Waters Synapt Mass Spectrometry System

Quick Start Guide

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Waters

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Safety considerations

Some reagents and samples used with Waters instruments and devices can pose chemical, biological, and radiological hazards. You must know the potentially hazardous effects of all substances you work with. Always follow Good Laboratory Practice, and consult your organization's safety representative for guidance.

When you develop methods, follow the "Protocol for the Adoption of Analytical Methods in the Clinical Chemistry Laboratory," *American Journal of Medical Technology*, 44, 1, pages 30–37 (1978). This protocol addresses good operating procedures and the techniques necessary to validate system and method performance.

Considerations specific to the Synapt MS System

Solvent leakage hazard

The source exhaust system is designed to be robust and leak-tight. Waters recommends you perform a hazard analysis, assuming a maximum leak into the laboratory atmosphere of 10% LC eluate.

Warning:

- To confirm the integrity of the source exhaust system, renew the source O-rings at intervals not exceeding one year.
- To avoid chemical degradation of the source O-rings, which can withstand exposure only to certain solvents, see Appendix B in the *Waters Synapt MS Operator's Guide* to determine whether any solvents you use that are not listed are chemically compatible with the composition of the seals.

Flammable solvents hazard

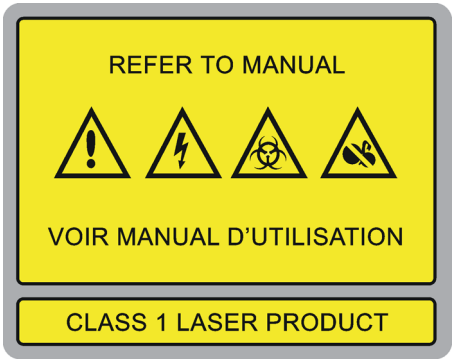
Warning: To prevent the ignition of accumulated solvent vapors inside the source, maintain a continuous flow of nitrogen through the source whenever significant amounts of flammable solvents are used during instrument operation.

Never let the nitrogen supply pressure fall below 400 kPa (4 bar, 58 psi) during analyses that require flammable solvents. Connect to the LC output with a gas-fail connector to stop the LC solvent if the nitrogen supply fails.

Laser radiation hazard

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

The MALDI Synapt™ MS system uses a solid-state laser, which produces a concentrated beam of invisible UV radiation. The instrument is a Class 1 laser product, as classified by EN60825-1: 1994, A1, A2 and indicated by a label affixed to the top of the instrument:



If you follow the operating procedures described in this manual, the laser beam will remain contained within the instrument, and no risk of personal exposure to laser radiation will ensue.

This system must be operated with all the exterior panels fitted. Removing any panels and defeating the laser safety interlocks creates the risk of personal exposure to a level of invisible radiation that exceeds Class 1.

Only Waters service personnel qualified to service the MALDI Synapt MS system may open the safety cover that surrounds the laser. When this cover is open and the interlocks are defeated, the instrument becomes a Class 3B laser hazard, indicated by a warning label affixed to the safety cover:

Output specification of enclosed laser

Item	Specification
Wavelength	355 nm
Average Power	20 mW @ 200 Hz
Repetition Rate	up to 200 Hz
Pulse Width	3 ns
Pulse Energy	100 µJ @ 200 Hz

Output specification of enclosed laser (continued)

Item	Specification
Peak Power	34 kW
Beam Divergence, Full Angle	< 2 mrad

Biological hazard

Warning: Waters instruments and software can be used to analyze or process potentially infectious human-sourced products, inactivated microorganisms, and other biological materials. To avoid infection with these agents, assume that all biological fluids are infectious, observe Good Laboratory Practices, and consult your organization's biohazard safety representative regarding their proper use and handling. Specific precautions appear in the latest edition of the US National Institutes of Health (NIH) publication, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL).

Chemical hazard

Warning: Waters instruments can be used to analyze or process potentially hazardous substances. To avoid injury with any of these materials, familiarize yourself with the materials and their hazards, observe Good Laboratory Practices (GLP), and consult your organization's safety representative regarding proper use and handling. Guidelines are provided in the latest edition of the National Research Council's publication, *Prudent Practices in the Laboratory: Handling and Disposal of Chemicals.*

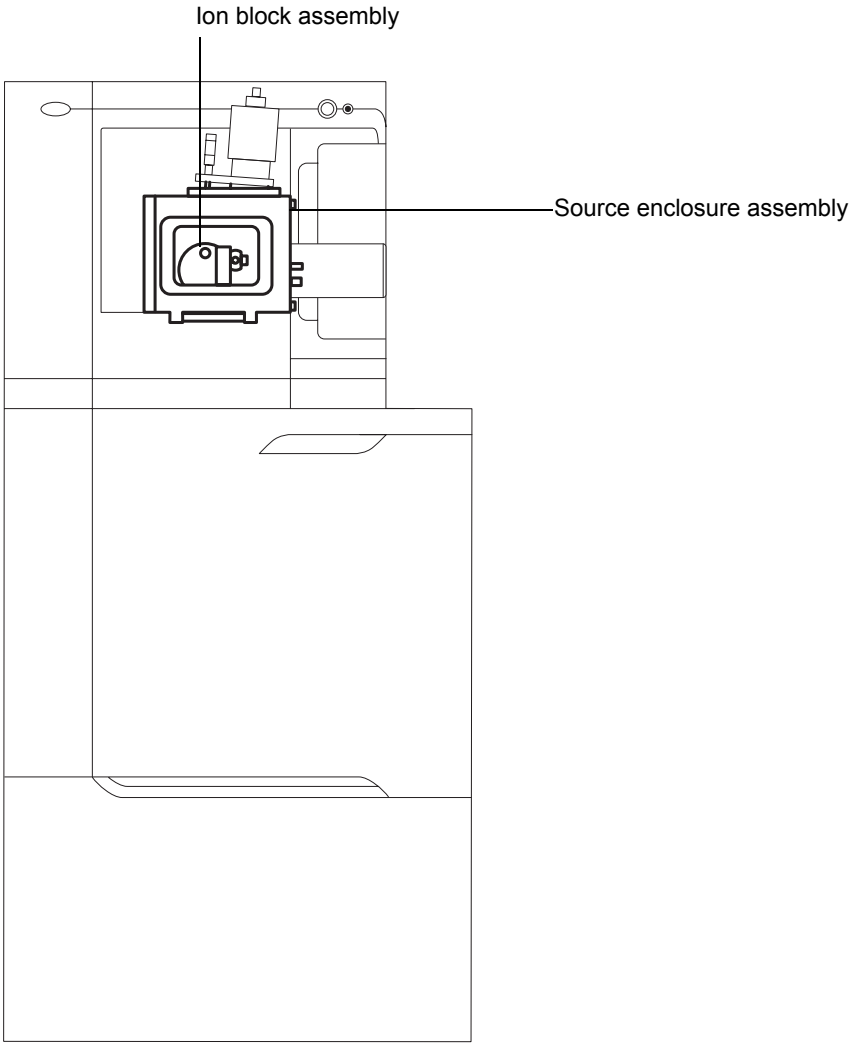
Pinch-point hazard

Warning: The MALDI Synapt MS source has moveable parts that can constitute a pinch point. When the source is moving keep away from the regions marked with yellow and gray labels.

High temperature hazard

Warning: To avoid burn injuries, avoid touching the source enclosure with your hand when operating or servicing the instrument.

Mass spectrometer high temperature hazard

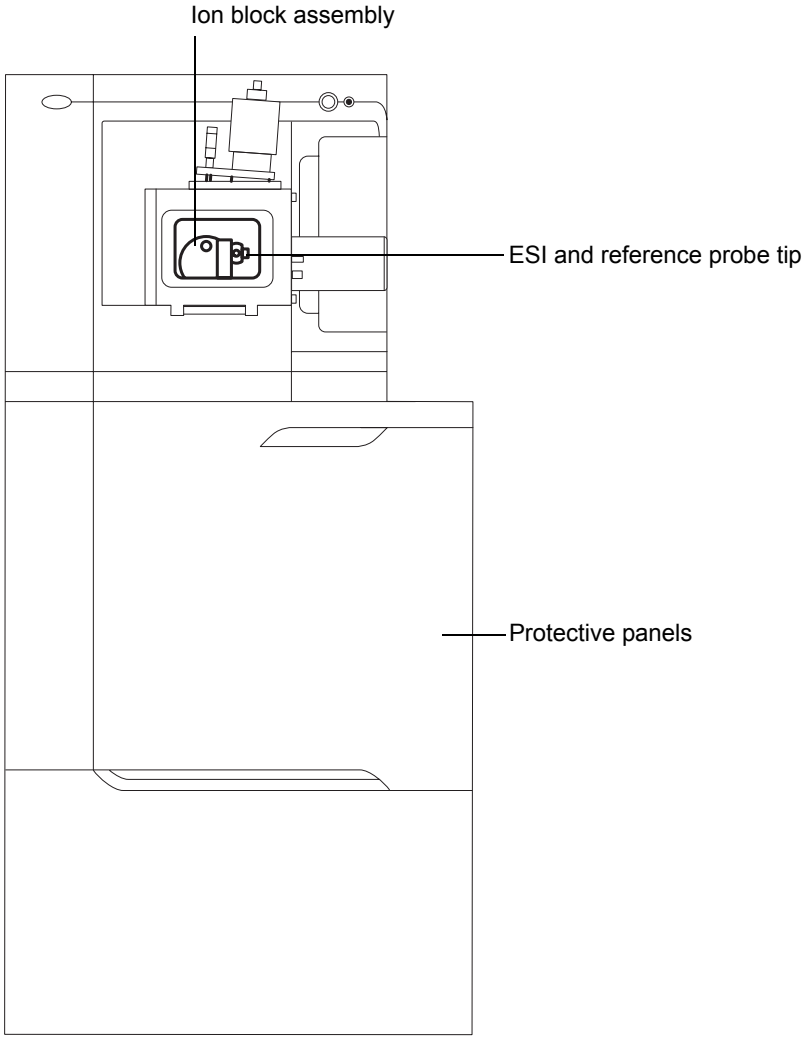


High voltage hazard

Warning:

- To avoid electric shock, do not remove the mass spectrometer's protective panels. The components they cover are not user-serviceable.
- To avoid nonlethal electric shock when the instrument is in Operate, avoid touching the areas marked with the high voltage warning symbol. To touch those areas, first put the instrument in Standby mode.

Mass spectrometer in ESI ionization mode



Safety advisories

Consult Appendix A of the *Waters Synapt MS System Operator's Guide* for a comprehensive list of warning and caution advisories.

Operating the system

When operating this system, follow standard quality control procedures and the guidelines presented in this section.

Audience and purpose

This guide is for spectrometrists with various levels of experience who want to quickly master the fundamental principles involved in operating the Synapt MS system.

Intended use

Waters designed this system to be used as a research tool to deliver authenticated exact mass measurement. It is not for use in diagnostic procedures.

Calibrating

To calibrate LC systems, follow acceptable calibration methods using at least five standards to generate a standard curve. The concentration range for standards must include the entire range of quality-control samples, typical specimens, and atypical specimens.

To calibrate the mass spectrometer, consult the calibration section in Waters Synapt MS system online Help.

Quality control

Routinely run three QC samples that represent subnormal, normal, and above-normal levels of a compound. Ensure that QC sample results fall within an acceptable range, and evaluate precision from day to day and run to run. Data collected when QC samples are out of range might not be valid. Do not report these data until you are certain that the instrument performs satisfactorily.

Table of Contents

1 Getting Started

This chapter describes how to prepare the instrument to ensure it is ready for operation.

Contents

Preparing the instrument for operation

Before you can use the instrument, you must prepare it for operation.

Using the rear-panel switches, power-on the vacuum pumps and electronics. Wait 3 minutes and then perform these actions in the order listed:

- Start MassLynx™ software, and open the Tune window.
- Pump down (evacuate) the instrument.
- Put the instrument in Operate mode.
- Condition the detector.

Starting MassLynx software and opening the Tune window

Before you can proceed, you must start the software and open the Tune window.

To start MassLynx software and open the Tune window

1. Double-click the MassLynx 4.1 icon on the desktop.

   Alternative: Click Start > All Programs (or Programs) > MassLynx > MassLynx 4.1.

2. In the MassLynx window, on the Instrument tab, click 

.

Pumping down the instrument

To pump down (evacuate) the instrument

In the Tune window, click Vacuum > Pump.

Result: When the instrument is pumped down, the Vacuum LED turns green.

Tip: To check vacuum pressures, in the Tune window, click Vacuum > Gauges.

See also: *Waters Synapt MS System Operator's Guide*.

Putting the instrument in Operate mode

Requirement: You must pump down (evacuate) the instrument so that the vacuum LED turns green (TOF vacuum < 3.9×10^{-6} mbar). Also, the API gas must be connected and set to deliver 690 kPA (6.9 bar, 100 psi) before you can put the instrument in Operate mode.

To put the instrument in Operate mode

From the MassLynx Tune window, click  .

Conditioning the detector

To condition the detector you must specify values in the MassLynx Detector Conditioning dialog box.

Caution: Do not start to condition the detector until the TOF vacuum is <1.2×10^{-6} mbar for at least 1 hour.

To condition the detector

1. From the MassLynx Tune window, ensure that the instrument is in Operate mode and that the detector is set to 0.
2. From the Tune window, click Setup > Detector Conditioning.
3. In the Detector Conditioning dialog box, enter these values:

Parameter	Value
Start	150
Stop	1800
Duration (mins)	60
Step (mins)	1
Delay (mins)	0

4. Click Start.

Leaving the instrument ready for operation

Leave the Synapt™ MS System in Operate mode except in the following cases:

- When performing routine maintenance
- When changing the source
- When leaving the instrument unused for a long period

In these instances, put the instrument in Standby mode.

To leave the instrument in Operate mode

1. From the Tune window's Source tab, turn off the syringe pump (ESI mode) or laser (MALDI mode).
2. From the Tune window's tool bar, switch off the API gas.

To put the instrument in Standby mode

1. From the Tune window's Source tab, turn off the syringe pump (ESI mode) or stop the laser (MALDI mode).
2. From the Tune window's tool bar, ensure you switched off the API, trap and IMS gases.
3. Click Standby  .

2 Acquiring Data

This chapter explains how to set up the instrument and acquire data via the Tune window.

Tip: You can also acquire data by using the MassLynx sample list. To learn how to do so, and for more complex experimental methods, batch acquisition, and multiple function support, see the MassLynx online Help.

Contents

Acquiring data, quick start

Requirement: To acquire data, the instrument must be in Operate mode. See "Putting the instrument in Operate mode" on page 1-3. If the instrument has been in Standby mode for more than 2 hours, allow 30 minutes in Operate mode for stabilization so that it can produce the most repeatable mass measurements.

The MassLynx Tune window's peak display runs continuously, providing a display of the instrument's live output (see "Using the Tune window's peak display" on page 2-6). However, these data are saved only when you start an acquisition.

You can acquire data using the Synapt MS System in one of two ways:

- From the Tune window – This process creates one data file. Only basic MS or MS/MS acquisitions are available. This type of acquisition is described below.
- From the MassLynx sample list – This process creates multiple data files in batch format. As well the basic MS and MS/MS acquisitions, you can perform data-directed, multiple function and mixed mode acquisitions. See the MassLynx online Help for details.

To acquire data, complete the following procedures:

- Select a source.
- Select an acquisition mode.
- Introduce or load a sample.
- Check the instrument parameters.
- Use the Tune window peak display to verify signal intensity.
- Start the acquisition:
  - Complete the acquisition dialog box.
  - Start the acquisition.
- Stop the acquisition.
- View the data.

Selecting the source

You use the instrument source simply to ionize your sample. Beyond the source configuration and operation, the principles of instrument use and data acquisition are the same for all sources.

You can acquire data using the following source types:

- ESI
- NanoFlow™
- ESCi®
- IonSABRE™
- APPI
- Dual APPI
- MALDI

You must choose the correct type of source to match your samples, fit the source onto the instrument, and then select the source from the Tune window. Sources are identified to the instrument by the heater/interlock connection on the front panel. The default source option is ESI. If no source is fitted, the instrument assumes you chose the ESI option.

To select the source

1. Install the source option appropriate for your application. See the *Waters Synapt MS System Operator's Guide*.
2. From the Tune window, click Source, and then select the appropriate source type.

   Result: The Source tab title reflects the source type you selected.

Selecting the polarity and optic configuration

The instrument can operate in different acquisition modes, which apply to all sources.

Positive or negative polarity

A sample produces positively or negatively charged ions in the instrument source. The instrument's polarity setting defines the type of ions that reach the detector.

Set the instrument polarity to match your sample; positive ion is the default polarity.

Note: The instrument response to the polarity is entirely sample-dependant. Selecting an inappropriate polarity can cause weak signals.

Optic configuration

You can chose one of two optic configurations—V-Optics™ or W-Optics™:

- V-Optics produces high resolution and high sensitivity data. It offers the largest mass range and is available in all modes and for all acquisition types. V-Optics is the default configuration.
- W-Optics produces higher resolution data—about one-and-a-half times greater than V-Optics—but with lower sensitivity than V-Optics. Typically, it is used for lower mass ranges when maximum accuracy is required and sample concentration is not a restriction. It offers a lower mass range and is available in all modes but is not compatible with every acquisition type.

To select the acquisition mode

From the Tune window, click Acq Mode, and specify the following settings:

- Positive ion or Negative ion
- V Optics or W Optics

Introducing or loading the sample

For ESI and other spray sources, you use a syringe or ACQUITY UPLC® system to introduce your sample. The simplest method of sample introduction is direct infusion via a syringe pump. For MALDI, load your sample onto a prepared sample plate.

To introduce a sample for ESI and other spray sources

1. Load a syringe with sample (or refer to the ACQUITY UPLC system's instructions).
2. Connect the syringe to the source. See the *Waters Synapt MS System Operator's Guide*.

3. From the Source tab, click Syringe  to start the flow.

   Note: Control of UPLC® systems is separate from the instrument control and specific to each model. Optimum source conditions vary with the sample introduction technique.

To load a sample for MALDI

1. Load a prepared sample plate into the plate carrier. See the *Waters MALDI Synapt MS System Operator's Guide*.

2. From the Tune window Source tab, click load  .

   Result: The system evacuates the plate carrier airlock, and the sample plate is transported into the source, a process that takes approximately 3 minutes.

3. From the Tune window Source tab, select the sample well containing your sample.

Checking the instrument parameters

You can save the configuration and settings of the instrument and subsequently apply them. The values you associate with the various parameters are stored in instrument parameter files (*.ipr) as part of a particular MassLynx project, which in turn groups all your data together. The title bar of the Tune window displays the name of a currently loaded .ipr file.

A single .ipr file contains parameter specifications for all available instrument modes and is not specific to any single acquisition mode. Instead, each file includes settings for all modes.

Note: When you specify parameter settings—for example, V-Optics, positive ion—the file configuration nevertheless includes settings for the other modes: W-Optics, negative ion. If you fail to specify values for these modes, the system default values or the values last used are adopted.

You need not load a different file when you change modes. However, you must use different .ipr files for different source conditions.

Tip: If you make changes to the instrument settings and do not select save, you can revert to the previously saved file.

The system is installed with the an example .ipr file, Example_Synapt.ipr, located in the default project folder (see "MassLynx Projects" on page 2-11). The file contains typical instrument settings for all available modes. Use this file as the starting point for creating your own .ipr files.

To load the instrument parameters

From the Tune window, click File > Open, and select an .ipr file.

Using the Tune window's peak display

The Tune window's peak display provides a live plot of detector output showing intensity against mass (see the figure "Peak display for leucine enkephalin" on page 2-7). When the instrument is in Operate mode, the peak display is continually refreshed at an update rate that you specify—the scan time.

By default, intensity on the vertical axis is automatically scaled so that the largest peak is full-scale and the horizontal axis shows the full mass range of the current acquisition.

Tip: When you use the MALDI source, signal is produced only when the laser is firing. If the laser is not firing, the peak display does not show any data.

To view a sample in the Tune window peak display

1. From the Tune window, click Gas > API On and Trap On.
2. From the Tune window, click Setup > Tuning Settings.
3. In the Tuning Setup dialog box, enter the values in the table below (or specify the mass range appropriate for your sample), and then click Update.

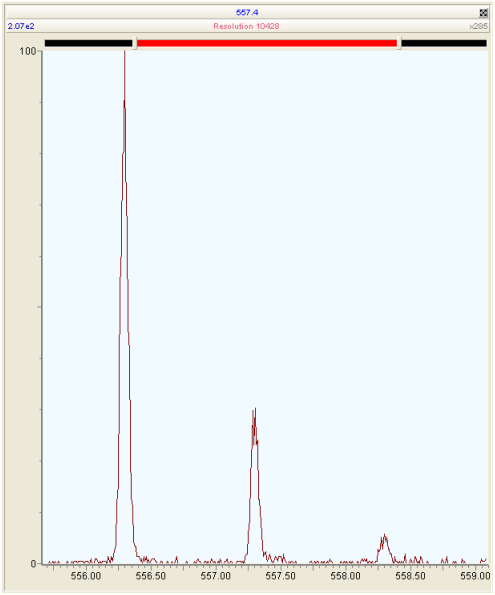
Tuning Setup dialog box

Parameter	Value
Data Format	Continuum
Scan Time (s)	1
Low Mass	200
High Mass	1000

4. If you are using a MALDI source,
   a. click Acquire > Fire Laser.
   b. from the MALDI control window, use the plate control function to select a sample well.
   c. from the MALDI control window, click Laser  to toggle the laser on.
5. To zoom the displayed mass range, click and drag in the Tune window's peak display.

   Tip: To undo zoom, right-click and select Undo.

Peak display for leucine enkephalin



Starting a Tune window acquisition

The MassLynx Tune window's peak display runs continuously, displaying the system's live output. To save these data so that you can view and process them later, you must start an acquisition.

This section describes how to start an acquisition from the Tune window. To start an acquisition from MassLynx sample list, see MassLynx online Help.

Before you start an acquisition, from the Start Acquisition Dialog box, you must specify the settings that accord with your sample and the acquisition type you choose. You start the acquisition from the Start Acquisition dialog box.

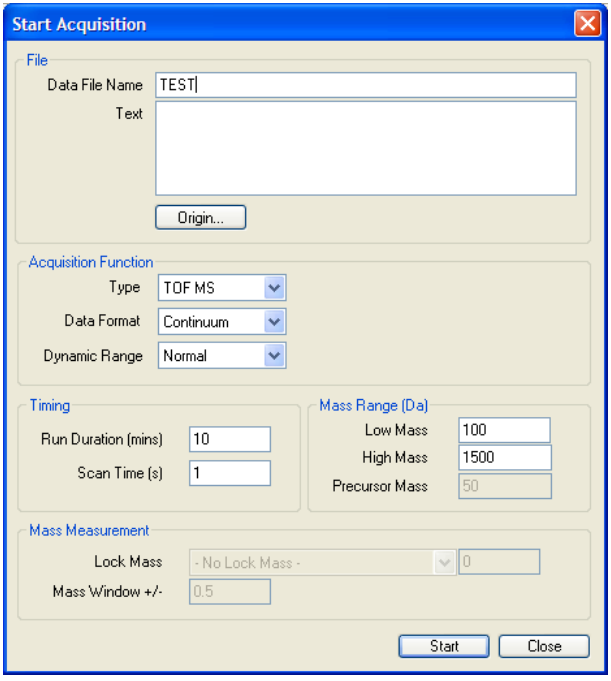
When you use a MALDI source, a MALDI control window opens simultaneously with starting an acquisition. The window permits you additional control specific to the MALDI source, and you access it to switch the laser on and off, and to start and stop the movement of the sample plate. The laser's firing consumes the sample, so you must move additional sample under the path of the laser. You can move the sample plate directly, using the Cross Hair option, or move it according to a defined pattern. See the *Waters MALDI Synapt MS System Operator's Guide*.

Note: For Mass Measurement Options see the Synapt MS System online Help.

To complete the Start Acquisition dialog box

1. From the MassLynx Tune Window, click Start  to open the Start Acquisition dialog box.

Start Acquisition dialog box



2. Enter a name for the file in the Data File Name box.
3. You can enter optional text in the Text box.

   Tip: This text appears in MassLynx file selection dialog boxes and when displaying data.
4. Select the type, data format, and dynamic range shown in the figure, above.
5. Enter the run duration (total acquisition time) and scan time (time for a single spectra) shown in the figure, above.
6. To determine the mass range, enter the low and high mass values, as shown in the figure, above, or that are appropriate to your sample.

   Tip: The values you select determine the size of the data file.
7. If you are using a MALDI source, select the sample plate control required. See the Synapt MS System online Help.

To start the acquisition using an ESI or other spray source

From the Start Acquisition Dialog box, click Start.

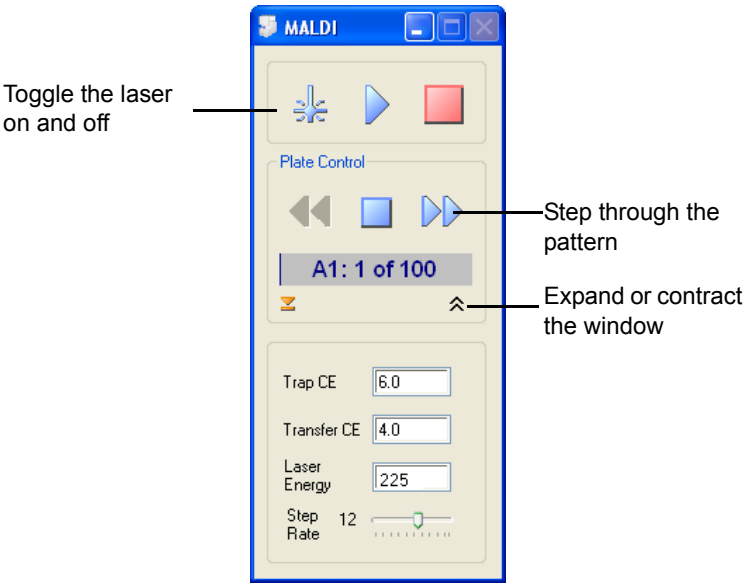
Result: The instrument begins to acquire data and saves the file in the Data folder of the current project.

To start the acquisition using a MALDI source

1. From the Start Acquisition Dialog box, click Start.

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

2. From the MALDI Control window, expand the window, and enter a laser energy of 225, as shown below.



3. From the MALDI control window, click Laser  to toggle the laser on.

   Result: The instrument begins to acquire data and saves the file in a folder called Data.

4. If you selected Cross Hair control in the Sample Acquisition dialog box, use the crosshairs in the MALDI Control window to move the sample under the path of the laser.

5. Adjust the laser energy to optimize peak intensity.

   Tip: Higher energy produces more noise in the data and reduces the time for which the sample is available. Lower energy results in lower peak intensity.

Stopping the acquisition

The acquisition continues until the time specified in Run Duration is reached, at which point the acquisition stops.

To stop the acquisition

From the MassLynx Tune window click  .

Tip: For MALDI source, you can stop the acquisition directly from the MALDI control window by pressing the Stop button. When the acquisition ends, the laser switches off, and the sample well position resets to the center of the current sample well.

Viewing acquired data

To view acquired data in MassLynx

In the MassLynx window, click File > Open Data File, and browse to the file you saved during the acquisition.

Result: The data you acquired appear in the Chromatogram and Spectra windows.

See also: *MassLynx 4.1 Getting Started Guide*.

Extended use, quick start

The preceding section outlined data acquisition from the MassLynx Tune window. The following features are available for more advanced data acquisition. These options, and their use, are described in the MassLynx online Help.

MassLynx Projects

All MassLynx data storage is organized into projects. When a MassLynx project is created, MassLynx creates a new directory called "project.pro", which includes a series of sub-directories.

MassLynx is supplied with several predefined projects that include example data. All data are stored in the default project (default.pro) until you select a new project. The default.pro project contains the following example data files:

File	Mode[1]	Description
ESI_TOF_EXAMPLE_01	MS, TOF	Sodium formate, ESI example calibration.
ESI_TOF_EXAMPLE_02	MS/MS, TOF	GFP[2], basic acquisition in MS/MS mode.
MALDI_SYNAPT_EXAMPLE_01	MS, TOF	PEG[3], MALDI example calibration
MALDI_SYNAPT_EXAMPLE_02	MS/MS, TOF	GFP, basic acquisition in MS/MS mode.

1. All the example files use positive ion, V-Optics.
2. Glu-fibrinopeptide B
3. Polyethylene glycol

The default.pro project also includes an example instrument parameter file (see "Checking the instrument parameters" on page 2-5).

Calibration

Use LockSpray™ or MALDI Lockmass as the routine mechanism for ensuring accurate mass measurement. However, you must nevertheless ascertain the effectiveness of the mass calibration regularly using an appropriate quality-control standard. To determine whether the instrument requires a new mass calibration, you can use the MassLynx Mass Difference Calculator to compare peak information obtained experimentally with standard reference peaks.

A Tuning Parameters

This appendix lists all the tuning parameters and their suggested starting values. You can adjust these values for maximum sensitivity and resolution.

Contents

Tuning parameters

Source tab tuning parameters

Parameters common to all sources and modes

Parameter	Value
Sampling cone	40
Extraction cone	4
Source temperature	80
Cone (Gas)	0
Desolvation (Gas)	500
Syringe pump	5

Parameters specific to ESI, NanoFlow, and ESCi

Parameter	ESI	NanoFlow	ESCi
Capillary, Positive ion mode	3	3	3
Capillary, Negative ion mode	2.5	2.5	2.5
Corona, Positive ion mode	N/A	N/A	5 µA
Corona, Negative ion mode	N/A	N/A	5 µA
Desolvation temperature	150	N/A	150

Parameters specific to IonSABRE, APPI, and Dual APPI

Parameter	IonSABRE	APPI	Dual APPI
Repeller, Positive ion mode	N/A	3	3
Repeller, Negative ion mode	N/A	2.5	2.5
Corona, Positive ion mode	5 µA	N/A	5 µA
Corona, Negative ion mode	5 µA	N/A	5 µA
Desolvation temperature	N/A	150	150
IonSABRE Probe	200	N/A	N/A

Parameters specific to MALDI

Parameter	Value
Sample plate	0
Extraction	10
Hexapole	10
Aperture 0	0
Cooling gas	10

Instrument tab tuning parameters

Parameters common to all sources and modes

Parameter	Value
Trap CE	6
Transfer CE	4
Source (Gas)	0
Mass Range	Auto
Trap (Gas)	1.5
IMS (Gas)	0

Parameters specific to source

Parameter	ESI and other spray sources	MALDI
Detector	1600	1700 (ESI + 100)

Note: The optimum Detector value for your instrument is determined by a System Gain Test as part of the scheduled maintenance.

Quadrupole parameters

Parameter	4k quad	8k quad	32k quad
LM Resolution	4.7	4.9	12

TriWave DC tab tuning parameters

Parameter	Value
Trap DC	
Entrance	2
Bias	4
Exit	5
IMS DC	
Entrance	-20
Exit	20
Transfer DC	
Entrance	5
Exit	15

Triwave tab tuning parameters

Parameter	Value
Source	
Wave Velocity	300
Wave Height	0.2
Trap	
Wave Velocity	300
Wave Height	0.5
IMS	
Wave Velocity	300
Wave Height	0.5
Transfer	
Wave Velocity	247
Wave Height	0.2

Note: Parameter values in the Triwave tab are set automatically.

Trapping tab tuning parameters

Parameter	Value
EDC trapping	
EDC mass	556

Index