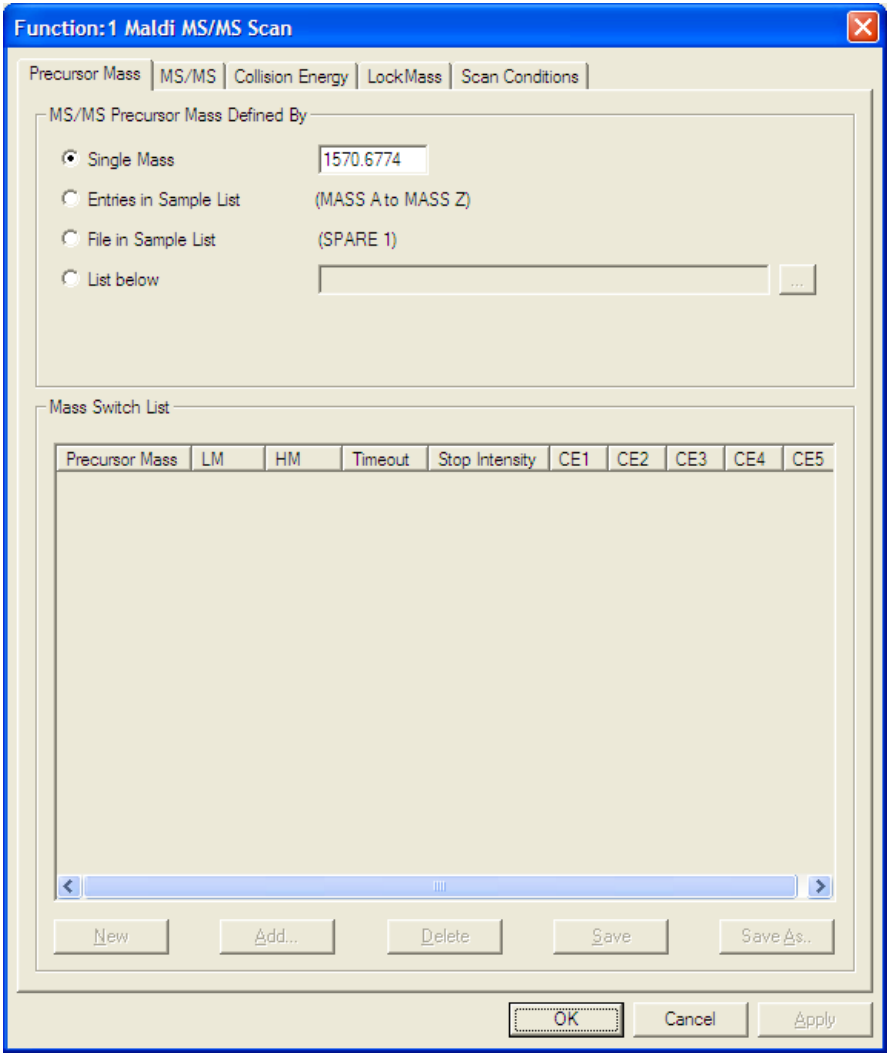
- The acquisition mass range.

  Use an Automatic Mass Range or enter the Start Mass and End Mass to define the acquisition mass range.

- When to stop each MSMS scan.

  This can be done after a fixed amount of time or after the intensity rises above a certain threshold. Precursor Mass Offset described in Peak detection on page 3-6 relates to the "Do not consider top % of mass range" parameter.

Precursor Mass page



The MS/MS Precursor Mass can be defined in the following ways:

- Single Mass - enter a single precursor mass that will be used by all the samples.
- Entries in Sample List - select this option if the precursor masses are defined individually in the Sample List. A maximum of 26 masses can be defined by inserting the MASS A to MASS Z fields into the Sample List. Enter an individual mass into each field.

- File in Sample List - select this option if you have several precursor masses defined in a text file. The Sample List references this file by using the SPARE 1 field. A different file can be used for each entry in the Sample List. The field must contain the full path description to the file and must be less than 50 characters in length.
- List below - a precursor mass list can be built in the Mass Switch list. Alternatively a text file can be loaded. By adding a list of masses each mass can have its own; LM and HM quad resolving setting; Timeout, Stop Intensity; collision energy settings - that supersede the general settings.

MALDI Survey method

For a MALDI Survey method you need to set conditions for the MS and MSMS portions of the experiment. These are the same as described for the MS and MSMS scans. In addition three extra pages are described below:

- MS to MSMS - defines the conditions at which to switch.

  See MS to MSMS page on page 3-16 for more details.

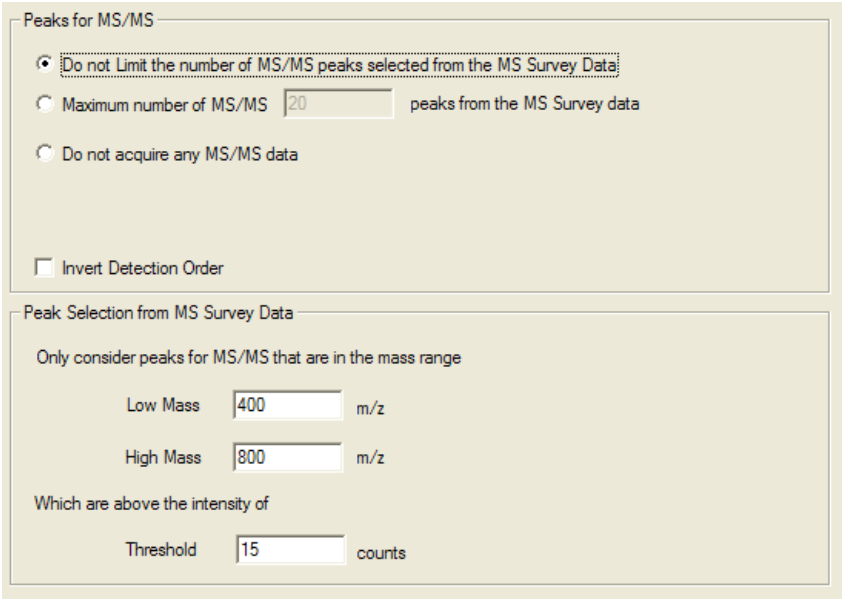
- Include - defines a set of masses to include.

  The options for this are identical to those described in the *Q-Tof Premier Operator's Guide*.

- Exclude- defines a set of masses to exclude.

  The options for this are identical to those described in the *Q-Tof Premier Operator's Guide,* except for Reject peaks using peptide mass sufficiency model. This is an extra filter that excludes mass peaks if their mass is outside the theoretical window for peptide masses (based on Mann, M. Possible Peptide Masses. Proceedings of the 43rd Conference on Mass Spectrometry and Allied Topics, Atlanta, GA, 21-25 May 1995, p. 639).

MS to MSMS page



Peaks for MS/MS

There are three options for determining how many peaks are selected for MSMS. Either:

- Attempt all peaks on the list by selecting 'Do not limit the number of MSMS peaks selected from the MS Survey Data'.
- Enter a maximum number of peaks to consider by selecting 'Maximum number of MS/MS peaks from the MS Survey data'.
- Do not acquire any MS/MS data.

You can also choose to invert the detection order. If this is the case the list selected for MSMS is run through backwards. The least intense peak from the selected list is surveyed first as opposed to the most intense.

Peak Selection from MS Survey Data

When switching from MS to MSMS, another window for peak selection is set - independent of that set for MS peak selection. This enables you to set a different mass range if required.

EDC for MALDI methods

MS method

EDC enhances a window of masses centered on the mass specified on the Scan conditions page. This mass can fall anywhere within the MS acquisition mass range.

If LockMass is selected then the enhanced LockMass mass range is centered at either the same mass as the MS data or, if Correct Auxiliary files is selected, centered at the specified LockMass mass.

MSMS method

EDC enhances a window of masses centered on the mass specified on the Scan conditions page. This mass can be any value below the MSMS precursor mass.

If LockMass is selected the enhanced LockMass mass range is centered at the specified LockMass precursor mass.

Survey method

EDC is not available with the Survey method.

EDC calibration

As part of any EDC method the instrument is automatically configured as though the acquisition end mass is 4000 m/z. However, data is only recorded over the mass range specified within the method, which may have a lower end mass than 4000. To ensure mass measurement accuracy with EDC data a LockMass should be used as part of the acquisition method. If this is not practical an instrument calibration should be created from data acquired with an End Mass of 4000 m/z so that the acquisition conditions are matched to the instrument configuration.

Auxiliary file output

The MS, MSMS and Survey methods automatically create peak list output files for use in applications outside of MassLynx. These Auxiliary files are created within the data file folder.

Method	MS peak list	MS peak list sorted by intensity	MSMS pkl per precursor	MSMS pkl of all precursors	List of detected precursors	List of acquired precursors
MS	Yes	Yes	No	No	No	No
MSMS	No	No	Yes	Yes	No	No
Survey	Yes	Yes	Yes	Yes	Yes	Yes

Each file contains a deisotoped mass-intensity pair peak list in a format applicable to the method from which it was produced.

MS output files

- _deisotoped.txt
- _deisotoped_sbi.txt

Consist of tab separated mass-intensity pairs. Each line also contains the sample well from which the data was acquired.

MSMS output files

- *_precursormass_counter*.pkl
- _total.pkl

Consist of PKL format mass-intensity pair list. Where _total.pkl is all the individual pkl files combined sequentially.

e.g. A method that acquires MSMS from 1570.6774, 1296.6853 and 2465.1989 would produce four auxiliary files:

- _1570_1.pkl
- _1296_2.pkl
- _2465_3.pkl

- _total.pkl

Survey output files

As MS and MSMS with the addition of:

- _peaksforMSMS.txt
- _switched.txt

Where _peaksforMSMS.txt contains the peaks that fulfill the peak selection criteria of the methods and _switched.txt contains the peaks that MSMS was actually recorded for.

4 Maintenance and Troubleshooting

In addition to the standard maintenance on Q-Tof Premier, described in the *Q-Tof Premier Operator's guide*, there are some additional maintenance procedures required for the MALDI source.

Contents:

Cleaning the hexapole

Warning: The instrument components are liable to be contaminated with biologically hazardous and toxic materials. Wear rubber gloves at all times while handling the components.

Warning: Ensure the instrument is in Standby mode and vented before removing the hexapole.

The hexapole will require cleaning on a regular basis. In most cases, gently washing the hexapole with a wash bottle filled with propan-2-ol (IPA) will be sufficient. In severe cases, the whole assembly can be placed in a large beaker of propan-2-ol and sonicated for 30 minutes.

To vent the instrument:

1. Put the instrument in Standby.
2. Click Vacuum > Vent to vent the instrument.

To remove and clean the hexapole:

1. Disconnect the MALDI cable and the Heater/Interlock cable from the front panel.
2. Disconnect the Hexapole Lid cable.
3. Release the four captive screws and two captive dowell screws.
4. Gently pull out, tilt forward, and lift out of the source.

   Caution: Ensure that the Hexapole Lid cable is refitted before switching the instrument into Operate.

Cleaning and replacing the vacuum lock O-ring

Warning: The instrument components are liable to be contaminated with biologically hazardous and toxic materials. Wear rubber gloves at all times while handling the components.

If the sample plate carrier fails to pump the plate carrier lid within the 3 minute system time-out period, a plate carrier error will occur.

The time out can be caused by either

- The lid failing to seat correctly on the vacuum lock o-ring
- The vacuum lock o-ring has failed or is contaminated.

To remove and clean the O-ring:

1. Unload the sample plate and open the plate carrier lid.
2. Lift the O-ring off the spigot.
3. Clean the O-ring and its seating surface with a lint-free cloth and suitable solvent, such as methanol.
4. Clean the inside surface of the lid as described in step 3. Ensure that no hairs or particles remain.

   Examine the integrity of the O-ring. If it is in good condition, refit it, seating it evenly around the spigot. If its condition is in any doubt, fit a new O-ring.

Plate carrier errors

The sample plate carrier generates primary state information which is displayed in the Tune window status bar and reflected in the behavior of the plate carrier control buttons. In an error condition the plate carrier generates additional secondary information which is displayed in a message box in front of the Tune window.

Secondary information is reported as either:

- Position Error
- Plate Carrier ERROR

In both cases the error must be cleared before normal operation can be resumed.

In the Position Error case, the control buttons will change state to automatically indicate the correct action to clear the error.

In the Plate Carrier ERROR case, the control buttons are disabled and the user must remove and refit the Heater/Interlock cable to clear the error.

Position errors are caused by either the plate carrier not reaching a requested position before a internal system time-out or the plate carrier requesting a move beyond its limit of travel. This latter case could be caused by an inappropriate plate definition.

Plate Carrier ERRORs indicate a system failure. If this state persists after the reset sequence contact Waters.

Support for alternative sample plates

MALDI sample plates not supplied by Waters may be used with the MALDI Q-Tof Premier.

- Where these plates are of the same dimensions as a Standard Waters plate only the sample plate definition needs to be modified.
- Where these plates are of a different size to the Standard Waters plate the sample plate carrier insert plate must be removed prior to using and the sample plate definition modified.

To remove the plate carrier insert:

1. Unload the sample plate
2. Open the plate carrier lid and remove the sample plate if fitted.
3. Using a suitable screwdriver release the captive screw in the center of the sample plate carrier insert.
4. Store the carrier insert in the source top cover directly above the plate carrier lid.

To revert back to Standard Waters plates the carrier insert must be refitted.

When alternative sample plates of larger dimensions to the Standard Plate are used it may not be possible to access all the rows and columns on these plates. If a request to move takes the plate carrier beyond the limit of movement, a plate carrier error will occur. In this instance refer to Plate carrier errors on page 4-4

Contacting Waters

You can easily correct many problems with the Q-Tof Premier. However, if this is not the case, you must contact Waters.

Customers in the USA and Canada should report maintenance problems they cannot resolve to Waters Technical Service (800 252-4752). All others should visit http://www.waters.com and click Offices, or phone their local Waters subsidiary or Waters corporate headquarters at 34 Maple Street, Milford, MA 01757, USA.

When contacting Waters, have the following information available:

- The nature of the symptom
- The Q-Tof Premier serial number

Depending on the nature of the fault, it may also be useful to have the following information available:

- Details about the flow rate, mobile phases, and sample concentrations
- Tune window settings
- The Software version update reference

5 Sample Preparation

Sample preparation is accepted as the most important step in mass analysis with MALDI mass spectrometers, and the impact of preparation quality on data quality cannot be over emphasized. This chapter gives sample and matrix details and protocols to act as a guideline. Waters suggests that you keep meticulous records of your procedures.

Contents:

Safety

Warning: Many procedures in this chapter involve using flammable or caustic agents. There is danger of contamination by biological agents that constitute a threat to humans. Personnel performing these operations should be aware of the risks. Refer to Material Safety Data Sheets and take all necessary precautions.

Calibration standards

To prepare calibration standards for peptide analysis:

For peptide analysis, calibrate using a polyethylene glycol (PEG) mixture

1. Prepare the following stock solutions:
   - 10 mg/mL of PEG 600, 1000, 1500, and 2000 in 1:1 water: acetonitrile.
   - 2 mg/mL of NaI in 1:1 water: acetonitrile.
2. Mix PEG oligomers: NaI in the ratio 10:10:10:10:6 ($^{v}/_{v}$).
3. Mix 1:1 with matrix.
4. Spot 1 µL of PEG mix/matrix onto the sample plate and air dry.

Washing stainless steel MALDI plates

To wash plates:

1. Scrub the sample plate in 2% Decon 90 with a soft nylon brush to remove matrix deposits. When analyzing synthetic polymers, use a suitable organic solvent such as dichloromethane.
2. Rinse the plate in distilled water.
3. Sonicate for 10 minutes in 1:1:1 dichloromethane: acetone: methanol or an alternative degreasing agent.
4. Sonicate for a further 10 minutes in methanol.

5. Dry under a dry nitrogen stream.

   Important: This cleaning procedure does not apply to MassPREP Pro or DIOS sample plates. See the relevant User's Guides.

Sample Preparation Considerations

Consider the following before preparing MALDI samples:

- Expected molecular mass range.
- Concentration or amount of sample supplied.
- Suitable solvent.
- Contaminants present, such as salts, buffers, glycerol. Higher purity samples will yield better spectra with less interference.

  Tip: You can de-contaminate samples before or after preparing the sample spots. For MassPREP Pro sample plates see the relevant User's Guide.
- Sample type. For example, peptide, protein, protein digest, oligonucleotide, oligosaccharide, synthetic polymer.
- Sample stability. For example, sensitivity to acid. If the sample is acid sensitive then omit TFA from the procedures outlined.
- Molecular structure or principal functional groups.

Matrices

Matrices and Substrates

Matrices and Substrates

Matrix		Typical Substrate
CHCA	α-cyano-4-hydroxy cinnamic acid	Peptides, polymers
Sinapinic acid	3,5-dimethoxy-4-hydroxycinnamic acid	Proteins, peptides, polymers
DHB	2,5-dihydroxybenzoic acid	Sugars, peptides, nucleotides, polymers

Matrices and Substrates

Matrix		Typical Substrate
CMBT	5-chloro-2-mercaptobenzothiazole	Proteins, peptides
Dithranol	1,8-Dihydroxy-9(10H)-anthracenone	Synthetic polymers
THAP	2,4,6-trihydroxyacetophenone	Oligonucleotides
HABA	2-(4-hydroxyphenylazo)benzoic acid	Glycolipids, peptides, proteins
HPA	Hydroxypicolinic acid	Oligonucleotides, peptides, glycoproteins
IAA	β-indole acrylic acid	Polymethyl methacylates
	3-aminoquinoline	Sugars, peptides, nucleotides, polymers
	4-hydroxy-α-phenylcinnamic acid	Proteins, glycoproteins
	All trans-retinoic acid	Synthetic polymers

Matrix Mixtures

Matrix Mixtures and Substrates

Matrix Mixture	Substrate
S-DHB = 5-methoxysalicilic acid and 2,5-dihydroxybenzoic acid (mixed 1:10)	Peptides, proteins
α-cyano-4-hydroxy cinnamic acid and 2,5-dihydroxybenzoic acid (mixed 3:5)	Synthetic polymers
Anthranilic acid and nicotinic acid	Oligonucleotides

Preparing Matrices

The matrix:sample molar ratio must be > 5000:1. Matrix solutions are light sensitive, so prepare fresh each day and keep in a dark tube. A possible exception is hydroxypicolinic acid (HPA), which can improve sensitivity for oligonucleotides / DNA when the matrix solution has ‘aged’ for up to two weeks before analysis, turning brown in color.

A washing stage or re-crystallization of the matrix before use can improve results, maybe due to a reduced contaminants, such as organic chemicals and metal ions.

Waters has a range of highly purified matrices to cover most application areas. These can ordered using the part numbers listed below.

Waters MALDI matrices and part numbers

Matrix	Waters Part Number
CHCA	186002331
DHB	186002333
Sinapinic acid	186002332
HPA	186002334
THAP	186002335

Premix Method

To mix the matrix:

1. Mix 2 µL of sample with 2 µL of matrix solution.
2. Spot 1 µL of this mixture onto the MALDI sample plate.
3. Repeat for the required samples and air dry.

Thin Film Technique

To improve signal strength, but with a reduction in sample longevity, reduce the matrix crystal size by seeding the sample spot with a thin film of matrix solution (dissolve the matrix in a volatile solvent such as acetone).

Tip: With this matrix preparation it may be beneficial to reduce the laser firing rate during the acquisition.

Use a low volume pipette tip such as a Gilson P2 pipette for reproducible 1 µL spotting of the volatile matrix solution. Deposit the sample/matrix mixture directly onto the thin film and air dry.

CHCA (α-cyano-4-hydroxy Cinnamic Acid)

Use a concentration of 2 to 10 mg/mL in 1:1 aqueous (aq) 0.1% TFA: acetonitrile.

For thin film CHCA use a concentration of 10 mg/mL in 495:495:10 ethanol: acetonitrile: 0.1% TFA (aq). Dilute this solution 4:1 in 10 mg/mL nitrocellulose.

CHCA can also be dissolved in other organic solvents for non-aqueous sample preparations, such as synthetic polymers.

For peptide analysis at the low fmol level it is beneficial to use CHCA at a concentration of 3.6 mg/mL, as this reduces the level of matrix clusters observed.

Sinapinic Acid (3,5-dimethoxy-4-hydroxycinnamic Acid)

Use a concentration of 10 mg/mL in 4:6 acetonitrile: 0.1% TFA (aq).

To improve the signal, mix sinapinic acid at a ratio of 3:1 matrix to sample.

Thin Film Sinapinic Acid

To obtain improved signal intensities with protein samples use a thin film sinapinic acid matrix.

To prepare thin film sinapinic acid matrix:

1. Prepare matrix as above and also at a concentration of 10 mg/mL in acetone
2. Apply 1 µL of the thin film solution to the sample plate and air dry.
3. Mix the sample 1:1 with the standard sinapinic acid matrix preparation and apply 1 µL of this mixture over the thin film.

You can also dissolve sinapinic acid in organic solvents to prepare non-aqueous samples such as synthetic polymers.

DHB (2,5-dihydroxybenzoic Acid)

Use a concentration of 10 mg/mL in 70:30 water: acetonitrile for general peptide analysis or prepare a saturated solution in 20:80 water:acetonitrile for the analysis of oligosaccharides.

You can also dissolve DHB in organic solvents to prepare non-aqueous samples such as synthetic polymers. Use a concentration of 10 mg/mL in 1:1 methanol: chloroform.

S-DHB ('Super' DHB)

Add 1 mg of 5-methoxysalicilic acid to 9 mg of DHB, and dissolve the mixture in 9:1 0.1% TFA (aq): acetonitrile.

CMBT (5-chloro-2-mercaptobenzothiazole)

Use a concentration of 10 mg/mL in 1:1:1 acetonitrile: methanol: aqueous 0.1% Formic acid.

CMBT is not very soluble and must be sonicated before use. This produces a saturated solution at the specified concentration.

Dithranol (1,8-Dihydroxy-9(10H)-anthracenone)

Use a concentration of 20 mg/mL in tetrahydrofuran, dichloromethane, methanol or hexafluoro-2-propanol (HFIP).

For synthetic polymer analysis use the matrix together with a trifluoroacetic acid salt such as sodium, potassium, or silver.

HABA (2-(4-hydroxyphenylazo)-benzoic Acid)

Use a concentration of 3.5 mg/mL in methanol.

Use this matrix for peptide and protein analysis. It is also effective in the analysis of glycolipids.

HPA (Hydroxypicolinic Acid)

Use a concentration of 25 mg/mL in 25:75 water: acetonitrile.

Use this matrix together with an ammonium salt solution or an ion exchange resin to analyze oligonucleotides.

IAA (β-indole acrylic Acid)

Use a concentration of 10 mg/mL in acetone.

Use this matrix to analyze acrylates.

THAP (2,4,6-trihydroxyacetophenone)

Use a concentration of 25 mg/mL in 1:1 water: acetonitrile.

Use this matrix together with an ammonium salt solution or an ion exchange resin to analyze oligonucleotides.

Anthranilic acid / Nicotinic Acid

Use a concentration of 27.9 mg of anthranilic acid and 12.3 mg of nicotinic acid in 500 µL acetonitrile: 300 µL aqueous ammonium citrate (100 mM): 300 µL water.

4-hydroxy-α-phenylcinnamic Acid

This matrix is used as an alternative to sinapinic acid. It is particularly useful for analyzing glycoproteins as it does not produce matrix adducts. However, the overall sensitivity is reduced compared to that of sinapinic acid.

3-aminoquinoline

Use a concentration of 10 mg/mL in 1:1 methanol: 0.1% TFA (aq).

All Trans-retinoic Acid

Use a concentration of 10 mg/mL in THF.

Especially useful for the analysis of high molecular weight polystyrenes.

Analysis of Peptides and Proteins

MALDI can be used to identify proteins from the unique masses of peptide fragments produced after specific digestion with protease enzymes. Trypsin is typically used to digest proteins that have been isolated electrophoretically from biological samples.

In most cases peptides and proteins are water soluble and stable in the presence of 0.1% TFA The presence of the acid prevents the analyte becoming associated with the walls of the tube.

Prepare each sample at a concentration of between 10 to 500 fmol/µL (peptides) and 1 to 10 pmol/µL (proteins). For unknown sample concentrations dilute over 4 orders of magnitude and load 4 separate spots on the sample plate. Dilution of the sample often enhances the higher molecular weight components, especially in a mixture.

Use the above concentrations as a starting point. For those samples that do not produce signals after trying various matrices, try diluting the sample rather than concentrating the sample as a second step.

You can improve the data from salt or buffer contaminated samples by on-spot washing.

Tip: For highly contaminated peptide samples, use a MassPREP Pro sample plate to allow rigorous on-spot washing without significant sample loss.

Analyze proteins above 12 kDa with a sinapinic acid matrix, for improved sensitivity use the thin film technique. Sinapinic acid produces adducts (M + 208 Da) that correspond to the addition of sinapinic acid with the loss of water. Multiply charged species and multimers are often observed, but the $[M+H]^+$ species typically predominates.

Preparing Peptide and Protein Standards

Prepare and store standards at a concentration of 1 mg/mL in 0.1% TFA (aq) in a freezer. Dilute to obtain a working concentration of approximately 10 pmol/µL.

Example standards are shown in the following table.

Dilution Factors for Peptide and Protein Standards

Substance Name	Average Molecular Mass (Da)	Dilution Factor *
Leucine enkephalin	555.6	180
Bradykinin	1060.2	95
Angiotensin I	1296.5	77
Glu-fibrinogen	1570.6	64
Renin	1759.0	57
ACTH (18-39 clip)	2465.7	40
Insulin (bovine)	5733.5	17
Cytochrome-C (Horse Heart)	12360	8
Myoglobin	16951	6
Trypsinogen	23980	4
Bovine Serum Albumin	66430	1.5

*Dilution factor of 1 mg/mL solution to give 10 pmol/µL.

Store the above solutions in a freezer and use as required, although solutions will deteriorate over time.

Preparing protein digests

Caution: Risk of sample contamination from keratin. Gloves (that have been rinsed with water) must be worn throughout the sample handling stages.

In-gel protein digests

To prepare in-gel protein digests:

The following procedure is for manual in-gel digestion of proteins separated by 2D-polyacryamide gel electrophoresis (Coomassie blue stain).

1. Rinse the gel with distilled water and excise the bands of interest with a clean scalpel cutting as close to the protein spot as possible. Chop the excised spot into pieces (1 x 1 mm).
2. If the gel pieces are still blue, rehydrate them in 100 to 150 µL 100 mM ammonium bicarbonate and heat to 37 °C. After 10 to 15 min. add an equal volume of acetonitrile.
3. Vortex the solution for 30 seconds, then centrifuge, remove supernatant and shrink the gel with acetonitrile. Dry gel fragments in a vacuum centrifuge. If the Coomassie stain persists, repeat destaining procedure.
4. Wash the gel fragments with 150 µL water for 5 min. Centrifuge and remove supernatant. Add acetonitrile (3 to 4 x volume of gel pieces and wait for 10 to 15 min), until gel pieces have shrunk and are white.
5. Dry gel fragments in a vacuum centrifuge.
6. Swell gel fragments in 10 mM DTT 100 mM ammonium bicarbonate using minimum volume to completely cover gel then incubate for 30 min at 56 °C to reduce protein (this stage is recommended even if the proteins were reduced before electrophoresis). Dehydrate gel with acetonitrile as above.
7. Centrifuge and remove supernatant then add 55 mM iodoacetamide dissolved in 100 mM ammonium bicarbonate using minimum volume to completely cover gel, leave for 30 minutes at room temperature in the dark.

8. Centrifuge and remove supernatant, wash with 150 µL 100 mM ammonium bicarbonate for 15 min, centrifuge and remove the supernatant.
9. Dehydrate gel with acetonitrile.
10. Remove supernatant and dry gel fragments in a vacuum centrifuge.
11. Rehydrate gel particles in a minimum volume of digest buffer 50 mM ammonium bicarbonate containing 12.5 ng/µL trypsin (w/v) and incubate at 4 °C for 30 to 45 min. After 15 to 20 min add more buffer to gel fragments if the gel fragments absorb all the liquid.
12. Incubate at 37 °C for 16 hr.
13. To recover the peptides add 10 µL of 50% acetonitrile / 5% formic acid to the digest mixture and sonicate for 10 minutes. Perform around 2 to 3 extractions of the digestion mixture with a suitable volume (double the volume necessary to immerse gel pieces).
14. Transfer the supernatant after each wash using gel loading pipette tips into micro-Eppendorf™ tubes.

    Note: The high acid concentration is used to minimize adsorptive sample loss.

    Tip: ZipTip™ extraction of samples into a lower volume can increase the concentration of the recovered peptides. Store the recovered peptides below -20 °C.

In-Solution Protein Digests

The first procedure describes a method for the tryptic digestion of non-covalently bound proteins using Waters RapiGest™ SF. RapiGest SF is a reagent used to enhance in-solution enzymatic digests of proteins by solubilizing the proteins, making them more susceptible to enzymatic proteolysis without inhibiting enzyme activity, allowing the rapid digestion of proteins.

If the protein has covalent links (disulfide bonds) these will need to be cleaved and acetylated.

To digest proteins without disulfide bonds:

1. Prepare 50 mM ammonium bicarbonate solution (39.6 mg ammonium bicarbonate in 10 mL H_2O).

2. Suspend 1 mg of RapiGest in 1 mL of 50 mM ammonium bicarbonate (resulting in 0.1% ($^{w}/_{v}$) RapiGest solution).
3. Dissolve protein in 25 to 50 µL of 0.1% RapiGest solution and vortex.
4. Equilibrate sample at 37 °C for 2 minutes.
5. Add enzyme at a ratio of 1:100 to 1:20 (enzyme: protein, $^{w}/_{w}$).
6. Incubate the sample at 37 °C for 20 to 60 minutes.
7. If the sample is particularly hydrophobic (for example, membrane proteins) boil the protein / RapiGest mixture at 100 °C for 5 minutes, cool the sample to room temperature before adding enzyme.

   Once the sample is digested the RapiGest should be removed from the sample before analysis by MALDI.
8. Prepare stock solution of 500 mM HCl.
9. Add 1:10 ($^{v}/_{v}$) HCl stock solution: sample (so that pH 2). The final concentration of HCl should be 30 to 50 mM.
10. Incubate sample at 37 °C for 30 to 45 minutes. A slight cloudiness is normal.
11. The sample can then be directly analyzed by MALDI (or diluted to a suitable concentration before analysis). Enhanced results may be obtained off a MassPREP Pro sample plate.

To digest proteins with disulfide Bonds:

1. Prepare 50 mM ammonium bicarbonate solution (39.6 mg ammonium bicarbonate in 10 mL H_2O).
2. Suspend 1 mg of RapiGest in 500 µL of 50 mM ammonium bicarbonate (resulting in 0.2% ($^{w}/_{v}$) RapiGest solution).
3. Dissolve protein in 25 to 50 µL of 0.2% RapiGest solution and vortex.
4. Add DTT to the protein sample to a final concentration of 5 mM.
5. Heat sample to 60 °C for 30 minutes.
6. Cool sample to room temperature.
7. Add iodoacetamide to the sample to the final concentration of 15 mM, place sample in the dark for 30 minutes.
8. Add enzyme at a ratio of 1:100 to 1:20 (enzyme: protein, $^{w}/_{w}$).

9. Incubate the sample at 37 °C for 20 to 60 minutes.
10. If the sample is particularly hydrophobic (e.g. membrane proteins) boil the protein / RapiGest mixture at 100 °C for 5 minutes, cool sample to room temperature before adding enzyme.

    Once the sample is digested the RapiGest should be removed from the sample before analysis by MALDI.
11. Prepare stock solution of 500 mM HCl.
12. Add 1:10 ($^{v}/_{v}$) HCl stock solution: sample (so that pH 2). The final concentration of HCl should be 30 to 50 mM.
13. Incubate sample at 37 °C for 30 to 45 minutes. A slight cloudiness is normal.

Analyze the sample directly by MALDI, or first dilute to a suitable concentration. To improve results use a MassPREP Pro sample plate.

Internal LockMass correction from trypsin autolysis peptides

The autolysis fragments of trypsin can be useful for internal LockMass correction of a protein digest spectrum. The masses of the observed autolysis fragments from porcine and bovine trypsin are shown in the following table.

Autolysis Fragment Monoisotopic Masses From Porcine and Bovine Trypsin

Porcine Trypsin	Bovine Trypsin
2211.1045	805.4168
	2163.0569

Analysis of other compounds

Phosphopeptides

MALDI analysis of phosphopeptides is compromised due to relative suppression by non-phosphopeptides, their relative instability (they readily lose H_3PO_4) and non-specific binding to glassware. Your sample preparation protocol must be meticulous.

To improve results you can fractionate digests or mixtures to enrich fractions containing the phosphopeptide, using either HPLC or stepwise elution from ZipTips. To enrich phosphopeptide samples use immobilized metal ion affinity chromatography (IMAC).

Phosphopeptides tend to give an improved response with the instrument in negative ion mode relative to positive ion mode. Another strategy to identify phosphopeptides is differential MALDI mapping, whereby a sample is analyzed before and after treatment with a phosphatase enzyme giving mass shifts of 80 Da (exact mass 79.9663, HPO_3) for a phosphate removal.

Oligonucleotides

Matrix of choice: 25 mg/mL hydroxypicolinic acid in 75:25 acetonitrile: water prepared as outlined below.

Sample: dissolved in deionized water at 1 to10 pmol/µL.

Analyze samples rapidly, do not leave them overnight as the sample / matrix will degrade.

Oligonucleotides readily form adducts with cations, leading to reduced resolution and sensitivity. It is therefore important to minimize these interactions. Improved data quality can be obtained from HPLC / SPE purified samples.

Oligonucleotides are sensitive to enzymes present on the hands, therefore wear gloves when handling oligonucleotides. Only use deionized water to prepare both the sample and matrix, as other grades, such as HPLC grade contain metal ions.

HPA Preparation with Desalting

First desalt the oligonucleotides to remove cations that form adducts and result in peak broadening. Desalt the sample is by adding strong cation exchange beads, which exchange the metal cation adduct for H^+. Use Dowex 50 W X8 beads, washed thoroughly and stored in deionized water.

Add a small amount of beads to the matrix solution (25 mg/mL hydroxypicolinic acid in 75:25 acetonitrile: water) and agitate for at least 4 hours (or over night). Centrifuge the matrix and remove the supernatant (the beads can be discarded). Mix the matrix and sample 1:1 and spot directly onto the sample plate.

Oligonucleotide Calibration

Use oligonucleotides of known masses.

Acquiring Data

Use a higher laser energy setting than for peptides.

Oligosaccharides and Sugars

Matrix of choice: saturated solution DHB in 8:2 acetonitrile: water.

Required sample concentration: usually at pmol/µL concentration.

Sample Preparation

Sugars readily form adducts with metal cations, which can result in a loss of resolution and mass accuracy. To reduce these effects, desalt the sample with ion exchange beads.

To prepare samples:

1. Mix 2 µL of matrix solution with 2 µL of sugar solution.
2. Spot 1 to 2 µL of the mixture onto the sample plate and air dry.
3. Add 0.5 µL of absolute ethanol to recrystallize the sample spot.

Oligosaccharide Calibration

Use sugars of known masses.

Acquiring Data

Use a higher laser energy setting than for peptides.

Glycoproteins

To resolve the glycoforms the sample must be rigorously desalted, as cations will adduct with the carbohydrate side-chains resulting in peak broadening. The ion exchange desalting procedure detailed for oligonucleotides can be applied to glycoproteins using 4-hydroxy-α-phenylcinnamic acid as the matrix.

Glycolipids

Matrix: HABA, 3.5 mg/mL in methanol.

Sample: 1 to 100 pmol/µL in methanol.

Sample Preparation

The premix method of sample preparation is suitable for glycolipids.

To prepare samples:

1. Mix 2 µL of the matrix solution with 2 µL of the sample solution.
2. Spot 1 µL of this mixture onto the sample well and air dry.

Glycolipids Calibration

Peptide mixtures are suitable for calibrating the instrument for glycolipid sample analysis.

Small Molecules / Pharmaceutical Products

Pharmaceutical products are best analyzed with a DIOS sample plate, although standard plates may also be used. See DIOS User's Guide for sample preparation / calibration guidelines.

Analysis of Synthetic Polymers

MALDI can provide useful information on synthetic polymers, such as repeat unit mass and end group mass(es). It is also possible to determine M_w and M_n values for monodisperse polymers that are in good agreement with other techniques such as GPC (gel permeation chromatography), viscometry and light scattering.

It is well documented, however, that M_w and M_n values determined by MALDI for polydisperse polymers, tend not to agree with values obtained using traditional methods. MALDI analysis tends to yield a polydispersity of approximately 1 and M_w and M_n skewed towards the low-mass end of the polymer distribution.

Several physical factors give rise to this effect, including more facile desorption / ionization of low-mass oligomers, 'dimerization' or multiple charging of long-chain polymers. The most common approach to overcoming

this fundamental limitation of MALDI is fractionation using GPC before analysis.

Matrices

Virtually all the common matrices used for MALDI have been used to analyze synthetic polymers. A general starting point is to dissolve the matrix and sample in the same solvent, using as volatile a solvent as possible. Matrices are typically employed at a concentration of 10 to 20 mg/mL.

Many synthetic polymers show good results by using methods containing the reagents 20 mg/mL dithranol in THF and either 1 mg/mL lithium, sodium, potassium or silver trifluoroacetate in THF.

To prepare samples:

1. Prepare a sample concentration of 10 mg/mL in THF.
2. Mix 10 µL of sample and 10 µL of matrix.
3. Add 1 µL of salt (i.e. Na^+, K^+ or Ag^+).
4. Spot 1µL of this mixture onto the sample plate.

   Tip: If there is no signal, vary the ratio of sample: matrix: salt. Diluting the sample may improve signal quality.

Common Solvents Suitable for Dissolving Synthetic Polymers and Matrices

Matrix	Solvents
CHCA	Acetone, MeOH, THF
Sinapinic acid	Acetone, methanol, THF
DHB	Acetonitrile, methanol, H_2O
β-indole acrylic acid	Acetone
HABA	THF
All trans-retinoic acid	THF
Dithranol	THF, $CHCl_3$, HFIP

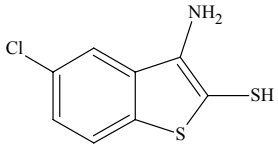
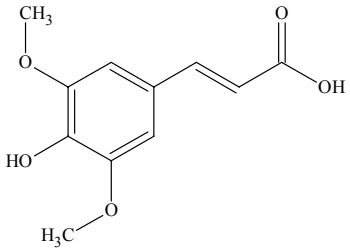
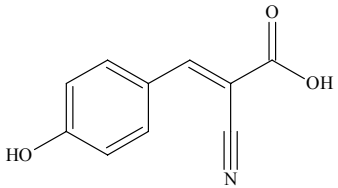
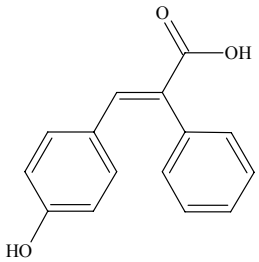
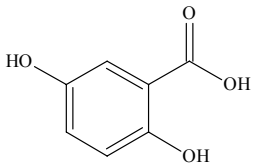
Polymer Classification and Suitable Matrices

Polymer	Suitable Matrix
Acrylates	β-indole acrylic acid, Dithranol
Unsaturated aromatic polyesters	Dithranol + silver trifluoroacetate
High molecular weight polystyrene	Retinoic acid + saturated ethnical silver nitrate
Resins	Dithranol
PEG	CHCA in acetone + sodium iodide

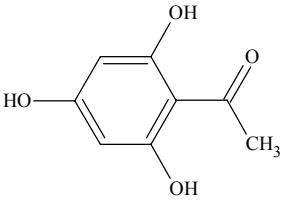
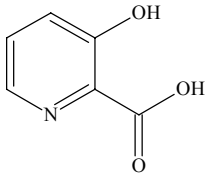
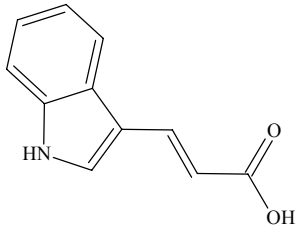
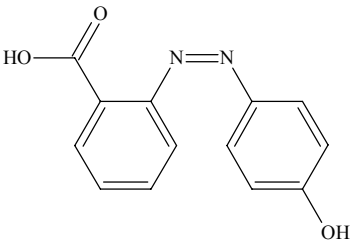
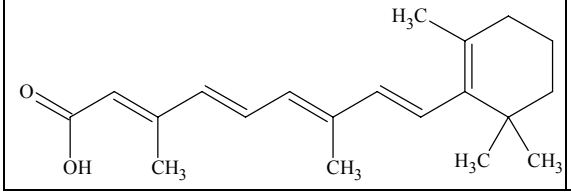
When running acrylates in *β*-indole acrylic acid the solvent of choice should be acetone. When applying the mixture of sample and matrix to the sample well, drag the pipette tip across the surface during the drying process whilst applying more sample / matrix solution, until about 2µL has been deposited.

Structures of MALDI Matrices

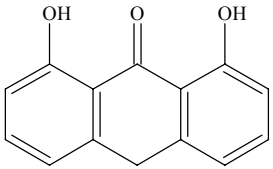
Structure and $[M+H]^+$ of Common MALDI Matrices

Structure	Name	$[M+H]^+$
	CMBT (5-chloro-2-mercapto benzothiazole)	Average: 216.7322 Mono: 215.9708
	Sinapinic acid (3,5-dimethoxy-4-hydroxycinnamic acid)	Average: 225.2212 Mono: 225.0763
	CHCA (α-cyano-4-hydroxy cinnamic acid)	Average: 190.1784 Mono: 190.0504
	4-hydroxy-α-phenyl cinnamic acid	Average: 241.2619 Mono: 241.0865
	DHB (2,5-dihydroxybenzoic acid)	Average: 155.1301 Mono: 155.0344

Structure and $[M+H]^+$ of Common MALDI Matrices

Structure	Name	$[M+H]^+$
	THAP (2',4',6'-trihydroxy benzoic acid)	Average: 169.1570 Mono: 169.0501
	HPA, Hydroxypicolinic acid (3-Hydroxy-2-pyridinec arboxylic acid)	Average: 140.1185 Mono: 140.0347
	IAA (β-indole acrylic acid)	Average: 188.2059 Mono: 188.0711
	HABA (2,-(4-hydroxyphenyl-azo)-benzoic acid)	Average: 243.2420 Mono: 243.0769
	All trans-retinoic acid	Average: 301.4431 Mono: 301.2168

Structure and $[M+H]^+$ of Common MALDI Matrices

Structure	Name	$[M+H]^+$
	Dithranol (1,8-Dihydroxy-9(10H)-anthracenone)	Average: 227.2395 Mono: 227.0708

Index

R

S

T

U

V

W