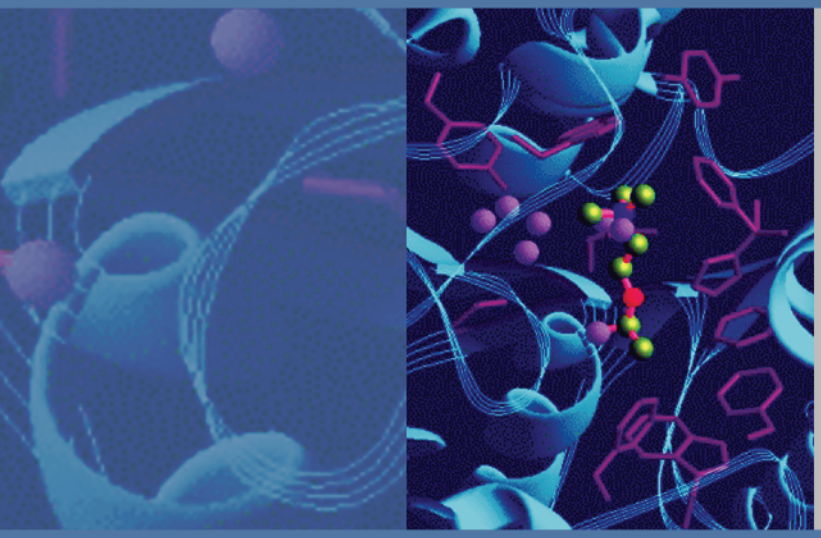

MALDI Source

Getting Started Guide

97155-97012 Revision A February 2008

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EN 61000-3-3: 1995, A1: 2001	EN 61000-4-5: 2001
EN 61326-1: 1998, A2: 2001, A3: 2003	EN 61000-4-6: 2003
EN 61000-4-2: 2001	EN 61000-4-11: 2001
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Contents

Preface

This guide describes how to calibrate and tune your MALDI LTQ XL™ system, and how to acquire matrix-assisted laser desorption/ionization (MALDI) data. It also provides a brief introduction to processing your MALDI MS/MS data using the BioWorks™ data analysis program.

Related Documentation

In addition to this guide, Thermo Fisher Scientific provides the following documents for the MALDI source:

- *MALDI Source Hardware Manual*
- *LTQ Series Preinstallation Requirements Guide*
- *LTQ Series Getting Connected Guide*

The software also provides Help.

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CAUTION Highlights hazards to humans, property, or the environment. Each CAUTION notice is accompanied by an appropriate CAUTION symbol.

IMPORTANT Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or contains information that is critical for optimal performance of the system.

Note Highlights information of general interest.

Tip Highlights helpful information that can make a task easier.

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Phone	800-685-9535
Fax	561-688-8736
E-mail	TechSupport.C+MS@thermofisher.com
Knowledge base	www.thermokb.com

Find software updates and utilities to download at www.mssupport.thermo.com.

❖ To contact Customer Service for ordering information

Phone	800-532-4752
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Introduction

The Thermo Fisher Scientific MALDI source produces ions from mixed matrix/analyte microcrystals under low-pressure conditions by irradiating these crystals with pulses of light from a nitrogen laser. When you use this ion source with the LTQ XL mass spectrometer, you can perform sensitive analyses of intact molecules, using only small amounts of sample material.

Contents

What is Matrix-Assisted Laser Desorption/Ionization?

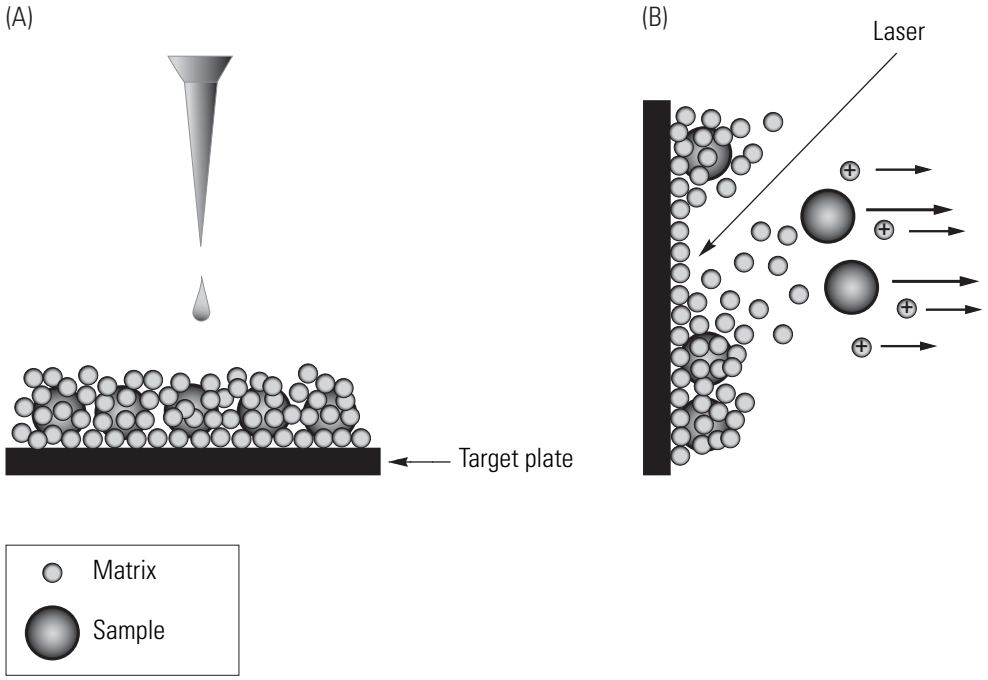
Matrix-assisted laser desorption/ionization (MALDI) is an effective mass spectrometry tool for a wide variety of large, labile biomolecules. By using the MALDI tool, you can transfer molecules directly from the solid phase to the gas phase as intact ions.

As Figure 1 shows, the MALDI method of introducing analyte ions into the mass spectrometer includes these basic steps:

1. You mix the analyte with a suitable matrix, dry this mixture on a target plate, and insert the target plate into the sample module of the MALDI LTQ XL system.
2. The XYZ mechanism of the sample module moves the target plate into a vacuum chamber where the remaining solvent evaporates.
3. The MALDI nitrogen laser fires pulses of UV light. The MALDI X-Y stage moves the selected spot on the target plate in line with the laser beam.
4. The energy from the UV light desorbs the analyte and matrix material from the target plate.

5. The MALDI source grounds the target plate and the LTQ XL mass spectrometer places an electrostatic charge on the aperture of the ion transfer optics. In the positive ion polarity mode, the LTQ XL mass spectrometer places a negative charge on the aperture. In the negative ion polarity mode, the LTQ XL mass spectrometer places a positive charge on the aperture.

Figure 1. MALDI sample preparation (panel A) and ionization (panel B)



Because the sample quality affects the production and quality of the ions introduced into the mass spectrometer, the most important step in the MALDI process is sample preparation. Variables that influence the production and quality of ions with the MALDI source include the concentration of the matrix and analyte, choice of matrix, analyte history (for example, exposure to strong ionic detergents or formic acid), and contaminants. For more information on preparing samples for MALDI analysis, see Appendix B, "Sample Preparation."

The preferred method for sample preparation is the dried-droplet method. Using this method, you mix a very dilute solution of the analyte (picomoles/microliter to femtomoles/microliter) with a solution of the matrix compound. The matrix compound is typically a small organic acid that absorbs energy at the wavelength of the laser. To form the sample, the analyst deposits approximately 0.5 to 1 µL of the mixture on a target plate. When the mixture dries, a solid deposit of analyte-doped matrix crystals remains. The large molar excess of matrix compound ensures that the analyte molecules are distributed throughout the matrix crystals.

During the desorption and ionization step, short pulses of laser light strike the target. Because the matrix is in excess and has a high molar absorptivity coefficient at the 337 nm wavelength produced by the laser, it absorbs essentially all of the incident radiation, minimizing analyte damage and fragmentation. While the actual mechanism of ionization is unknown, the predominant theory is that the laser light causes intense heating in the matrix. This heating

leads to vibrational excitation of the matrix and localized deterioration of the solid solution. The matrix molecules vaporize and form a rapidly expanding plume of ejected material. This matrix plume carries some analyte molecules into the vacuum. The ion-molecule reactions in the gas phase or simple desorption of preformed ions from solution then ionize the analyte molecules.

After the analyte molecules are vaporized and ionized, they are transferred electrostatically to the mass spectrometer to be analyzed.

Why Use the LTQ XL Mass Spectrometer?

The LTQ XL mass spectrometer provides multiple stages of mass analysis for positive compound identification. The LTQ XL also provides features that improve mass resolution and enhanced structural information.

The various stages of mass analysis provided by the LTQ XL are as follows:

- Mass analysis (molecular mass information): Single-stage mass analysis provides molecular mass information for the identification of analytes of interest.
- MS/MS (structural information): Two-stage mass analysis provides even more positive compound identification. MS/MS analysis monitors how precursor ions fragment when exposed to an additional stage of ionization.

  There are two types of MS/MS analysis:

  – Full-scan MS/MS: monitors the production of all product ions from a specific precursor ion.

  – SRM MS/MS: monitors a specific reaction path involving the production of a specific product ion from a specific precursor ion.

  Using MS/MS analysis, you can easily quantitate target analytes in complex matrices such as plant or animal tissue, plasma, urine, groundwater, or soil. Because of the specificity of MS/MS measurements and the ability to eliminate interferences by an initial mass selection stage, you can easily accomplish quantitative target compound analysis using the LTQ XL mass spectrometer. Due to the complex nature of matrix assisted laser desorption, you typically use isotopic labels for quantitation. A discussion on using isotope labels to improve quantitation is beyond the scope of this manual.
- MS^n (structural information): Multi-stage mass analysis provides a unique capability to obtain structural information that can be useful in structure elucidation of peptides, carbohydrates, lipids, metabolites, and natural products. MS^n techniques on the LTQ mass spectrometer allow for stepwise fragmentation pathways, making interpretation of MS^n spectra relatively straightforward. The LTQ mass spectrometer has several advanced features that make its MS^n capabilities extremely powerful for qualitative analysis. (See "What Types of Experiments Can I Perform?" on page 6.)

The following features provide higher mass resolution information:

- ZoomScan™ analysis (higher mass resolution information): You collect ZoomScan data by using slower scans at higher resolution. In the electrospray ionization mode, you can use ZoomScan to determine the charge state, which in turn allows for the correct determination of molecular mass. Since MALDI produces almost exclusively singly-charged ions, ZoomScan with MALDI is primarily used to provide greater mass resolution.
- UltraZoomScan analysis (the highest mass resolution information) produces the highest resolution data of the three scan rates (Normal, Zoom, and UltraZoom).

In addition to providing multi-stage mass analysis and high resolution scans, the LTQ XL can perform Wideband Activation™, a technique that applies collision energy to ions during MS/MS fragmentation over a fixed mass range of 20 u. This technique enables the LTQ XL mass spectrometer to apply collision energy to both the precursor ion and the product ions created as a result of non-specific losses of water (18 u) or ammonia (17 u), for example, or formed from the loss of fragments less than 20 u.

Use the Wideband Activation technique for qualitative MS/MS when you want enhanced structural information in MS/MS. Because the collision energy is applied to a broad mass range, this option reduces signal sensitivity. To compensate for the reduction in sensitivity, you can increase the value of the collision energy (Activation Amplitude).

With the MALDI source, you can set up high throughput, automated analyses of target plates spotted with 96 or 384 samples. You can also set up tissue imaging experiments on tissues fixed to glass or stainless steel slides. Because samples are not completely consumed when acquiring data with the MALDI source, they are available for future experiments.

What Are the Data Acquisition Features of the MALDI Source?

To automate data acquisition and improve the quality of your data, the MALDI source offers these features:

- The Crystal Positioning System (CPS)
- The Automatic Spectrum Filter (ASF)
- The Automatic Gain Control (AGC)

The Crystal Positioning System (CPS)

The Crystal Positioning System (CPS) automatically selects crystals of the sample/matrix for targeting by the laser during data acquisition. When you enable the CPS, the MALDI program processes the camera image of the sample well and identifies the location of the crystals in the sample/matrix mixture. The program then calculates a path for the sample plate motion that keeps the laser targeted at the crystals and skips over those areas of the well that

contain little sample deposit. The CPS eliminates the need to manually select the location within the sample well where the laser fires, and keeps signal levels consistent even when the sample is not deposited evenly in the well.

In addition to CPS, you can select from a number of predefined plate motion patterns. These raster options are spiral outwards, spiral inwards, and zig zag. When using Tune Plus, you can also manually select where to fire the laser.

The Automatic Spectrum Filter (ASF)

The Automatic Spectrum Filter (ASF) feature sets the MALDI program to discard spectra that do not have a peak height, total area, or signal-to-noise ratio above a specified threshold over a specified mass range. When the ASF discards a spectrum, the MALDI source moves the sample plate so that the laser targets the next MALDI crystal in the path. ASF ensures the acquisition of optimal-signal spectra and speeds data collection by going to fresh crystals when the MALDI signal is not optimal. Spectrum filtering, in conjunction with crystal positioning, allows for intelligent data acquisition. The ASF feature is not available in Ion Mapping™ experiments.

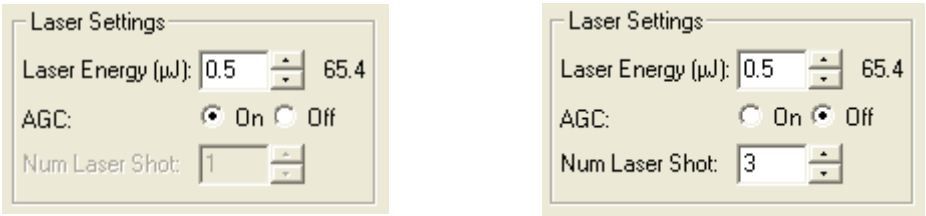
The Automatic Gain Control (AGC)

The Automatic Gain Control (AGC) feature helps you to maintain the quality of the MALDI spectra. When you enable AGC, the MALDI data system adjusts the number of laser shots to produce the same number of ions for each scan. If the number of ions produced per laser shot is low, the laser fires more shots. If the number of ions produced per laser shot is high, the laser fires fewer shots.

AGC in MALDI excludes masses below 500 u when it calculates the ion intensity. When AGC is On, MALDI spectra are normalized using the number of laser shots and a normalization factor. For this reason, you cannot deduce the actual ion counts directly from the parameters in the spectrum. To compare the ion counts between spectra collected with AGC On or Off requires a series of calculations beyond the scope of this manual.

You have the option of enabling AGC when you operate the MALDI source from the Tune Plus program. When collecting data automatically using an acquisition sequence in Xcalibur, the experiment uses the AGC mode (either On or Off) from the tune file specified in the instrument method. Figure 2 shows the AGC options on the Control page of the MALDI Source dialog box, accessed from Tune Plus.

Figure 2. AGC controls on the Control page of the MALDI Source dialog box in Tune Plus



What Types of Experiments Can I Perform?

You can perform the following types of experiments with the MALDI LTQ XL system:

- General MS or MS^n Experiments
- Data Dependent Experiments
- Ion Mapping Experiments

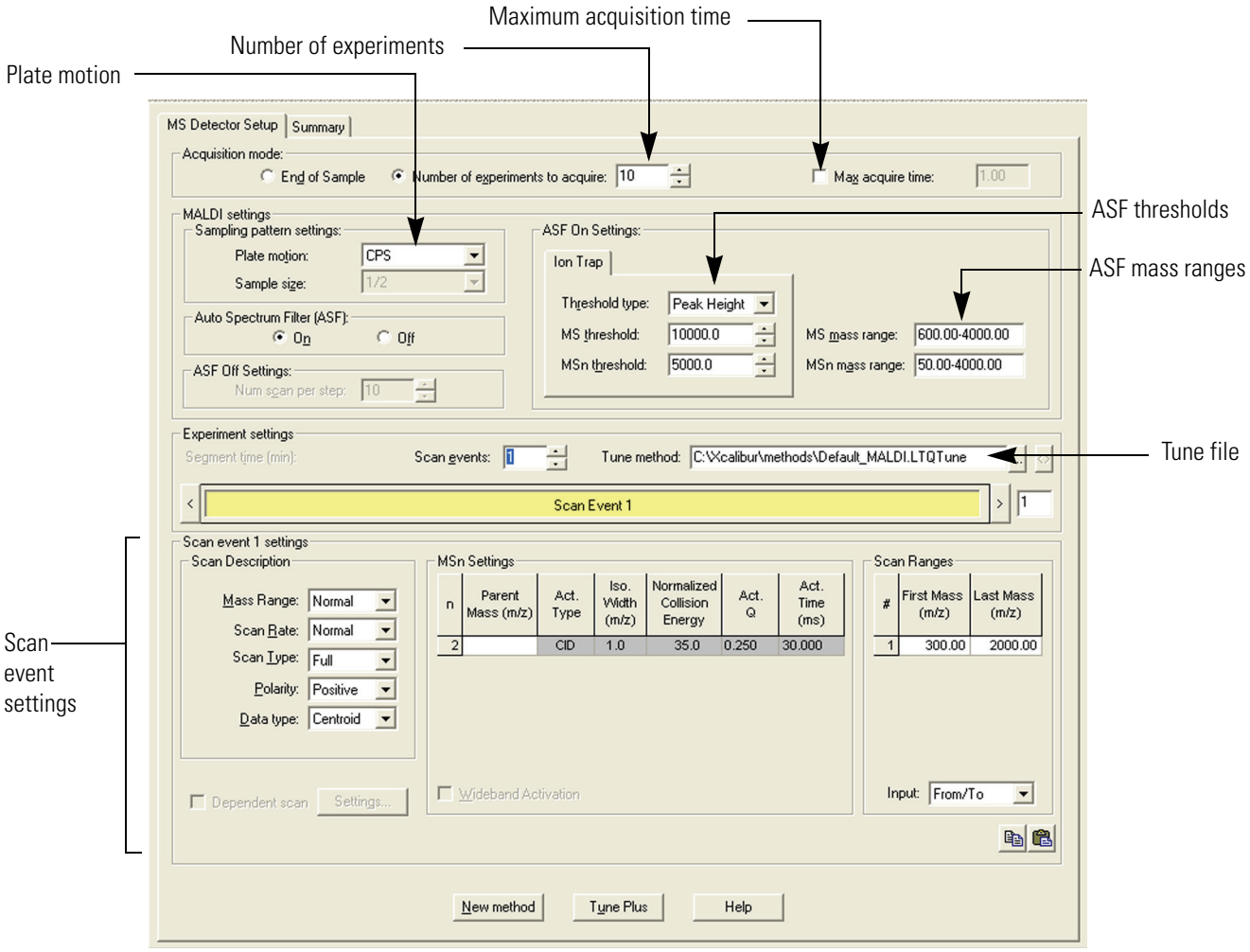
General MS or MS^n Experiments

General MS or MSn

A General MS or MS^n experiment acquires one type of spectrum: either a full MS or an MS^n acquisition. The Xcalibur data system includes an instrument method template for a General MS or MS^n experiment in Instrument Setup. Use a general MS experiment to acquire full scan MS data from a whole MALDI plate (96 or 384 sample wells).

Figure 3 shows an example of an MS experiment with one scan event.

Figure 3. General MS or MSn template with the default settings



You can set the following parameters for an MS experiment:

- Number of experiments to acquire (how many times a scan event is repeated)
- Maximum acquisition time per sample spot (either the number of experiments or a time limit, whichever is achieved first)
- Plate motion (CPS, Spiral Outwards, Spiral Inwards, or Zig Zag)
- Thresholds for ASF settings and their mass ranges
- Tune file (laser energy, AGC option, number of microscans, and number of sweep shots)
- Mass range for the analyte of interest (low, normal, or high)
- Scan rate (normal, Zoom, or UltraZoom)

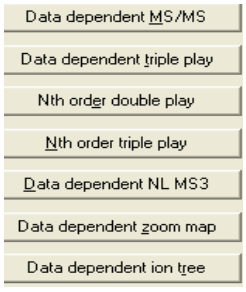
  Typical scan rates with MALDI are normal and Zoom. Use UltraZoom when scanning the normal mass range of up to *m/z* 2000. (The Turbo scan rate is used with an LC run in electrospray mode to increase the number of scans across a chromatographic peak. With ESI, the Enhanced scan rate provides charge state identification up to charge state 3.)
- Scan type (full or selected ion monitoring, SIM)
- Profile or centroid, positive or negative ion mode
- Scan range values, as defined by the first mass and last mass in the scan range or the center mass and width

In Xcalibur, click **Help** to learn more about each of these parameters.

For an MS^n experiment, you also specify the mass-to-charge ratios of all the precursor ions of interest, the isolation width, and the collision energy in the MS^n settings. The LTQ XL mass spectrometer collects data on the ions in the range or on the product ions of the precursor ion(s) that you specify.

The LTQ XL mass spectrometer can generate analyte-specific spectra that you can reproduce from laboratory to laboratory. Consequently, you can use reference spectra generated with the LTQ XL mass spectrometer to confirm structures of compounds generated with other LTQ XL systems.

Data Dependent Experiments



Because they can provide structure elucidation, Data Dependent experiments are useful for the qualitative analysis of unknown compounds. A Data Dependent experiment can produce a composite spectrum of MS^2, MS^3, and MS^4 data and the program can store the MS^n fingerprint data in a custom MS^n library spectrum. The data is valuable for use in process control, quality assurance, or research.

You can approach the setup of Data Dependent experiments in two ways:

- If you know the precursor ion, or if you expect a certain kind of precursor ion, you can set up a list of possible precursor ions so that when the mass spectrometer detects one of the specified precursor ions, it acquires product spectra. You can also set up a list of ions that you do not want the mass spectrometer to fragment.
- If you have little information about your compound, you can set up the parameters of a Data Dependent experiment so that the LTQ XL mass spectrometer generates product spectra when the intensity of the ion signal is above a specified threshold. Parameters that you can specify include threshold values for the intensity of the MS or MS^n ion signal. The threshold values you choose should successfully isolate the precursor ions of interest. These threshold values differ from the ASF thresholds found in the Xcalibur Instrument Setup.

You can use the templates in the Xcalibur Instrument Setup in a Data Dependent experiment to do the following:

- Identify low-level peptides in complex mixtures of enzymatically digested proteins (using Data Dependent MS/MS or Nth Order Double Play).
- Identify post-translational modifications (such as phosphorylation and glycosylation) automatically from intense precursors in a complex peptide mixture (Data Dependent Neutral Loss).
- Identify post-translational modifications through a hypothesis-driven approach by adding the mass of the modification to the identified peptides that could harbor such modifications and placing these in a Precursor List.
- Identify proteins by peptide mass fingerprinting using full MS at ZoomScan rate or using the first full MS from the N^{th} Order Double Play experiment, using the deisotoping option in BioWorks. (See Chapter 9, “Identifying Proteins Using BioWorks.”)
- Quantitate isotopically labeled peptides from a ratio of peaks in the same MALDI spectrum.

Instrument Setup contains the following templates for setting up Data Dependent experiments:

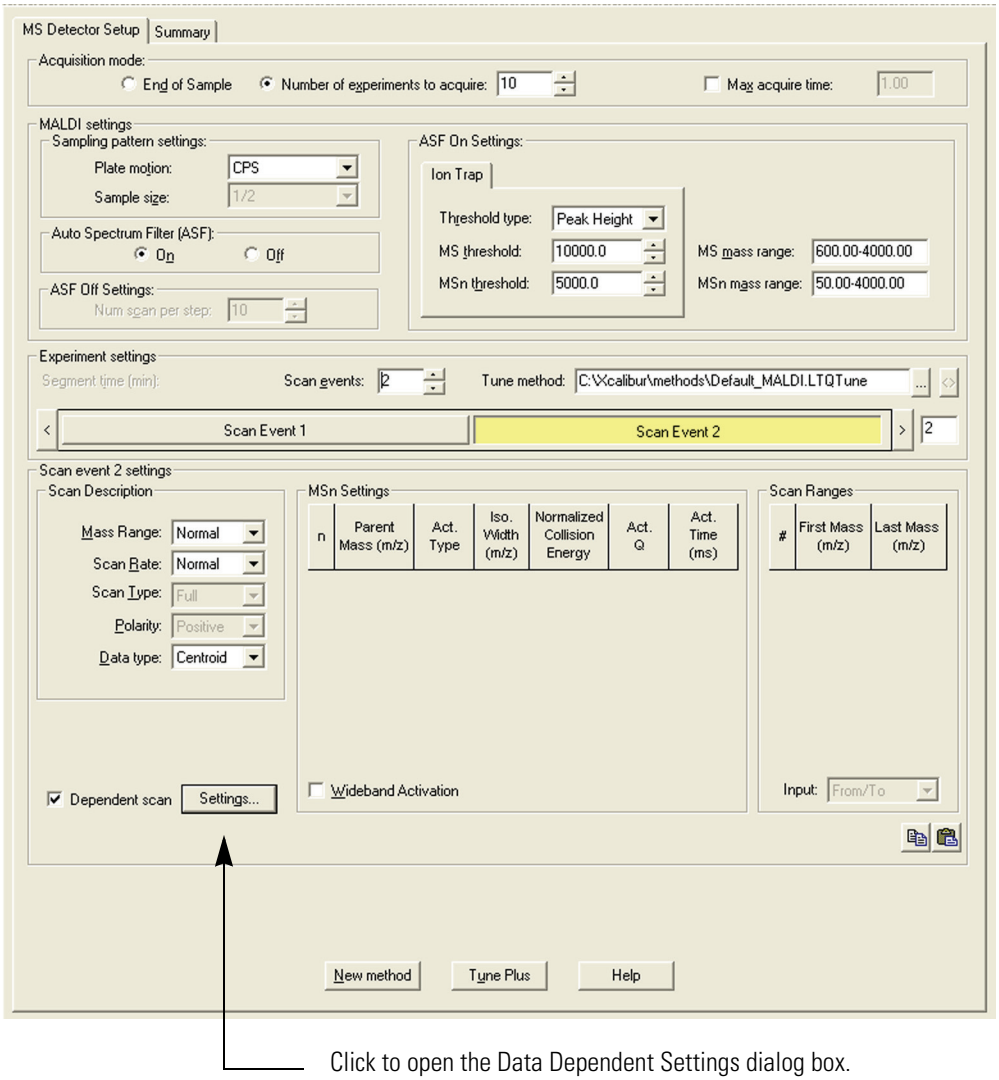
- Data Dependent MS/MS Template
- Data-Dependent Triple Play Template
- Nth Order Double Play Template
- Nth Order Triple Play Template

Data Dependent MS/MS Template

Even with the simplest data-dependent experiment (Data Dependent MS/MS template) in which you specify the MS scan range but do not specify a precursor ion, you can automatically find useful structural information about your compound. With these parameters, the LTQ XL mass spectrometer collects full-scan MS data over the specified range, picks the most intense precursor ion in the spectrum, and then fragments the ion to generate product ions.

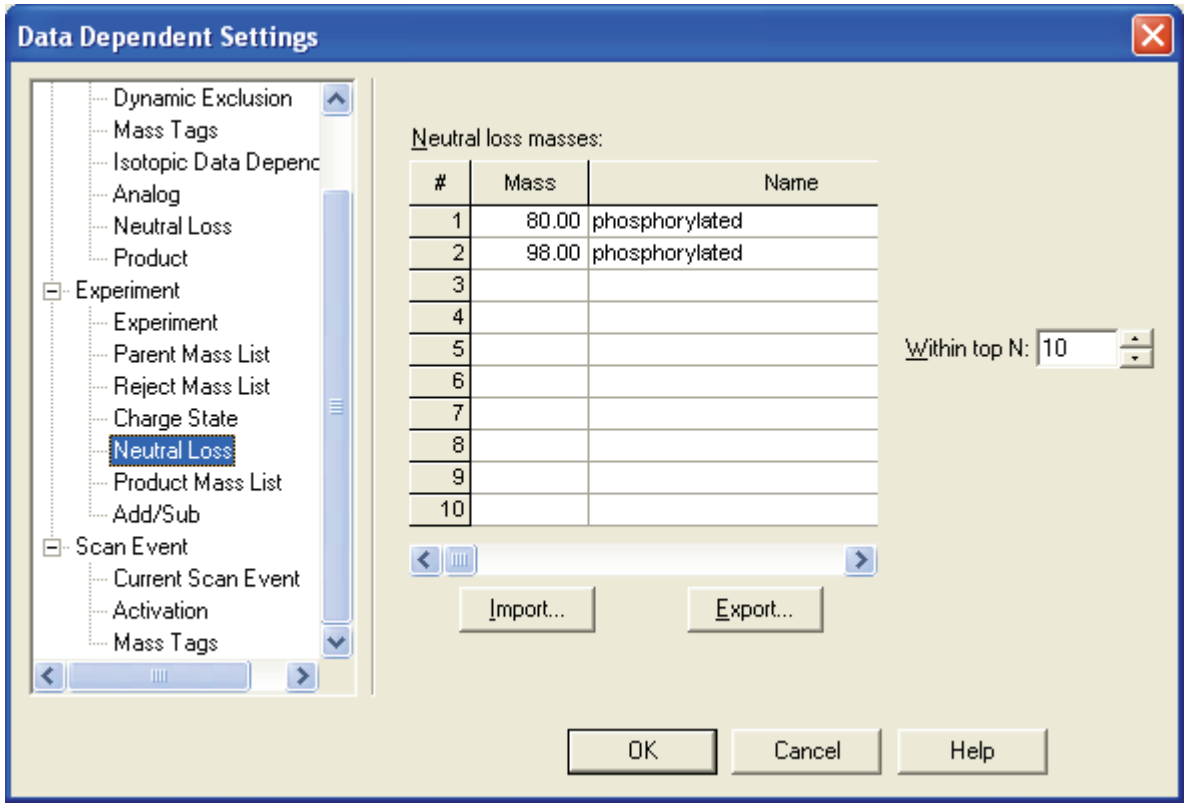
Figure 4 shows the default settings for the Data Dependent MS/MS template. With the default settings, this template has two scan events. The second scan event is data dependent.

Figure 4. Data Dependent MS/MS template with the default settings



As Figure 5 shows, you can use the Data Dependent MS/MS template to set up neutral loss experiments.

Figure 5. Data dependent settings for a Data Dependent MS/MS experiment

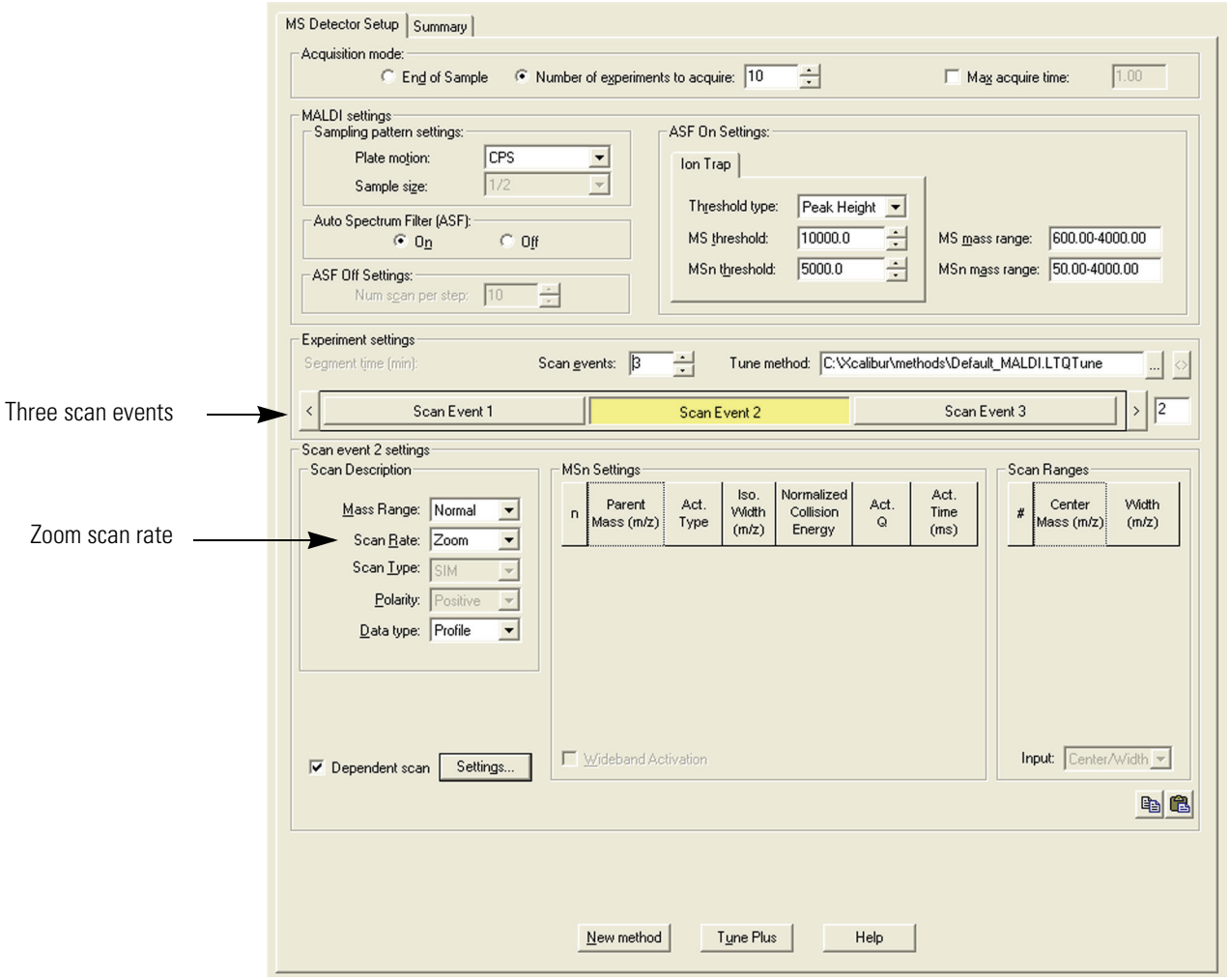


Data-Dependent Triple Play Template

A Data Dependent Triple Play experiment collects full-scan MS data, and then uses ZoomScan to determine the charge state of the precursor ion. With the *m/z* information obtained from the full-scan MS data and the charge state information obtained from the Zoomscan, the program calculates the molecular mass of the precursor ion. The precursor ion is then fragmented into product ions (MS/MS). Because MALDI ions are predominantly singly charged, this experiment type is more useful for the ESI mode.

Figure 6 shows the Data Dependent Triple Play template with the default settings.

Figure 6. Data Dependent Triple Play template with the default settings



Nth Order Double Play Template

Because the Nth Order Double Play Data Dependent experiment automatically performs MS/MS scans on multiple ions with intensities above a specified threshold, it is the most common type of experiment performed with the LTQ XL mass spectrometer in both the ESI and MALDI modes. For this type of experiment, specify the number of times that Scan Event 2 repeats. The data-dependent scans (Scan Event 2) are performed on the N most intense ions detected in Scan Event 1, starting with the most intense ion and proceeding in order of decreasing intensity. You can collect up to 50 MS/MS scans in a single experiment and you can acquire up to 10000 experiments per data file. For MALDI, limit the number of experiments per data file to two. See Figure 7 and Figure 8.

Figure 7. Nth Order Double Play dialog box

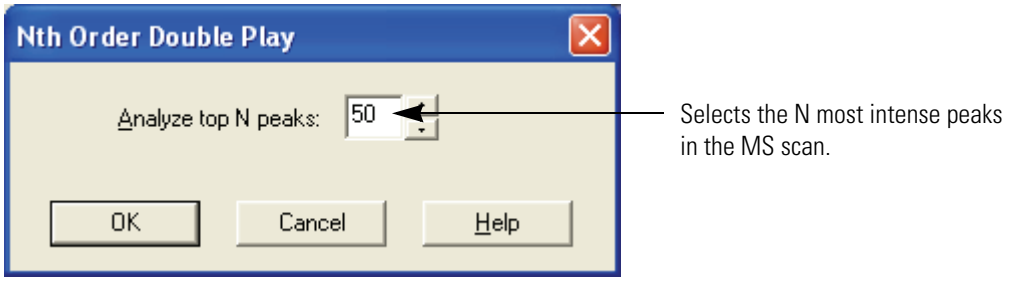


Figure 8. Nth Order Double Play template (with advanced features)

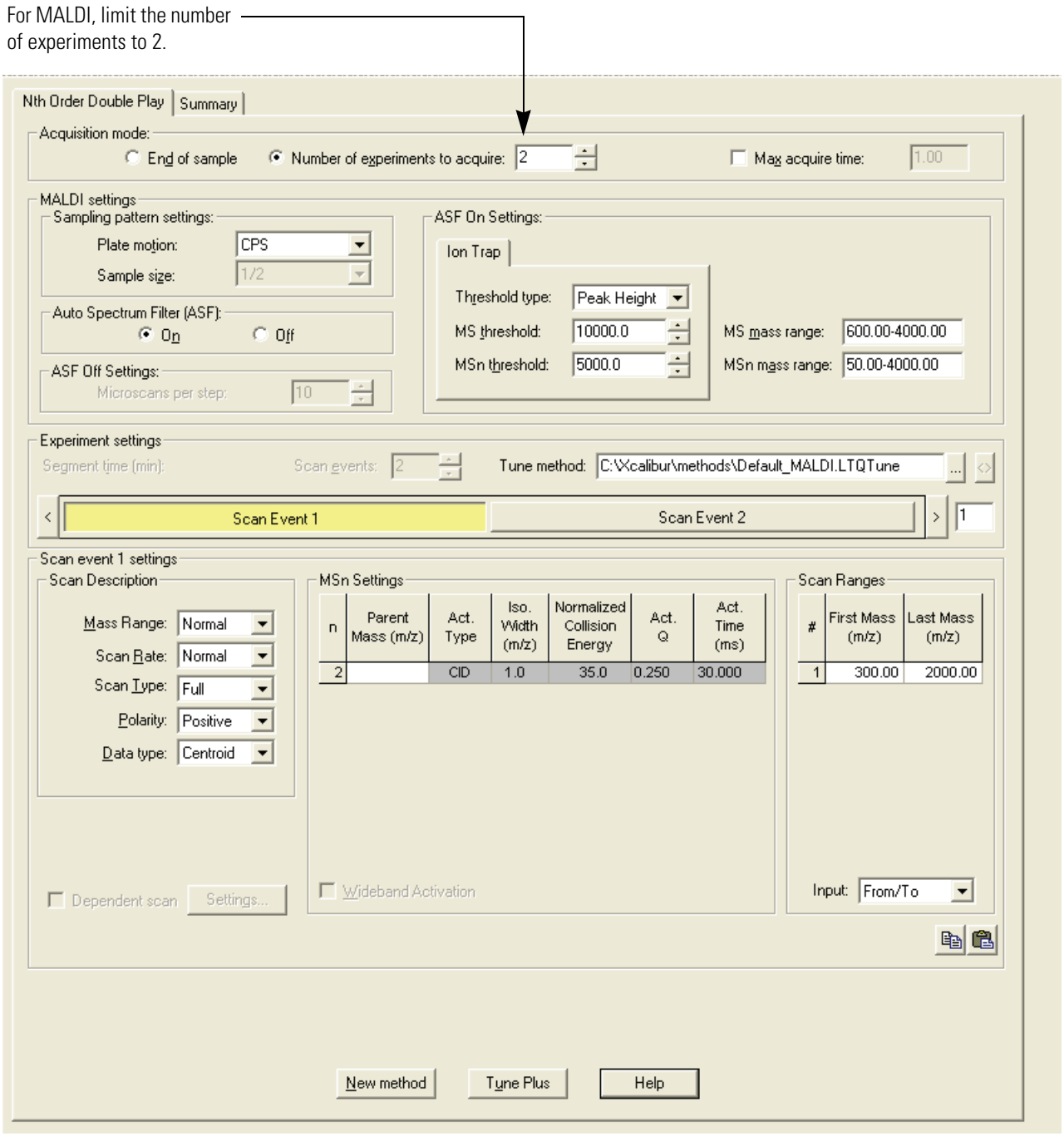


Figure 9 shows some common settings for the Dynamic Exclusion™ parameters in an Nth Order Double Play experiment. You can use the dynamic exclusion parameters to maximize the number of compounds analyzed in the MS/MS scans.

Exclusion duration and Exclusion mass width (by mass) are the two most important dynamic exclusion parameters. Set the exclusion duration to a value greater than the expected time to perform the method. Set the exclusion mass width to an *m/z* range that includes the whole isotopic envelope of the precursor ion to ensure each MS/MS scan is of a unique (by molecular weight) precursor. Figure 10 shows an example of the isotopic envelope of Melittin, a peptide in the High Mass Calibration Mix. With an exclusion mass width of 1.50 *m/z* on the low-end and 4.0 *m/z* on the high-end of the mass range, the LTQ XL mass spectrometer performs an MS/MS scan on the most intense peak at 2846.82 *m/z* and ignores the remaining peaks between 2845.32 *m/z* and 2850.82 *m/z*.

Repeat count and Repeat duration are used with LC-ESI-MS experiments.

Figure 9. Nth Double Play experiment (Dynamic Exclusion parameters)

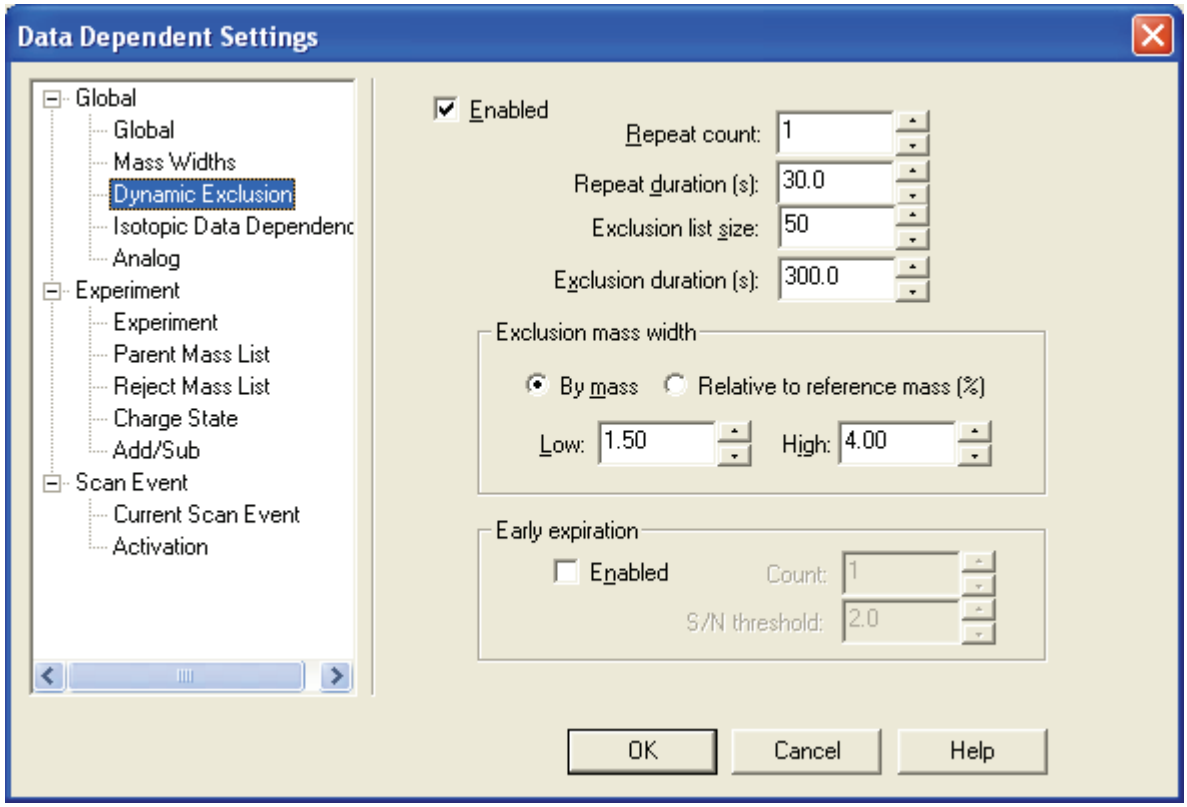


Figure 10. Isotopic envelope of Melittin (peptide in the High Mass Calibration Mix)

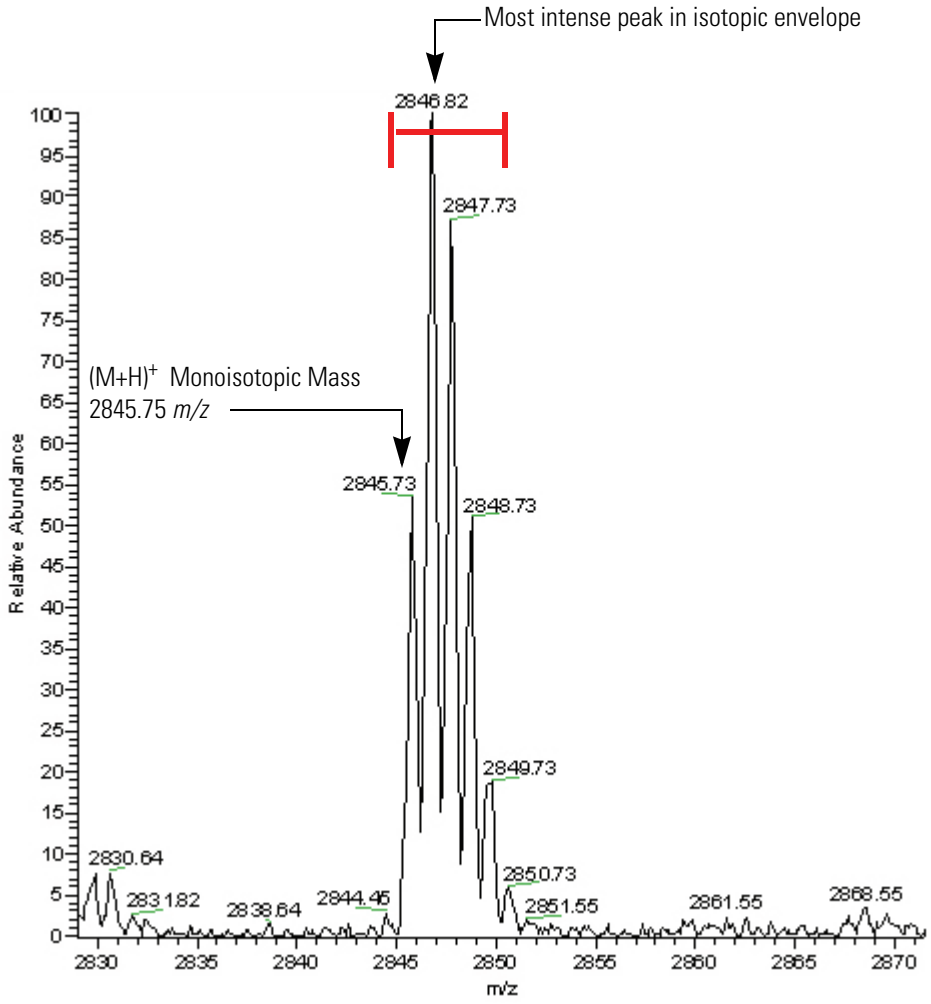


Figure 11 shows the Experiment parameters for an Nth Order Double Play experiment. By default, none of the check boxes are selected. These features are generally not used for a MALDI experiment.

Figure 11. Nth Order Double Play (Experiment parameters)

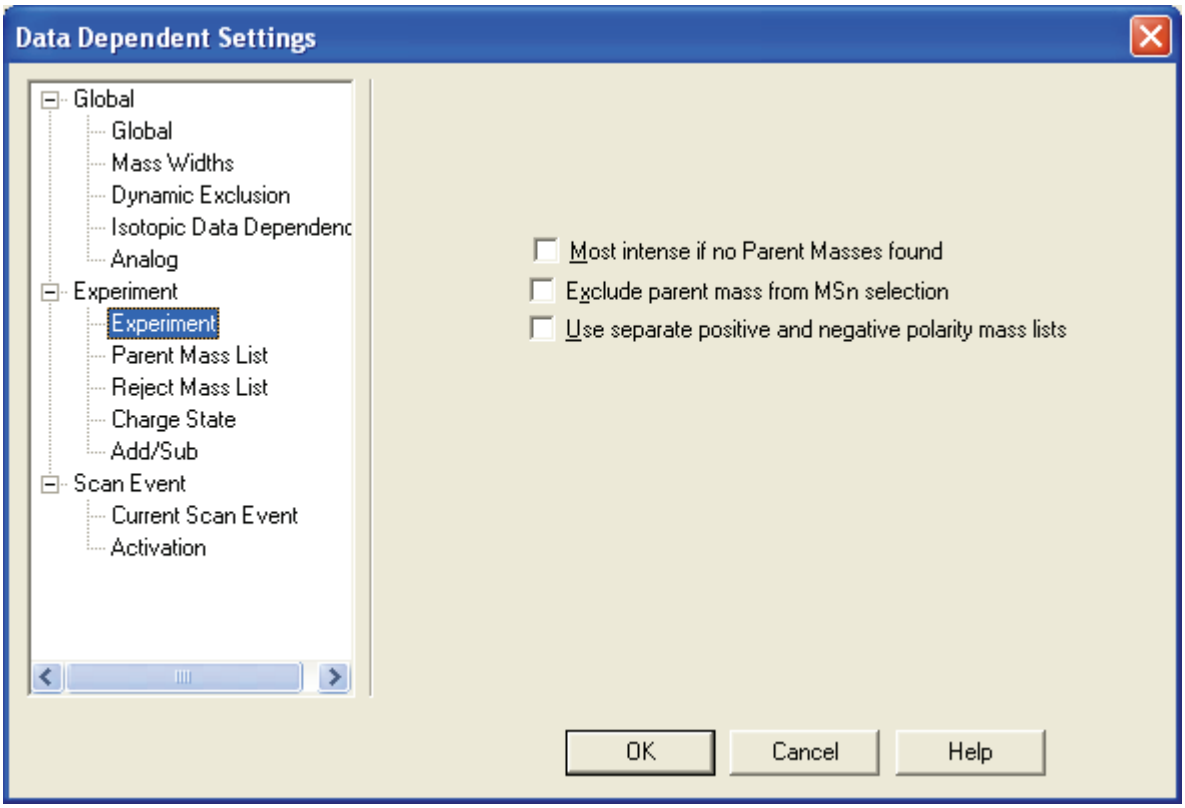
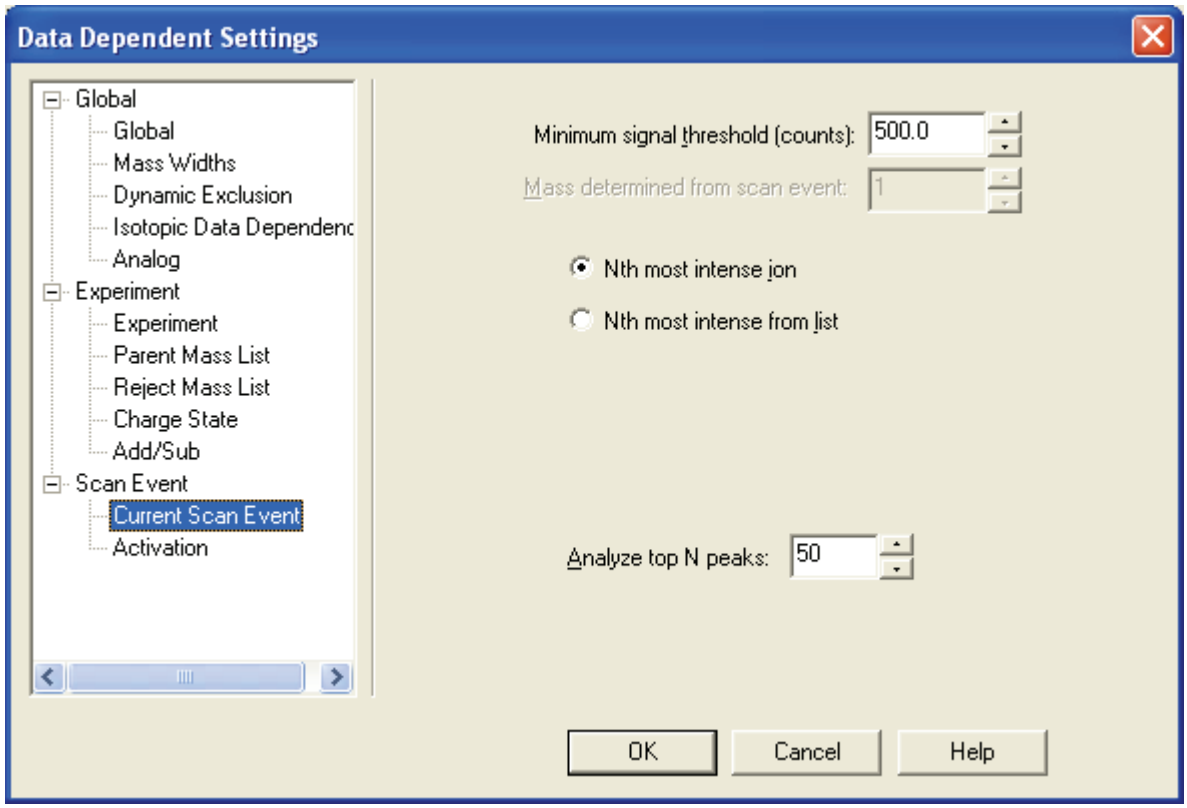


Figure 12 shows the default Current Scan Event parameters in an Nth Double Play template. The settings in the Current Scan Event parameters page define the criteria by which the dependent scan is executed or not executed. The dependent scan event is only executed if the selected ion satisfies all specified criteria in a prior scan event.

Figure 12. Nth Double Play experiment (Current Event parameters)



Nth Order Triple Play Template

Use this template to set up a special type of Data Dependent Triple-Play experiment in which you specify the number of ions (N) you want to further analyze in the independent scan performed in Scan Event 1.

The dependent scans are performed on the N most intense ions detected in independent Scan Event 1. These start with the most intense ion (N=1) and proceed in the order of intensity (N=2, 3, 4, and so on.) The maximum number of ions in Scan Event 1 that can be selected is 9.

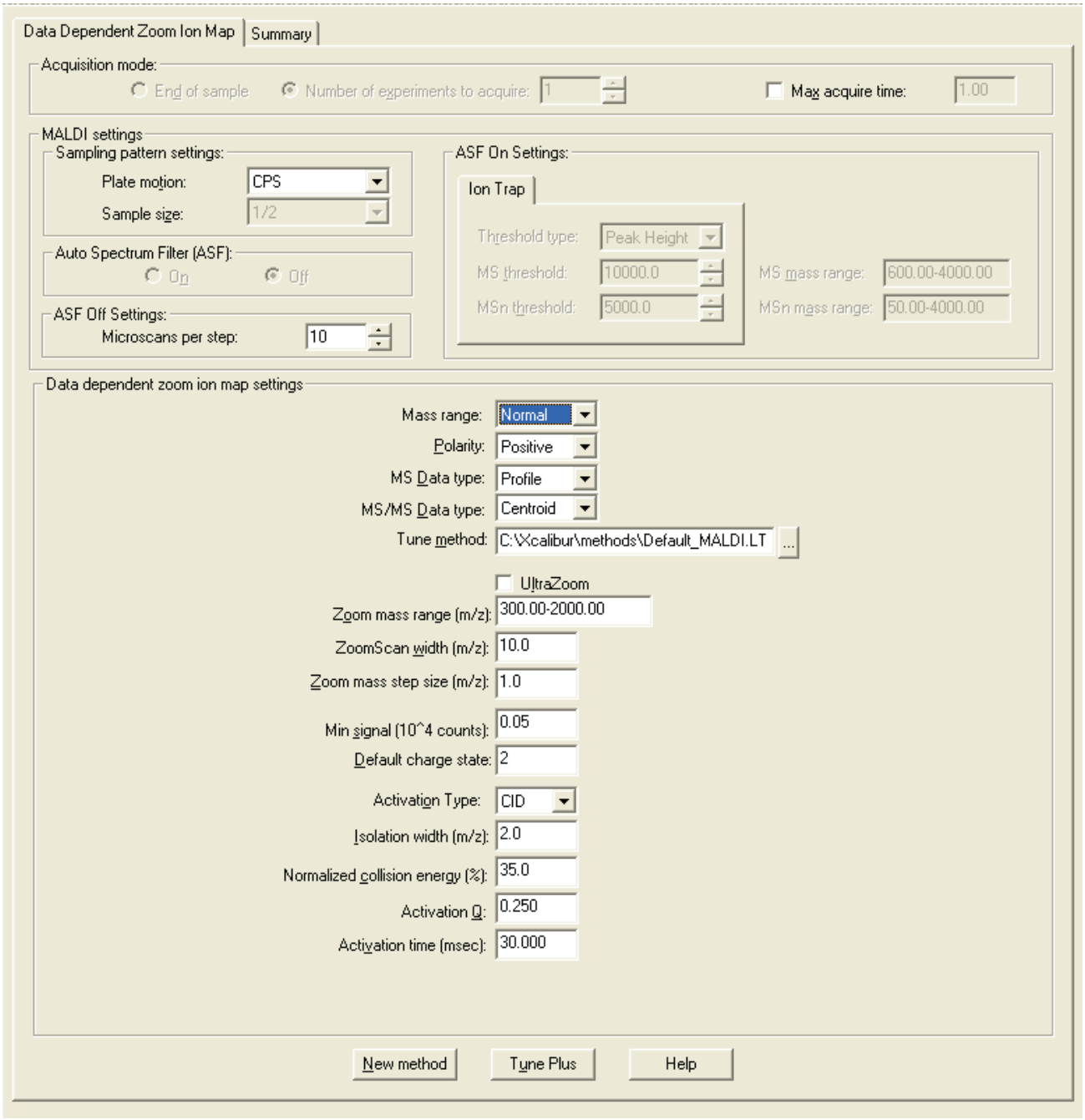
Data-Dependent Zoom Map Experiments

A Data Dependent Zoom Map is an Ion Mapping™ experiment that collects ZoomScan data on every scan interval in a specified mass range, as well as data-dependent MS/MS product spectra on every mass above an intensity threshold.

A Data Dependent Zoom Ion Map experiment consists of an independent MS ZoomScan scan with isolation of precursor masses in the Zoom mass range, which is the range of precursor masses isolated as specified by the ZoomScan width. During subsequent cycles through this step, the parent (precursor) mass is incremented by the Zoom mass step size. ASF settings are not available in this experiment.

Figure 13 shows the Data Dependent Zoom Ion Map template with default settings.

Figure 13. Data Dependent Zoom Ion Map template with default settings



Tip To review the data, open the raw data file in Xcalibur Qual Browser, and view the ion map by choosing **View > Ion Map**.

Data-Dependent Ion Tree Experiments

The Data Dependent Ion Tree experiment provides methods for automatically collecting and interpreting MS^n data and arranging the data in formats that are easy to manipulate. You can specify a particular precursor ion for fragmentation, or you can let the LTQ XL mass spectrometer find the precursor ions automatically and fragment them to any level between MS^2 and MS^{10}. The LTQ XL mass spectrometer automates the collection of data by determining what actions need to occur next for the experiment to progress. These methods allow you to perform Data Dependent MS^n scans on up to 25 ions per scan.

In an Ion Tree experiment, use the Breadth Focus or Depth Focus option to prioritize how the LTQ mass spectrometer gathers information. Independent of the focus type (Depth or Breadth), specify a maximum depth and a maximum breadth for the experiment. The maximum depth is the maximum MSn levels analyzed. The maximum breadth is the maximum number of ions analyzed at each MS^n level.

Breadth Focus characterizes the specified number of ions at the same MSn level before advancing to the next MSn level. For example, if you specify a Max Breadth of 3 and a Max Depth of 3, the mass spectrometer performs MS/MS scans on the first, second, and third most intense peaks in the MS scan. It then performs MS^3 scans on the first second, and third most intense peaks in the MS/MS scan.

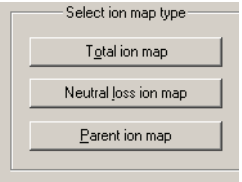
Depth Focus characterizes an ion by performing a series of MS^n-level fragmentations (for example, MS/MS, MS^3, MS^4) before characterizing the next most intense ion in the MS scan. For example, if you specify a Max Depth of 4 [MS^4] and a Max Breadth of 2, the mass spectrometer performs the MS/MS, MS^3, and MS^4 scans on the first most intense peak of the MS scan. Then it performs the MS/MS, MS^3, and MS^4 scans on the second most intense peak of the MS scan.

You can review the results of a Data Dependent Ion Tree experiment in the Xcalibur Qual Browser window. The results are displayed as a structure tree that originates from a particular precursor ion.

Ion Mapping Experiments

Note The Total Ion Map, Neutral Loss Ion Map, and Precursor Ion Map experiments described in this topic are *not* Data Dependent.

Use an Ion Mapping experiment to get full structural characterization of unknown molecules in complex mixtures. In an Ion Mapping experiment, you can get product ion scans on every precursor ion over a specified mass range. An Ion Mapping experiment can help to identify automatically which precursor ions were fragmented to yield a specified product ion. The experiment maps one or more precursor ions by using the information from product ion scans.



The LTQ XL program includes the following Ion Mapping templates in Instrument Setup:

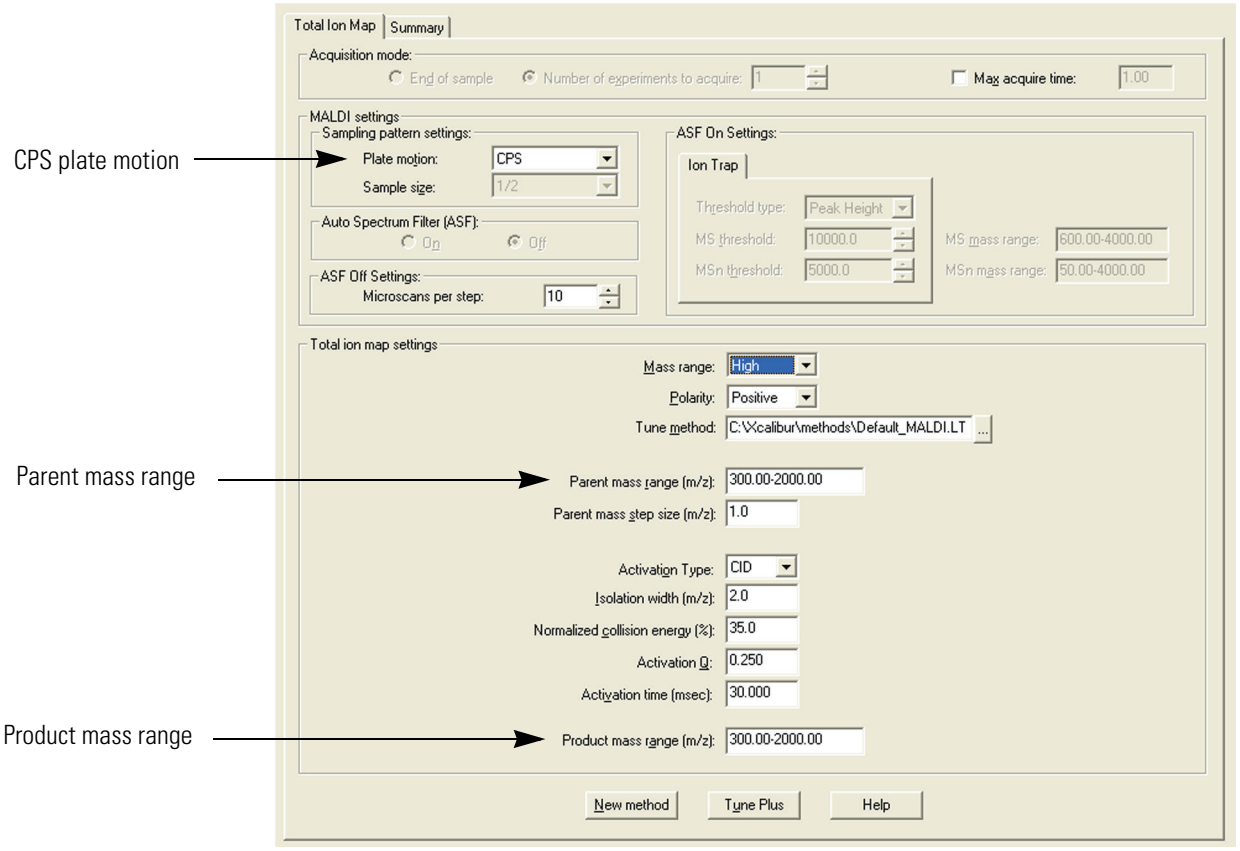
- Total (Full Scan) Ion Mapping Experiments
- Neutral Loss Ion Mapping Experiments
- Precursor Ion Mapping Experiments

These Ion Mapping experiments require a stable signal throughout the length of the experiment. To achieve signal stability, it is important to prepare homogeneous sample spots. For best results, use the Crystal Positioning System (CPS) feature of the MALDI source to help maintain signal stability. The Automatic Spectrum Filtering (ASF) feature is not available for Ion Mapping experiments, because it could interfere with maintaining a stable signal. See "What Are the Data Acquisition Features of the MALDI Source?" on page 4 for more information about these features.

Total (Full Scan) Ion Mapping Experiments

In a total (or full-scan) Ion Mapping experiment, you get product ion scans for each precursor ion. These scans provide information that you can use to determine which precursor ions lost a particular fragment to yield a particular product ion. You can also determine which precursor ions are related to specific product ions. For example, you can map the spectral peaks in a mass range from *m/z* 600 to *m/z* 2000 and scan for MS/MS product ions in incremental steps of every mass-to-charge ratio, every fifth mass-to-charge ratio, or every tenth mass-to-charge ratio. Figure 14 shows an example of the settings for a total Ion Mapping experiment.

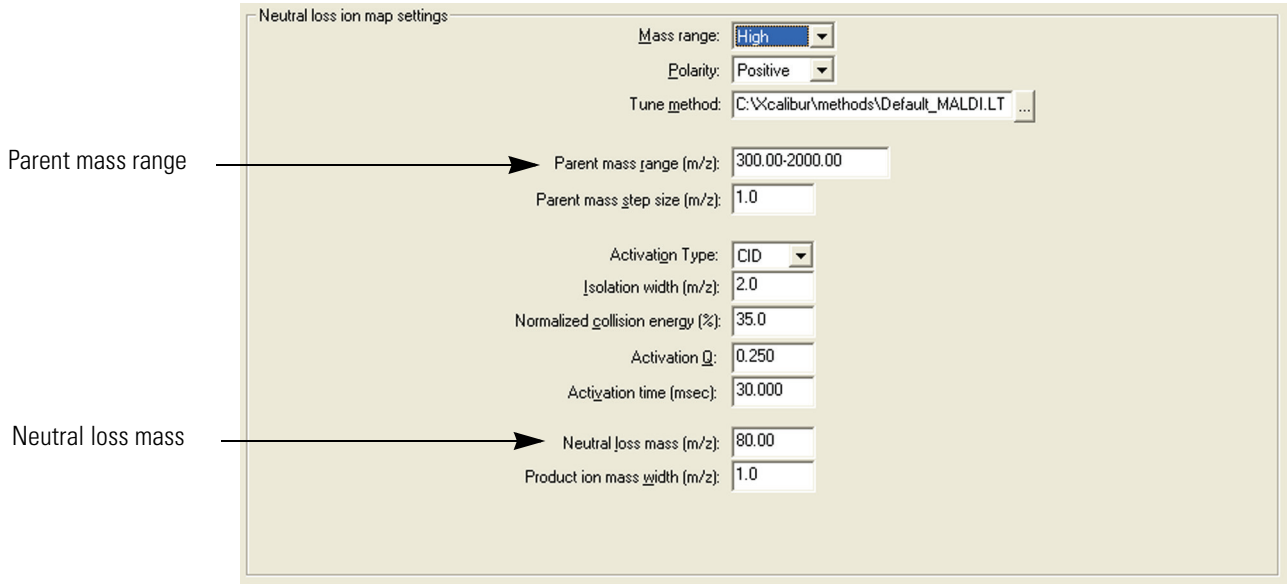
Figure 14. Total Ion Map settings



Neutral Loss Ion Mapping Experiments

A Neutral Loss Ion Mapping experiment collects scans for masses that have lost neutral fragments. As with full scan Ion Mapping, you can get product ion scans on every precursor ion. However, a neutral loss ion map experiment identifies which precursor ions lost a neutral fragment of a particular mass. For example, you can specify a neutral loss of 80 u (as in the case of a phosphorylated peptide in a tryptic digest). A neutral loss ion mapping experiment can step through each product mass in the mixture searching for evidence of the loss of a neutral moiety of mass 80 u. See Figure 15.

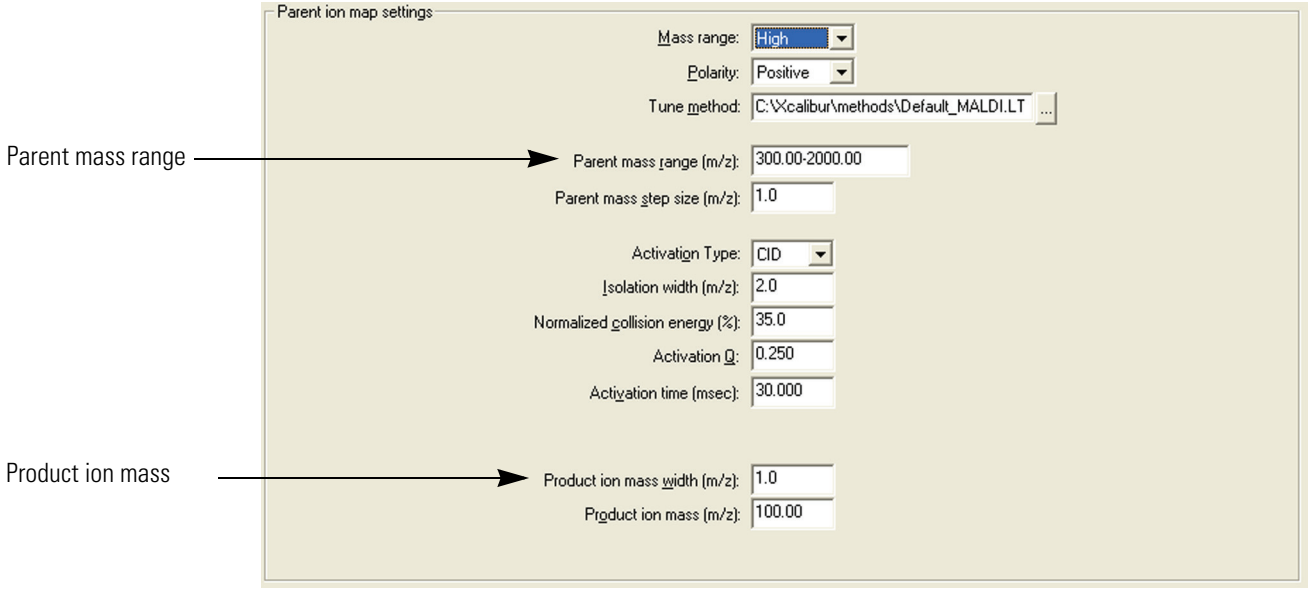
Figure 15. Neutral loss ion map settings



Precursor Ion Mapping Experiments

A Precursor Ion Mapping experiment identifies all the ions that produce a particular molecular ion. For example, if you specify a product ion mass of *m/z* 100, a precursor ion map includes all the precursor ions (parent ions) that yielded that product ion. See Figure 16.

Figure 16. Parent ion mass settings



What is Tuning and Calibration of the LTQ Mass Spectrometer?

Tune parameters are instrument parameters (for example, laser energy) whose values can vary with the type of experiment or analyte being mass analyzed. You tune the instrument on an analyte of interest to ensure sensitivity and mass resolution.

Calibration parameters are instrument parameters whose values do not vary with the type of experiment. You calibrate the mass spectrometer to ensure mass accuracy. Thermo Fisher Scientific recommends that you calibrate the mass spectrometer at least once every three months, or whenever you notice a decrease in system performance.

To tune and calibrate the MALDI LTQ XL system, you perform these basic steps:

1. Prepare a fresh calibration mixture and spot it onto a target plate.
2. Acquire a mass spectrum of the calibration mixture to make sure that the mass spectrometer is operating properly and that you can get a good signal.
3. Use the automatic tuning procedure in Tune Plus to ensure a laser energy that is high enough to produce a good signal-to-noise ratio for the ions in the calibration mixture, but not so high that it causes space charging in the ion trap. Space charging, caused by too many ions in the trap, reduces mass resolution and causes mass shifts.
4. Use the automated calibration procedure to calibrate the mass spectrometer.

See Chapter 5, “Tuning and Calibrating in the MALDI Mode,” for detailed instructions on tuning and calibrating the MALDI LTQ XL system.

Setting Up the Xcalibur Instrument Configuration

This chapter describes how to add the LTQ XL mass spectrometer and the MALDI source to the Xcalibur Instrument Configuration and specify some of their configuration options.

Contents

Adding the MALDI LTQ XL to the Xcalibur Instrument Configuration

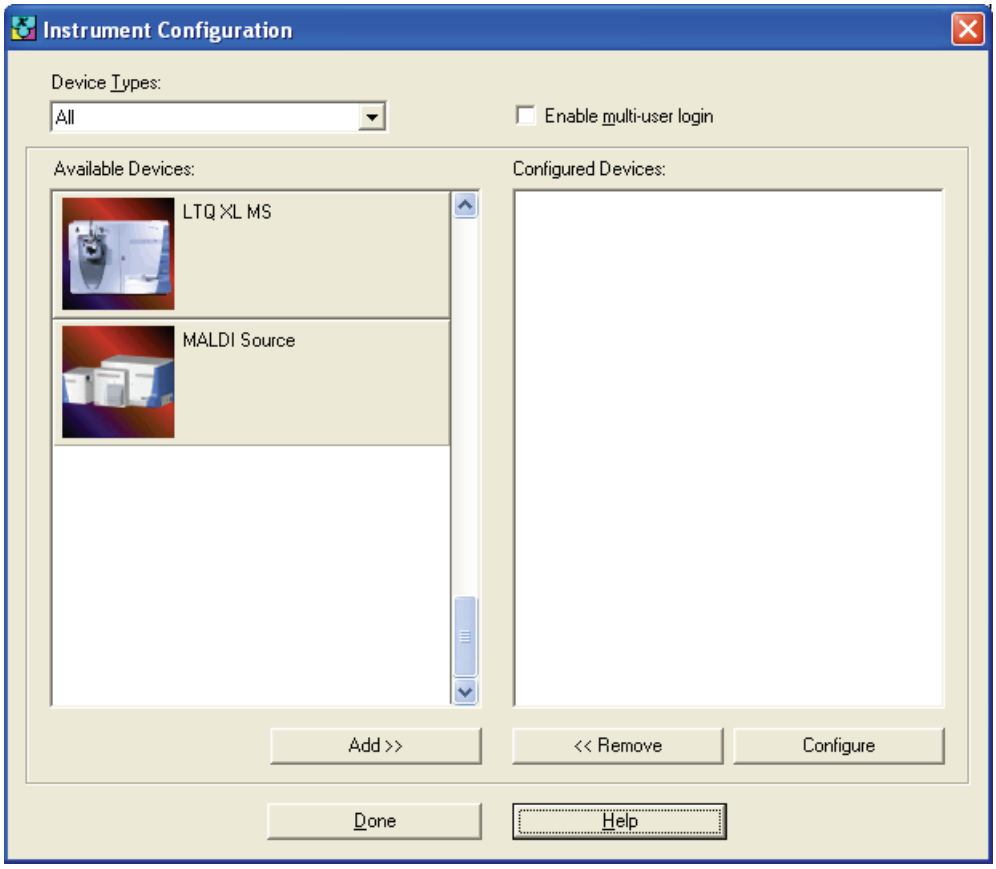
To control the MALDI source and the LTQ XL mass spectrometer from the Xcalibur data system, add these devices to the software configuration using the Xcalibur Instrument Configuration program.

❖ **To add the LTQ XL MS and MALDI source to the list of Configured Devices**

1. Because the Xcalibur Instrument Configuration program cannot be open simultaneously with the Xcalibur data system or the Tune Plus program, close the Xcalibur data system and the Tune Plus program if they are open.
2. Open the Xcalibur Instrument Configuration program:
   - Choose **Start > All Programs > Xcalibur > Instrument Configuration**.
   - From the computer desktop, double-click the  Xcalibur Instrument Configuration icon.

   The Xcalibur Instrument Configuration dialog box appears. See Figure 17.

Figure 17. Xcalibur Instrument Configuration dialog box

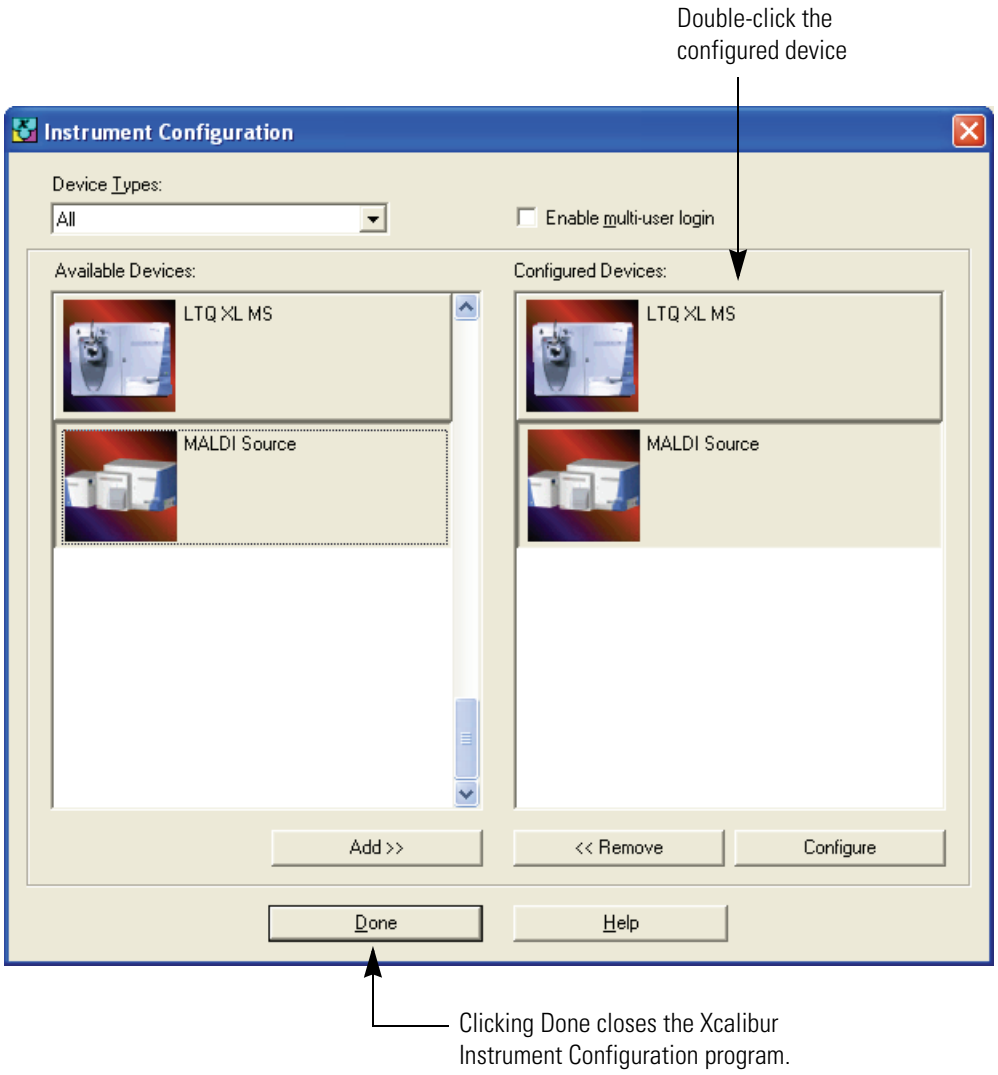


3. Select the devices that you want to control from the Xcalibur data system:
   a. In the Device Types list, select **ALL**.
   b. In the Available Devices list, double-click **LTQ XL MS**.

      A copy of the LTQ XL MS button appears in the Configured Devices list. See Figure 18.
   c. In the Available Devices list, double-click **MALDI Source**.

      A copy of the MALDI Source button appears in the Configured Devices list. See Figure 18.

Figure 18. Instrument Configuration dialog box with configured devices



Xcalibur now recognizes the LTQ XL mass spectrometer and the MALDI source as devices currently in use.

4. Do not close the Instrument Configuration dialog box. Go to the next topic, "Specifying the Configuration Options for the LTQ XL."

Specifying the Configuration Options for the LTQ XL

Use the Xcalibur Instrument Configuration program to specify the configuration options for the LTQ XL mass spectrometer. This topic describes how to specify the MALDI source as the default ion source.

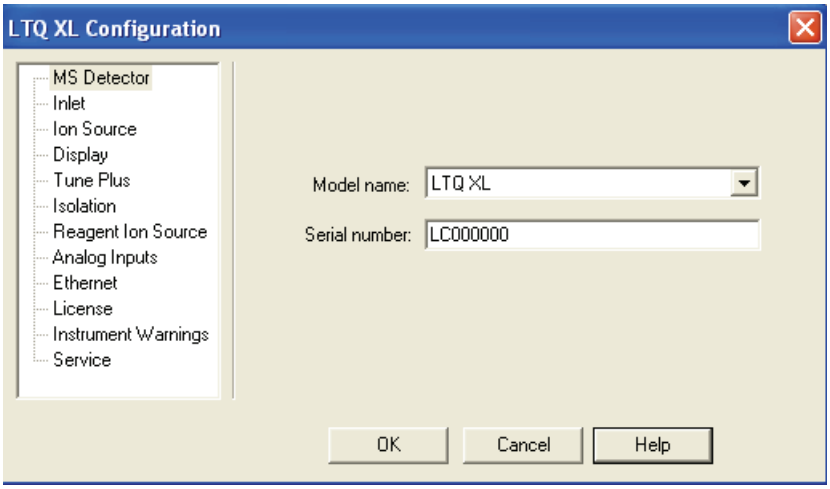
❖ To specify the configuration options for the LTQ XL instrument

1. Add the LTQ XL mass spectrometer to the list of configured devices as described in the previous topic, "Adding the MALDI LTQ XL to the Xcalibur Instrument Configuration" on page 25.
2. Double-click 

LTQ XL MS in the Configured Devices list.

   The LTQ XL Configuration dialog box appears. See Figure 19.

   Figure 19. LTQ XL Configuration dialog box

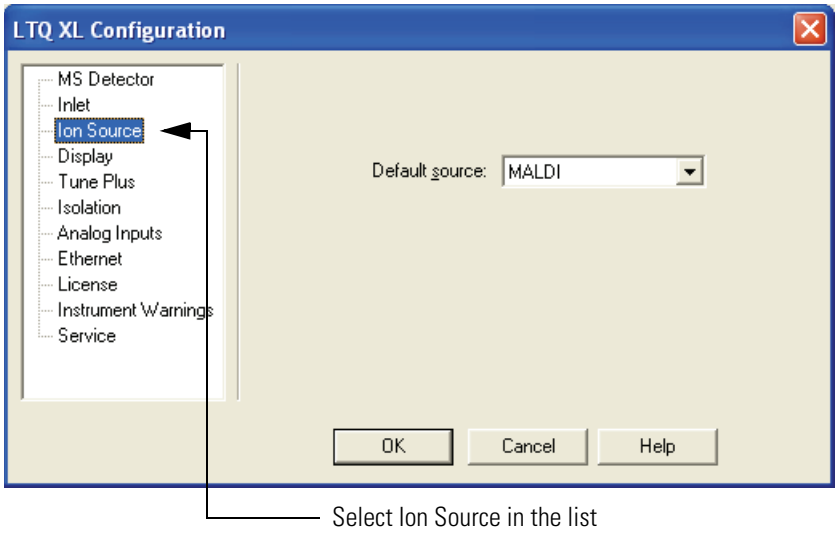


3. In the list on the left side of the dialog box, select **Ion Source**.

   The Default source list appears.

4. In the Default source list, select **MALDI** as shown in Figure 20.

Figure 20. LTQ XL Configuration dialog box with the Default source list

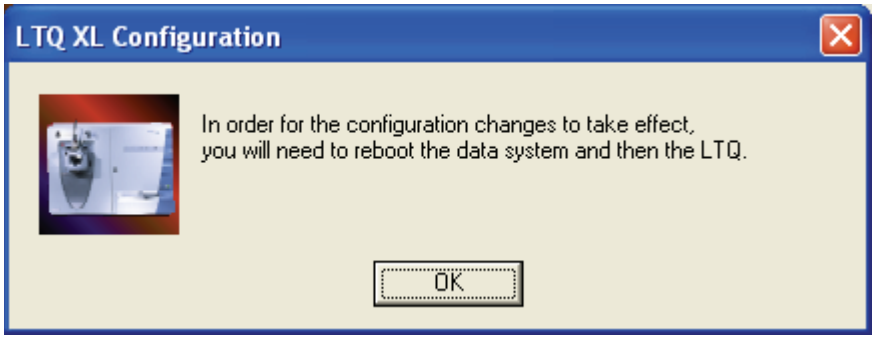


See the LTQ XL Help for information about specifying the other configuration options.

5. When you finish selecting the configuration options for the LTQ XL mass spectrometer, click **OK**.

Figure 21 shows the LTQ XL Configuration dialog box that appears when you change any of the configuration settings.

Figure 21. Confirmation message to change configuration settings



6. Click **OK** to save the settings and close the LTQ XL Configuration dialog box.
7. Do one of the following:
   - To enter the serial number of the MALDI source, go to the next topic, "Specifying the Configuration Options for the LTQ XL."
   - To exit the Xcalibur Instrument Configuration program, click **Done**. See Figure 18 on page 27.

Specifying the Configuration Options for the MALDI Source

You do not need to configure the ion source for operation. However, you can use the MALDI Source Configuration dialog box to view the serial number and firmware version for the MALDI source, and to change the serial number.

❖ To change a serial number or view the firmware version

1. From the computer desktop, choose **Start > All Programs > Xcalibur > Instrument Configuration**.

   The Xcalibur Instrument Configuration dialog box appears.

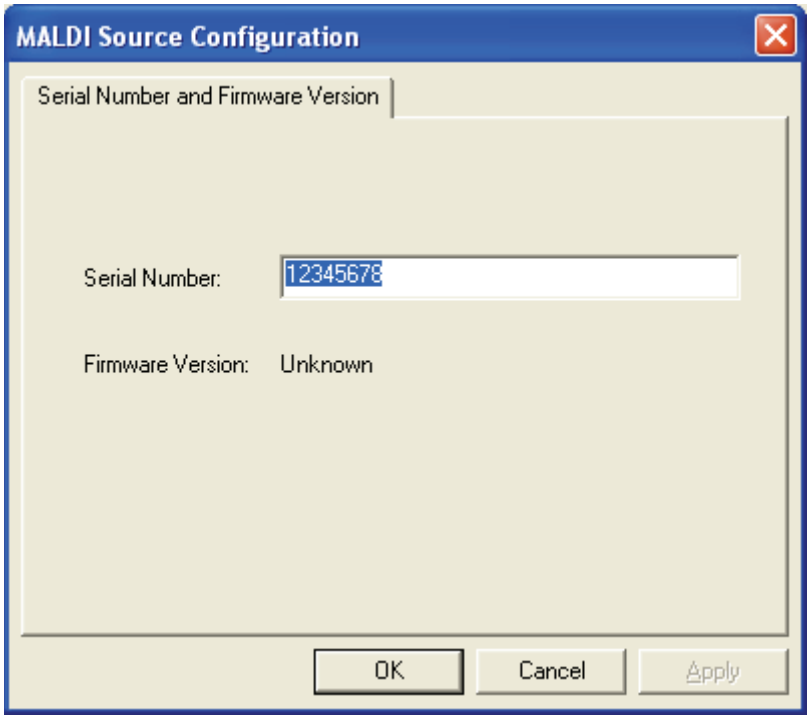
2. In the Configured Devices list, double-click 

MALDI Source.

   The MALDI Source Configuration dialog box appears. See Figure 22.

   The Serial Number box contains the current entry for the serial number, and the Firmware Version readback displays the firmware version of the MALDI source. The Firmware Version readback displays a value of Unknown until you run the Tune Plus MALDI diagnostics tool for the first time.

Figure 22. MALDI Source Configuration dialog box



3. To change the serial number, type a new number in the Serial Number box.
4. Click **OK** to save your changes and close the dialog box.
5. Click **Done** to exit the Xcalibur Instrument Configuration program.

Preparing for Daily Operation

This chapter describes how to prepare the MALDI LTQ XL system for daily operation.

Contents

Equipment and Materials Checklist

Check that your laboratory has the following equipment, consumables, and chemicals (see Table 1). You must have these items to prepare samples and clean the MALDI target plates. In addition, you must also have the ProteoMass™ MALDI Calibration Kit to prepare the calibration and sensitivity samples.

For information on preparing the calibration mixtures, see Appendix A, "ProteoMass Calibration Kit."

Table 1. Equipment and Materials checklist

Equipment
❑ Sonicator bath, approved for use in a chemical hood in the presence of flammable vapor, large enough to hold a 1 L beaker or a flat tray that can hold the MALDI plate. (The tray must be inert to acetonitrile, methanol, and dilute ammonium hydroxide.)
❑ Microcentrifuge
❑ Vortexer
❑ Pipettors (2.5 µL, 200 µL, and 1000 µL recommended; additional sizes might be useful.)
❑ Analytical balance
Consumables
❑ Microcentrifuge tubes (500 µL and 1.5 mL non siliconized)
❑ Tips for pipettors, non siliconized
❑ Optical lens wipes or soft clean room wipes
❑ Can of compressed difluoroethane (sold as "compressed air" for dust removal) or ultra high purity nitrogen
Chemicals/Solvents (all HPLC grade or better)
❑ Water
❑ Isopropyl alcohol
❑ Methanol
❑ Acetonitrile
❑ Acetone
❑ Trifluoroacetic acid (TFA)
❑ 28 to 30% ammonium hydroxide solution

Checking the Vacuum Pressure

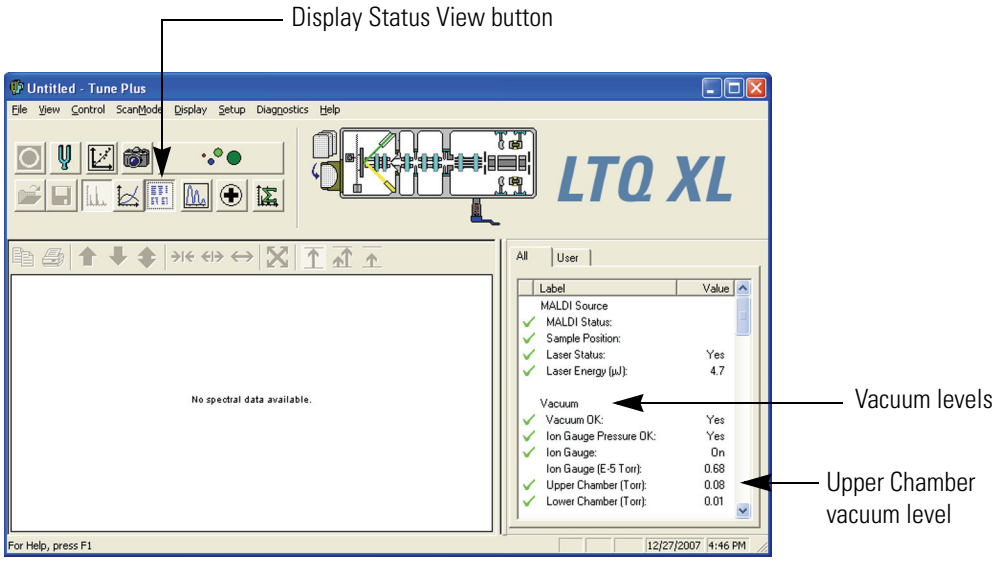
If the Thermo Fisher Scientific field service engineer has just installed the MALDI source, or if you have vented the source to atmosphere recently, allow sufficient time for the ion source to reach operating vacuum levels before you use the system.

❖ To check the vacuum pressures

1. On the LTQ workstation desktop, choose **Start > Programs > Xcalibur > LTQ Tune**.
2. If the status view is not displayed, click  (Display Status View).

   The status view appears on the right side of the window. The values for Vacuum OK and Ion Gauge Pressure OK should be Yes. The value for Upper Chamber should be approximately 0.08 Torr (70 to 80 mTorr). See Figure 23.

Figure 23. Tune Plus window with the Status view



Checking the Helium and Nitrogen Gas Supplies

Be sure to check the helium and nitrogen gas supplies before operating the MALDI LTQ XL system. The LTQ XL mass spectrometer uses helium as a damping gas and collision activation partner in the mass analyzer. When the LTQ XL mass spectrometer is operated in the MALDI mode, the MALDI source uses nitrogen gas to maintain a pressure of approximately 75 mTorr in the sample chamber.

❖ To check the helium and nitrogen gas supplies

1. Check that the helium and nitrogen lines are properly connected to the back of the LTQ XL mass spectrometer.

   For information on checking the nitrogen line connection to the MALDI sample compartment, contact your Thermo Fisher Scientific field service engineer.

2. Check that gases are flowing and that the pressure for each gas is correct.

   - The helium pressure should be 40 ± 10 psig (275 ± 70 kPa).
   - The nitrogen pressure should be 100 ± 20 psig (690 ± 140 kPa).

3. Make sure that you have sufficient gas to complete your analysis. If necessary replace the helium or nitrogen tank.

   For more information on gas requirements, see the documentation for the LTQ XL mass spectrometer.

Placing the LTQ XL System in the On Mode

When you are not using the MALDI LTQ XL system, place the LTQ XL mass spectrometer in Standby mode. When you put the LTQ XL mass spectrometer in Standby mode, the electron multipliers, conversion dynode, main RF voltage, and ion guide RF voltages turn off. The LTQ XL also turns off the nitrogen gas flow to the MALDI sample module. The System LED on the front panel of the LTQ XL mass spectrometer is amber when the system is in Standby mode.

Before beginning an analysis, take the LTQ XL mass spectrometer out of Standby mode. Because the MALDI laser has a limited lifespan, place the MALDI source in the Standby mode when it is not in use, even for brief periods.

❖ To place the LTQ XL mass spectrometer in the On mode

1. On the LTQ workstation desktop, choose **Start > All Programs > Xcalibur > LTQ Tune**.
2. In the Tune Plus window, click **On/Standby**  to place the LTQ XL mass spectrometer in the On mode.

   In the On mode, a green triangle  appears in the top-left corner of the Tune Plus window.

   The nitrogen supply to the MALDI source automatically turns on. The System LED on the front panel of the LTQ XL mass spectrometer turns green. The high voltage to the electron multipliers turns on and the MALDI source goes into Standby mode unless you have just rebooted the mass spectrometer by pressing the Reset button on the power entry panel of the instrument (see "Rebooting the MALDI LTQ XL System" on page 38).

Placing the MALDI Source in the On Mode

Table 2 describes the three operation modes for the MALDI source.

Table 2. MALDI source operation modes

Mode	Description	
Off	 	When the LTQ XL mass spectrometer is in the Off or Standby modes, the MALDI source is in the Off mode. In the Off mode, the Turn on/Turn off MALDI source and the Start/Stop Acquisition buttons at the bottom of the MALDI Source dialog box are unavailable.
Standby	 	When the LTQ XL mass spectrometer is in the On mode, the MALDI source is in Standby mode, by default.
On	 	Clicking the Standby button, places the MALDI source in the On mode. In the On mode, the laser is firing. Clicking the Start/Stop Acquisition button, starts the laser fire, if it is not already firing, and starts data acquisition to an Xcalibur raw file. Clicking the Start/Stop Acquisition button a second time stops data acquisition.

❖ To place the MALDI Source in the On mode

Click  **Turn on MALDI Source** at the bottom of the MALDI Source dialog box.

The laser starts firing. If the MALDI source has been in Standby mode for more than 15 minutes, the laser initializes before firing, resulting in a 10 second delay. During this delay period, the Standby button flashes on and off and the Start/Stop Acquisition button is unavailable.

❖ To place the MALDI Source in the Standby mode

Click  at the bottom of the MALDI Source dialog box.

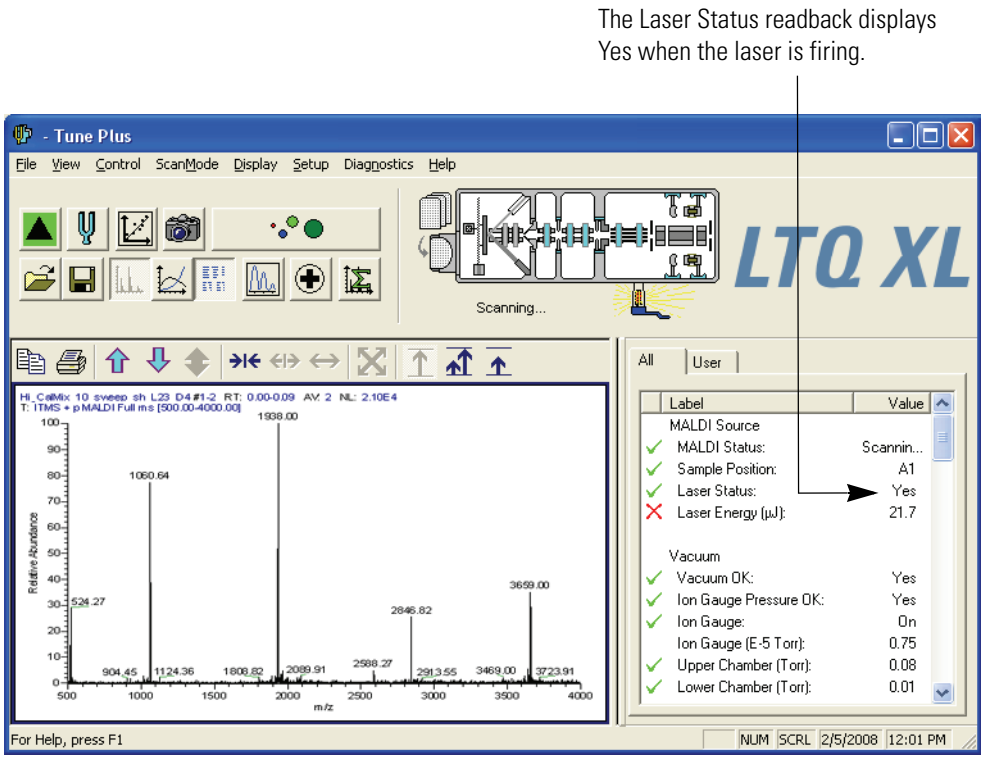
When you are acquiring an Xcalibur raw data file, stopping data acquisition also places the MALDI source in the Standby mode.

Because the MALDI laser has a limited lifespan, place the MALDI source in the Standby mode when not in use, even for brief periods.

The Status view displays the following readback states for the MALDI laser:

- When the MALDI source has been in Standby mode for more than 15 minutes, the status readback displays Off. When the laser is in the Off mode, it must go through an initialization process before firing.
- When the laser is not firing (but has fired shots within the last 15 minutes), the status readback displays Standby.
- When the laser is firing, the status readback displays Yes (see Figure 24).

Figure 24. Tune Plus window with the Status view showing the laser firing



Rebooting the MALDI LTQ XL System

Occasionally, you might need to reboot the LTQ XL mass spectrometer. When you reboot the LTQ XL mass spectrometer, the high voltage to the electron multipliers does not turn on until you take the LTQ XL mass spectrometer out of Standby mode and place the MALDI source in the On mode.

❖ To reboot the MALDI LTQ XL system

1. Press the Reset button (see Figure 25) on the power entry panel of the LTQ XL mass spectrometer.

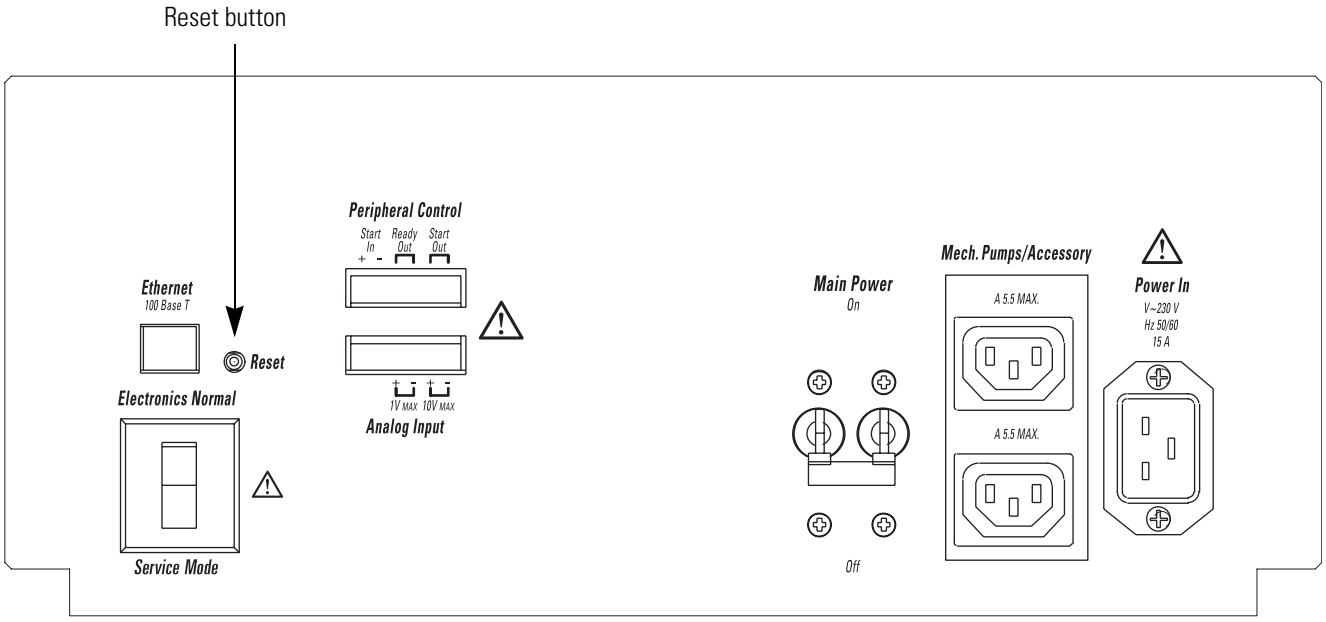
   The instrument goes through an initialization process that you can monitor in the LTQ XL console window.

2. In the Tune Plus window, click **On/Standby**  to turn on the LTQ XL mass spectrometer.

3. Click  at the bottom of the MALDI Source dialog box to place the MALDI source in the On mode.

4. Click  to place the MALDI source back in the Standby mode.

Figure 25. Power entry panel located on the lower-right side of the MALDI LTQ XL system



Using the Diagnostics Tool Box

The Thermo Fisher Scientific field service engineer uses the Diagnostics tool box during installation of the MALDI LTQ XL system. You can also use this feature to provide diagnostic information to your Thermo Fisher Scientific field service engineer.

The following procedures describe how to open the Diagnostics tool box, check the current calibration settings, and check the sample plate type:

- Opening the Diagnostics Tool Box
- Displaying the Currently Saved Calibration Settings
- Checking or Selecting the Sample Plate Type
- Adjusting the Video Camera Contrast and Brightness

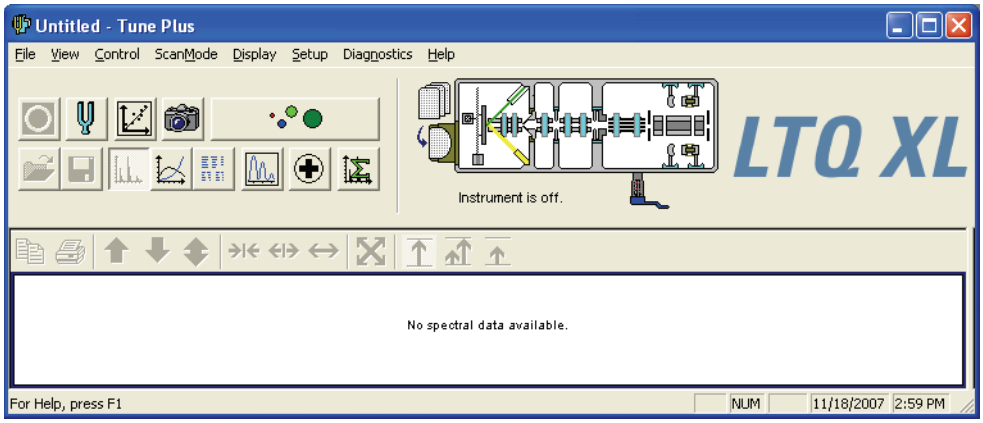
Opening the Diagnostics Tool Box

❖ **To open the Diagnostics tool box**

1. Open Tune Plus by doing one of the following:
   - From the computer desktop, choose **Start > All Programs > Xcalibur > LTQ Tune**.
   - From the computer desktop, double-click the  **LTQ Tune** icon.

   The Tune Plus window appears. See Figure 26.

   Figure 26. Tune Plus window



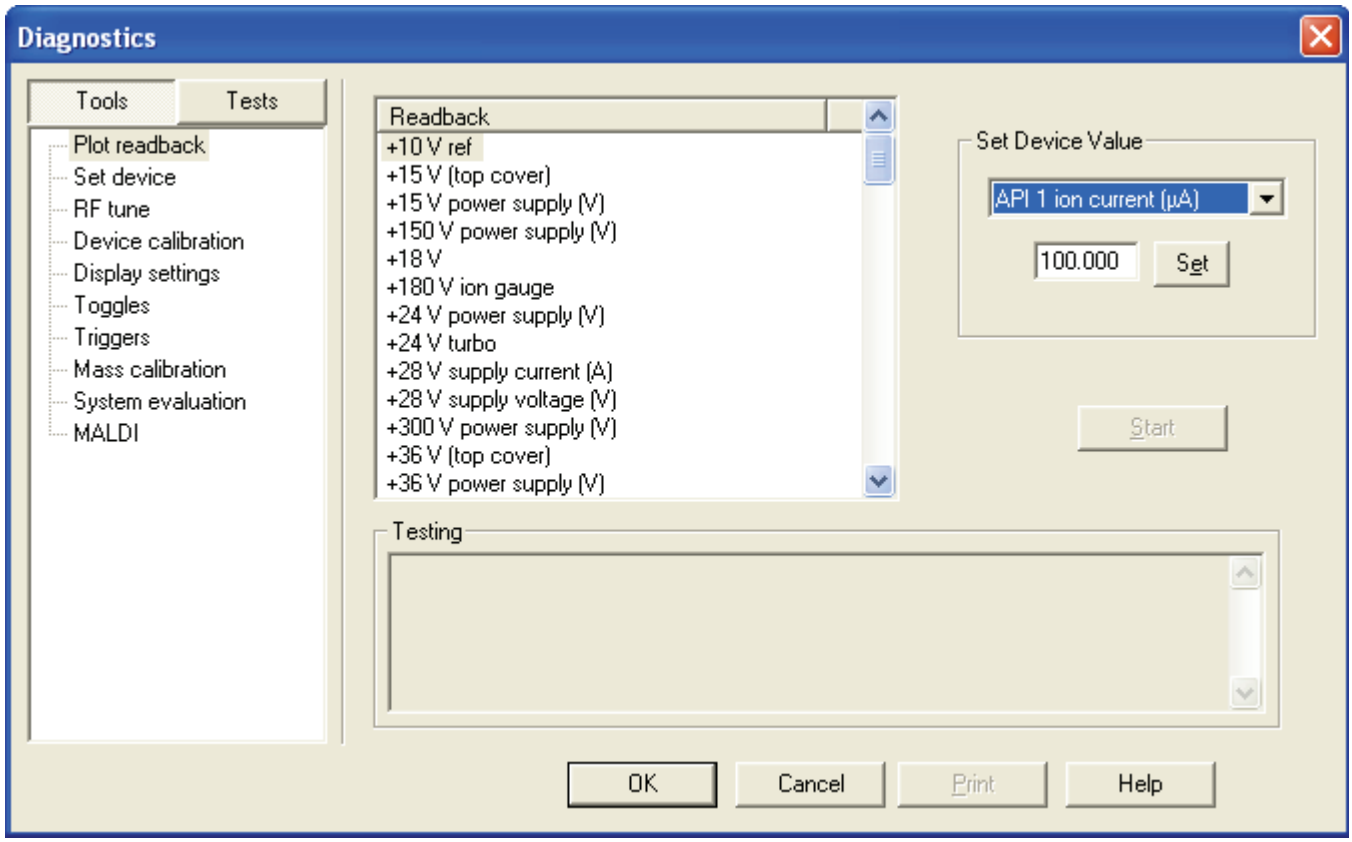
2. Choose **Diagnostics > Diagnostics** from the menu bar.

   The Diagnostics dialog box appears.

3. Click **Tools**.

   Figure 27 shows the Plot readback tool option.

Figure 27. Plot Readback tools selection in the Diagnostics dialog box



Displaying the Currently Saved Calibration Settings

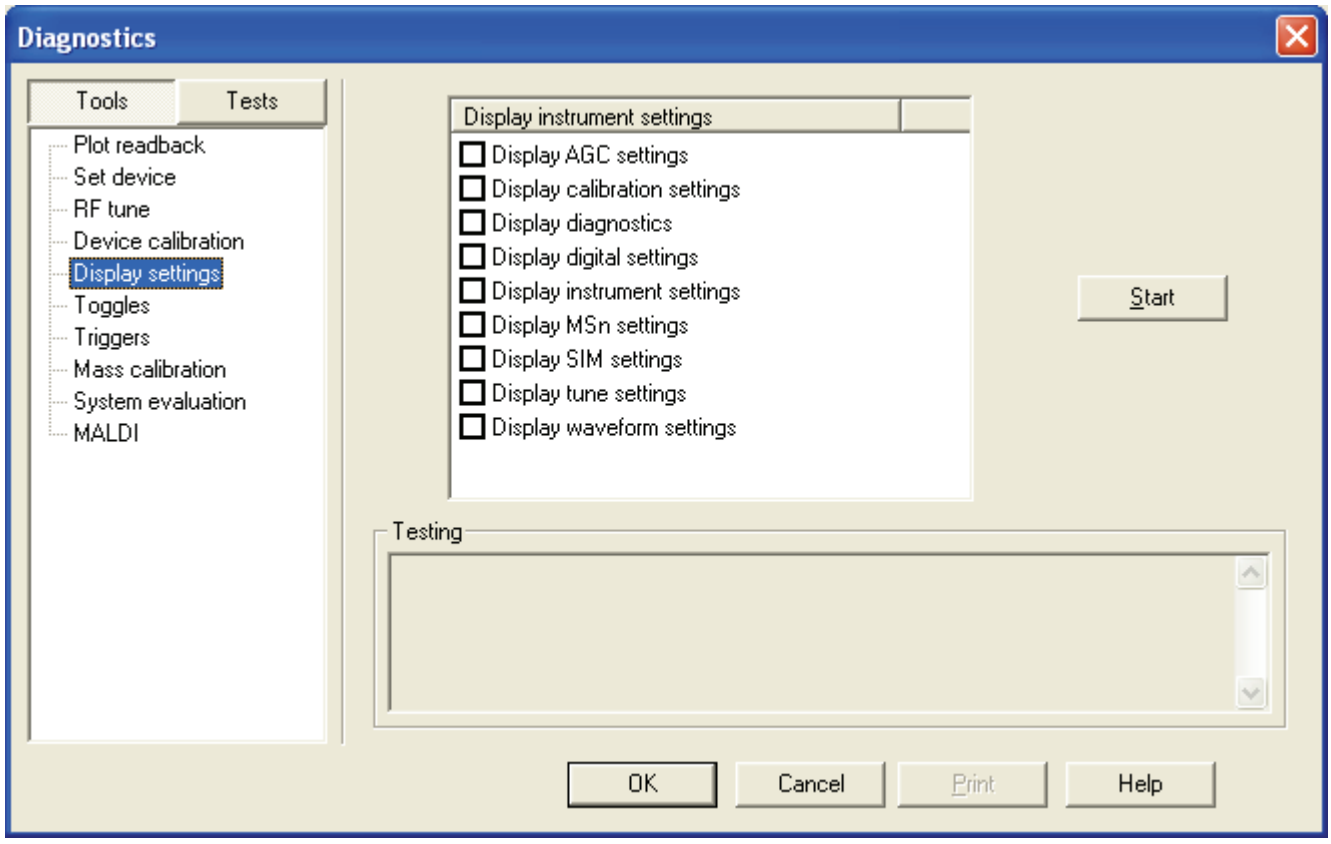
You can view the currently saved calibration settings from the Diagnostics dialog box.

❖ **To display the currently saved calibration settings**

1. Open the Diagnostics tool box as described in the previous topic, "Opening the Diagnostics Tool Box."
2. In the Tools list, select **Display settings**.

   Figure 28 shows the Display settings options.

Figure 28. Display settings options in the Diagnostics dialog box



3. Select the **Display calibration settings** check box.
4. Click **Start**.

   The calibration settings appear in the Testing pane. To print the current calibration values, click **Print**.

   The current calibration settings are stored in the MasterLTQ.cal file. You can find this file in the following directory:

   drive:\Xcalibur\system\LTQ\msx

Checking or Selecting the Sample Plate Type

If the MALDI system cannot locate the identification marks on the sample plate that you have loaded into the sample compartment, it defaults to the Thermo 384 Well Plate type. If the data system does not recognize the sample plate type that you have loaded into the MALDI sample compartment, you can perform the following diagnostic test.

Table 3 lists the identification marks for the various types of Thermo Scientific plates you can use with the MALDI source.

Table 3. Sample plate identification mark

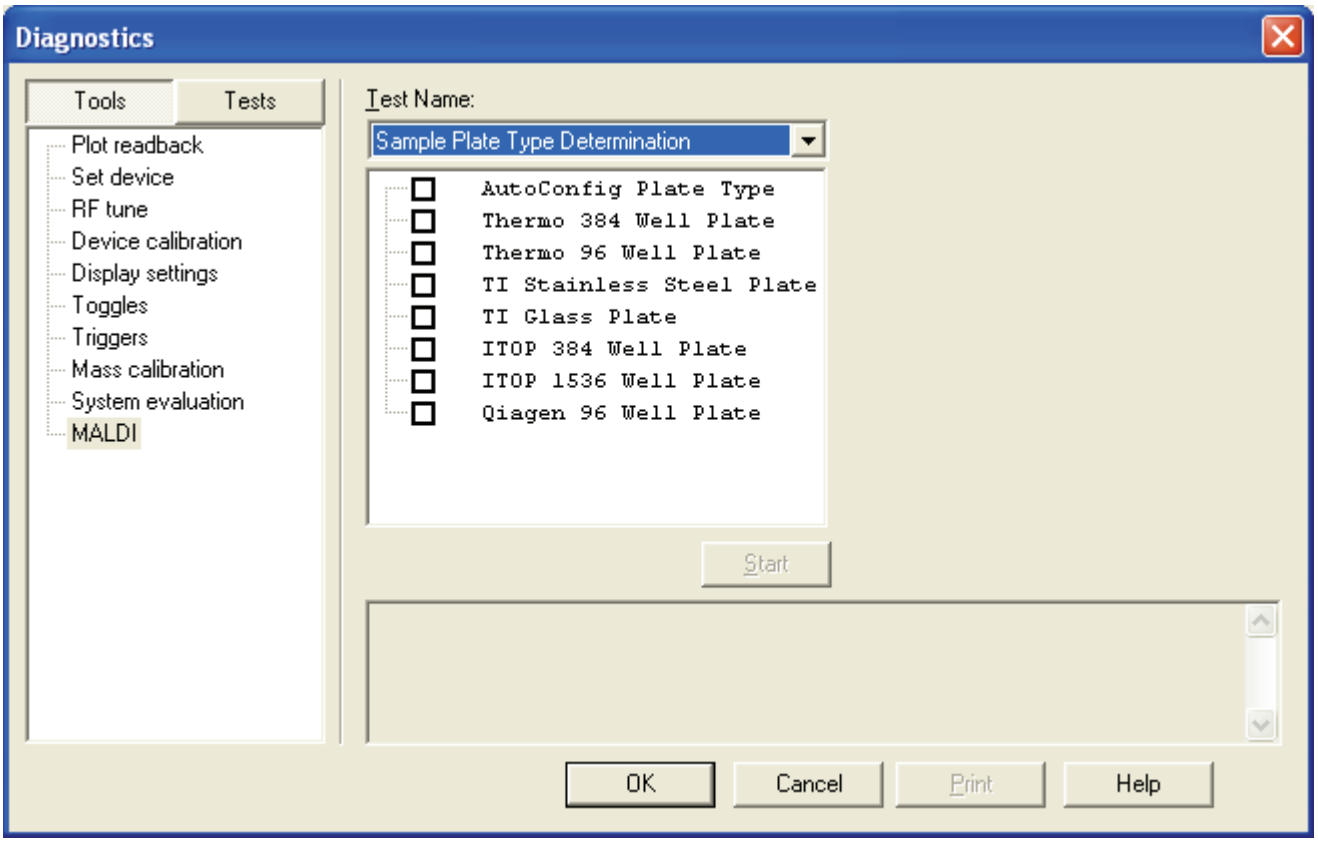
Plate type	Identification mark	
96-well plate		Four squares in a plus sign arrangement
384-well plate		Five squares in an X arrangement
Adapter plate for stainless steel slides (tissue imaging)		Capital T
Adapter plate for glass slides (tissue imaging)		Capital I

❖ To check the sample plate type

1. Open the Diagnostics tool box as described in "Opening the Diagnostics Tool Box" on page 39.
2. In the Tools list, select **MALDI**.
3. In the Test Name list, select **Sample Plate Type Determination**.

   A set of check boxes for the sample plate types appears. See Figure 29.

Figure 29. Sample Plate Type Determination test options



4. Select the **AutoConfig Plate Type** check box.
5. Click **Start**.
6. If the camera cannot locate the plate identification marks, do one of the following:
   - Increase the video camera contrast and brightness as described in the next procedure, "Adjusting the Video Camera Contrast and Brightness."
   - Select the check box that corresponds to the plate type you have inserted into the MALDI sample module, and then click **Start**.

Adjusting the Video Camera Contrast and Brightness

If the sample plate looks too dark or blurry in the image view of the MALDI Source dialog box, or if the camera cannot locate the plate identification marks on the plate, try adjusting the video camera contrast and brightness.

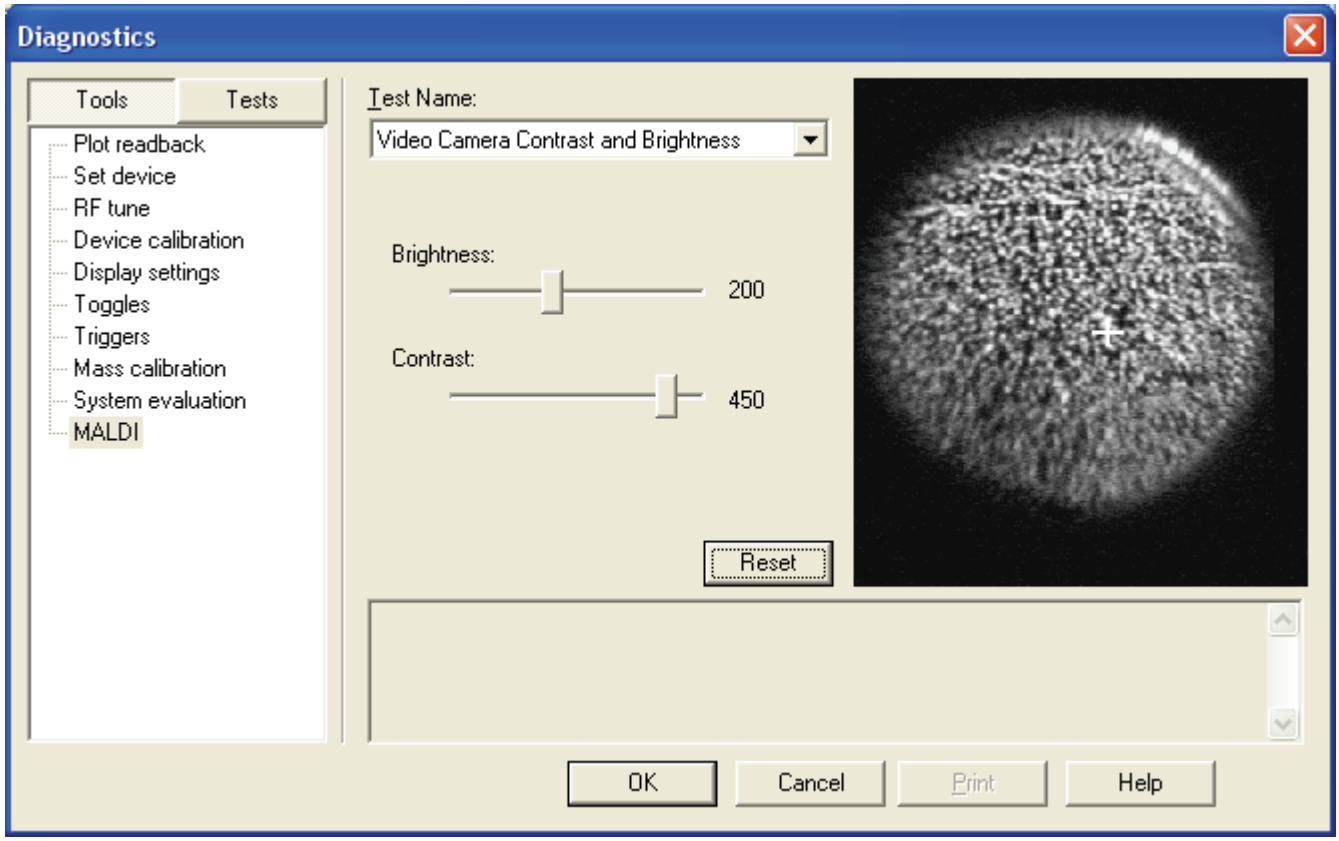
❖ To adjust the video camera contrast and brightness

1. Open the Diagnostics tool box as described in "Opening the Diagnostics Tool Box" on page 39.

2. In the Tools list, select **MALDI**.
3. In the Test Name list, select **Video Camera Contrast and Brightness**.

   The parameters for the Video Camera Contrast and Brightness test appear. See Figure 30.

Figure 30. Video Camera Contrast and Brightness settings



4. Increase the brightness by approximately 20%.
5. Click **OK**.

   Note Clicking the Reset button restores the factory default values for brightness and contrast.

6. Repeat step 4 and step 5.
7. If the camera cannot locate the plate identification marks after you increase the video brightness, remove the plate from the system and clean it.

For more information on the Diagnostics tool box, refer to the *MALDI Source Hardware Manual*.

Working with Sample Plates

This chapter describes how to clean a sample plate before you spot it and how to load the sample plate into and remove the sample plate from the MALDI sample module.

Contents

Preparing the Sample Plate

For accurate analyses, you must clean the sample plate before you spot it with the calibration mixtures, the sensitivity test solutions, or your samples.

To prepare the sample plate, follow these procedures:

1. Disassembling the Sample Plate
2. Cleaning the Top Plate of the Sample Plate
3. Assembling the Sample Plate

Disassembling the Sample Plate

The MALDI sample plate consists of two parts: a top plate, which is engraved in either a 96-well or 384-well format, and a base plate, on which the top plate is mounted. You only need to clean the top plate.

❖ **To remove the top plate from the base plate**

IMPORTANT When handling a sample plate, be sure not to scratch the plate surface. Hold the sample plate by the edges. Never touch the plate surface, even when wearing gloves.

1. Wearing gloves, hold the sample plate upside down. Grasp the top plate by the edges, being careful not to touch its surface.
2. Pull the latch on the back of the base plate in the direction of the arrow to disengage the top plate from the base plate.
3. Pull the top plate away from the base plate.

Cleaning the Top Plate of the Sample Plate

Before you prepare the calibration mixture and spot it on the sample plate, clean the surface of the sample plate to remove contaminants and ensure a good surface for sample deposition.

CAUTION Using sample plates other than those designed or certified by Thermo Fisher Scientific for use with the MALDI source can severely damage the source.

Thermo Fisher Scientific recommends the following procedures to clean the surface of the sample plate:

- Routine Cleaning
- Deep Cleaning

If the routine cleaning procedure does not remove the surface contamination, try the deep cleaning procedure.

Routine Cleaning

Before beginning the routine cleaning procedure, make sure you have the following items available:

- Soft wipes (optical lens wipes or soft clean room wipes are recommended to avoid scratching the plate surface)
- Compressed difluoroethane (sold as compressed air for removing dust) or ultra high quality nitrogen

IMPORTANT It is best to use gas from an oil-free compressed gas system. Gas from an aerosol can contaminate the plate with propellant (liquid difluoromethane). If you must use gas from an aerosol can, be sure to prevent the propellant from contaminating the plate surface. If you contaminate the plate with propellant, you must clean it again. Take the following precautions to avoid getting the propellant on the sample plate:

- Do not shake the compressed gas can.
- Keep the can upright when spraying.
- Spray in a series of short bursts rather than in a single continuous stream.
- When the can is close to being empty, replace it with a fresh can.

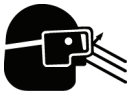
- Sonicator (ultrasonic cleaner)

CAUTION The sonicator (ultrasonic cleaner) must be approved for use with flammable vapors. Perform the sonication in a chemical fume hood.

This procedure also requires the following solvents, which must be HPLC grade or better:

- HPLC grade acetonitrile
- HPLC grade methanol
- 18 MΩ-cm deionized water or HPLC grade water

CAUTION This procedure uses chemicals that are toxic and volatile. Wear gloves, work in a fume hood, and take other appropriate precautions while cleaning the sample plate.



CAUTION AVOID EXPOSURE TO POTENTIALLY HARMFUL MATERIALS. Always wear safety glasses when you use solvents or corrosives. Refer to your supplier's Material Safety Data Sheet (MSDS) for the proper handling of a particular solvent or compound.

❖ To clean the sample plate using the routine cleaning procedure

1. Sequentially rinse the plate with the following solvents and use a soft wipe to wipe the surface of the plate dry between each solvent rinse:
   a. Acetonitrile
   b. Methanol
   c. Water
2. Place the plate in a 1000-mL beaker or similar container. The beaker or container must be inert to acetonitrile.
3. Add enough acetonitrile to cover the entire plate.
4. Cover the beaker with aluminum foil, place it in the sonicator, and sonicate for 5 minutes.

CAUTION The sonicator (ultrasonic cleaner) must be approved for use with flammable vapors. Perform the sonication in a chemical fume hood.

5. Replace the acetonitrile with methanol and sonicate for 5 minutes.
6. Replace the methanol with water and sonicate for 5 minutes.
7. Remove the plate from the beaker.
8. Dry the plate completely with ultra high quality compressed air or ultra high quality nitrogen.
9. Store the plate under vacuum for a few hours to ensure complete dryness.

   Storing the plate under vacuum is especially important when spotting calibration samples, since the high organic content of the calibration mix can cause it to spread if the plate is not completely dry.

Deep Cleaning

Before beginning the deep cleaning procedure, make sure that you have the following items available:

- Sonicator

CAUTION The sonicator (ultrasonic cleaner) must be approved for use with flammable vapors. Perform the sonication in a chemical fume hood.

- Soft wipes (optical lens wipes or soft clean room wipes to avoid scratching the plate surface
- Ultra high quality nitrogen or compressed air in a can, sold as compressed air for removing dust

IMPORTANT It is best to use gas from an oil-free compressed gas system. Gas from an aerosol can contaminate the plate with propellant (liquid difluoromethane). If you must use gas from an aerosol can, be sure to prevent the propellant from contaminating the plate surface. If you contaminate the plate with propellant, you must clean it again. Take the following precautions to avoid getting the propellant on the sample plate:

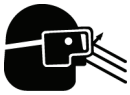
- Do not shake the compressed gas can.
- Keep the can upright when spraying.
- Spray in a series of short bursts rather than in a single continuous stream.
- When the can is close to being empty, replace it with a fresh can.

You must use the following solvents (HPLC grade or better) with this procedure:

- Isopropyl alcohol
- Acetonitrile
- Methanol
- Water
- 30% ammonium hydroxide solution

  Vapors from a solution of 30% ammonium hydroxide can irritate the respiratory tract and eyes. Skin contact with this solution can cause burns.

CAUTION This procedure uses chemicals that are toxic, corrosive, and/or volatile. Wear gloves, work in a fume hood, and take other appropriate precautions while cleaning the sample plate.



CAUTION AVOID EXPOSURE TO POTENTIALLY HARMFUL MATERIALS. Always wear safety glasses when you use solvents or corrosives. Refer to your supplier's Material Safety Data Sheet (MSDS) for the proper handling of a particular solvent or compound.

❖ To clean the sample plate using the deep cleaning procedure

1. Prepare a 3% ammonium hydroxide/acetonitrile cleaning solution by mixing 450 mL of acetonitrile, 450 mL of water, and 100 mL of 30% ammonium hydroxide. Mix the solution well.

   You can prepare this solution in advance and store it in a glass container with a securely fitted screw cap.

2. Wet the plate surface with isopropyl alcohol while wiping the surface gently with a soft wipe. Repeat this step several times.
3. Rinse the plate surface with methanol.
4. Place the plate in a 1000 mL beaker or other suitable container, and add the cleaning solution prepared in step 1 until the plate is covered.
5. Place the beaker in a sonicator. Cover the beaker with aluminum foil to prevent evaporation of the cleaning solution.

CAUTION The sonicator (ultrasonic cleaner) must be approved for use with flammable vapors. Perform the sonication in a chemical fume hood.

6. Sonicate a fully immersed plate for 5 minutes.
7. Using forceps, remove the plate from the cleaning solution, and place it in another container. Rinse several times with water to remove the cleaning solution.
8. Rinse the plate thoroughly with methanol.
9. Rinse the plate thoroughly with water.
10. Dry the plate completely with ultra high quality compressed air or ultra high quality nitrogen.
11. Store the plate under vacuum for a few hours to ensure complete dryness.

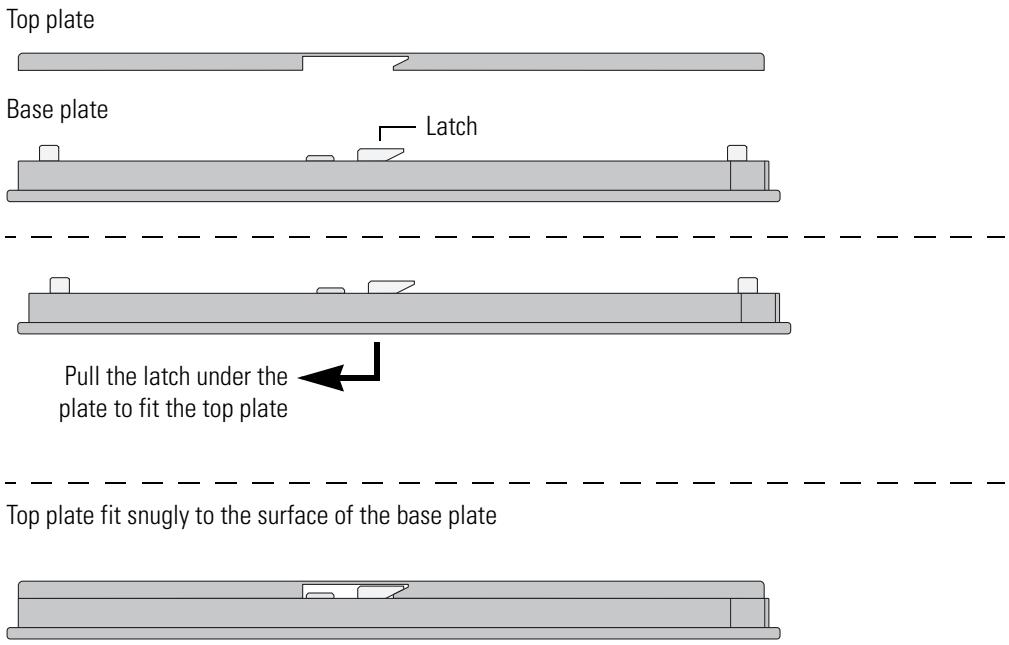
    Drying the plate under vacuum is especially important when spotting calibration samples since the high organic content of the calibration mix can cause it to spread if the plate is not completely dry.

Assembling the Sample Plate

❖ **To reassemble the clean sample plate**

1. Hold the base plate upside down.
2. Pull the latch on the back of the base plate in the direction of the arrow.
3. Fit the top plate snugly to the surface of the base plate. There must be no space between the top plate and the base plate. See Figure 31.

Figure 31. Sample plate assembly



CAUTION The top plate must be fit snugly to the base plate. Loading a misaligned plate assembly into the MALDI sample module can damage the MALDI source.

4. Release the latch on the back of the plate. Do not touch the surface of the wells.
5. (Optional) Place an identifying label on the plate with a marker pen. Do not tape anything to the sample plate.

CAUTION Do not tape anything to the sample plate. Loading sample plates with taped objects into the MALDI sample module can damage the MALDI source.

Loading a Sample Plate into the MALDI Sample Module

Always use sample plates approved by Thermo Fisher Scientific. Make sure that you have assembled the sample plate correctly and have not taped anything to the plate before you load the plate into the MALDI sample module. A grinding noise coming from the MALDI sample module usually indicates that a sample plate is jammed somewhere in the XYZ mechanism. If this situation occurs, turn off the power to the LTQ XL mass spectrometer and contact Thermo Fisher Scientific Technical Support.

CAUTION If you hear a grinding noise coming from the MALDI sample module and you suspect that a sample plate is jammed somewhere in the XYZ mechanism, turn off the power to the LTQ XL mass spectrometer.

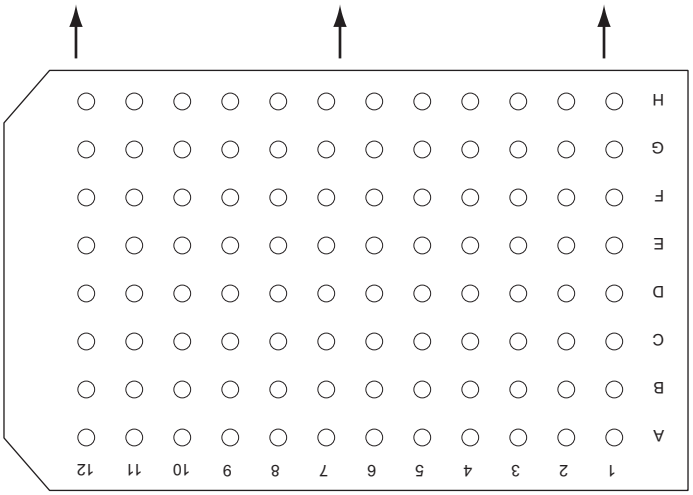
❖ To load the sample plate into the MALDI sample module

1. Start Tune Plus:
   a. From the LTQ workstation desktop, choose **Start > All Programs > Xcalibur > LTQ Tune**.
   b. Or, double-click the  **LTQ Tune** shortcut icon on the computer desktop.
2. If there is currently a sample plate loaded in the MALDI source, remove it before proceeding. See "Removing a Sample Plate from the MALDI Sample Module" on page 56.
3. Make sure that there is no gap between the top plate and the base plate and that nothing is taped to the sample plate.

CAUTION Inserting a misaligned sample plate assembly or a sample plate assembly with anything extra attached to it, such as a piece of tape, can damage the MALDI source.

4. Insert the sample plate into the loading slot in the front of the MALDI sample module. The slot accepts the plate in one orientation only. Ensure that the top edge of the plate with the row of numbers engraved on it faces outward. See Figure 32.

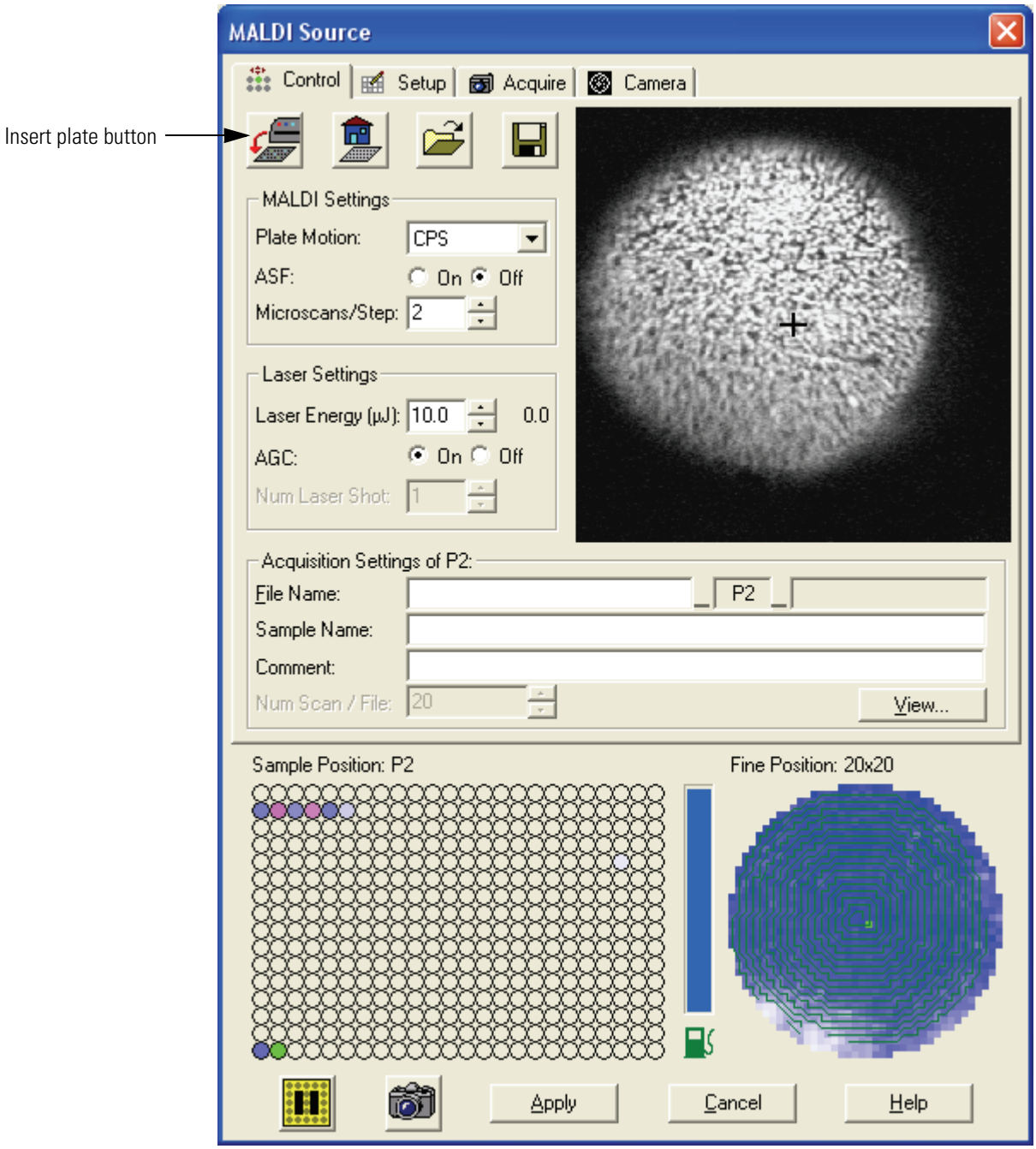
Figure 32. Schematic of MALDI sample plate with arrows showing the orientation of plate loading



5. In the Tune Plus window, choose **Setup > MALDI Source**.

   The MALDI Source dialog box appears with the Control tab shown by default. See Figure 33.

Figure 33. Control page of the MALDI Source dialog box



6. Click **Insert Plate** .

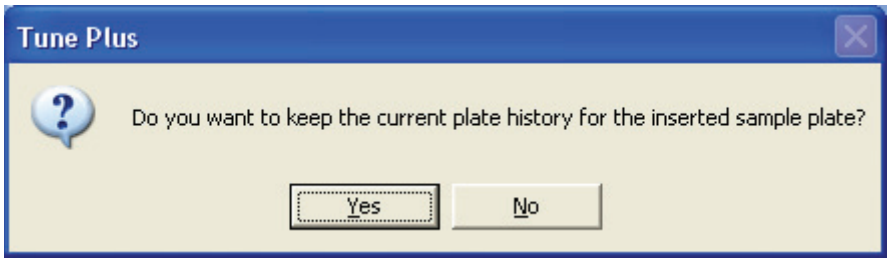
   The Instrument Messages dialog box appears. This dialog box displays messages indicating the progress of the plate insertion; the MALDI source retracts the plate into the sample chamber, pumps the chamber down to operating pressure, and calibrates the plate position. The plate insertion process takes approximately two minutes to complete.

Figure 34 shows the series of messages that appear when you insert a plate into the MALDI sample module.

Figure 34. Instrument Messages dialog box during the plate loading process

When the MALDI source completes the plate insertion process, this message appears (see Figure 35).

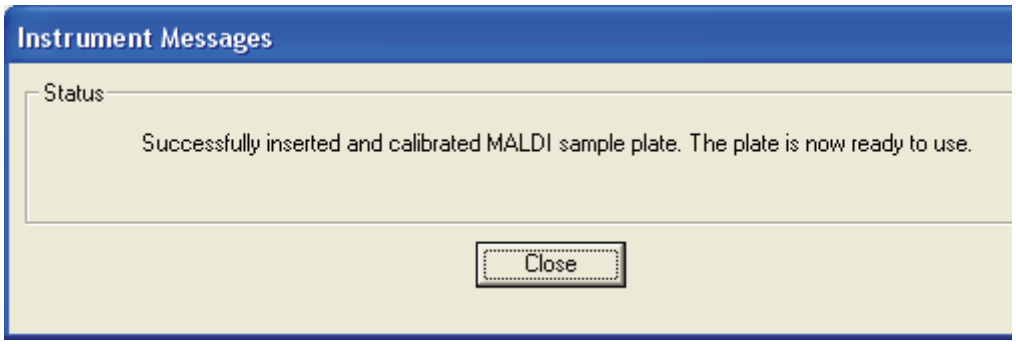
Figure 35. Query about keeping the current plate history



7. Do one of the following:
   - If you want to keep the current plate history, click **Yes**. The Open dialog box appears. Select the appropriate file (*.MALDIPlate) and click **Open**.
   - If you do not want to keep the current plate history, click **No**.

   When the MALDI source completes the insertion process, the Instrument Messages dialog box displays the message shown in Figure 36.
8. Click **Close** to close the dialog box.

Figure 36. Instrument Messages dialog box when plate insertion is complete



Removing a Sample Plate from the MALDI Sample Module

❖ **To remove the sample plate from the MALDI sample module**

1. If Tune Plus is not running, start it by doing one of the following:
   - From the LTQ workstation desktop, click the **LTQ Tune**  icon.
   - In the Instrument Setup window, choose **LTQ > Start Tune Plus**.
2. Choose **Setup > MALDI Source**.

   The MALDI Source dialog box appears.

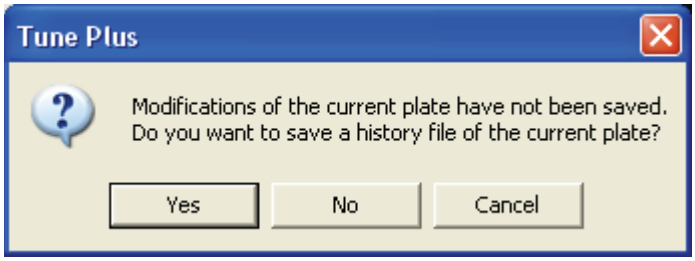
3. If the Control page is not visible, click the **Control** tab.

   The MALDI source must be in Standby mode for the buttons at the top of the page to be available.

4. Click **Eject Plate**  .

   The dialog box shown in Figure 37 appears.

   Figure 37. Prompt for saving history file

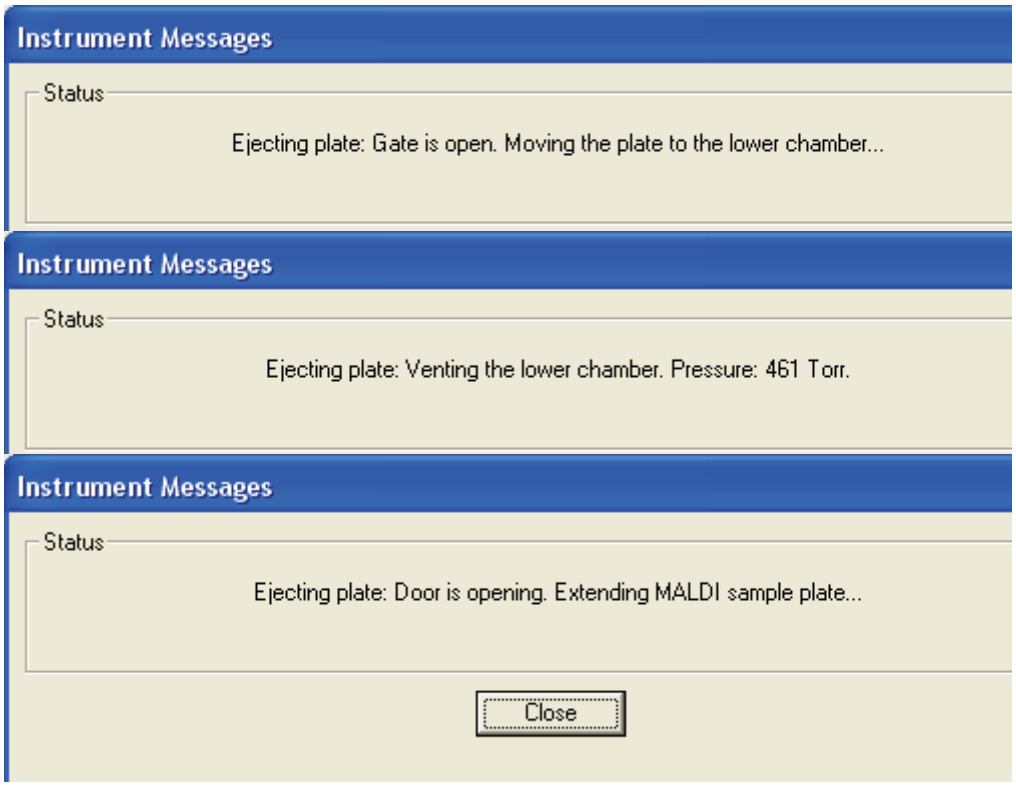


5. Do one of the following:

   - To save a history file, click **Yes**. The Save As dialog box appears. Save the file with an appropriate name.
   - To eject the plate without saving a history file, click **No**.
   - To stop the plate ejection process, click **Cancel.**

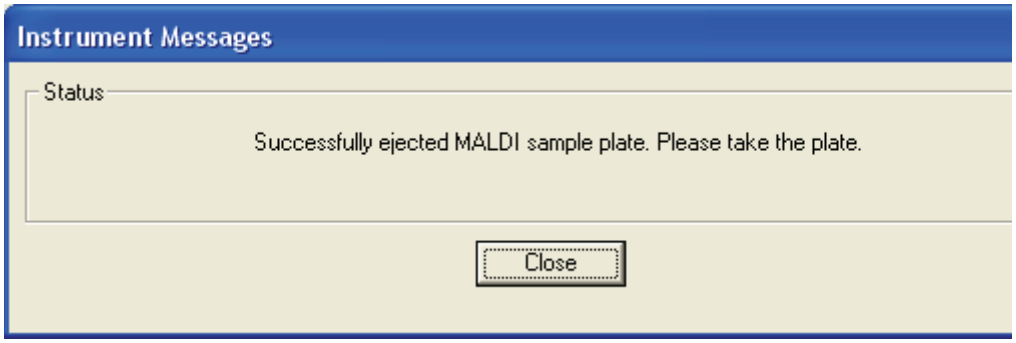
6. Observe the messages displayed in the dialog box as the MALDI source goes through the ejection process. The plate ejection process usually takes between two and three minutes to complete. Figure 38 shows the instrument messages that appear during the injection process.

Figure 38. Instrument messages that appear during the ejection process



When the ejection process is complete, the Instrument Messages dialog box displays the message shown in Figure 39.

Figure 39. Instrument Messages dialog box after plate ejection



7. Click **Close** to close the dialog box.
8. Remove the plate.

Tuning and Calibrating in the MALDI Mode

This chapter describes how to tune and calibrate and test the sensitivity of the MALDI LTQ XL system.

IMPORTANT These procedures use specially prepared calibrant/matrix mixtures. Before you spot the sample plate with the calibrant/matrix mixtures, ensure that the sample plate is clean and dry. To ensure dryness, store the sample plate under vacuum in the MALDI sample module until you are ready to spot the plate with the calibrant/matrix mixtures.

Contents

To tune and calibrate the LTQ XL mass spectrometer in the MALDI mode, perform these tasks:

1. Spot the calibrant/matrix mixtures on a plate that has been freshly cleaned for calibration.

   For information on preparing the calibration and sensitivity test targets, see the instructions sheet supplied in the Proteomass Calibration Kit or Appendix A, "ProteoMass Calibration Kit."

2. Load the spotted calibration plate into the MALDI sample module.

   For information on loading the sample plate, see "Loading a Sample Plate into the MALDI Sample Module" on page 52.

3. Tune the laser energy to optimize ionization as described in "Tuning the Laser Energy with the Semi-Automatic Function" on page 70.

4. Obtain a quick spectrum of the calibration mix to ensure that all of the peptides are observed.

5. With the tune file that contains the optimized laser energy open in the Tune Plus window, calibrate the mass spectrometer as described in "Calibrating the Mass Spectrometer" on page 76.

Use the Check page of the Calibrate dialog box to check the calibration on your mass spectrometer once a month, and recalibrate if it does not pass the calibration check tests or if you notice a decrease in mass accuracy.

Tuning on an Analyte

This topic describes how to create a tune file for your analyte of interest. For the tuning and calibration procedure, the analyte of interest is the calibrant/matrix mixture prepared by following the procedure in "Preparing the Mass Calibration Targets" on page 170.

To create a tune file, follow these procedures:

1. Setting the LTQ XL Parameters for Tuning
2. Setting the MALDI Source Parameters for Tuning
3. Viewing Mass Spectra in the Tune Plus Window
4. Tuning the Laser Energy with the Semi-Automatic Function or Adjusting the Laser Energy Manually

Table 4 lists the parameters stored in a MALDI tune file.

Table 4. Parameters stored in a tune file (Sheet 1 of 2)

Parameter	Description
Optimal laser energy for a mass range	The laser energy focused on a target spot determines the number of ions that enter the ion trap. A laser energy that is too low causes a poor signal-to-noise ratio for the ions of interest. A laser energy that is too high can cause space charging. Space charging, which is caused by too many ions in the ion trap, can result in poor mass resolution and mass shifts. Use the MALDI Source dialog box to set the laser energy.
AGC setting (On or Off), including the number of laser shots with AGC Off	• With the AGC On option selected, the MALDI source uses a prescan to determine the number of laser shots per microscan. • With the AGC Off option selected, you manually set the number of laser shots. Use the MALDI Source dialog box, to turn AGC On or Off.

Table 4. Parameters stored in a tune file (Sheet 2 of 2)

Parameter	Description
Number of microscans	A microscan is one mass analysis (ion injection and storage/scan out of ions followed by ion detection). Microscans are summed to produce one analytical scan. Summing microscans improves the signal-to-noise ratio of the mass spectral data. Increasing the number of microscans increases the time required to perform each scan. Use the Define Scan dialog box to set the number of microscans. Set the number of microscans in the Define Scan dialog box to a multiple of the microscans/step (enabled when ASF is Off) setting in the MALDI Source dialog box.
Number of sweep shots	The plate history file contains information about each sample spot, including a record of the fine positions within a sample spot that have been fired on by the laser. If the laser has not previously fired on a spot, the laser fires the specified number of sweep shots to remove contaminants from a spot. A sweep shot carries the same energy as a standard laser shot. Use the MALDI Source dialog box - Setup page to set the number of sweep shots.

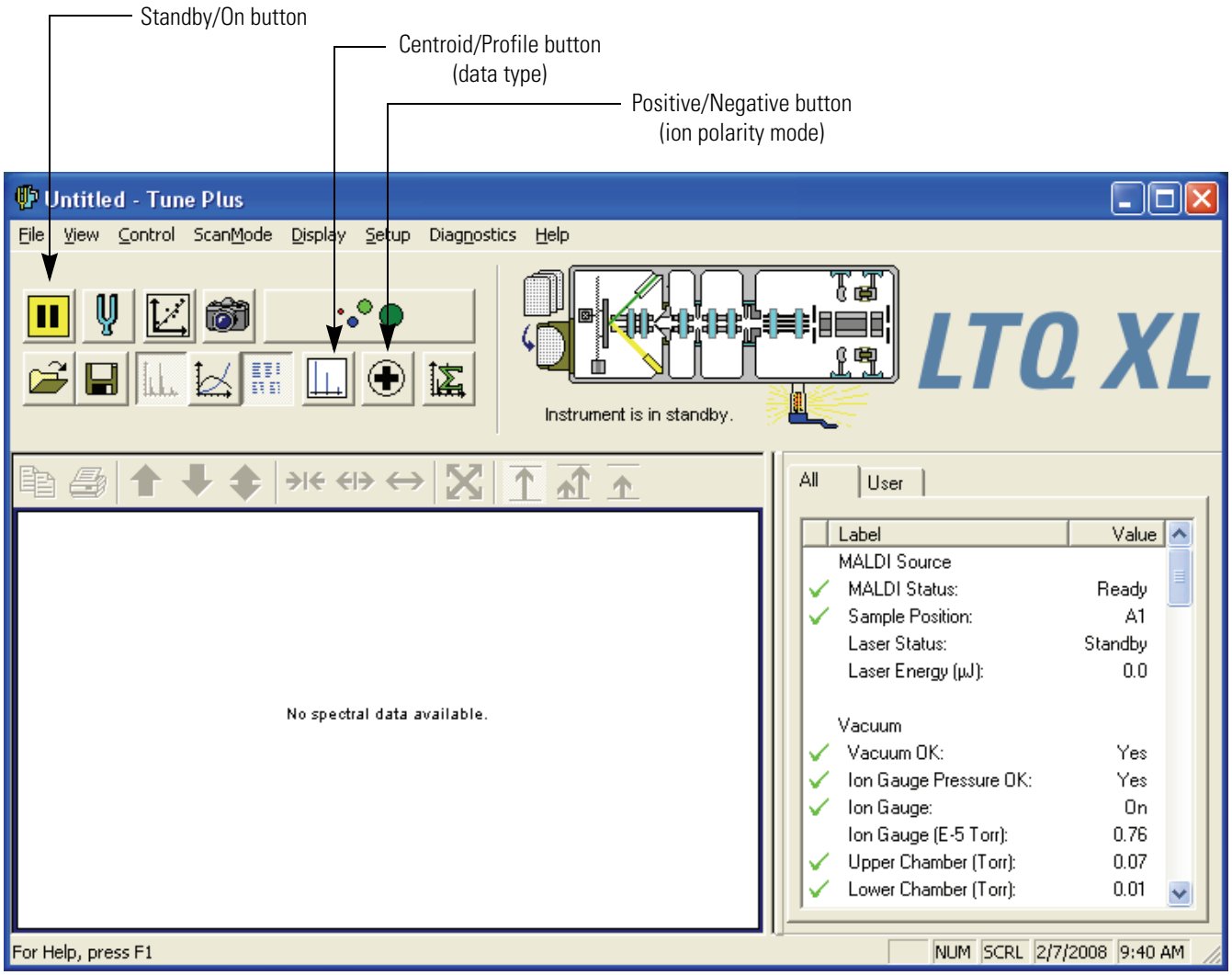
Setting the LTQ XL Parameters for Tuning

❖ **To set the LTQ mass spectrometer parameters for tuning and calibration**

1. Start Tune Plus:
   a. From the computer desktop, choose **Start > All Programs > Xcalibur > LTQ Tune**.
   b. Or, double-click the  **LTQ Tune** shortcut icon on the computer desktop.

   The Tune Plus window appears (see Figure 40).

Figure 40. Tune Plus window with a view of the LTQ XL mass spectrometer in Standby mode



2. On the Control/Scan Mode toolbar, click the **On/Standby** button to take the mass spectrometer out of Standby mode.
3. Open the default MALDI tune method:
   a. Choose **File > Open** to display the Open dialog box.
   b. Browse to the following folder:

      C:\Xcalibur\methods

   c. Select the **Default_MALDI.LTQtune** file or a tune file with an appropriate laser energy setting for the calibrant/matrix mixture.
   d. Click **Open** to open the file.

      The title bar displays the name of the tune file.


Profile


Centroid

4. On the Control/Scan Mode toolbar, ensure that the data type is set to Profile.

   Because it retains resolution information, use the profile data type for MALDI. In profile mode, the peak has an area and a width. In centroid mode, the whole peak area is contained in a spike. Internal calculations by the program use centroid peaks regardless of the acquisition mode selected.

5. Click **Positive/Negative**  to switch the ion polarity mode to positive.

6. Specify the scan parameters for the LTQ XL mass spectrometer:

   a. Ensure that the advanced settings are turned on by choosing **Scan Mode** from the menu bar.

      If the Advanced Scan Mode command in the drop-down menu has a check mark in front of it, the advanced settings are turned on. If not, choose **Advanced Scan Mode** to turn on the advanced settings.

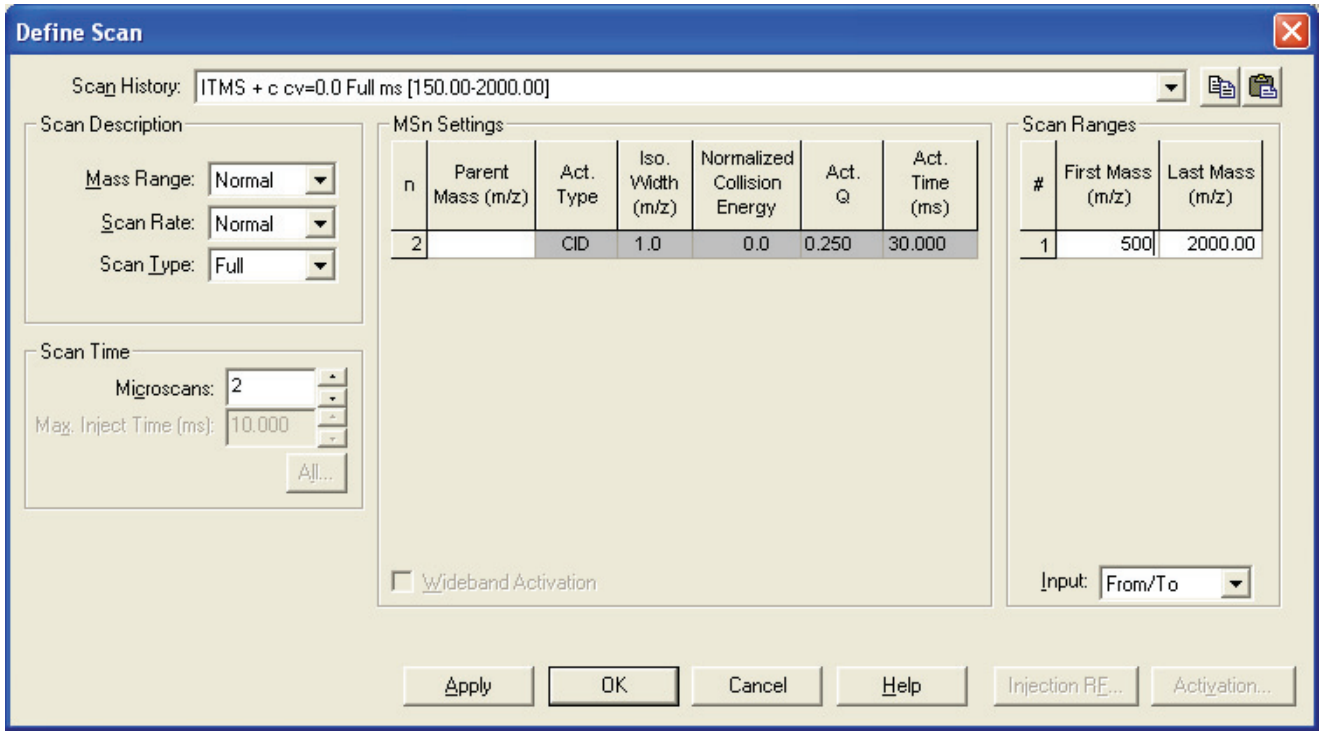
   b. On the Control/Scan Mode toolbar, click **Define Scan**  .

      The Define Scan dialog box appears.

   c. Set the parameters in the Define Scan dialog box. With the exception of the following settings, use the default settings. Figure 41 shows the settings for viewing the mass spectrum of the Normal Mass Range Calibration Mix.

Parameter	Setting	Result
Scan Description		
Mass Range	Normal	Specifies that the scan range for this scan falls within the Normal mass range (*m/z* 50 to 2000).
Scan Rate	Normal	Specifies Normal Scan Rate for this scan.
Scan Type	Full	Specifies the Full scan type.
Scan Time		
Microscans	2	Specifies 2 microscans per scan.
Scan Ranges		
First Mass (*m/z*)	500.00	Specifies that the first mass in the scan range is *m/z* 500.00.
Last Mass (*m/z*)	2000.00	Specifies that the last mass in the scan range is *m/z* 2000.00.

Figure 41. Define Scan dialog box with settings for MALDI tuning and calibration



d. Click **OK** to save the settings and to close the dialog box.

After you finish setting the LTQ XL parameters, set the MALDI source parameters as described in the next topic.

Setting the MALDI Source Parameters for Tuning

With modifications, you can use the following procedure to tune on your analyte of interest. The analyte of interest for tuning and calibration is either the Normal Mass Calibration Mix/matrix mixture or the High Mass Calibration Mix/matrix mixture. Because the calibrant/matrix mixture contains a high concentration of each of the calibration peptides, the Automatic Gain Control (AGC) feature, which automatically determines the number of laser shots required for a target spot, is not used. AGC is a useful feature for low concentration samples.

❖ To set the MALDI source parameter to tune on the calibrant/matrix mixture

1. In the Tune Plus window, choose **Setup > MALDI Source**.

   The MALDI Source dialog box appears with the Control page shown by default. See Figure 42 on page 66.

2. Click the **Control** tab.

3. For the calibrant/matrix mixture, use the following settings. Figure 42 shows the MALDI Source - Control page settings for tuning on the calibrant/matrix mixture. Figure 43 shows the Number Sweep Laser Shots setting. The laser energy setting for your MALDI LTQ XL system might differ.

Parameter	Setting
MALDI Settings	
Plate Motion	CPS
ASF	Off
Microscans per step	2
Laser Settings	
Laser Energy	current setting
AGC	Off
Num Laser Shot	3
Number Sweep Laser Shots (MALDI Source - Setup page)	5 to 10

Note Microscans per step are the number of microscans acquired at one crystal location before moving to a fresh MALDI crystal.

IMPORTANT For best results when creating tune files for low concentration samples in the attomole (10^{-18}) to femtomole (10^{-15}) range, select the On option for AGC. For best results when creating tune files for samples in the picomole (10^{-12}) to nanomole (10^{-9}) range, select the Off option for AGC and manually set the number of laser shots.

IMPORTANT Use laser sweep shots to remove contaminants from the target spots. Contaminants rise to the surface of target spots prepared with alpha-cyano-4-hydroxycinnamic acid (CHCA), the matrix used to prepare the calibrant/matrix mixtures.

4. Click **Apply**.

Figure 42. Control page of the MALDI Source dialog box

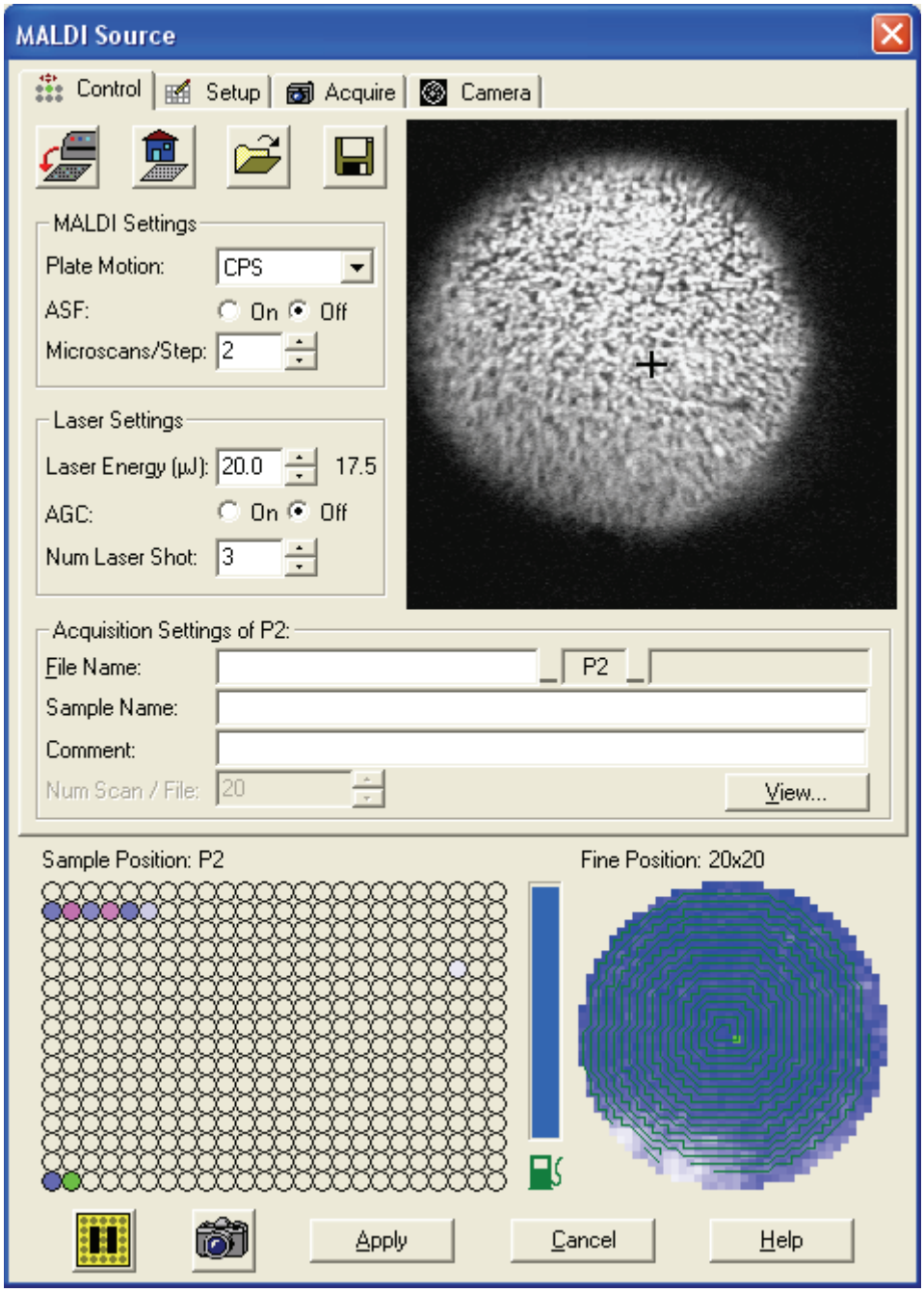
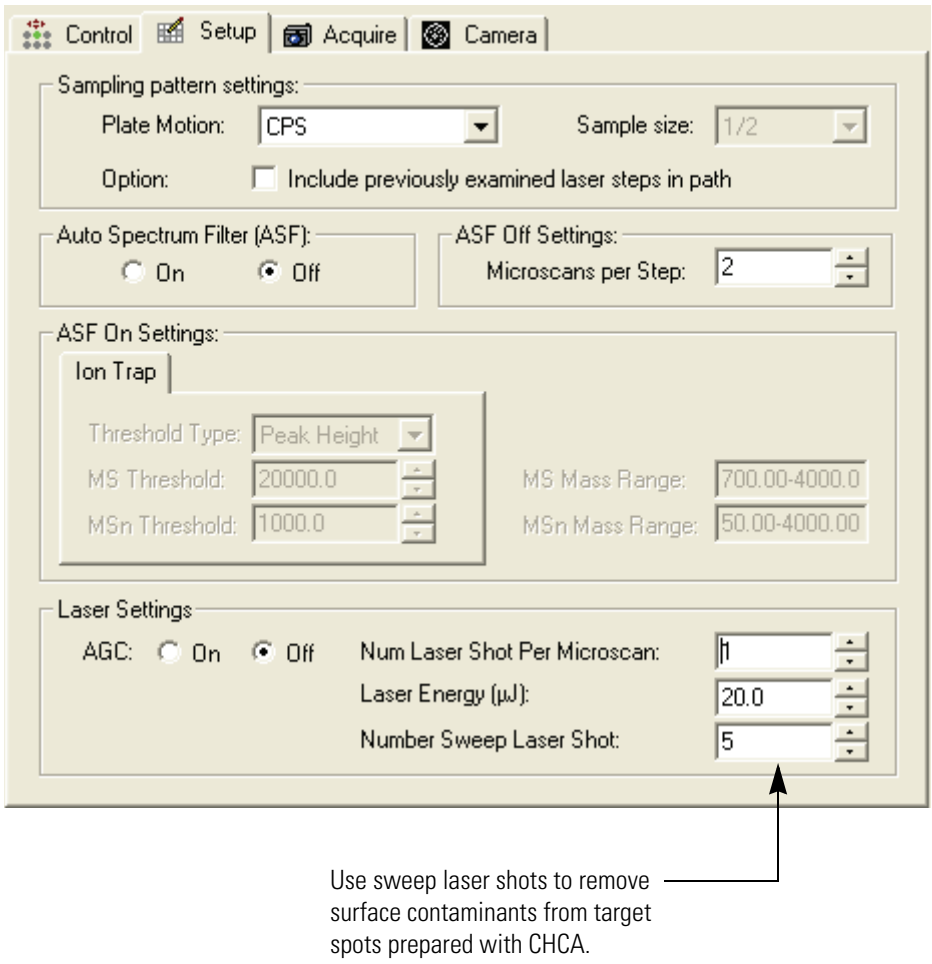


Figure 43. Setup page of the MALDI Source dialog box



Viewing Mass Spectra in the Tune Plus Window

Before you tune on an analyte or calibrate the mass spectrometer, view the mass spectrum of a test spot.

The following procedure describes how to view the mass spectrum of the Normal Mass Calibration Mix/matrix mixture. For information on preparing samples for MALDI analysis, see Appendix B, "Sample Preparation." Adjust the LTQ XL and MALDI source parameters for your sample/matrix mixtures.

❖ To view a mass spectrum in the Tune Plus window

1. Prepare the Normal Mass Calibration Mix/matrix mixture and spot the target plate as described in "Preparing the Mass Calibration Targets" on page 170.
2. Load the plate into the MALDI sample module as described in "Loading a Sample Plate into the MALDI Sample Module" on page 52.
3. Set the LTQ XL parameters as described on "Setting the LTQ XL Parameters for Tuning" on page 61.

   Adjust the parameters for your analyte.
4. Set the MALDI source parameters as described on "Setting the MALDI Source Parameters for Tuning" on page 64.

   Adjust the parameters for your analyte.
5. In the MALDI Source dialog box, select a spot on the target plate that has a homogeneous coating of the calibrant/matrix. To select a target spot, click a sample spot on the sample position map.

   When you click a sample spot on the position map, the spot turns red. The sample plate moves so that the laser is positioned to fire at the selected sample spot, and the spot turns green.
6. Click  in the lower left corner of the MALDI Source dialog box.

   The laser begins firing and the LTQ XL mass spectrometer begins scanning. The laser takes approximately 15 seconds to begin firing after the MALDI source has been in Standby mode for more than 15 minutes.
7. Position the Control page so that you can observe it and the Tune Plus window simultaneously.
8. On the File/Display toolbar in the Tune Plus window, click the  **Display Spectrum View** button to open the Spectrum view.
9. Monitor the spectrum for the masses of interest.

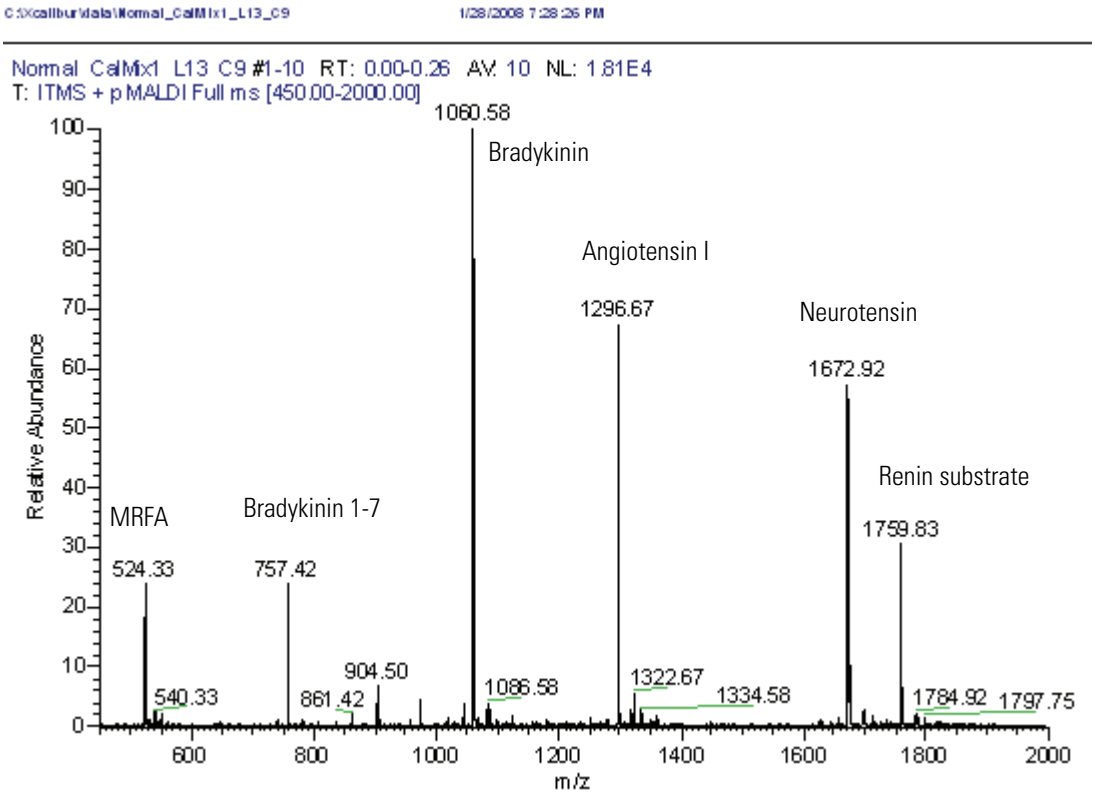
When the MALDI LTQ XL system is mass analyzing the Normal Mass Range Calibration Mix/matrix mixture, you should see a mass spectrum consisting of the singly charged ions listed in Table 5.

Table 5. Monoisotopic masses of the six peptides in the Normal Mass Range Calibration Mix

Peptide	$(M+H)^+$ Monoisotopic Mass
MRFA	524.27
Bradykinin 1-7	757.40
Bradykinin	1060.57
Angiotensin I	1296.69
Neurotensin	1672.92
Renin substrate	1758.93

Figure 44 shows the mass spectrum of the Normal Mass Calibration Mix.

Figure 44. Mass spectrum of the Normal Mass Calibration Mix



Tuning the Laser Energy with the Semi-Automatic Function

The laser energy is the most important parameter to tune with your MALDI source. Properly tuned laser energy is essential to obtaining high quality spectra. You do not need to tune the ion optics parameters, such as the multipole offset voltages and lens voltages.

Optimize the laser energy with the analyte of interest and save the value in a tune file. For the Normal Mass Calibration procedure, create a tune file by tuning on the normal mass range calibration mix/matrix mixture. For the High Mass Calibration procedure, create a tune file by tuning on the high mass calibration mix/matrix mixture. The optimum laser energy for these two samples differs by a few microJoules.

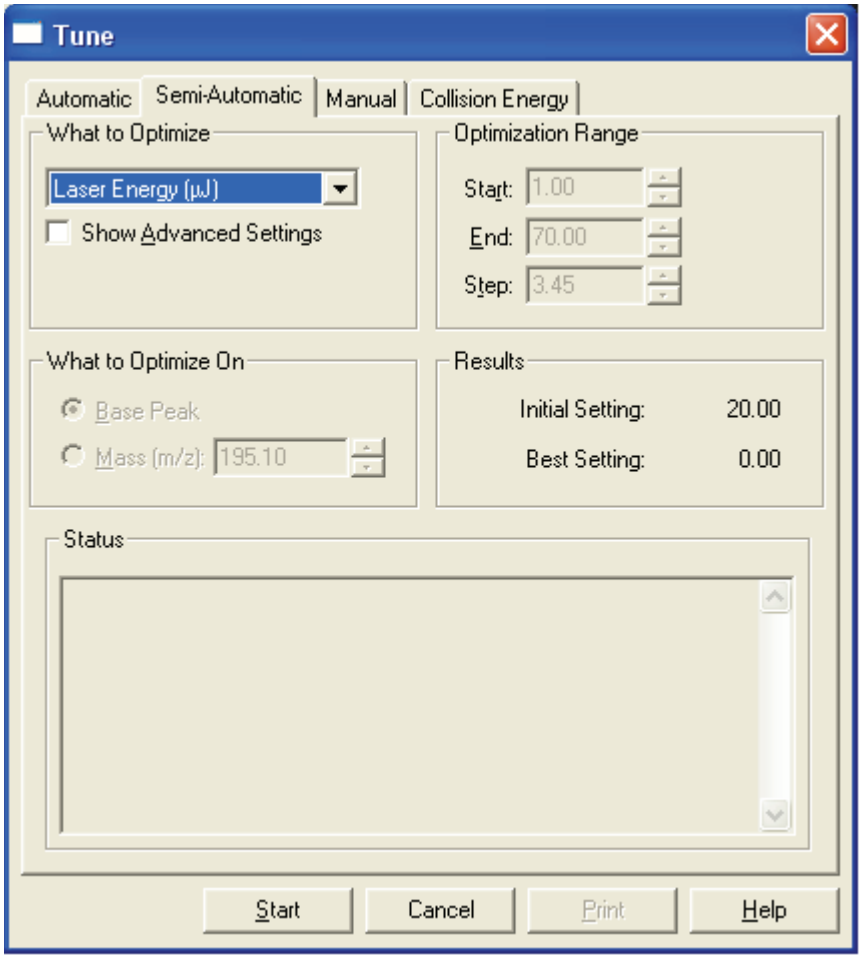
Before you start the automated tune procedure, you must set the LTQ XL mass spectrometer scan parameters. During the semi-automatic laser energy optimization procedure, the MALDI program automatically sets the MALDI source parameters.

❖ To tune the mass spectrometer

1. Select a sample spot and view the mass spectrum as described in the previous topic "Viewing Mass Spectra in the Tune Plus Window."
2. In the Tune Plus window, choose **Control > Tune**.

   The Tune dialog box appears with the Automatic tab shown by default.
3. Click the **Semi-Automatic** tab to open the Semi-Automatic page. See Figure 45.

Figure 45. Semi-Automatic page of the Tune dialog box

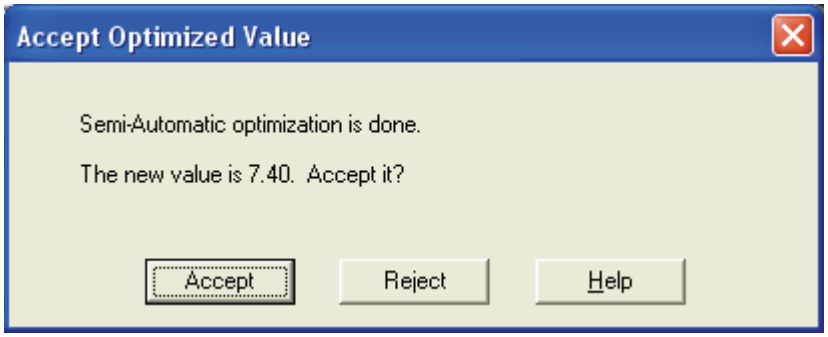


4. In the What to Optimize list, select **Laser Energy**.
5. Click **Start** to begin the tuning procedure.
6. Observe the Tune Plus window and the Tune dialog box.

   While the tuning procedure is in progress, the Spectrum and Graph views display various tests and the Status box in the Tune dialog box displays status messages.

   When the system determines the optimal laser energy for the target sample, the Accept Optimized Value dialog box appears. See Figure 46.

Figure 46. Accept Optimized Value dialog box with the new laser energy value



7. Click **Accept** to accept this laser energy value.

Note When the laser energy setting is optimized, the spectrum view displays all ions with a resolved isotopic pattern. The monoisotopic masses are within 0.15 to 0.20 u of the theoretical *m/z* for a particular peak (unless the mass calibration has shifted) and the signal-to-noise level for the mass peaks is reasonable. See Figure 44 on page 69.

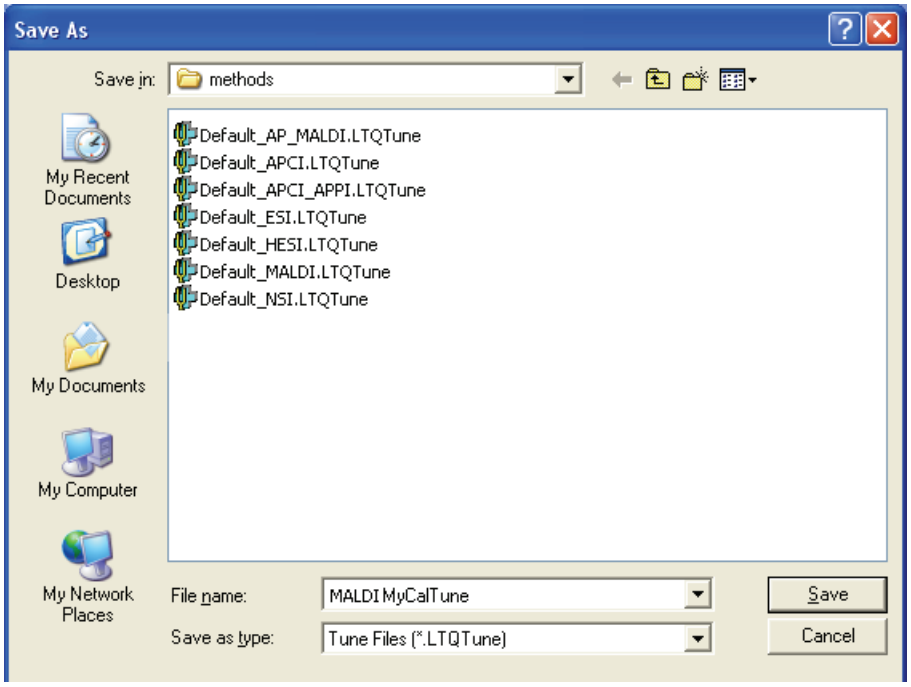
8. Save your tune method:
   a. Choose **File > Save As** to open the Save As dialog box.
   b. Browse to the following folder:

      C:\Xcalibur\methods.

   c. In the File name box, type a descriptive name for the file, such as *MALDIMycCalTune* or the date so that you can keep track of laser energy and conditions used on a specific day. See Figure 47.

      Tune files have a LTQtune file extension.

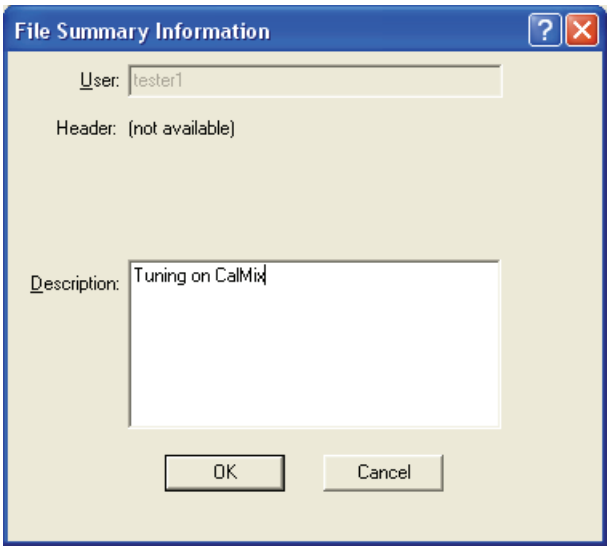
Figure 47. Save as file with a view of the Xcalibur\methods folder contents



d. Click **Save** to save the tune file.

The File Summary Information dialog box appears. See Figure 48.

Figure 48. File Summary Information dialog box



e. In the Description box, type a description for the tune file, and then click **OK** to save the file and return to the Tune Plus window.

The accepted laser energy value is stored in the saved tune file.

Adjusting the Laser Energy Manually

Correct adjustment of the laser energy is essential to obtain the best possible spectra with the MALDI source. If the laser energy is set too low, below the MALDI threshold, the laser desorbs primarily neutral molecules rather than ions, resulting in low signal or no signal. When the threshold level for ionization is achieved, analyte ions appear in the spectrum in amounts that increase with increasing laser energy. If the laser energy is set too high, the laser desorbs too many ions from the sample plate, including multiple clusters. This leads to high baseline, high chemical noise, and space charge effects. These in turn causes shifts in *m/z* that affect mass accuracy and reduce resolution. In addition, the higher the laser energy, the faster the consumption of sample. If you want to conserve sample, use the lowest laser energy setting that still gives good spectra.

The following procedures describes how to manually adjust the laser energy for the Normal Mass Calibration Mix/matrix mixture.

❖ To adjust the laser energy manually

1. View the mass spectrum of the Normal Mass Calibration Mix/matrix mixture as described in "Viewing Mass Spectra in the Tune Plus Window" on page 68.
2. Monitor the spectrum for the masses of interest.

   When the MALDI LTQ XL system is mass analyzing the Normal Mass Range Calibration Mix, you should see a mass spectrum consisting of the singly charged ions listed in Table 5 on page 69.
3. In the Control page, adjust the value in the Laser Energy (μJ) box to improve the signal according to guidelines given in Table 6.

   Table 6. Guidelines for MALDI laser power adjustment

Problem	Solution
No signal	Increase laser energy.
Low signal	Increase laser energy.
High baseline	Decrease laser energy.
High signal, low baseline, and poor mass resolution	Decrease laser energy in small increments.
High chemical noise	Decrease laser energy.
Peak broadening and shifting to higher mass values (space charge effects)	Decrease laser energy.

   Make initial adjustments in increments or decrements of 5, and then make finer adjustments in smaller increments.

If your signal is weak or noisy, make sure that the calibration sample is fresh. If the calibration sample is fresh and you cannot improve the signal by adjusting the laser energy, try another sample spot.

If the signal is too high, even when the laser energy is low, follow these steps to fix the problem:

1. Reduce the number of laser shots per spectrum: On the Control page of the MALDI Source dialog box, type or select a smaller value in the Num Laser Shot box. You can only access the Num Laser Shot box when AGC is Off.
2. If the signal is too strong, even with a single laser shot per spectrum, attenuate the ion beam by adjusting the front lens voltage:
   a. Choose **Setup > Ion Optics** to display the Ion Optics dialog box.
   b. Decrease the value in the Front Lens (V) box by 1 to 2 V. (Make the voltage more negative.)
   c. Click **Apply** to apply the changes.
   d. Observe the spectrum, and if necessary, make additional adjustments to the Front Lens voltage.
   e. Click **OK** to close the dialog box.

If you are not able to resolve the signal problems by taking these steps, consult the *MALDI Source Hardware Manual* for more information on diagnostics and troubleshooting, or contact technical support.

Calibrating the Mass Spectrometer

Calibrating the mass spectrometer ensures that the mass assignments and the number of ions in the ion trap are correct. The semi-automatic calibration procedure calibrates all of the calibration parameters, such as the multipole RF frequency and the electron multiplier gain.

If you want to calibrate the mass spectrometer in the high mass range, calibrate the normal mass range first, and then calibrate the high mass range.

To calibrate the mass spectrometer, follow these procedures as necessary:

- Calibrating the Normal Mass Range
- Recalibrating a Failed Item
- Calibrating the High Mass Range

Calibrating the Normal Mass Range

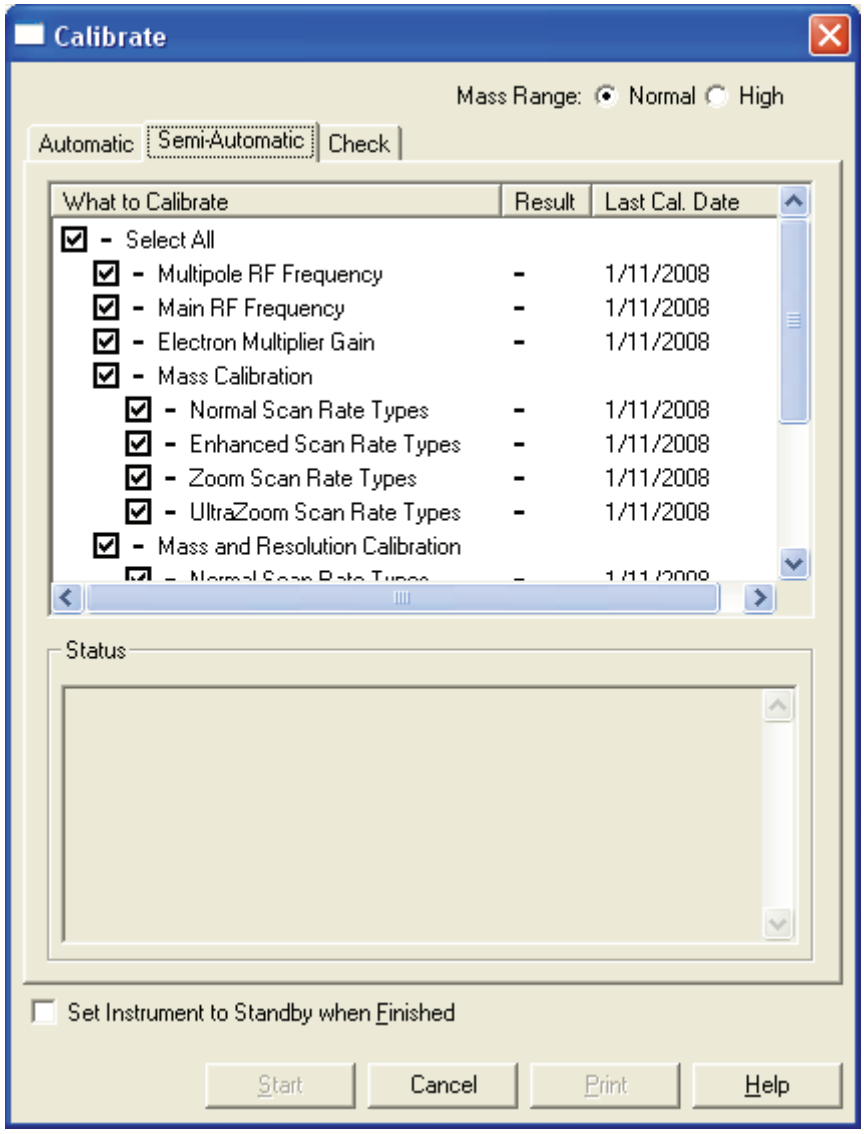
❖ **To calibrate the normal mass range of the mass spectrometer automatically**

1. If you have not already done so, optimize the laser energy for the normal mass calibration sample (see "Tuning the Laser Energy with the Semi-Automatic Function" on page 70).
2. In the Tune Plus window, ensure that a tune file with an appropriate laser energy is open. The title bar displays the current tune file.
3. View the mass spectrum of the Normal Mass Calibration Mix/matrix mixture (see "Viewing Mass Spectra in the Tune Plus Window" on page 68).
4. When you observe a stable mass spectrum, choose **Control > Calibrate**.

   The Calibrate dialog box appears with the Automatic page shown by default.
5. Click the **Semi-Automatic** tab.

   The Semi-Automatic page appears (see Figure 49).

Figure 49. Semi-Automatic page of the Calibrate dialog box



6. For the Mass Range, select the **Normal** option.
7. To select all items for calibration, select the **Select All** check box.
8. To start the semi-automatic calibration procedure, click **Start**.

   The program automatically downloads the appropriate scan settings for the normal mass range to the mass spectrometer, and the mass spectrometer uses the laser energy specified in the current tune file.
9. Observe the Tune Plus window and the Calibrate dialog box.

   While calibration is in progress, the Spectrum and Graph views display a variety of test results, and the Status box of the Calibrate dialog box displays status messages.

   The calibration procedure takes approximately 30 minutes to complete.

When the calibration procedure finishes, the Instrument Messages dialog box appears and indicates whether the calibration for each item was successful. If the calibration for an item is successful, the mass spectrometer saves the new calibration parameter automatically to the master calibration file on the hard disk.

10. If the calibration for a particular item fails, try recalibrating on that item individually as described in the next topic, "Recalibrating a Failed Item." Before recalibrating, check the spectrum again to make sure that all ions in the calibration mix are present with adequate signal-to-noise ratios.

Recalibrating a Failed Item

❖ **To recalibrate a failed item**

1. In the MALDI Source dialog box, Control page, select a new spot as necessary.
2. In the What to Calibrate list, select only the check box next to the item you want to recalibrate.
3. Click **Start** to recalibrate the selected parameters.
4. Repeat step 1 to step 3 until all calibration items have passed.

When all calibration items are successful, your mass spectrometer is properly calibrated in the normal mass range.

Calibrating the High Mass Range

Before you operate the mass spectrometer in the high mass range (up to 4000 u), calibrate the mass spectrometer in the high mass range.

Note When you operate the MALDI LTQ XL system in the high mass range, you might observe artifact peaks. These peaks are produced when some ions of a particular *m/z* are held in the ion trap and ejected later. This produces an artifact peak at a higher *m/z* value than the true *m/z* value of the ions. When seen, these artifact peaks are typically present at low intensities and cannot be isolated or fragmented.

❖ **To calibrate the high mass range of the mass spectrometer**

1. Prepare the High Mass Calibration Mix/matrix mixture and spot the target plate (see "Preparing the Mass Calibration Targets" on page 170).
2. Load the plate into the MALDI sample module (see "Loading a Sample Plate into the MALDI Sample Module" on page 52).
3. Open a tune file with an appropriate laser energy, or optimize the laser energy for the high mass calibration sample (see "Tuning the Laser Energy with the Semi-Automatic Function" on page 70).

4. View the mass spectrum of the High Mass Calibration Mix/matrix mixture:

   a. Set the LTQ XL parameters as described on "Setting the LTQ XL Parameters for Tuning" on page 61. with the following exceptions: select **High** in the Mass Range list and type **4000** in the Last Mass (*m/z*) box.

   b. Set the MALDI source parameters as described on "Setting the MALDI Source Parameters for Tuning" on page 64.

   c. In the MALDI Source dialog box, select a spot on the target plate that has a homogeneous layer of the calibrant/matrix.

   d. Click the **On/Standby** button at the lower-left corner of the MALDI Source dialog box.

      The laser begins firing.

   e. On the File/Display toolbar in the Tune Plus window, click the **Display Spectrum View** button to open the Spectrum view.

5. Monitor the spectrum for the masses of interest.

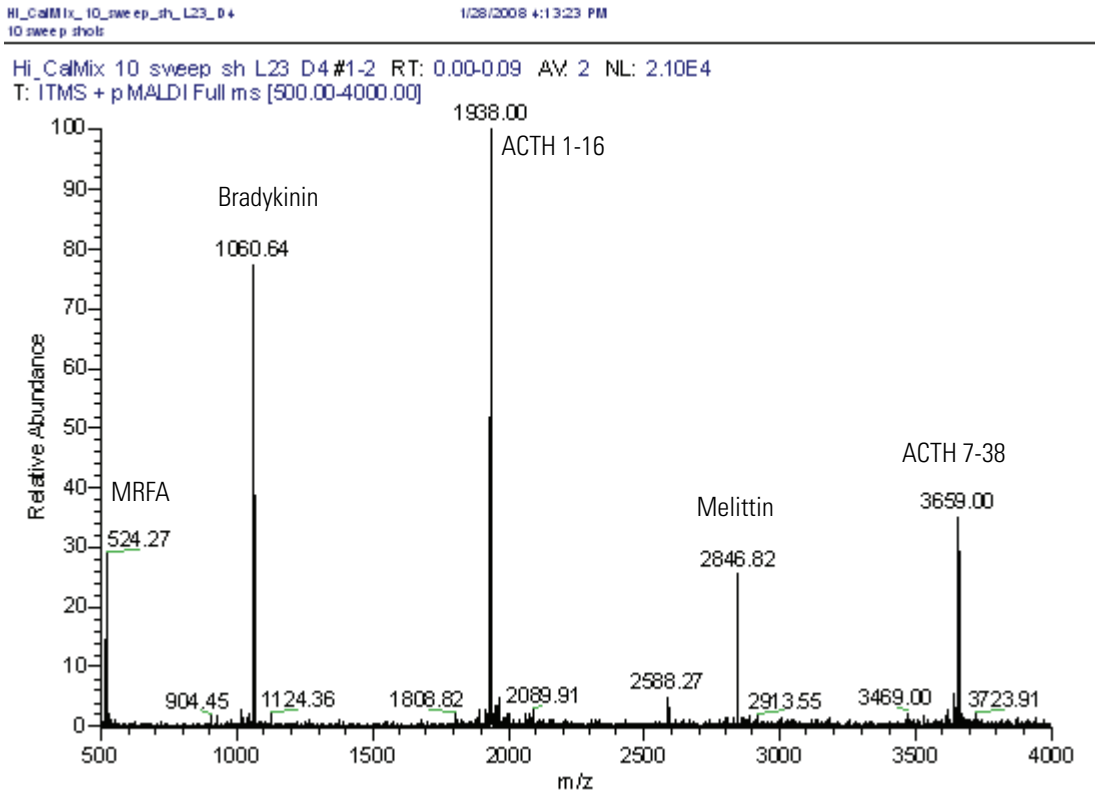
   The High Mass Calibration Mix contains the five peptides listed in Table 7.

Table 7. Monoisotopic masses of the five peptides in the High Mass Range Calibration Mix

Peptide	$(M+H)^+$ Monoisotopic Mass
MRFA	524.27
Bradykinin	1060.57
ACTH 1-16	1936.99
Melittin	2845.75
ACTH 7-38	3657.93

Figure 50 shows the mass spectrum of the peptides in the High Mass Calibration Mix.

Figure 50. Mass spectrum of the High Mass Calibration Mix

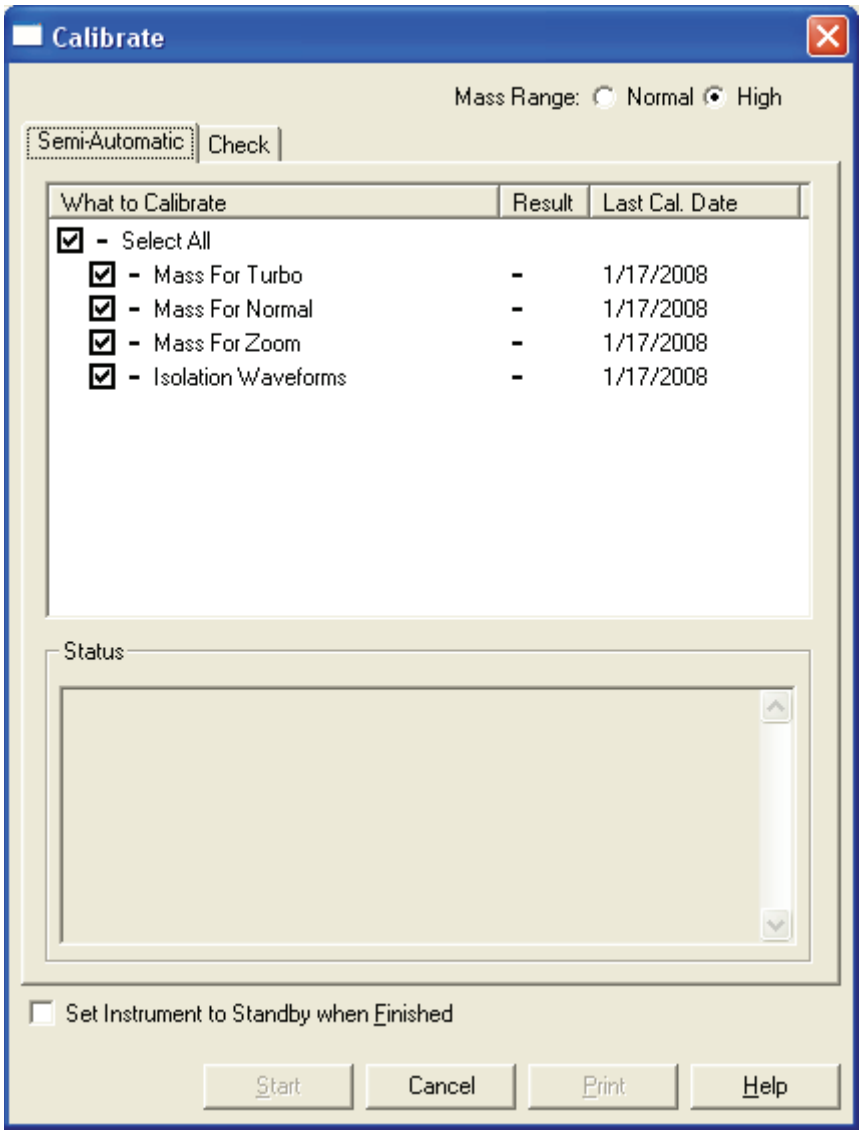


6. When you observe a stable mass spectrum, choose **Control > Calibrate** to open the Calibrate dialog box.

7. For the Mass Range, select the **High** option.

   The High Mass Range Calibration page appears. See Figure 51.

Figure 51. Calibrate dialog box with the parameters for high mass range calibration



8. In the What to Calibrate column, select the **Select All** check box.
9. Click **Start** to begin the high mass calibration procedure.

Checking the Sensitivity of the MALDI LTQ XL System

You can check the sensitivity of the MALDI LTQ XL system by evaluating the signal-to-noise ratio of the 1046 *m/z* peak for a 1 fmol angiotensin II target spot.

❖ To test the sensitivity of the MALDI LTQ XL system

1. Specify the acquisition parameters for the LTQ XL mass spectrometer:
   a. Start Tune Plus.
   b. On the Control/Scan Mode toolbar, click the **On/Standby**  button to switch the mass spectrometer from Standby to On.
   c. If you have not already done so, create a tune file for angiotensin II. For instructions on tuning the mass spectrometer for a specific analyte, see "Tuning on an Analyte" on page 60.
   d. On the Control/Scan Mode toolbar, click **Centroid/Profile**  to switch the data type to profile.
   e. Click **Positive/Negative**  to switch the ion polarity mode to positive.
   f. On the Control/Scan Mode toolbar, click **Define Scan**  .
   The Define Scan dialog box appears.
   g. With the exception of the following settings, use the default settings in the Define Scan dialog box.

Parameter	Setting	Result
Scan Description		
Mass Range	Normal	Specifies that the scan range for this scan falls within the Normal mass range (*m/z* 50 to 2000).
Scan Rate	Normal	Specifies Normal Scan Rate for this scan.
Scan Type	Full	Specifies the Full scan type.
Scan Range		
First Mass (*m/z*)	500.00	Specifies that the first mass in the scan range is *m/z* 500.00.
Last Mass (*m/z*)	2000.00	Specifies that the last mass in the scan range is *m/z* 2000.00.

2. Specify the acquisition parameters for the MALDI source:
   a. In the Tune Plus window, choose **Setup > MALDI Source**.

      The MALDI Source dialog box appears.

   b. Click the **Setup** tab.

   c. With the exception of the settings specified in the following table, use the default settings on the Setup page of the MALDI Source dialog box.

      Use the Semiautomatic page of the Tune dialog box to determine the optimal laser energy for the angiotensin II target spots. For information on optimizing the laser energy, see "Tuning the Laser Energy with the Semi-Automatic Function" on page 70.

Parameter	Setting
Sampling pattern settings	
Plate Motion	Manual
Option check box	Cleared
ASF Spectrum Filer (ASF)	
	Off
ASF Off Settings	
Microscans per step	5
Laser Settings	
AGC	Off
Number Laser Shots Per Microscan	5
Laser Energy	Varies (30 to 50)
Number Sweep Laser Shot	5

   d. Click **Apply**.

   e. Click the **Acquire** tab.

   f. Specify an appropriate file name and folder for the raw data file.

   g. In the Acquisition mode area, type **10** in the Number Scan box.

   h. Make sure that the **Go to Standby when Finished** check box is clear.

3. Insert the target plate with the sensitivity target spots into the MALDI sample module.

   For information on preparing the sensitivity targets, see "Preparing the Sensitivity Test Targets" on page 173. For information on loading a sample plate, see "Loading a Sample Plate into the MALDI Sample Module" on page 52.

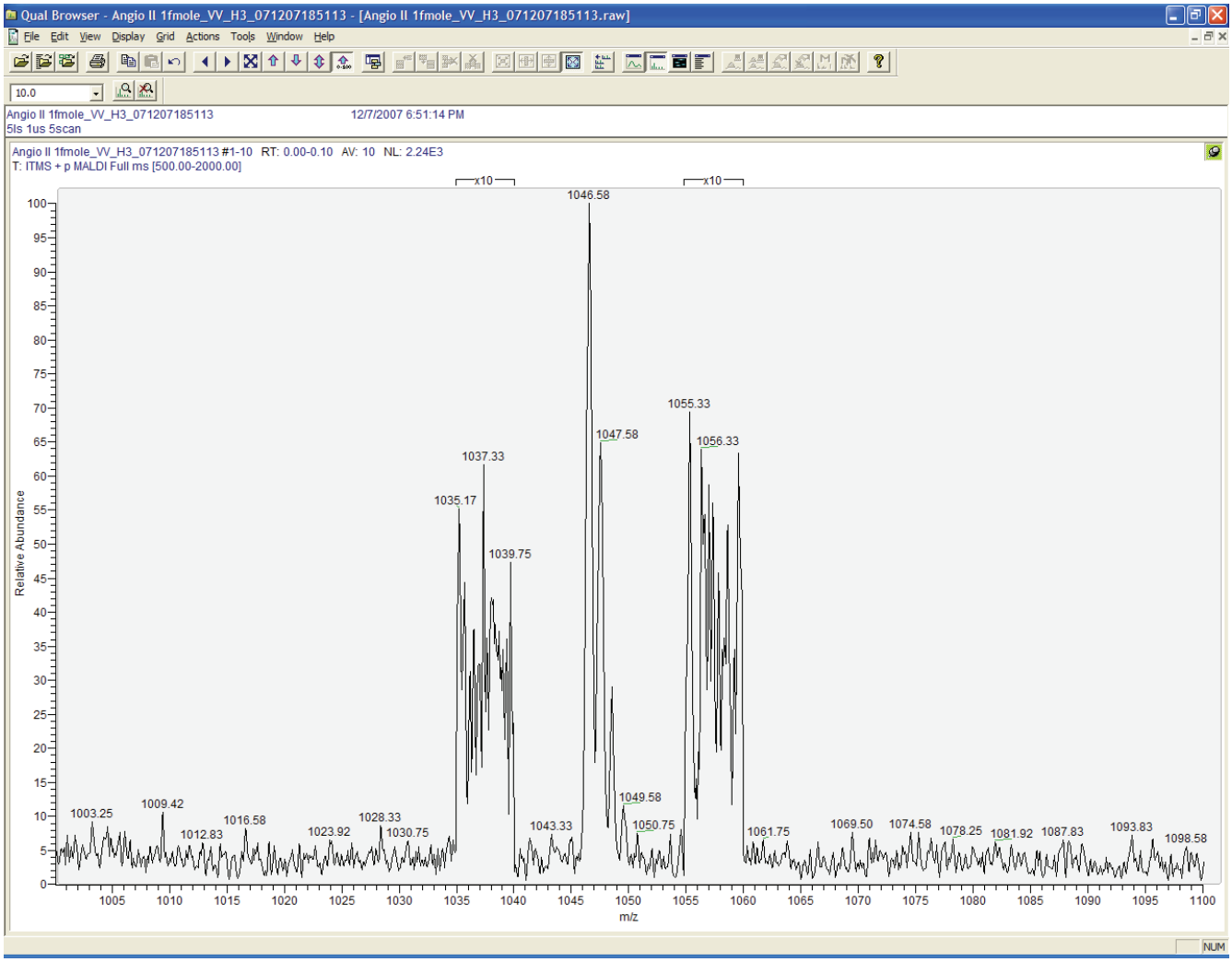
4. Acquire data at several positions within the well spot.

For information about acquiring sample data in Tune Plus, see Chapter 6, "Acquiring Sample Data in Tune Plus."

5. Review the raw data file in Qual browser. See Figure 52.

   The intensities of the noise peaks on either side of the 1046 *m/z* peak should be no greater than one-tenth the intensity of the 1046 *m/z* peak.

Figure 52. Qual Browser with a raw data file acquired from an angiotensin II target spot



Checking the Calibration

To verify the performance of the mass spectrometer, check the calibration periodically. Checking the calibration does not update the calibration settings in the master calibration file. The date stamp of the master calibration file changes when you perform a calibration check because the master calibration file stores a record of the calibration check.

Because the mass spectrometer performs an internal calibration check of the multipole RF frequency and the main RF frequency, these calibration checks do not require external calibration samples. The Normal Mass Calibration Mix/matrix mixture is used for the rest of the calibration checks.

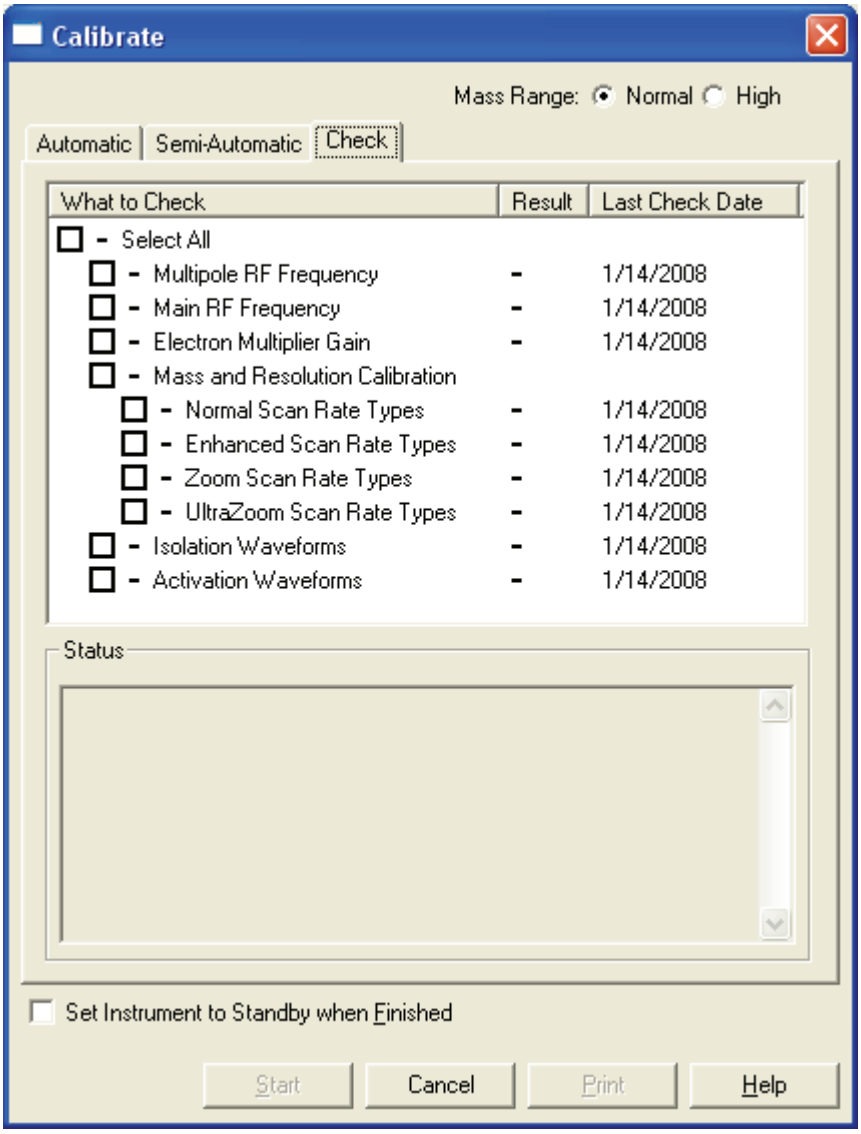
❖ To perform a calibration check

1. Prepare the Normal Mass Calibration Mix/matrix mixture and spot the target plate as described in "Preparing the Mass Calibration Targets" on page 170.
2. Load the plate into the MALDI sample module as described in "Loading a Sample Plate into the MALDI Sample Module" on page 52.
3. View the mass spectrum of the Normal Mass Calibration Mix/matrix mixture:
   a. Set the LTQ XL parameters as described in "Setting the LTQ XL Parameters for Tuning" on page 61.
   b. Set the MALDI source parameters as described in "Setting the MALDI Source Parameters for Tuning" on page 64.
   c. In the MALDI Source dialog box, select a spot on the target plate that has a homogeneous coating of the calibrant/matrix.
   d. Click  to turn on the MALDI laser.
   e. Observe the Spectrum view in Tune Plus.
4. In the Tune Plus window, choose **Control > Calibrate**.

   The Calibrate dialog box appears.
5. Click the **Check** tab.

   The Check page appears. See Figure 53.
6. Select the check boxes for the calibration parameters of interest, and then click **Start**.

Figure 53. Check page of the Calibration dialog box



Acquiring Sample Data in Tune Plus

This chapter describes how to view and acquire mass spectral data in Tune Plus. When you acquire sample data using Tune Plus, you can set the data acquisition parameters in an interactive manner or use a stored instrument method.

For information on creating instrument methods, see "Creating an Instrument Method" on page 105.

Contents

Acquiring Sample Data Interactively in Tune Plus

This procedure shows typical settings for acquiring mass spectral data on a BSA digest/DHB matrix sample in the positive ion polarity mode and the high mass range with the MALDI LTQ XL system.

To acquire data interactively in Tune Plus, follow these procedures:

1. Setting the LTQ XL Parameters for Sample Data
2. Setting the MALDI Source Parameters
3. Acquiring a Raw Data File in Tune Plus

Setting the LTQ XL Parameters for Sample Data

❖ **To set the LTQ XL mass spectrometer parameters**

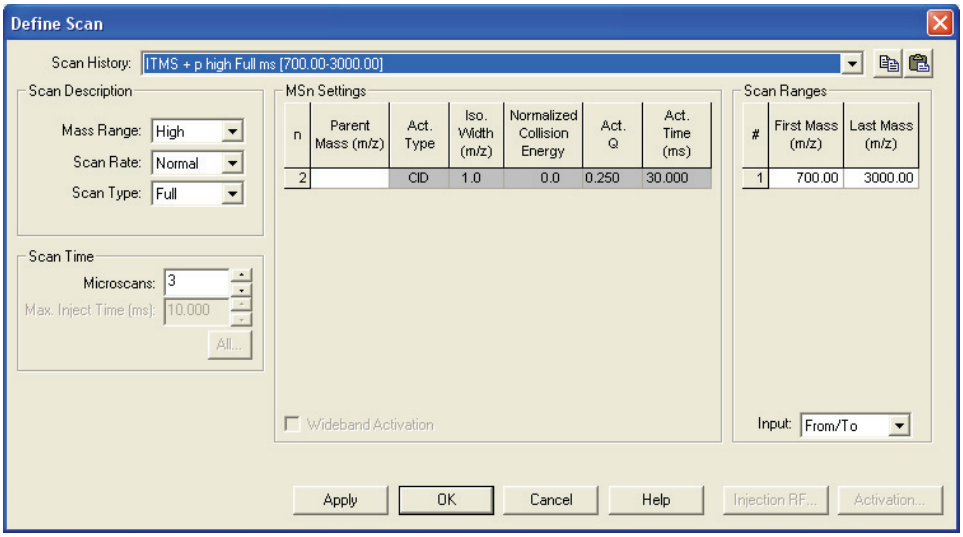
1. Start Tune Plus:
   - From the LTQ workstation desktop, choose **Start > All Programs > Xcalibur > LTQ Tune**.
   - Or, double-click the  **LTQ Tune** shortcut icon on the computer desktop.

The Tune Plus window appears (see Figure 40 on page 62).

2. On the Control/Scan Mode toolbar, click the **On/Standby** button to switch the mass spectrometer from Standby to On.
3. Open a MALDI tune method:
   a. Choose **File > Open** to display the Open dialog box.
   b. Navigate to and select the tune file you created in "Tuning the Laser Energy with the Semi-Automatic Function" on page 70.
   c. Click **Open** to open the file.
4. On the Control/Scan Mode toolbar, click the **Centroid/Profile** button to switch the data type to profile.
5. Click the Positive/Negative button to switch the ion polarity mode to positive.
6. Specify the scan parameters for the mass spectrometer:
   a. On the Control/Scan Mode toolbar, click the **Define Scan** button to open the Define Scan dialog box.
   b. With the exception of the settings listed in the following table, use the default settings in the Define Scan dialog box. Figure 54 shows the parameters settings for the Define Scan dialog box.

Parameter	Setting	Result
Scan Description		
Mass Range	High	Specifies that the scan range for this scan falls within the High mass range (*m/z* 100 to 4000).
Scan Rate	Normal	Specifies Normal Scan Rate for this scan.
Scan Type	Full	Specifies the Full scan type.
Scan Ranges		
First Mass (*m/z*)	700.00	Specifies that the first mass in the scan range is *m/z* 700.00. 'First Mass' values for protein digest samples of 600 to 800 are common in a MALDI scan range, depending on the matrix used. For calibration mix use a value of 500. For small molecules adjust accordingly.
Last Mass (*m/z*)	3000.00	Specifies that the last mass in the scan range is *m/z* 3000.00.
Microscans	Varies	Use 2 to 3 or more if needed.

Figure 54. Define Scan dialog box with settings for data acquisition in the Full scan mode



c. Click **OK** to save the settings and close the dialog box.

You have now finished setting the LTQ XL mass spectrometer parameters. Continue to the next topic, which shows you how to set the MALDI source parameters in the Tune Plus window.

Setting the MALDI Source Parameters

The next step in acquiring data with the MALDI LTQ XL system is to set the parameters for the MALDI source.

❖ To set the MALDI parameters in Tune Plus

1. In the Tune Plus window, choose **Setup > MALDI Source**.

   The MALDI Source dialog box appears with the Control page shown by default.

2. Use the information in Table 8 to set the parameters for the MALDI source.

Table 8. Typical MALDI source settings based on the matrix compound

Parameters	DHB matrix	CHCA matrix
Laser energy (µJ)	20 (approximately 15 to 20 µJ higher than for CHCA)	5
Number Laser Shots (with AGC in the Off mode)	3 to 5	3 to 5
Number Sweep Laser Shots (Setup page)	0 to 3	3 to 5
ASF On settings		
Microscans per Step for ASF Off (This setting must be a multiple of the Microscans setting in the Define Scan dialog box)	3	3
Threshold type (Setup page)	Peak height	
MS threshold (Setup page)	≥5000	≥5000
MS^2 threshold (Setup page)	300 to 1000	300 to 1000

For a BSA digest/DHB matrix mixture, Figure 55 shows typical Control page settings and Figure 56 shows typical Setup page settings. For information on determining the optimal laser energy for your samples, see "Adjusting the Laser Energy Manually" on page 74.

Figure 55. Control page with settings for a BSA digest/DHB matrix sample

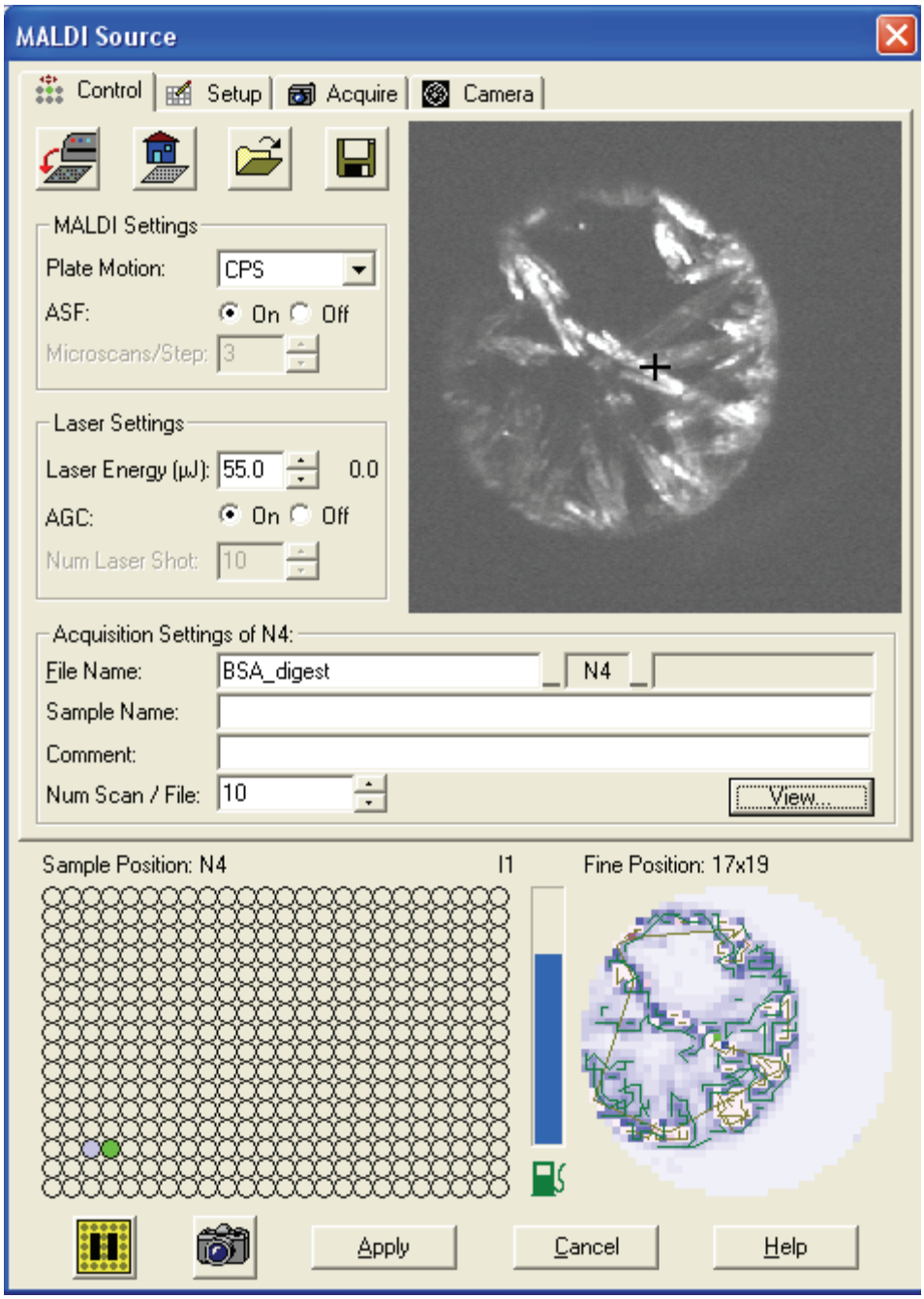
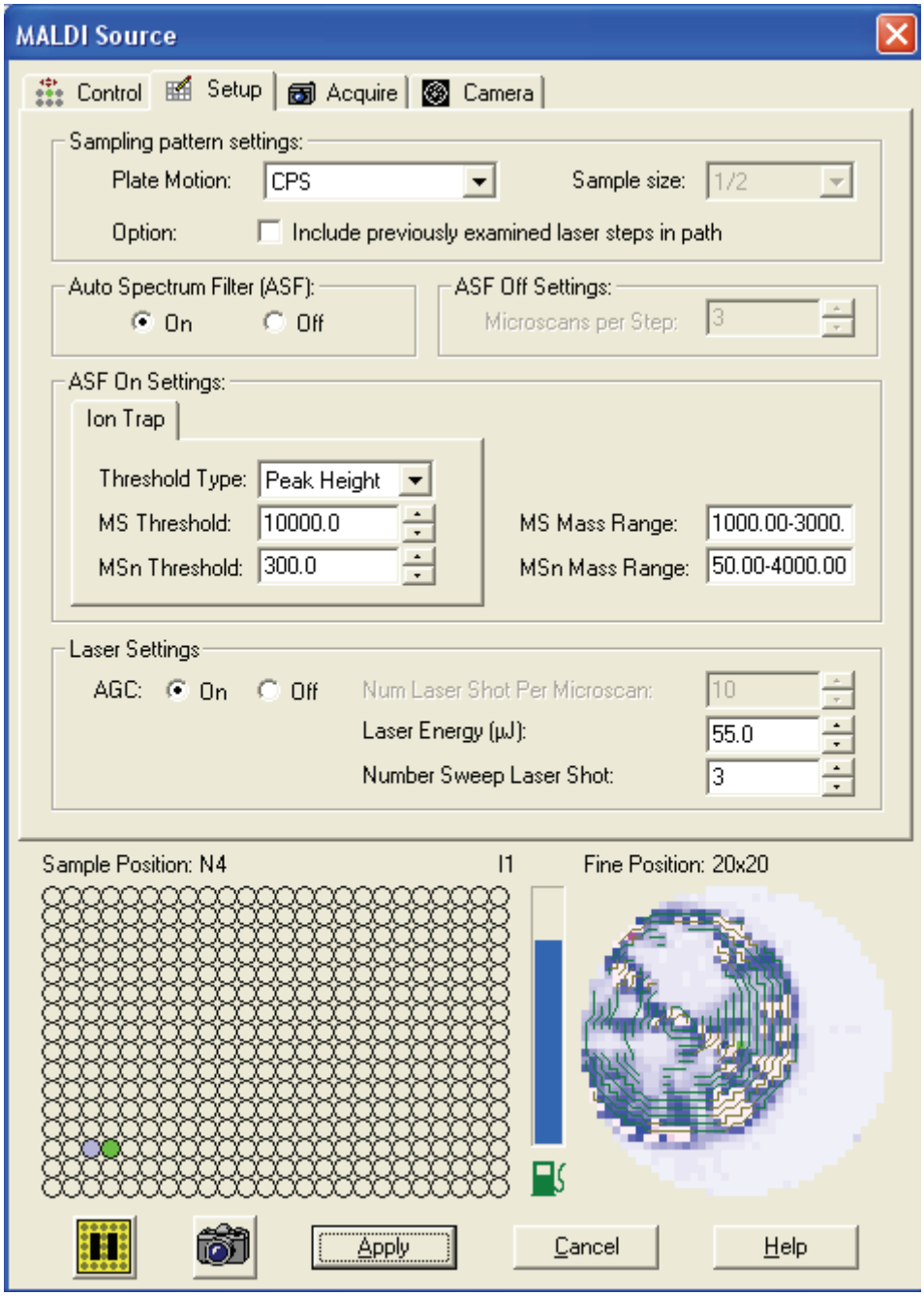


Figure 56. Setup page with typical settings for a BSA digest/DHB matrix sample



3. Click **Apply**.

You have now set the MALDI parameters for data acquisition. With the MALDI Source dialog box open, go to the next topic, "Acquiring a Raw Data File in Tune Plus."

Acquiring a Raw Data File in Tune Plus

❖ To acquire data in Tune Plus

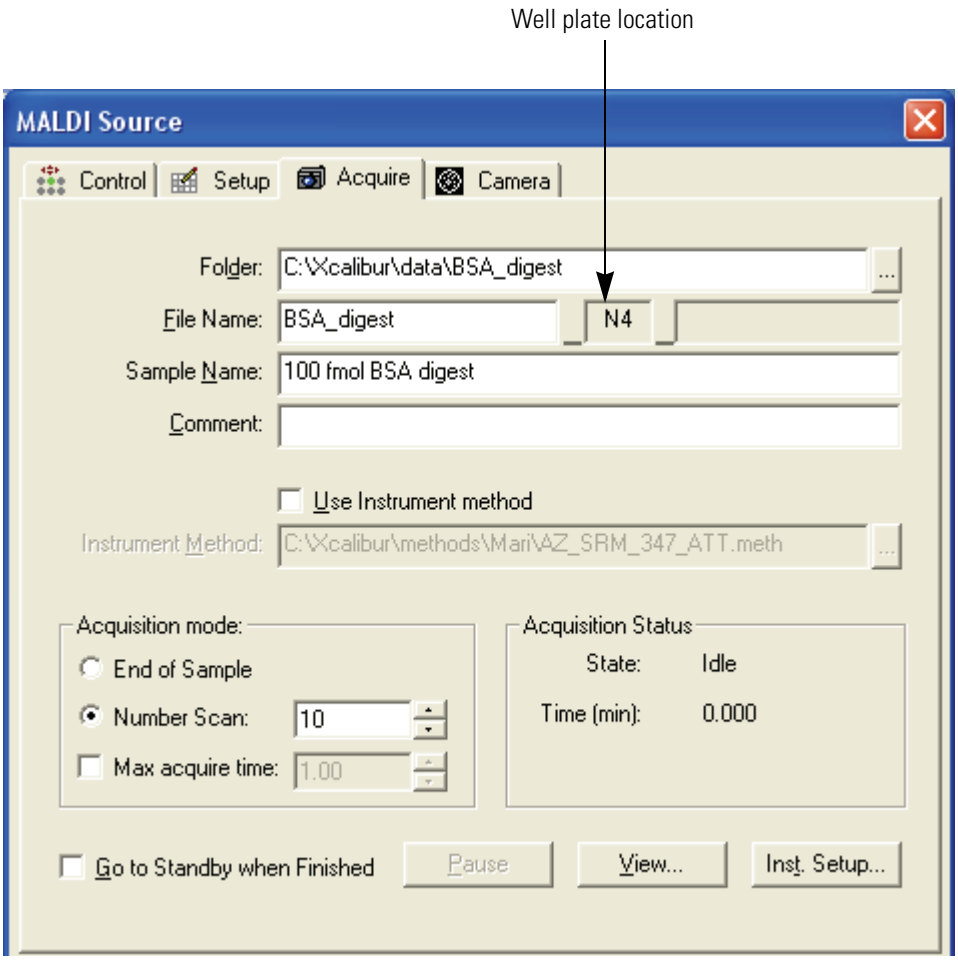
1. Ensure that the On/Standby button on the Tune Plus toolbar is set to On  , and that the MALDI Source dialog box is open.
2. In the MALDI Source dialog box, click the **Acquire** tab.

   The Acquire page appears (see Figure 57).
3. Select a well plate spot by clicking a spot in the sample position map at the bottom of the MALDI Source dialog box.

   The X-Y mechanism of the MALDI source moves the sample plate to position the laser over the requested sample position. After you select it, the position is highlighted in red until the plate moves to the specified position. Then the position is highlighted in green.
4. Specify the file location and file name:
   - Select the file folder by clicking  and browsing to the appropriate directory.
   - Specify the file name by typing a name in the File name box.

     Three separate boxes separated by underscores constitute the file name: the base file name box, the sample position box, and the suffix box. The program automatically fills in the sample position box with the current well plate location and the suffix box with a date stamp.
   - (Optional) In the Sample Name box, type the sample name.
   - (Optional) In the Comment box, type additional comments about the sample or experiment.
5. Under Acquisition mode, select the **Number Scan** option and type a value for the number of scans to be collected per data file in the adjacent box.

Figure 57. Acquire page



6. Click the button at the lower-left corner of the MALDI Source dialog box.

   The laser begins firing, and the mass spectrum appears in the Tune Plus window.

Note The LTQ XL mass spectrometer and the MALDI source have their own independent On/Standby or On/Off buttons.

The LTQ On/Standby button is located in the Tune Plus toolbar. Clicking this button places the LTQ XL mass spectrometer in the On mode, making it ready to acquire data. The MALDI source switches from the Off mode to the Standby mode.

The MALDI source (Turn on/Turn off MALDI source) button is located at the lower-left corner of the MALDI Source dialog box. Clicking this button starts the laser firing.

For more information on the operation modes for the MALDI source, see "Placing the MALDI Source in the On Mode" on page 36.

7. Click the **Start/Stop Acquisition** button at the bottom of the MALDI Source dialog box to turn on the MALDI source and begin saving data to the specified file.

   The instrument continues to acquire data and save it to the specified file until you click the **Start/Stop Acquisition** button again or it acquires the specified number of scans per file. The data file is stored in the specified folder and has a .raw file extension.

Using an Instrument Method to Acquire Sample Data in Tune Plus

Before you use an instrument method to acquire a sequence of data files in Xcalibur Sequence Setup, you can test the method by acquiring a sample data file in Tune Plus.

❖ To use a stored instrument method to acquire sample data in Tune Plus

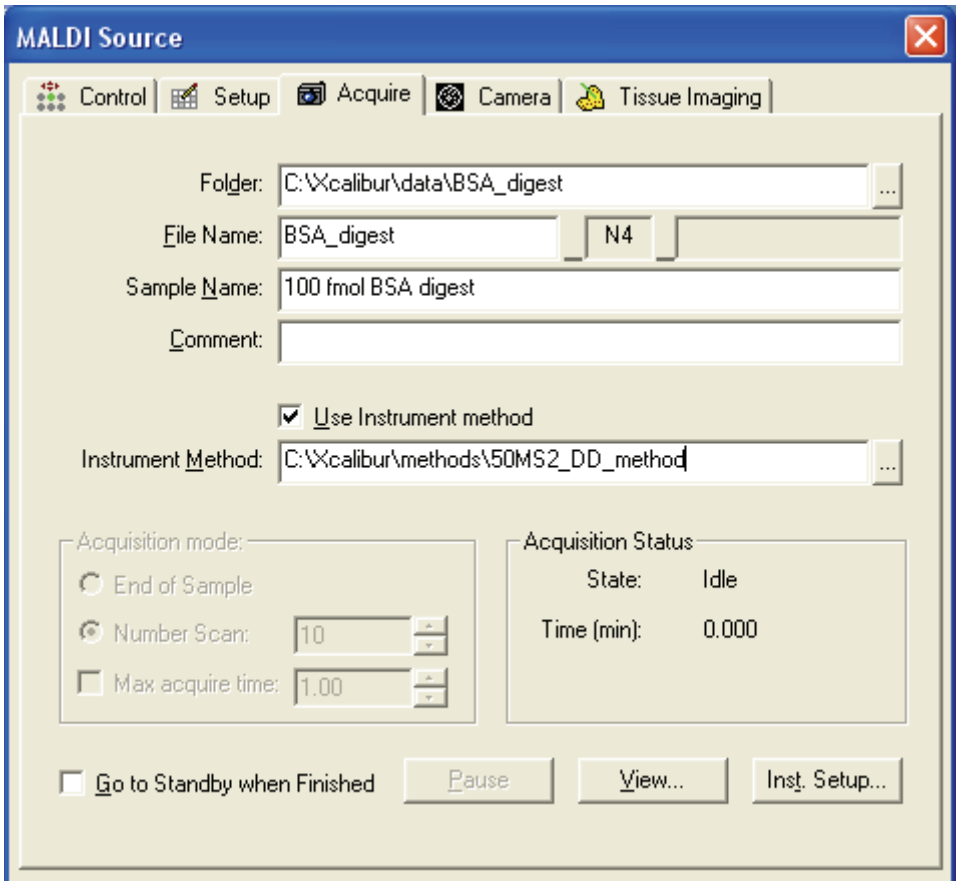
1. Start Tune Plus:
   - From the LTQ workstation desktop, choose **Start > All Programs > Xcalibur > LTQ Tune**.
   - Or, double-click the **LTQ Tune** shortcut icon on the computer desktop.
2. On the Control/Scan Mode toolbar, click the **On/Standby** button to switch the mass spectrometer from Standby to On.
3. In the Tune Plus window, choose **Setup > MALDI Source**.

   The MALDI Source dialog box appears with the Control tab shown by default.
4. Click the **Acquire** tab.

   The Acquire page appears.
5. Make the appropriate entries and selections for the data file location and name (see step 4 on page 93).

6. Select the **Use Instrument method** check box, and then select an instrument method (see Figure 58).

Figure 58. Acquire page with selected instrument method



Note Selecting the Go to Standby when Finished check box places the LTQ XL mass spectrometer in Standby mode at the end of data acquisition. When the LTQ XL mass spectrometer is in Standby mode, the high voltages and nitrogen gas flow are Off.

7. Click **Apply** (at the bottom of the MALDI Source dialog box).
8. To start data acquisition, click  .

   The laser starts firing. The system starts scanning and acquiring data (based on the settings in the instrument method) to an Xcalibur raw data file. When the system completes the instrument method, the MALDI source returns to the Off mode.

Setting Up to Acquire MS/MS Data

Before you perform full-scan MS/MS experiments, for best results, optimize the isolation width and the relative collision energy parameters for your analytes. You visually determine an appropriate isolation width to ensure the effective isolation of the ion of interest, and then you optimize the relative collision energy to ensure efficient fragmentation of the parent ion (precursor ion). The relative collision energy for a particular analysis depends on the type of sample you are analyzing. You can optimize the collision energy manually or use the automated process provided by the program.

To determine an appropriate isolation width and optimal collision energy for the ions of interest, follow these procedures:

1. Determining the Optimum Isolation Width
2. Manually Optimizing the Collision Energy
3. Automatically Optimizing the Collision Energy

Determining the Optimum Isolation Width

For this experiment, and for most applications, use the default settings for the advanced scan features, Activation Q and Activation Time. For more information about these parameters, refer to the Help. The Activation Q and Activation Time parameters appear in the Define Scan dialog box when you choose **Scan Mode > Advanced Scan Features**.

❖ **To manually optimize the isolation width for an MS/MS experiment**


On


Standby

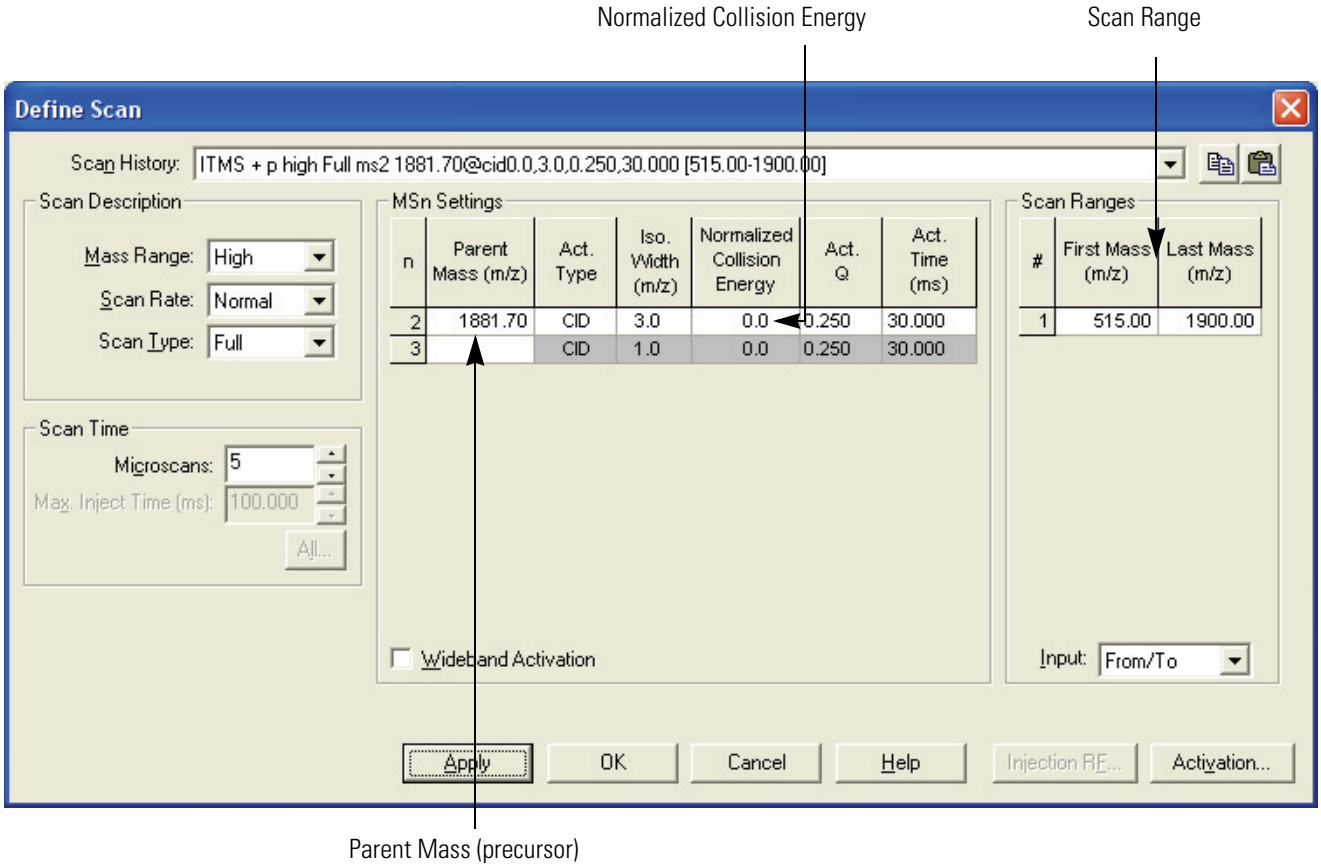
1. From the Tune Plus window, click the **On/Standby** button to take the MS detector out of Standby mode and turn it on.
2. Ensure that the **Profile**  data type is selected.
3. Set the scan parameters to acquire full-scan MS/MS data for your analyte of interest:
   a. Click the **Define Scan**  button.

      The Define Scan dialog box appears. Figure 59 shows the scan settings for the 1881.7 *m/z* precursor ion in a BSA digest/DHB matrix sample.

   b. Ensure that the Parent Mass box contains the correct *m/z* value and that the scan range is appropriate.

   c. Ensure that the value in the Normalized Collision Energy column is set to 0.

Figure 59. Define Scan dialog box with initial settings to optimize the isolation width for *m/z* 1881.7



4. In the MSn Settings area of the Define Scan dialog box, type **3.0** to specify an isolation width of *m/z* 3.0 in the Isolation Width column, and then click **Apply**. Do not close the Define Scan dialog box.

   Note The optimum value for the Isolation Width is the smallest *m/z* width that gives a mass spectrum of maximum intensity for only the ions of interest. When the optimum Isolation Width is obtained, the value for the normalized intensity (NL) in the mass spectrum display is stable, and the mass peak for the parent ion is at its maximum intensity and appears symmetrical. An Isolation Width value that is less than the optimum value causes a substantial drop in the NL reading. A significant drop in sensitivity indicates that the ions of interest are not effectively isolated.

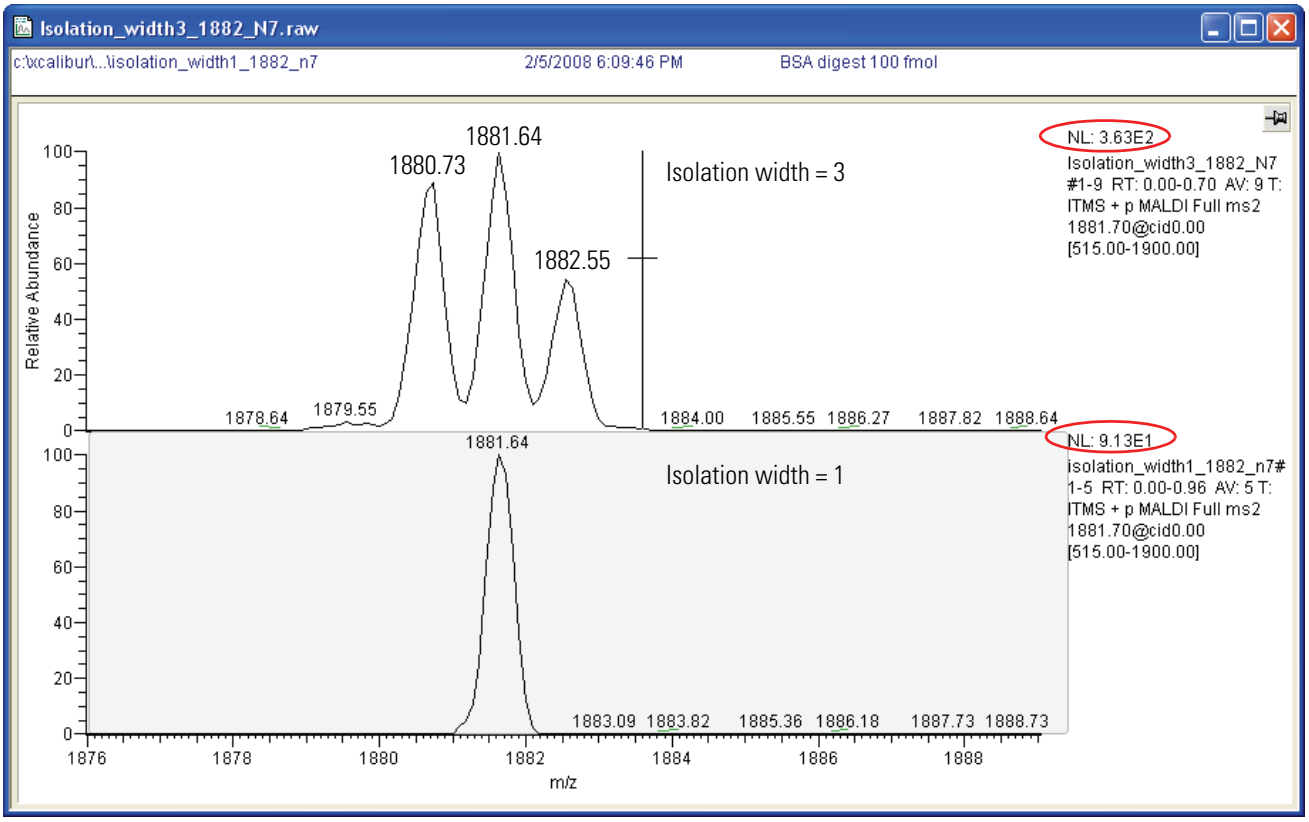
5. In the Tune Plus window, observe the mass spectrum for the parent ion of your analyte. Ensure that the readback value for the normalized intensity (NL) is relatively stable.

6. Repeat step 4 and step 5, entering successively smaller values for the isolation width. Continue to observe the intensity of the mass spectrum of the parent ion, and ensure that the value for the normalized intensity (NL) is stable with each change you make to the isolation width.

Note The optimal isolation width is typically between 1 to 3. After you optimize the isolation width, you can compensate for minor changes in tune stability by increasing the isolation width value a small amount. This adjustment should be no larger than *m/z* = 1.

Figure 60 shows the MS/MS precursor isolation spectra isolation widths of 1 and 3.

Figure 60. MS/MS spectrum for *m/z* 1881.7 with an isolation width of 3 (collision energy = 0)

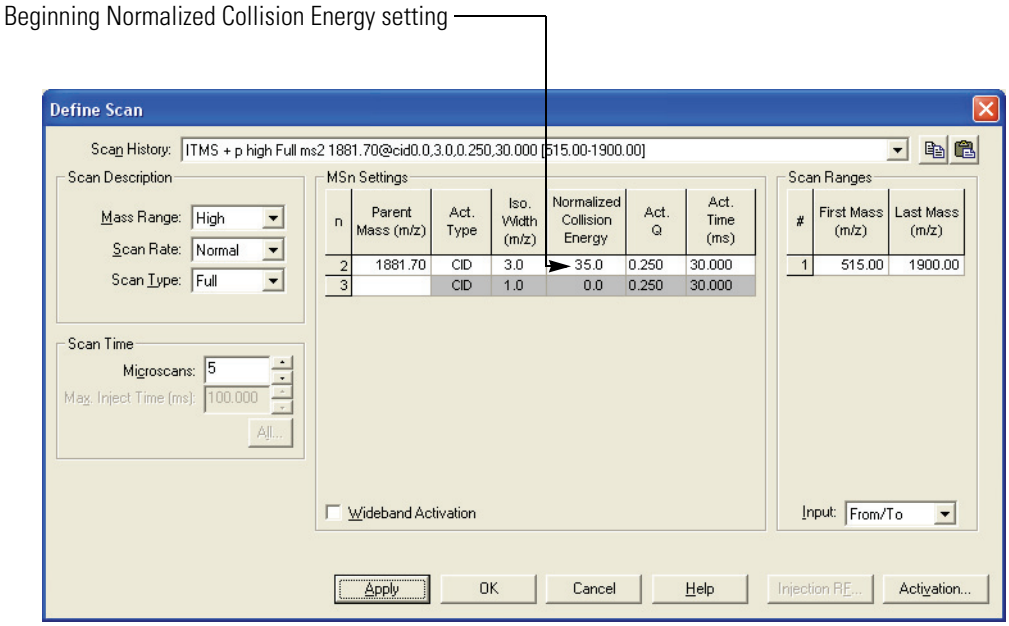


Manually Optimizing the Collision Energy

❖ To manually optimize the collision energy

1. In the Tune Plus window, ensure that the MS detector is in the On mode.
2. Ensure that the  **Profile** data type is selected.
3. Click the **Define Scan**  button to open the Define Scan dialog box.
4. Set the scan parameters for your analyte of interest. You must type a value for the parent mass in the Parent Mass box in the MSn Settings area. See Figure 61.

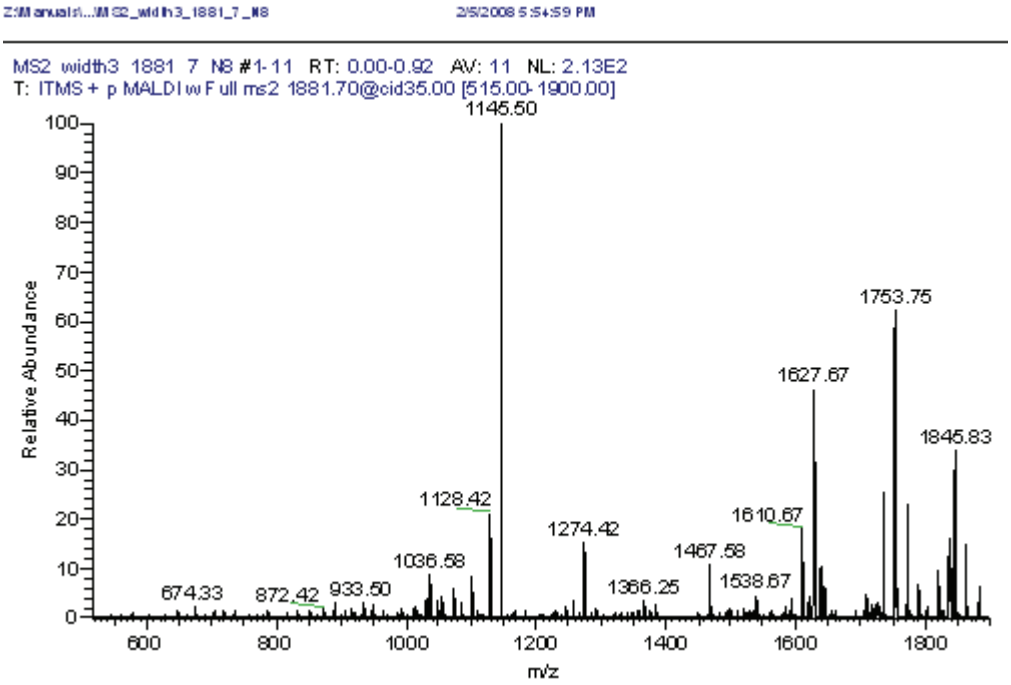
Figure 61. Define Scan dialog box with typical settings for acquiring full-scan MS/MS data



5. In the Normalized Collision Energy box under MSn Settings, type or select **35**.
6. Click **Apply**.
7. In the Tune Plus window, observe the mass spectrum of the product ions of your analyte of interest.

   Figure 62 shows the MS/MS spectrum for the *m/z* 1881.7 precursor ion.

Figure 62. MS/MS spectrum for the *m/z* 1881.7 precursor ion



8. If necessary, increase the value for the normalized collision energy in increments of 5% and click **Apply**, until the intensity of the precursor ion is less than 5% of the intensity of the product ions.

Automatically Optimizing the Collision Energy

The optimum relative collision energy maximizes the product ion intensity.

❖ **To automatically optimize the relative collision energy for an analyte of interest**

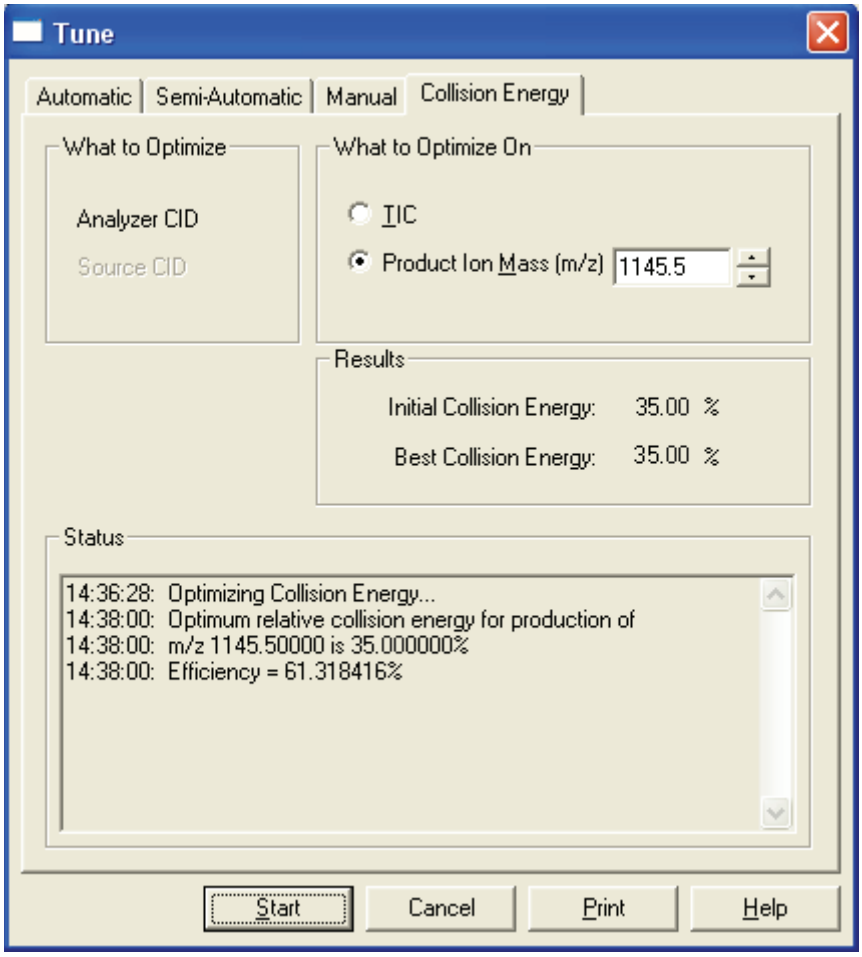
1. When you have clearly identified a mass-to-charge ratio of a product ion for your analyte of interest, click the **Tune** button.

   The Tune dialog box appears with the Automatic tab shown by default.

2. Click the **Collision Energy** tab.

   The Collision Energy page appears (see Figure 63).

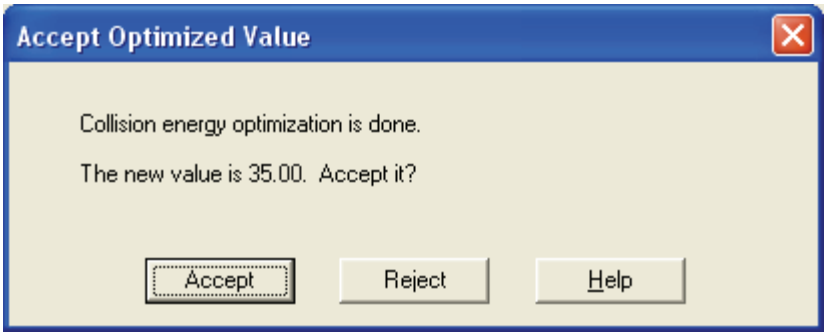
Figure 63. Collision Energy page



3. Select the **Product Ion Mass** option.
4. In the Product Ion Mass box, type a value for one of the product ions of your analyte.
5. Click **Start** to start the optimization procedure.
6. In the Spectrum view of Tune Plus, observe the full-scan MS/MS spectrum of your analyte of interest.

   When the collision energy is optimized, the Accept Optimized Value dialog box appears. See Figure 64.

Figure 64. Accept Optimized Value dialog box



7. Click **Accept** to accept the new collision energy value and return to Tune Plus.

   The new value appears in the Define Scan dialog box.

8. Click **Cancel** to close the Tune dialog box.

After you optimize the relative collision energy, the MALDI LTQ XL system is ready to acquire MS/MS data on your analyte of interest.

Automatically Acquiring Sample Data with Xcalibur

This chapter describes how to automate data acquisition for a set of samples analyzed with the MALDI LTQ XL system.

When you automate data acquisition with the Xcalibur data system, you create an instrument method specifying how the instrument collects spectra, and an acquisition sequence specifying which sample positions are to be examined and where the data is to be stored.

The procedures in this chapter describe how to set up a data-dependent MS/MS experiment that can be used to identify the components of an enzyme digest. Refer to Xcalibur Help for more information on how to customize the Instrument Setup and Sequence Setup parameters for your experiment.

Contents

Creating an Instrument Method

The instrument method defines the experimental parameters for data acquisition. When you use Xcalibur to acquire data, you select an instrument method for each sample that you run in the Sequence Setup view. You can also use instrument methods in Tune Plus.

This procedure describes how to create an instrument method with the Nth Order Double Play template. This template is useful for setting up an experiment in which you acquire MS/MS data for the most intense precursor peaks in the MS spectrum.

When you create an instrument method, you import a tune file for your analytes of interest. The tune file becomes part of the method and contains parameters that optimize the ion transmission for your analytes. For information on creating a tune file for your analytes, see "Tuning on an Analyte" on page 60.

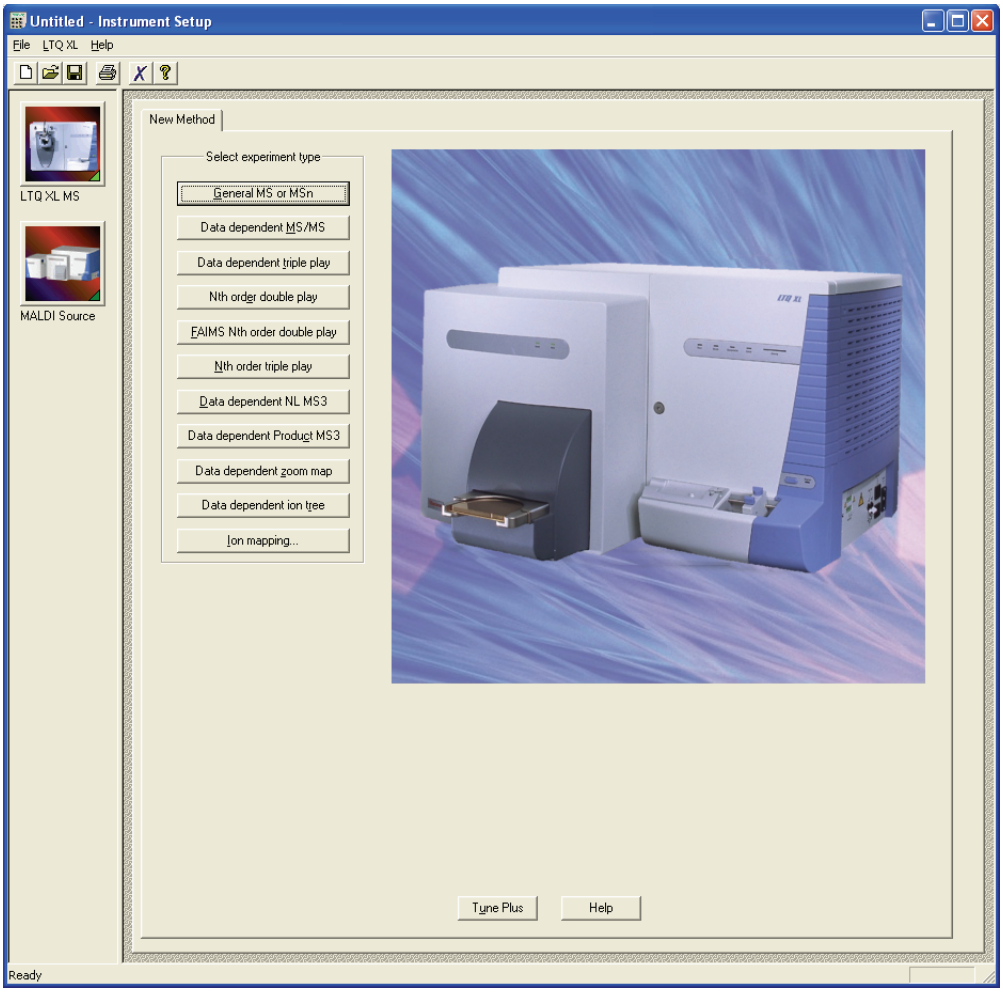
❖ To set up an instrument method based on the Nth Order Double Play template

1. Open the Instrument Setup window:
   a. From the taskbar, choose **Start > All Programs > Xcalibur > Xcalibur**.
      The Xcalibur Home Page - Roadmap view appears.
   b. In the Roadmap view, click the **Instrument Setup** 

button.
      The Instrument Setup window appears.
2. In the left pane of the Instrument Setup window, click the **LTQ MS**  button.

The New Method page appears. See Figure 65.

Figure 65. New Method page with the list of templates for the LTQ XL MS

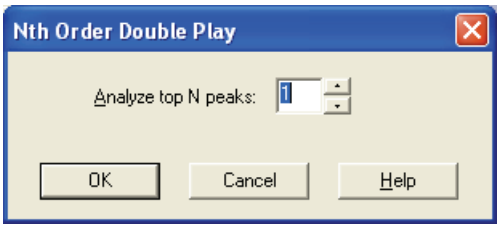


The remaining steps in this procedure describe how to set up an Nth order double play experiment suitable for an enzyme digest.

3. Click **Nth order double play**.

The Nth Order Double Play dialog box appears (see Figure 66).

Figure 66. Nth Order Double Play dialog box with the default setting of 1



4. In the Analyze top N peaks box, type or select **50**.

   When you run this experiment, the MALDI LTQ XL acquires data on the 50 most intense peaks in the mass spectrum.

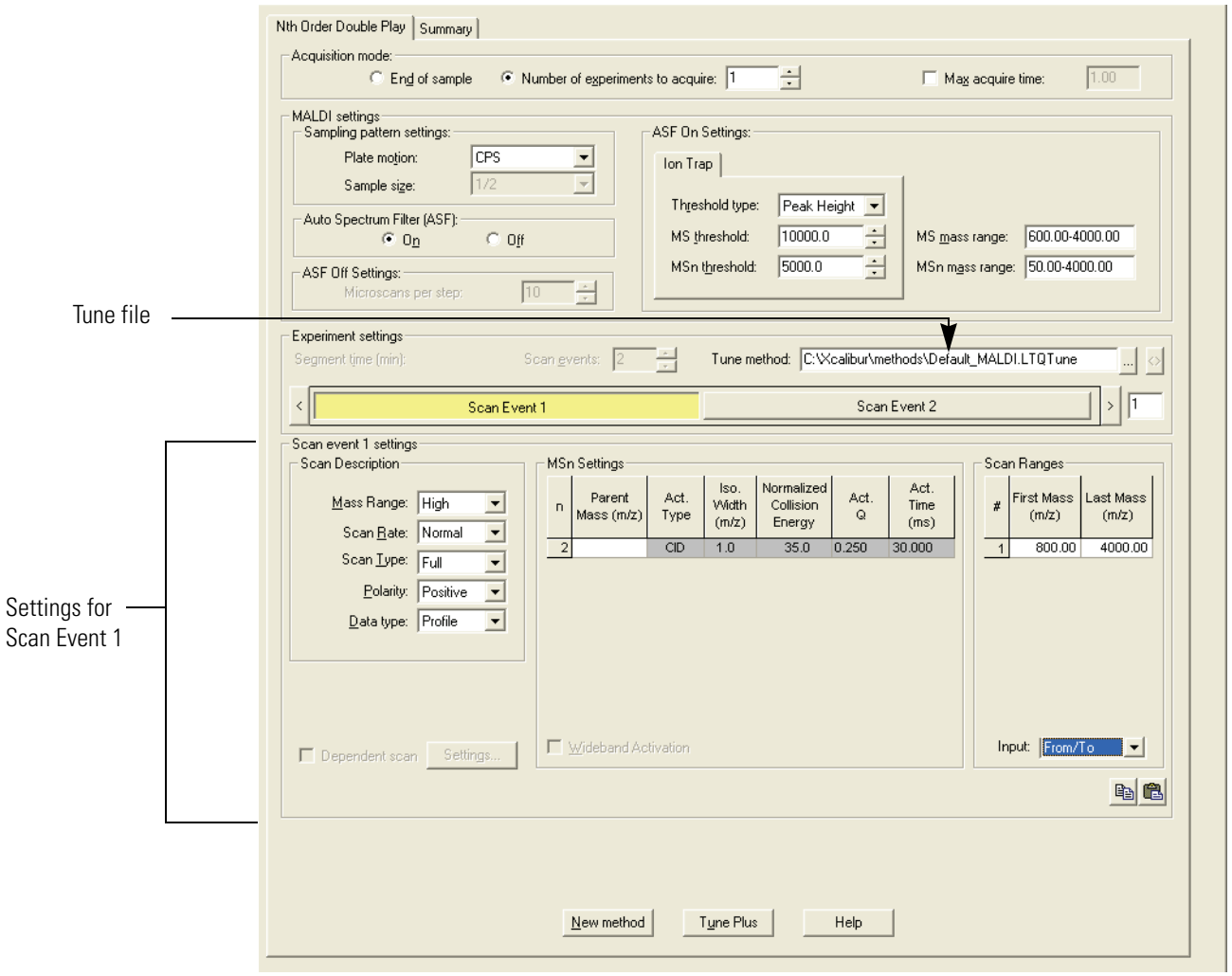
5. Click **OK**.

   The template for an Nth Order Double Play experiment appears.

   For the purpose of this tutorial, use the default settings in the Nth Order Double Play template with the exception of the parameters listed in the following table and shown in Figure 67.

Parameter	Setting	Result
Acquisition Mode		
Number of Experiments to Acquire	1	The system performs one acquisition experiment.
Experiment Settings		
Tune method	The tune file for the analyte of interest	The tune file contains the parameters listed in Table 4 on page 60.
Scan Event 1 Settings		
Scan Description		
Mass Range	High	The scan range for this scan falls within the high mass range.
Data type	Profile	The scan collects Profile data.
Scan Ranges		
First Mass (*m/z*)	800.00	Sets the first mass in the scan range to 800.00.
Last Mass (*m/z*)	4000.00	Sets the last mass in the scan range to 4000.00.

Figure 67. Nth Order Double Play page with the Scan Event 1 settings



6. To select a tune file, click the ... button to the right of the Tune method box, browse to the directory where you stored the tune file, and double-click the appropriate *.LTQtune file.

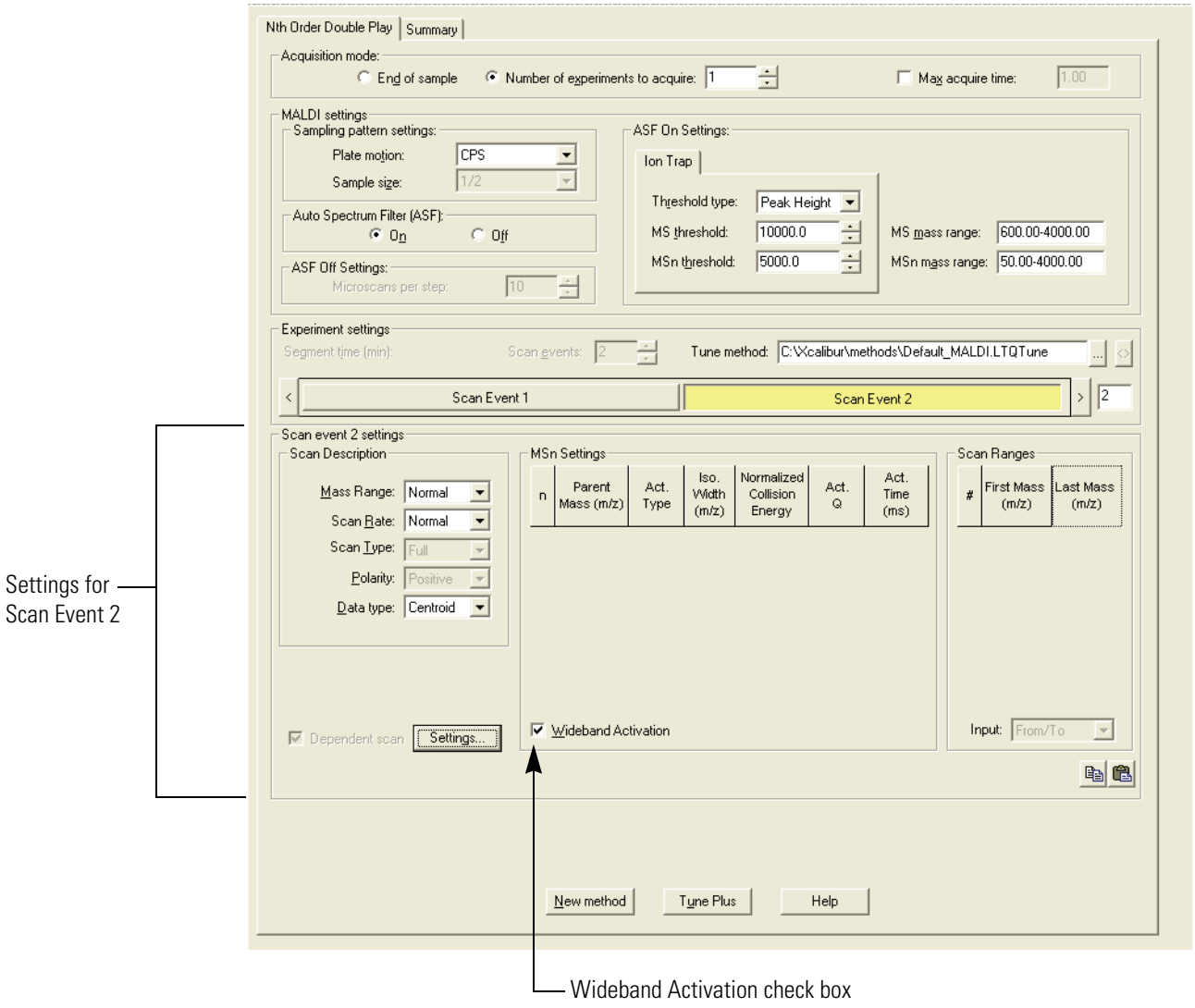
7. Set up the dependent scan parameters for Scan Event 2:

   a. Click **Scan Event 2** to display the Scan Event 2 Settings area in the bottom portion of the window. See Figure 68.

   b. Select the **Wideband Activation** check box.

      Selecting the wideband activation check box increases the amount of fragmentation during mass analyzer collision-induced dissociation (CID).

Figure 68. Nth Order Double Play page with the Scan Event 2 settings



c. Click **Settings**.

   The Data Dependent Settings dialog box appears.

d. In the list on the left side of the dialog box, select **Dynamic Exclusion**.

   The Dynamic Exclusion page appears (see Figure 69).

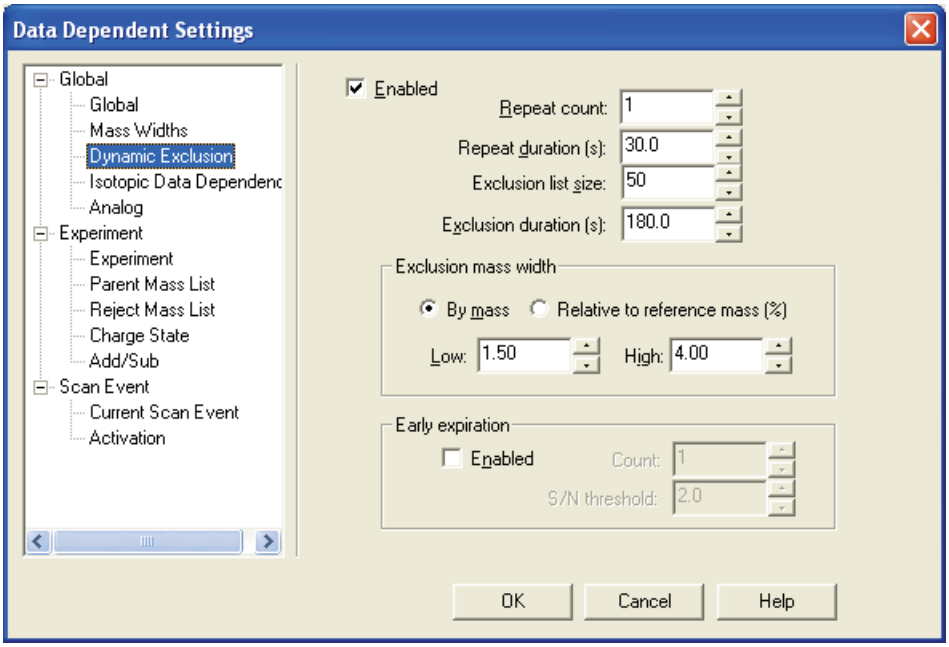
e. Select the **Enabled** check box.

f. With the exception of the High value, use the default settings in the Dynamic Exclusion page. See the following table and Figure 69.

The exclusion mass width of 1.50 to 4.00 ensures that the mass spectrometer does not perform an MS^n experiment on both the monoisotopic and most intense isotope peaks in the MS spectrum.

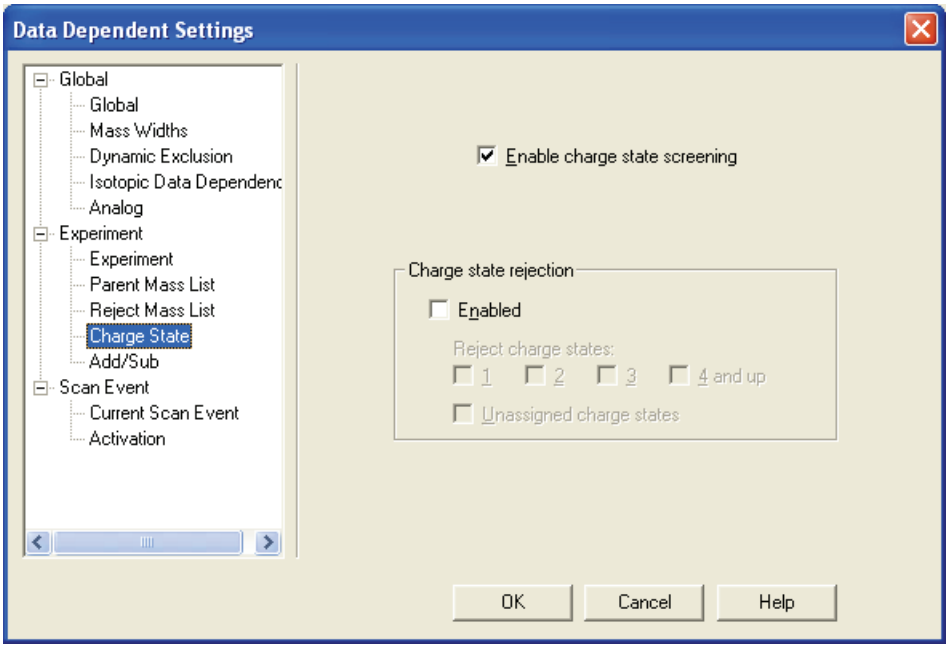
Parameter	Setting	Result
Enabled	☑	This scan uses the Dynamic Exclusion feature.
Exclusion Mass Width		
Low	1.50	An ion does not trigger a dependent scan if its mass falls within a range of 1.50 *m/z* units less than a mass on the exclusion list.
High	4.00	An ion does not trigger a dependent scan if its mass falls within a range of 4.00 *m/z* units greater than a mass on the exclusion list.

Figure 69. Dynamic exclusion settings



g. Under Experiment, select **Charge State** to open the Charge State page. Then select the Enable charge state screening check box. See Figure 70.

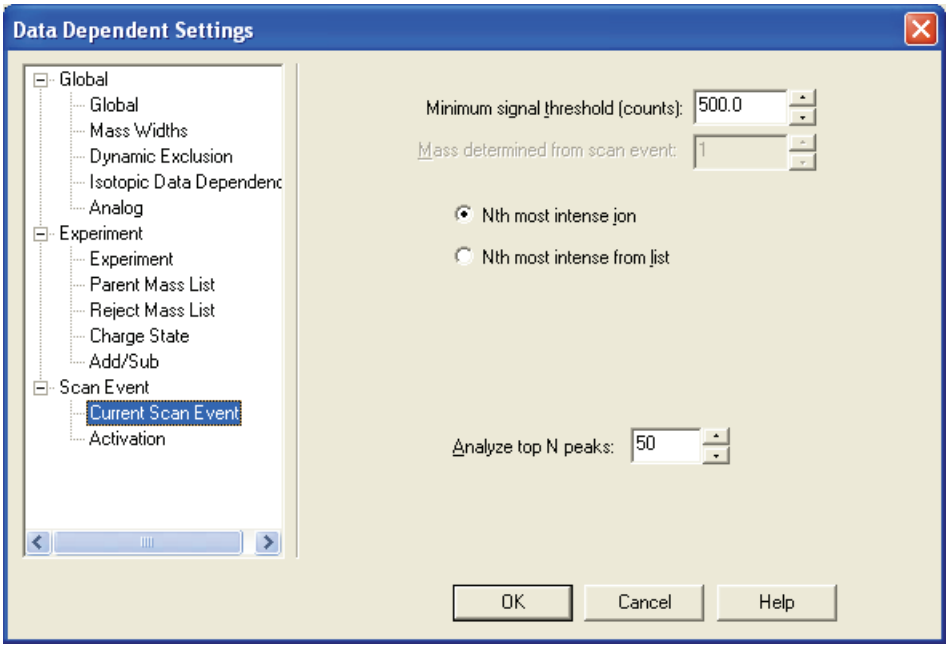
Figure 70. Charge State settings



h. Under Scan Event, select **Current Scan Event** to open the Current Scan Events page. With the exception of the setting listed in the following table, use the default settings on the Current Scan Events page. Figure 71 shows typical settings for the parameters in the Current Scan Events page.

Parameter	Setting	Result
Minimum signal threshold (counts)	300	Signals above 300 counts trigger MS/MS. Values from 100 to 500 are common. If an experiment quits before collecting all the MS/MS you expect, the threshold might be set too high.

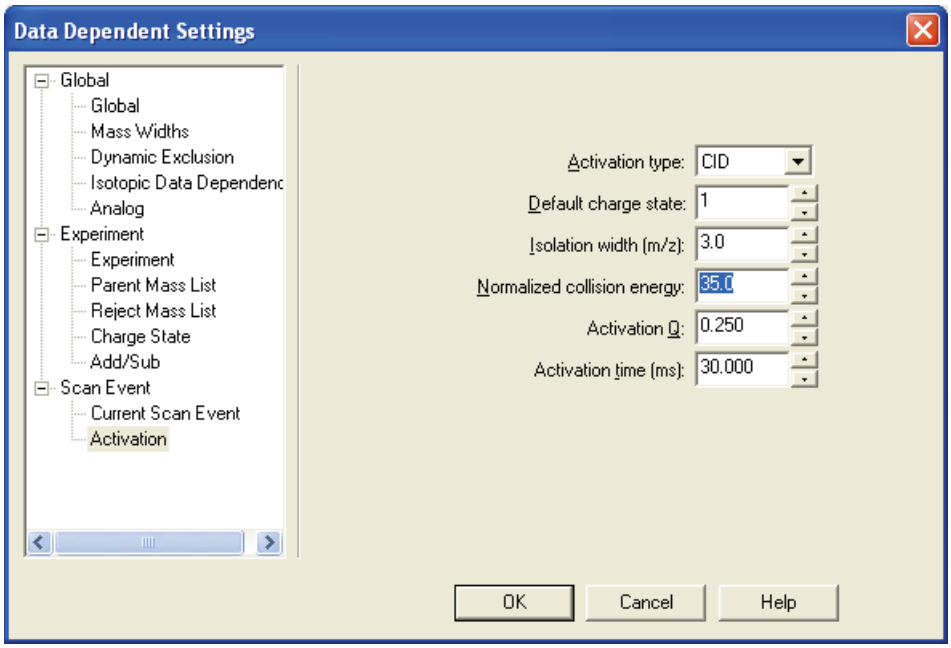
Figure 71. Current Scan Event settings



i. Under Scan Event, select **Activation** to display the Activation page. With the exception of the settings listed in the following table, use the default settings. Figure 72 shows typical settings for the Activation page.

Parameter	Setting	Result
Default charge state	1	The default charge state for this scan is 1.
Isolation Width (*m/z*)	3.0	The isolation width for this scan is *m/z* 3.0
Normalized Collision Energy	The default setting is 35.	Use the normalized collision energy value determined by following the procedure in "Setting Up to Acquire MS/MS Data" on page 97.

Figure 72. Activation settings



8. Click **OK** to close the Data Dependent Settings dialog box.
9. Choose **File > Save As** to save your instrument method file.
10. In the File name box, type a descriptive file name for your instrument method.
11. Click **Save**.

    The File Summary Information dialog box appears.
12. Type a comment in the Comment box, and then click **OK** to save the instrument method and close the dialog box.

    Instrument method files have the file extension .meth.

Creating an Acquisition Sequence

Creating an acquisition sequence allows you to keep all the data you collect from a single sample plate together and organized. The sequence (*.sld) file you create stores the data file path and the location on the plate for each sample, plus the sample name and any comments you add.

❖ To create an acquisition sequence

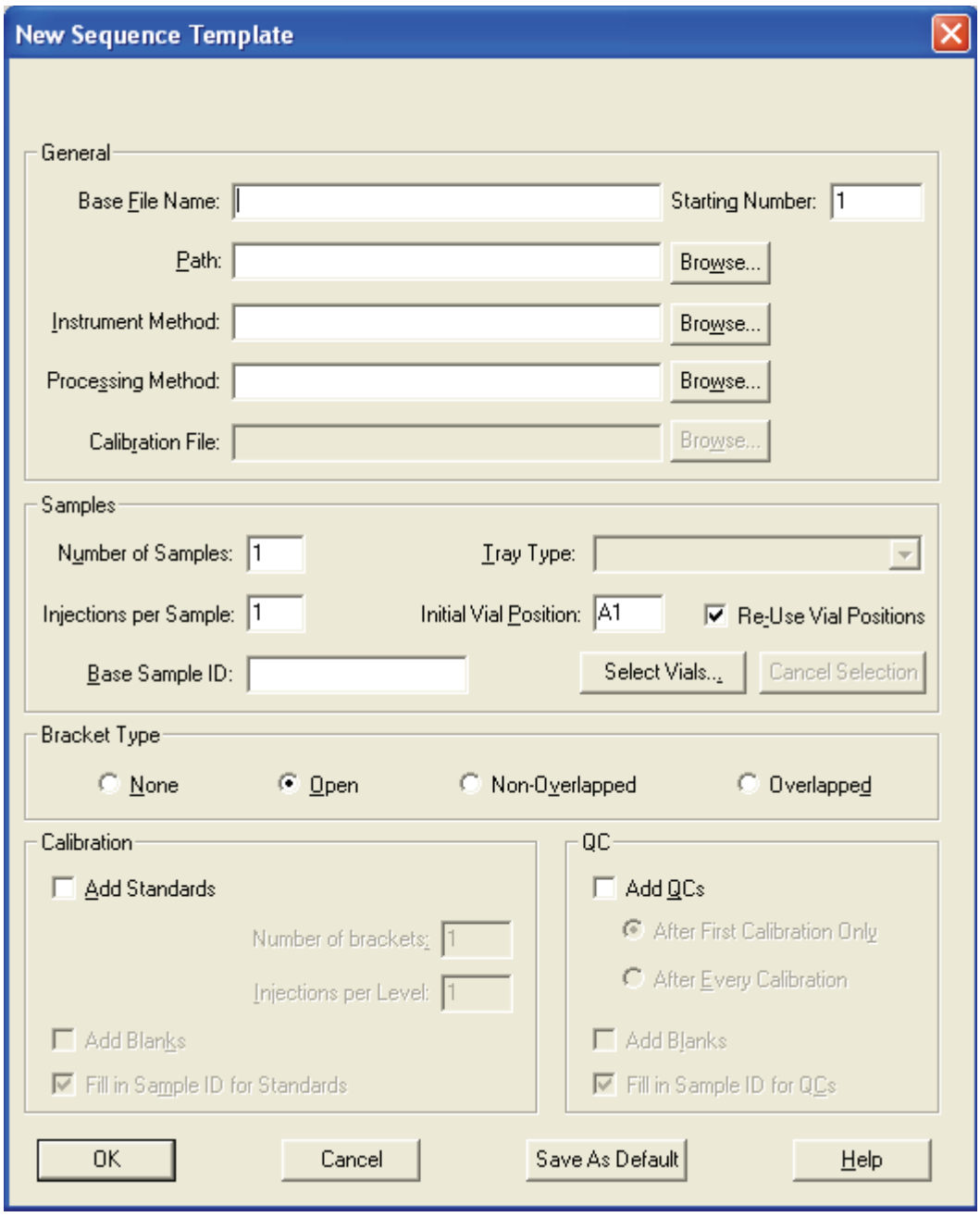
1. Open Xcalibur by choosing **Start > All Programs > Xcalibur > Xcalibur**.
2. On the Xcalibur Home Page - Roadmap view, click the Sequence Setup button.

   The Sequence Setup Home Page window appears.

3. Choose **File > New**.

   The New Sequence Template dialog box appears. See Figure 73.

Figure 73. New Sequence Template dialog box



4. Fill in the parameters in the General area:

   a. In the Base File Name box, type a base file name.

      Xcalibur appends a suffix to this base file name to create a data file name for each sample in the sequence.

b. In the Path box, select a directory path for storing your data files.

c. Click **Browse** next to the Instrument Method box. Locate and open the instrument method that you created in the previous procedure, "Creating an Instrument Method."

d. Do not specify a processing method or a calibration file (leave these selections blank) unless you want the program to process the raw data files as it acquires them. You do not need a processing method or a calibration file to acquire raw data files.

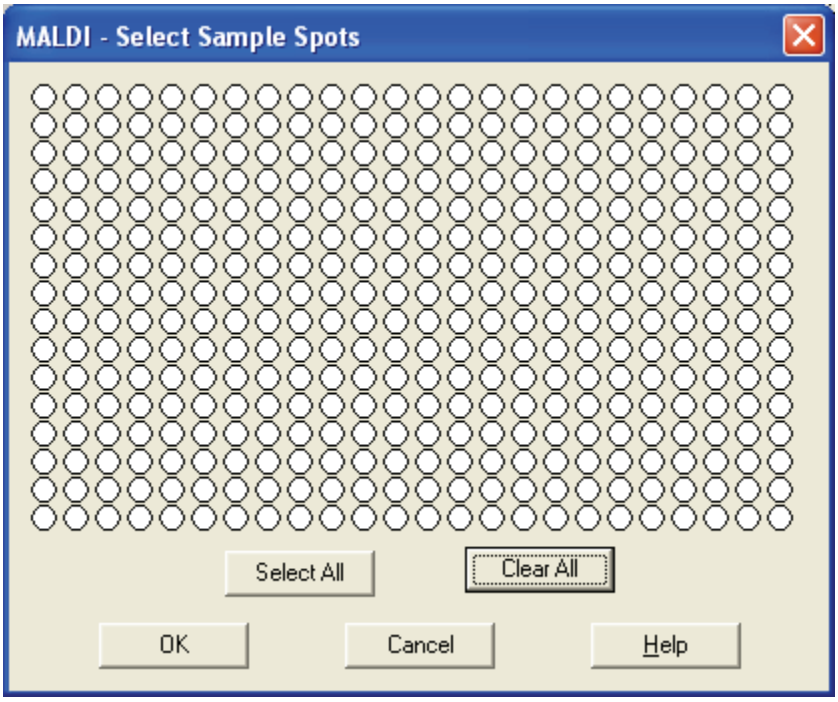
For information on creating processing methods and calibration files, refer to the Xcalibur data system Help.

5. Select the sample positions:

a. In the Samples area, click **Select Vials.**

The MALDI - Select Sample Spots dialog box appears. See Figure 74.

Figure 74. MALDI – Select Sample Spots dialog box



b. Select the positions in which you have placed samples on the plate. Click individual positions to select them, or click and drag over a range to select a range of positions.

Note Data acquisition proceeds by rows rather than by columns.

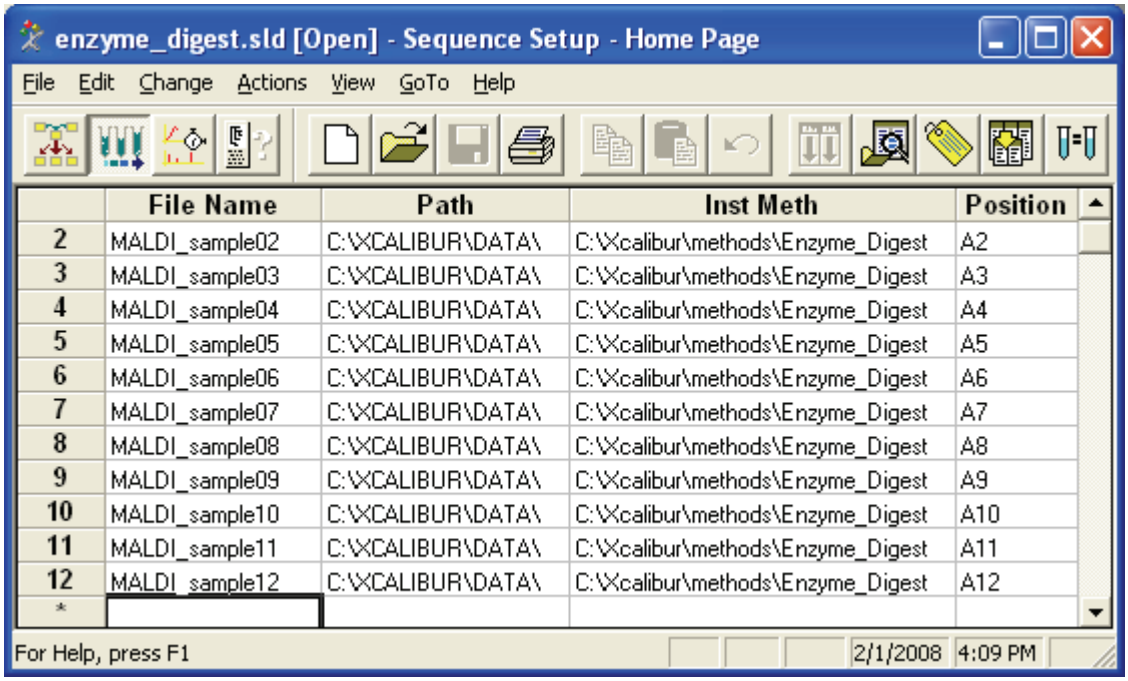
c. Click **OK** to close the MALDI - Select Sample Spots dialog box.

6. If you are using this sequence to acquire data files (rather than acquire and process, or process only), use the default settings for the rest of the parameters in the New Sequence Template dialog box.

7. Click **OK**.

A sample list appears in the Sequence Setup window. See Figure 75.

Figure 75. Xcalibur Sequence Setup view with a typical MALDI acquisition sequence

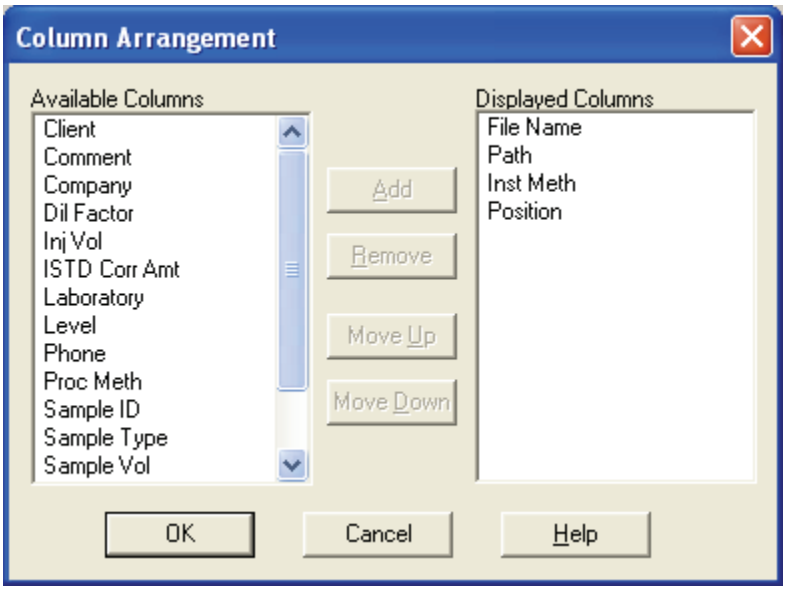


	File Name	Path	Inst Meth	Position
2	MALDI_sample02	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Enzyme_Digest	A2
3	MALDI_sample03	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Enzyme_Digest	A3
4	MALDI_sample04	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Enzyme_Digest	A4
5	MALDI_sample05	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Enzyme_Digest	A5
6	MALDI_sample06	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Enzyme_Digest	A6
7	MALDI_sample07	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Enzyme_Digest	A7
8	MALDI_sample08	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Enzyme_Digest	A8
9	MALDI_sample09	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Enzyme_Digest	A9
10	MALDI_sample10	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Enzyme_Digest	A10
11	MALDI_sample11	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Enzyme_Digest	A11
12	MALDI_sample12	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Enzyme_Digest	A12
*				

8. To display more columns, change the column arrangement:
   a. Click the **Column Arrangement** button on the toolbar.

      The Column Arrangement dialog box appears. See Figure 76.

      Figure 76. Column Arrangement dialog box with the columns used for a MALDI sequence



   b. In the Available Columns list, select the columns of interest and click **Add** to add them to the Displayed Columns list.

c. When you have added the columns of interest, click **OK**.

9. Save the sequence:

   a. Choose **File > Save** to display the File Summary Information dialog box.

   b. Type a description or summary of the sequence in the Description box, and then click **OK**.

      The Save As dialog box opens.

   c. Specify the path and file name for the acquisition sequence and click **Save**.

      The file extension for sequence files is .sld.

The Acquisition Sequence is now saved. You are ready to run the sequence and acquire data. With the Acquisition Sequence open in the Sequence Setup view, go to the next topic, "Acquiring Raw Data Files in Sequence Setup."

Acquiring Raw Data Files in Sequence Setup

❖ **To run your sequence and acquire data**

1. Prepare your samples as described in Appendix B, "Sample Preparation."
2. Ensure that the sample plate is loaded and the MALDI source has pumped down to the operating vacuum level.

   For information on loading a sample plate into the MALDI sample module, see "Loading a Sample Plate into the MALDI Sample Module" on page 52.

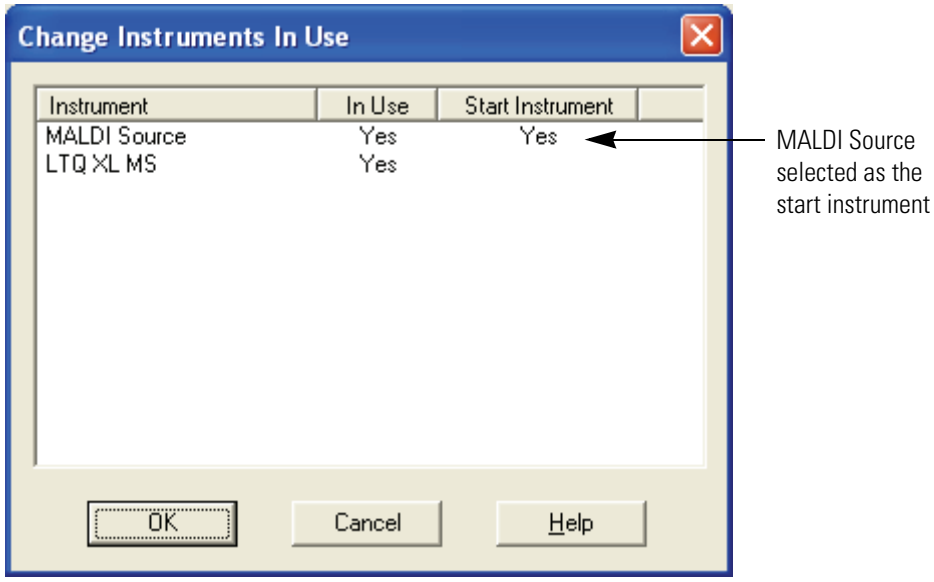
3. In the Sequence Setup view, do one of the following:

   - Choose **Actions > Run Sequence**.
   - Or, in the view toolbar, click  .

4. If the instruments in use has changed, the Change Instruments In Use dialog box appears on top of the Run Sequence dialog box.

   By default the MALDI Source is listed as the start instrument. See Figure 77.

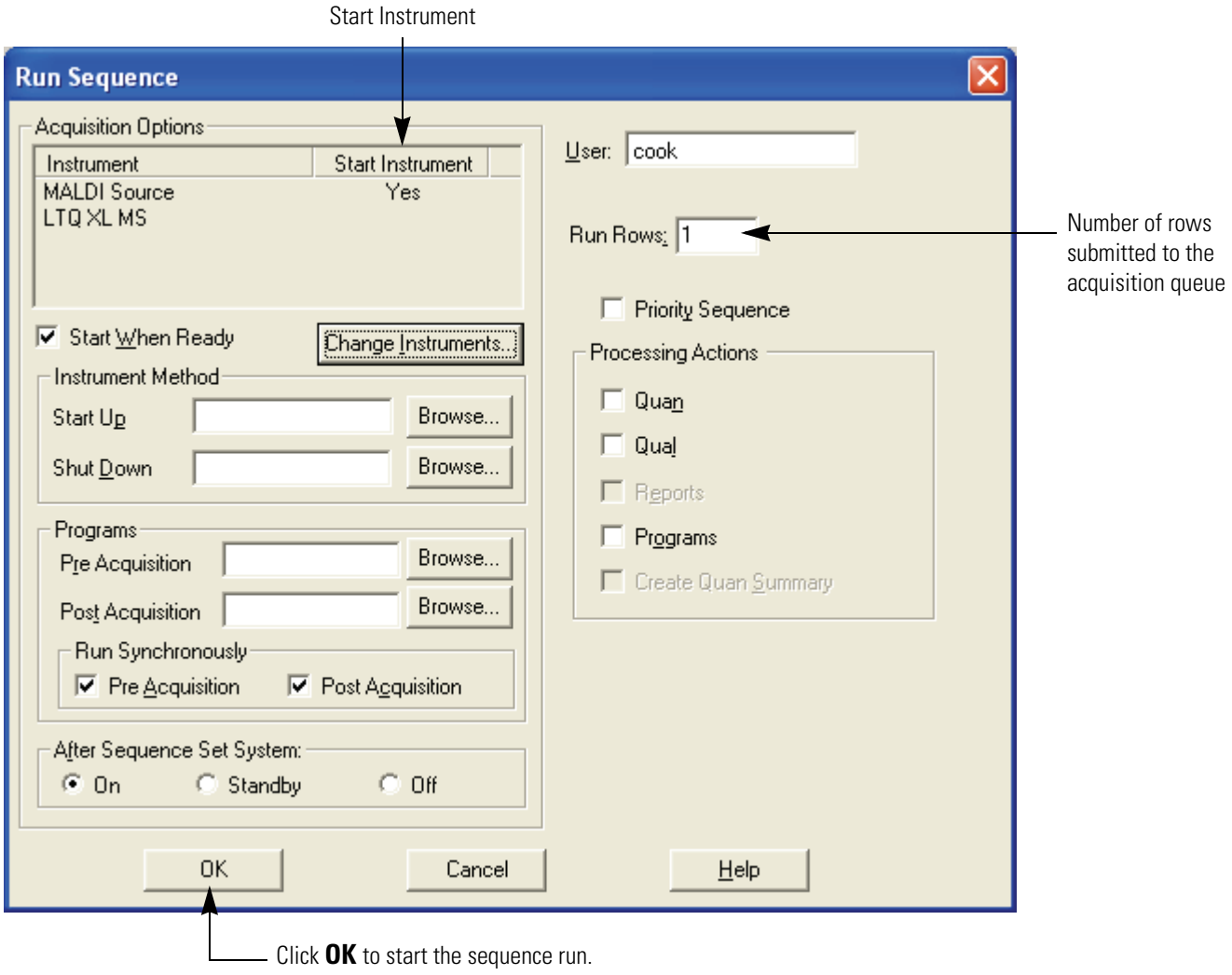
Figure 77. Change Instruments In Use dialog box.



5. Click **OK** to save the settings and close the Change Instruments In Use dialog box.
6. In the Run Sequence dialog box, with the exception of the settings listed in the following table, use the default settings. Figure 78 shows typical settings for the Run Sequence dialog box.

Parameter	Setting	Result
Acquisition Options		
Start When Ready	☑	Xcalibur adds the sequence to the end of the acquisition queue when you click OK.
After Sequence Set System		
Set System	◉ Standby	The mass spectrometer returns to Standby mode when the sequence finishes.
User	*user name*	Specifies the name of the operator.
Run Rows	*range of rows to run*	Specifies the sequence rows to run.
Priority Sequence	☐	Xcalibur places the sequence at the end of the acquisition queue.

Figure 78. Run Sequence dialog box with settings for acquiring MALDI data



7. Click **OK**.

   If there are no other runs in the acquisition queue, data acquisition for this sequence begins. For tips about running acquisition sequences to collect tissue imaging sequences, see Chapter 8, "Tissue Imaging."

8. Check the acquisition queue. If the Information view is not visible, click  (Info View button) and then click the **Acquisition Queue** tab.

You can analyze the MS/MS data you acquire using the procedures in this chapter using BioWorks. See Chapter 9, "Identifying Proteins Using BioWorks."

Tissue Imaging

This chapter describes how to prepare tissue samples for imaging with the MALDI LTQ XL system and how to take optical and mass spectral scans of the tissue samples.

Using MALDI mass spectrometry, you can use the tissue imaging feature to acquire mass spectral data on the spatial distribution of small molecules or peptides in a tissue sample. After data acquisition, using ImageQuest, you can visualize and process the data. You must get a license to enable the tissue imaging feature.

Contents

Getting a License for the Tissue Imaging Feature

❖ **To get a license for the MALDI tissue imaging feature**

1. Install the LTQ XL instrument control program according to the instructions on the CD cover.
2. Get a new license code from Thermo Fisher Scientific. See the next topic "Getting a New License Code."
3. Install the new license code as described in "Installing a New License Code" on page 124.

Getting a New License Code

To get a license code, use e-mail.

❖ **To get a license code**

1. From the desktop of the data system computer, choose **Start > All Programs > Xcalibur > Instrument Configuration**.

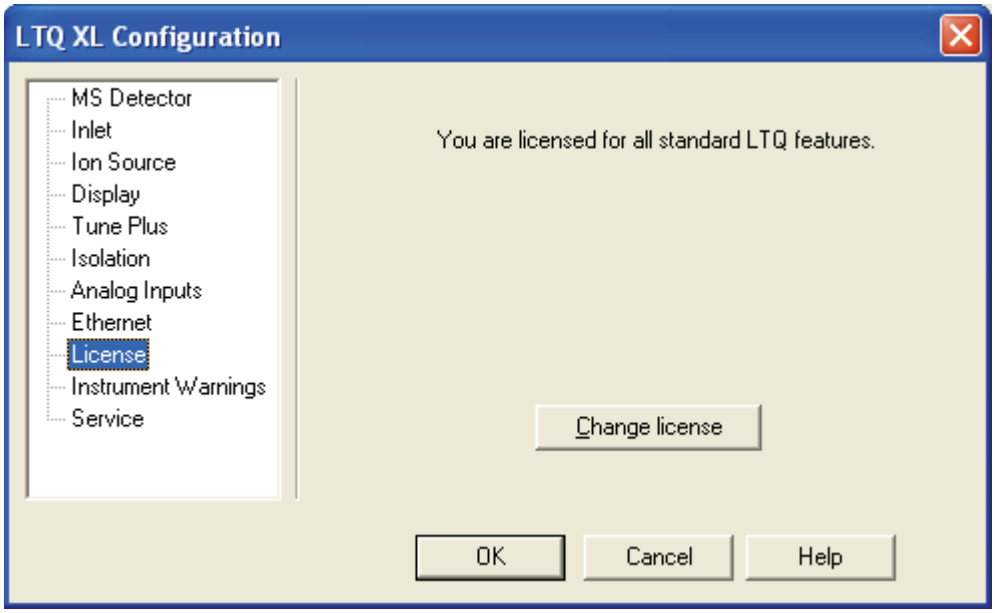
   The Xcalibur Instrument Configuration dialog box appears.
2. In the Configured Devices list, double-click the **LTQ XL MS** button.

   The LTQ XL Configuration dialog box appears (see Figure 79).
3. Select **License** in the list on the left side of the dialog box.

   If you do not have a license for the tissue imaging feature, the following message appears:

   You are licensed for all standard LTQ features.

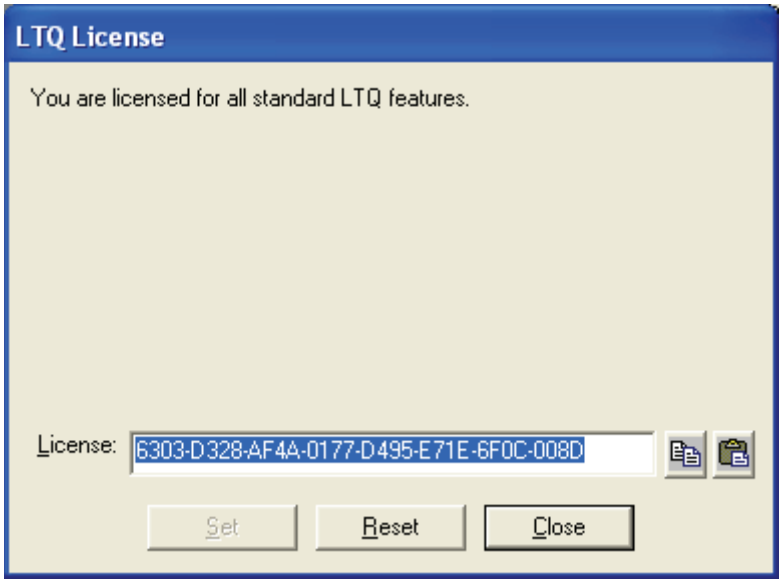
Figure 79. LTQ XL Configuration dialog box with License selected



4. Click **Change license**.

   The LTQ License dialog box appears. See Figure 80.

Figure 80. LTQ License dialog box



5. Click **Reset**.

   A dialog box with the following prompt appears:

   > If you reset the license, your existing license information will be invalidated and you will need to obtain a new license for this product. Do you wish to continue?

6. Click **Yes** to reset the license key.

A new license key appears in the License box.

7. Highlight the license key in the License box.
8. Press CTRL+C to copy the license key to the Windows clipboard.
9. Send an e-mail message to licenses.ms@thermo.com:
   a. In the subject line, type **License Request**.
   b. In the body of the e-mail message, press CTRL + V to paste the license key, and then type the following:
      - Your name
      - Your company name
      - Your company address
      - Your company phone number with your extension
      - The serial number that appears below the bar code on the back of the LTQ 2.5 software CD case

When Thermo Fisher Scientific Customer Support sends you a new license code, install it as described in the next topic.

Installing a New License Code

After you receive your new license code from Thermo Fisher Scientific Customer Support, install it as described in the following procedure.

❖ To install the license code

1. From the desktop of the data system computer, choose **Start > All Programs > Xcalibur > Instrument Configuration**.

   The Xcalibur Instrument Configuration dialog box appears.

2. In the Configured Devices list, double-click the **LTQ XL MS** button.

   The LTQ XL Configuration dialog box appears.

3. Select **License** in the list on the left side of the dialog box.
4. Click **Change license**.

   The LTQ License dialog box appears.

5. In the License box, type the new license number, or copy the license number from the e-mail sent from Thermo Fisher Scientific and paste it in the box.

   To avoid copying extra spaces, you can copy the license number from the e-mail and paste it into Notepad or Wordpad. Then carefully select the number, and copy it from Notepad or Wordpad and paste it in the License box.

   Tip Take care not to copy and paste any extra spaces. Pasting a space in the License box causes the license number to be declined.

6. Once you have pasted the correct number in the License box (without any extra spaces), click **Set**.
7. Click **OK** to accept the change in license.
8. Click **OK** to close the LTQ License MALDI dialog box.

   IMPORTANT Do not click Reset after you install the new license code. Reset invalidates your license.

9. Click **Done** to close the Instrument Configuration dialog box.

   A dialog box appears with the following message:

   > The new license number has been set.

10. Click **OK**.

    A dialog box appears with the following prompt:

    > In order for the configuration change to take effect, you will need to reboot the data system and then the LTQ.

11. Click **OK**.
12. Reboot the data system computer.
13. Reboot the MALDI LTQ XL system:
    a. Press the Reset button on the power entry panel.
    b. Take the LTQ XL mass spectrometer out of Standby mode.
    c. Take the MALDI source out of Standby mode.
    d. Place the MALDI source back in Standby mode.

    For more information on rebooting the MALDI LTQ XL system, see "Rebooting the MALDI LTQ XL System" on page 38.

Preparing Tissue Slides

For best results, section and prepare the sample just prior to MS analysis.

❖ **To prepare the tissue sample and apply it to a slide**

1. Flash freeze the sample in liquid nitrogen or isopentane, and then store the sample at -80 °C until you are ready to section the tissue.
2. Slice the sample and place it on a slide.

   The most commonly used thickness for a tissue sample is 10 to 12 µm. Sections thinner than 10 µm are too close to cell size and might not provide optimal results.

   You can use either conductive or non-conductive slides with the MALDI LTQ XL system. Because linear ion traps do not use high ion extraction potentials, they are not as susceptible to inhomogeneities in the field that would affect the ion packet, as opposed to axial MALDI-TOF instruments.
3. Prior to applying the MALDI matrix, place the slide with the sample in a desiccator for about 30 minutes.

   IMPORTANT Before you spray the matrix on the tissue sections ("Acquiring a Bitmap Image with an External Scanner" on page 129), acquire an optical image of the tissue slides. The matrix spray coats the tissue with a layer of white crystals. These crystals looks like a white cloud on top of the tissue and make it very difficult to see the tissue below, especially once the sample plate is inside the MALDI sample module.
4. Fix or wash the sample to remove compounds such as salts and lipids that can interfere with the results, as necessary.

   Note Take care not to remove the analyte of interest when rinsing tissue sections with solvents. When possible, select an appropriate wash solvent based on the relative solubilities of the analyte and contaminant.
5. (Optional) Outline the sample areas on the back of the slide with a marker. Once you coat the tissue with the matrix, distinguishing the tissue and the matrix becomes difficult.

CAUTION Only use a marker pen to place an identifying label on your plates. Do not tape anything to the sample plates, as doing so can cause the sample plate to jam inside the MALDI sample module.

Loading the Tissue Slides onto the Adapter Plate

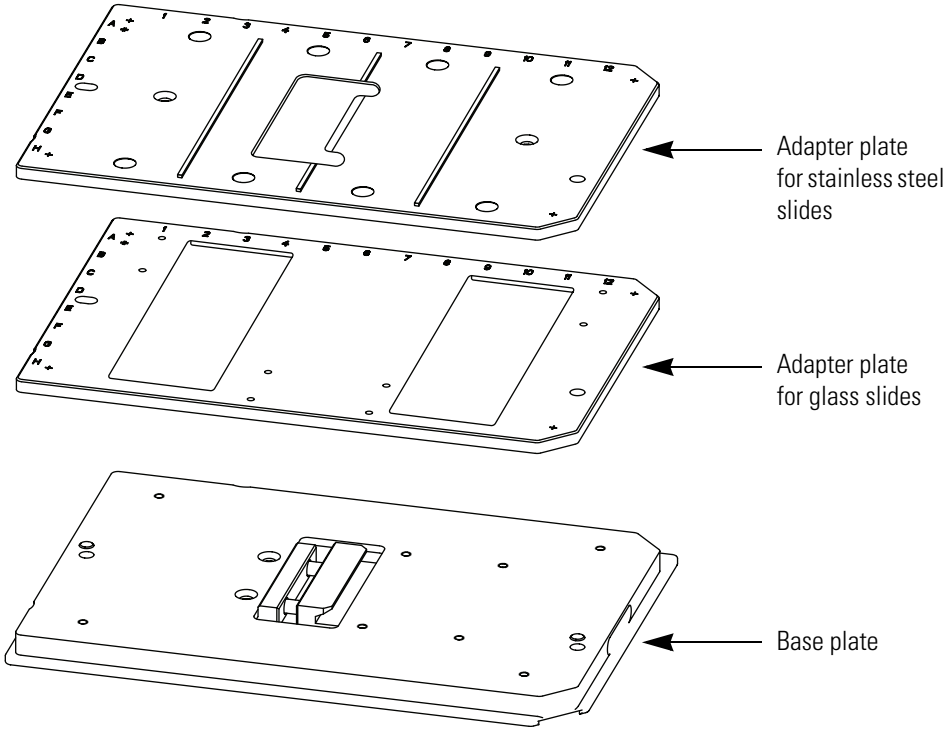
Two adapter plates are provided with the MALDI tissue imaging kit: one for holding glass slides and one for holding stainless steel slides. The adapter plate for stainless steel slides incorporates magnets to secure the slides. The adapter plate for glass slides holds two slides secured with two retaining clips each.

The adapter plates for tissue imaging use the same base plate as the 96- and 384-well plates. You must connect the plate to the base plate before loading it into the MALDI sample module. For instruction on connecting the top plate (adapter or well plate) to the base plate, see "Assembling the Sample Plate" on page 51.

When you load the plate assembly into the MALDI sample compartment (see "Loading a Sample Plate into the MALDI Sample Module" on page 52), the MALDI imaging system recognizes the plate you have inserted as a tissue plate by the marks on the adapter plate.

Figure 81 shows the two adapter plates for slides and the base plate.

Figure 81. Adapter plates for glass slides and stainless steel slides



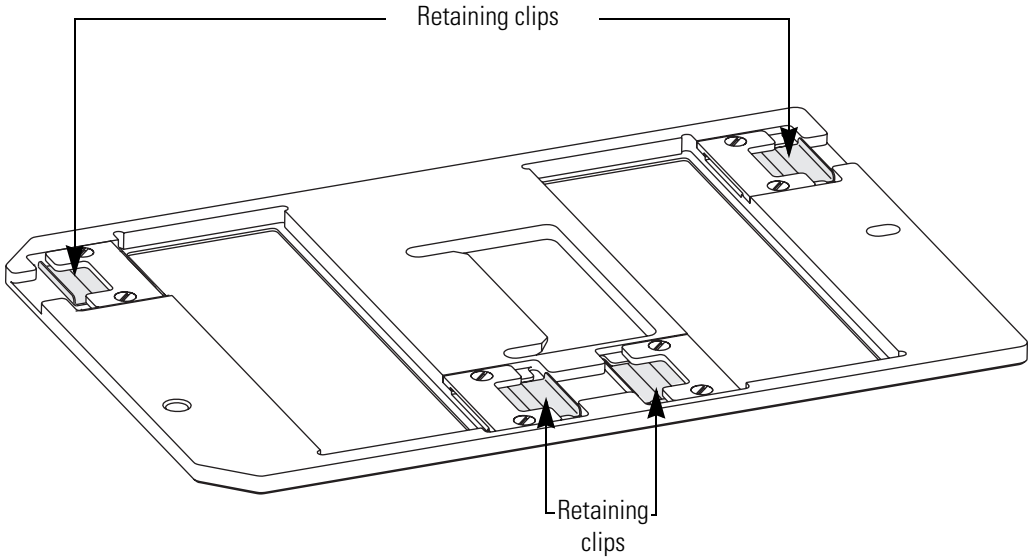
CAUTION Fit the top plate snugly to the base plate. Loading a misaligned plate assembly into the MALDI sample module can damage the MALDI source.

CAUTION Do not tape anything to the sample plate. Once the plate is loaded into the upper chamber of the XYZ mechanism, there is very little space between the plate and the ion transfer optics. Loading sample plates with taped objects into the MALDI sample module can damage the MALDI source.

❖ To secure the glass slides to the adapter plate

1. Turn the adapter plate over so that the retaining clips are visible. See Figure 82.

Figure 82. Back side of the adapter plate for glass slides



2. Pull the retaining clips away from the slide slots.
3. With the tissue samples facing the front of the adapter plate, place the glass slides in the slide slots.
4. Push the retaining clips toward the slide slots to retain the slides.

Acquiring a Bitmap Image with an External Scanner

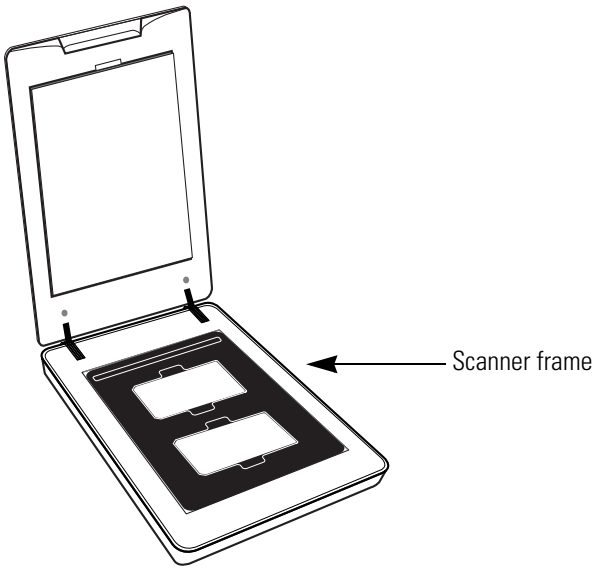
The MALDI tissue imaging kit includes an HP optical scanner. After you create a bitmap image of your tissue slides with the scanner, you can import the images onto the Tissue Imaging page of the MALDI Source dialog box in Tune Plus.

Tip Before applying the matrix, use a marker on the back side of the glass microscope slide to outline the tissue. The marker always shows through (whether you scan the image with the HP scanner or the MALDI camera), even after coating the tissue with matrix.

❖ To create a bitmap image of your tissue slides with the optical scanner

1. Load your slides onto the adapter plate.
2. Place the scanner frame on top of the scanner glass. See Figure 83.

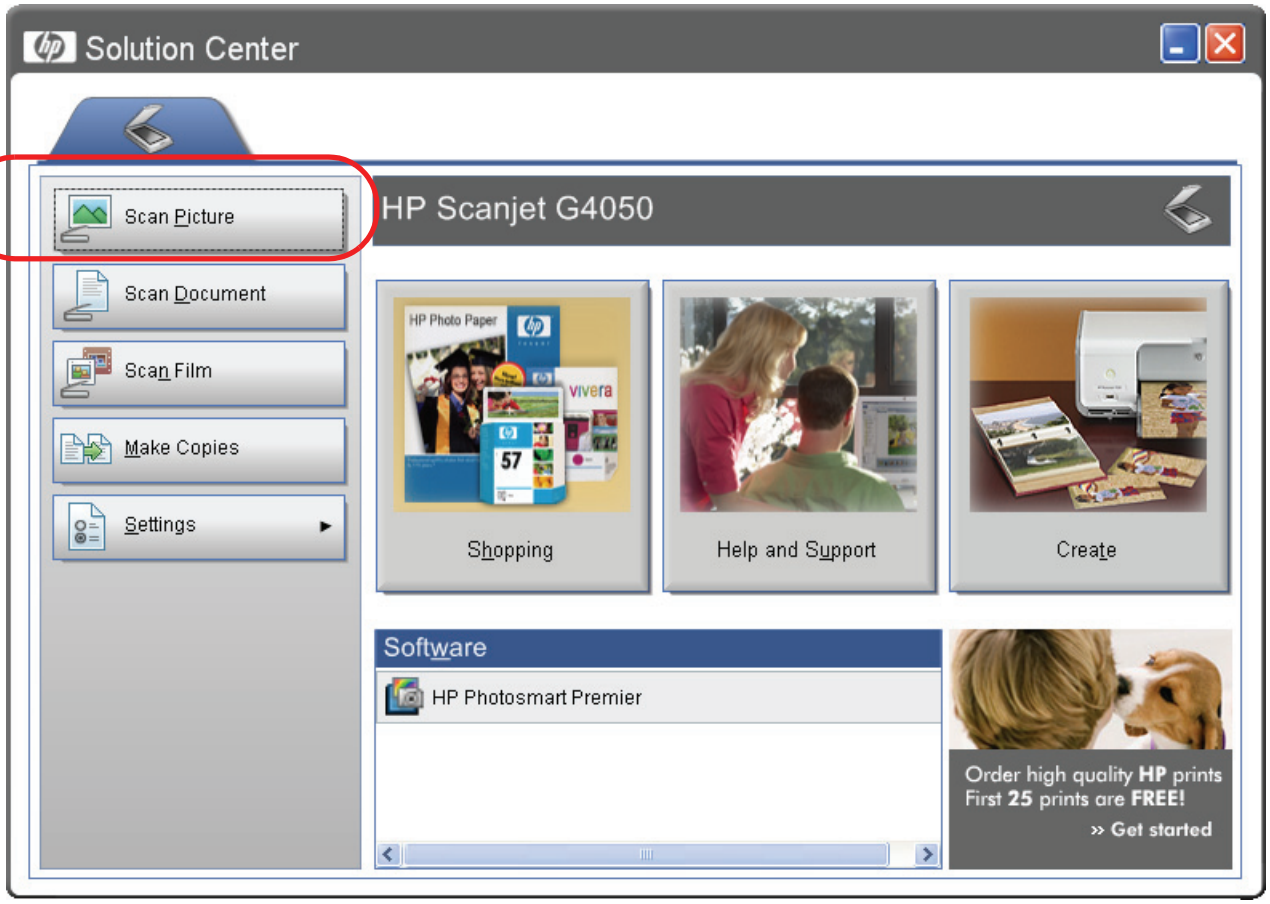
   Figure 83. HP scanner with scanner frame on top of the scanner glass



3. Place the adapter plate into the scanner frame. The tissue slides must be facing the scanner glass.
4. From the computer desktop, click the **HP Solution Center** icon.

   The Solution Center window appears. See Figure 84.

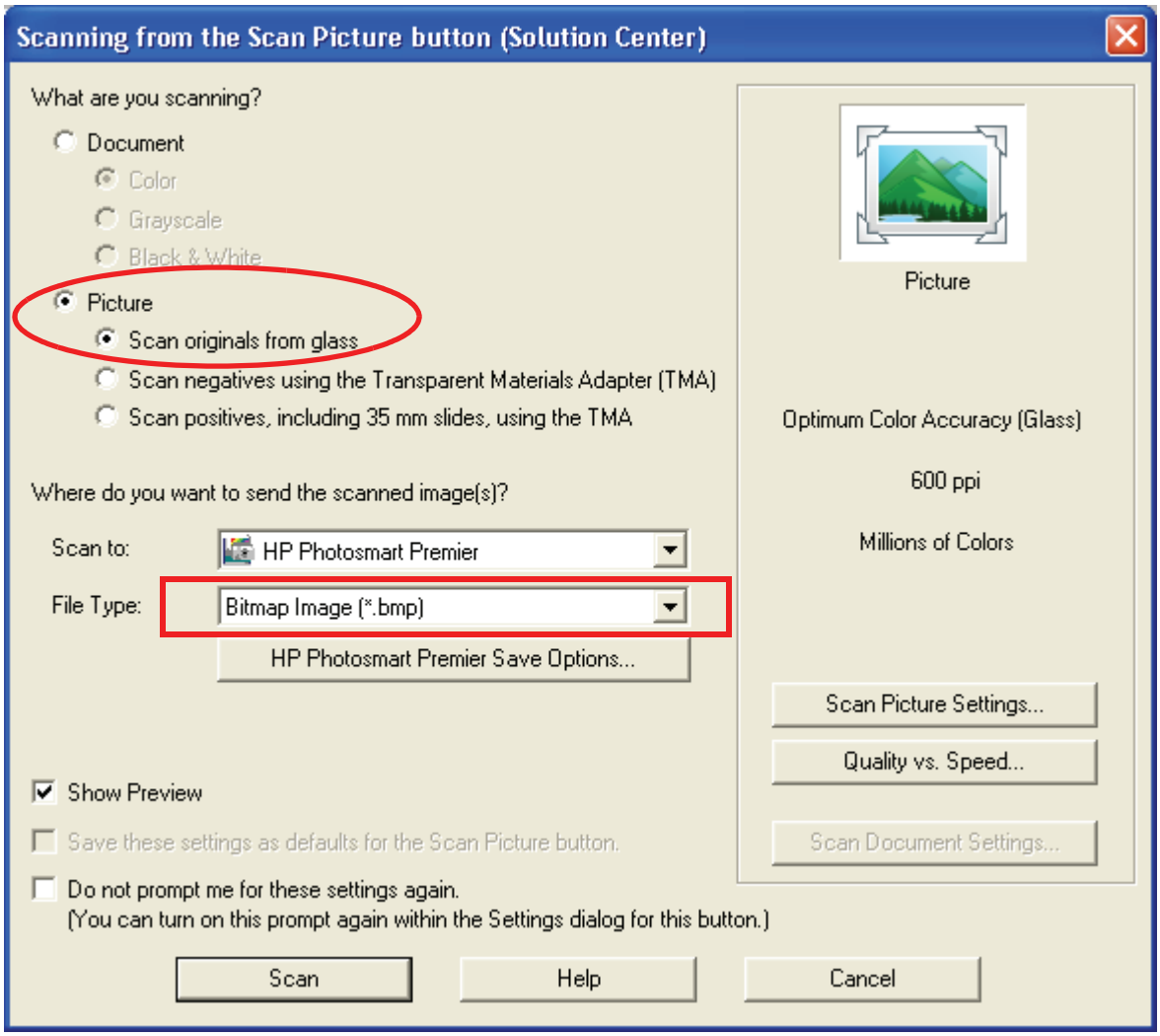
Figure 84. HP Solution Center



5. Click **Scan Picture**.

   The Scanning from the Scan Picture button dialog box appears (see Figure 85).

Figure 85. Scanning from the Scan Picture button dialog box

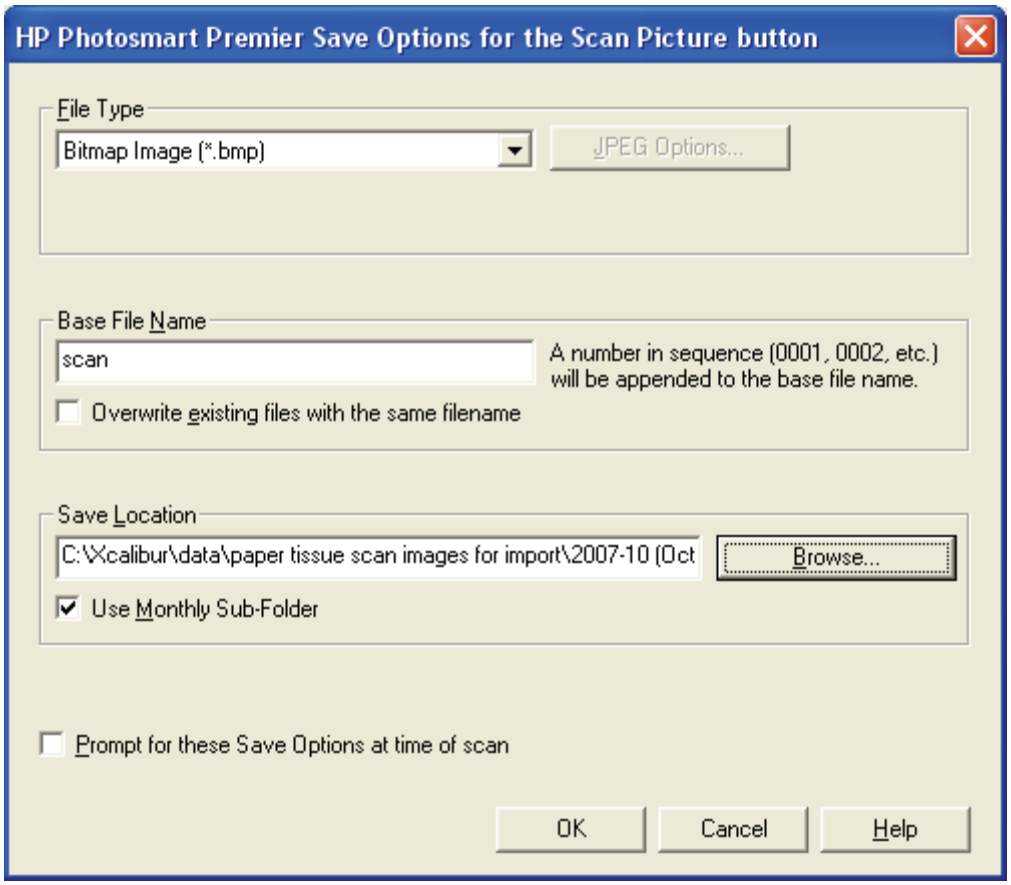


6. Make the following selections:
   - Under What are you scanning?, select the **Picture** option and the **Scan originals from glass** option.
   - Under Where do you want to send the scanned images?, select **Bitmap Image (*.bmp)** in the File Type list.

     On the Tissue Imaging page of the MALDI Source dialog box in Tune Plus, you can only open image files saved as bitmap images.
   - Select the **Show Preview** check box.
7. Click **HP Photosmart Premier Save Options**.

   The HP Photosmart Premier Save Options for the Scan Picture button dialog box appears. See Figure 86.

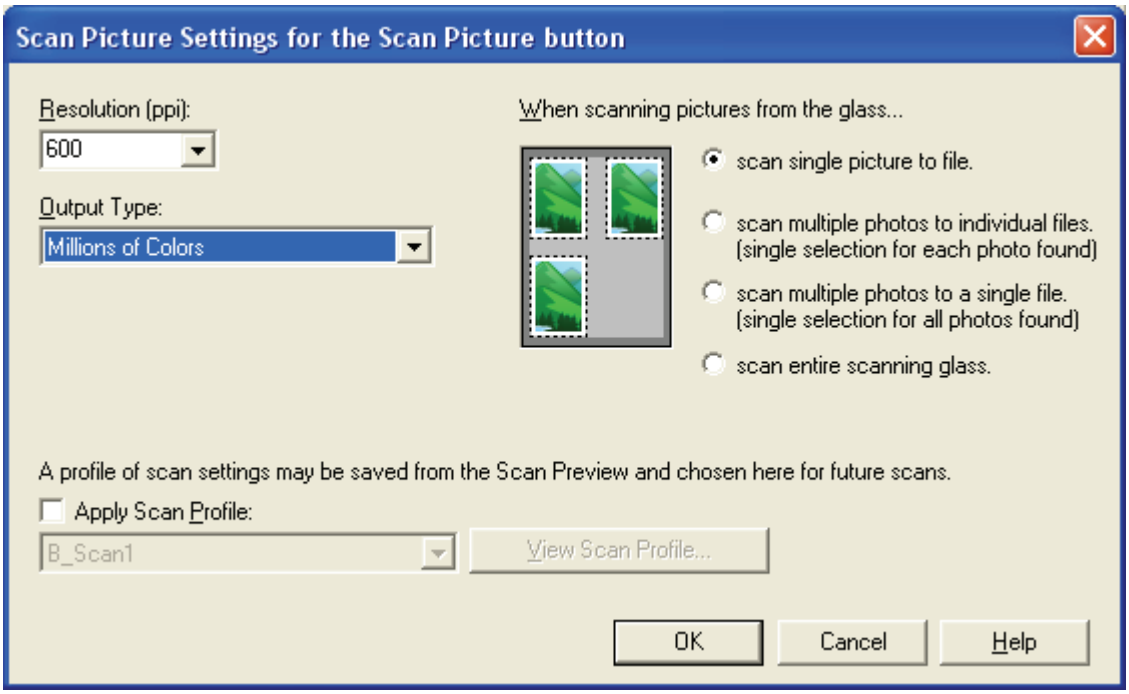
Figure 86. HP Photosmart Premier Save Options for the Scan Picture button dialog box



8. Make the following selections and entries:
   - Under File Type, select **Bitmap Image (*.bmp)**.
   - Under Base File Name, type an appropriate name to identify the file.
   - Under Save Location, browse to or type the location where you want to store the bitmap image files.
9. Click **OK** to accept the settings and return to the Scanning from the Scan Picture button dialog box. See Figure 85.
10. Click **Scan Picture Settings**.

    The Scan Picture Settings for the Scan picture button dialog box appears. See Figure 87.

Figure 87. Scan Picture Settings for the Scan Picture button



11. Make the following selections:
    - In the Resolution (ppi) list, select **600**.
    - In the Output Type list, select **Millions of Colors**.
    - Under When scanning picture from the glass, select the **scan single picture to file** option.
12. Click **OK** to accept the settings and return to the Scanning from the Scan Picture button dialog box.
13. Click **Scan**.

    The scanning process begins. The image is stored to the specifed file location as a bitmap image (*.bmp).

Testing the MALDI Matrix

For best results, before coating the entire tissue, test the MALDI matrix you plan to use by analyzing a test spot on a tissue sample. For information on selecting an appropriate MALDI matrix, see Appendix B, "Sample Preparation."

❖ To test the MALDI matrix

1. Prepare the tissue slide (see "Preparing Tissue Slides" on page 126).
2. Use a spotter to spot the tissue with one or more MALDI matrix compounds. Mark the spotted areas and label the spots if you are testing more than one type of MALDI matrix.
3. Load the tissue slide onto the adapter plate (see "Loading the Tissue Slides onto the Adapter Plate" on page 127).
4. Connect the adapter plate to the base plate (see"Assembling the Sample Plate" on page 51).
5. Load the sample plate into the MALDI sample module (see "Loading a Sample Plate into the MALDI Sample Module" on page 52).
6. As you view the mass spectrum produced by the test spot (see "Interactively Acquiring Data for Tissue Samples in Tune Plus" on page 145), manually optimize the tune parameters. After you determine the optimum tune parameters (see Table 4 on page 60) for your sample, save the tune parameters in a tune file.
7. Eject the sample plate from the MALDI sample module and prepare the tissue sample for analysis.

Applying the MALDI Matrix to the Tissue Samples

However you choose to apply the MALDI matrix, the key to matrix application for determining compound distribution in a tissue is to apply the matrix as uniformly as possible, forming the smallest crystals possible when the matrix co-crystallizes with the analyte. You can apply the matrix with one of the following tools:

- Airbrush
- Commercial nebulizer
- Spotters

Several commercial airbrushes suitable for applying the matrix are available from manufacturers such as Aldrich (thin layer chromatography), Testors (Aztek airbrush), and Meinhard (nebulizers).

IMPORTANT Before applying the matrix, use the HP scanner to scan a bitmap image of your tissue

Tip Before applying the matrix, use a marker on the back side of the glass microscope slide to outline the tissue. The marker always shows through (whether you scan the image with the HP scanner or the MALDI camera), even after coating the tissue with matrix.

❖ **To apply the matrix with an airbrush**

1. Connect the airbrush to a high-quality, compressed nitrogen tank with a regulator for controlled air flow.
2. Set the airbrush on a stand.
3. Clamp on the air flow regulation lever.
4. Select the drop size.
5. Fill the liquid reservoir with the solvent used as a diluent for the matrix and test the drop size.
6. Adjust the drop size if necessary to obtain the correct uniformity.
7. Fill the liquid reservoir with the matrix solution and test the drop size again.
8. Apply the matrix.

   The matrix should be a fine mist that covers the tissue and is not too dry or too wet.

Importing a Bitmap Image into the Tissue Imaging Page

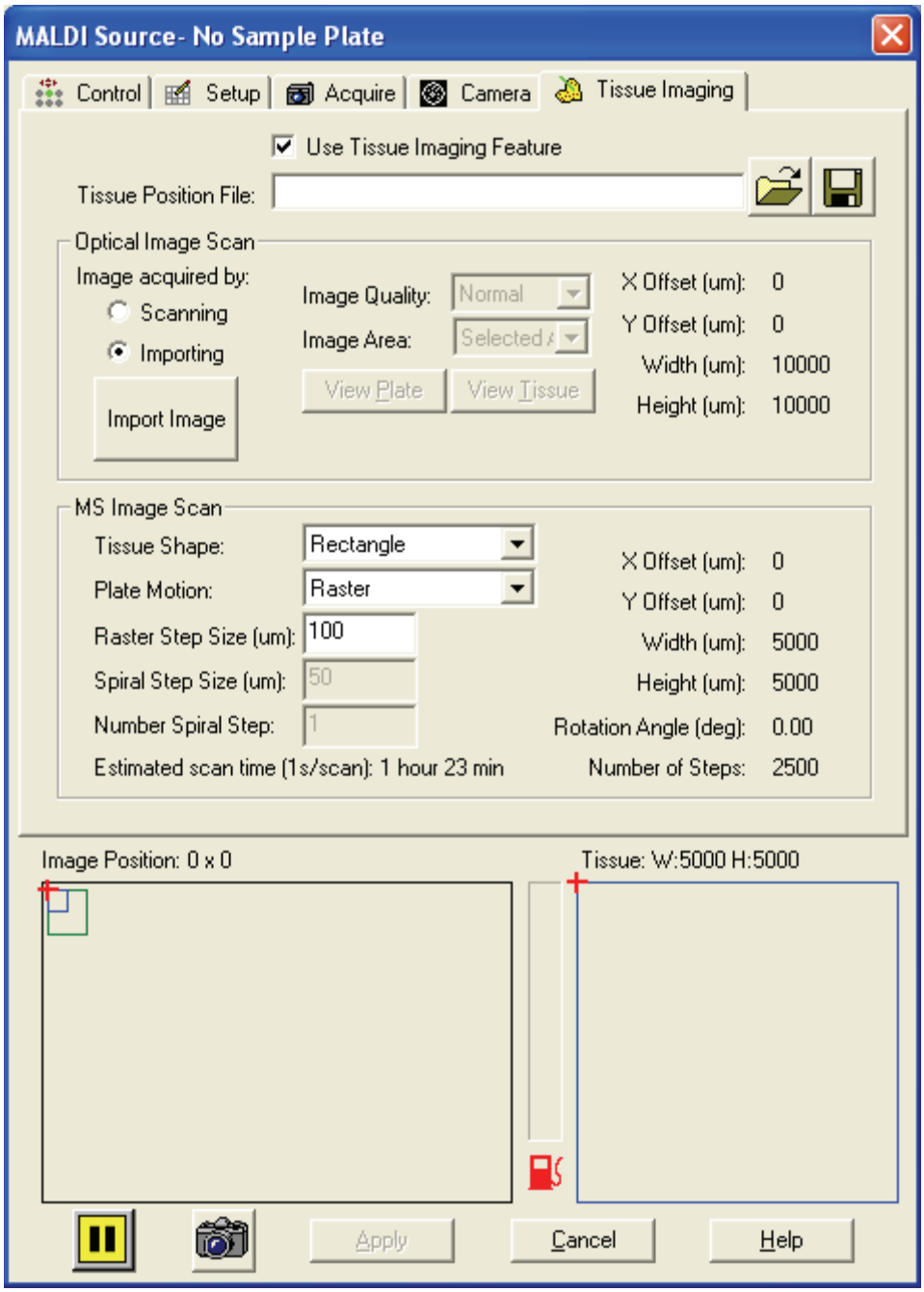
You can import the bitmap image acquired by scanning the tissues slides with the HP scanner into the Tissue Imaging page.

❖ **To import a bitmap image (600 ppi) created with the external scanner**

1. Open Tune Plus by doing one of the following:
   - From the Windows™ Start menu, choose **All Programs > Xcalibur > LTQ Tune**.
   - From the computer desktop, double-click the LTQ Tune shortcut icon.
2. In Tune Plus, choose **Setup > MALDI Source**.
3. Click the **Tissue Imaging** tab

   The Tissue Imaging page opens.
4. Select the **Use Tissue Imaging Feature** check box.
5. In the Optical Image Scan area, select the **Importing** option. See Figure 88.

Figure 88. View of the Importing option in the Tissue Imaging page



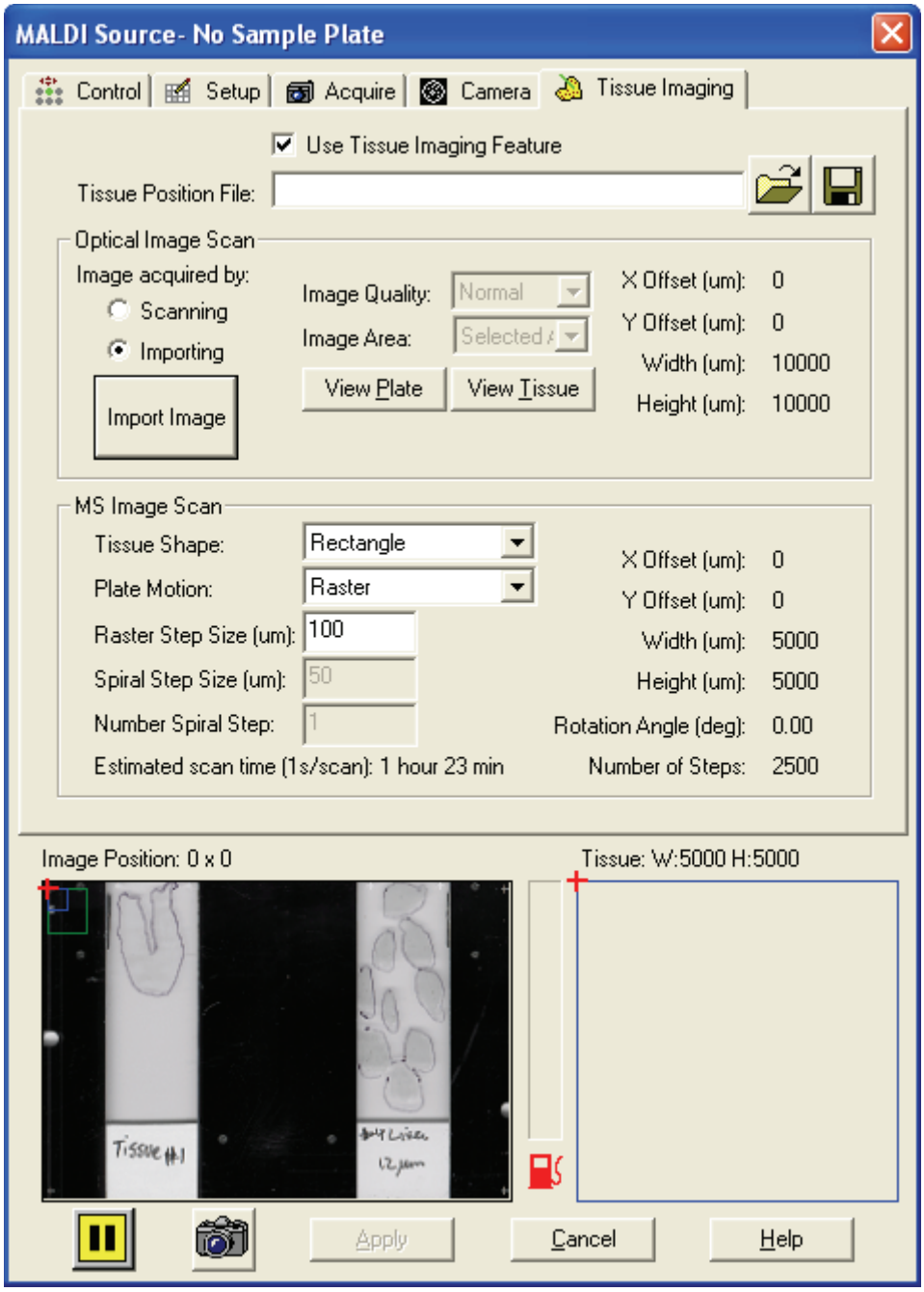
6. Click **Import Image**.

   The Open dialog box appears.

7. Browse to the folder where you saved the scanned bitmap image, select the bitmap image of interest, and click **Open**.

The image created with the HP scanner appears in the Image Position box at the bottom of the MALDI Source dialog box (see Figure 89).

Figure 89. View of an imported bitmap image in the Image Position box



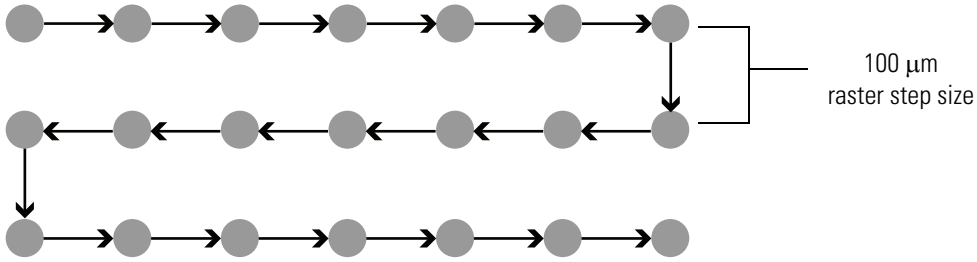
Creating a MALDI Position File Using an Imported Bitmap Image

MALDI position files (*.MALDIpos) contain the bitmap image of the tissue and the MS scanning selections. If you do not make any MS scanning selections, the MALDI program uses the settings listed in Table 9.

Table 9. Default MALDI position file settings

Parameter	Default setting
Tissue Shape	Shape of the selected tissue area
Plate Motion	Raster
Raster Step Size (µm)	100

With the above settings (assuming a rectangular tissue shape), the MALDI X-Y stage moves the sample plate in the pattern shown below. The circles represent the areas that the laser fires on. The distance between each circle is the step size.



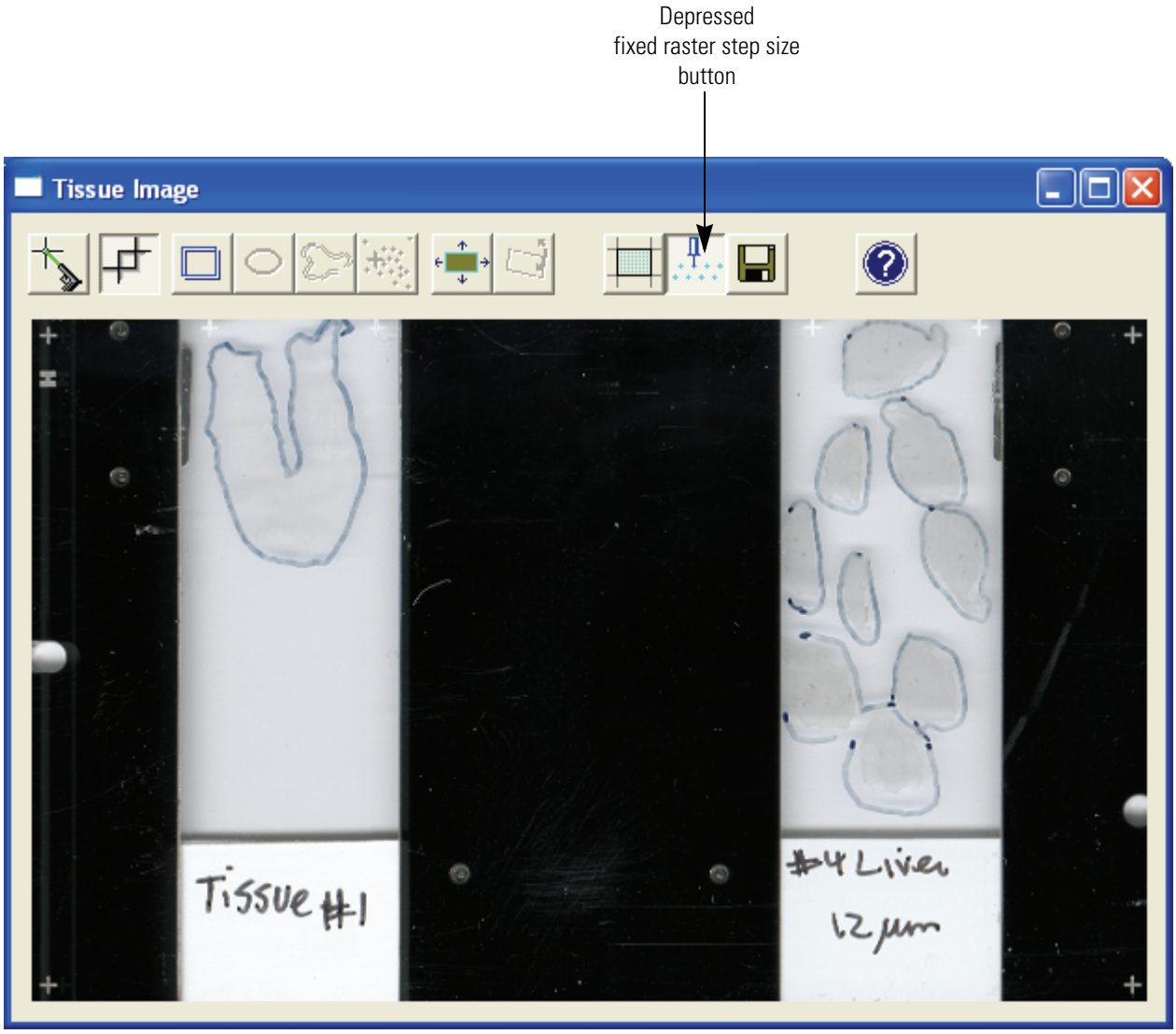
MALDI position files for tissue imaging plates are analogous to well plate locations of well plates. To acquire data with a sequence file, create MALDI position files of the areas interest.

❖ To create a MALDI position file of a selected tissue area from an imported image

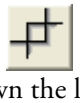
1. Import the bitmap image as described in "Importing a Bitmap Image into the Tissue Imaging Page" on page 135.
2. Select the tissue area where you want to acquire MS scan data, by doing one of the following:
   - Crop and select the tissue area from a magnified view of the plate as described in step 3 and step 4.
   - (Optional for large tissues) In the Image position box at the bottom of the MALDI Source dialog box, hold down the SHIFT key and drag the cursor to select the tissue area of interest. The selected rectangular area appears in the Image Position area at the bottom of the MALDI Source dialog box.
3. Crop the tissue area from a magnified view of the plate:
   a. Click **View Plate**.

      The Tissue Image window opened by clicking the View Plate button appears (see Figure 90).

Figure 90. Tissue Image window opened by clicking the View Plate button



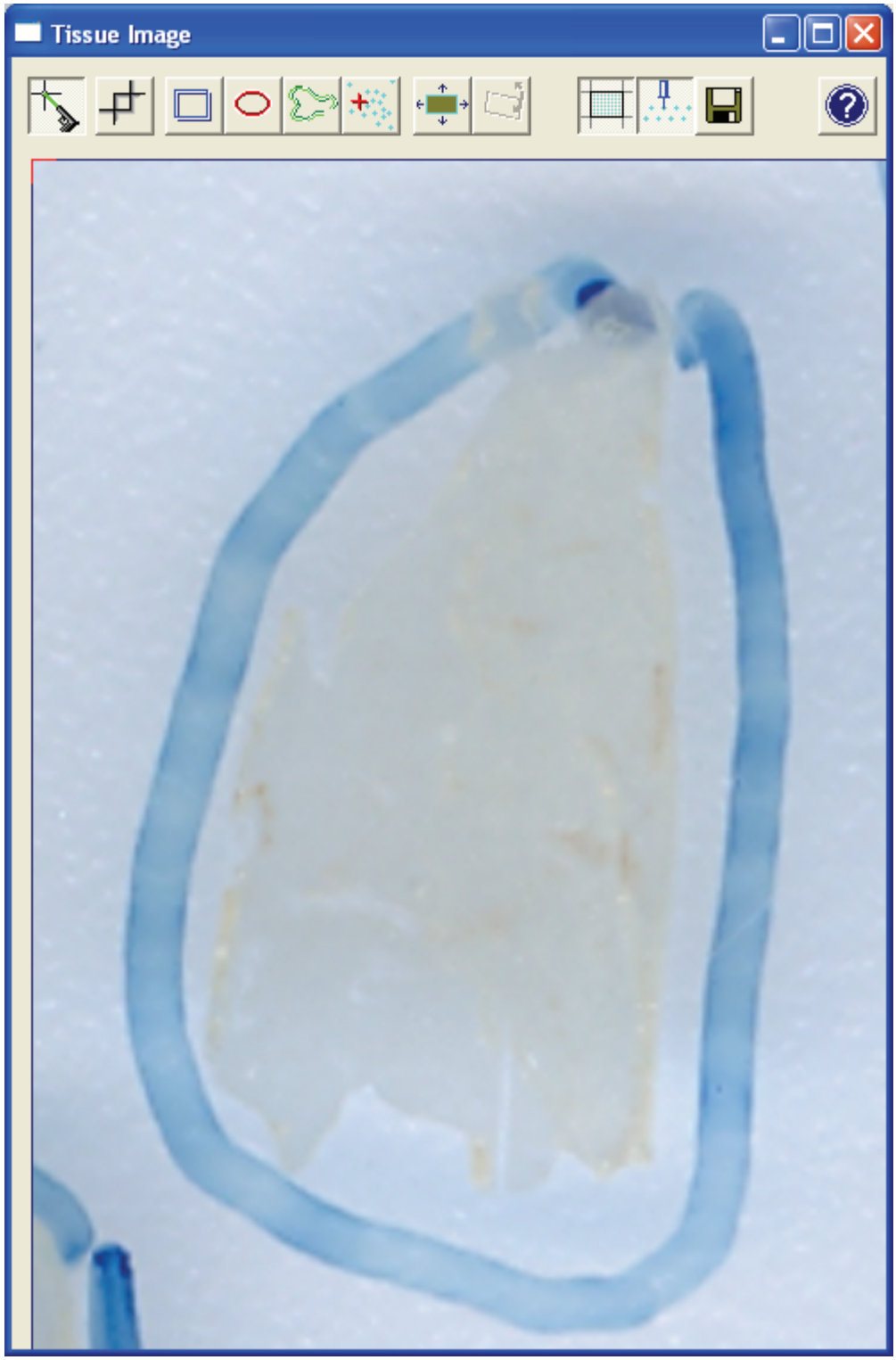
b. Ensure that the Fixed raster step size while selecting a MS scan area button is depressed (see Figure 90).

c. Click the  **Crop current image to a selected rectangle** button.

d. Hold down the left mouse button and drag the curser to crop the tissue area of interest.

   The cropped area appears in the Tissue Image window and all the toolbar buttons become available (see Figure 91).

Figure 91. Tissue Image window with cropped tissue area



4. In the cropped tissue image, further refine the tissue area to be scanned:

   a. Click the appropriate toolbar button.

Toolbar button	Selection	Toolbar button	Selection
	Rectangle		Free form area
	Ellipse		Individual points

   b. To select a rectangular, elliptical, or free form area selection, hold down the left mouse key and drag the cursor. To select individual points click the points of interest in the image.

5. To save a bitmap image of the selected tissue, click  in the Tissue Image window. The Save As dialog box appears. Type a file name in the File name box and click **Save**. The MALDI program automatically saves the file as a bitmap image (*.bmp).

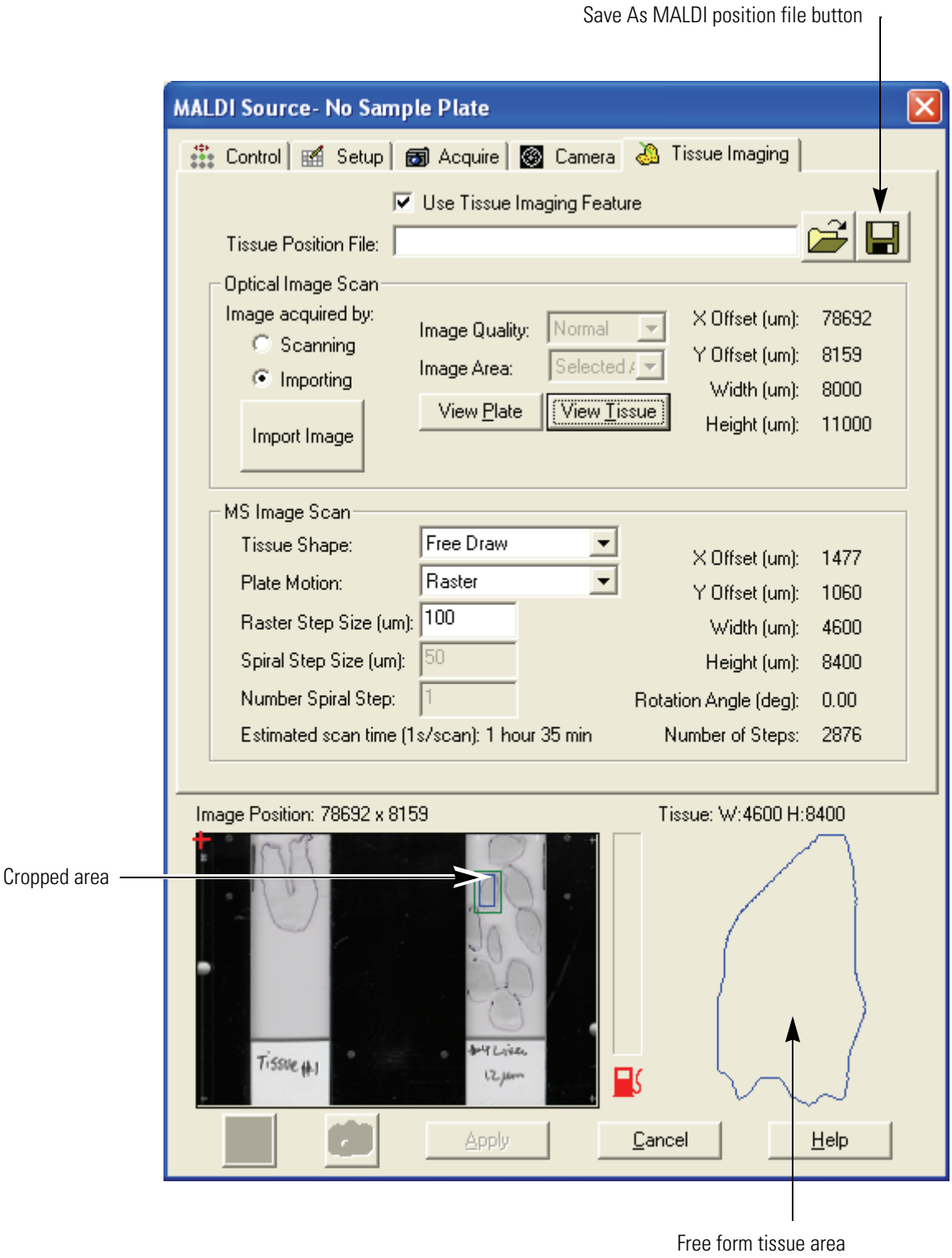
   Note Clicking  in the Tissue Image window saves the image as a bitmap file, not as a MALDI position file.

6. Click  to close the Tissue Image window.

   The cropped area is outlined in the Image Position box at the bottom of the MALDI Source dialog box. An outline of the selected tissue area appears to the right of the Image Position box. Figure 92 shows a tissue area selected with the Free Form area tool.

   Under MS image scan, the Tissue Shape list displays the shape of the selected tissue. The Plate Motion list displays the Raster selection, and the Raster Step Size box display a value of 100 µm.

Figure 92. Tissue Imaging page with a view of the cropped tissue area



7. In the MS Image Scan area of the Tissue Imaging page, for most applications, use the plate motion and raster step size automatically selected by the MALDI program (see Table 9 on page 138).

   An estimate appears of the time required to complete the scan, assuming a scan rate of 1 scan/second.

8. Click  in the Tissue Imaging dialog box.

   The Save Tissue Position File As dialog box appears.

9. Type a name in the File name box.

10. Click **Save** to save the file and close the dialog box.

    By default, the program stores MALDI position files (*.MALDIpos) in the following folder:

    drive:\Xcalibur\methods

Creating a MALDI Position File Using the MALDI Camera

You can create a MALDI position file by scanning the sample plates with the MALDI camera.

❖ **To create a MALDI position file by scanning the image with the MALDI camera**

1. Open Tune Plus by doing one of the following:
   - From the Windows Start menu, choose **All Programs > Xcalibur > LTQ Tune**.
   - Or, from the computer desktop, double-click the **LTQ Tune** shortcut icon.
2. In Tune Plus, choose **Setup > MALDI Source**.

   The MALDI Source dialog box opens with the Control tab shown by default.
3. Load the sample plate with the tissue slides into the MALDI sample module as described in "Loading a Sample Plate into the MALDI Sample Module" on page 52.
4. Click the **Tissue Imaging** tab.

   The Tissue Imaging page opens (see Figure 88 on page 136).
5. Select the **Use Tissue Imaging Feature** check box.
6. In the Optical Image Scan area, select the **Scanning** option.

   The image scanning features become available.

7. In the Image Quality list, select an appropriate resolution value (see Table 10). Increasing the image quality increases both the file size of the image and the time required to scan it.

Table 10. Image quality selections

Image Quality	Description
Survey	A very fast, low-resolution scan that reveals the position of the tissue sample on the plate
Coarse	A fast scan that is appropriate if the image quality is not important
Normal	A typical scan that is good for most applications
Fine	A high-resolution scan that yields the best possible image

8. In the Image Area list, select the area of the plate to be scanned.

   The available selections (see Table 11) depend on the plate type in the MALDI sample module.

Table 11. Image Area selections

Plate type	Selections
Stainless steel slides	selected area, slide 1, slide 2, slide 3, and slide 4
Glass slides	selected area, slide 1, and slide 2
Well plate	selected area and whole plate

9. If you selected Selected Area for the image area, hold down the SHIFT key and drag the cursor in the Plate Image portion of the MALDI Source dialog box to select the tissue area of interest.

   The selected area is outlined in the Plate Image portion of the dialog box. An estimate appears of the time required to complete the optical scan.

10. Click **Scan Image**.

    The program scans the image. When the scan ends, the Tissue Image window appears.

11. Select the tissue area of interest. (The area for which you want to collect MS scan data.)

12. Click  to close the Tissue Image window.

    The MALDI Source dialog box appears open to the Tissue Imaging page.

13. In the MS Image Scan area of the Tissue Imaging page, select the scanning options.

    An estimate appears of the time required to complete the scan assuming a scan rate of 1 scan/second.

14. Save the image as a MALDI position file (*MALDIpos):

    a. Click  in the Tissue Imaging dialog box.

    The Save Tissue Position File As dialog box appears.

b. Type a name in the File name box and click **Save**.

The program automatically saves the file as a MALDI position file. By default, the program stores MALDI position files (*.MALDIpos) in the following folder:

drive:\Xcalibur\methods

Acquiring Mass Spectral Data from Tissue Samples Using Tune Plus

You can acquire a data file in Tune Plus by interactively defining the scan parameters for the LTQ XL mass spectrometer and setting the MALDI source parameters or you can acquire a data file by using an instrument method.

This topic contains these procedures:

- Interactively Acquiring Data for Tissue Samples in Tune Plus
- Using an Instrument Method to Acquire Data for Tissue Samples in Tune Plus

Interactively Acquiring Data for Tissue Samples in Tune Plus

❖ **To interactively acquire mass spectral data from tissue samples in Tune Plus**

1. Prepare the tissue slides (see "Preparing Tissue Slides" on page 126) and load them onto the adapter plate (see "Loading the Tissue Slides onto the Adapter Plate" on page 127).
2. Use the HP scanner to scan the sample plate and acquire a bitmap image.

   See "Acquiring a Bitmap Image with an External Scanner" on page 129.
3. Apply the MALDI matrix to the tissue slides.

   See "Applying the MALDI Matrix to the Tissue Samples" on page 134.
4. Load the sample plate into the MALDI sample module.

   See "Loading a Sample Plate into the MALDI Sample Module" on page 52.
5. Start Tune Plus:
   - From the computer desktop, choose **Start > All Programs > Xcalibur > LTQ Tune**.
   - Or, double-click the  **LTQ Tune** shortcut icon on the computer desktop.
6. In the Tune Plus window, do the following:
   a. Choose **File > Open** and select the tune file for your analyte of interest.

      For best results, prepare a test sample spot and determine the optimal tune parameters (see Table 4 on page 60) for your sample as described in "Testing the MALDI Matrix" on page 134.

   b. Set up the scan parameters for the LTQ XL mass spectrometer.

See "Acquiring Sample Data Interactively in Tune Plus" on page 87.

IMPORTANT The setting for Microscans in the Define Scan dialog box must match the setting for the Microscans per Step in the MALDI Source dialog box - Control page.

c. Choose **Setup > MALDI Source**.

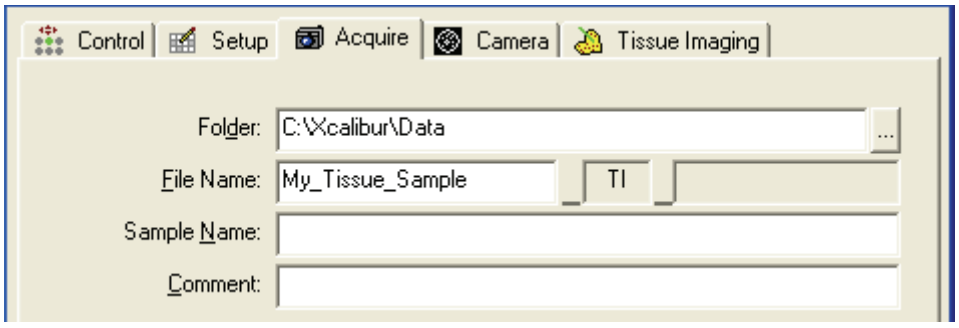
The MALDI Source dialog box appears with the Control page shown by default.

7. Click the **Tissue Imaging** tab.
8. On the Tissue Imaging page, do the following:
   a. Select the **Use Tissue Imaging Feature** check box.
   b. Click  to the right of the Tissue Position File box. The Open Tissue Position File dialog box appears. Select the MALDI position file for the sample plate you are analyzing, and then click **Open**.

      The image stored in the MALDI position file appears in the image view at the bottom of the MALDI Source dialog box.
   c. Select the tissue area that you want to scan. If you make changes that affect the MALDI position file, save the changes to a new MALDI position file.
   d. Click **Apply**.
9. In the MALDI Source dialog box, click the **Acquire** tab.

   The Acquire page appears. See Figure 93.

Figure 93. Top portion of the Acquire page



10. Specify the file location and file name:
    - Select the file folder by clicking  and browsing to the appropriate directory.
    - Specify the file name by typing a name in the File Name box.

      Three separate boxes separated by underscores constitute the file name: the base file name box, the sample position box, and the suffix box. The program automatically fills in the sample position box with TI (for tissue imaging) and the suffix box with a date stamp.

- (Optional) In the Sample Name box, type the sample name.
- (Optional) In the Comment box, type additional comments about the sample or experiment.

11. In the MALDI Source dialog box, click the **Control** tab.
12. On the Control page, do the following:
    a. Under MALDI Settings, make sure ASF is Off.
    b. Under Acquisition settings, type a name for the data file to be acquired.
    c. Click **Apply**.
    d. To start data acquisition, click  .

       The laser starts firing. The system starts scanning and acquiring data to an Xcalibur raw data file.
    e. To stop data acquisition before the end of the sample, click  .

       The MALDI source goes into Standby mode.

Using an Instrument Method to Acquire Data for Tissue Samples in Tune Plus

❖ To acquire a data file using a stored instrument method

1. Prepare the sample plate and load it into the MALDI sample module.
2. Start Tune Plus.
3. Choose **Setup > MALDI Source**.

   The MALDI Source dialog box appears with the Control page shown by default.
4. Click the **Tissue Imaging** tab.

   The Tissue Imaging page appears.
5. Select the **Use Tissue Imaging Feature** check box.
6. Open a Tissue Position File:
   a. Click  to the right of the Tissue Position File box.

      The Open Tissue Position File dialog box appears.
   b. Select the MALDI position file for the sample plate you are analyzing, and then click **Open**.

      The image stored in the MALDI position file appears in the image view at the bottom of the MALDI Source dialog box.
7. Click the **Acquire** tab.

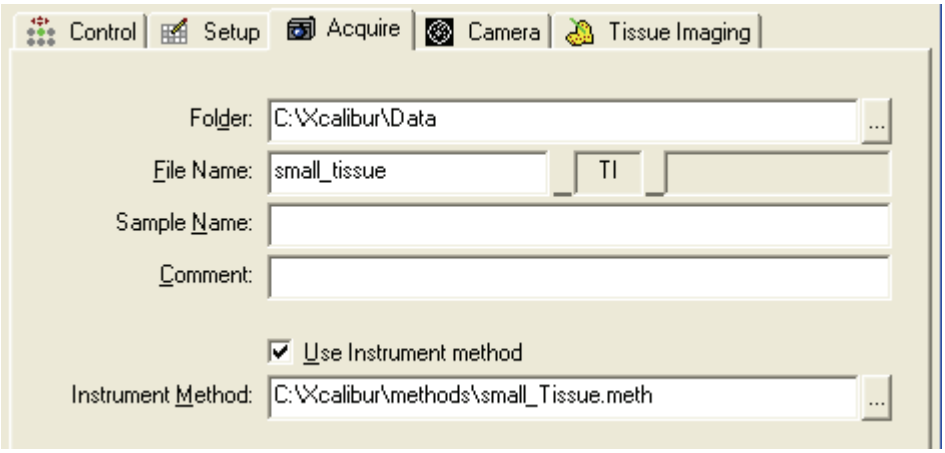
   The Acquire page appears.

8. Make the appropriate entries and selections for the data file location and name. See step 10 on page 146.

9. Select the **Use Instrument Method** check box, and then select a tissue imaging method (see Figure 94).

   For information on creating an instrument method, see "Creating an Instrument Method for Tissue Imaging" on page 150.

   Figure 94. Top portion of the Acquire page



10. In the Tissue view at the bottom of the page, select the area of interest.

11. Click **Apply**.

12. To start data acquisition, click  .

    The laser starts firing. The system starts scanning and acquiring data (as specified in the instrument method) to an Xcalibur raw data file. When the system completes the method, the MALDI source returns to Standby mode.

Acquiring Mass Spectral Data from Tissue Slides Using Xcalibur

You can automate the collection of mass spectral data for a tissue sample by creating a MALDI position file, a tissue imaging instrument method, and an acquisition sequence. You use Tune Plus to create MALDI position files and tune files. You use the Xcalibur data system to create instrument methods and acquisition sequences.

The MALDI position file contains a bitmap image of the tissue slides and the MS scanning parameters, including the plate motion. The instrument method contains the tune file created for the analytes of interest (see "Tuning on an Analyte" on page 60) and the experiments to be performed by the LTQ XL mass spectrometer. Each row of the acquisition sequence contains information to acquire a data file, including the data file name, the instrument method, and the MALDI position file.

❖ To automatically acquire a set of (*.raw) data files in Xcalibur

1. Create a tune file for your analytes (see "Tuning on an Analyte" on page 60).
2. Prepare the tissue slides (see "Preparing Tissue Slides" on page 126) and load them onto the adapter plate (see "Loading the Tissue Slides onto the Adapter Plate" on page 127).

   Because a tissue imaging instrument method must be accompanied with a MALDI position file, perform step 3, step 8, or both to create a MALDI position file.
3. Take a (600 ppi) scan of the tissue slides (see "Acquiring a Bitmap Image with an External Scanner" on page 129). Import this image into the Tissue Imaging page of the MALDI Source dialog box and create a MALDI position file.
4. (Optional) Test the matrix and optimize the laser energy, number of microscans and microscans per step (see "Testing the MALDI Matrix" on page 134)
5. Apply the MALDI matrix to your tissue sample (see "Applying the MALDI Matrix to the Tissue Samples" on page 134).
6. Take a second optical scan of the sample plate with the matrix applied.
7. Load the sample plate into the MALDI sample compartment (see "Loading a Sample Plate into the MALDI Sample Module" on page 52).
8. (Optional) Take a scan of the target area with the MALDI camera and create a MALDI position file.

   Taking a scan of the entire tissue using the MALDI camera can be quite time consuming. To reduce the amount of time required to produce an image of the desired target area, acquire a bitmap image (600 ppi) of the entire tissue using the external scanner. Import this image onto the Tissue Imaging page, and then select a portion of the plate to be scanned with the MALDI camera.
9. (Optional) Acquire a test data file. See "Interactively Acquiring Data for Tissue Samples in Tune Plus," on page 145.
10. Create an instrument method for tissue imaging. See the next topic.

11. Create an acquisition sequence (see "Creating an Acquisition Sequence for Tissue Imaging" on page 151).
12. Run the sequence (see "Acquiring Raw Data Files in Sequence Setup" on page 117).

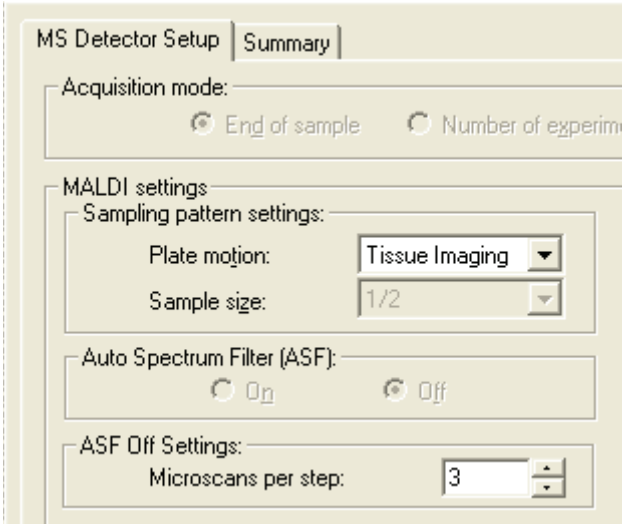
Creating an Instrument Method for Tissue Imaging

"Creating an Instrument Method" on page 105 provides more information on creating instrument methods. The following procedure describes the settings required to create a tissue imaging instrument method.

❖ To create a tissue imaging instrument method

1. In the Xcalibur Instrument Setup window, select the template for the type of experiment you want to perform. See Figure 95.

   Figure 95. General MS or MSn template with Tissue Imaging selected in the Plate motion list



2. In the instrument method template, select **Tissue Imaging** in the Plate motion list.

   When you select Tissue Imaging, ASF is unavailable.

3. In the Microscans per step box, type or select the number of microscans per step.

   A step is a change in the plate position. The position of the laser is static. The MALDI X-Y stage moves the tissue plate as specified by the plate motion settings in the MALDI position file.

   When you create a tissue imaging instrument method, you can overwrite the microscans setting in the tune file. When you increase the number of microscans per step, you also increase the acquisition time.

   IMPORTANT The number of microscans (in the Tune file) must match the number of microscans per step (in the instrument method).

4. Set up the scan events.
5. Save the instrument method. Use a name that you can identify as a tissue imaging instrument method.

Creating an Acquisition Sequence for Tissue Imaging

You must specify a position file for each row in the sequence that contains a tissue imaging instrument method. You can use the same position file or different position files for every row of the sequence.

These procedures describe how to specify the position file in an Xcalibur sequence list:

- Specifying the Same Position File for the Entire Sequence
- Specifying a Different Position File for Each Sequence Row

Specifying the Same Position File for the Entire Sequence

❖ **To specify the same position file for the entire sequence**

1. Open the Tissue Imaging page:
   a. In Tune Plus, choose **Setup > MALDI Source**.

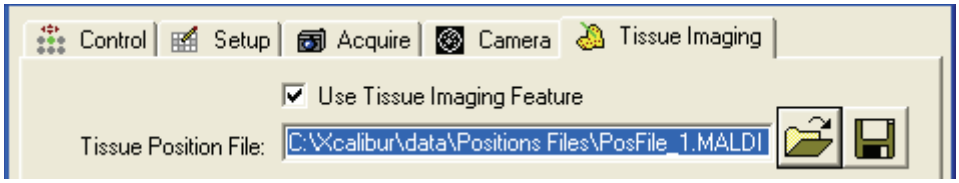
      The MALDI Source dialog box opens with the Control page shown by default.

   b. Click the **Tissue Imaging** tab.

      The Tissue Imaging page opens.

   c. Select the **Use Tissue Imaging Feature** check box.
2. Click to the right of the Tissue Position File box, and open the MALDI position file of interest. See Figure 96.

   Figure 96. Highlighted tissue position file



3. Highlight the entire path and file name of the file including the .MALDIpos file extension.
4. Press CRTL + C to copy the name of the MALDI position file, including its file extension, to the clipboard.
5. Open the New Sequence Template dialog box:
   a. Open Xcalibur by choosing **Start > All Programs > Xcalibur > Xcalibur**.

b. In the Xcalibur Home Page - Roadmap view, click the **Sequence Setup** button.

The Sequence Setup Home Page window appears.

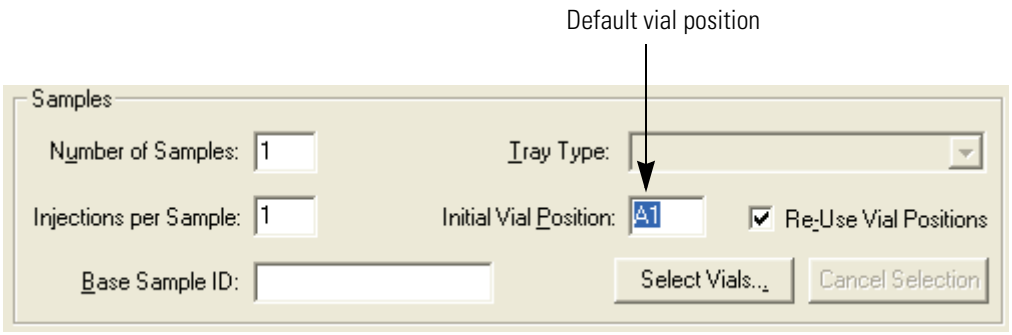
c. Choose **File > New**.

The New Sequence Template dialog box appears.

6. Under Samples, highlight **A1** in the Initial Vial Position box. See Figure 97.

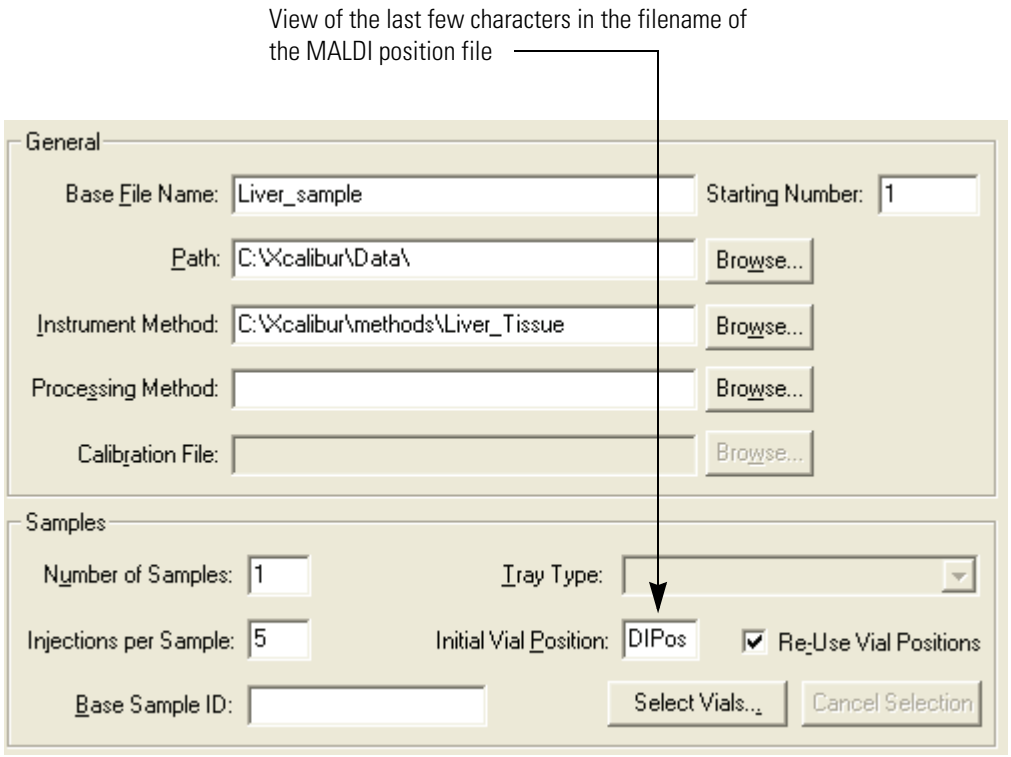
The default vial position for the New Sequence Template is A1.

Figure 97. Highlighting the text in the Initial Vial Position box



7. Press CRTL + V to paste the name of the MALDI position file from the clipboard into the Initial Vial Position box. See Figure 98.

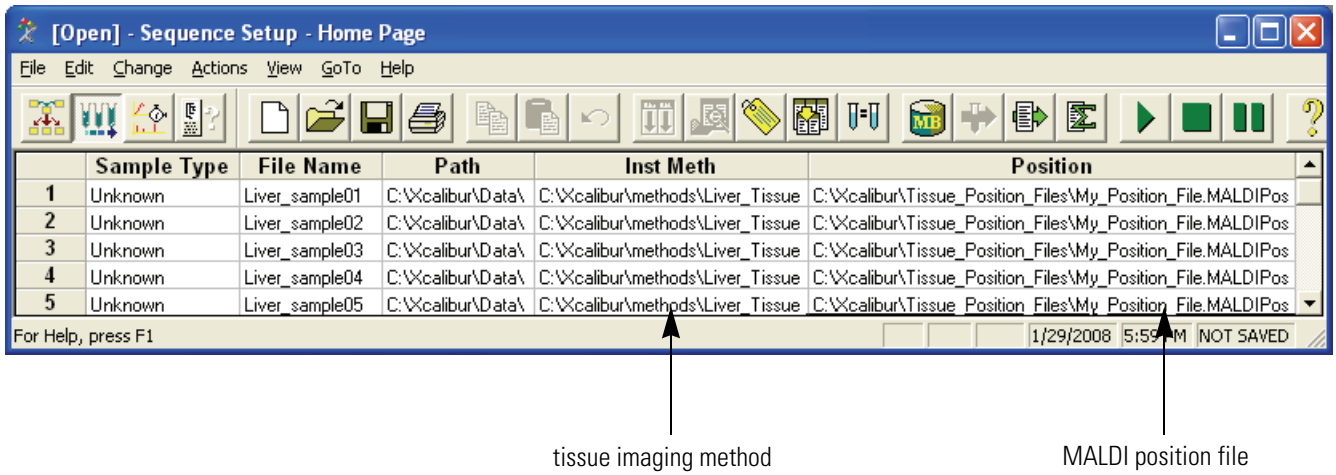
Figure 98. Pasting the name of the MALDI position file in the Initial Vial Position box



8. Under Samples, in the Injections per Sample box, type a number to specify the number of data files you want to acquire (see Figure 98).
9. Under General (see Figure 98), make the following entries and selections:
   - In the Base File Name box, type a file name. Xcalibur does not accept spaces within base file names.
   - In the Path box, type or browse to an appropriate directory.
   - In the Instrument Method box, browse to the folder where you store your instrument methods, and select a tissue imaging method.
10. In the New Sequence Template dialog box, use the default settings in the remaining areas of the template.
11. Click **OK** to accept the settings and exit the New Sequence Template dialog box.

    The sequence list appears in the Sequence Setup view. See Figure 99.

Figure 99. Sequence file with tissue imaging method and position file



12. Save the sequence file.

Specifying a Different Position File for Each Sequence Row

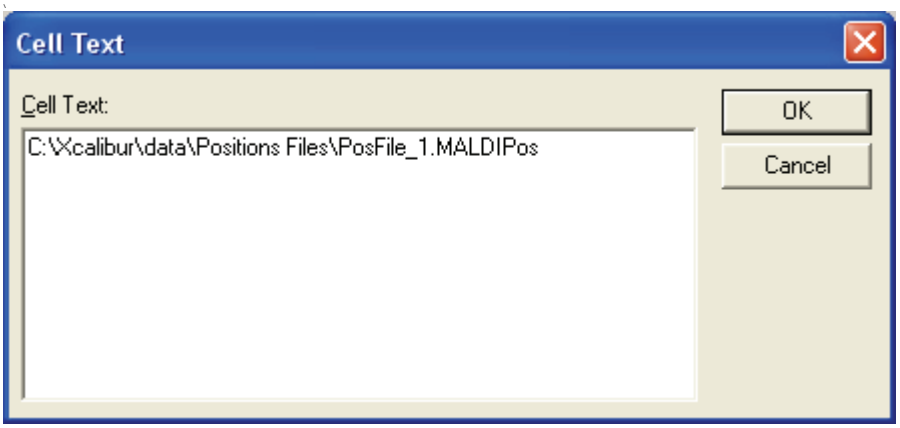
❖ **To specify a different position file for each row of the sequence**

1. Create an acquisition sequence as described in "Creating an Acquisition Sequence" on page 113.
2. Follow step 1 to step 4 on page 151 to copy the name of the position file from the Tissue Position File of the MALDI Source dialog box to the clipboard.
3. In the sequence row of interest, place the cursor in the Position box.
4. Press F2 twice.

   The Cell Text dialog box appears.
5. Paste the text into the Cell Text box.

   Figure 100 shows the text string pasted in the Cell Text box.

   Figure 100. Cell Text dialog box



6. Click **OK**.

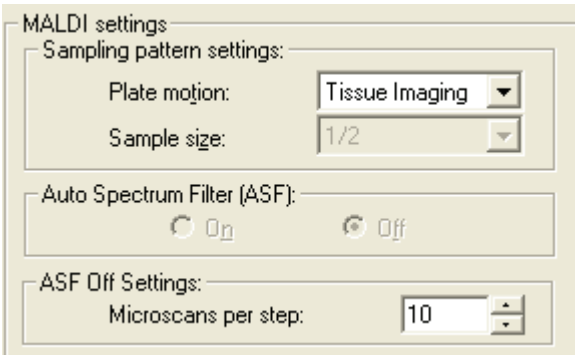
   The text string appears in the Position cell of the sequence list.

Checking the Acquisition Sequence

For the MALDI LTQ XL system, sequence rows contain standard runs or tissue imaging runs:

- A tissue imaging run includes a tissue imaging instrument method and a MALDI position file. A tissue imaging method is an instrument method that has Tissue Imaging selected for the plate motion. Figure 101 shows the MALDI settings area of the Instrument Setup view with Tissue Imaging selected as the Plate motion.
- A standard run includes a non-tissue imaging instrument method and a well plate location.

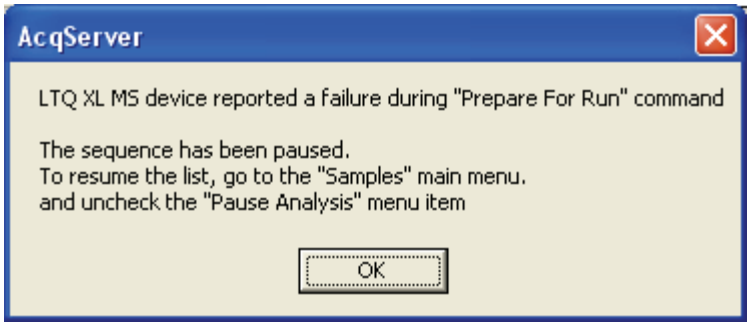
Figure 101. MALDI settings in the Instrument Setup view



When you select a tissue imaging method for a sequence row, you must specify a MALDI position file in the Position column for that row. See Figure 99 on page 153.

When you submit a sequence to the acquisition queue, Xcalibur does not check the sequence to verify that each row contains a valid instrument method and position type. When a sequence row reaches the front of the queue, the acquisition server sends out a Prepare for Run command. If the sequence row contains a mismatched instrument method and position type, the sequence pauses and the program generates an error message. Figure 102 shows the error message that the program generates when you do not match the instrument method to the position type.

Figure 102. Acquisition Server error message



To obtain more information about errors, open the LTQ XL Instrument Console using the following procedure.

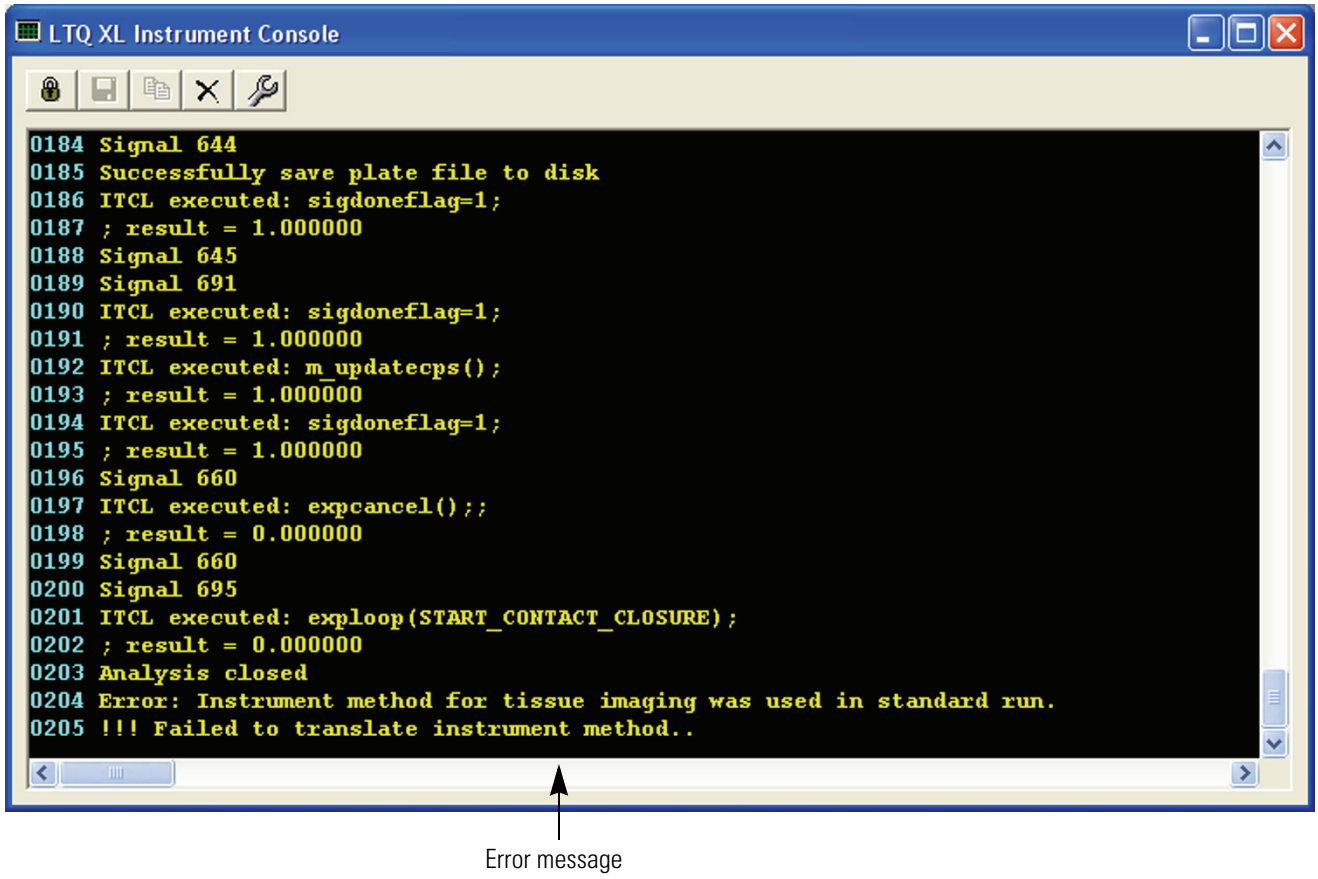
❖ To open the LTQ XL Instrument Console

1. Double-click the **LTQ XL Instrument Console** icon on the right side of the task bar.

   The LTQ XL Instrument Console opens.

   Figure 103 shows the error message that the system generates when it attempts to initiate a run for a sequence row containing a tissue imaging instrument method and a well position.

Figure 103. LTQ XL Instrument Console



Identifying Proteins Using BioWorks

This chapter briefly describes how to use the BioWorks™ 3.3.1 program to analyze your MALDI MS/MS data. It also highlights the search parameters that are specific for ions generated by the MALDI source.

After you have completed the automated analysis of your protein digest samples, you can use BioWorks to search protein, nucleotide, and genomic databases from your RAW file. Use BioWorks to cross-correlate MS/MS mass spectra from peptides with the theoretical mass spectra produced from sequences generated from either a protein or translated nucleotide FASTA database.

For more information about BioWorks, see the *BioWorks User Guide* and Help.

Contents

Using BioWorks to Identify Proteins

To analyze your MALDI MS/MS data with BioWorks, complete the following procedures:

1. Opening the RAW Data File
2. Defining the Search Parameters
3. Modifying the DTA Generation Parameters
4. Setting SEQUEST Search Parameters
5. Searching and Viewing the Results

Opening the RAW Data File

❖ **To start BioWorks and open a MALDI data file**

1. From the Windows taskbar, choose **Start > All Programs > Xcalibur > BioWorks Browser** to open the BioWorks Browser window.
2. Choose **File > Open Analysis file (.RAW)** to display the Open RAW File dialog box.
3. Select a RAW file to be analyzed.
4. Click **Open**.

   The BioWorks Browser window changes to display the chromatogram of the RAW file with the mass spectrum below it. The chromatogram of a MALDI experiment displays relative or absolute abundance of the total ion current versus time (min.).

You are now ready to define the search parameters as described in the procedure "Defining the Search Parameters" on page 158.

Defining the Search Parameters

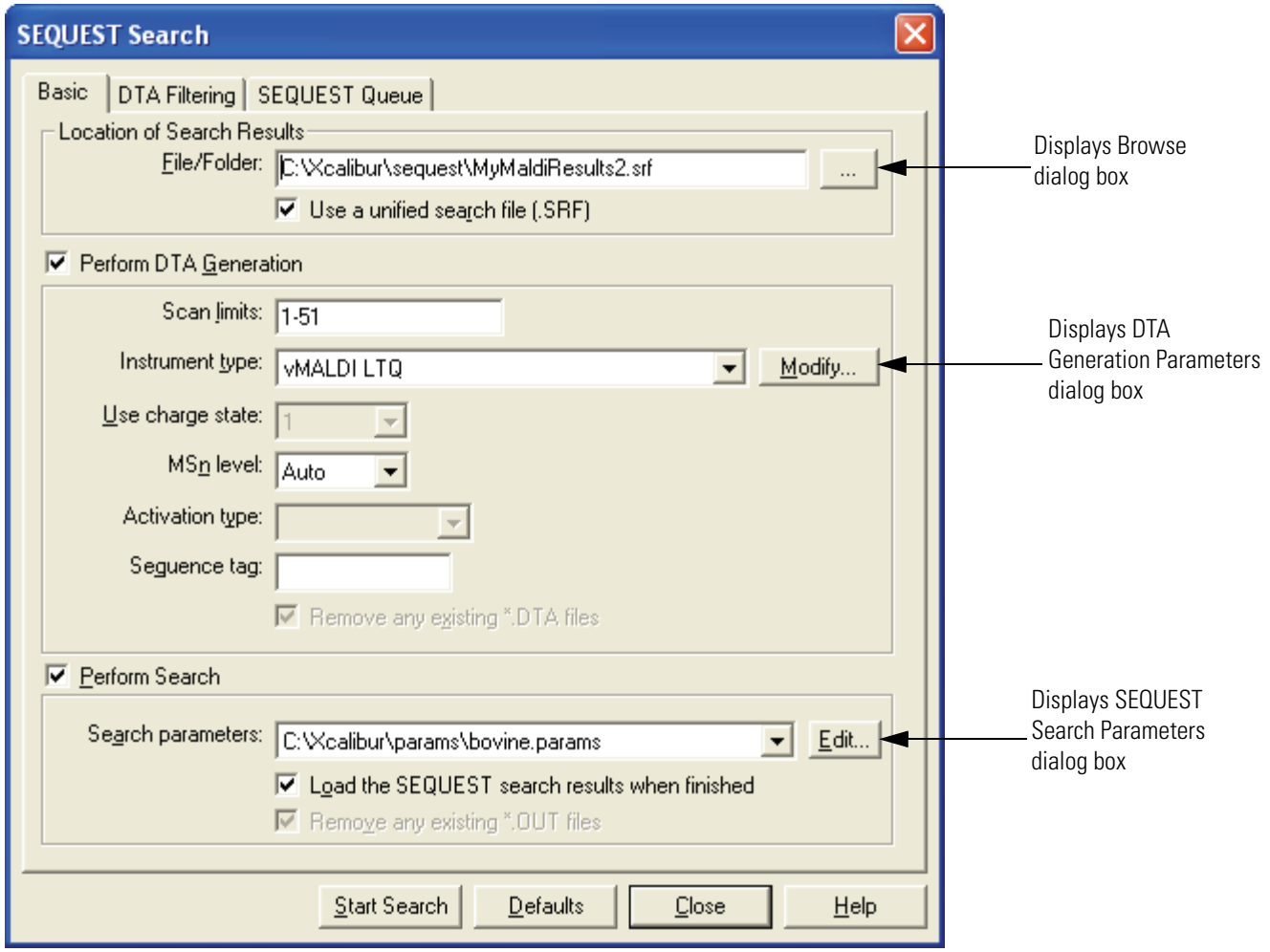
BioWorks contains the SEQUEST™ application that enables you to search protein and peptide databases. Use SEQUEST to cross-correlate uninterpreted MS/MS mass spectra of peptides with single-letter-coded amino acid sequences from protein databases or with three-letter-coded nucleotide sequences from nucleotide databases.

❖ **To set and save search parameters**

1. Choose **Actions > SEQUEST Search**. The SEQUEST Search dialog box opens with the Basic page shown (Figure 104).

   Note The default SRF file name is the name of your RAW file with the .srf extension. Thermo Fisher Scientific recommends you create a unique name for your SRF file. The default location for your search results is: \Xcalibur\sequest\.

Figure 104. MALDI settings for SEQUEST Search Basic page



2. In the Location of the Search Results area, type your SRF file name in the File/Folder box.
3. From the Instrument type list, select **vMALDI LTQ**.

   Automatically the Use charge state, MSn level, and Activation type settings are changed to match the vMALDI LTQ instrument type.
4. Modify the DTA Generation settings as defined in the procedure "Modifying the DTA Generation Parameters" on page 160.
5. Define and save the search parameters as defined in the procedure "Setting SEQUEST Search Parameters" on page 161.
6. In the SEQUEST Search dialog box, click **Start Search** to begin the search analysis.
   - The SEQUEST Search box closes.
   - The SEQUEST Progress box opens, showing the progress of the search.

- When the parameters in the SEQUEST Search dialog box are saved and the search is started, these parameters are written to the .params file in the Xcalibur results directory.

To view and interpret the SEQUEST search results, see "Searching and Viewing the Results" on page 164.

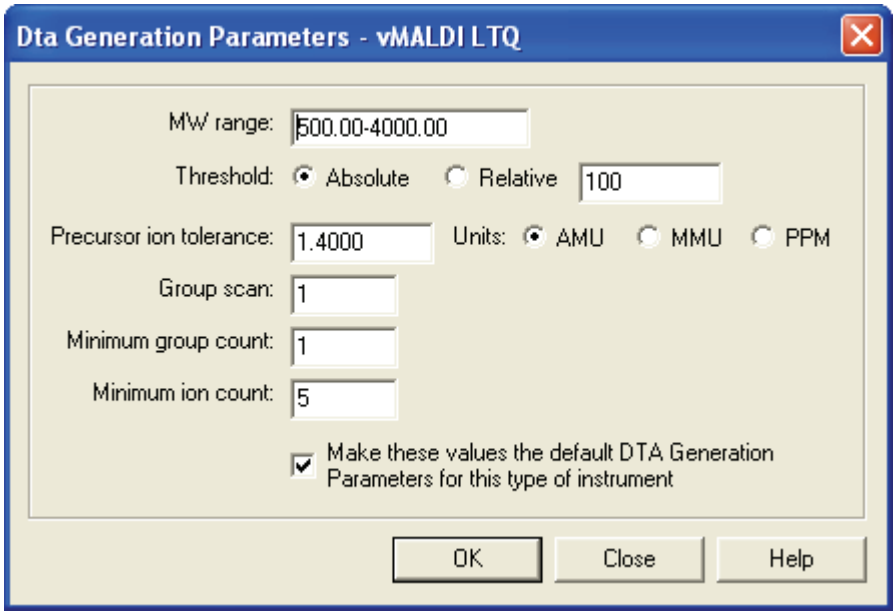
Modifying the DTA Generation Parameters

Use the DTA Generation Parameters dialog box to modify the .dta generation parameters. The DTA Generation settings described in this procedure are typically used for MALDI data.

❖ To modify the .dta generation parameters

1. On the Basic page of the SEQUEST Search dialog box, click **Modify** (Figure 104 on page 159). The Dta Generation Parameters dialog box appears as shown in Figure 105.

   Figure 105. DTA Generation Parameters settings



2. In the Minimum ion count box, type **5**.
3. In the MW range box, type **500.00-4000.00**.
4. When the Absolute threshold is selected, type **100** in the Threshold box.
5. Select **Make these values the default DTA Generation Parameters for this type of instrument** check box. Your settings are saved so that they can be reused the next time you select this instrument type for your search.
6. Click **OK** to save the settings and close the Dta Generation Parameters dialog box.

   The SEQUEST Search parameters dialog box appears (Figure 104 on page 159).

7. Continue setting the parameters for SEQUEST Search dialog box. See "Setting SEQUEST Search Parameters" on page 161.

Setting SEQUEST Search Parameters

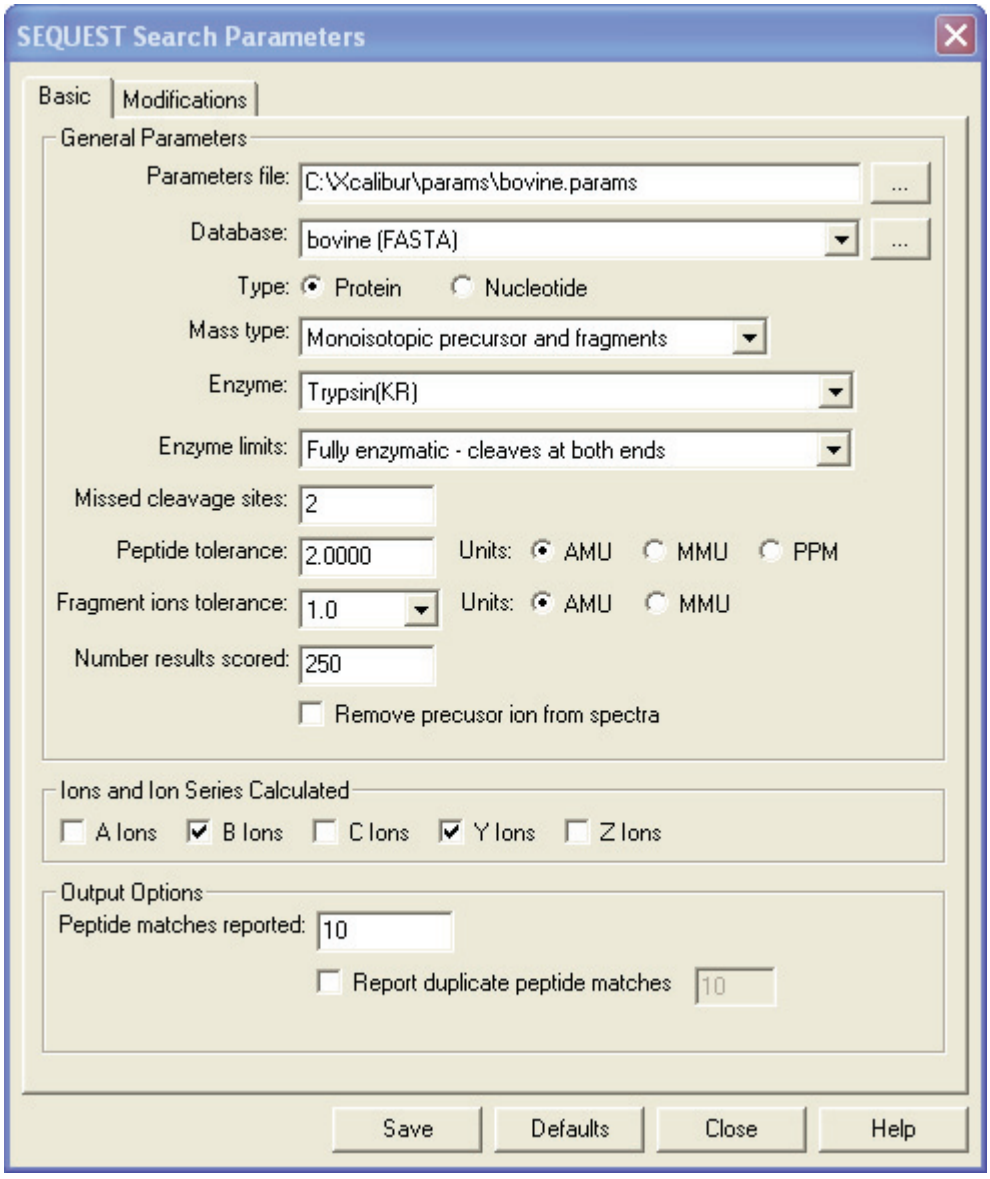
The search parameters described in this procedure are typically used for tryptic digests of proteins.

❖ To set SEQUEST Search Parameters

1. On the Basic page of the SEQUEST Search dialog box, in the Search Parameters area, click **Edit** (Figure 104 on page 159).

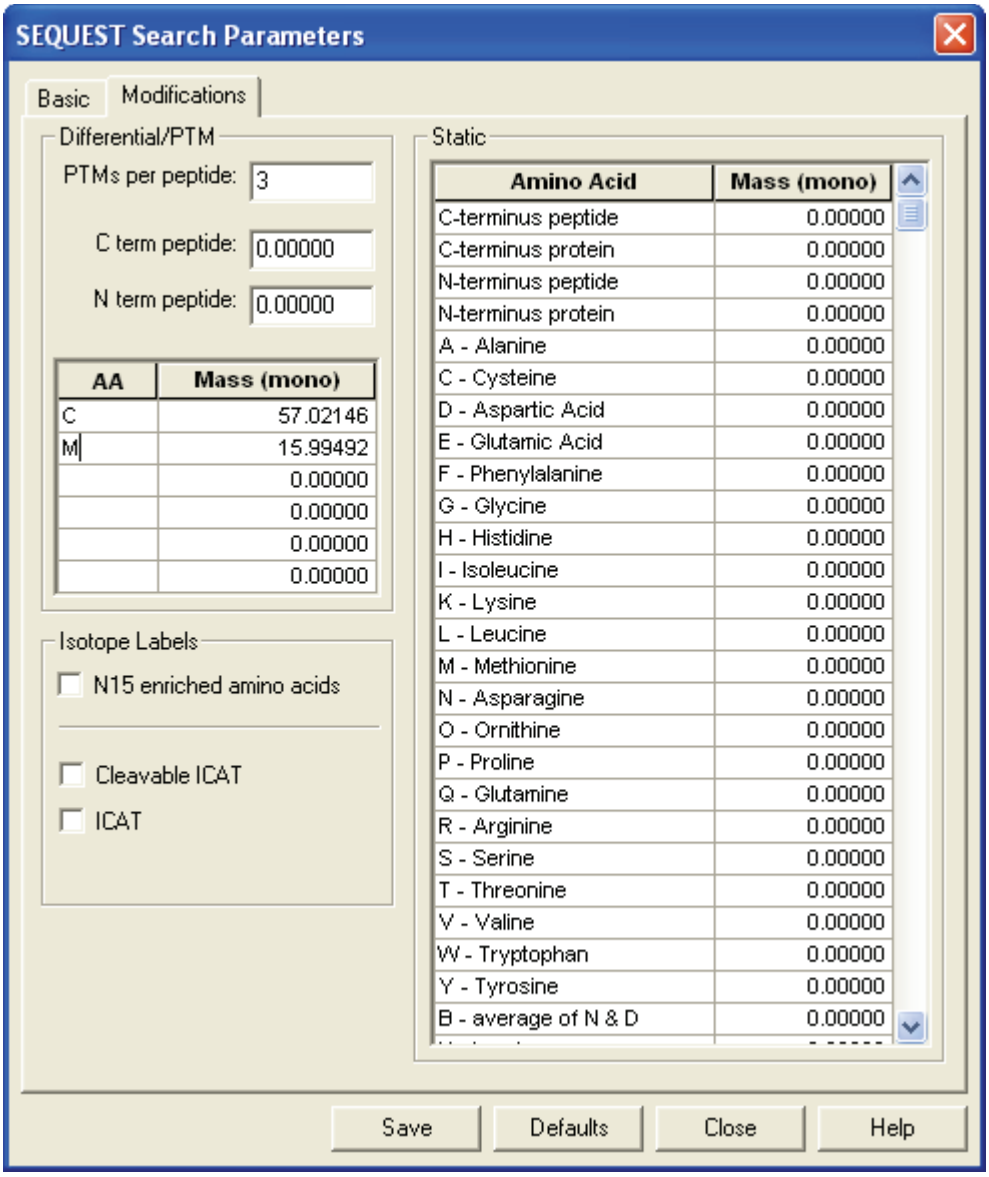
   The Basic page of the SEQUEST Search Parameters dialog box appears as shown in Figure 106.

Figure 106. MALDI settings for SEQUEST search parameters



2. In the Number results scored box, type **250**.
3. From the Enzyme list, select **Trypsin (KR)** if this enzyme was used to digest your protein.
4. From the Database list, select a FASTA file. This search example uses bovine.
5. In the Missed cleavage sites box, type **2**.
6. In the Peptide tolerance box, type **2.000.**
7. From the Fragment ions tolerance list, select **1.0**.
8. In the Parameters file box, type a file name for this parameters file.
9. Click **Modifications** to display the page for chemical modifications. See Figure 107.

Figure 107. MALDI settings for chemical modifications



10. In the Differential/PTM area in the AA column, type an amino acid. Type the one-letter abbreviation for the amino acid. See Figure 107.

Note Differential modifications, also known as *variable* or *amino acids modifications*, are mainly used for determining post translational modifications (PTMs). Some phosphorylated peptide serines will be modified and some will not be modified. Static modifications apply the same specific mass to all occurrences of that named amino acid, as in an exhaustive chemical modification.

11. For each amino, click the corresponding cell in the Mass column and type the mass of the modification, or right-click the cell and choose a specific modification. The mass of the modification or the selected item appears. See Figure 107.

12. Click **Save** to save your settings and close the Search Parameters dialog box.

    The SEQUEST Search dialog box appears.

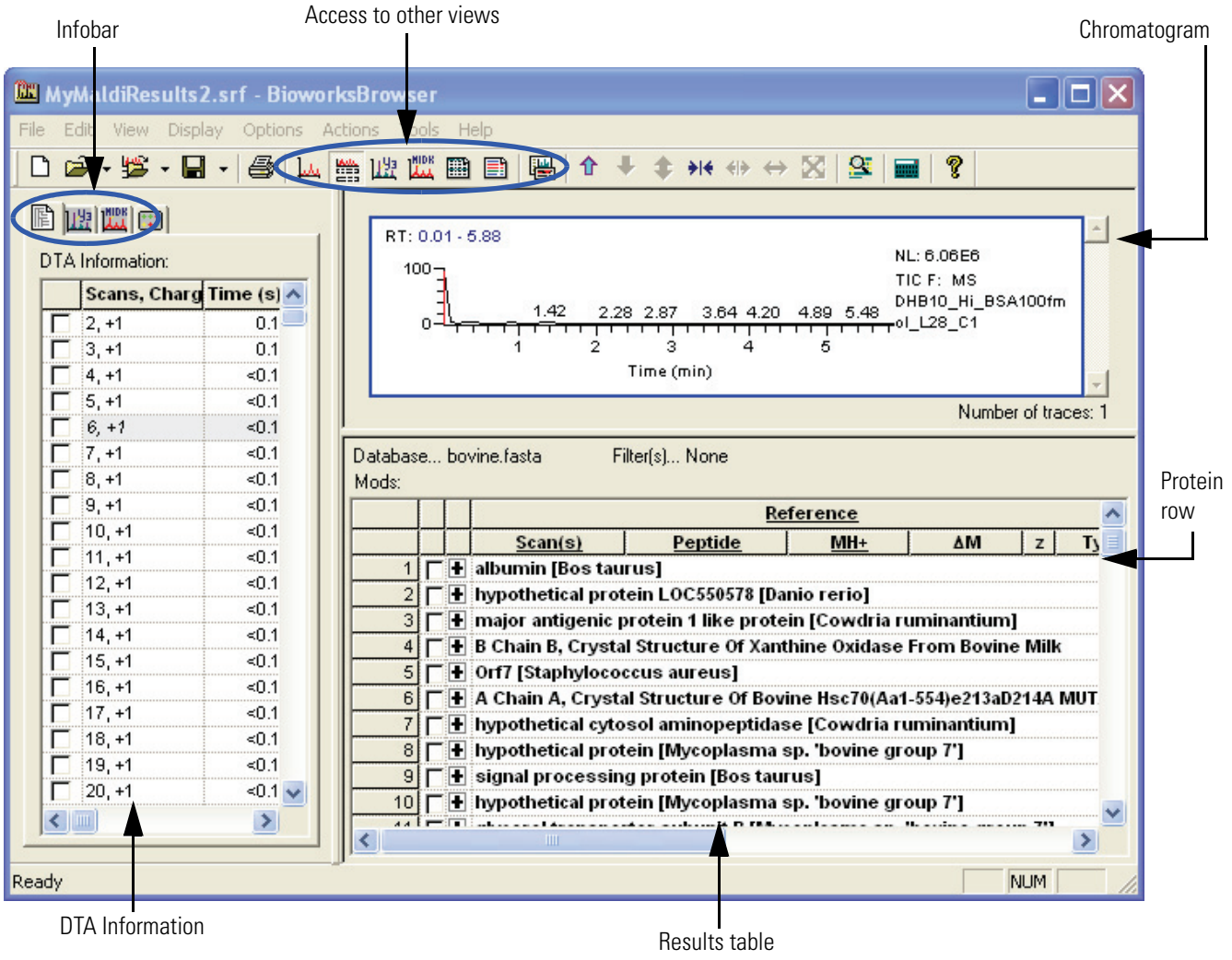
13. Continue setting the parameters for SEQUEST from step 6 in the procedure "Defining the Search Parameters" on page 158.

Searching and Viewing the Results

When the search is complete, the SEQUEST Progress message box closes. BioWorks Browser changes to display the chromatogram (top pane), the search results in a summary table (bottom pane), and .dta files in the Info Bar (left pane). See Figure 108. Each pane provides unique information you can use to analyze your search results. The panes contain the following:

- The Chromatogram window displays a visual depiction of the MALDI experiment with total ion current versus the time for the experiment.
- The Results table shows the probability calculations, scores, and other values for the matching peptides and proteins from the SEQUEST search.
- The DTA Information in the Info Bar pane displays the scans, the charge states, and the time required to process each .dta file.

Figure 108. BioWorks Browser window showing the MALDI data search results



The SEQUEST Results report displays a list of matching peptide and protein sequences with various probability calculations indicating how good the match is for your peptide or protein. Also, the protein and peptide summaries can be exported to Microsoft™ Excel™ for further analysis or archiving.

Refer to the *BioWorks User Guide* for additional information on setting filters, accessing other views, and interpreting your search results.

ProteoMass Calibration Kit

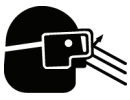
This appendix describes how to prepare the calibration and sensitivity test spots.

IMPORTANT Use clean and dry sample plates to prepare the calibration and sensitivity test spots. To ensure dryness, store the sample plate under vacuum in the MALDI sample module as you prepare the calibration mixes.

Contents

CAUTION Store and handle all chemicals in accordance with standard safety procedures. The Material Safety Data Sheets (MSDSs) describing the chemicals being used are to be freely available to lab personnel for them to examine at any time. Material Safety Data Sheets (MSDSs) provide summarized information on the hazard and toxicity of specific chemical compounds. MSDSs also provide information on the proper handling of compounds, first aid for accidental exposure, and procedures for the remedy of spills or leaks. Producers and suppliers of chemical compounds are required by law to provide their customers with the most current health and safety information in the form of an MSDS. Read the material safety data sheets for each chemical you use.

CAUTION AVOID EXPOSURE TO POTENTIALLY HARMFUL MATERIALS. Always wear protective gloves when you use solvents or corrosives. Refer to your supplier's Material Safety Data Sheet (MSDS) for the proper handling of a particular solvent or compound.



CAUTION AVOID EXPOSURE TO POTENTIALLY HARMFUL MATERIALS. Always wear safety glasses when you use solvents or corrosives. Refer to your supplier's Material Safety Data Sheet (MSDS) for the proper handling of a particular solvent or compound.

Calibration Kit Description

The Sigma ProteoMass™ Calibration Kit (MS CAL4) contains a range of standard peptides for the purpose of calibrating and testing the MALDI LTQ XL mass spectrometer. The kit provides high-purity, low-alkali metal solvents, and recrystallized matrices, and provides standards which are ideal for most applications.

Storage/Stability

The kit is shipped at ambient temperature. Store the kit at 2 to 8 °C. Matrices, after reconstitution in solvent, are stable for approximately one week at room temperature if protected from light. Some methionine oxidation can occur over time in MRFA [a small peptide (methionine-arginine-phenylalanine-alanine)], which is one of the peptides in the Normal Mass Calibration Mix.

Matrix and Solvents

Recrystallized alpha-cyano-4-hydroxycinnamic acid comes in 2.0 mL amber vials. Solvents come in high-density polyethylene bottles. See Table 12 for more details.

Table 12. Matrix and solvents

Product code	CAS number	Product	Amount
C 8982	28166-41-8	alpha-cyano-4-hydroxycinnamic acid	5 × 10 mg
T 3693	76-05-1	1% trifluoroacetic acid (TFA) solution	4 mL
A 8596	75-05-8	Acetonitrile	30 mL
E 7023	64-17-5	Ethanol, 200 proof, molecular biology grade	10 mL

ProteoMass Normal Mass Calibration Mix (Product Code C 9241)

The normal mass calibration mix is supplied in clear 0.5-mL vials, containing optimal quantities of the standard peptides indicated in Table 13. Each kit contains five vials.

Table 13. ProteoMass Normal Mass Calibration Mix for the normal mass range

Peptide	CAS number	$(M+H)^+$ Monoisotopic mass
MRFA	67368-29-0	524.27
Bradykinin 1-7	23815-87-4	757.40
Bradykinin	5979-11-3	1060.57
Angiotensin I	70937-97-2	1296.69
Neurotensin	58889-67-1	1672.92
Renin Substrate	20845-02-7	1758.93

ProteoMass High Mass Calibration Mix (Product Code C 9366)

The High Mass Calibration Mix is supplied in clear 0.5-mL vials, containing optimal quantities of the standard peptides indicated in Table 14. Each kit contains five vials of the High Mass Calibration Mix.

Table 14. ProteoMass High Mass Calibration Mix for the high mass range

Peptide	CAS number	$(M+H)^+$ Monoisotopic mass
MRFA	[67368-29-0]	524.27
Bradykinin	[5979-11-3]	1060.57
ACTH 1-16	[5576-42-1]	1936.99
Melittin	[20449-79-0]	2845.75
ACTH 7-38	[68563-24-6]	3657.93

ProteoMass Angiotensin II Sensitivity Standard (A 9854)

The sensitivity standard is supplied in clear 0.5 mL vials. Each vial contains 500 pmol of angiotensin II.

Preparing the Mass Calibration Targets

In addition to the solvents, reagents, and protein mixes included in the ProteoMass Calibration kit, you must have extra 1.7-mL centrifuge tubes and pipettes to prepare and spot the calibration samples.

To prepare the calibration targets used to calibrate the normal and high mass range of the MALDI LTQ XL system, follow these procedures:

1. Preparing the Solvent Mixtures
2. Reconstituting the Calibration Mixes
3. Preparing the Matrix Solution for the Calibration Targets
4. Preparing the Calibrant/Matrix Mixtures
5. Spotting the Calibrant/Matrix Mixtures on the Sample Plate

Preparing the Solvent Mixtures

Use these solvent mixtures to prepare the calibrant/matrix mixtures:

- 0.1% by volume TFA in water (10-fold dilution of the 1% TFA solution in the kit)
- Solvent 1: a solution of 80% HPLC grade water, 20% acetonitrile (v/v)
- Solvent 2: a solution of 3% (0.1% TFA in water), 13% ethanol, 84% acetonitrile (v/v/v)

❖ To prepare 1 mL of 0.1% TFA in water

Mix 100 µL of 1% TFA (provided in the kit) with 900 µL of HPLC-grade water (or 18 MΩ-cm deionized water) in a 1.7-mL microcentrifuge tube.

❖ To prepare 1 mL of Solvent 1

Mix 800 µL of HPLC-grade water (or 18 MΩ-cm deionized water) with 200 µL of acetonitrile in a 1.7-mL microcentrifuge tube.

❖ To prepare 1 mL of Solvent 2

Mix 30 µL of the 0.1% TFA solution prepared above with 130 µL of 200-proof ethanol (provided in the kit) and 840 µL of acetonitrile (provided in the kit).

Reconstituting the Calibration Mixes

The reconstituted calibration mixes must sit at room temperature for 30 minutes before you can use them. To save time, reconstitute the calibration mixes that you plan to use before you prepare the matrix solutions.

- To calibrate the MALDI LTQ XL system in the normal mass range, reconstitute the Normal Mass Calibration Mix.
- To calibrate the MALDI LTQ XL system in the high mass range, reconstitute both the Normal and High Mass Calibration Mixes.

❖ To reconstitute the Normal Mass Calibration Mix

1. Add 58 µL of Solvent 1 to a vial containing the Normal Mass Calibration Mix.
2. Incubate the vial for 30 minutes at room temperature.

❖ To reconstitute the High Mass Calibration Mix

1. Add 150 µL of Solvent 1 to a vial containing the High Mass Calibration Mix.
2. Incubate the vial for 30 minutes at room temperature.

Preparing the Matrix Solution for the Calibration Targets

❖ To prepare the 10 mg/mL matrix stock solution

1. Add 0.5 mL of Solvent 2 to one vial of alpha-cyano-4-hydroxycinnamic acid (5 mg in a 1.7-mL microcentrifuge tube).
2. Vortex or sonicate the mixture until the matrix is completely dissolved.

❖ To prepare the 3.5 mg/mL matrix solution

1. Transfer 35 µL of the 10 mg/mL matrix stock solution prepared above to a microcentrifuge tube.
2. Add 65 µL of Solvent 2 to the microcentrifuge tube.
3. Label the tube, and briefly vortex the tube.

Preparing the Calibrant/Matrix Mixtures

Depending on the mass range that you want to calibrate, prepare one or both of the following calibrant/matrix mixtures.

❖ To prepare the calibrant/matrix mixture for the normal mass range

1. If you have not already done so, reconstitute a vial of the Normal Mass Calibration Mix as described in "Reconstituting the Calibration Mixes" on page 171.
2. Transfer 5 µL of the reconstituted normal mass calibration mix to a microcentrifuge tube.
3. Add 45 µL of the 3.5 mg/mL matrix solution to the microcentrifuge tube.
4. Label the tube, and briefly vortex the tube.

❖ To prepare the calibrant/matrix mixture for the high mass range

1. If you have not already done so, reconstitute a vial of the High Mass Calibration Mix as described in "Reconstituting the Calibration Mixes" on page 171.
2. Transfer 5 µL of the reconstituted High Mass Calibration Mix to a microcentrifuge tube.
3. Add 45 µL of the 3.5 mg/mL matrix solution to the microcentrifuge tube.
4. Label the tube, and briefly vortex the tube.

Spotting the Calibrant/Matrix Mixtures on the Sample Plate

❖ To spot the calibrant/matrix mixtures on the sample plate

1. For each calibrant/matrix mixture that you plan to use, spot 5 or more sample wells on the sample plate with 1.5 µL of the calibrant/matrix mixture per well.
2. Gently use the pipette tip to spread the calibration solution out to the edges of the sample well.

   The sample must touch the edges of the well engraving to ensure even drying and uniform distribution. You can spread the solution slightly outside of the sample circle if necessary.

IMPORTANT If the sample does not spread homogeneously over the well, do not use the spot for calibration.

3. Continue spotting the sample wells until you have created 5 or more homogeneous spots.
4. Allow the sample spots to dry at room temperature for 1 to 2 minutes.

Preparing the Sensitivity Test Targets

Thermo Fisher Scientific recommends that you check the sensitivity of the MALDI LTQ XL system before performing analyses on low-concentration samples. The following procedure describes how to prepare the sensitivity test targets.

❖ To prepare the sensitivity test targets

1. Prepare Solvent 3, a solution of 50:50 acetonitrile /0.1% TFA in water (v/v):
   a. Transfer 100 µL of 0.1% TFA in water to a microcentrifuge tube.

      For instructions on preparing a solution of 0.1% TFA in water, see "Preparing the Solvent Mixtures" on page 170.

   b. Add 100 µL of acetonitrile to the tube and mix.
2. Reconstitute a vial of angiotensin II:
   a. Add 625 µL of HPLC-grade water (or 18 MΩ-cm deionized water) to a vial of angiotensin II.

      The 0.5-mL vials contain 500 pmol of angiotensin II. The concentration of the reconstituted solution is 800 fmol/µL angiotensin II.

   b. Incubate the vial for 30 minutes at room temperature.
3. Prepare a 40 fmol/µL solution of angiotensin II:
   a. Transfer 5 µL of the reconstituted angiotensin II to a clean, dry microcentrifuge tube.
   b. Add 95 µL of water to the tube and vortex briefly.

      The final concentration of this solution is 40 fmol/µL angiotensin II.
4. Prepare a 4 fmol/µL solution of angiotensin II:
   a. Transfer 5 µL of the 40 fmol/µL angiotensin II solution from step 3 to a clean, dry microcentrifuge tube.
   b. Add 45 µL of water to the tube and vortex briefly.

      The final concentration of this solution is 4 fmol/µL angiotensin II.
5. Prepare a 1 mg/mL matrix solution:
   a. Transfer 10 µL of the stock matrix solution (10 mg/mL) to a clean, dry microcentrifuge tube.
   b. Add 90 µL of Solvent 3 and vortex.

      The final concentration of this solution is 1 mg/mL matrix.
6. Prepare a 10 fmol angiotensin II target:
   a. Transfer 5 µL of the 40 fmol/µL angiotensin II solution prepared in step 3 to a clean, dry microcentrifuge tube.

b. Add 5 µL of the 1 mg/mL matrix solution prepared in step 5 to the tube.

c. Vortex briefly, and spot 0.5 µL (10 fmol of angiotensin II) onto the sample plate.

7. Prepare a 1 fmol angiotensin II target:

a. Transfer 5 µL of the 4 fmol/µL Angiotensin II solution prepared in step 4 to a clean, dry microcentrifuge tube.

b. Add 5 µL of the 1 mg/mL matrix solution prepared in step 5 to the tube.

c. Vortex briefly, and spot 0.5 µL (1 fmol angiotensin II) onto the sample plate.

Sample Preparation

The quality of your MALDI spectra is highly dependent on sample preparation. The optimal conditions can vary considerably from analyte to analyte. Experiment to find the best way to prepare your analyte of interest. This appendix contains guidelines on how to prepare and deposit samples to be analyzed with the MALDI LTQ XL system.

Contents

CAUTION Store and handle all chemicals in accordance with standard safety procedures. The Material Safety Data Sheets (MSDSs) describing the chemicals being used are to be freely available to lab personnel for them to examine at any time. Material Safety Data Sheets (MSDSs) provide summarized information on the hazard and toxicity of specific chemical compounds. MSDSs also provide information on the proper handling of compounds, first aid for accidental exposure, and procedures for the remedy of spills or leaks. Producers and suppliers of chemical compounds are required by law to provide their customers with the most current health and safety information in the form of an MSDS. Read the material safety data sheets for each chemical you use.

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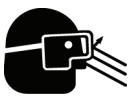


CAUTION AVOID EXPOSURE TO POTENTIALLY HARMFUL MATERIALS. Wear safety glasses when you use solvents or corrosives. Refer to your supplier's Material Safety Data Sheet (MSDS) for the proper handling of a particular solvent or compound.

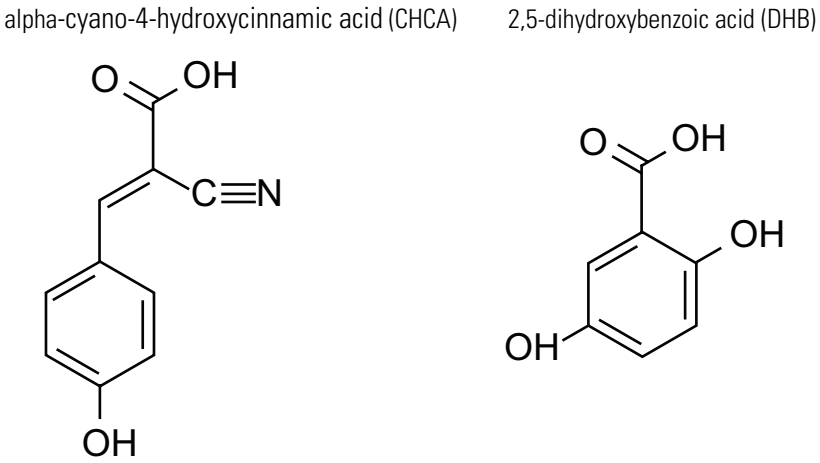
Matrix

The compound used as the MALDI matrix must meet the following requirements:

- Be capable of co-crystallization with the analyte
- Be soluble in a solvent that is compatible with the analyte
- Have a high absorptivity coefficient at the UV wavelength of 337.7 nm emitted by the MALDI laser
- Be chemically non-reactive with the analyte
- Must not sublimate at a pressure of 70 mTorr, the pressure maintained within the sample chamber of theMALDI source

Figure 109 shows two compounds commonly used as matrices for peptide analysis with MALDI, alpha-cyanohydroxycinnamic acid (CHCA) and 2,5-dihydroxybenzoic acid (DHB).

Figure 109. Matrix compounds



DHB has a lower internal energy upon desorption than CHCA, and produces less fragmentation of analytes. Consequently, DHB is sometimes preferred to CHCA, particularly for use with labile biological molecules, such as carbohydrates and proteins with post translational modifications. Recrystallized matrices are preferred. Table 15 lists suggested suppliers of recrystallized matrices.

Table 15. Ordering information for CHCA and 2, 5-DHB matrixes

Matrix	Supplier	Part number
CHCA	Laser Biolabs, France	M001
CHCA	Waters	186002331
2,5-DHB	Laser Biolabs, France	M003
2,5-DHB	Waters	186002333

Matrix Solvent

The solvent used to make the matrix solution can affect the crystallization of the matrix and sample. In general, solvent mixtures with higher organic content evaporate more quickly, leading to faster crystallization and thinner sample coverage. (Refer to Cohen and Chait, 1996[1] for a more detailed description of solvent effects on matrix crystallization.) The solvent commonly used for CHCA is 50:50 (0.1% TFA in water)/acetonitrile (v/v). You can use another common solvent, 3:13:84 (0.1% TFA in water)/ethanol/acetonitrile (v/v/v), when you need a faster evaporation rate. Chromasolv™ or HPLC-grade solvents are recommended.

Matrix-to-Analyte Ratio

The matrix-to-analyte ratio is an important factor in the quality of spectra obtained using the MALDI ion source. Many commonly used sample-preparation protocols for MALDI employ saturated or near-saturated CHCA. Such concentrations are too high to use with the MALDI source, and result in matrix ion signals overwhelming the spectrum. A baseline "hump" in the low-mass region (<*m/z* 1000) might be an indication that too much matrix has been used, that the matrix is contaminated, or that the sample plate is dirty. In the case of CHCA, adduct peaks (also known as matrix clusters) can appear in the spectrum as a series of peaks with an *m/z* difference of 211 to 212. These adducts are comprised of several molecules of CHCA with sodium/and or potassium[2,3].

For samples in the 100 to 500 fmol/µL range, a solution of <2.5 mg/mL CHCA is often a good starting point. Lower analyte concentrations require lower matrix concentrations.

Sample Purification

Many common buffers and additives used in handling biological samples can reduce the quality of MALDI spectra when present above a particular concentration. These include sodium and potassium salts, phosphate buffers, and many detergents and denaturants. Many samples need purification to remove these substances before MALDI analysis. (Refer to Mock et al., 1992[4] for more information on problematic compounds.)

A popular purification technique is the use of C-18 containing micro extraction pipette tips (such as the Varian OMIX™ tips or Millipore ZipTip™), which allow rapid reverse phase purification of small volume protein or peptide samples. Samples can be eluted directly onto the MALDI sample plate with the matrix of choice, or can be eluted into a microcentrifuge tube with a suitable solvent. The latter option allows for more flexibility in experimenting with different matrices and matrix concentrations.

---

[1]Cohen, S.; Chait, B. *Anal. Chem.* **1996**, 68, 31-37.

[2]Keller, B.O. and Li, L. *J Am Soc Mass Spectrom* 2000, 11, 88-93.

[3] Harris, W.A.; Janecki, D.J., and Reilly, J.P. *Rapid Commun. Mass Spectrom* 2002, 16, 1714-1722.

[4]Mock, K.; Sutton, C.; Cottrell, J. *Rapid Commun. Mass Spec*. **1992**, 6, 233-238.

Most samples from in-gel digests need a purification step after digestion, but in-gel digests in the high picomolar range might not need the purification step.

Lyophilized protein digests are usually resuspended in 10% to 20% acetonitrile in 0.1% TFA. Subsequent dilutions can be made with 18 MΩ -cm or HPLC-grade water.

Sample Deposition Techniques

Many sample deposition techniques have been used with MALDI. This topic describes two common sample deposition techniques: the dried-droplet method and the analyte first method.

The dried-droplet method is used in the sample preparation protocols given in this manual. It is also the preferred sample deposition method for use with DHB, as well as CHCA. With this method, you mix the sample solution and matrix solution, and then load the mixture onto a sample plate. You typically use a 1:1 ratio for the sample/matrix mixture, and load the sample plate with 0.5 to 1.0 µL of the mixture per spot. You can vary the matrix-to-sample ratio. It is important to load the sample/matrix mixture as soon as possible after mixing because crystals might form in the mixture.

The analyte first method is used to improve sensitivity. With this method, you apply 0.3 to 0.5 µL of sample solution to the sample spot and allow it to dry. Then you apply an approximately equal amount of matrix solution to the spot and allow it to dry.

MALDI Sample Preparation Guidelines

This topic describes sample preparation with either the 2,5-DHB (2,5-dihydroxybenzoic acid) or the CHCA (alpha-cyano-4-hydroxycinnamic acid) matrices and contains the following procedures:

- Preparing Samples with the 2, 5-DHB Matrix
- Preparing Samples with the CHCA Matrix
- Preparing Phosphorylated Peptides for a Neutral Loss Experiment

Preparing Samples with the 2, 5-DHB Matrix

The preferred matrix for protein digests and labile molecules is 2,5-dihydroxybenzoic acid (2,5-DHB) because it causes less fragmentation than CHCA. You can use 50:50 acetonitrile/0.1% TFA in water (v/v), or 20:80 acetonitrile/0.1% TFA in water (v/v) as the solvent for a 2,5-DHB matrix. Adding 0.1% TFA maintains the acidity of the solution below pH 4.

❖ To prepare samples with the 2,5-DHB matrix

1. Prepare a stock solution of 50 mg/mL 2, 5-DHB in 50:50 acetonitrile/0.1%TFA.

2. To prepare the target spots, do one of the following:

   - For peptides, prepare a 1:1 sample/matrix mixture, and then spot the sample wells of the sample plate with 0.3 to 0.5 µL of this mixture.
   - Spot the sample wells with 0.3 to 0.5 µL of the analyte solution. Allow the spot to dry, and then apply the same amount of matrix solution on top of the analyte spots.
   - Spot a sample well with the analyte solution. Before the analyte solution drys, spot the well with the matrix solution. Using a pipette with a disposable tip, mix the analyte and matrix solutions by drawing the solution into and expelling the solution from the pipette tip. Make sure the dry sample is no larger than 2 mm in diameter, as this is the size of the camera view.

Preparing Samples with the CHCA Matrix

Alpha-cyano-4-hydroxycinnamic acid (CHCA) is a universal type of matrix that can be used with non-volatile, thermally labile molecules.

If needed, you can add ammonium citrate dibasic (Sigma P/N 09833 or 09831) to the 0.1% TFA solution, for a total ammonium citrate concentration of 2 mM in the 50/50 acetonitrile/0.1%TFA solvent. Signal suppression might occur above 5 mM ammonium citrate. This additive has been shown to help with certain contaminants in samples (high salt concentrations, certain buffers, and ionic detergents).

❖ To prepare samples with the alpha-cyano-4-hydroxycinnamic acid (CHCA) matrix

1. Prepare a stock solution of 10 mg/mL CHCA in 50:50 acetonitrile/0.1%TFA.

   This stock CHCA solution can be stored for up to a few weeks in a light resistant container or in a dark location at 20 °C.

2. Prepare a working solution of 1.0 to 2.5 mg/mL CHCA in the same solvent used to prepare the stock solution. If samples are at the attomole level, use the lower CHCA solution concentration of 0.5 to 1.0 mg/mL.

   Use the working CHCA solution to prepare the target spots.

3. To prepare the target spots, do one of the following:

   - Spot the sample wells with 0.5 µl of the analyte solution. Allow the spots to dry. and then apply 0.5 µL of the CHCA working solution (1 mg/mL to 2.5 mg/mL) on top of the analyte spots.
   - Use the dried droplet method. Prepare a 1:1 sample/matrix (CHCA working solution, 1.0 to 2.5 mg/mL) solution, and then spot the sample wells with 0.5 µL of this mixture.

Preparing Phosphorylated Peptides for a Neutral Loss Experiment

The recommended matrix for observing phosphorylated peptides is 2,5-DHB, making it the preferred matrix for the neutral loss, data-dependent method. Samples prepared with CHCA tend to show a reduced phosphorylated precursor. Phosphoric acid has been shown to improve the sensitivity of the phosphorylated group[5].

❖ Preparation of samples containing phosphorylated peptides:

1. Dissolve 2,5-DHB in 50/50 (ACN/0.1% phosphoric acid) solvent
2. Prepare a 1:1 (v/v) matrix to analyte mixture.
3. Load 0.3 to 0.5 µL of this mixture onto each sample well.

[5]Kjellström S. and Jensen O.N. *Anal. Chem*. 2004, 76, 5109-5117.

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