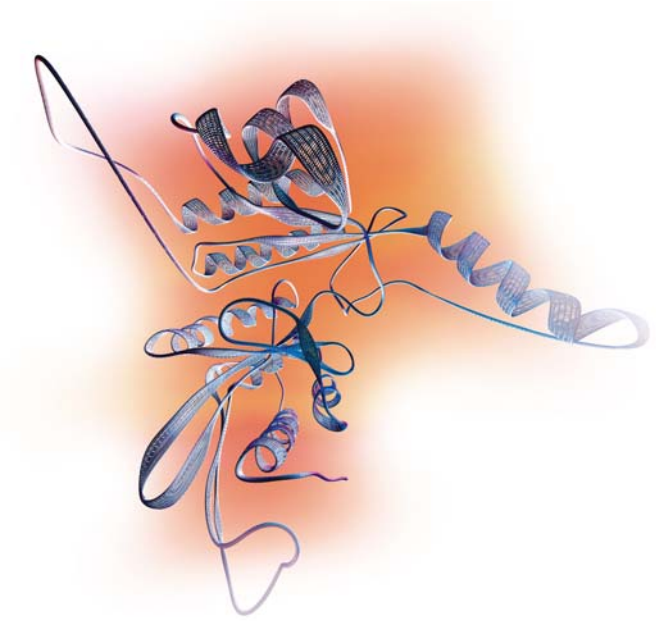


Applied Biosystems/MDS SCIEX 4800 MALDI TOF/TOF™ Analyzer

Getting Started Guide

Version 3.0 Series Software

Applied Biosystems | MDS SCIEX

For Research Use Only. Not for use in diagnostic procedures.

Information in this document is subject to change without notice. Applied Biosystems assumes no responsibility for any errors that may appear in this document. This document is believed to be complete and accurate at the time of publication. In no event shall Applied Biosystems be liable for incidental, special, multiple, or consequential damages in connection with or arising from the use of this document.

Applied Biosystems supplies or recommends certain configurations of computer hardware, software, and peripherals for use with its instrumentation. Applied Biosystems reserves the right to decline support for or impose charges for supporting non-standard computer configurations that have not been supplied or recommended by Applied Biosystems. Applied Biosystems also reserves the right to require that computer hardware and software be restored to the standard configuration prior to providing service or technical support.

The 4800 MALDI TOF/TOF™ Analyzer, 4700 Proteomics Analyzer, and other Delayed Extraction® products are covered by one or more of the following U.S. patents: 5,625,184; 5,627,369; 5,760,393; 6,002,127; 6,057,543; 6,281,493; 6,348,688; 6,541,765; and 6,770,870. Additional U.S. and foreign patents are pending.

TRADEMARKS:

Applied Biosystems is a registered trademark, and AB (Design) and Applera are trademarks of Applera Corporation or its subsidiaries in the U.S. and/or certain other countries.

Data Explorer and Delayed Extraction are registered trademarks, and Explorer, MALDI TOF/TOF, Opti-TOF, and TOF/TOF are trademarks of Applied Biosystems/MDS SCIEX, a joint venture between Applera Corporation and MDS Inc.

Microsoft and Windows are registered trademarks of Microsoft Corporation.
Oracle is a registered trademark of Oracle Corporation.
AutoSense and QuickScan are registered trademarks of PSC Inc.

All other trademarks are the sole property of their respective owners.

Part Number 4352078 Rev. B
11/2004

Contents

Preface

Chapter 1 Before You Begin

Chapter 2 4000 Series Explorer™ Software Basics

Chapter 3 Loading Sample Plates

Chapter 4 Acquiring MS Spectra from Calibration Standards

Chapter 5 Processing Spectra

Chapter 6 Acquiring MS Spectra from Unknown Samples

Chapter 7 Acquiring MS/MS Spectra from Unknown Samples

Chapter 8 Performing Interpretation on MS Spectra

Chapter 9 Acquiring Multiple Spectra in Batch Mode

Index

Preface

How to Use This Guide

Purpose of This Guide

The *4800 MALDI TOF/TOF™ Analyzer Getting Started Guide* provides brief, step-by-step procedures for preparing and analyzing a sample. It is designed to help you quickly learn how to use the 4800 MALDI TOF/TOF™ Analyzer. For more detailed procedures, refer to the *4000 Series Explorer™ Software Online Help*.

Audience

This guide is intended for novice 4800 MALDI TOF/TOF™ Analyzer users.

Assumptions

This guide assumes that your 4800 MALDI TOF/TOF™ Analyzer has been installed by an Applied Biosystems Technical Representative, that a Source Model Calibration has been performed, and that the source vacuum pressure is less than 5×10^{-7} torr. Source pressure is displayed in the status bar (Figure 2-2 on page 2-5).

Text Conventions

This guide uses the following conventions:

- **Bold** indicates user action. For example:

  Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.
- A right arrow bracket (>) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File > Open > Spot Set**.

  Right-click the sample row, then select **View Filter > View All Runs**.

User Attention Words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

- Notes provide information that may be of interest or help, but is not critical to the use of the product. For example:

  Note: The size of the column affects the run time.

  Note: The Calibrate function is also available in the Control Console.

- Importants provide information that is critical to the use of the product or completion of a procedure. Importants can also emphasize the safe use of chemicals. For example:

  IMPORTANT! To verify your client connection to the database, you need a valid Oracle® user ID and password.

  IMPORTANT! You must create a separate Sample Entry Spreadsheet for each 96-well microtiter plate.

Safety Alert Words

IMPORTANT! Safety alert words also appear in user documentation. For more information about safety, see the *4800 MALDI TOF/TOF™ Analyzer Hardware Guide*.

How to Obtain More Information

Related Documentation

The following related documents are shipped with the system:

- ***4800 MALDI TOF/TOF™ Analyzer Hardware Guide*** – Describes the 4800 MALDI TOF/TOF™ Analyzer hardware, and provides information on preparing, maintaining, and troubleshooting the system.
- ***4000 Series Explorer™ Software Quick Reference Card*** – Provides abbreviated but key information.
- ***4000 Series Explorer™ Software Online Help*** – Describes the software used to run the 4800 instrument, and provides detailed procedures for common tasks. Help is available from the software Help menu or by pressing F1.
- ***Peak Explorer™ Software Online Help*** **–** Describes the LC/MALDI visualization and analysis software included with the 4800 instrument, and provides procedures for common tasks. Help is available from the Help menu or by pressing F1.
- ***DeNovo Explorer™ Software Online Help*** **–** Describes the denovo sequencing and database search software included with the 4800 instrument, and provides procedures for common tasks. Help is available from the Help menu or by pressing F1.
- ***4000 Series Database Tools Online Help*** **–** Describes the archive, restore, backup, and recovery tools for the 4000 Series database, and provides procedures for using the tools. Help is available by pressing F1 in the 4000 Series Database Tools.
- ***Data Explorer® Software Online Help*** **–** Describes Data Explorer® software, and provides procedures for command tasks. Help is available from the Data Explorer software Help menu or by pressing F1.

Portable document format (PDF) versions of the Hardware Guide, Quick Reference Card, and this Getting Started Guide are also included on the 4800 MALDI TOF/TOF™ Analyzer software installation CD. You can also access the PDF files from the 4000 Series Explorer™ software Help menu.

Note: For additional documentation, see "How to Obtain Support" on page x.

Send Us Your Comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

How to Obtain Support

To contact Applied Biosystems Technical Support from North America by telephone, call **1.800.899.5858**.

For the latest services and support information for all locations, go to **http://www.appliedbiosystems.com**, then click the link for **Support**.

At the Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

Before You Begin 1

This chapter contains the following sections:

Introducing the 4800 MALDI TOF/TOF™ Analyzer

Overview

The 4800 MALDI TOF/TOF™ Analyzer is a floor-standing MALDI TOF/TOF™ mass spectrometer that includes a reflector analyzer.

Figure 1-1 4800 MALDI TOF/TOF™ Analyzer

The 4800 MALDI TOF/TOF™ Analyzer can be used for high-throughput proteomics research. The system can identify proteins by determining accurate masses of peptides formed by enzymatic digestion.

Additionally, the system can more definitively identify and characterize proteins by isolating and fragmenting a molecular ion of interest and measuring the fragment ion masses.

For more information about the 4800 MALDI TOF/TOF™ Analyzer, refer to the *4800 MALDI TOF/TOF™ Analyzer Hardware Guide*.

Workflow in This Guide

Overview

This Getting Started Guide describes how to analyze a peptide standard and a mock peptide sample using the 4800 MALDI TOF/TOF™ Analyzer.

Figure 1-2 summarizes the procedure described in this guide.

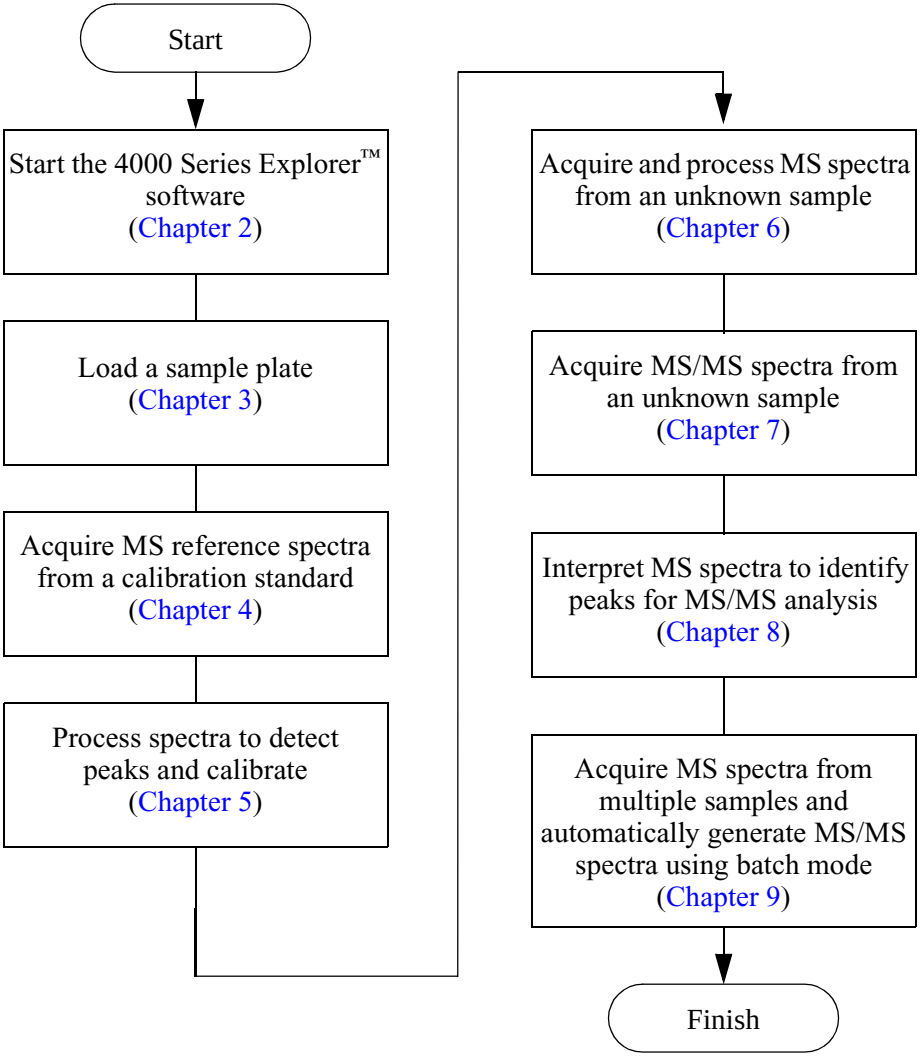


Figure 1-2 Workflow in this Getting Started Guide

Note: This guide provides brief procedures. For more detailed procedures and reference information, refer to the *4000 Series Explorer™ Software Online Help* and the *4800 MALDI TOF/TOF™ Analyzer Hardware Guide*.

Accessing Online Help

To access the *4000 Series Explorer™ Software Online Help*, press the **F1** key on the keyboard of the 4800 MALDI TOF/TOF™ Analyzer, or select **Help > Contents and Index** in the 4000 Series Explorer™ software.

Accessing Online Documentation

You can access online (.PDF) versions of the Hardware Guide, Quick Reference Card, and this Getting Started Guide from the 4000 Series Explorer™ software Help menu.

Assumptions

This guide assumes that your 4800 MALDI TOF/TOF™ Analyzer has been installed by an Applied Biosystems Technical Representative, that a Source Model Calibration has been performed, and that the source vacuum pressure is less than 5×10^{-7} torr. Source pressure is displayed in the status bar (Figure 2-2 on page 2-5).

Required Materials

To perform the experiment in this guide, you need:

- One 3 × 5 in. Opti-TOF™ magnetic holder (part number: 4350840).
- One pre-spotted Mass Standards Calibration Opti-TOF™ insert (part number: 4358092)

Figure 1-3 shows the standards spotted on the Mass Standards Calibration Opti-TOF™ insert.

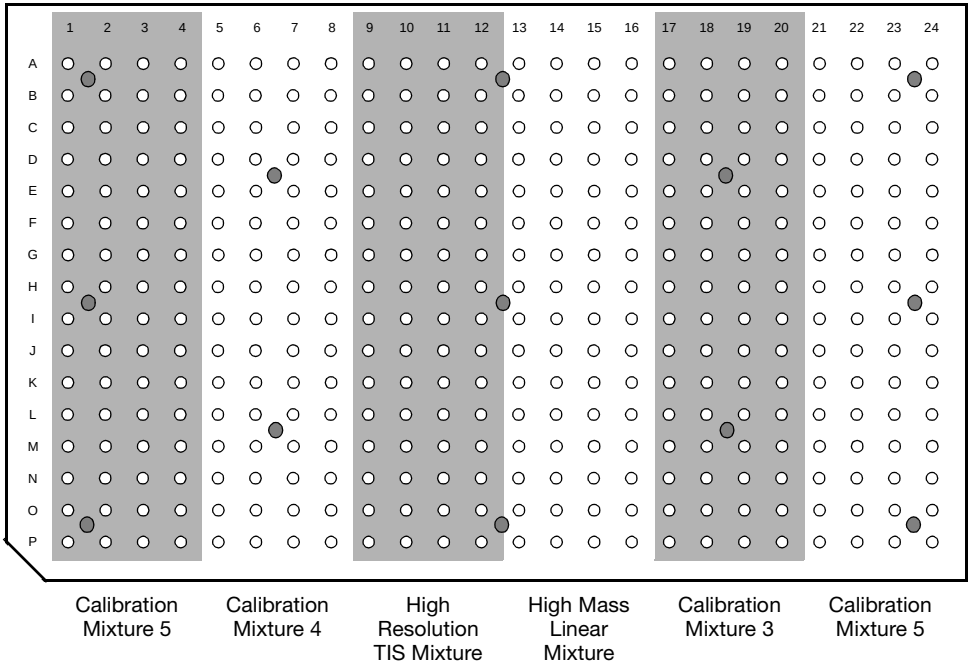


Figure 1-3 Standard Mixtures Spotted on the Opti-TOF™ Mass Standards Calibration Insert

Note: The 13 calibration spots (CAL 1 through CAL 13) on the insert are not spotted.

4000 Series Explorer™ Software Basics

2

This chapter contains the following sections:

Overview

In This Chapter

In this chapter, you will:

- Start the 4000 Series Explorer™ software.
- Review the features and parts of the software in interactive mode and batch mode.
- Learn the basics of the software, control pad, and handheld bar code scanner.

For More Information

Refer to the *4000 Series Explorer™ Software Online Help* for more information on:

- The 4000 Series Explorer™ software
- Using the 4000 Series Explorer™ software
- Using the control pad
- Using the bar code scanner

Refer to the *4800 MALDI TOF/TOF™ Analyzer Hardware Guide* for more information on:

- The 4800 MALDI TOF/TOF™ Analyzer hardware
- Using the control pad

Refer to the *Applied Biosystems Handheld Bar Code Scanner Installation Guide* for more information on:

- Installing the bar code scanner
- Adjusting the height of the bar code scanner holder

Starting the 4000 Series Explorer™ Software

Powering On

Typically, the 4800 MALDI TOF/TOF™ Analyzer should be powered on at all times.

Starting the 4000 Series Explorer Software

To start the 4000 Series Explorer™ software:

1. Log on to the 4800 MALDI TOF/TOF™ Analyzer using your User Name and Password. See your system administrator for your User Name and Password.
2. Double-click the **4000 Series Explorer** icon on the Microsoft® Windows® XP desktop. The 4000 Series Explorer™ software starts (Figure 2-1), and the hardware is automatically initialized.

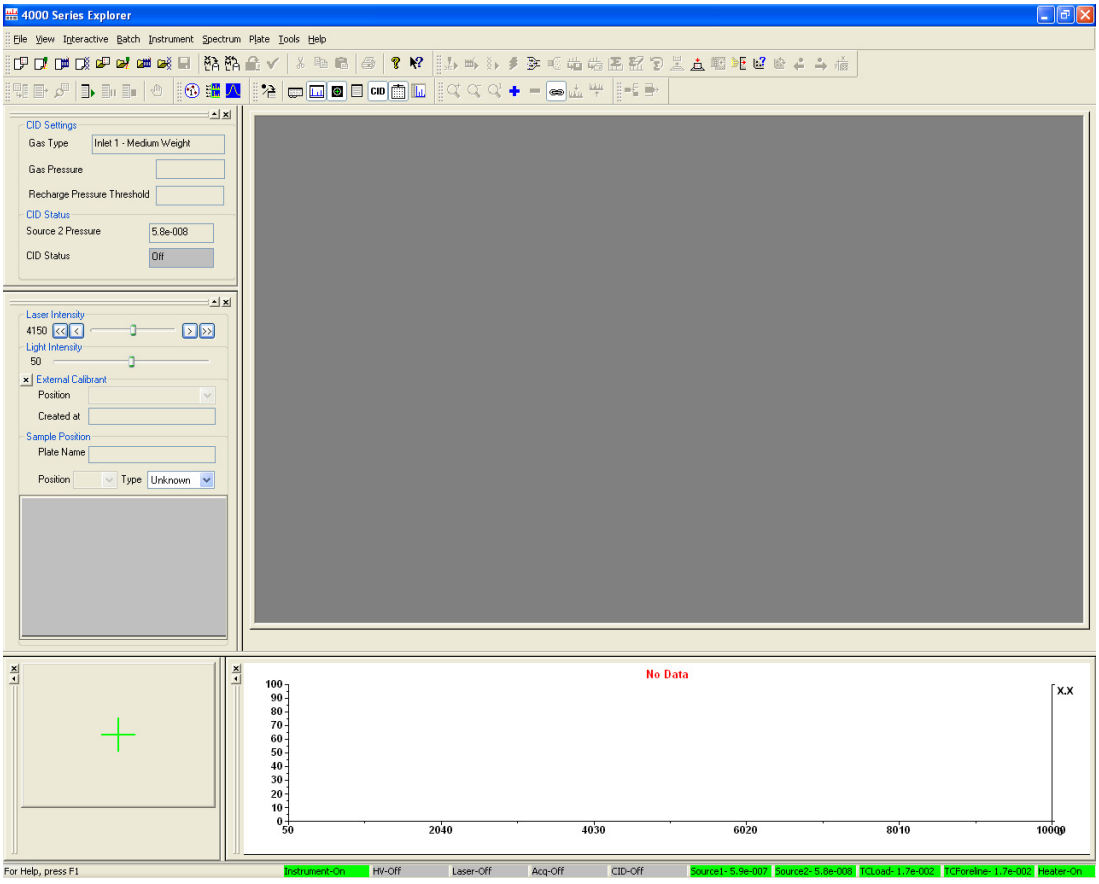


Figure 2-1 4000 Series Explorer™ Software

Overview of the 4000 Series Explorer™ Software

Overview

The 4000 Series Explorer™ software is the interface you use to perform tasks and access functions of the 4800 MALDI TOF/TOF™ Analyzer. You can use the 4000 Series Explorer™ software in either interactive mode or batch mode.

Interactive Mode

The 4000 Series Explorer™ software always opens in interactive mode. Interactive mode allows you to:

- Open, edit, and save multiple acquisition, processing, and interpretation methods.
- Acquire data using the settings specified in the active acquisition method.
- Detect peaks, and process and calibrate spectra using the settings specified in the active processing method.
- Interpret MS spectra, and generate a list of peaks for MS/MS analysis using the settings specified in the active interpretation method.
- Print and save data from acquired samples.
- Open spot sets, then view and process data from previous acquisitions.

Parts of the Software in Interactive Mode

The default layout of the 4000 Series Explorer™ software in interactive mode is shown in Figure 2-2.

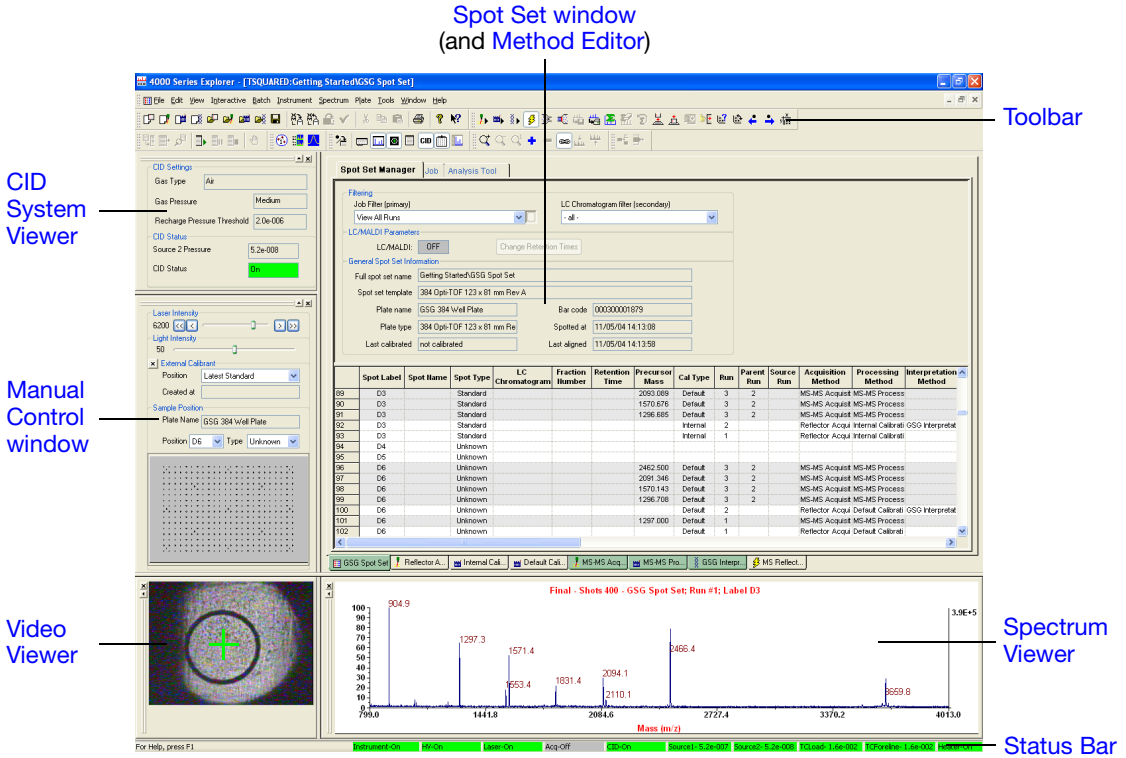


Figure 2-2 4000 Series Explorer™ Software Interactive Mode

Batch Mode

The 4000 Series Explorer™ software batch mode allows you to:

- Acquire data from multiple samples using different acquisition methods.
- Process data from multiple samples using different processing methods.
- Automatically acquire MS/MS spectra from multiple peaks in each MS spectrum using interpretation methods.
- Acquire, process, and interpret data from remote locations, using the optional 4000 Series Explorer™ software – Remote Access Client or GPS Explorer™ software.

Parts of the Software in Batch Mode

The default layout of the 4000 Series Explorer™ software in batch mode is shown in (Figure 2-3).

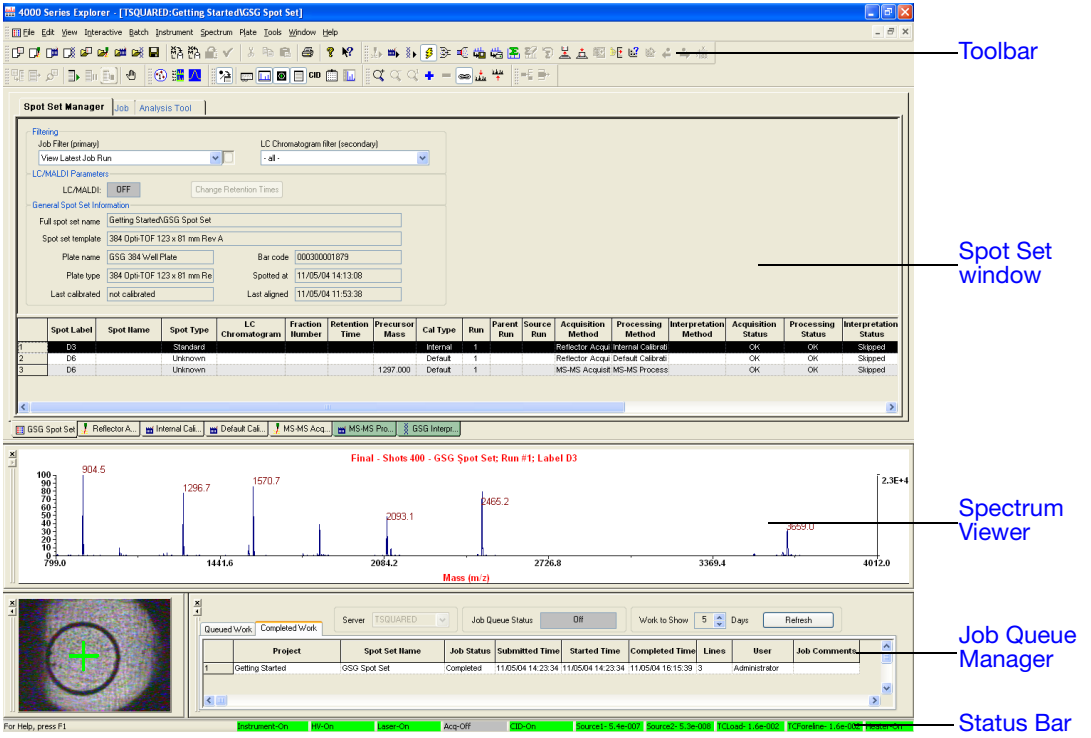


Figure 2-3 4000 Series Explorer™ Software Batch Mode

4000 Series Explorer™ Software Basics

Online Help

The *4000 Series Explorer™ Software Online Help* provides context-sensitive help for most windows in the software, as well as more general information about the software and procedures for common tasks.

Press **F1** on the keyboard to display information about the currently active window.

Select **Help > Contents and Index** to display the default Help topic.

Switching Between Interactive and Batch Modes

To switch between interactive mode and batch mode, either:

- Click  in the toolbar.
- Select **View > Switch to Batch Mode**.

Summary of Software Components

Table 2-1 describes the function of the 4000 Series Explorer™ software components.

Table 2-1 Summary of Software Components

Component	Description
Toolbar	Contains buttons that control the software and the instrument. For a brief description of a toolbar button, place the cursor on the button. The description (tooltip) is displayed below the button.
Spot Set window	Displays information about each sample position in a spot set. Each time data is saved for a sample position, an additional row is added to the Spot Set Manager for that position. You can use the Spot Set window to view data from the spot set, create spot set jobs to acquire or process spectra, and set other spot set parameters.
Method Editor	Allows you to create, edit, and view acquisition, processing, and interpretation methods. A tab is displayed at the bottom of the Method Editor for each open method. The tabs of all active methods are green.
Spectrum Viewer	Displays spectra during and after acquisition, and peak labels after processing. You can use the Spectrum Viewer to select peaks in an MS spectrum as precursors for MS/MS acquisition.

Table 2-1 Summary of Software Components *(continued)*

Component	Description
Output Window	Displays the spectrum peak list, calibration results, interpretation results, and other information regarding the instrument and the active spectrum.
Manual Control window	Allows you to select the active sample position and specify spot types in interactive mode. You can also use the Manual Control window to adjust laser intensity, light intensity, and sample position during manual acquisition.
CID System Viewer	Displays the status of the collision-induced-dissociation (CID) gas system and collision cell. All parameters in the CID System Viewer are read only.
Job Queue Manager	Displays the current status of the job queue, any currently queued spot set jobs, and recently completed spot set jobs.
Video Viewer	Displays real-time video of the sample spot from the instrument camera. A crosshair on the video viewer shows the laser position. You can use the video viewer to adjust the position of the sample under the laser during manual acquisition and when aligning the sample plate.
Status Bar	Displays the status of various system components.

Using the Control Pad

Overview

When working in interactive mode, you can use the Control Pad provided with the 4800 MALDI TOF/TOF™ Analyzer to:

- Adjust sample position under the laser beam
- Start and stop acquisition
- Adjust laser intensity
- Save a spot (methods, spectrum, and peak list) to the database

Figure 2-4 Control Pad

Using the Handheld Bar Code Scanner

LASER HAZARD. Class 2 (II) lasers can cause damage to eyes. Avoid looking into a Class 2 (II) laser beam or pointing a Class 2 (II) laser beam into another person's eyes.

The Opti-TOF™ plates provided with the 4800 MALDI TOF/TOF™ Analyzer have a bar code label on the bottom of the insert. When the Opti-TOF insert is in the magnetic holder, you can scan the bar code through the hole in the bottom of the holder.

You can scan bar codes in two ways:

- Holding the scanner by hand
- With the scanner mounted in the holder

Scanning by Hand

To scan by hand:

1. Hold the scanner about 23 cm (9 in.) from the plate.
2. Point the scanner at a slight angle to the bar code.
3. Press the trigger. The laser beam must illuminate the entire length of the bar code to correctly read the bar code. A beep indicates a successful read.

Scanning in the Holder

The bar code scanner must be in the AutoSense® scan mode to scan when mounted in the holder. To enable the AutoSense® scan mode, refer to the *QuickScan® 6000/6000plus AutoSense® Stand Instructions* included with the scanner.

Note: The scanner must be approximately 23 cm (9 in.) from the plate to properly read the bar code.

The trigger is automatically depressed when the scanner is mounted in the holder. To scan a bar code:

1. Slide the plate onto the base of the scanner. Do not hold the plate at an angle.
2. Pass the bar code label below the scanner laser beam.
3. When the laser beam illuminates the entire length of the bar code, tip the plate *very slightly.* The scanner is sensitive; you may need to move the plate *slightly* under the beam to get the scanner to read the bar code. A beep indicates a successful read.

Loading Sample Plates 3

This chapter contains the following sections:

Overview

In This Chapter

In this chapter, you will:

- Create a new project folder.
- Create a new spot set in the software.
- Load the sample plate into the 4800 MALDI TOF/TOF™ Analyzer.

For More Information

Refer to the *4000 Series Explorer™ Software Online Help* for more information about:

- Creating and managing project folders in the database.
- Creating new plates and spot sets.
- Creating new spot sets for existing plates.
- Creating spot set templates
- Loading and ejecting plates.
- Aligning sample plates.
- Calibrating sample plates.

Creating a New Project Folder

Creating a project folder allows you to save the data from an experiment in a specific folder within the database.

To create a new project folder:

1. Select **File > Database Management**. The Database Management dialog box opens (Figure 3-1).

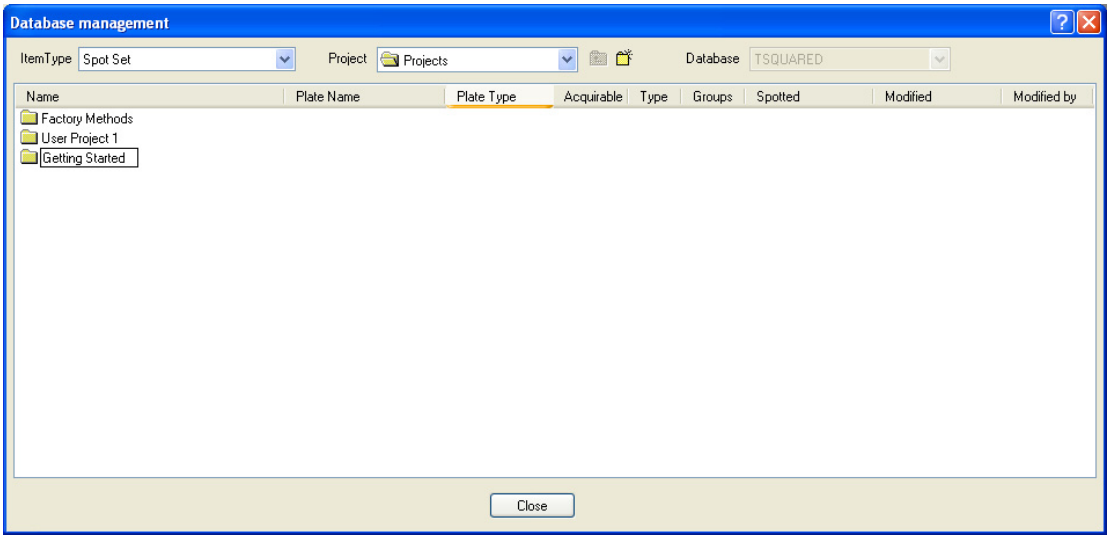


Figure 3-1 Database Management Dialog Box

2. In the Project drop-down list, select **Projects**.
3. Click (Create New Project) at the top right of the dialog box. A New Project folder is added to the database.
4. Type **Getting Started** for the name of the project folder.
5. Click **Close**. The project folder is created in the database.

Creating a New Spot Set

Overview

Before you load a new sample plate into the 4800 MALDI TOF/TOF™ Analyzer, you must create a new *spot set* in the software, create a new *plate* associated with the spot set, and choose a *spot set template* for the spot set.

- The *plate* stores the name and unique bar code of a specific MALDI plate in the database. Alignment and calibration information is also stored with the plate.
- The *spot set* contains information on a specific set of samples spotted onto the plate. The system stores the data generated from these samples in the spot set within the database.
- The *spot set template* specifies the number and layout of spots on the plate, as well as attributes for each spot.

Each MALDI plate that you load into the 4800 MALDI TOF/TOF™ Analyzer must have a unique spot set associated with it. If you wash and re-spot a plate, you must create a new spot set for the plate.

Creating a New Spot Set

To create a new spot set:

1. Select **File > New** > **Spot Set**. The Create New Spot Set dialog box opens (Figure 3-2).

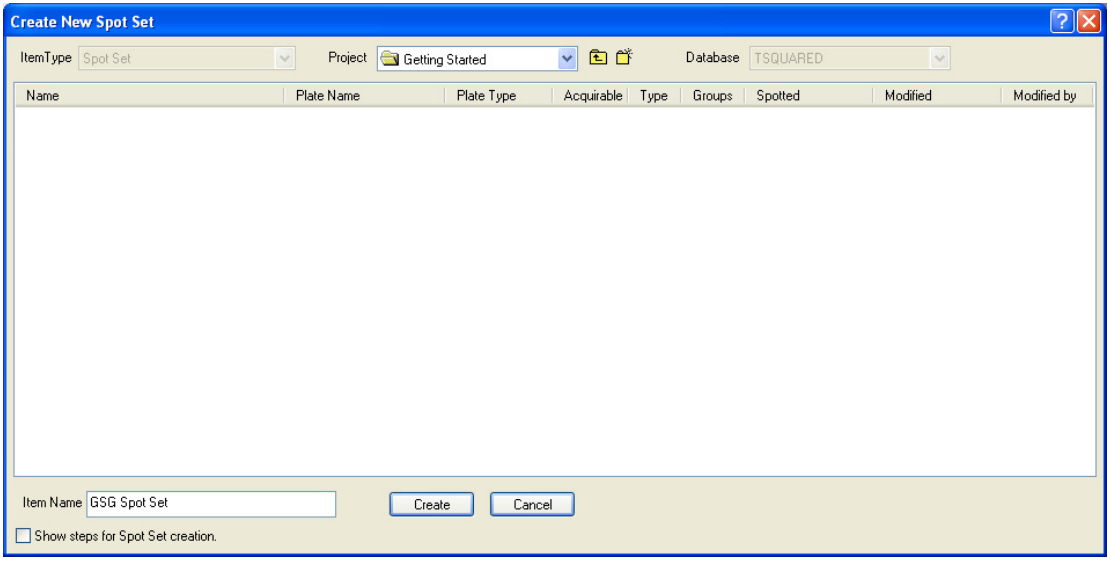


Figure 3-2 Create New Spot Set Dialog Box

2. Select **Getting Started** in the Project drop-down list.
3. In the Item Name field (at the bottom of the dialog box), type **GSG Spot Set**.
4. Click **Create**. The Select/Create Plate for New Spot Set dialog box opens (Figure 3-3).

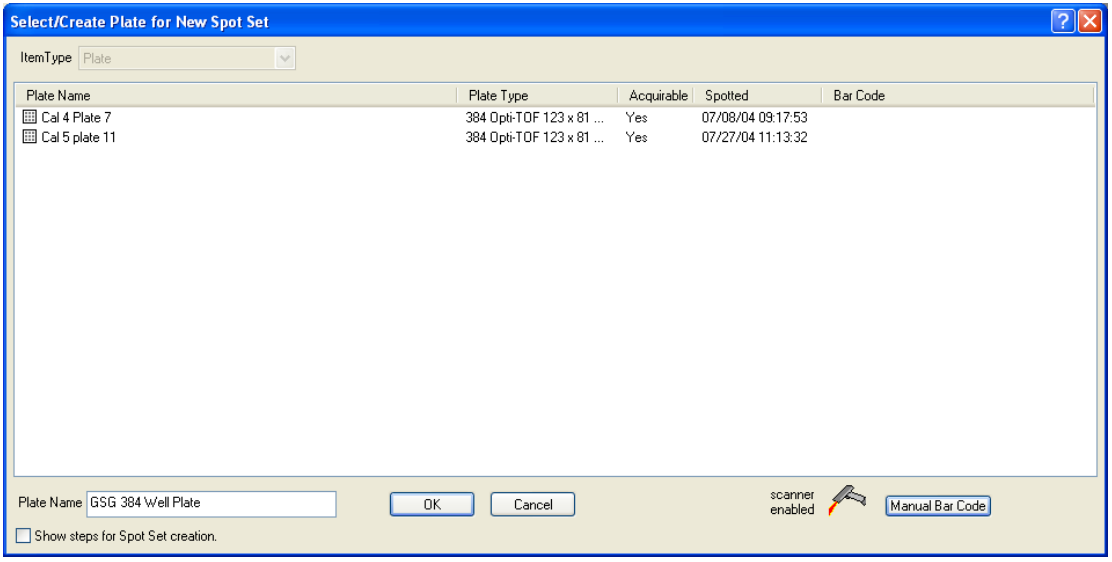


Figure 3-3 Select/Create Plate for New Spot Set Dialog Box

Creating a New Plate

5. If your plate has a bar code:
   - Scan it with the handheld bar code scanner

     or
   - Click **Manual Bar Code**, enter the number in the Manual Bar Code dialog box, then click **OK**.

   If your plate does not have a bar code, type **GSG 192 Well Plate** in the Plate Name field at the bottom of the dialog box.

Selecting a Spot Set Template

6. Click **OK**. The Select Spot Set Template for New Spot Set dialog box opens (Figure 3-4).

   Note: Each time you create a new spot set in the 4000 Series Explorer™ software, you must select a spot set template from which to create the spot set. The spot set template specifies the number and layout of spots on the sample plate associated with the spot set, as well as attributes for each spot on the plate.

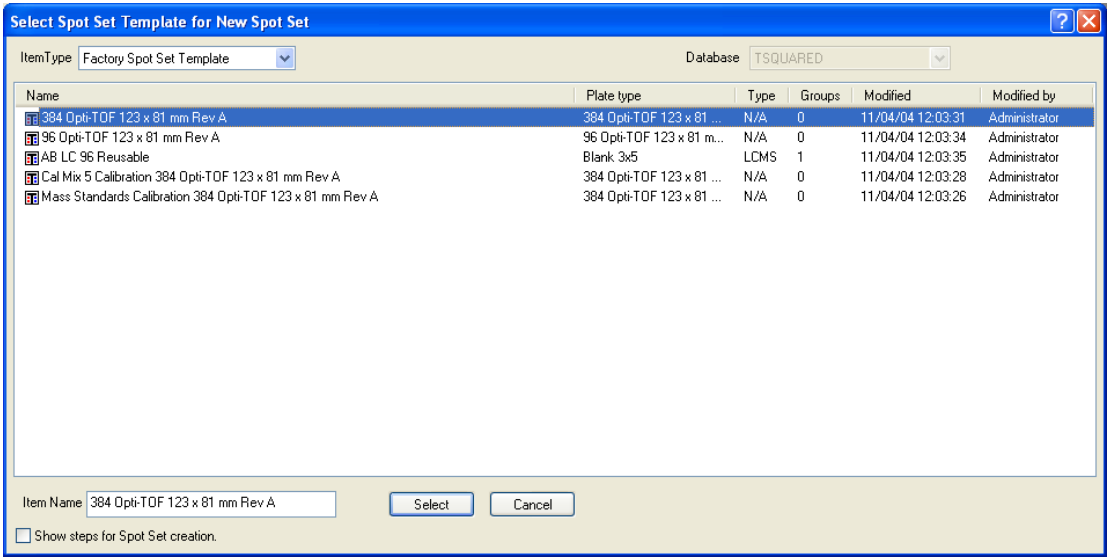


Figure 3-4 Select Spot Set Template for New Spot Set Dialog Box

7. Select **Factory Spot Set Template** in the Item Type drop-down list.

   Note: In addition to the Factory Spot Set Templates supplied by Applied Biosystems, you can create User Defined spot set templates for your own samples. For more information, refer to the *4000 Series Explorer™ Software Online Help*.

8. Select **384 Opti-TOF 123 x 81 mm Rev A**, then click **Select**. The Spot Set Window opens in the 4000 Series Explorer™ software (Figure 2-2 on page 2-5).

Loading Sample Plates in the Mass Spectrometer

Assembling the Opti-TOF™ Plate

IMPORTANT! Wear powder-free gloves when handling inserts to avoid contaminating the hydrophobic surface or your samples.

To assemble the Opti-TOF™ plate:

1. Remove the pre-spotted mass standards calibration insert from the shipping tray by gently bending the tray to lift a corner of the insert out of the tray, then grasping the insert with a gloved hand and removing.
2. Hold the magnetic holder in one hand.
3. In the other hand, hold the insert at an angle. Handle the insert by the edges to avoid touching the spotted surface.
4. Align the notch in the insert with the notch in the holder, then drop the plate into the holder (Figure 3-5).

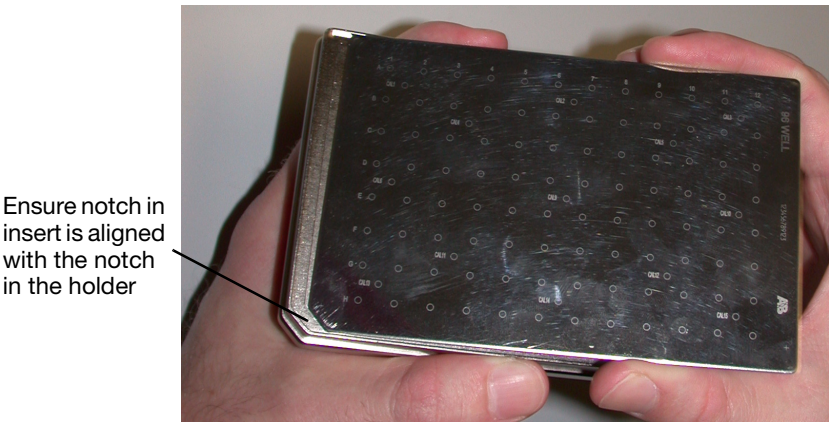


Figure 3-5 Assembling an Opti-TOF™ Plate

5. Ensure the face of the insert is flush with the face of the holder.

   CAUTION If the notch in the insert is not aligned with the notch in the holder, the plate will jam when you load it into the system.

6. If the notched edge of the insert does not fit snugly into the notch in the holder, push your finger through the hole in the bottom of the holder, then move the insert by pushing on the edges of the insert.

Loading the Sample Plate

CAUTION Before placing the plate onto the load pad, always use dry, compressed gas to blow off any fibers that may be on the plate. Fibers on the plate can be transferred to the ion optics components during sample acquisition and severely and adversely affect sensitivity.

To load the sample plate:

1. Use dry, compressed gas to blow off any fibers that may be on the plate.
2. Open the sample loading chamber (Figure 3-6).

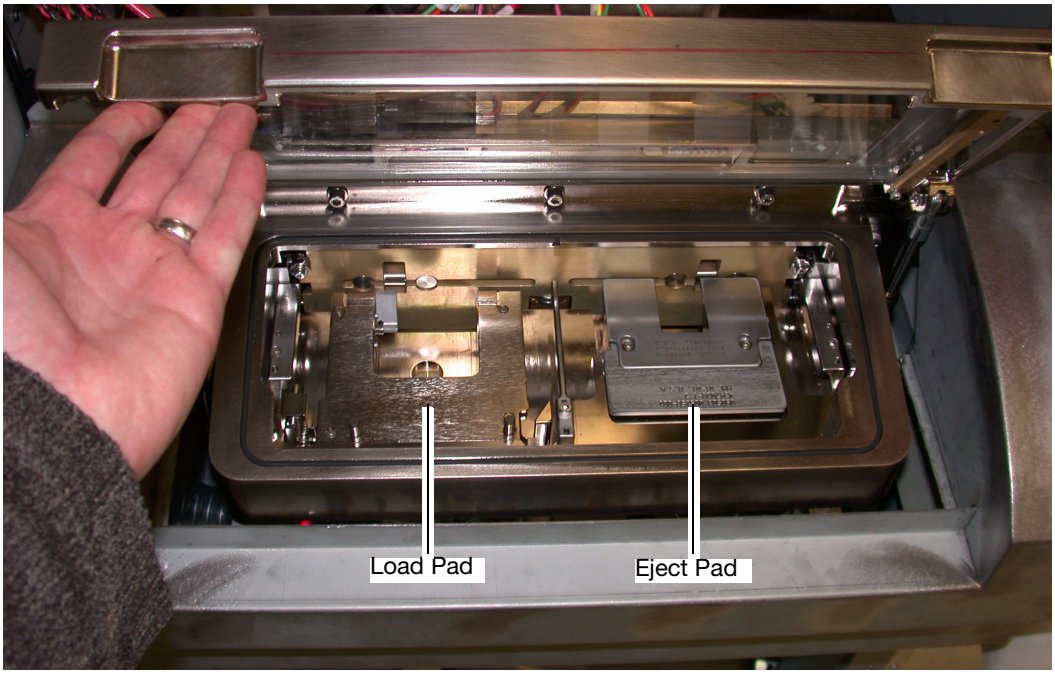


Figure 3-6 Sample Loading Chamber

3. If a sample plate is on the eject pad, remove it.
4. Place the sample plate on the load pad, with the notched corner of the plate facing the lower left, and the front edge of the plate flush against the pins on the front of the load pad (Figure 3-7).

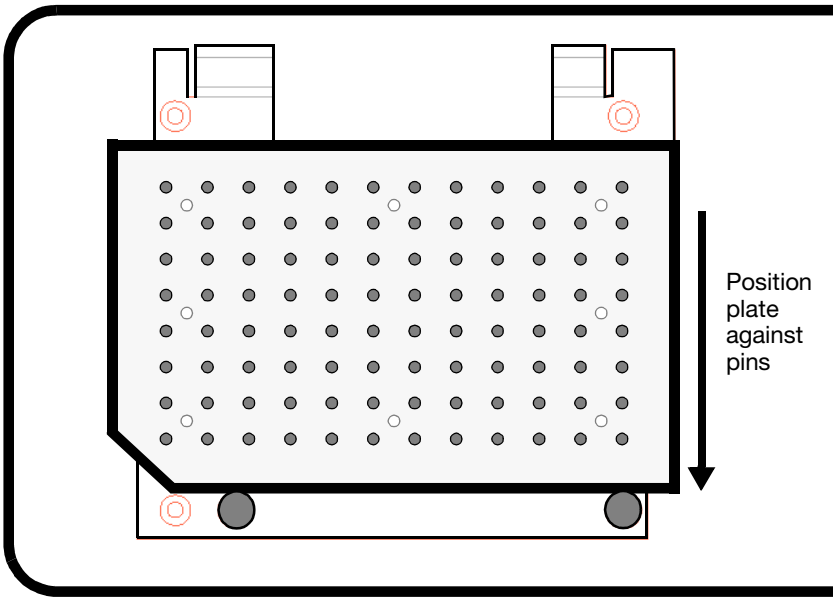


Figure 3-7 Loading Sample Plate

5. Close the sample loading chamber.
6. Select **Plate > Load Plate**. The Select Spot Set dialog box opens (Figure 3-8).

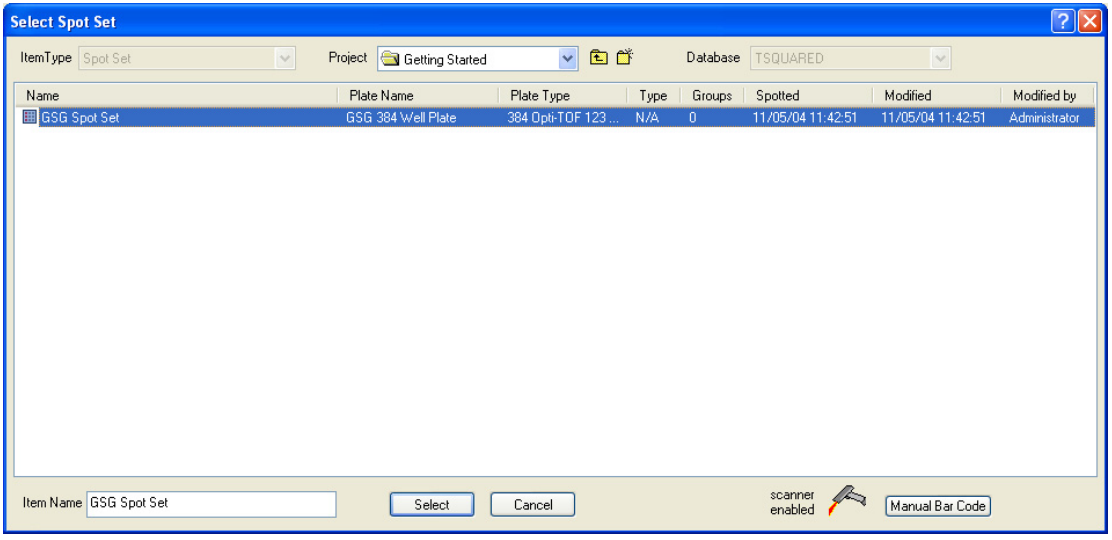


Figure 3-8 Select Spot Set Dialog Box

7. Select **GSG Spot Set**, then click **Select**.
   The plate and spot set information appears in the Load Sample Plate dialog box (Figure 3-9).

   Note: If you entered a bar code when you created the plate (step 5 on page 3-5), you can select the plate by scanning it with the handheld bar code scanner, or by clicking **Manual Bar Code**, then manually entering the bar code number in the Manual Bar Code dialog box.

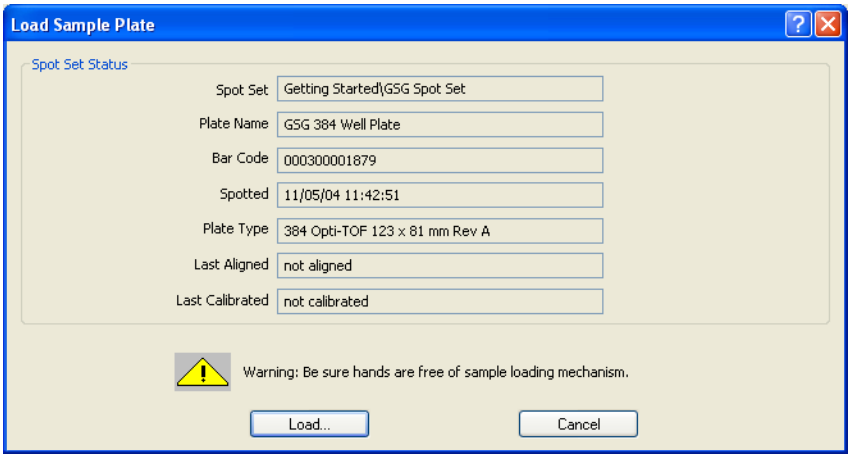


Figure 3-9 Load Sample Plate Dialog Box

8. Click **Load**. The sample plate is loaded into the main source chamber.

   Note: While the sample plate is moving into position (about 1 minute), the Load/Eject Status dialog box displays the status of the hardware.

Acquiring MS Spectra from Calibration Standards

4

This chapter contains the following sections:

Overview

In This Chapter

In this chapter, you will:

- Create a new acquisition method.
- Set the active acquisition method.
- Align the sample plate.
- Select the sample position to acquire.
- Start an acquisition.
- Observe the signal during and after acquisition.

Note: Use Calibration Mixture 5 as the calibration standard (see Figure 1-3 on page 1-5).

For More Information

Refer to the *4000 Series Explorer™ Software Online Help* for more information about:

- Creating acquisition methods.
- Acquisition method parameters.
- Using the Manual Control window.
- Acquiring spectra.
- Aligning sample plates.
- Aligning the laser target (crosshair).
- Troubleshooting.

Creating an Acquisition Method

Before you can acquire data, you must create an *acquisition method*. Acquisition methods specify the instrument settings needed to acquire data on the 4800 MALDI TOF/TOF™ Analyzer.

To create an acquisition method:

Creating a New Acquisition Method

1. Select **File > New > Acquisition Method**. The Create New Acquisition Method from Default dialog box opens (Figure 4-1).

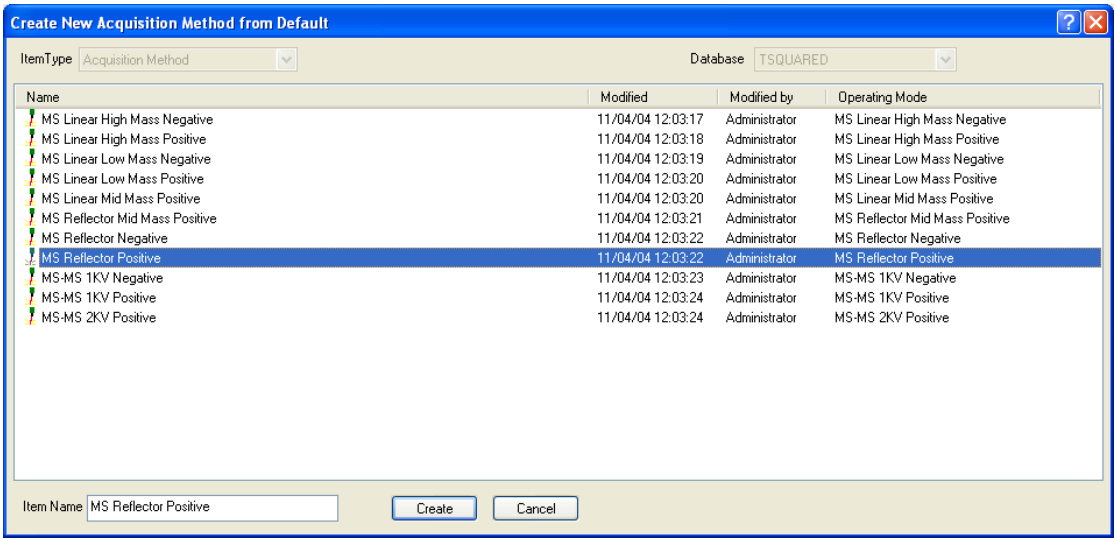


Figure 4-1 Create New Acquisition Method Dialog Box

2. Select **MS Reflector Positive** in the list of acquisition methods.
3. Click **Create**. The Acquisition Method Editor Instrument tab opens (Figure 4-2).

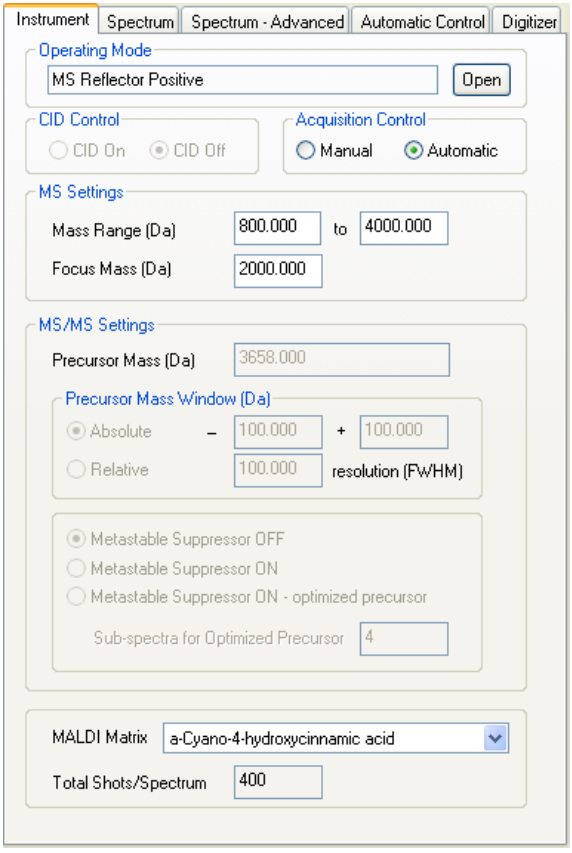


Figure 4-2 Acquisition Method Editor Instrument Tab

Setting Acquisition Parameters

The default acquisition methods specify instrument settings that have been optimized for each operating mode. Typically, you do not need to change these settings to acquire the calibration standard.

Note: When acquiring samples, you use the default acquisition methods as starting points to create custom acquisition methods.

Saving the Acquisition Method

4. Select **File > Save Acquisition Method**.
5. Type **Reflector Acquisition Method** for Item Name, then click **Save**.

Preparing for Acquisition

Before you start an acquisition, perform the following procedures:

- "Warming Up the High-Voltage Power Supplies"
- "Setting the Active Acquisition Method"
- "Aligning the Sample Plate"
- "Selecting the Sample Position"

Warming Up the High-Voltage Power Supplies

To ensure maximum mass accuracy, allow the high-voltage power supplies to warm up for at least 30 minutes before starting acquisition.

To turn on the high-voltage power supplies, select **Instrument > Turn on High Voltage**, or click  in the toolbar.

Note: You must have a plate loaded and an acquisition method open before you can turn on the high-voltage power supplies.

Setting the Active Acquisition Method

Overview

Although you can open and edit multiple acquisition methods within the 4000 Series Explorer™ software, only one of the open acquisition methods is the *active method*, the method used to acquire data.

Before performing an acquisition, you must specify the acquisition method you want to use to acquire data.

Note: You can determine which method is active by checking the tabs at the bottom of the Method Editor. The tab for the active method is shaded green.

Setting the Active Acquisition Method

To set the active acquisition method:

1. Select the **Reflector Acquisition Method** tab at the bottom of the Method Editor.
2. Select **File > Set as Active Acquisition Method**, or click  in the toolbar.

   The tab for the active method appears green in the Method Editor.

Aligning the Sample Plate

Overview

The first time you load a plate into the 4800 MALDI TOF/TOF™ Analyzer, you must align the plate so that the center of each sample position is aligned with the laser beam. After you align a plate, the alignment is used as the default alignment for future sample plates of the same type.

Using the Sample Plate Alignment Wizard

To align the sample plate:

1. Select **Plate > Align Sample Plate**. The Sample Plate / Laser Target Alignment wizard opens (Figure 4-3).

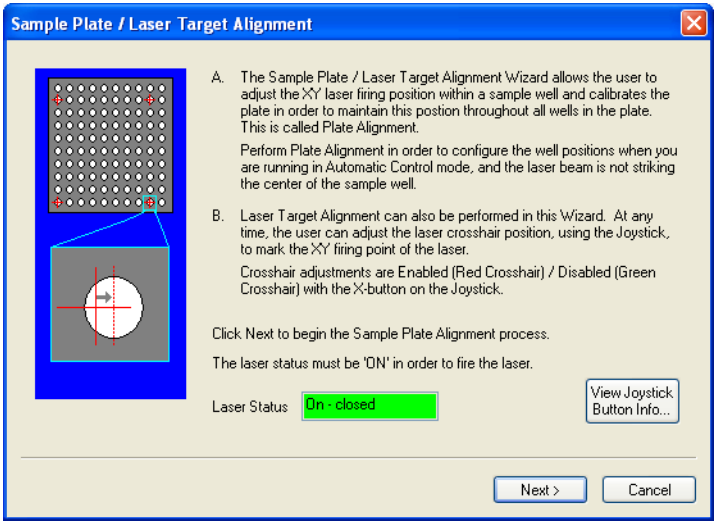


Figure 4-3 Sample Plate / Laser Target Alignment Wizard

2. Click **Next**. The Sample Plate / Laser Target Alignment - Step 1 page opens (Figure 4-4).

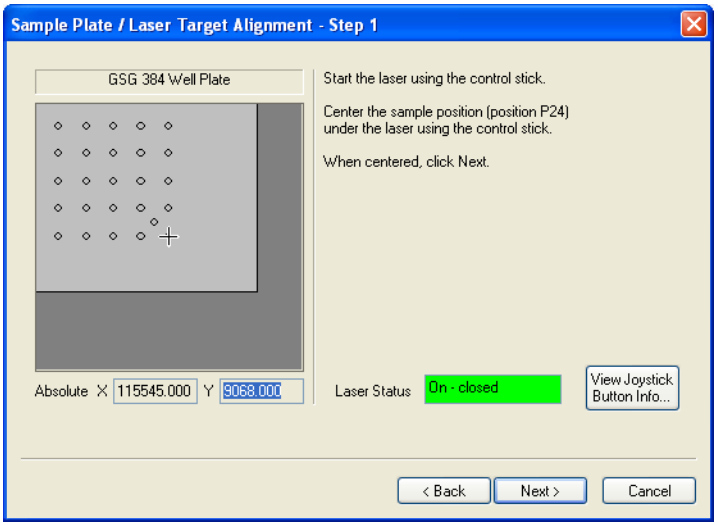


Figure 4-4 Sample Plate Alignment - Step 1 Page

3. Start the laser by pressing the **1** button on the control pad (Figure 4-7 on page 4-10).

   Note: When aligning the sample plate, starting the laser does not start acquisition.

4. Center the specified sample position under the laser target using the control pad.

5. When the position is centered, click **Next**.

6. Repeat steps 3 through 5 for the other three alignment positions on the sample plate.

   The software calculates the alignment and uses the settings to ensure that all sample positions on the plate are centered under the laser.

7. Click **Finish** to exit the Sample Plate Alignment wizard.

   Note: If the alignment fails, you may have aligned the incorrect spots on the plate. Repeat the procedure, but use the control pad to verify that you are aligning the spots specified in each page of the Sample Plate Alignment Wizard (Figure 4-4).

Selecting the Sample Position

You select the sample plate position from which to acquire spectra in the Manual Control window of the 4000 Series Explorer™ software.

To select the sample position:

1. If the Manual Control window is not displayed, select **View > Manual Control**.

   The Manual Control window opens (Figure 4-5), displaying the Plate Name you selected when you loaded the sample plate.

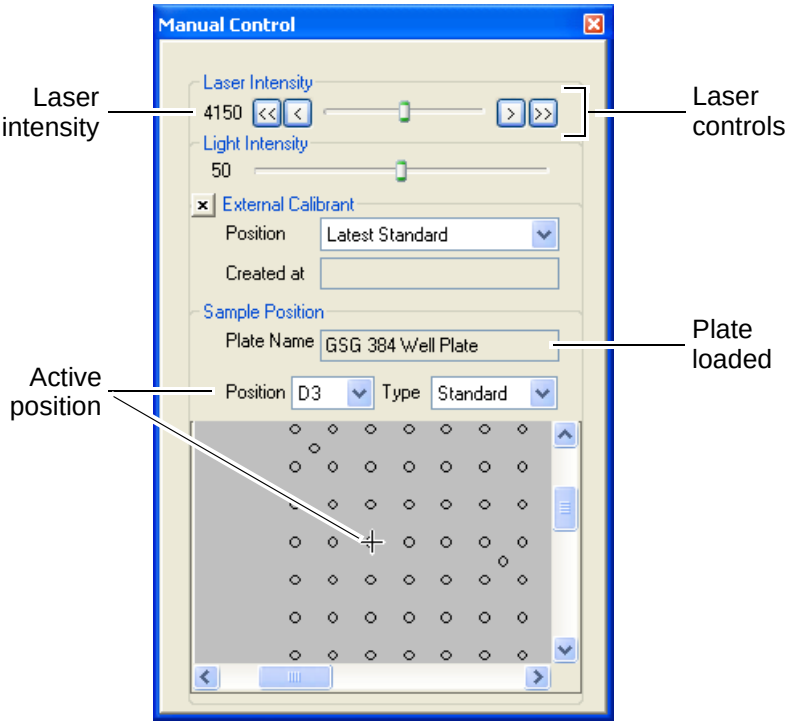


Figure 4-5 Manual Control Window (Partial Plate View)

2. Select one of the spots in the first four columns of the sample plate (the spots containing Calibration Mixture 5) by doing one of the following:
   - Select the spot label of the position from the Position drop-down list. For example: **D3**.
   - Click the sample position in the Plate view.

   Note: See Figure 1-3 on page 1-5 for a plate layout diagram.

3. Select **Standard** from the Type drop-down list.
4. Select **Plate > Show Sample View** to display an expanded view of the selected sample position (Figure 4-6).

   Note: You can also right-click the sample position to switch between Full Plate, Partial Plate, and Sample views.

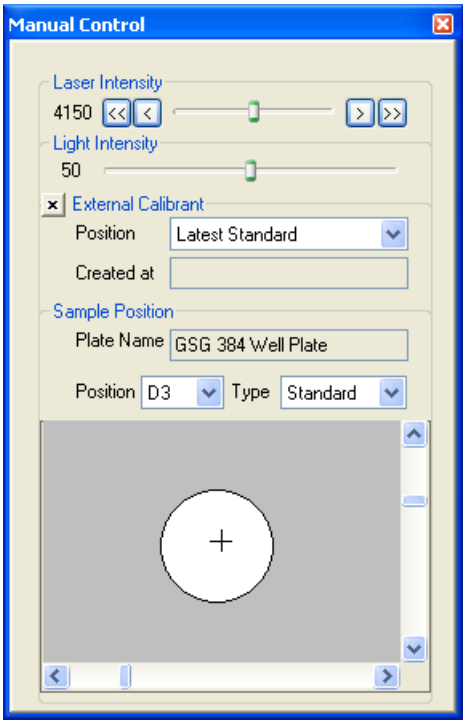


Figure 4-6 Manual Control Window (Sample View)

5. You can fine-tune the sample plate position under the laser by doing one of the following:
   - Click the spot in sample view.
   - Use the control pad (Figure 4-7 on page 4-10).
   - Drag the scroll bars in sample view.

Starting Acquisition

To start acquisition, do any one of the following:

- Click  in the toolbar.
- Select **Interactive > Start Active Acquisition Method**.
- Press the **1** button on the control pad (Figure 4-7).

  Note: If the laser is turned off, there may be up to a 2-minute delay for laser warmup before acquisition begins.

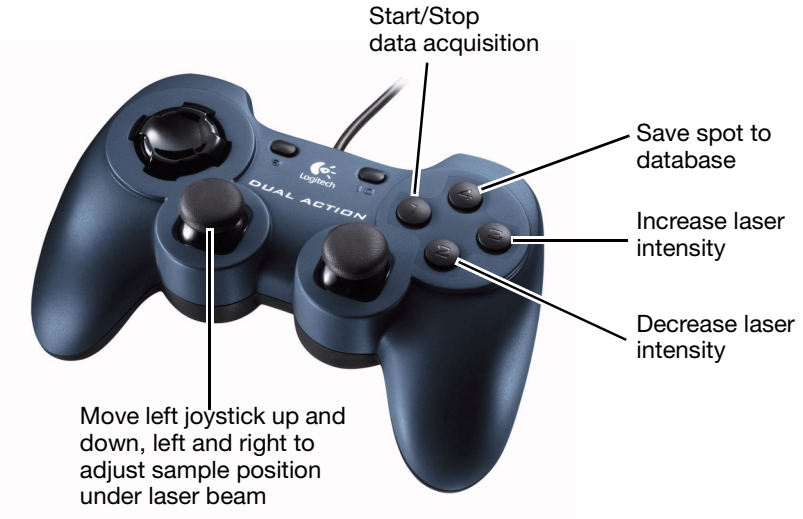


Figure 4-7 Control Pad

Note: For more information on using the control pad, see "Using the Control Pad" on page 2-9.

Observing the Signal

During acquisition, a Live trace is displayed and updated in the Spectrum Viewer.

When acquisition is complete, a Final trace is displayed in the Spectrum Viewer similar to the spectrum in Figure 4-8.
The spectrum should contain the following peaks:

- **des-Arg1-Bradykinin**: 904.5 Da
- **Angiotensin**: 1,296.7 Da
- **Glu1-Fibrinopeptide B**: 1,570.7 Da
- **ACTH (1–17)**: 2,093.1 Da
- **ACTH (18–39)**: 2,465.2 Da
- **ACTH (7–38)**: 3,657.9 Da

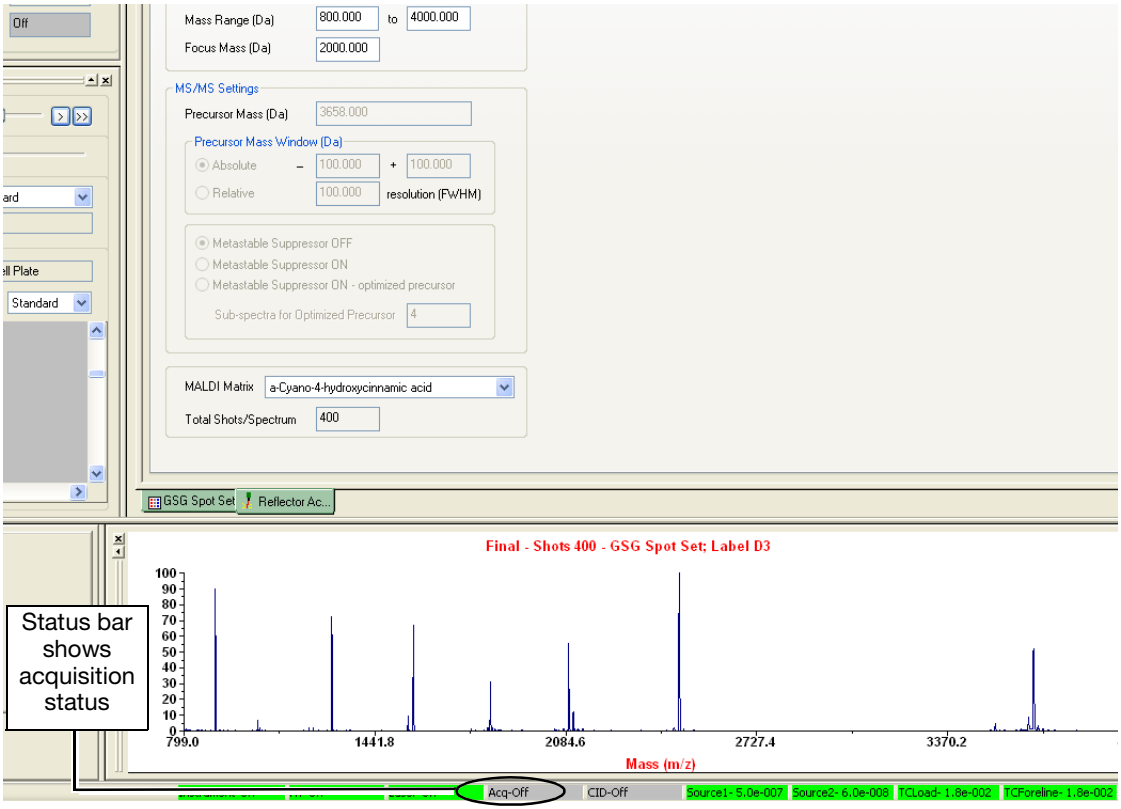


Figure 4-8 Final Trace in the Spectrum Viewer

If You Do Not See Signal

If you do not see any signal during acquisition, or if signal intensity is low, perform the following in sequence:

- Verify that you selected a sample position that contains the calibration standard. See "Selecting the Sample Position" on page 4-8.
- Increase the laser intensity in the Manual Control window. See Figure 4-5 on page 4-8.
- Realign the sample plate. If the sample plate is not properly aligned, the laser may not hit the sample. See "Aligning the Sample Plate" on page 4-6.
- Refer to Troubleshooting in the *4000 Series Explorer™ Software Online Help*.

Stopping Acquisition

Acquisition continues until the Stop Conditions specified in the Acquisition Method Spectrum tab are satisfied.

Alternatively, you can stop acquisition when you observe an acceptable signal by doing any of the following:

- Click  in the toolbar.
- Select **Interactive > Stop Active Acquisition Method**.
- Press the **A** button on the control pad (Figure 4-7 on page 4-10).

CAUTION Check if acquisition has already stopped automatically before you try to manually stop acquisition. If acquisition has stopped, the Spectrum Viewer displays "Final", and the Status Bar displays "Acq-OFF" (it displays "Acq-ON" during acquisition). See Figure 4-8 on page 4-11.

If acquisition has stopped, and you restart acquisition with the software or control pad, a new acquisition begins and overwrites the previously acquired Final spectrum.

Processing Spectra 5

This chapter contains the following sections:

Overview

In This Chapter

In this chapter, you will:

- Create a new processing method.
- Set the active processing method.
- Run the processing method.
- Evaluate data.
- Internally calibrate the spectrum.
- Save the spectrum.

For More Information

Refer to the *4000 Series Explorer™ Software Online Help* for more information about:

- Creating processing methods.
- Processing method parameters.
- Processing spectra.
- Labeling peaks.
- Performing internal and external calibration.
- Creating calibration reference files.
- Viewing and analyzing data.
- Updating the default calibration.

Creating a Processing Method

After you acquire data, you can process the spectrum to detect peaks and perform calibration. A *processing method* specifies the parameters needed to smooth and baseline-correct a spectrum, detect peaks, and calibrate.

To create a processing method:

Creating a New Processing Method

1. Select **File > New > Processing Method**. The Create New Processing Method from Default dialog box opens (Figure 5-1).

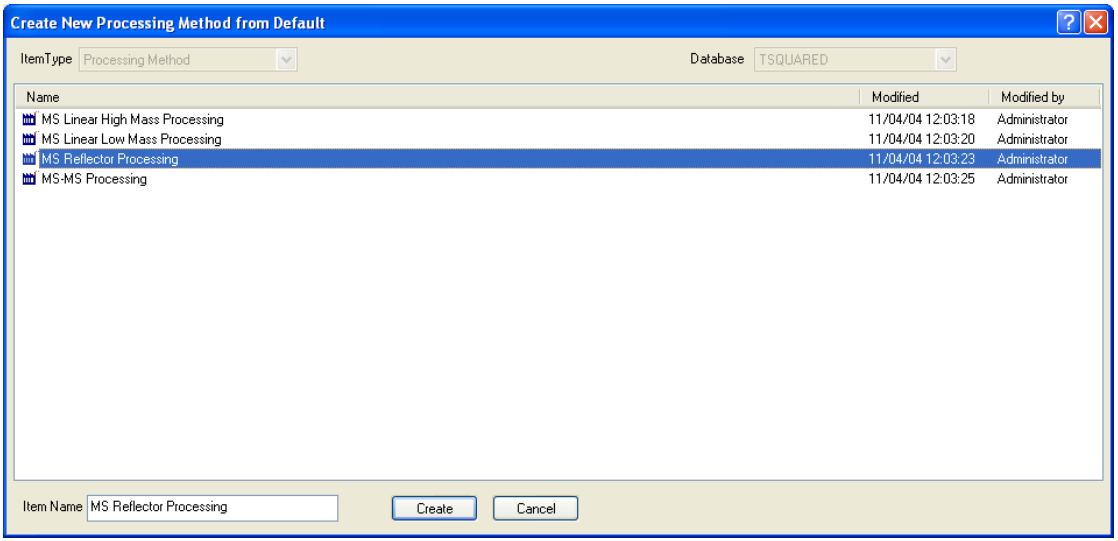


Figure 5-1 Create New Processing Method from Default Dialog Box

2. Select **MS Reflector Processing**.
3. Click **Create**. The Processing Method Editor opens (Figure 5-2).

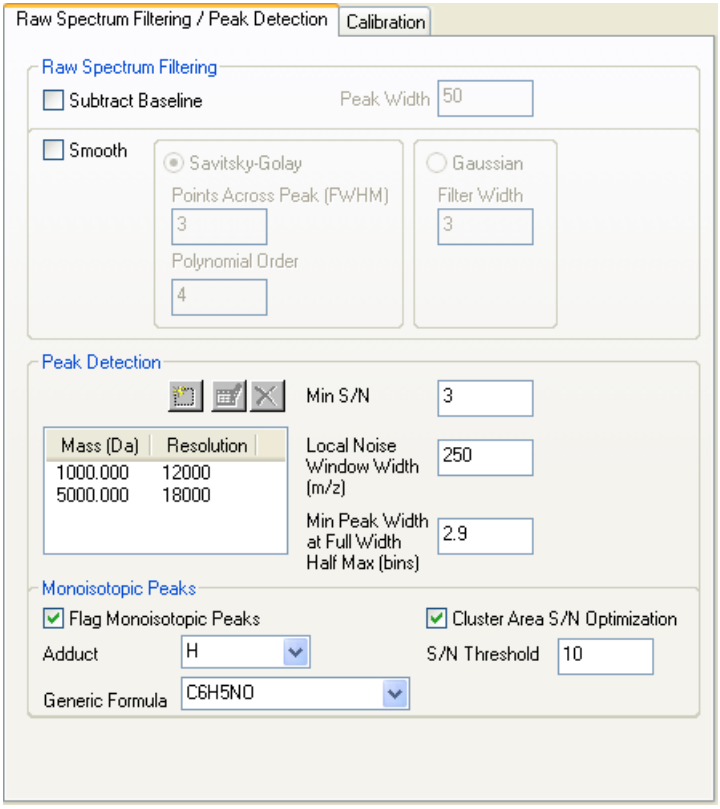


Figure 5-2 Processing Method Editor

Processing Parameters

The default processing methods specify settings that detect peaks and perform a default (automatic) calibration.

Saving the Processing Method

4. Select **File > Save Processing Method**.
5. Type **Default Calibration Processing Method** for the Item Name, then click **Save**.

Setting the Active Processing Method

Overview

Although you can open and edit multiple processing methods within the 4000 Series Explorer™ software, only one of the open processing methods is the *active method*, the method used to process data.

Before processing, you must specify the processing method you want to use to process data.

Note: You can determine which method is active by checking the tabs at the bottom of the Method Editor. The tab for the active method is shaded green.

Setting the Active Processing Method

To set the active processing method:

1. Select the **Default Calibration Processing Method** tab at the bottom of the Method Editor.
2. Select **File > Set as Active Processing Method**, or click  in the toolbar.

   The tab for the active method appears green in the Method Editor.

Running the Processing Method

Processing the spectrum using the settings in the default processing method detects peaks in the spectrum and performs a default calibration.

Running the Processing Method

To run the processing method, do either of the following:

- Click  in the toolbar.
- Select **Interactive > Run Active Processing Method**.

Peaks are detected and labeled in the Spectrum Viewer (Figure 5-3).

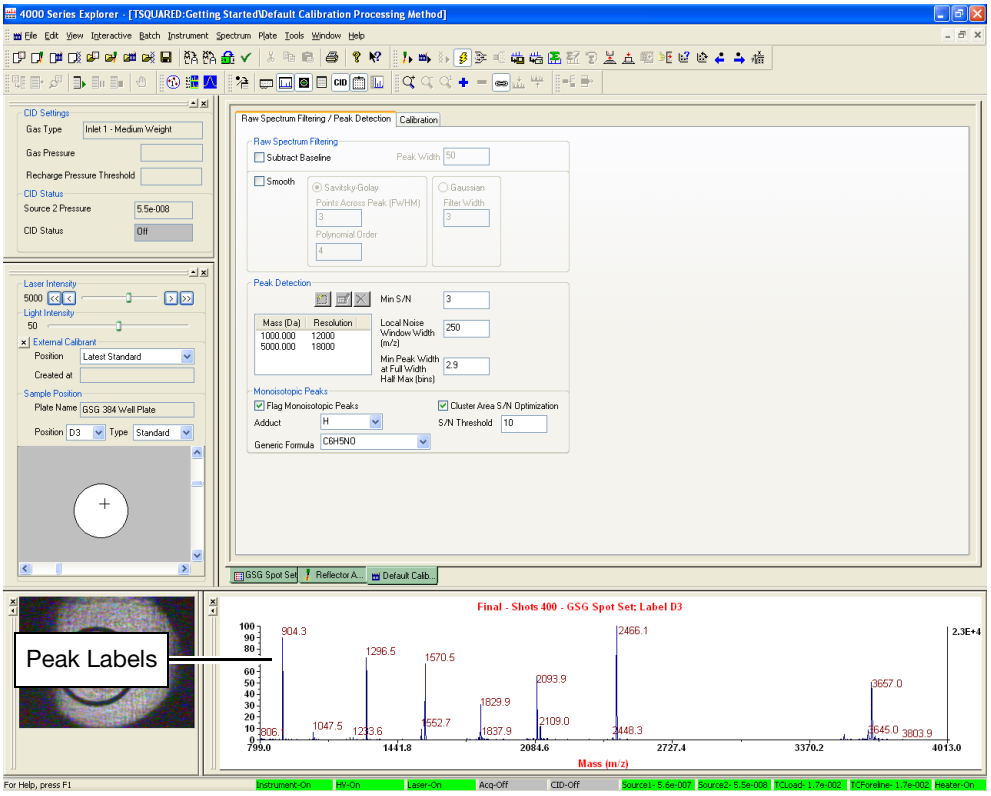


Figure 5-3 Peak Detection

If Peaks Labels Do Not Appear

If peak labels do not appear, peak labeling may not be enabled. Select **Spectrum > Peak Label**, then select **Enable** in the Spectrum Peak Label dialog box.

Evaluating Data

Overview After you run the processing method, evaluate the data by:

- Zooming on Peaks
- Eliminating Unwanted Peaks
- Examining the Spectrum
- Viewing the Peak List
- Checking Resolution and Signal-To-Noise

Zooming on Peaks

To zoom in on the spectrum trace, drag a box around the area of the spectrum you want to enlarge. Make sure to drag only within the Spectrum Viewer (Figure 5-4).

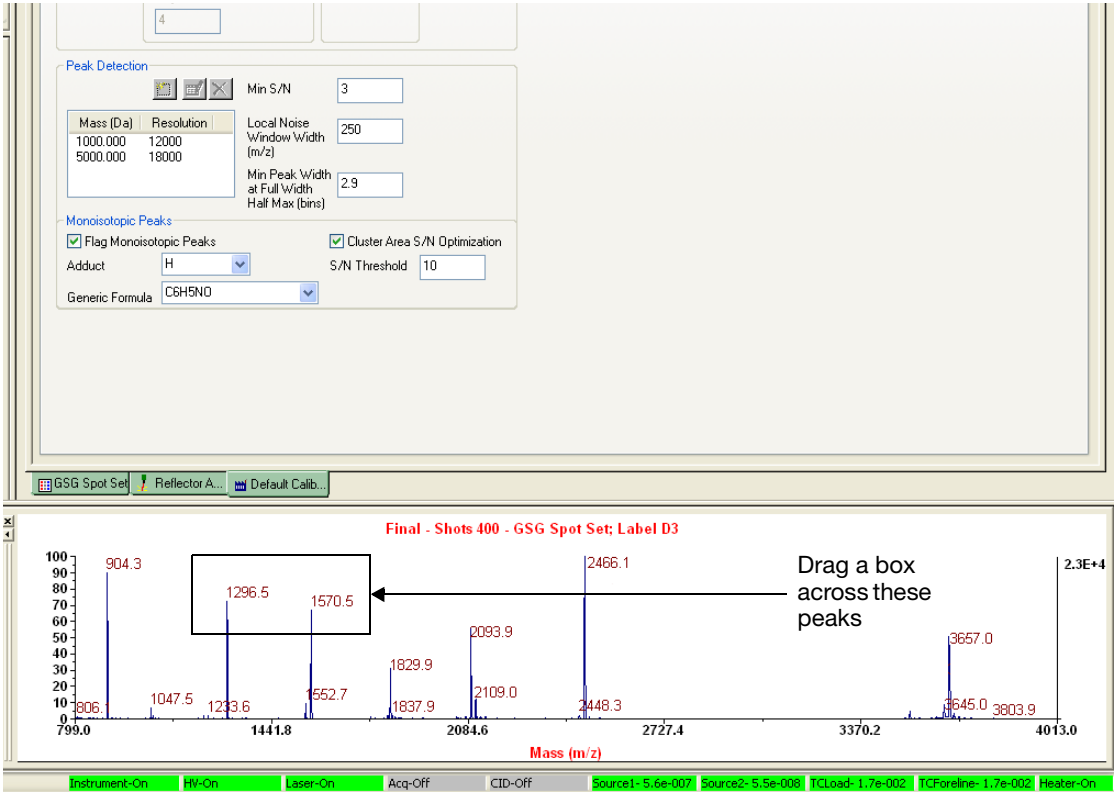


Figure 5-4 Zooming on Peaks

The selected area is magnified when you release the left mouse button (Figure 5-5).

You can also right-click in the Spectrum Viewer, or use the following toolbar buttons to zoom:

- Click  to Zoom in.
- Click  to Zoom out.
- Click  for Full Unzoom.

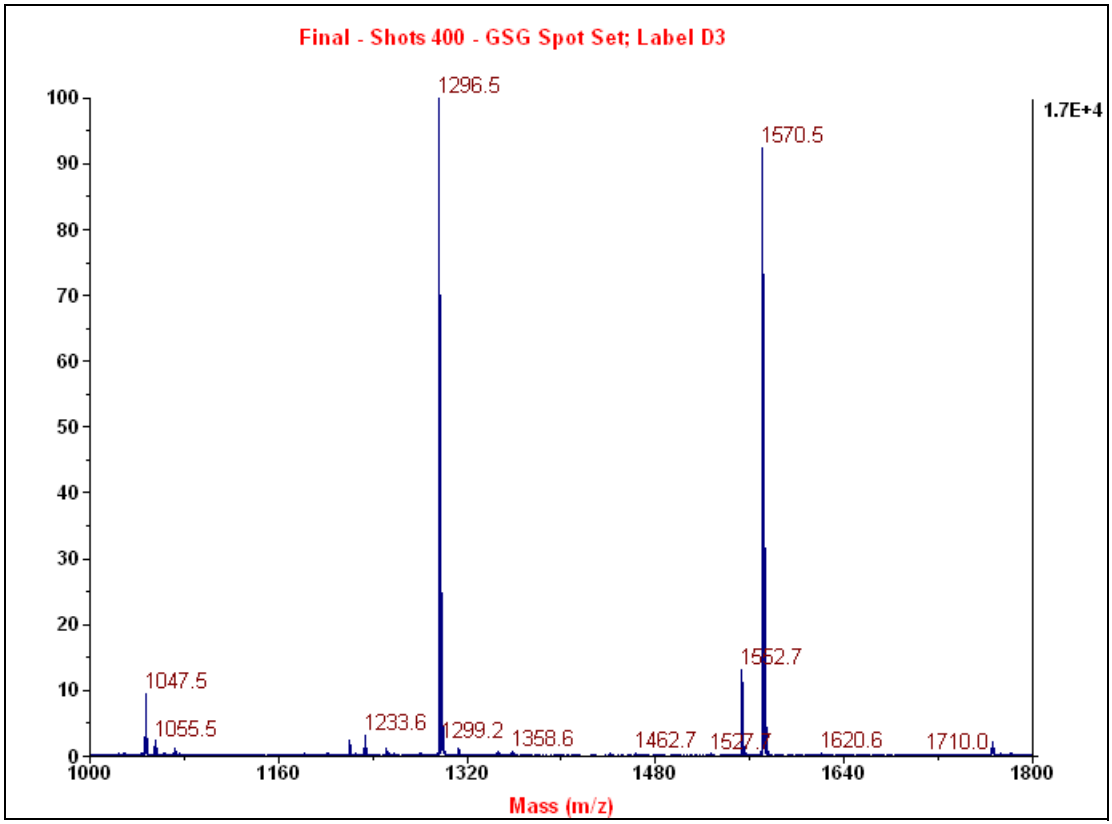


Figure 5-5 Magnified Spectrum

Eliminating Unwanted Peaks

To avoid detecting lower-intensity (noise) peaks:

1. Select the **Raw Spectrum Filtering / Peak Detection** tab in the Processing Method Editor (Figure 5-2 on page 5-4).
2. In the Peak Detection section, increase the S/N Threshold to an appropriate filtering value.
3. Click  in the toolbar, or select **Interactive > Run Active Processing Method** to rerun the processing method with the new settings.

   Peaks with a signal-to-noise ratio below the specified value are no longer detected or labeled (Figure 5-6).

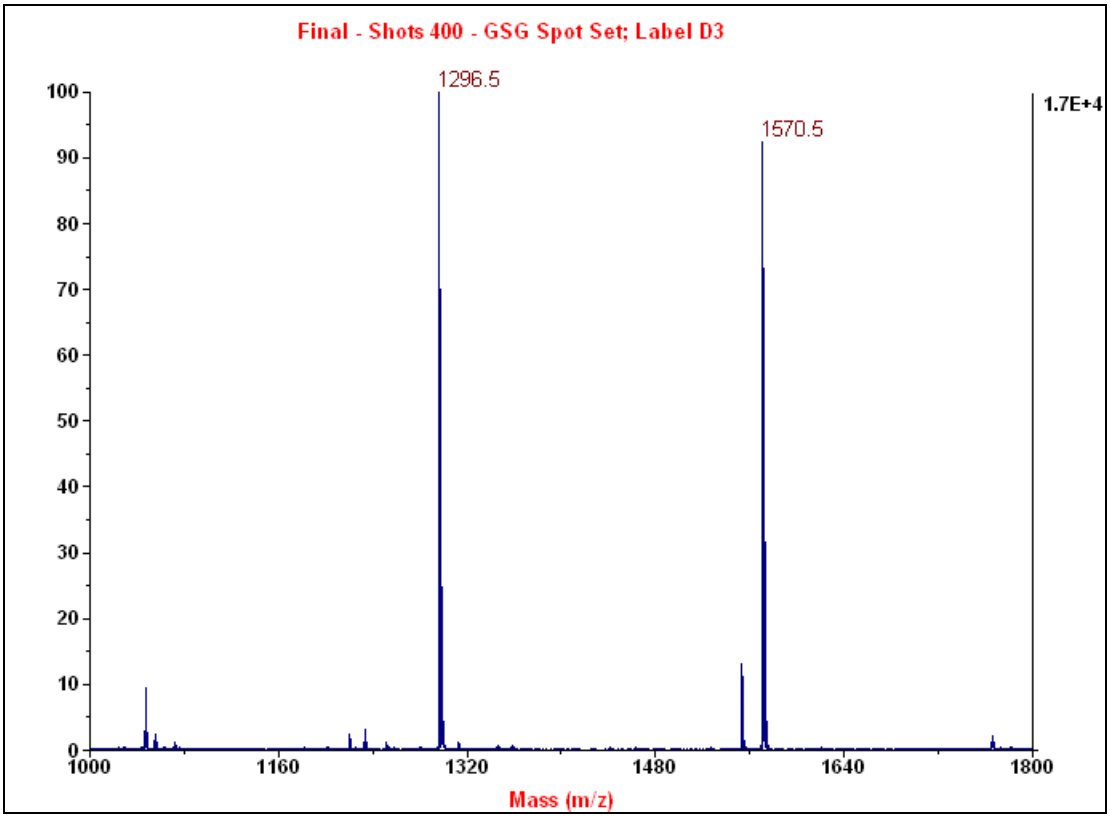


Figure 5-6 Spectrum with Noise Peaks Eliminated

Examining the Spectrum

When the noise peaks are eliminated, you can more easily evaluate the remaining masses. Examine the spectrum to check that:

- Peaks of interest are present, and masses are within the expected range for the standard (Table 5-1 on page 5-13).
- Peaks are narrow and well resolved (Table 5-1 on page 5-13).
- The signal is not saturated (not greater than 9.0E+4).

Figure 5-7 shows an acceptable signal in the Spectrum Viewer.

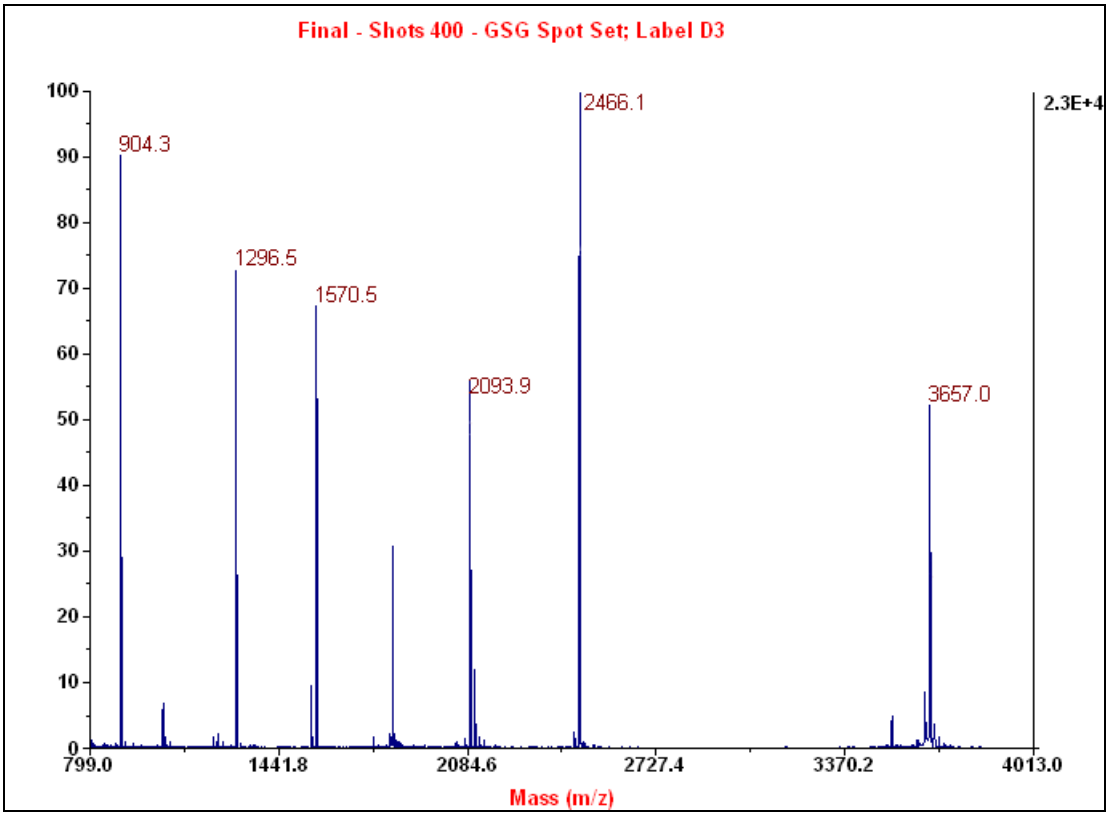


Figure 5-7 Acceptable Signal in the Spectrum Viewer

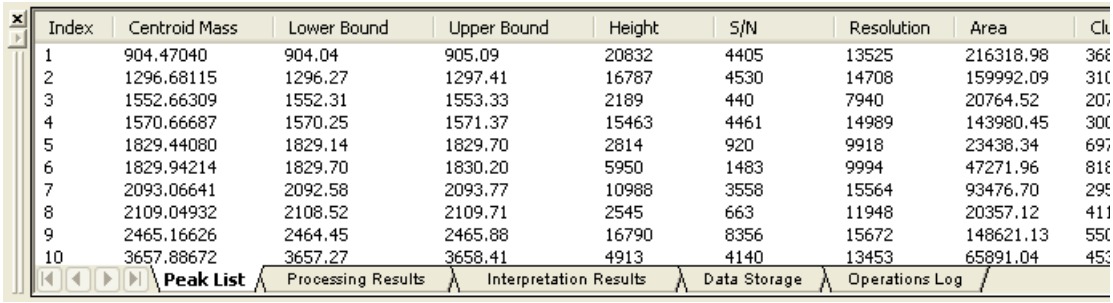
Viewing the Peak List

When you run a processing method, the system creates a peak list containing the peaks detected in the spectrum. The peak list displays information about each peak, including:

- Mass (centroid, lower and upper bounds)
- Height
- Signal-to-noise ratio
- Resolution
- Area
- Cluster area
- Reference Mass (for internally calibrated spectra)

To view the peak list:

1. If the Output Window is not displayed, select **View > Output Window**. The Output Window opens.
2. Select the **Peak List** tab. The peak list for the active spectrum opens (Figure 5-8).



Index	Centroid Mass	Lower Bound	Upper Bound	Height	S/N	Resolution	Area	Clu
1	904.47040	904.04	905.09	20832	4405	13525	216318.98	368
2	1296.68115	1296.27	1297.41	16787	4530	14708	159992.09	310
3	1552.66309	1552.31	1553.33	2189	440	7940	20764.52	207
4	1570.66687	1570.25	1571.37	15463	4461	14989	143980.45	300
5	1829.44080	1829.14	1829.70	2814	920	9918	23438.34	697
6	1829.94214	1829.70	1830.20	5950	1483	9994	47271.96	818
7	2093.06641	2092.58	2093.77	10988	3558	15564	93476.70	295
8	2109.04932	2108.52	2109.71	2545	663	11948	20357.12	411
9	2465.16626	2464.45	2465.88	16790	8356	15672	148621.13	550
10	3657.88672	3657.27	3658.41	4913	4140	13453	65891.04	453

Peak List | Processing Results | Interpretation Results | Data Storage | Operations Log

Figure 5-8 Peak List Displayed in the Output Window

Note: You can sort the peak list by clicking any of the column headings.

Checking Resolution and Signal-To-Noise

Overview

When you run a processing method, mass resolution and signal-to-noise ratios are automatically calculated for all detected peaks and displayed in the Peak List tab of the Output Window.

If enabled in the Spectrum Peak Label dialog box, mass resolution and signal-to-noise values are also displayed in the Spectrum Viewer Final trace next to the mass value for the peak. The peaks are labeled with (R*xxxx*, S*xxx*), where R*xxxx* is the resolution and S*xxx* is the signal-to-noise ratio (Figure 5-9).

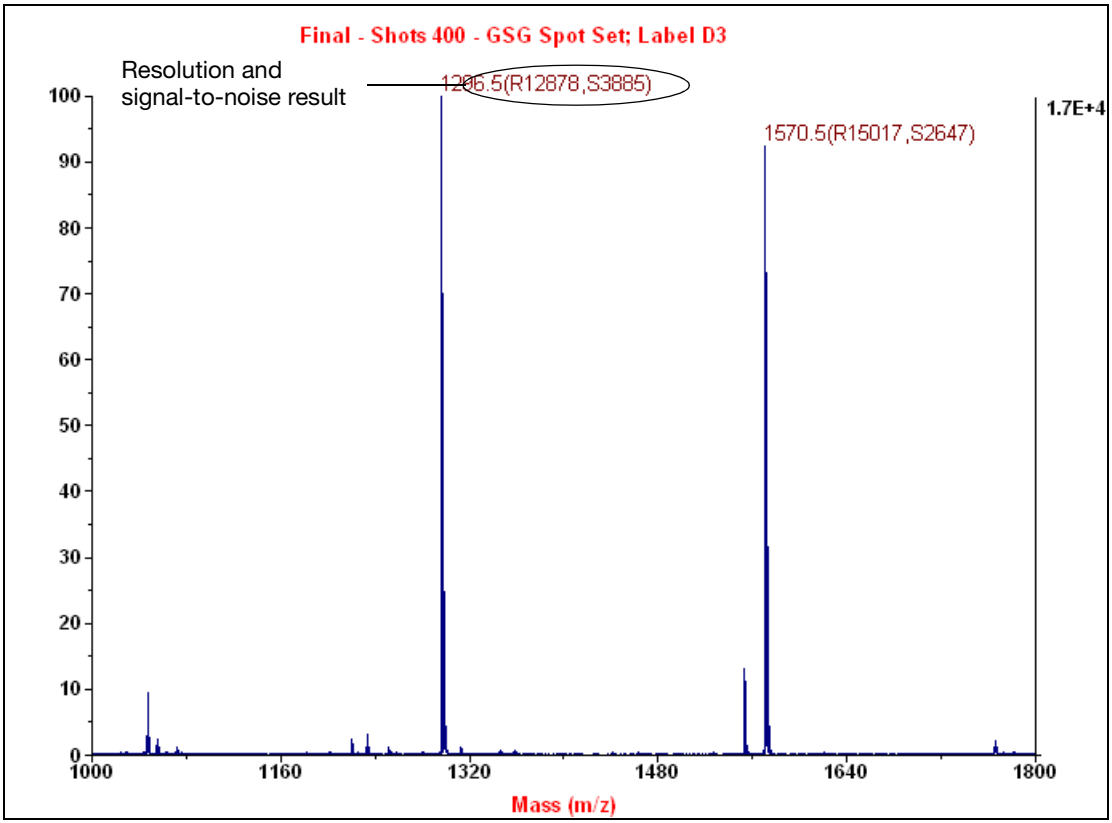


Figure 5-9 Resolution and Signal-to-Noise Values in the Spectrum Viewer Final Trace

If Resolution and Signal-to-Noise are Not Displayed

If mass resolution and signal-to-noise values are not displayed in the Final trace, these parameters may not be selected in the Spectrum Peak Label dialog box.

To enable resolution and signal-to-noise labeling:

1. Select **Spectrum > Peak Label**. The Spectrum Peak Label dialog box opens.
2. In the Other Label Attributes section, select **Resolution** and **Signal to Noise**.
3. Click **OK**.

Determining If Resolution Is Acceptable

Compare the resolution values you obtain to the values in Table 5-1.

Table 5-1 Acceptable Resolution Values for Reference Masses

Compound	Mass (Da)	Acceptable Resolution
des-Arg1-Bradykinin	904.5	>12,000
Angiotensin	1,296.7	>12,000
Glu-1-Fibrinopeptide B	1,570.7	>12,000
ACTH (1-17)	2,093.1	>15,000
ACTH (18-39)	2,465.2	>15,000
ACTH (7-38)	3,657.9	>12,000

Internally Calibrating Spectra

To internally calibrate spectra:

Setting Calibration Parameters

1. Select the **Calibration** tab of the Processing Method Editor. The Calibration tab opens (Figure 5-10).

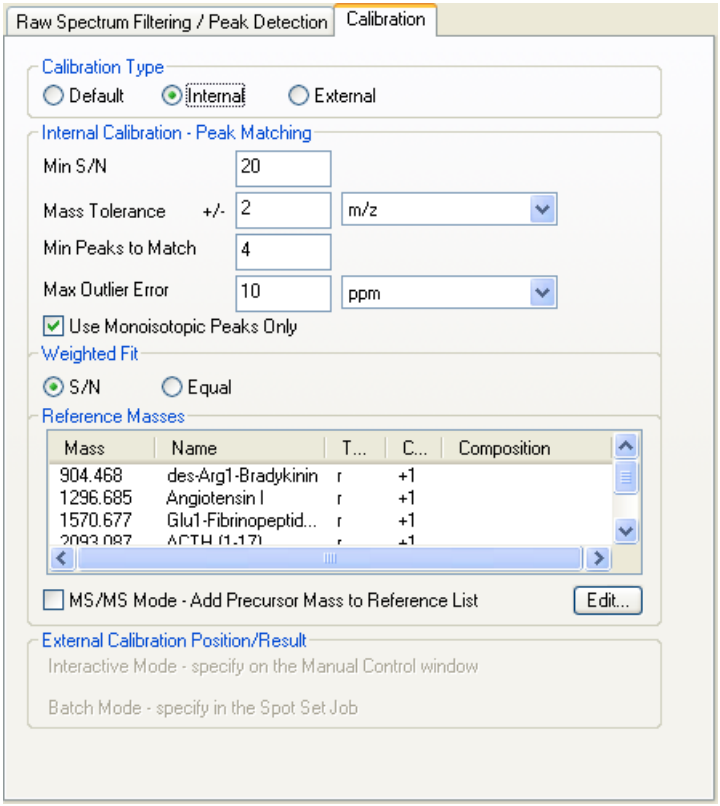


Figure 5-10 Processing Method Editor Calibration Tab

2. Select **Internal** for Calibration Type.

Selecting Reference Masses

3. Click **Edit** in the Reference Masses section. The Select Calibration Reference Masses dialog box opens (Figure 5-11).

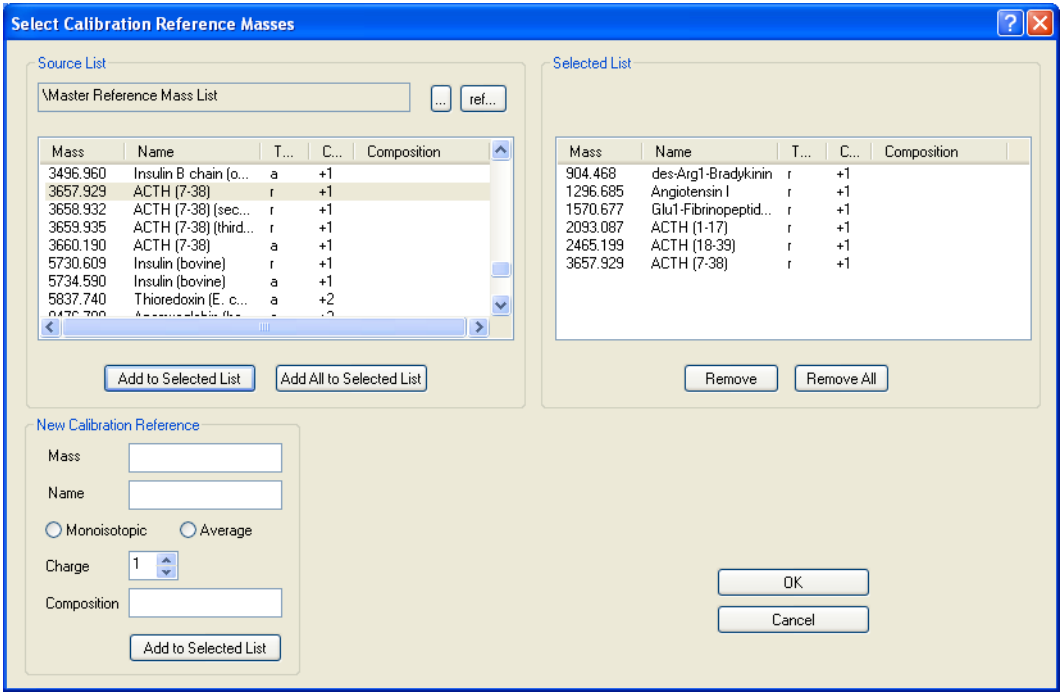


Figure 5-11 Select Calibration Reference Masses Dialog Box

4. Click ... in the Source List section. The Select Calibration Reference Mass List dialog box opens.
5. Select **All Objects** in the Project drop-down list.
6. Select the **Master Reference Mass List**, then click **Select**. The reference masses appear in the Source List on the left of the Select Calibration Reference Masses dialog box (Figure 5-11).

   Note: You can also click ref... in the Source List section to select a reference mass file outside the database. Refer to the *4000 Series Explorer™ Software Online Help* for information on creating reference mass files.

7. Select the following reference mass in the Source List:

   3,657.929 ACTH (7-38)

   Note: The default processing method automatically includes des-Arg1-Bradykinin, Angiotensin, Glu1-Fibrinopeptide B, ACTH (1-17), and ACTH (18-39) as reference masses in the Selected List.

8. Click **Add to Selected List**. The selected masses are added to the Selected List on the right of the dialog box (Figure 5-11).

9. Click **OK**. The selected reference masses appear in the Calibration tab of the Processing Method Editor (Figure 5-10 on page 5-14).

Saving the Method

10. Select **File > Save Processing Method As**.

11. Type **Internal Calibration Processing Method** for Item Name, then click **Save**.

Calibrating the Spectrum

12. Start the processing method by selecting **Interactive > Run Active Processing Method**, or clicking  .

    The spectrum is calibrated based on the reference masses that are identified. Figure 5-12 shows the spectrum after calibration.

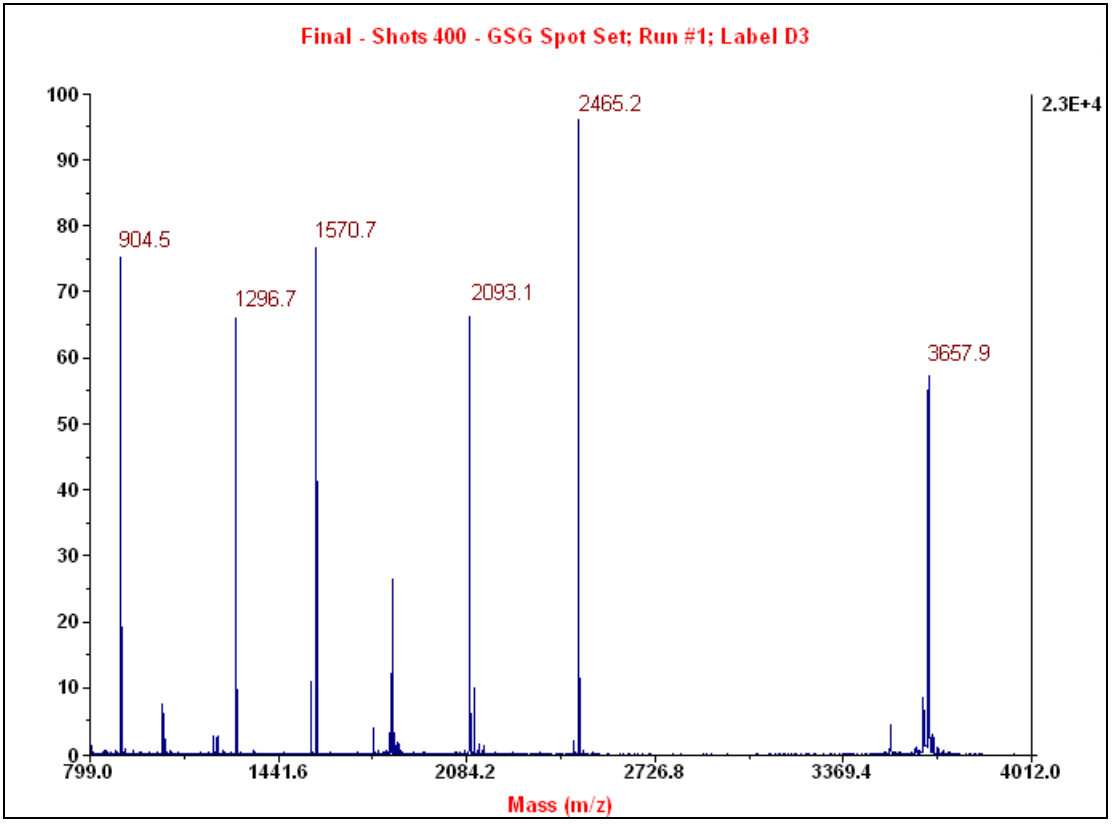


Figure 5-12 Spectrum After Calibration

Note: After calibration, the reference mass for each peak used in the internal calibration is listed in the Reference Mass column of the Peak table.

Saving the Spectrum

To save the calibrated spectrum, select **Interactive > Save Spot**.

The calibrated spectrum and peak list are saved into the spot set, along with copies of the acquisition and processing methods.

Note: If you did not save the acquisition or processing method used for the spectrum, the system prompts you to save the method.

Acquiring MS Spectra from Unknown Samples

6

This chapter contains the following sections:

Overview

After you acquire, internally calibrate, and save the spectrum from the calibration standard, you can use the standard as a reference spectrum to update the default calibration.

When you acquire and process the mock sample, the updated default calibration ensures optimum mass accuracy for the mock sample spectrum.

Use Calibration Mixture 4 as the mock unknown sample (see Figure 1-3 on page 1-5)

In This Chapter

In this chapter, you will:

- Update the default calibration.
- Create a default calibration processing method.
- Acquire and process the mock unknown sample.
- Optimize acquisition parameters for the unknown.
- Evaluate the data.
- Save the spectrum.

For More Information

Refer to the *4000 Series Explorer™ Software Online Help* for more information about:

- Updating the default calibration.
- Acquiring and processing spectra.
- Externally calibrating spectra.
- Using the Manual Control window.
- Optimizing acquisition and processing parameters.
- Viewing and analyzing data.

Refer to the *Data Explorer® Software Online Help* for more information about analyzing your data using the Data Explorer® Software.

Updating Default Calibration

Overview

After you acquire, internally calibrate, and save the reference spectrum, you can update the default calibration to improve the mass accuracy of future default calibrations. Updating the default calibration compares the observed masses in the internally calibrated spectrum to the reference masses, then uses the results to optimize the default calibration equations.

IMPORTANT! Update the default calibration regularly to ensure that you obtain optimum mass accuracy when you use the default calibration equations built into the 4000 Series Explorer software.

Updating the Default Calibration

To update the default calibration:

1. Select the **Reflector Acquisition Method** tab at the bottom of the method editor.
2. Select the acquisition method **Instrument** tab.
3. In the Operating Mode section, click **Open**. The operating mode associated with the method opens (Figure 6-1).

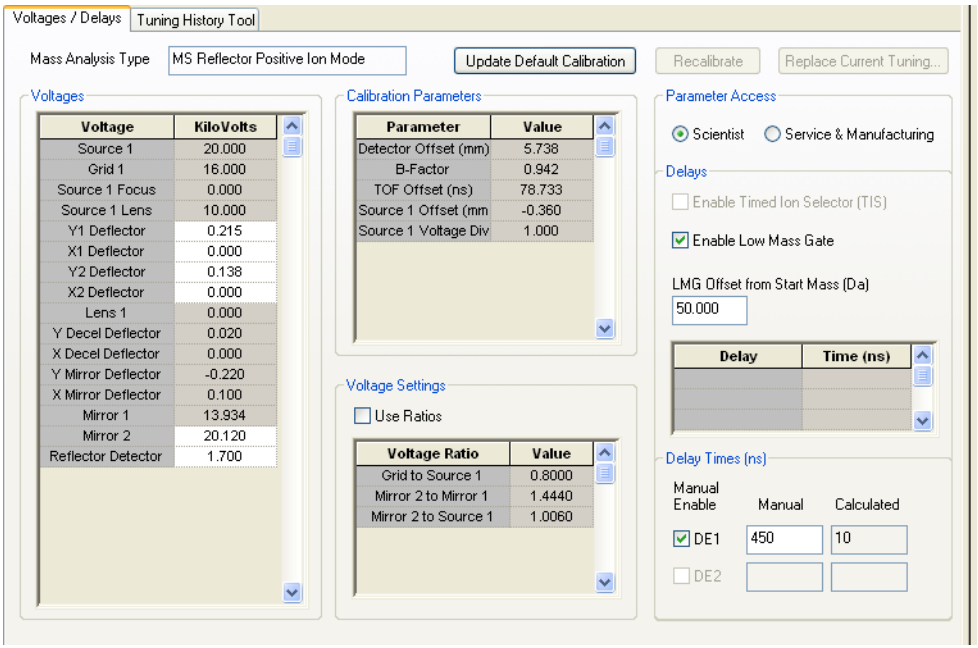


Figure 6-1 Operating Mode Voltages/Delays Tab

4. At the top of the Voltages/Delays tab, click **Update Default Calibration**.

   The system compares the observed masses in the spectrum to the reference masses, and uses the results to optimize the following operating mode parameters:

   - Detector Offset
   - B-Factor
   - TOF Offset

   Note: To update all three calibration parameters, the internal calibration must match at least four peaks in the spectrum.

5. Select **File > Save Operating Mode**. The operating mode is saved with the new settings.

6. Select **File > Close Operating Mode**. The operating mode is closed.

   IMPORTANT! Default calibration is updated only for the operating mode you are using. You must update the default calibration for each operating mode separately.

Modifying Settings to Acquire the Unknown

To modify instrument settings for MS acquisition of the mock unknown sample:

Selecting Default Calibration

1. In the Calibration tab of the Processing Method Editor, select **Default** for Calibration Type.
2. Select **File > Open > Processing Method**. The Open Processing Method dialog box opens.
3. Select the **Default Calibration Processing Method** you saved in Chapter 5, then click **Open**. The processing method opens.
4. Select the **Calibration** tab in the Processing Method Editor.
5. Ensure that the selected Calibration Type is **Default**.

Setting the Active Processing Method

To set the active processing method:

1. Select the **Default Calibration Processing Method** tab at the bottom of the Method Editor.
2. Select **File > Set as Active Processing Method**, or click  in the toolbar.

   The tab for the active method appears green in the Method Editor.

Selecting Sample Position

3. In the Manual Control Window, select one of the spots in columns 5 through 8 of the sample plate (the spots containing Calibration Mixture 4) by doing either of the following:
   - Select the spot label of the position from the Position drop-down list. For example: **D6**.
   - Click the sample position in the Plate view.

   For more information on selecting the sample position, refer to "Selecting the Sample Position" on page 4-8.

   Note: See Figure 1-3 on page 1-5 for a plate layout diagram.
4. Select **Unknown** in the Spot Type drop-down list.

Acquiring and Processing the Unknown Sample

Starting Acquisition

To start acquisition of the mock unknown sample, do any one of the following:

- Click  in the toolbar.
- Select **Interactive > Start Active Acquisition Method**.
- Press the **1** button on the control pad (Figure 4-7 on page 4-10).

Acquisition starts and continues until the Stop Conditions specified in the Acquisition Method Spectrum tab are met, or until you manually stop the acquisition. For more information, see "Stopping Acquisition" on page 4-12.

During Acquisition

During acquisition:

- The Live trace in the Spectrum Viewer is updated to display the spectrum that results from each subspectrum.
- The system averages all subspectra acquired after the start of acquisition.

Processing After Acquisition

When acquisition is complete, the software automatically runs the active processing method on the spectrum. During processing:

- Peaks are detected.
- The spectrum is externally calibrated based on the previously acquired spectrum from the calibration standard.

After processing, the software displays the data in the Final trace in the Spectrum Viewer (see Figure 6-2), and a peak list is displayed in the Peak List tab of the Output Window.

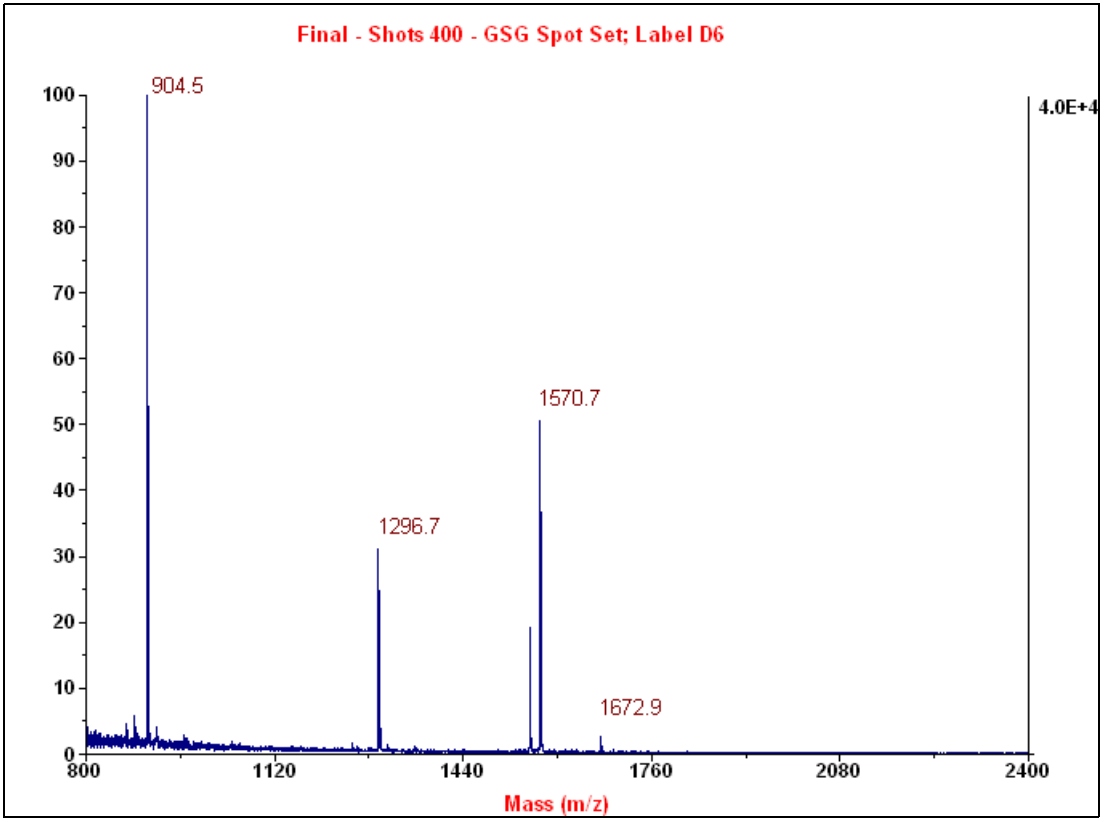


Figure 6-2 Sample Spectrum

Optimizing Acquisition Parameters

One of the sample peaks appears to be at about 1297 Da. Because you now know the mass of the peak, you can optimize the acquisition parameters and reacquire for best intensity and resolution of this sample peak.

To optimize acquisition parameters:

1. Select the **Reflector Acquisition Method** tab at the bottom of the Method Editor.
2. Select the **Instrument** tab. The Acquisition Method Editor Instrument tab opens (Figure 6-3).

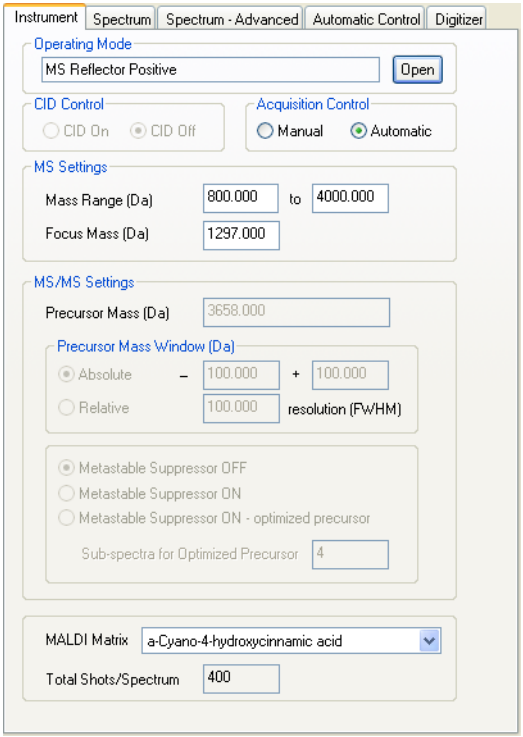


Figure 6-3 Instrument Tab of the Acquisition Method Editor

3. Set the Focus Mass to **1297** Da.
4. Select **File > Save Acquisition Method**.
5. Select **Interactive > Start Active Acquisition Method**.

   During acquisition, the system optimizes ion extraction delay time and other parameters for best resolution of the specified mass.

Evaluating Data

Evaluate the data as described in "Evaluating Data" on page 5-7.

You can also examine the data in the Data Explorer® software. See the *Data Explorer® Software Online Help* for more information.

Saving the Spectrum

To save the spectrum, select **Interactive > Save Spot**.

The calibrated spectrum and peak list are saved into the spot set, along with copies of the acquisition and processing methods.

Acquiring MS/MS Spectra from Unknown Samples

7

This chapter contains the following sections:

Overview

Acquiring data in MS/MS mode uses the 4800 MALDI TOF/TOF™ Analyzer collision-induced dissociation (CID) system and TOF/TOF™ optics to fragment and analyze individual peaks in the original MS spectrum.

In This Chapter

In this chapter, you will:

- Create MS/MS acquisition and processing methods.
- Set the active methods.
- Specify CID settings for the MS/MS acquisition.
- Acquire and process MS/MS data.
- Evaluate the data.
- Save the spectrum.

For More Information

Refer to the *4000 Series Explorer™ Software Online Help* for more information about:

- Creating MS/MS acquisition and processing methods.
- Configuring the CID system.
- CID system parameters.
- Acquiring and processing MS/MS spectra.

Refer to the *4800 MALDI TOF/TOF™ Analyzer Hardware Guide* for more information about the CID system hardware.

Creating MS/MS Methods

Overview

Before you can acquire MS/MS spectra, you must create methods optimized for MS/MS operation. Creating these methods requires:

- Creating MS/MS Acquisition Methods
- Creating MS/MS Processing Methods
- Setting Active Methods

Creating MS/MS Acquisition Methods

To create an MS/MS acquisition method:

Creating a New Acquisition Method

1. Select **File > New** > **Acquisition Method**. The Create New Acquisition Method from Default dialog box opens (Figure 4-1 on page 4-3).
2. Select **MS-MS 1KV Positive**.
3. Click **Create**. The Acquisition Method Editor Instrument tab opens (Figure 7-1).

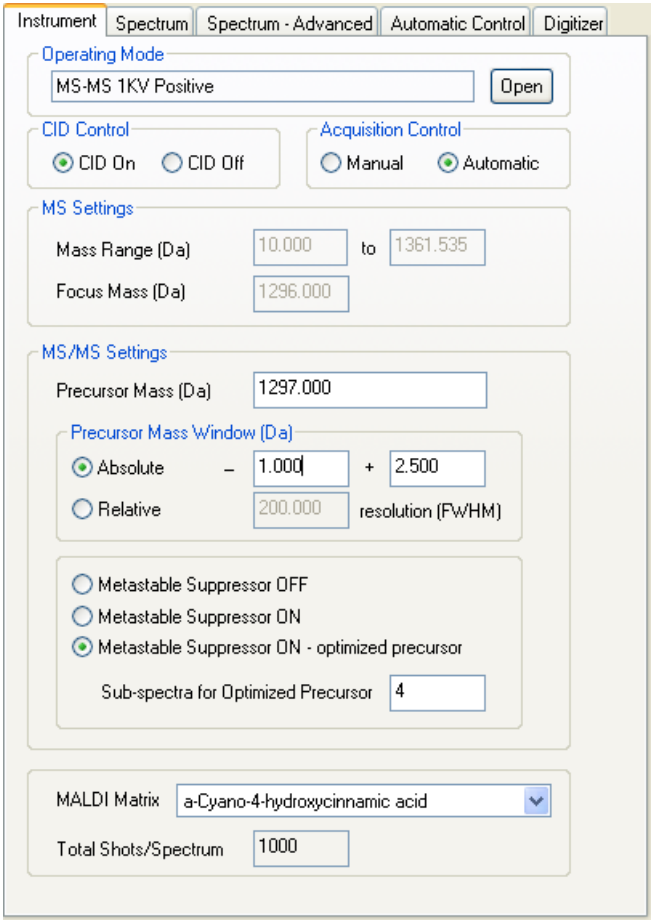


Figure 7-1 Instrument Tab for MS/MS Acquisition

Setting Acquisition Parameters

4. Set the Precursor Mass to **1297** Da, which is the mass of one of the mock unknown sample peaks you acquired in Chapter 6.
5. Select **Metastable Suppressor ON – optimized precursor**.

Saving the Acquisition Method

6. Select **File > Save Acquisition Method**.
7. Type **MS-MS Acquisition Method** for Item Name, then click **Save**.

Creating MS/MS Processing Methods

To create an MS/MS processing method:

Creating a New Processing Method

1. Select **File > New > Processing Method**. The Create New Processing Method from Default dialog box opens (Figure 5-1 on page 5-3).
2. Select **MS-MS Processing**.
3. Click **Create**. The Processing Method Editor Raw Spectrum Filtering / Peak Detection tab opens (Figure 7-2).

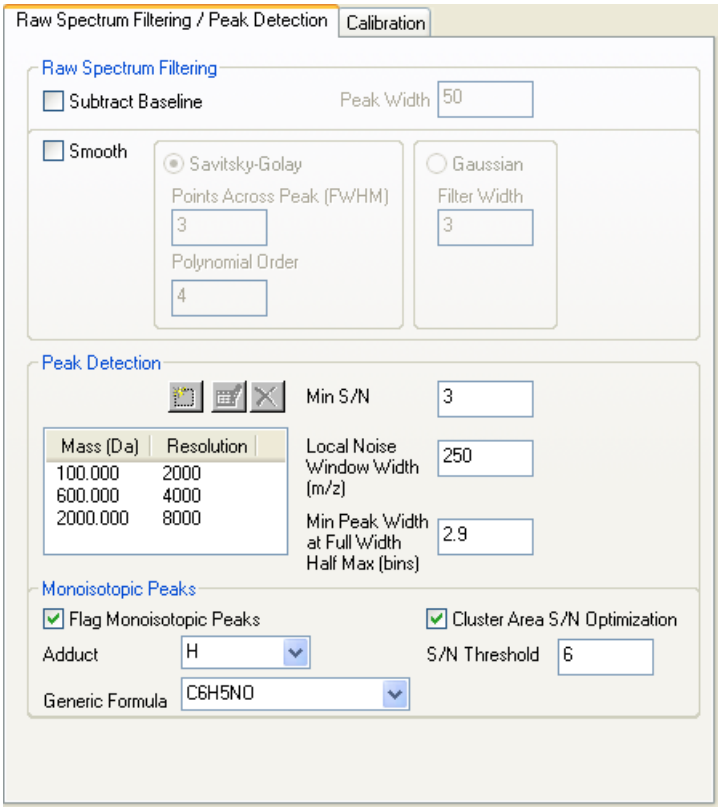


Figure 7-2 Raw Spectrum Filtering / Peak Detection Tab

Saving Processing Method

4. Select **File > Save Processing Method**.
5. Type **MS-MS Processing Method** for Item Name, then click **Save**.

Setting Active Methods

Overview

Before you acquire an MS/MS spectrum, you must specify the MS/MS acquisition and processing methods as the active methods.

Note: You can determine which methods are active by looking at the tabs at the bottom of the Method Editor. The tabs for active methods are shaded green.

To set the active acquisition and processing methods:

Setting Active Acquisition Method

1. Select the **MS-MS Acquisition Method** tab at the bottom of the Method Editor.
2. Select **File > Set as Active Acquisition Method**, or click  in the toolbar.

   The tab for the active acquisition method appears green.

Setting Active Processing Method

3. Select the **MS-MS Processing Method** tab at the bottom of the Method Editor.
4. Select **File > Set as Active Processing Method**, or click  in the toolbar.

   The tab for the active processing method appears green.

Specifying CID Settings for MS/MS Acquisition

MS/MS acquisitions use the 4800 MALDI TOF/TOF™ Analyzer collision-induced dissociation (CID) system.

The CID system automatically turns on when you run an MS/MS acquisition method. Before starting an MS/MS acquisition however, you can specify CID system settings.

Configuring the CID System

To specify CID settings for MS/MS acquisition:

1. Select the **GSG Spot Set** tab at the bottom of the Method Editor. The Spot Set window opens.
2. Select the **Job** tab (Figure 7-3).

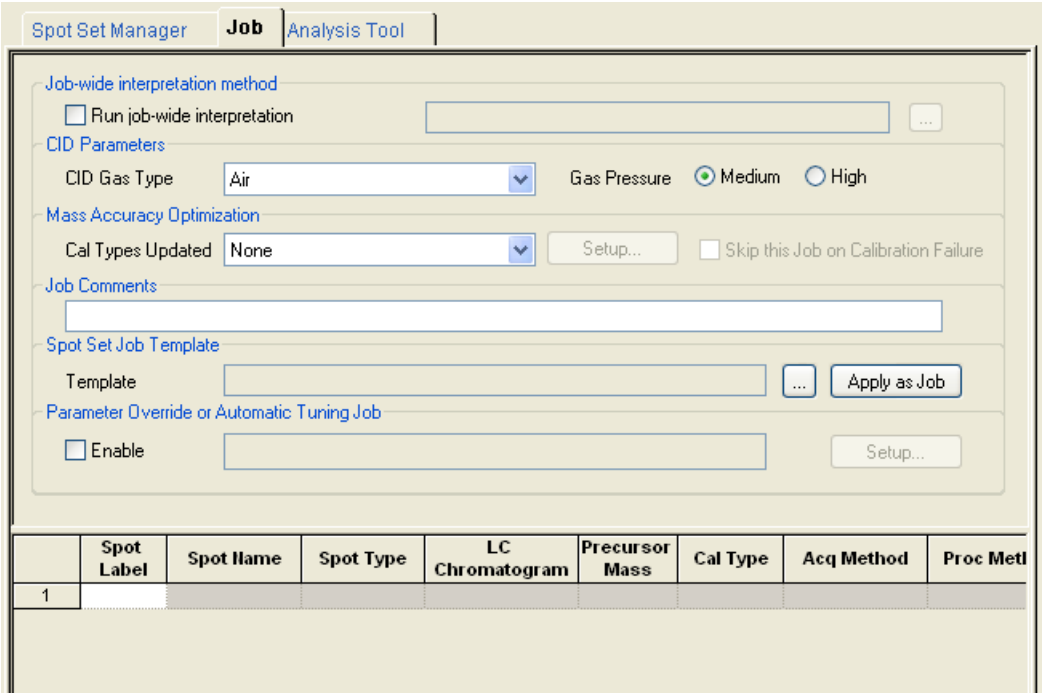


Figure 7-3 Job Tab

3. Select **Air** in the CID Gas Type drop-down list.
4. Select **Medium** Gas Pressure.
5. Select **None** in the Cal Types Updated drop-down list.

Acquiring and Processing MS/MS Data

To acquire and process MS/MS data:

Selecting Sample Position

1. Verify that the plate position of the *mock unknown sample* is selected in the Manual Control window. Refer to "Selecting Sample Position" on page 6-5.

Starting Acquisition

2. Start acquisition by doing any one of the following:
   - Click  in the toolbar.
   - Select **Interactive > Start Active Acquisition Method**.
   - Press the **1** button on the control pad (Figure 4-7 on page 4-10).

   A message box alerts you that the current CID status does not match the CID status specified in the acquisition method.

3. Click **Yes** to continue.

   Note: The CID system may require up to 5 minutes to turn on.

   The CID system turns on and begins to pressurize. When the CID system reaches the appropriate pressure, acquisition starts and continues until the Stop Conditions are met, or until you manually stop the acquisition. For more information, see "Stopping Acquisition" on page 4-12.

During Acquisition

During acquisition:

- The Live trace in the Spectrum Viewer is updated to display the spectrum that results from each subspectrum.
- The system averages all subspectra acquired after the start of acquisition.

Processing After Acquisition

After acquisition, the software automatically runs the active processing method on the spectrum. During processing:

- Peaks are detected.
- The spectrum is calibrated based on the default calibration equations.

After processing, the software displays the data in the Final trace in the Spectrum Viewer (see Figure 7-4), and a peak list is displayed in the Peak List tab of the Output Window.

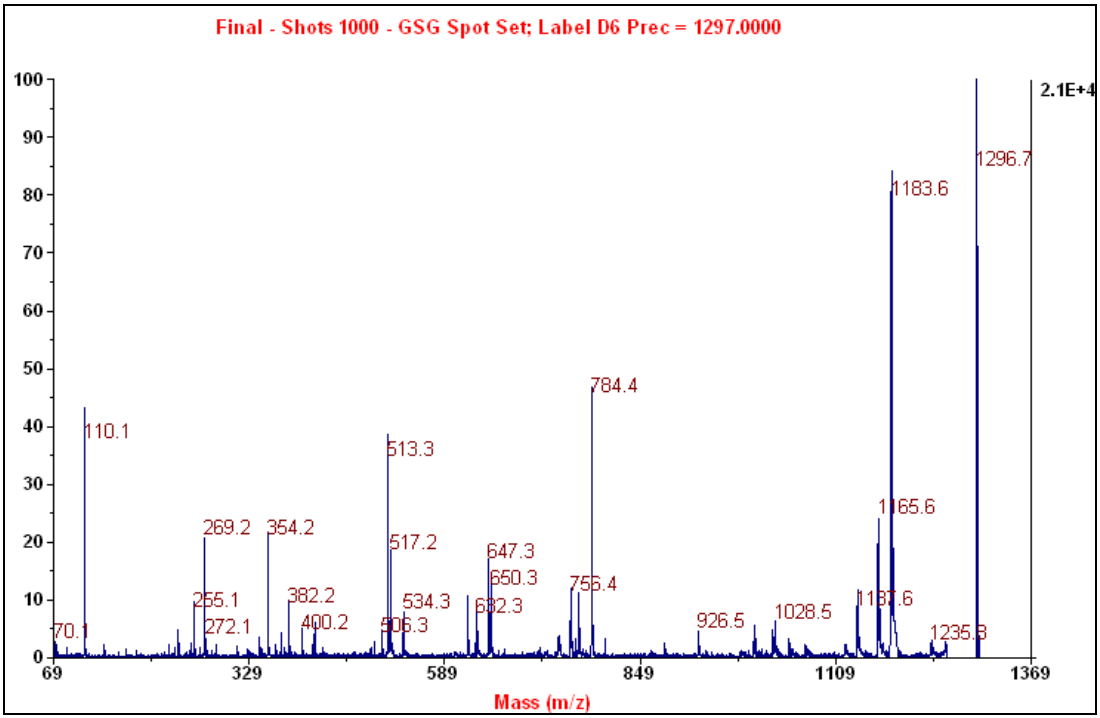


Figure 7-4 Sample MS/MS Spectrum

Evaluating Data

Evaluate the data as described in "Examining the Spectrum" on page 5-10.

Saving the Spectrum

To save the spectrum, select **Interactive > Save Spot**.

The calibrated spectrum and peak list are saved into the spot set, along with copies of the acquisition and processing methods.

Performing Interpretation on MS Spectra

8

This chapter contains the following sections:

Overview

In This Chapter

In this chapter, you will:

- Create a new interpretation method.
- Set the active interpretation method.
- View a spectrum to interpret.
- Run the interpretation method.
- View the interpretation results.

For More Information

Refer to the *4000 Series Explorer™ Software Online Help* for more information about:

- Creating interpretation methods.
- Interpretation method parameters.
- Performing interpretation on spectra.
- Performing interpretation on LC/MALDI data (job-wide interpretation).
- Viewing and analyzing data.

Creating an Interpretation Method

After you acquire and process data, you can perform interpretation on an MS spectrum to automatically identify peaks for MS/MS acquisition. An *interpretation method* specifies the parameters needed to identify peaks of interest from an MS spectrum and to acquire and process MS/MS data on those peaks.

To create an interpretation method:

Creating a New Interpretation Method

1. Select **File > New** > **Interpretation Method**. The Create New Interpretation Method from Default dialog box opens (Figure 8-1).

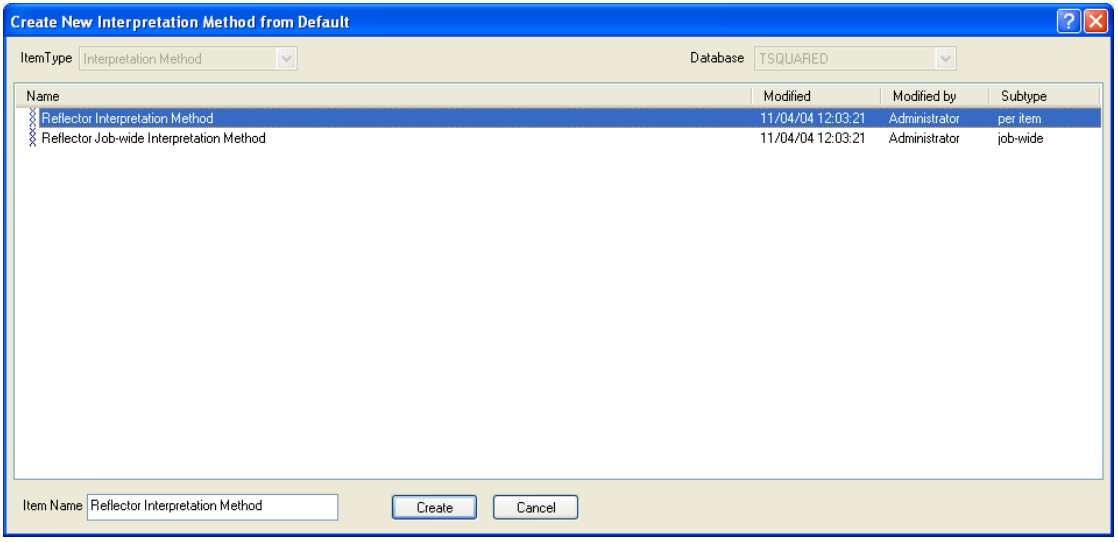


Figure 8-1 Create New Interpretation Method Dialog Box

2. Select **Reflector Interpretation Method**.
3. Click **Create**. The Interpretation Method Editor opens (Figure 8-2).

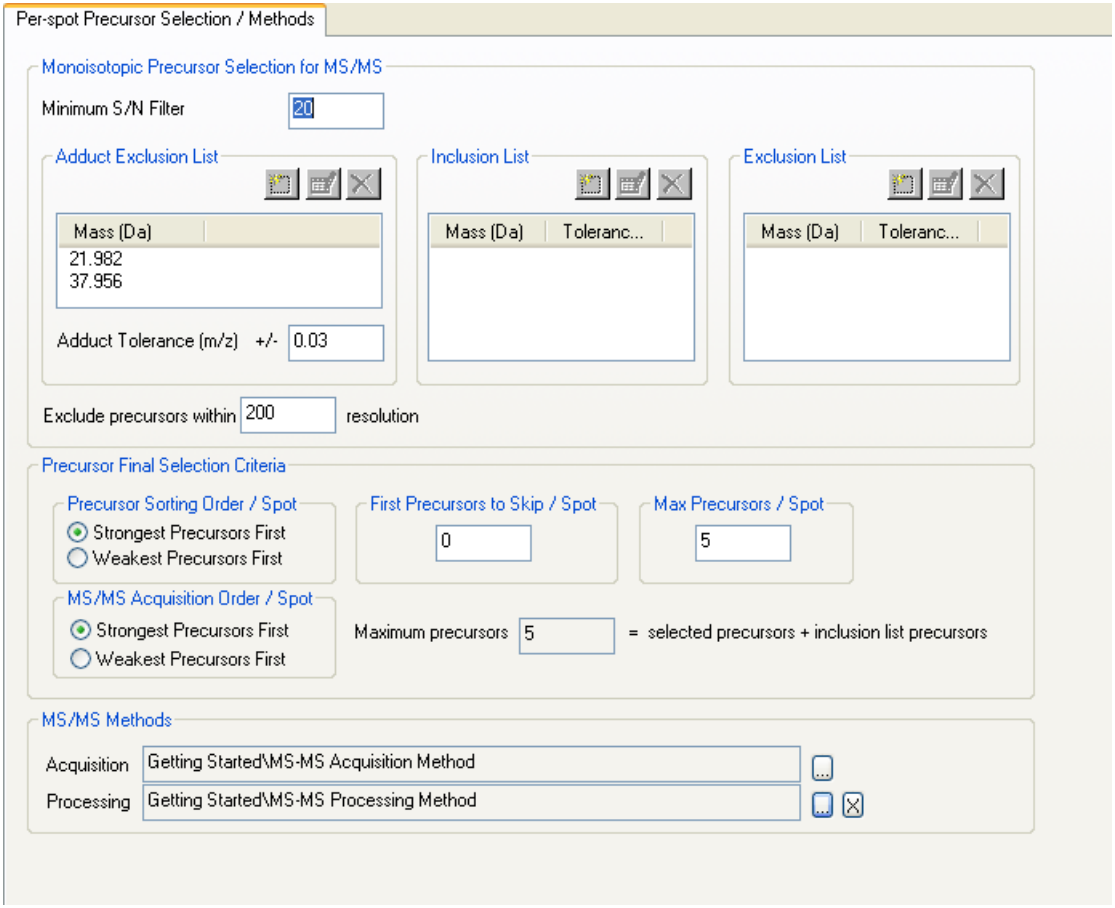


Figure 8-2 Interpretation Method Editor

Interpretation Parameters

The default interpretation method specifies settings that identify the five strongest peaks in the MS spectra. However, you must specify the acquisition and processing methods used to acquire the MS/MS spectra.

Specifying MS/MS Acquisition and Processing Methods

4. In the MS/MS Methods section of the Interpretation Method Editor, Click ... to the right of Acquisition. The Open Acquisition Method dialog box opens.
5. Select **MS-MS Acquisition Method**, then click **Open**.

   The name of the selected MS/MS acquisition method is displayed.

6. In the MS/MS Methods section of the Interpretation Method Editor, Click … to the right of Processing. The Open Processing Method dialog box opens.
7. Select **MS-MS Processing Method**, then click **Open**.

   The name of the selected MS/MS processing method is displayed.

Saving the Interpretation Method

8. Select **File > Save Interpretation Method**.
9. Type **GSG Interpretation Method** for Item Name, then click **Save**.

Setting the Active Interpretation Method

Overview

Although you can open and edit multiple interpretation methods within the 4000 Series Explorer™ software, only one of the open interpretation methods is the *active method*, the method used to interpret the MS spectrum.

Before performing interpretation, you must specify the interpretation method you want to use to identify peaks in the MS spectrum.

Note: You can determine which method is active by looking at the tabs at the bottom of the Method Editor. The tab for the active method is shaded green.

Setting Active Interpretation Method

To set the active interpretation method:

1. Select the **GSG Interpretation Method** tab at the bottom of the Method Editor.
2. Select **File > Set as Active Interpretation Method**, or click  in the toolbar.

   The tab for the active method appears green in the Method Editor.

Performing Interpretation on an MS Spectrum

Performing interpretation requires:

- Viewing a Spectrum you want to interpret.
- Running the Interpretation Method.
- Viewing the Interpretation Results.

Viewing a Spectrum

To view the spectrum of the calibration standard that you saved in Chapter 6:

1. Select the **GSG Spot Set** tab at the bottom of the Spot Set window. The Spot Set Manager opens.
2. Select the **Spot Set Manager** tab.
3. In the Spot Set Manager, select the row corresponding to the position of the calibration standard.
4. Right click the row, then select **View Spectrum**. The spectrum for the calibration standard appears in the Spectrum Viewer.

Running the Interpretation Method

To start the interpretation method:

- Click  in the toolbar.

  or
- Select **Interactive > Run Active Interpretation Method**.

  Note: Interpreting the MS spectrum with the settings in the default interpretation method identifies the five strongest peaks in the spectrum.

Viewing the Interpretation Results

To view the interpretation results, select the **Interpretation Results** tab in the Output Window.

The interpretation results for the spectrum appear in the Output Window (Figure 8-3).

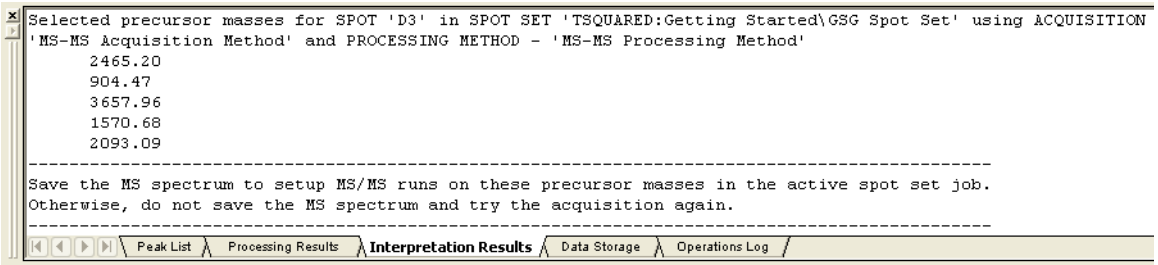


Figure 8-3 Output Window Interpretation Results Tab

Automatically Acquiring MS/MS Data for Selected Peaks

If you save the spot after running an interpretation method in interactive mode, the system automatically creates a spot set job that you can run to acquire MS/MS data for the selected peaks. However, the job is not run until you manually submit it and start the job queue.

For more information on creating and running spot set jobs, refer to Chapter 9, “Acquiring Multiple Spectra in Batch Mode.”

Acquiring Multiple Spectra in Batch Mode

9

This chapter contains the following sections:

Overview

The 4000 Series Explorer™ software batch mode allows you to:

- Acquire data from multiple samples using different acquisition methods.
- Process data from multiple samples using different processing methods.
- Automatically acquire MS/MS spectra from multiple peaks in each MS spectrum using interpretation methods.

In This Chapter

In this chapter, you will:

- Switch to batch mode.
- Create a spot set job.
- Run a spot set job.
- Use the Job Queue Viewer to check the status of a spot set job.
- Use the Spot Set Manager to display spot set information.
- View spectra and peak lists from completed spot set jobs.

For More Information

Refer to the *4000 Series Explorer™ Software Online Help* for more information about:

- Using batch mode.
- Using the Spot Set Manager.
- Using spot set jobs.
- Using spot set job templates.
- Using the job queue.
- Viewing and analyzing data.

Switching Between Interactive and Batch Modes

To switch between interactive mode and batch mode:

- Click  in the toolbar.

  or

- Select **View > Switch to Batch Mode**.

The 4000 Series Explorer™ software switches to batch mode (Figure 9-1).

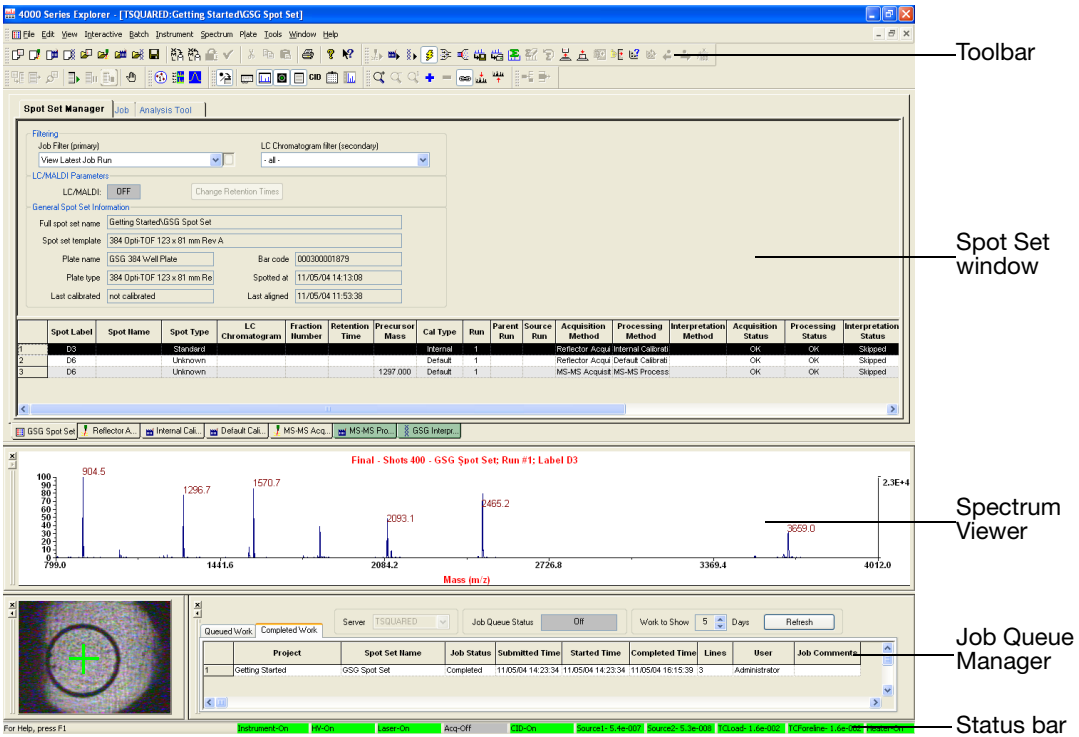


Figure 9-1 4000 Series Explorer™ Software Batch Mode

Acquiring MS and MS/MS Spectra in Batch Mode

Overview

Automatically acquiring MS and MS/MS spectra in batch mode requires:

- Creating a Spot Set Job
- Running a Spot Set Job

Creating a Spot Set Job

Overview

A *spot set job* is a list of samples (spots) to run (acquire, process, or interpret). For each row in a spot set job, you can specify different acquisition, processing, and interpretation methods to run. You can also specify Spot Type, Precursor Mass (for MS/MS acquisition), External Calibrant Position, and other parameters for each row.

To create a spot set job:

Selecting Spots to Acquire

1. In the Spot Set Manager, select the two rows corresponding to the sample plate positions of the calibration standard and mock unknown sample.

   Note: The rows for your samples contain information in the Spot Type, Cal Type, Run, Method, and Status columns.

2. Right-click either of the selected rows, then select **Copy Spots to Job > Using Latest Methods** (Figure 9-2).

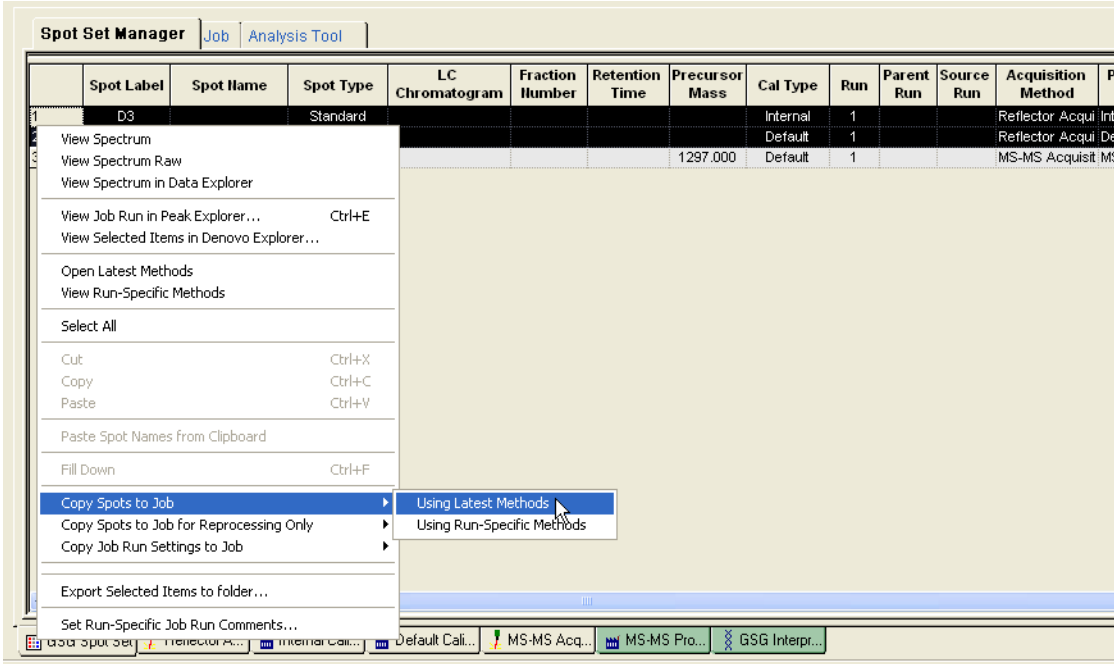


Figure 9-2 Copying Spots to a Spot Set Job

The selected spots are copied to the spot set job (Figure 9-3).

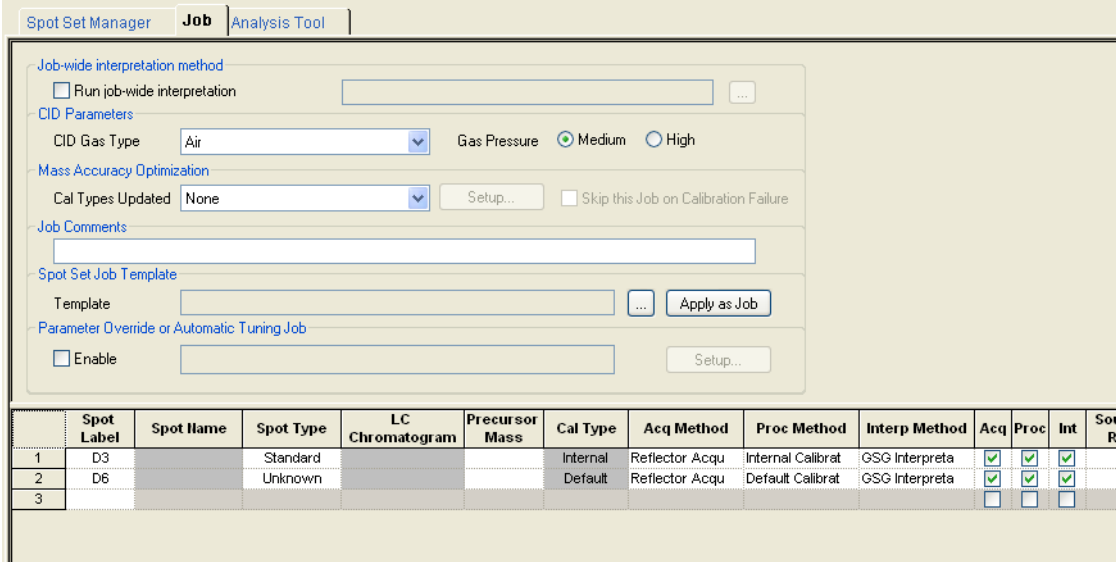


Figure 9-3 Spot Set Job

Selecting the Interpretation Method

3. In the first row of the spot set job, select the **Interp Method** column. The Open Interpretation Method dialog box opens.
4. Select the **GSG Interpretation Method** you created in Chapter 8, then click **Open**.

   "GSG Interpretation Method" appears in the Interp Method column.
5. Right-click the Interp Method column, then select **Fill Down**.

   "GSG Interpretation Method" appears in each row in the spot set job.
6. Ensure that the Acq, Proc, and Int check boxes are selected for each row in the spot set.

Setting Other Parameters

7. In the row of the spot set that contains the calibration standard, type **Calibration Standard** in the Comments column.
8. In the row of the spot set that contains the mock unknown sample, type **Mock Sample** in the Comments column.
9. In the Mass Accuracy Optimization section of the Job tab, select **None** from the Cal Types Updated drop-down list.

Running a Spot Set Job

Overview

After you create a spot set job, you need to run the job to acquire and process the samples in the job. Running a spot set job requires:

- Submitting a Spot Set Job to the Job Queue
- Starting the Job Queue

Submitting a Spot Set Job to the Job Queue

Before you run a spot set job, you must submit it to the job queue.

Note: You can submit multiple spot set jobs to the job queue.

To submit a spot set job to the job queue:

- Select **Batch > Submit Spot Set Job**.

  or

- Click  in the toolbar.

The software validates the spot set job. After validation, the job appears in the Job Queue Manager (Figure 9-4), and the Spot Set Manager displays Acquisition, Processing, and Interpretation Status as "Submitted."

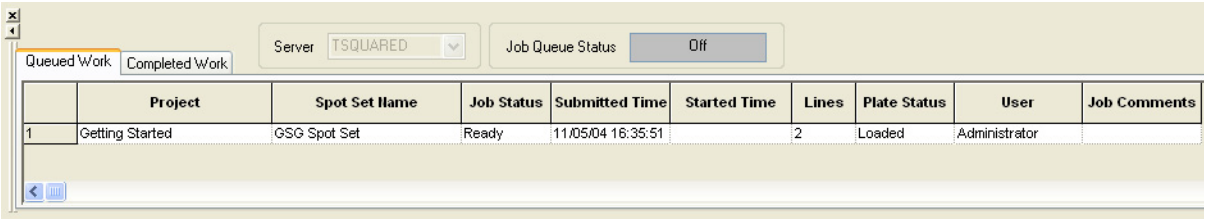


Figure 9-4 Job Queue Manager

Starting the Job Queue

When you start the job queue, all spot set jobs in the job queue are run.

To start the job queue:

- Select **Batch > Start Job Queue**.

  or

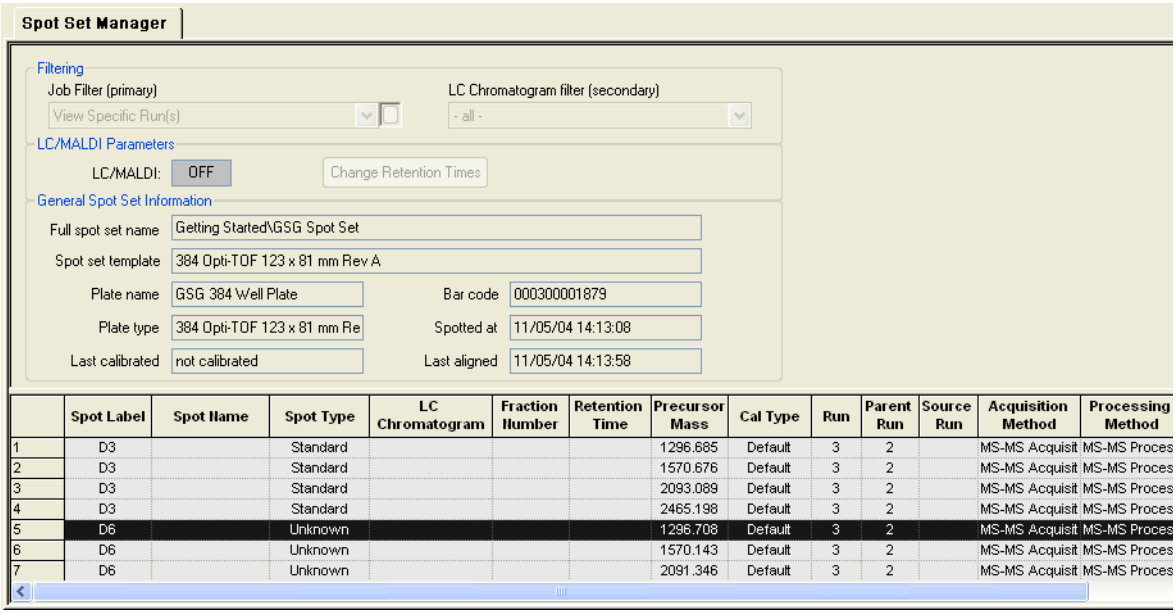
- Click  in the toolbar.

The Job Queue Status displays "On" (Figure 9-4), and the samples are acquired and processed.

Interpretation After Acquisition and Processing

After each row in the spot set job is acquired and processed, the system interprets each spectrum based on the parameters specified in the interpretation method. Then, the system automatically generates a spot set job to acquire MS/MS spectra for peaks that meet the specified criteria.

The automatically generated MS/MS spot set job appears in the Spot Set Manager (Figure 9-5), and is submitted to the job queue and run.



	Spot Label	Spot Name	Spot Type	LC Chromatogram	Fraction Number	Retention Time	Precursor Mass	Cal Type	Run	Parent Run	Source Run	Acquisition Method	Processing Method
1	D3		Standard				1296.685	Default	3	2		MS-MS Acquisit	MS-MS Proces
2	D3		Standard				1570.676	Default	3	2		MS-MS Acquisit	MS-MS Proces
3	D3		Standard				2093.089	Default	3	2		MS-MS Acquisit	MS-MS Proces
4	D3		Standard				2465.198	Default	3	2		MS-MS Acquisit	MS-MS Proces
5	D6		Unknown				1296.708	Default	3	2		MS-MS Acquisit	MS-MS Proces
6	D6		Unknown				1570.143	Default	3	2		MS-MS Acquisit	MS-MS Proces
7	D6		Unknown				2091.346	Default	3	2		MS-MS Acquisit	MS-MS Proces

Figure 9-5 Automatically Generated Spot Set Job

Pausing and Continuing the Job Queue

Pausing the job queue allows you to temporarily stop a spot set job run. You can then resume the job run at a later time.

To pause the job queue, select **Batch > Pause Job Queue**.

The spot set job stops running after the current row is acquired, processed, and interpreted.

To resume the spot set job, select **Batch > Continue Job Queue**.

Stopping the Job Queue

Stopping the job queue immediately stops the job run and removes the currently running spot set job from the queue.

To stop the job queue, select **Batch > Stop Job Queue**.

The spot set job stops running as soon as the current processing function can safely stop.

CAUTION If you stop the job queue while a spot set job is running, you cannot resume the stopped job. When you stop the job queue, the currently running job is removed from the job queue and appears in the Completed Work tab of the Job Queue Viewer (see "Checking Spot Set Job Status" on page 9-10). Any rows that did not finish running are marked "Stopped." To run a stopped job again, you must copy the information to a new spot set job.

Viewing and Evaluating Data

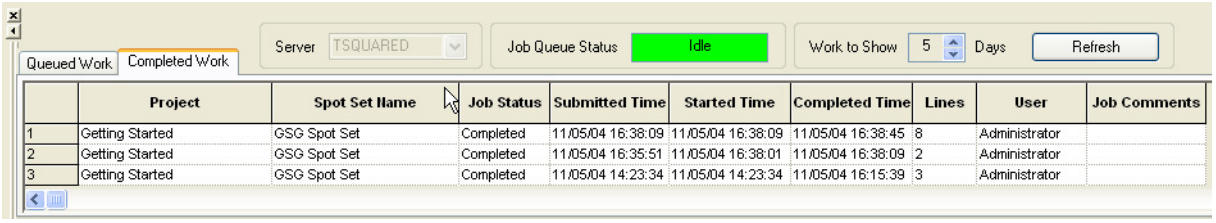
Overview After you acquire data in batch mode, you can check the status of spot set job runs and evaluate data by:

- Checking Spot Set Job Status
- Filtering the Spot Set View
- Viewing Spectra and Peak Lists

Checking Spot Set Job Status

To check the status of completed spot set jobs:

1. Select the **Completed Work** tab in the Job Queue Manager to display the status of all the jobs you have run. (Figure 9-6).



	Project	Spot Set Name	Job Status	Submitted Time	Started Time	Completed Time	Lines	User	Job Comments
1	Getting Started	GSG Spot Set	Completed	11/05/04 16:38:09	11/05/04 16:38:09	11/05/04 16:38:45	8	Administrator	
2	Getting Started	GSG Spot Set	Completed	11/05/04 16:35:51	11/05/04 16:38:01	11/05/04 16:38:09	2	Administrator	
3	Getting Started	GSG Spot Set	Completed	11/05/04 14:23:34	11/05/04 14:23:34	11/05/04 16:15:39	3	Administrator	

Figure 9-6 Job Queue Manager Completed Work Tab

The first row displays the spot set job that was automatically generated from the interpretation method. The second row displays the spot set job you created in batch mode. Row 3 displays the three acquisitions you performed in interactive mode (Chapter 5, Chapter 6, and Chapter 7).

Note: Acquisitions performed in interactive mode are considered part of the same spot set job, and have the same run number, until you do one of the following:

- Close the software
- Switch to batch mode
- Load a new plate
- Start a new interactive session

For more information on interactive sessions, see the *4000 Series Explorer™ Software Online Help.*

2. Ensure that the Job Status column of each row displays "Completed."

Filtering the Spot Set View

Overview

You can specify which interactive mode acquisitions and spot set jobs are displayed in the Spot Set Manager using the Job Filter feature. Use this feature to check the status of the acquisition, processing, and interpretation performed on each spot.

There are five Job Filter options:

- **View All Runs** – Displays the status of all runs for each spot label in the spot set. If a spot label has not been acquired, the corresponding row appears empty.

  Note: View All Runs is the default display in the Spot Set Manager for a new spot set.

- **View Latest Run Per Spot** – Displays the status of the last run for each spot label in the spot set. If a spot label has not been acquired, the corresponding row appears empty.
- **View Latest Job Run** – Displays the status of the spot labels acquired in the last spot set job.

  Note: View Latest Job Run is the default display in the Spot Set Manager after you run spot set jobs.

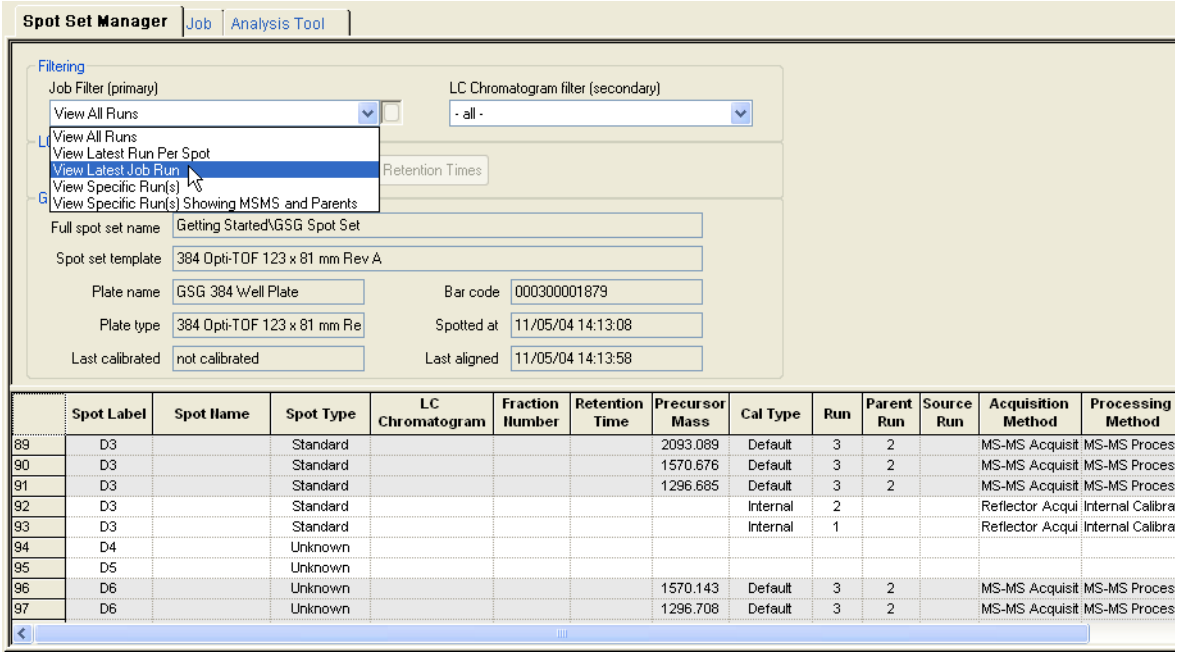
- **View Specific Run(s)** – Displays the status of the spot labels acquired in specified spot set jobs.
- **View Specific Run(s) Showing MSMS and Parents** – Displays the status of the spot labels acquired in specified MS/MS spot set jobs, as well as the parent spot set jobs from which the MS/MS spot set jobs were generated.

  Note: You must select an MS/MS spot set job to view MSMS and Parents.

Selecting View Filter

To display the status of the last spot set job:

1. Select the **Spot Set Manager** tab.
2. In the Job Filter (primary) drop-down list, select **View Latest Job Run** (Figure 9-7).



	Spot Label	Spot Name	Spot Type	LC Chromatogram	Fraction Number	Retention Time	Precursor Mass	Cal Type	Run	Parent Run	Source Run	Acquisition Method	Processing Method
89	D3		Standard				2093.089	Default	3	2		MS-MS Acquisit	MS-MS Proces
90	D3		Standard				1570.676	Default	3	2		MS-MS Acquisit	MS-MS Proces
91	D3		Standard				1296.685	Default	3	2		MS-MS Acquisit	MS-MS Proces
92	D3		Standard					Internal	2			Reflector Acqui	Internal Calibra
93	D3		Standard					Internal	1			Reflector Acqui	Internal Calibra
94	D4		Unknown										
95	D5		Unknown										
96	D6		Unknown				1570.143	Default	3	2		MS-MS Acquisit	MS-MS Proces
97	D6		Unknown				1296.708	Default	3	2		MS-MS Acquisit	MS-MS Proces

Figure 9-7 Selecting View Latest Job Run

The latest spot set job appears in the Spot Set Manager (Figure 9-8).

	Spot Label	Spot Name	Spot Type	LC Chromatogram	Fraction Number	Retention Time	Precursor Mass	Cal Type	Run	Parent Run	Source Run	Acquisition Method	Processing Method
1	D3		Standard				1296.685	Default	3	2		MS-MS Acquisit	MS-MS Proces
2	D3		Standard				1570.676	Default	3	2		MS-MS Acquisit	MS-MS Proces
3	D3		Standard				2093.089	Default	3	2		MS-MS Acquisit	MS-MS Proces
4	D3		Standard				2465.198	Default	3	2		MS-MS Acquisit	MS-MS Proces
5	D6		Unknown				1296.708	Default	3	2		MS-MS Acquisit	MS-MS Proces
6	D6		Unknown				1570.143	Default	3	2		MS-MS Acquisit	MS-MS Proces
7	D6		Unknown				904.466	Default	3	2		MS-MS Acquisit	MS-MS Proces
8	D6		Unknown				1672.933	Default	3	2		MS-MS Acquisit	MS-MS Proces

Figure 9-8 Latest Job Run

The latest job run displays the spot set job that was automatically generated from the interpretation method. The run should include MS/MS acquisitions for up to five peaks from each spot. The mass of each peak that was analyzed is displayed in the Precursor Mass column.

3. Ensure that the Acquisition Status and Processing Status columns for each row display “OK.”

Viewing Spectra and Peak Lists

Overview

You can view any spectrum (as well as associated peak list, interpretation peaks, and operating log) that you acquired in the spot set using the Spot Set Manager.

To view a specific spectrum:

Selecting a Run

1. Select the **Spot Set Manager** tab.
2. In the Job Filtering (primary) drop-down list, select **View Specific Run(s)**.

   The Run Number Selection dialog box opens (Figure 9-9).

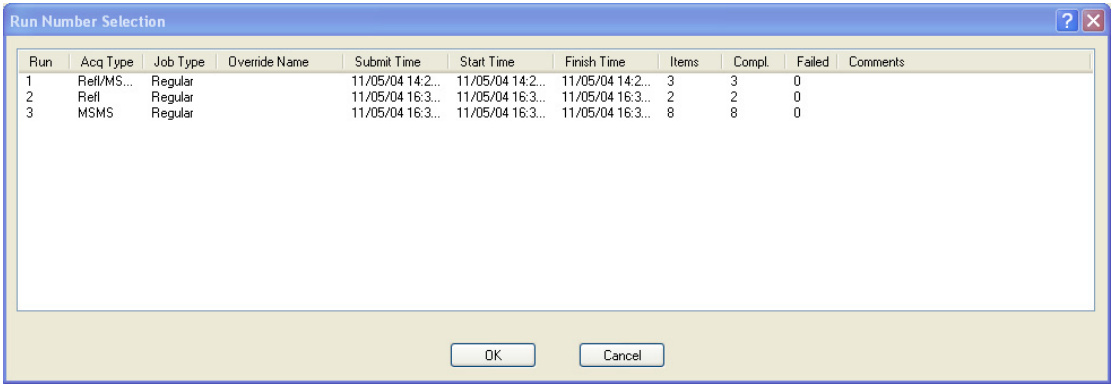


Figure 9-9 Run Number Selection Dialog Box

3. Select Run Number **2** (the spot set job that you submitted in "Running a Spot Set Job" on page 9-7), then click **OK**.

   The two spot labels that you acquired appear in the Spot Set Manager (Figure 9-10).

	Spot Label	Spot Name	Spot Type	LC Chromatogram	Fraction Number	Retention Time	Precursor Mass	Cal Type	Run	Parent Run	Source Run	Acquisition Method	Processing Method
1	D3		Standard					Internal	2			Reflector Acqui	Internal Calibrat
2	D6		Unknown					Default	2			Reflector Acqui	Default Calibrat

Figure 9-10 Viewing a Specific Run

Holding Job Queue Updates

4. Select **Batch > Hold Job Updates** to stop displaying job queue updates.

   Note: You cannot view spectra while the job queue is being updated.

Viewing the Spectra

5. Right-click the row containing the mock unknown sample, then select **View Spectrum**. The spectrum for the mock sample appears in the Spectrum Viewer.
6. Select **Spectrum > Add Trace**. A second trace opens in the Spectrum Viewer.
7. Right-click the row containing the calibration standard, then select **View Spectrum**. The spectrum for the calibration standard appears in the Spectrum Viewer.

Viewing the Peak List

8. Select the spectrum for the mock sample in the Spectrum Viewer.
9. Select **View > Output Window**. The Output Window opens at the bottom of the screen.
10. Select the **Peak List** tab in the Output Window.

    The peak list for the mock sample appears in the Output Window (Figure 9-11).

Index	Centroid Mass	Lower Bound	Upper Bound	Height	S/N	Resolution	Area	Clust. Area	Ref. Mass	Label
1	904.46698	904.11	905.05	22952	3909	7747	169302.13	288535.50	904.46802	
2	1296.68628	1296.37	1297.41	17679	3329	10731	113587.49	226411.23	1296.68506	
3	1552.67102	1552.44	1553.14	3079	610	12759	18518.80	33435.40	0.00000	
4	1570.67810	1570.27	1571.41	19622	3884	12140	126291.44	276234.94	1570.67700	
5	1829.47034	1829.22	1829.72	3816	747	13347	24736.32	73625.30	0.00000	
6	1829.96997	1829.72	1830.28	7247	1418	12505	46280.75	82971.10	0.00000	
7	2093.08887	2092.44	2093.82	9444	1892	12180	70203.72	226361.06	2093.08691	
8	2110.08203	2109.82	2110.47	2018	407	12749	13080.17	13080.17	0.00000	
9	2465.19409	2464.82	2465.91	13484	3826	12286	114004.51	429665.91	2465.19897	
10	3657.93042	3657.30	3658.44	2795	1019	10139	39027.76	276088.63	3657.92896	

Peak List | Processing Results | Interpretation Results | Data Storage | Operations Log

Figure 9-11 Output Window Peak List Tab

Evaluating Data

Evaluate the data as described in "Examining the Spectrum" on page 5-10.

Index

Numerics

A

B

C

D

E

F

G

H

I

J

L

M

N

O

P

Q

R

S

T

U

V

W

Z

iScience. To better understand the complex interaction of biological systems, life scientists are developing revolutionary approaches to discovery that unite technology, informatics, and traditional laboratory research. In partnership with our customers, Applied Biosystems provides the innovative products, services, and knowledge resources that make this new, **Integrated Science** possible.

Worldwide Sales Offices

Applied Biosystems vast distribution and service network, composed of highly trained support and applications personnel, reaches 150 countries on six continents. For international office locations, please call our headquarters or see our Web site at **www.appliedbiosystems.com**.

Applera is committed to providing the world's leading technology and information for life scientists. Applera Corporation consists of the Applied Biosystems and Celera Genomics businesses. Applied Biosystems/MDS SCIEX is a joint venture between Applera Corporation and MDS Inc.

Headquarters
850 Lincoln Centre Drive
Foster City, CA 94404 USA
Phone: +1 650.638.5800
Toll Free (In North America): +1 800.345.5224
Fax: +1 650.638.5884

Technical Support
In North America, call +1 800.899.5858.
Outside North America, see our Web site at **www.appliedbiosystems.com**.

11/2004

Part Number 4352078 Rev. B