Voyager™ Biospectrometry™ Workstation

With Delayed Extraction® Technology

User Guide

Version 5.1 Series Software

Applied Biosystems

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Glossary

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Safety and Compliance Information

In this section This section includes:

- Instrument safety
- Safety and EMC standards
- Laser safety

Instrument Safety

In this section This section includes:

- Notes, Hints, Cautions, and Warnings
- Safety symbols
- Before operating this instrument
- Material Safety Data Sheets (MSDSs)
- General Warnings

Notes, Hints, Cautions, and Warnings

Notes, Hints, Cautions, and Warnings are used in this document as follows.

A Note provides important information to the operator. For example:

NOTE: *If you are prompted to insert the boot diskette into the drive, insert it, then press any key.*

A Hint provides helpful suggestions not essential to the use of the system. For example:

Hint: *To avoid complicated file naming, use Save First to Pass or Save Best Only modes.*

A Caution provides information to avoid damage to the system or loss of data. For example:

CAUTION

Do not touch the lamp. This may damage the lamp.

A Warning provides specific information essential to the safety of the operator. For example:

WARNING

CHEMICAL HAZARD. *Wear appropriate personal protection and always observe safe laboratory practices when operating your system.*

Remarques, recommandations et avertissements

Une remarque fournit une information importante à l'opérateur er se présente ainsi:

REMARQUE: *Si on vous demande d'insérer la disquette de démarrage dans le lecteur, insérez-la puis appuyez sur n'importe quelle touche.*

Une recommandation fournit une information destinée à éviter des détériorations du système ou la perte de données:

RECOMMANDATION

La lampe peut être endommagée. N'y touchez pas.

Un avertissement fournit une information indispensable à la sécurité de l'operateur et se présente ainsi:

AVERTISSEMENT

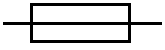
Conformez-vous toujours aux règlements du laboratoire quand vous utilisez votre système.

Safety symbols

The following symbols may be displayed on the system. These symbols may also appear next to associated warnings in this document.

Electrical Symbols

The following chart is an illustrated glossary of electrical symbols that may be displayed on your instrument. Whenever such symbols appear on instruments, please observe appropriate safety procedures.

	This symbol indicates the **on** position of the main power switch.
	This symbol indicates the **off** position of the main power switch.
	This symbol indicates the **on/off** position of a push-push main power switch.
	This symbol indicates that a terminal may be connected to another instrument's signal ground reference. This is not a protected ground terminal.
	This symbol indicates that this is a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
	A terminal marked with this symbol either receives or delivers alternating current or voltage.
	A terminal marked with this symbol can receive or supply an alternating and a direct current or voltage.
	This symbol appears next to the values of the fuses required by the system.

	**WARNING**: This symbol indicates the presence of high voltage and warns the user to proceed with caution.
	**WARNING**: This symbol alerts you to consult the manual for further information and to proceed with caution.

Non-electrical Symbols

The following is an illustrated glossary of non-electrical safety alert symbols that may be displayed on your instrument.

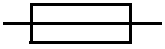
	**WARNING**: This symbol illustrates a heater hazard. Proceed with caution when working around these areas to avoid being burned by hot components.
	This symbol indicates that a laser is present inside the instrument.

Symboles des alertes de sécurité

Les symboles suivants peuvent être affichés sur le système. Dans ce document, ces symboles peuvent aussi apparaître à côté des avertissements auxquels ils s'associent.

Symboles électriques

Le tableau suivant donne la signification de tous les symboles électriques qui figurent sur les appareils. En présence de l'un de ces symboles, il est impératif de se conformer aux consignes de sécurité appropriées.

	Position *MARCHE* de l'interrupteur d'alimentation principal.
	Position *ARRÊT* de l'interrupteur d'alimentation principal.
	Positions MARCHE-ARRÊT de l'interrupteur d'alimentation principal à bouton poussoir.
	Borne pouvant être reliée à la mise à la terre d'un autre appareil. Ce n'est pas une borne de mise à la terre protégée.
	Borne de mise à la terre de protection devant être reliée à la terre avant d'effectuer tout autre raccordement électrique à l'appareil.
	Borne recevant ou fournissant une tension ou un courant de type alternatif.
	Borne pouvant recevoir ou fournir une tension ou un courant de types alternatif et continu.
	Ce symbole apparaît à côté des valeurs des fusibles requis par le système.

	**AVERTISSEMENT**: Indique la présence d'une haute tension et avertit l'utilisateur de procéder avec précaution.
	**AVERTISSEMENT**: Avertit l'utilisateur de la nécessité de consulter le manuel pour obtenir davantage d'informations et de procéder avec précaution.

Symboles non électriques

Le tableau suivant donne la signification des symboles d'alertes de sécurité non électriques qui figurent sur les appareils.

	**AVERTISSEMENT**: Danger associé à la présence d'un appareil de chauffage. Procéder avec précaution pour éviter de se brûler au contact de pièces ou d'éléments chauds.
	Indique que l'appareil renferme un laser.

Before operating this instrument

Ensure that anyone involved with the operation of the instrument is instructed in both general safety practices for laboratories and specific safety practices for the instrument. Make sure you have read and understood all related Material Safety Data Sheets.

Material Safety Data Sheets (MSDSs)

Some of the chemicals that may be used with your system are listed as hazardous by their manufacturer. When hazards exist, they are prominently displayed on the labels of all chemicals. In addition, MSDSs supplied by the chemical manufacturer provide information about:

- Physical characteristics
- Safety precautions
- Health hazards
- First-aid
- Spill clean-up
- Disposal procedures

WARNING

CHEMICAL HAZARD. *Before handling any chemicals, refer to the Material Safety Data Sheet provided by the manufacturer, and observe all relevant precautions.*

AVERTISSEMENT

RISQUE CHIMIQUE. *Avant de manipuler des produits chimiques, veuillez consulter la fiche de sécurité du matériel fournie par le fabricant, et observer les mesures de précaution qui s'imposent.*

To order MSDSs...	Then...
Over the Internet	Go to our Web site at www.appliedbiosystems.com/techsupport. Click **MSDSs**. Enter keywords (or partial words), or a part number, or the MSDSs Documents on Demand index number. Click **Search.** Click the Adobe Acrobat symbol to view, print, or download the document, or check the box of the desired document and delivery method (fax or e-mail).
By telephone in the United States	Dial 1-800-327-3002, then press **1.**

General Warnings

WARNING

FIRE HAZARD. *Using a fuse of the wrong type or rating can cause a fire. Replace fuses with those of the same type and rating.*

AVERTISSEMENT

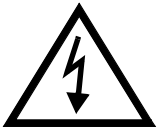
DANGER D'INCENDIE. *L'usage d'un fusible de type ou de valeur nominale différents risque de provoquer un incendie. Il convient donc de remplacer les fusibles usagés par des fusibles du même type et de la même valeur nominale.*

WARNING

LASER HAZARD. *The laser emits ultraviolet radiation. Lasers can burn the retina and leave permanent blind spots. Do not remove any instrument panels or look directly into the laser beam or allow a reflection of the beam to enter your eyes. Wear proper eye protection if any panels are removed for service.*

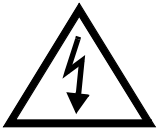
AVERTISSEMENT

DANGER LASER. *Le laser émet des radiations ultraviolettes. Les lasers peuvent brûler la rétine et laisser des points aveugles permanents. Il convient de ne pas retirer le panneau avant ou les panneaux latéraux de l'appareil et de ne pas regarder directement dans le faisceau laser ou laisser une réflexion du faisceau entrer dans les yeux. Portez des protections adéquates pour les yeux si le panneau avant ou les panneaux latéraux ont été retirés afin d'effectuer l'entretien.*



WARNING

ELECTRICAL SHOCK HAZARD. *Severe electrical shock can result by operating the instrument without the panels in place. Do not remove instrument panels. High voltage contacts are exposed with panels removed.*



AVERTISSEMENT

RISQUE DE DÉCHARGE ÉLECTRIQUE. *Des décharges électriques sérieuses peuvent résulter du fonctionnement de l'appareil lorsque le panneau avant et les panneaux latéraux sont retirés. Ne pas retirer le panneau avant ou les panneaux latéraux. Des contacts haute tension sont exposés lorsque les panneaux sont retirés.*

WARNING

CHEMICAL HAZARD. *To prevent eye injury, always wear eye protection when working with solvents.*

AVERTISSEMENT

RISQUE CHIMIQUE. *Pour éviter les blessures aux yeux, porter toujours des protections pour les yeux lorsque vous manipulez des solvants.*

WARNING

PHYSICAL INJURY HAZARD. *Use the Voyager Biospectrometry Workstation only as specified in this document. Using this system in a manner not specified may result in injury or damage to the system.*

AVERTISSEMENT

DANGER DE BLESSURES CORPORELLES.*Veuillez suivre avec attention les indications figurant dans ce document lorsque vous utilisez la Station de Travail de Biosptectrométrie Voyager. Un usage différent de la station pourrait causer un accident ou endommager le système.*

Safety and EMC (Electromagnetic Compliance) Standards

US Safety and EMC Standards

Safety

This instrument has been tested to and complies with standard ANSI/UL 3101-1, “Electrical Equipment for Laboratory Use; Part 1: General Requirements”, 1st Edition. It is an ETL Testing Laboratories listed product.

EMC

This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

WARNING

Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user’s authority to operate the equipment.

NOTE: *This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.*

NOTE: *Shielded cables must be used with this unit to ensure compliance with the Class A FCC limits.*

Canadian Safety and EMC Standards

Safety

This instrument has been tested to and complies with standard CSA 1010, “Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use; Part 1: General Requirements”. It is an ETL Testing Laboratories listed product.

Sécurité

Cet instrument a été vérifié avec la norme CSA 1010, «Spécifications de sécurité du matériel électrique utilisé pour les mesures, les contrôles et dans les laboratoires ; Partie 1 : Spécifications générales», et il est conforme à cette norme. C’est un produit homologué par les ETL Testing Laboratories.

EMC

This Class A digital apparatus meets all requirements of the Canadian Interference-Causing Equipment Regulations.

Cet appareil numérique de la classe A respecte toutes les exigences du Règlement sur le materiel brouilleur du Canada.

European Safety and EMC Standards

CE

Safety

This instrument meets European requirements for safety (EMC Directive 73/23/EEC). This instrument has been tested to and complies with standard EN61010-1 “Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use”.

EMC

This instrument meets European requirements for emission and immunity (EMC Directive 98/336/EEC). This product has been evaluated to the EN61326:1998, “Electrical Equipment for Measurement, Control and Laboratory Use—EMC Requirements”. Radiated Emissions were evaluated to Group 1, Class B requirements.

Laser Safety

Laser classification

The Voyager™ Biospectrometry™ Workstation uses a standard nitrogen laser and an optional Nd:YAG laser. Under normal operating conditions, the instrument laser is categorized as a Class I laser. Under certain conditions during servicing, when interlocks have been circumvented, the lasers fall into the following categories (can cause permanent eye damage):

- Nitrogen—Class IIIb
- Nd:YAG—Class IV

The Voyager Biospectrometry Workstation complies with Title 21, U.S. Government DHEW/BRH Performance Standards, Chapter 1, Subchapter J, Section 1040, as applicable.

Laser safety features

The following safety features are included on the Voyager Biospectrometry Workstation:

- Cabinet is designed to prevent access to collateral laser radiation exceeding the accessible emission limits in Performance Standards for Laser Products, 21 CFR 1040.10.
- Front and side panels have interlock switches that disable the laser when panels are removed.
- Safety labels for Class I standards are affixed to the unit.

Laser safety requirements

To ensure safe laser operation, note the following:

- The system must be installed and maintained by an Applied Biosystems Technical Representative.
- All panels must be installed during operation. When all panels are installed, there should be no detectable radiation present. If any panels are removed when the laser is operational, you may be exposed to laser emissions in excess of Class 1 rating.
- Do not remove labels or disable safety interlocks.

Additional safety information

Refer to the users manual provided with the laser for additional information on government and industry safety regulations.

How to Use This Guide

Purpose of this guide

The Applied Biosystems *Voyager™ Biospectrometry™ Workstation User's Guide* describes the procedures for installing, using, maintaining, and troubleshooting Voyager™ Biospectrometry™ workstations.

Audience

This guide is intended for novice and experienced Voyager workstation users who are analyzing biomolecules.

Structure of this guide

The Applied Biosystems *Voyager Biospectrometry Workstation User's Guide* is organized in chapters and appendixes. Each chapter page is marked with a tab and a header to help you find information.

The table below describes the material covered in each chapter.

Chapter/Appendix	Content
Chapter 1, Introducing the Voyager™ Biospectrometry™ Workstations	Describes the parts of the system and software, and gives background information on MALDI-TOF and Delayed Extraction® technology.
Chapter 2, Installing the Voyager Biospectrometry Workstations	Provides procedures for installing the system, attaching components (such as the video monitor), installing the software, starting up and shutting down.
Chapter 3, Preparing Samples	Describes how to prepare matrix and sample, how to load sample on sample plates, and how to load plates in the mass spectrometer. Also describes sample plate types and plate (.PLT) files.
Chapter 4, Voyager Instrument Control Panel Basics	Describes the parts of the Instrument Control Panel, how to manipulate traces in the Spectrum window, how to control the workstation from the software, and how the Instrument Control Panel works with the Sequence Control Panel.

Chapter/Appendix	Content
Chapter 5, Optimizing Instrument Settings	Describes instrument settings (.BIC) files and how to optimize them.
Chapter 6, Acquiring Spectra from the Instrument Control Panel	Describes how to acquire mass spectra from single samples using the Instrument Control Panel in Manual Control mode and Automatic Control mode.
Chapter 7, Acquiring Spectra from the Sequence Control Panel	Describes how to acquire mass spectra from multiple samples using the Sequence Control Panel.
Chapter 8, PSD Analysis	Describes using PSD analysis software, and exploring the impact of system settings on the quality of data obtained.
Chapter 9, Maintenance and Troubleshooting	Lists routine maintenance procedures performed by Applied Biosystems. Contains troubleshooting information and error codes.
Appendix A, Specifications	Includes system specifications.
Appendix B, Warranty/Service Information	Contains warranty, service, return, and spare parts information.
Appendix C, Matrixes	Lists chemical structures and preparation information for common matrixes.
Appendix D, Log Sheets	Contains blank log sheets that you can copy and use to record sample location.
Appendix E, Grid Voltage% and Delay Time Settings	Contains graphs that illustrate the relationship between these Delayed Extraction tuning parameters.

Chapter/Appendix	Content
Appendix F, Reference Standard Information	Includes molecular weights and mass to time conversions for standard calibration compounds. Also lists theoretical cleavages and observed fragment masses for angiotensin.
Appendix G, Maintenance Log	Includes a log sheet for tracking routine maintenance.
Appendix H, Continuous Extraction Mode	Includes information for running the system in Continuous Extraction mode for diagnostic purposes.
Appendix I, Using the Oscilloscope and Control Stick	Describes the front panel controls of the oscilloscope hardware and how to use the Control stick to move sample position, start and stop the laser, and start and stop acquisition.

Related documentation

The related documents shipped with your system are:

- ***Voyager™ Biospectrometry™ Workstation Getting Started Guide*** —Use this guide to learn the basics of operating the system. It provides step-by-step information for running your first experiment.
- ***Data Explorer™ Software User's Guide*** —Refer to this guide for functions in the Data Explorer software that are not described in the *Voyager Biospectrometry Workstation User's Guide.*

Reference documentation

The reference documents shipped with your system are:

- **Printer documentation** (depends on the printer you purchase)—Use this documentation to set up and service your printer.

- ***Microsoft® Windows NT® User's Guide*** and related documents—Use this guide to learn detailed information about the Microsoft Windows NT user interface.
- *Varian Turbopump Instruction Manual*
- *Varian Turbocontroller Instruction Manual*
- *Varian Multigauge Controller Manual*
- *Mechanical Pump Operating Instructions*
- *GAST Compressor Operating and Maintenance Instructions*

You also receive the appropriate manual for the digitizer included with your system.

Send us your comments

We welcome your comments and suggestions for improving our manuals. You can send us your comments in two ways:

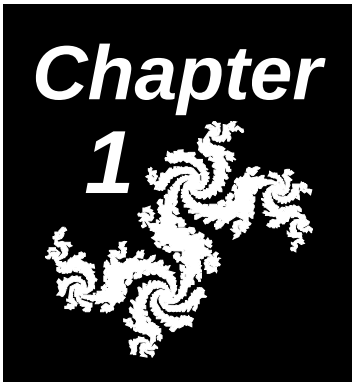
- Use the Technical Publications Customer Survey at:

  www.pbio.com/techsup/survey.htm

- Send e-mail to:

  TechPubs@pbio.com

1 Introducing the Voyager™ Biospectrometry™ Workstations



This chapter contains the following sections:

1.1 Voyager-DE™ and Voyager-DE PRO System Overview 1-2

1.2 Voyager-DE™ STR System Overview 1-5

1.3 MALDI-TOF MS Technology Overview 1-7

1.4 Voyager-DE™ (Delayed Extraction®) Technology 1-11

1.5 Parts of the Voyager-DE and Voyager-DE PRO Systems 1-17

1.6 Parts of the Voyager-DE STR System 1-31

1.7 Software Overview 1-42

1

1.1 Voyager-DE™ and Voyager-DE PRO System Overview

The Voyager-DE™ and Voyager-DE PRO Biospectrometry™ Workstations are designed for use by mass spectrometrists, biochemists, molecular biologists, and life scientists.

Voyager-DE

The Applied Biosystems Voyager-DE Biospectrometry Workstation (Figure 1-1) is a benchtop MALDI-TOF (matrix-assisted laser desorption ionization time-of-flight) mass spectrometer. Voyager Delayed Extraction® technology provides improved resolution and mass accuracy.

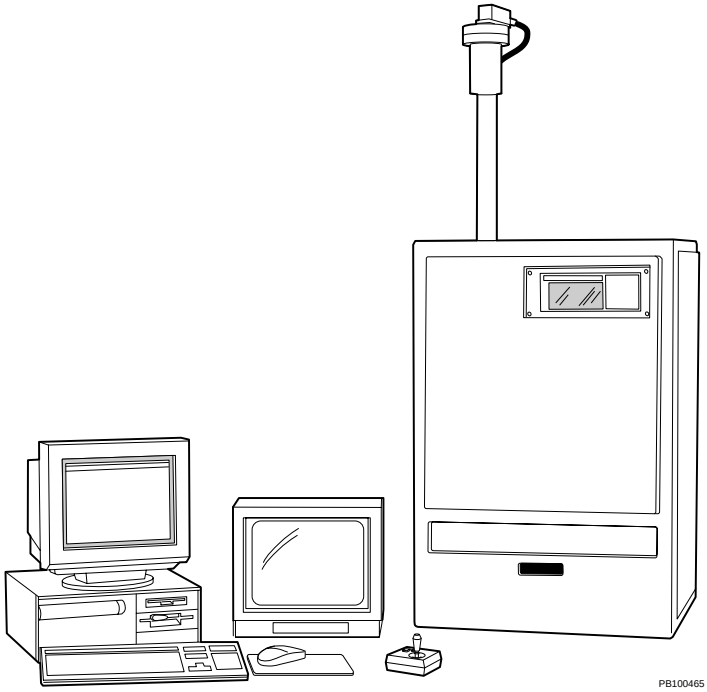


Figure 1-1 Voyager-DE Biospectrometry Workstation

Voyager-DE PRO

The Voyager-DE™ PRO Biospectrometry™ Workstation (Figure 1-2) is a benchtop MALDI-TOF (matrix-assisted laser desorption time-of-flight) mass spectrometer that includes a reflector analyzer. Voyager Delayed Extraction® technology provides improved resolution and mass accuracy.

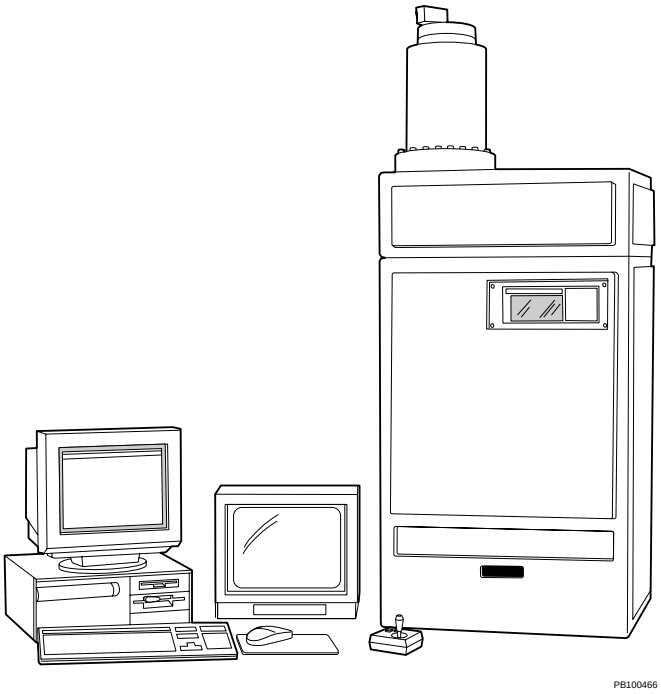


Figure 1-2 Voyager-DE PRO Biospectrometry Workstation

Biospectrometry

Biospectrometry is the application of mass spectrometry in the field of the life sciences.

This field uses fast chromatographic techniques, enzymatic chemistries, and surface chemistries and combines them with mass spectrometry and advanced software to better enable biomolecular research and facilitate data interpretation.

Features

Features of the Voyager-DE and Voyager-DE PRO Biospectrometry Workstations include:

- High-speed digitizer options and laser rates for optimum sample throughput
- Positive or negative ion detection
- m/z range in excess of 300 kDa
- Sensitivity to less than 5 femtomoles with dried droplet application
- Ion path length:
  - Voyager-DE—1.2 meter
  - Voyager-DE PRO in linear mode—1.3 meter
  - Voyager-DE PRO in reflector mode—2.0 meter
- Variable acceleration voltage
- Compact benchtop design
- Variable two-stage ion source
- Automated single-plate sample loading system, sample plates with various formats available
- Beam guide technology for high mass sensitivity
- Video camera and monitor for sample viewing
- Low mass gate matrix suppression
- Intuitive Microsoft® Windows NT®-based software

Features unique to the Voyager-DE PRO Biospectrometry Workstation include:

- High-performance reflector
- Timed ion selector
- Post-source decay (PSD) analysis capability
- Linear or reflector mode operation
- Collision-induced dissociation (CID) option

1.2 Voyager-DE™ STR System Overview

Voyager-DE STR

The Voyager-DE™ STR Biospectrometry™ Workstation (Figure 1-3) is a floor-standing MALDI-TOF (matrix-assisted laser desorption ionization time-of-flight) mass spectrometer that includes a reflector analyzer.

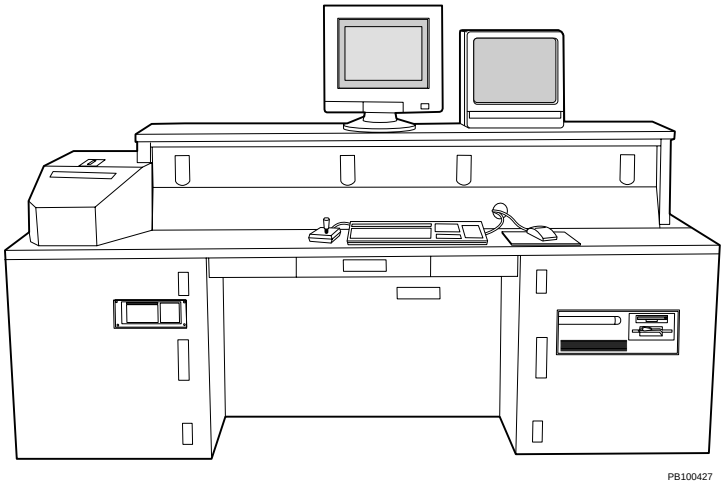


Figure 1-3 Voyager-DE STR Biospectrometry Workstation

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Biospectrometry

Biospectrometry is the application of mass spectrometry in the field of the life sciences.

This field uses fast chromatographic techniques, enzymatic chemistries, and surface chemistries and combines them with mass spectrometry and advanced software to better enable biomolecular research and facilitate data interpretation.

Features

Features of the Voyager-DE STR Biospectrometry Workstation include:

- High-speed digitizer options and laser rates for optimum sample throughput
- Positive and negative ion detection
- Modular horizontal design
- High-performance reflector
- Post-source decay (PSD) analysis capability
- Linear or reflector mode operation
- Ion path length:
  - Linear mode—2.0 meter
  - Reflector mode—3.0 meter
- Timed ion selection
- m/z range in excess of 300 kDa
- Sensitivity to less than 5 femtomoles with dried droplet application
- Variable two-stage ion source
- Automated single-plate sample-loading system; sample plates of various formats available
- Ion focusing lens technology for increased sensitivity (serial number 4154 and later)
- Video camera and monitor for sample viewing
- Low mass gate matrix suppression
- Intuitive Microsoft® Windows NT®-based software
- Collision-induced dissociation (CID) option
- Second laser option

1.3 MALDI-TOF MS Technology Overview

Mass spectrometry

Mass spectrometry measures the mass of molecules by measuring the mass-to-charge ratio (*m/z*). Mass is a molecular attribute that can help identify or confirm the identity of a molecule.

Molecular weight measurements by mass spectrometry are based upon the production, separation, and detection of molecular ions. A typical mass spectrometer includes:

- **Ion source**—Ionizes sample and generates gas phase ions
- **Analyzer**—Separates ions according to individual mass-to-charge ratios
- **Detector**—Detects and amplifies ions
- **Data system**—Converts detection of ions into a readable or graphic display

Advantages of mass spectrometry technology include:

- Can directly measure an absolute physical constant
- Provides highly sensitive detection
- Can analyze mixtures and different classes of biopolymers, including peptides, oligonucleotides, and oligosaccharides

MALDI-TOF mass spectrometry

The Voyager Biospectrometry Workstations are MALDI-TOF mass spectrometry systems:

- **MALDI**—Matrix-Assisted Laser Desorption Ionization sample ionization mechanism
- **TOF**—Time-of-Flight analysis and detection mechanism

1

Matrix-Assisted Laser Desorption Ionization (MALDI)

In Matrix-Assisted Laser Desorption Ionization (MALDI), sample is embedded in a low molecular weight, UV-absorbing matrix that enhances intact desorption and ionization of the sample.

The matrix is present in vast excess of sample, and therefore isolates individual sample molecules.

Figure 1-4 illustrates the ionization of sample.

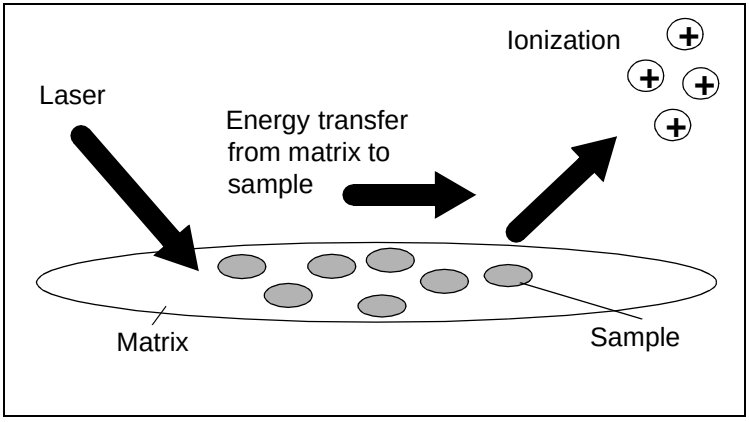

Figure 1-4 Matrix-Assisted Laser Desorption Ionization

Time-of-Flight (TOF)

Time-of-flight mass spectrometry works on the principle that if ions are accelerated with the same potential from a fixed point and at a fixed initial time and are allowed to drift, the ions will separate according to their mass-to-charge ratios. Lighter ions drift more quickly to the detector. Heavier ions drift more slowly (Figure 1-5).

Ions generated by MALDI exhibit a broad energy spread after acceleration in a stationary electric field. By forming ions in a field-free region, and then applying a high voltage pulse after a predetermined time delay to accelerate the ions, this energy spread can be minimized. See Section 1.4, Voyager-DE™ (Delayed Extraction®) Technology, for more information.

For acquiring TOF spectra, time measurement depends on extraction mode:

- **Delayed Extraction mode**—Measurement of the ion signal starts when the extraction pulse is applied. The time at which the extraction pulse is applied is user-settable. See Section 1.4, Voyager-DE™ (Delayed Extraction®) Technology for more information.
- **Continuous Extraction mode**—The extraction field is continuously applied. Measurement of the ion signal starts when the laser pulses.

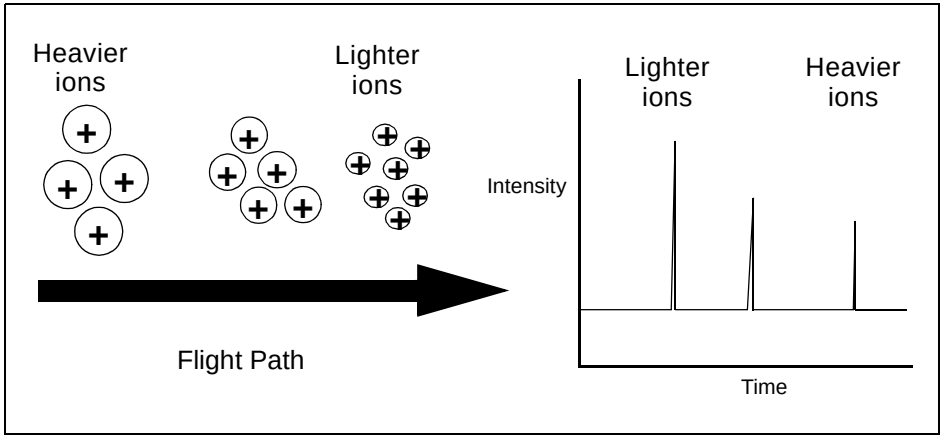

Figure 1-5 Time of Flight Analysis

The time required for ions to reach the detector at the opposite end of the flight tube (drift time) is measured. The number of ions reaching the detector at any given time is also measured, and is referred to as ion intensity (abundance) or signal intensity.

Drift time is proportional to the square root of the mass as defined by the following equation:

$$t = s\left(\frac{m}{(2KE)z}\right)^{1/2}$$

where:

t	=	drift time
s	=	drift distance
m	=	mass
KE	=	kinetic energy
z	=	number of charges on ion

Approximate ion mass is determined using the equation above. A calibration procedure using a reference standard of known mass can be used to establish a more accurate relationship between flight time and the mass-to-charge ratio of the ion.

Advantages of MALDI-TOF

Advantages of MALDI-TOF technology include:

- Can analyze a theoretically unlimited mass range, greater than 300,000 daltons (Da). Mass range is limited by ionization ability, not analyzer physics.
- Can obtain complete mass spectrum from a single ionization event. This is also referred to as multiplexing, or parallel (versus serial) detection.
- Is compatible with buffers normally used in biological assays, reducing the need for sample cleanup.
- Can analyze mixtures and different classes of biopolymers, including peptides, oligonucleotides, glycoconjugates, and synthetic polymers.
- Provides very high sensitivity and requires only femtomoles of sample.

1.4 Voyager-DE™ (Delayed Extraction®) Technology

In this section

This section describes:

- Limitations of MALDI technology
- Delayed Extraction
- Delayed Extraction versus Continuous Extraction
- Benefits of Delayed Extraction
- Velocity focusing

Limitations of MALDI technology

In traditional MALDI, ions exhibit a broad kinetic energy distribution which is largely due to the initial velocity imparted to ions during the desorption/ionization process. This initial velocity of desorbed analyte ions is nearly independent of mass of the analyte and the initial kinetic energy is proportional to the mass. In addition, when desorption occurs in a strong electrical field, energy is lost by collisions with the neutral plume, and further mass-dependent energy dispersion results.

According to the theory of “time-lag energy focusing” as originally developed by Wiley and McLaren[1], the dependence of ion flight time on initial velocity can be corrected, to the first order, by delaying the extraction of ions from the source. If higher order terms are insignificant, then the mass resolution should be determined by the ratio of the total flight time to the uncertainty in the time measurement. The observed mass resolution should increase in proportion to the effective length of the ion flight path.

1. W. C. Wiley and I. H. McLaren, *Rev. Sci. Instrum.*, **1953**, ***26***, 1150–1157, W. C. Wiley, U.S. Patent 2,685,035.

1

Delayed Extraction

With Voyager-DE™ (Delayed Extraction®) technology, ions form in a field-free region, and then are extracted by applying a high voltage pulse to the accelerating voltage after a predetermined time delay.

Figure 1-6 compares Delayed and Continuous Extraction.

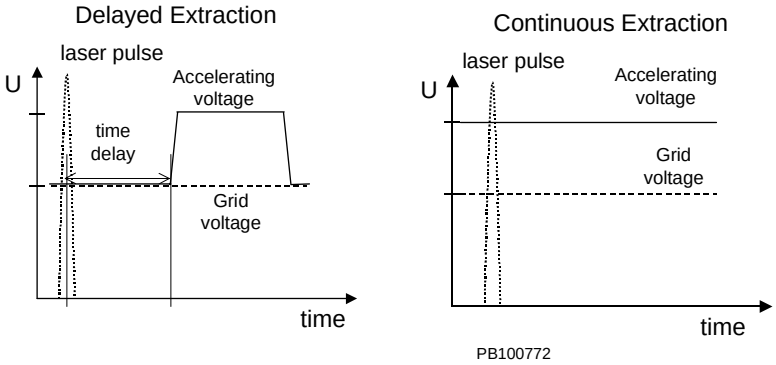

Figure 1-6 Delayed Versus Continuous Extraction

Delayed Extraction versus Continuous Extraction

The Voyager-DE systems can be operated in Delayed Extraction (DE) or Continuous Extraction mode.

NOTE: *Due to the superior results obtained with Delayed Extraction (DE) mode, use Continuous Extraction mode for diagnostic purposes only. See Appendix H, Continuous Extraction Mode, for more information on optimizing methods and obtaining good spectra.*

In Delayed Extraction mode:

- Potential gradient does not exist when sample is ionized (sample plate and grid are at similar potentials)
- Accelerating voltage is pulsed after a user-set time delay (potential gradient is applied) and ions are accelerated

In Continuous Extraction mode:

- Accelerating voltage is continuously applied, and the potential gradient exists when sample is ionized
- Ions are immediately accelerated

Figure 1-7 and Figure 1-8 show the improved resolution obtained in Delayed Extraction mode.

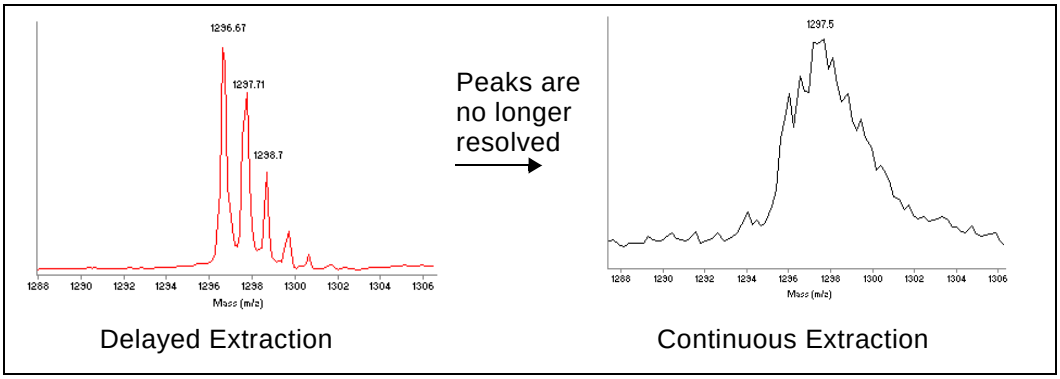

Figure 1-7 Comparison of Angiotensin Resolution with Delayed and Continuous Extraction (Linear Mode)

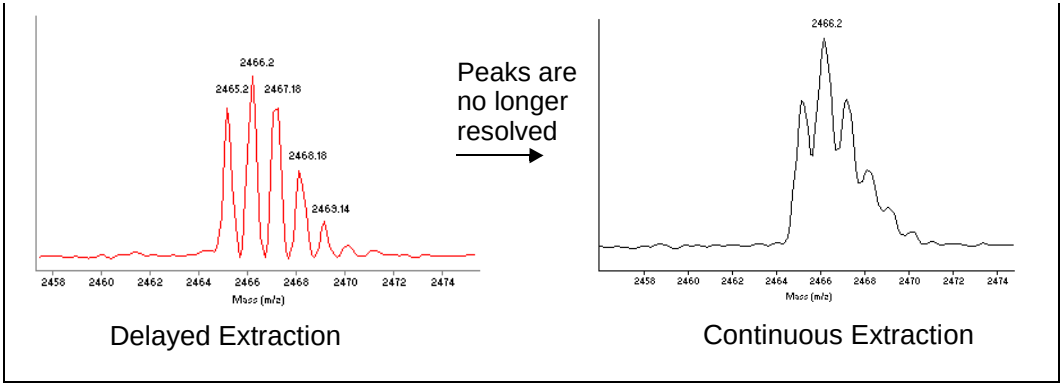

Figure 1-8 Comparison of ACTH (Clip 18–39) Resolution with Delayed and Continuous Extraction (Reflector Mode)

Benefits of Delayed Extraction

Delayed Extraction of ions overcomes many of the adverse effects of Continuous Extraction:

Benefits of Delayed Extraction	Adverse Effects of Continuous Extraction
Velocity focusing of ions is controlled by variable-voltage grid in the ion source and the delay time applied to acceleration. See “Velocity focusing” on page 1-15. Resolution and mass accuracy are improved.	Initial velocity distribution of ions.
Minimal energy loss from collision of ions. Ion acceleration is delayed, the ion/neutral plume becomes less dense, and ion collision is minimized.	Substantial energy loss from collision of ions. Ions are accelerated immediately after sample is ionized and collide in the dense ion/neutral plume.
Fragmentation is greatly reduced by acceleration delay. Background noise is reduced, and the effect of laser intensity on performance is minimized.	Background noise from fragmentation in the source (prompt fragmentation) related to ion collision.
Less analyte/matrix-dependent performance because initial velocity distribution and energy loss is corrected or minimized.	Analyte/matrix-dependent performance, because initial velocity distribution and energy loss conditions are specifically related to the sample and matrix used.

Velocity focusing

Delayed Extraction technology facilitates tuning modes, when the time-of-flight of an ion is independent of the initial velocity.

After ions are released from the sample surface, their position in the ion source is correlated with their initial velocity.

When the extraction voltage pulse is applied, initially slower ions acquire slightly higher energy from the accelerating field than initially faster ions. By careful tuning of variable-voltage grid in the source and the delay time applied to acceleration, slow and fast ions of the same mass reach the detector plane at the same time. This is referred to as “velocity focusing”.

Linear mode

Figure 1-9 and Figure 1-10 illustrate velocity focusing in linear mode. Ions are focused at the detector.

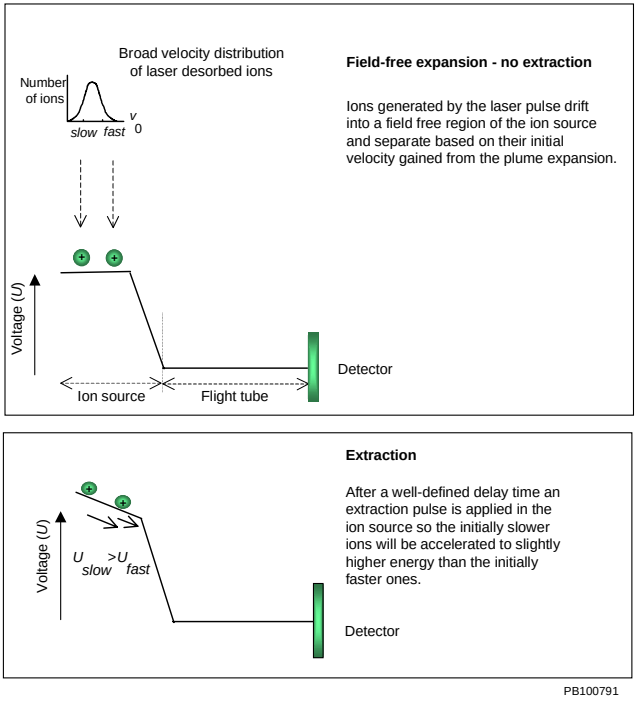

Figure 1-9 Velocity Focusing of Ions in Linear Mode—Field Free Expansion and Extraction Phases

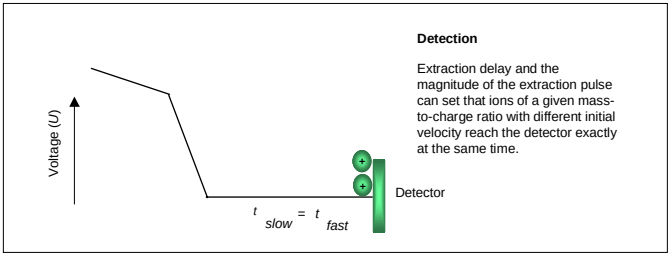

Figure 1-10 Velocity Focusing of Ions in Linear Mode—Detection Phase

Reflector mode

In Reflector mode, ions are velocity-focused at the exit of the ion source instead of at the detector. By tuning the variable-voltage grid and the acceleration delay time, you can alter the point at which ions are focused, and enhance resolution. This initial focus is refocused by the reflector, which you can tune for second-order velocity focusing.

1.5 Parts of the Voyager-DE and Voyager-DE PRO Systems

This section describes:

- System components
- Mass spectrometer
- Vacuum system
- Computer components

1.5.1 System Components

Voyager-DE The Voyager-DE Biospectrometry Workstation is shown in Figure 1-11.

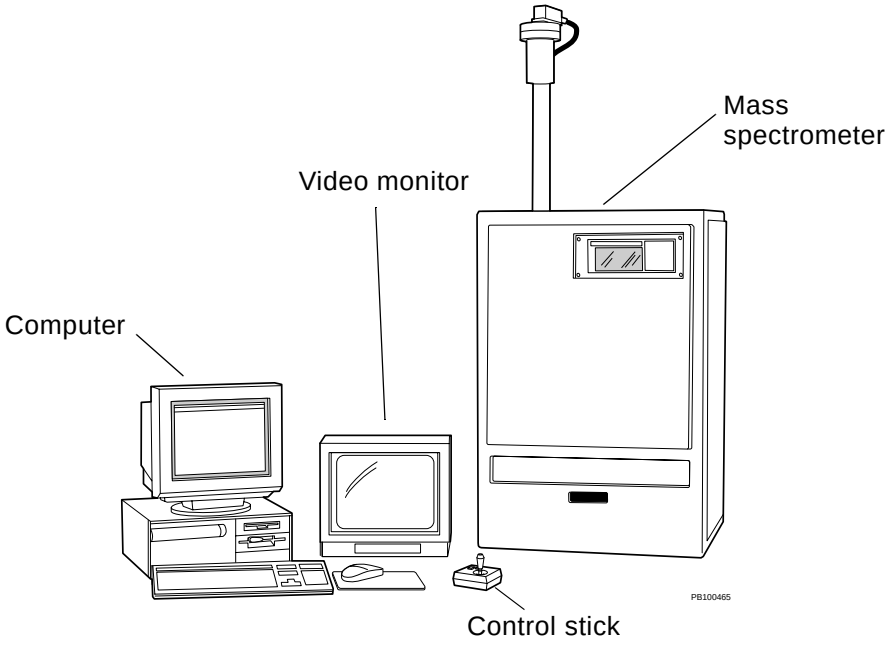

Figure 1-11 Voyager-DE Biospectrometry Workstation

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Voyager-DE PRO

The Voyager-DE PRO Biospectrometry Workstation is shown in Figure 1-12.

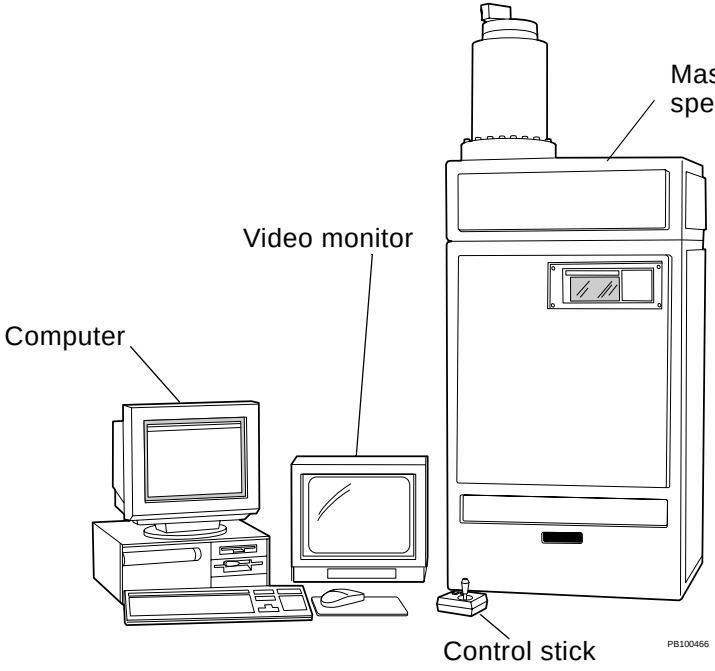

Figure 1-12 Voyager-DE PRO Biospectrometry Workstation

Parts of the Voyager-DE and Voyager-DE PRO systems include:

- **Mass spectrometer**—A time-of-flight mass spectrometer, described in Section 1.5.2, Mass Spectrometer. The high-vacuum system of the mass spectrometer is described in Section 1.5.3, Vacuum System.
- **Computer/Data System**—A computer that operates the Voyager control software and the Voyager processing software. You control the mass spectrometer using the computer.
- **Digitizer (not shown)**—An analog-to-digital converter that allows the signal from the mass spectrometer to be transferred to the computer.

  The standard digitizers provided are:

  - Voyager-DE—500 MHz
  - Voyager-DE RP—2 GHz

  Optional 2 to 4 GHz oscilloscopes are also available.
- **Video monitor**—A monitor that displays a real-time video image of the sample spot for examination during acquisition.
- **Control stick**—A device that controls position of the sample plate in the mass spectrometer. Allows you to start and stop acquisition and transfer data to the processing software.

NOTE: *You can also control sample position, start and stop acquisition, and transfer data using the Voyager Instrument Control Panel software.*

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1.5.2 Mass Spectrometer

Voyager-DE The parts of the Voyager-DE Biospectrometry Workstation mass spectrometer are shown in Figure 1-13.

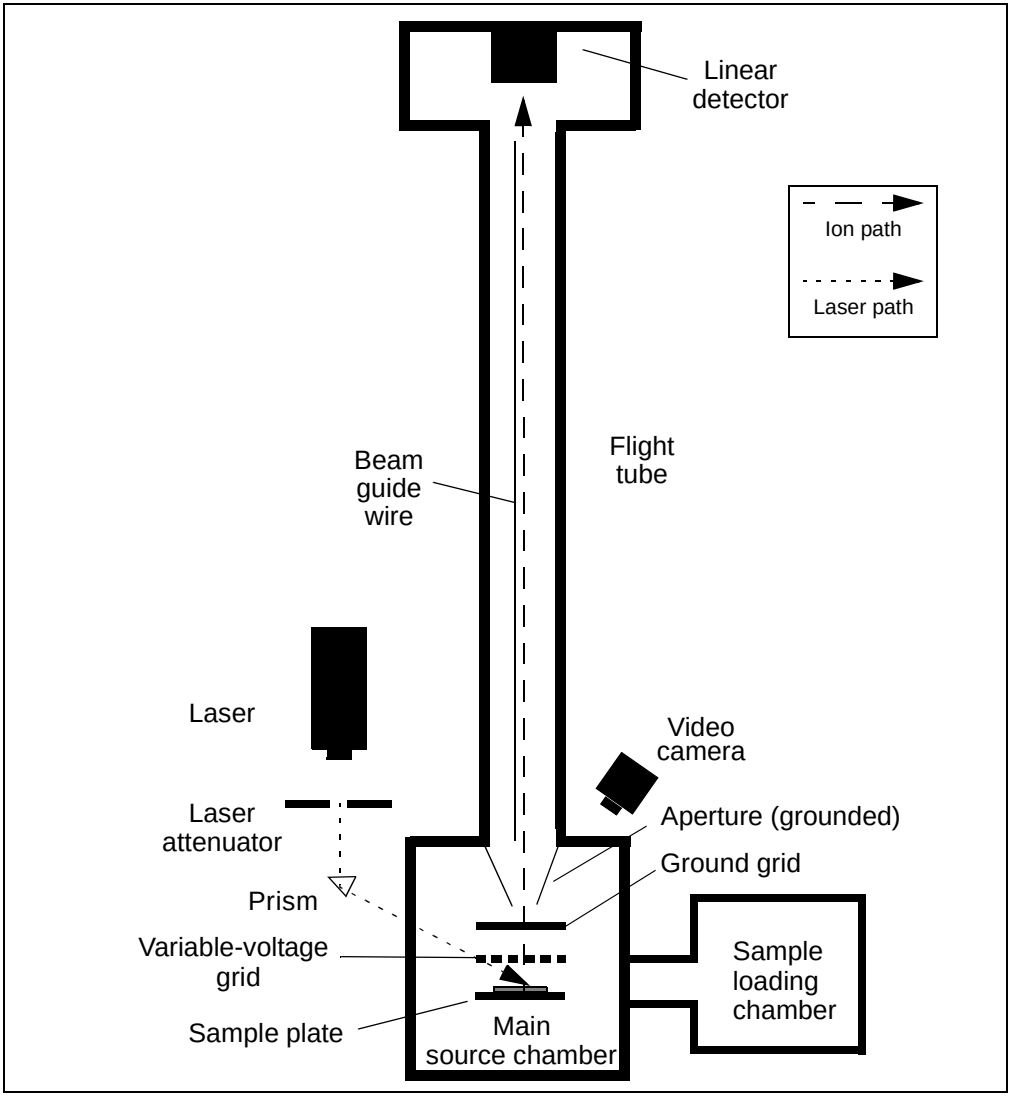

Figure 1-13 Voyager-DE Mass Spectrometer

Voyager-DE PRO

The parts of the Voyager-DE PRO Biospectrometry Workstation mass spectrometer are shown in Figure 1-14.

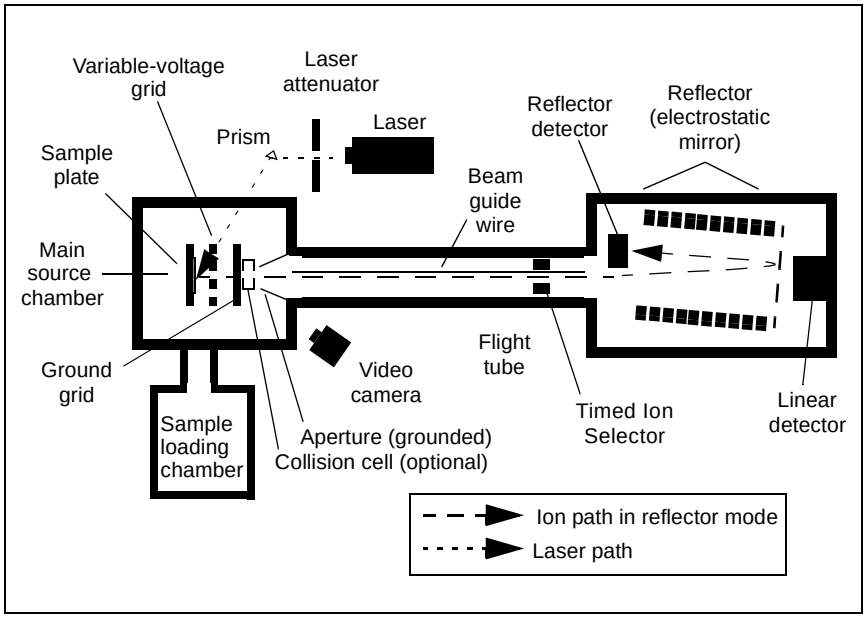

Figure 1-14 Voyager-DE PRO Mass Spectrometer (Single-Stage Reflector)

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Parts of the mass spectrometer

The Voyager-DE and Voyager-DE PRO mass spectrometers include:

- **Laser, attenuator, and prism**—A nitrogen laser that operates at 337 nm and ionizes sample. It produces 3-nanosecond-duration pulses. Laser rate can be set to Default (3 Hz) or Optimized (see page 5-26). Maximum possible rate is 20 Hz; actual rate depends on the digitizer installed and the digitizer Bin Size setting.

NOTE: *Optimized laser rate is available only on systems with the following serial numbers: Voyager-DE—1171 and later; Voyager-DE PRO—6131 and later.*

  The laser attenuator varies the intensity of the laser beam reaching the sample.

  The prism deflects the laser beam into the ion source.

- **Ion Source**—A high voltage region used to accelerate ions. Includes:

  - **Sample plate and sample stage**—An area supplied with voltage (0 to 25,000 V) for acceleration of ions into the flight tube.
  - **Variable-voltage grid**—A grid supplied with additional voltage to fine-tune ion acceleration.
  - **Ground grid**—Ground surface for formation of potential gradient.
  - **Grounded aperture**—Entrance to flight tube.

  For more information on the ion source and voltages, see Section 5.4.4.2, Setting Accelerating Voltage, and Section 5.4.3.5, Optimizing Grid Voltage%.

- **Video camera**—A camera that displays a real-time sample image (100 times magnification) on the video monitor.

- **Vacuum system**—A pumping system and sealed enclosure that creates and maintains a high-vacuum environment for unobstructed ion drift. Refer to Section 1.5.3, Vacuum System, for more information.
- **Flight tube and beam guide wire**—A field-free region (no additional accelerating forces are present) in which ions drift at a velocity inversely proportional to the square root of their masses. The voltage applied to the beam guide wire overcomes the dispersion effect from the ion source and refocuses ions on the detector.
- **Linear detector**—A device that detects ions that travel down the flight tube. The linear detector measures ion abundance over time and sends a signal to the digitizer for conversion.

  On the Voyager-DE PRO system, the linear detector is used in Linear mode only. It is not used in Reflector or PSD mode.

  Linear detectors are hybrid high-current detectors consisting of a single microchannel plate, a fast scintillator, and a photomultiplier. These detectors have superior tolerance for high ion currents.

NOTE: *High current detectors are standard on Voyager-DE systems with serial number 1128 and later, and Voyager-DE PRO systems with serial number 6007 and later.*

Additional parts of the Voyager-DE PRO

Parts unique to the Voyager-DE PRO mass spectrometer:

- **Collision Cell**—Provided with CID (Collision-Induced Dissociation) option for enhanced fragmentation in PSD analysis.
- **Reflector**—A single-stage gridded mirror that focuses energy. In reflector mode, a uniform electric field is applied to the mirror to reflect ions. Ion reflection:
  - Filters out neutral molecules
  - Corrects time dispersion due to initial kinetic energy distribution
  - Provides greater mass accuracy and resolution

  The single-stage design provides high mass resolution across a wide mass range and highly accurate mass measurements. Accurate calibration formulas for the single-stage reflector allow you to vary instrument conditions without degrading mass accuracy. Also, easy calibration of PSD data is facilitated by single-stage reflectors. For more information, see Chapter 8, PSD Analysis.
- **Reflector detector**—The reflector detector measures ions reflected by the mirror. The reflector detector sends a signal to the digitizer for conversion.

  Two multichannel plate reflector detectors optimized for response time are included.

- **Timed Ion Selector**—A device that allows only ions of a selected mass of interest to pass to the detector.

  The Timed Ion Selector (TIS) device is a Bradbury-Nielson gate positioned approximately 676 mm from the ion source. This prevents ions deflected by the gate from entering the reflector and reduces background noise in the detector in PSD experiments.

  When the Timed Ion Selector is turned on, voltage is applied to the Timed Ion Selector to deflect ions. At the time that corresponds to the ion of interest, voltage is turned off, and the ion of interest passes to the detector. After the ion passes through the Timed Ion Selector, voltage is turned on again.

1.5.3 Vacuum System

The Voyager-DE and Voyager-DE PRO Biospectrometry Workstations provide a high-vacuum environment for time-of-flight analysis. The high vacuum environment:

- Allows unobstructed ion drift
- Provides conditions needed to maintain the high potential difference between the ion source and ground

1

1.5.3.1 Voyager-DE Vacuum System

Vacuum chambers

The Voyager-DE Biospectrometry Workstation includes two vacuum chambers (Figure 1-15):

- Main source chamber
- Sample loading chamber

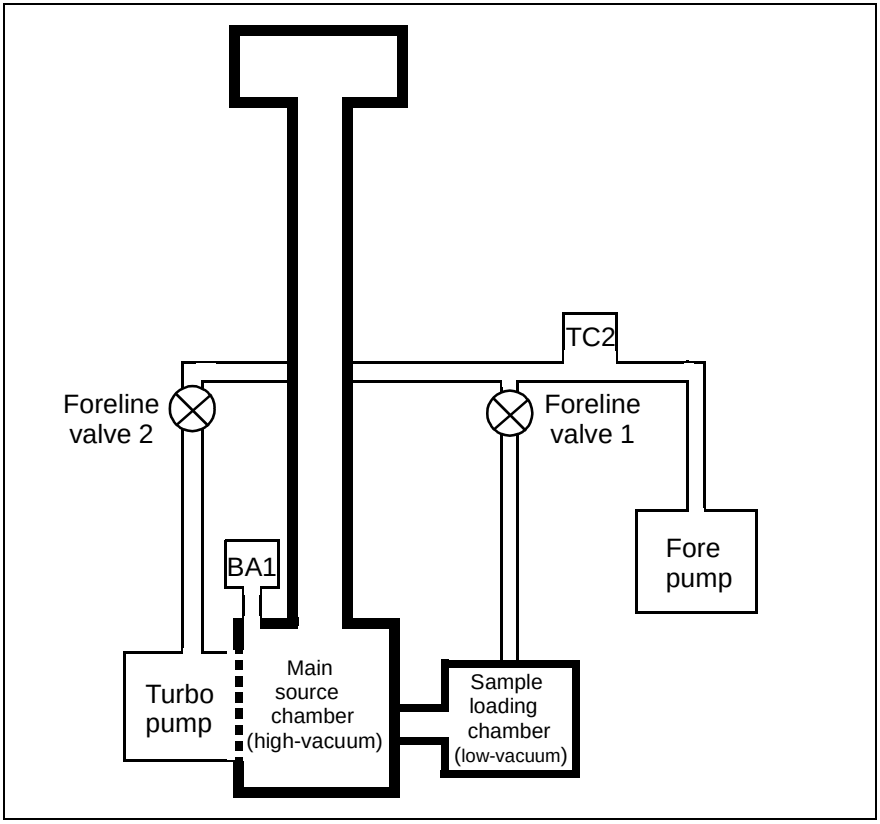

Figure 1-15 Voyager-DE Biospectrometry Workstation Vacuum Chambers

Vacuum pumps

Two vacuum pumps create the vacuum environment:

- **Fore pump**—Creates a vacuum in the sample loading chamber, creates a lower-than-atmospheric-pressure condition before the turbo pump starts, and provides backing pressure to the turbo pump.
- **Turbo pump**—Creates a high vacuum condition in the main source chamber.

Vacuum is maintained in the chambers by valves that isolate the chambers.

Vacuum gauges

The Voyager-DE Biospectrometry Workstation includes two vacuum gauges:

- **BA1 (Bayard-Alpert Gauge)**—Monitors pressure in the main source chamber.
- **TC2**—Monitors pressure in the sample chamber.

Readings from the vacuum gauges are displayed in the System Status Control page in the Instrument Control Panel. See Section 2.11, Checking System Status and Pressures.

1

1.5.3.2 Voyager-DE PRO Vacuum System

Vacuum chambers

The Voyager-DE PRO Biospectrometry Workstation includes three vacuum chambers (Figure 1-16):

- Main source chamber
- Sample loading chamber
- Mirror chamber

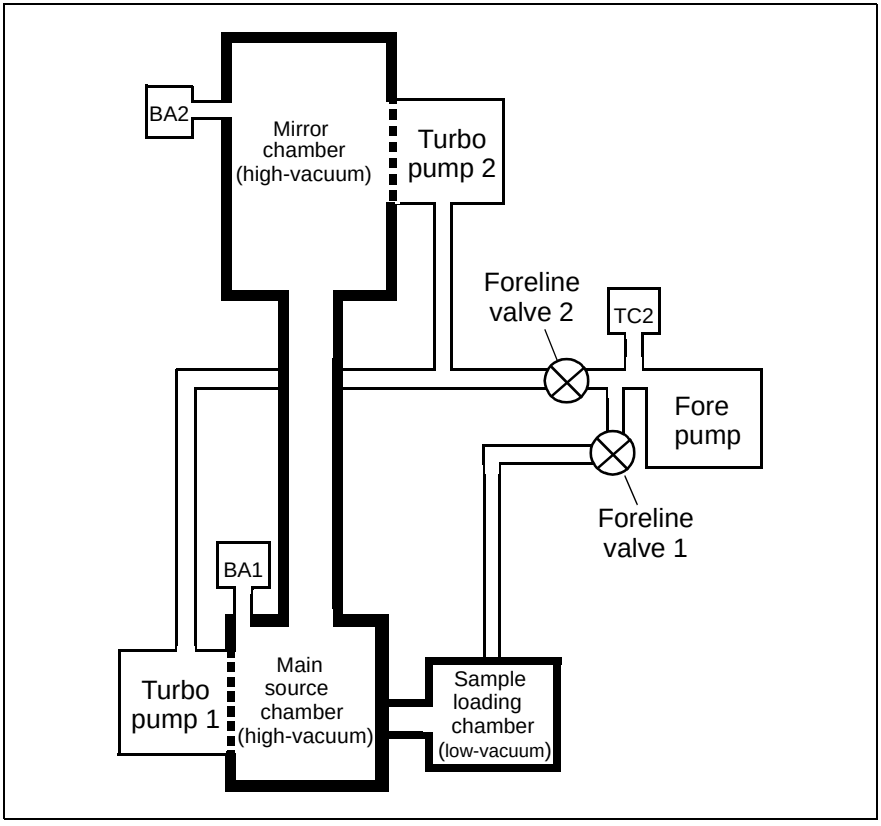

Figure 1-16 Voyager-DE PRO Biospectrometry Workstation Vacuum Chambers

Vacuum pumps

Three vacuum pumps create the vacuum environment:

- **Fore pump**—Creates a vacuum in the sample loading chamber, creates a lower-than-atmospheric-pressure condition before the turbo pumps start, and provides backing pressure to the turbo pumps.
- **Turbo pump 1**—Creates a high vacuum condition in the main source chamber.
- **Turbo pump 2**—Creates a high vacuum condition in the mirror chamber.

Vacuum is maintained in the main source chamber and sample loading chamber by valves that isolate the chambers. Vacuum is maintained in the mirror chamber by a differential pumping baffle.

Vacuum gauges

The Voyage-DE PRO Biospectrometry Workstation includes three vacuum gauges:

- **BA1 (Bayard-Alpert Gauge)**—Monitors pressure in the main source chamber.
- **BA2 (Bayard-Alpert Gauge)**—Monitors pressure in the mirror chamber.
- **TC2**—Monitors pressure in the sample loading chamber.

Readings from the vacuum gauges are displayed in the System Status Control page in the Instrument Control Panel. See Section 2.11, Checking System Status and Pressures for more information.

1

1.5.4 Computer Components

Hardware

The Voyager-DE and Voyager-DE PRO Biospectrometry Workstations include the following IBM®-compatible computer hardware components:

- Minimum computer configuration of Pentium® III 500 MHz CPU, 9 GB hard disk, and 128 MB RAM (random access memory)
- 3.5-inch HD (high-density), 1.44 MB floppy disk drive
- CD-ROM drive, integrated sound card
- 17-inch color monitor
- WIN95 Spacesaver Quiet Key, 104-key keyboard
- Microsoft-compatible mouse
- Control stick
- Optional laser printer

Software

The Voyager-DE and Voyager-DE PRO Biospectrometry Workstations include the following software components:

- Microsoft® Windows NT® version 4.0 or later
- Voyager software (includes Voyager Instrument Control Panel, Voyager Sequence Control Panel, and Data Explorer™ software)
- Microsoft Office 2000

Problems using screen savers

Do not enable screen savers on the Voyager computer. Screen savers use computer memory and may decrease system performance or cause other system problems.

1.6 Parts of the Voyager-DE STR System

This section describes:

- System components
- Mass spectrometer
- Vacuum system
- Front panel indicators
- Computer components

1.6.1 System Components

The Voyager-DE STR Biospectrometry Workstation is shown in Figure 1-17.

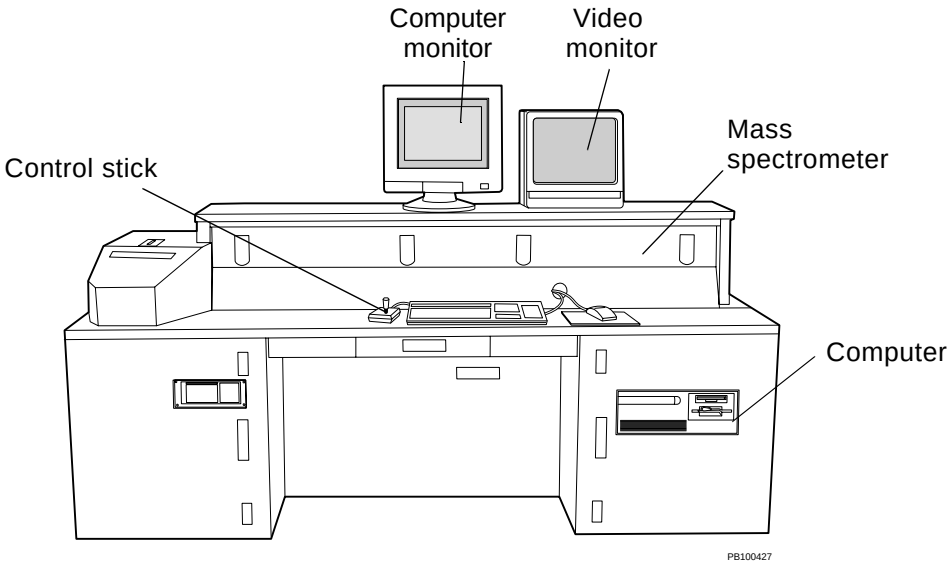

Figure 1-17 Voyager-DE STR Biospectrometry Workstation

Parts of the Voyager-DE STR system include:

- **Mass spectrometer**—A time-of-flight mass spectrometer, described in Section 1.6.2, Mass Spectrometer. The high-vacuum system of the mass spectrometer is described in Section 1.6.3, Vacuum System.
- **Computer/Data System**—A computer that operates the Voyager control software and the Voyager processing software. You control the mass spectrometer using the computer.
- **Digitizer (not shown)**—An analog-to-digital converter that allows the signal from the mass spectrometer to be transferred to the computer.

  The standard digitizer provided is a 4 GHz digitizer.
- **Video monitor**—A monitor that displays a real-time video image of the sample spot for examination during acquisition.
- **Control stick**—A peripheral device that controls position of the sample plate in the mass spectrometer. Allows you to start and stop acquisition and transfer data to the processing software.

NOTE: *You can also control sample position, start and stop acquisition, and transfer data using the Voyager Instrument Control Panel software.*

1.6.2 Mass Spectrometer

The parts of the Voyager-DE STR Biospectrometry mass spectrometer are shown in Figure 1-18.

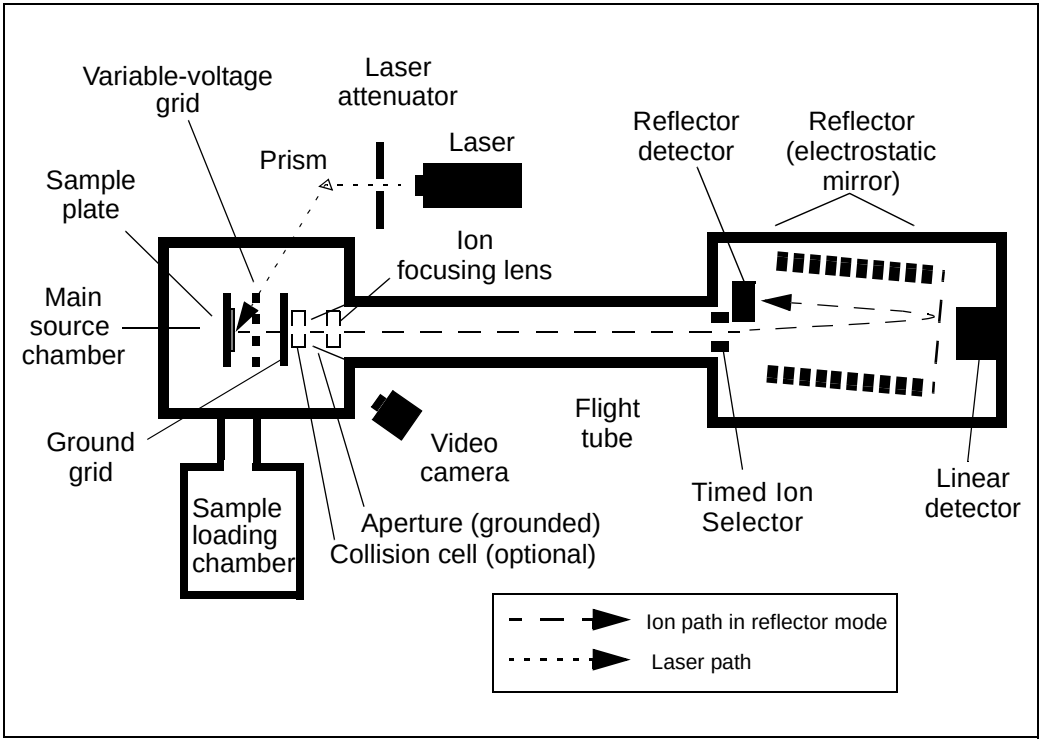

Figure 1-18 Voyager-DE STR Mass Spectrometer

NOTE: *Voyager-DE STR models with serial number 4153 and earlier include a Beam Guide Wire instead of an Ion Focusing lens.*

1

Parts of the mass spectrometer

The Voyager-DE STR mass spectrometer includes:

- **Laser, attenuator, and prism**—A nitrogen laser that operates at 337 nm and ionizes sample. It produces 3-nanosecond-duration pulses. Laser rate can be set to Default (3 Hz) or Optimized (see page 5-26). Maximum possible rate is 20 Hz; actual rate depends on the digitizer installed and the digitizer Bin Size setting.

NOTE: *Optimized laser rate is available only on Voyager-DE STR systems with serial number 4161 and later.*

  The laser attenuator varies the intensity of the laser beam reaching the sample.

  The prism deflects the laser beam into the ion source.

- **Ion Source**—A high voltage region used to accelerate ions. Includes:
  - **Sample plate and sample stage**—An area supplied with voltage (0 to 25,000 V) for acceleration of ions into the flight tube.
  - **Variable-voltage grid**—A grid supplied with additional voltage to fine-tune ion acceleration.
  - **Ground grid**—Ground surface for formation of potential gradient.
  - **Collision cell**—Provided with CID (Collision-Induced Dissociation) option for enhanced fragmentation in PSD analysis.
  - **Grounded aperture**—Entrance to flight tube.

  For more information on the ion source and voltages, see Section 5.4.4.2, Setting Accelerating Voltage, and Section 5.4.3.5, Optimizing Grid Voltage%.

- **Video camera**—A camera that displays a real-time sample image (100 times magnification) on the video monitor.
- **Vacuum system**—A pumping system and a sealed enclosure that creates and maintains a high-vacuum environment for unobstructed ion drift. For more information, see Section 1.6.3, Vacuum System.
- **Flight tube and ion focusing lens**—A field-free region (no additional accelerating forces are present) in which ions drift at a velocity inversely proportional to the square root of their masses. The fixed voltage (50 percent of the Accelerating Voltage) applied to the ion focusing lens refocuses ions on the detector.

NOTE: *Voyager-DE STR models with serial number 4153 and earlier include a beam guide wire instead of an ion focusing lens.*

- **Linear detector**—A device that detects ions that travel down the flight tube. The linear detector measures ion abundance over time and sends a signal to the digitizer for conversion.

  The linear detector is used in Linear mode only. It is not used in Reflector or PSD mode.

  Linear detectors are hybrid high-current detectors consisting of a single microchannel plate, a fast scintillator, and a photomultiplier. These detectors have superior tolerance for high ion currents.

NOTE: *High current detectors are standard on Voyager-DE STR systems with serial numbers 4112, 4113, and 4116 and later.*

- **Reflector**—A single-stage, gridded mirror that focuses energy. In reflector mode, a uniform electric field is applied to the mirror to reflect ions. Ion reflection:
  - Filters out neutral molecules
  - Corrects time dispersion due to initial kinetic energy distribution
  - Provides greater mass accuracy and resolution

  The single-stage design provides high mass resolution across a wide range and highly accurate mass measurements. Accurate calibration formulas for the single-stage reflector allow the user to vary instrument conditions without degrading mass accuracy. Also, easy calibration of PSD data is facilitated by single-stage reflectors. For more information, see Chapter 8, PSD Analysis.

- **Reflector detector**—The reflector detector measures ions reflected by the mirror. The reflector detector sends a signal to the digitizer for conversion.

  Two multichannel plate reflector detectors optimized for response time are included.

- **Timed Ion Selector**—A device that allows only ions of a selected mass of interest to pass to the detector.

  The Timed Ion Selector (TIS) device is a Bradbury-Nielson gate positioned approximately 676 mm from the ion source. This prevents ions deflected by the gate from entering the reflector and reduces background noise in the detector in PSD experiments.

  When the Timed Ion Selector is turned on, voltage is applied to the Time Ion Selector to deflect ions. At the time that corresponds to the ion of interest, voltage is turned off, and the ion of interest passes to the detector. After the ion passes through the Timed Ion Selector, voltage is turned on again.

1.6.3 Vacuum System

Overview

The Voyager-DE STR Biospectrometry Workstation provides a high-vacuum environment for time-of-flight analysis. The high vacuum environment:

- Allows unobstructed ion drift
- Provides conditions needed to maintain the high potential difference between the ion source and ground

Vacuum chambers

The Voyager-DE STR Biospectrometry Workstation includes three vacuum chambers (Figure 1-19):

- Main source chamber
- Sample loading chamber
- Mirror chamber

Vacuum pumps

Three vacuum pumps create the vacuum environment:

- **Fore pump**—Creates a vacuum in the sample loading chamber, creates a lower-than-atmospheric-pressure condition before the turbo pumps start, and provides backing pressure to the turbo pumps.
- **Turbo pump 1**—Creates a high vacuum condition in the main source chamber.
- **Turbo pump 2**—Creates a high vacuum condition in the mirror chamber.

Vacuum is maintained in the main source chamber and sample loading chamber by valves that isolate the chambers. Vacuum is maintained in the mirror chamber by a differential pumping baffle.

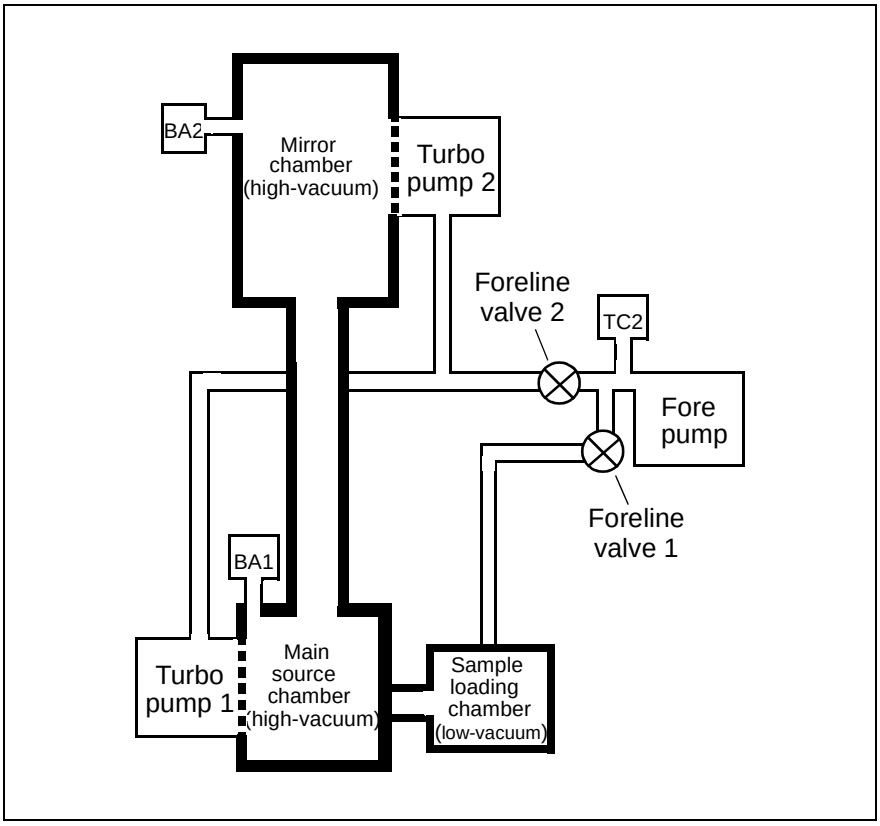

Figure 1-19 Voyager-DE STR Biospectrometry Workstation Vacuum Chambers (Top View)

Vacuum gauges

The Voyager-DE STR Biospectrometry Workstation include three vacuum gauges:

- **BA1 (Bayard-Alpert Gauge)**—Monitors pressure in the main source chamber.
- **BA2 (Bayard-Alpert Gauge)**—Monitors pressure in the mirror chamber.
- **TC2**—Monitors pressure in the sample loading chamber.

Readings from the vacuum gauges are displayed in the System Status Control page in the Instrument Control Panel. See Section 2.11, Checking System Status and Pressures, for more information.

1.6.4 Front Panel Indicators

The front panel of the Voyager-DE STR system is shown in Figure 1-20.

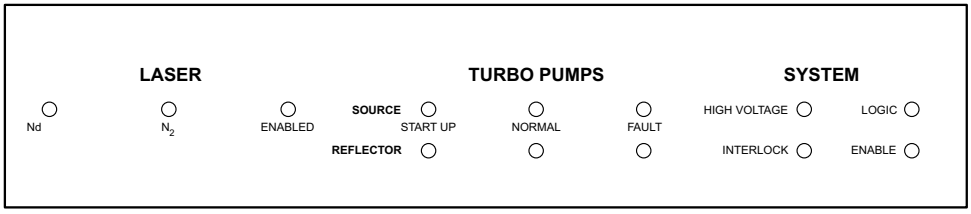

Figure 1-20 Voyager-DE STR Front Panel Indicators

Front panel indicators are described in Table 1-1.

Table 1-1 Front Panel Indicators

Indicator	Indication When Lit
Laser	
Laser N_2 (Red)	Laser power is on.
Enabled (Red)	Laser is firing.
Turbo Pumps	
Startup (Yellow)	One or both turbo pumps are initializing.
Normal (Green)	Both turbo pumps are operating at normal speed.
Fault (Red)	One or both turbo pumps are off. Should not be lit during normal operation.
System	
High Voltage	High voltage is on.
Interlock	An interlock error (door open or panel off). Automatically disables laser and high voltage.
Logic	Internal control board in mass spectrometer has been powered up.
Enabled	Computer is controlling mass spectrometer.

1.6.5 Computer Components

Hardware

The Voyager-DE STR Biospectrometry Workstation includes the following IBM®-compatible computer hardware components:

- Minimum computer configuration of Pentium® III 500 MHz CPU, 9 GB hard disk, and 128 MB RAM (random access memory)
- 3.5-inch HD (high density), 1.44 MB floppy disk drive
- CD-ROM drive, integrated sound card
- 17-inch color monitor
- WIN95 Spacesaver Quiet Key, 104-key keyboard
- Microsoft-compatible mouse
- Control stick
- Optional laser printer

Software

The Voyager-DE STR Biospectrometry Workstation includes the following software components:

- Microsoft® Windows® NT version 4.0 or later
- Voyager software (includes Voyager Instrument Control Panel, Voyager Sequence Control Panel, and Data Explorer software)
- Microsoft Office 2000

Problems using screen savers

Do not enable screen savers on the Voyager computer. Screen savers use computer memory and may decrease system performance or cause other system problems.

1

1.7 Software Overview

The Voyager Biospectrometry Workstation software includes control software (Voyager Instrument Control Panel, Voyager Sequence Control Panel) and post-processing software (Data Explorer software):

- **Voyager Instrument Control Panel**—Controls the mass spectrometer for calibration and acquisition of single samples.
- **Voyager Sequence Control Panel**—Works in conjunction with the Instrument Control Panel to allow you to acquire multiple samples using different instrument settings (.BIC) files.
- **Data Explorer processing software**—Allows post-processing analysis of mass spectral data.

1.7.1 Control Software (Instrument and Sequence Control Panels)

Instrument Control Panel

The Voyager Instrument Control Panel allows you to directly control the Voyager mass spectrometer to acquire mass spectra one at a time.

The Instrument Control Panel provides the following features:

- Mass calibration, mass resolution calculator, and signal-to-noise ratio calculator functions.
- Direct control of instrument hardware including high voltages, load/eject, and acquisition start/stop.
- Real-time viewing and manipulation of mass-calibrated (default or external calibration) and peak-labeled spectral traces as data is acquired with Acqiris®, LeCroy®, and Signatec digitizers.

- Ability to zoom in on up to four different areas of a trace.
- Ability to acquire single samples in Manual or Automatic Control mode.
- Manual accumulation of mass spectra from multiple acquisitions into a single data file.

The Instrument Control Panel (Figure 1-21) is displayed when you start the Voyager Control Panel software.

The Instrument Control software is described in Chapter 4, Voyager Instrument Control Panel Basics.

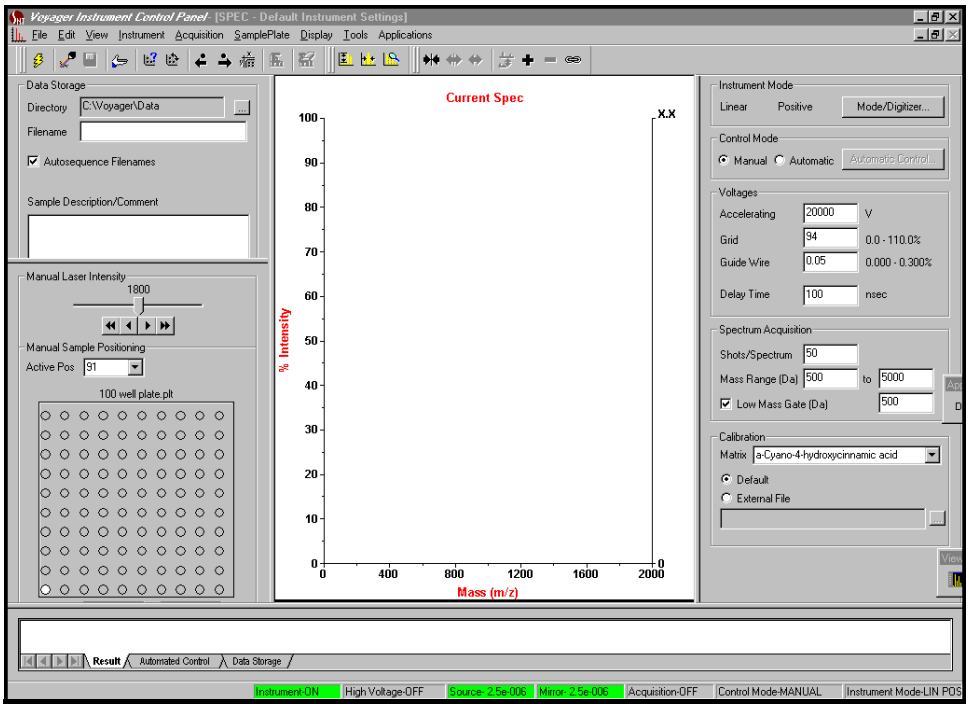

Figure 1-21 Voyager Instrument Control Panel

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Sequence Control Panel

The Sequence Control Panel works with the Instrument Control Panel to allow you to acquire multiple samples using different instrument settings (.BIC) files.

The Sequence Control Panel (Figure 1-22) is displayed when you start the Voyager Sequence Control software, or click a toolbar button in the Instrument Control Panel.

The Sequence Control software and how the Instrument and Sequence Control panels interact are described in Chapter 4, Voyager Instrument Control Panel Basics.

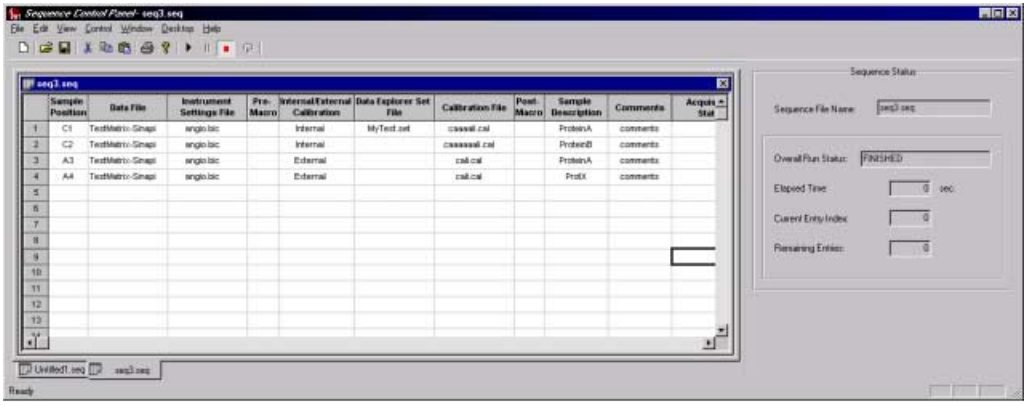

Figure 1-22 Sequence Control Panel

Data (.DAT) file format

Data (.DAT) file format incorporates all information into a single file about how a data file is acquired and processed. This format improves data processing and data storage efficiency. Voyager .DAT files can contain one or more spectra.

You can also store parameters in separate files by extracting information from a .DAT file as needed for use with other files. For more information, *the Data Explorer Software User's Guide,* Section 1.2, File Formats and Types.

1.7.2 Post-Processing Software (Data Explorer™)

The Data Explorer™ software is a powerful software module that allows you to graphically and interactively manipulate spectral and chromatogram (multispectrum) data.

Using the Data Explorer software, you can:

- Automatically and manually calibrate spectrum data.
- Set peak detection parameters and custom labels for regions of the trace.
- Calculate centroid mass, elemental composition, theoretical isotope distributions, resolution, and signal-to-noise ratio.
- Customize windows, toolbars, and traces.

Figure 1-23 shows the Data Explorer main window.

The Data Explorer processing software is described in the *Data Explorer™ Software User's Guide*.

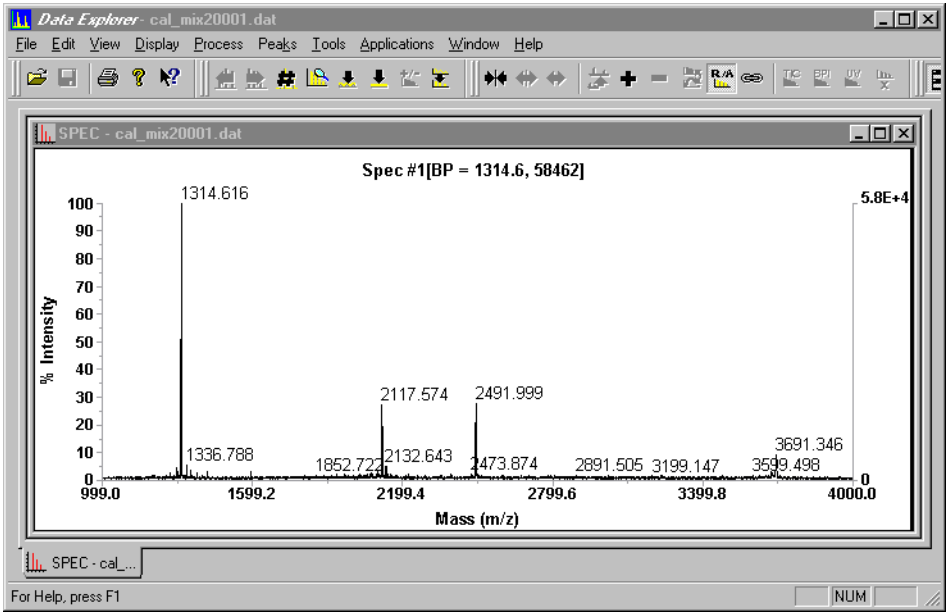

Figure 1-23 Data Explorer Window

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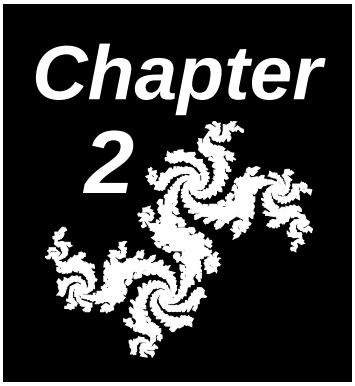
2 Installing the Voyager Biospectrometry Workstations



This chapter contains the following sections:

2.1 Installing the System 2-2

2.2 Selecting the Site 2-2

2.3 Connecting Voyager-DE and Voyager-DE PRO Workstations 2-8

2.4 Connecting the Voyager-DE STR Workstation 2-23

2.5 Installing Software 2-29

2.6 Hardware Configuration 2-35

2.7 Aligning the Sample Plate 2-46

2.8 Running OptiPlate to Optimize Mass Accuracy 2-53

2.9 Resetting the Optional External Laser 2-71

2.10 Startup and Shutdown 2-73

2.11 Checking System Status and Pressures 2-76

2.1 Installing the System

Your Voyager Biospectrometry Workstation is initially installed by an Applied Biosystems Technical Representative. Do not use the Voyager system before it is properly installed. Use the information in this chapter if you move the Voyager system after initial installation.

2.2 Selecting the Site

This section includes:

- Voyager-DE and Voyager-DE PRO Workstations
- Voyager-DE STR Workstation

2.2.1 Voyager-DE and Voyager-DE PRO Workstations

In this section

This section includes:

- Required space
- Weight
- Power/voltage requirements
- Selecting input voltage

Required space

The Voyager-DE and Voyager-DE PRO mass spectrometers measure:

- 27 inches (69 cm) deep
- 25 inches (64 cm) wide
- 65 inches (165 cm) high, includes cabinet and flight tube

Allow an additional 10 inches (26 cm) above the top of the flight tube for service.

Allow 4 inches (10 cm) at the back of the mass spectrometer for fans and cables.

Allow 4 inches (10 cm) on the right side of the mass spectrometer for cables.

Allow an additional 40 inches (102 cm) to the right side of the mass spectrometer for:

- Video monitor for sample chamber
- Computer, monitor, control stick, and keyboard
- Printer
- Optional oscilloscope or external digitizer

Weight

The Voyager-DE system weighs approximately 250 pounds (113 kg).

The Voyager-DE PRO system weighs approximately 350 pounds (159 kg).

Power/voltage requirements

The Voyager-DE and Voyager-DE PRO systems require:

Location	Power/Voltage Required
US	120 V, 20 A, single-phase dedicated circuit, 60 Hz power source
European	230 V, 10 A, single-phase, 50/60 Hz power source

You must be able to quickly disconnect the main power supply to the instrument, if necessary.

In addition, you need grounded outlets for the:

- Computer CPU
- Computer monitor
- External digitizer or oscilloscope (if your system includes)
- Printer (optional)

Selecting input voltage

If the system is set for a voltage that does not accommodate your power source, select a different input voltage setting on the Voyager system. If you select a different input voltage setting, install fuses of the proper rating. Extra fuses for different voltage settings are supplied with the system.

Perform this procedure before plugging in and powering up the system for the first time. This procedure is required for Voyager-DE and Voyager-DE PRO systems only.

To select input voltage:

1. Remove the power cord from the mass spectrometer.
2. Carefully remove the voltage selector/fuse holder from the system (Figure 2-1) using a small flat-blade screw driver.
3. Carefully remove the voltage selector from the holder and insert the selector with the proper voltage displayed in the window of the holder.

CAUTION

The plastic tabs that hold the voltage selector in place are fragile. Do not exert force when removing this piece.

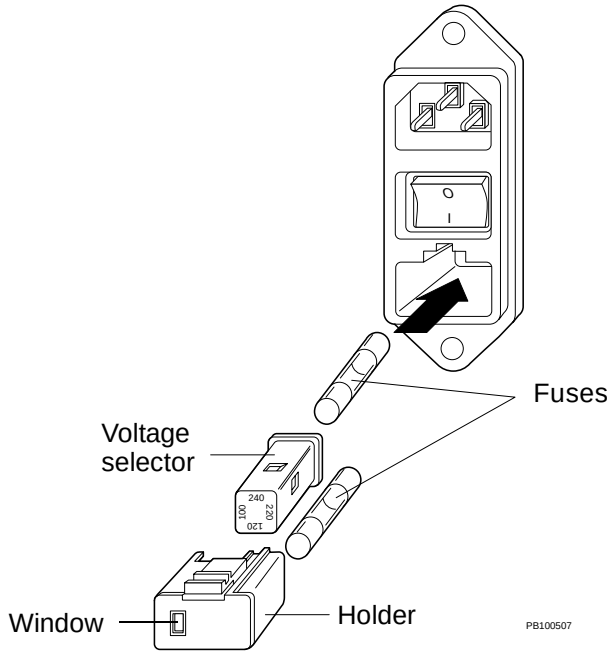


Figure 2-1 Selecting Input Voltage and Installing Fuses

WARNING

FIRE HAZARD. *Using a fuse of the wrong type or rating can cause a fire. Replace fuses with those of the same type and rating.*

4. Insert two fuses of the proper rating for the selected voltage.

Electrical Rating	
Volts/Amps	**Fuse** (5 x 20 mm)
100 V~10A	T10A 250V
120 V~10A	T10A 250V
220 V~6.3A	T6.3A 250V
240 V~5A	T5A 250V

5. Insert the voltage selector/fuse holder into the receptacle.

6. Plug in the mass spectrometer. Connect devices to the mass spectrometer as described in Section 2.3, Connecting Voyager-DE and Voyager-DE PRO Workstations, then power up.

2.2.2 Voyager-DE STR Workstation

In this section

This section includes:

- Required space
- Weight
- Power/voltage requirements

Required space

The Voyager-DE STR system is a floor-standing unit that measures:

- 34 inches (87 cm) deep
- 94 inches (239 cm) wide
- 46 inches (117 cm) high

The Voyager-DE STR Workstation is constructed on a rolling base. For service, you must have enough space to pull the workstation forward 24 inches (61 cm) to allow access at the rear of the workstation. Allow an additional 18 inches (48 cm) on the sides for service access.

Weight

The Voyager-DE STR system weighs approximately 1,100 pounds (499 kg).

Power/voltage requirements

The Voyager-DE STR systems can be configured to operate at 110 V (voltage is ±10%).

Operating voltage is selected by an internal jumper, which must be set by an Applied Biosystems Technical Representative.

NOTE: *Workstations are shipped with the internal voltage selector jumper disconnected.*

The Voyager-DE STR systems require a single-phase, 50/60 Hz power source at one of the following voltages:

- 100 V, 16 A
- 120 V, 16 A
- 220 V, 10 A
- 240 V, 10 A

CAUTION

Before operation, internal jumpers must be set to accommodate your power source. Do not plug in or power up the Voyager-DE STR Biospectrometry Workstation unless it has been configured correctly by an Applied Biosystems Technical Representative.

In addition, you need grounded outlets for:

- Computer monitor
- External digitizer (if your system includes one)
- Printer (optional)

2.3 Connecting Voyager-DE and Voyager-DE PRO Workstations

This section describes:

- Side panel diagrams for mass spectrometer and computer
- Connecting the mass spectrometer to the computer
- Connecting the Signatec 500 MHz digitizer board
- Connecting the LSA1000 LeCroy digitizer
- Connecting the Acqiris digitizers
- Connecting the Tektronix® oscilloscope
- Connecting the video monitor
- Connecting devices to the computer

Before connecting devices to the mass spectrometer, power down the mass spectrometer.

When you plug in cables, make sure they are securely connected and screwed in place, if connectors include screws.

2.3.1 Side Panel Diagrams for Mass Spectrometer and Computer

This section includes diagrams for:

- Mass spectrometer
- Computer

Mass spectrometer

Figure 2-2 shows the connections on the right side panel of the Voyager-DE and Voyager-DE PRO mass spectrometer cabinet.

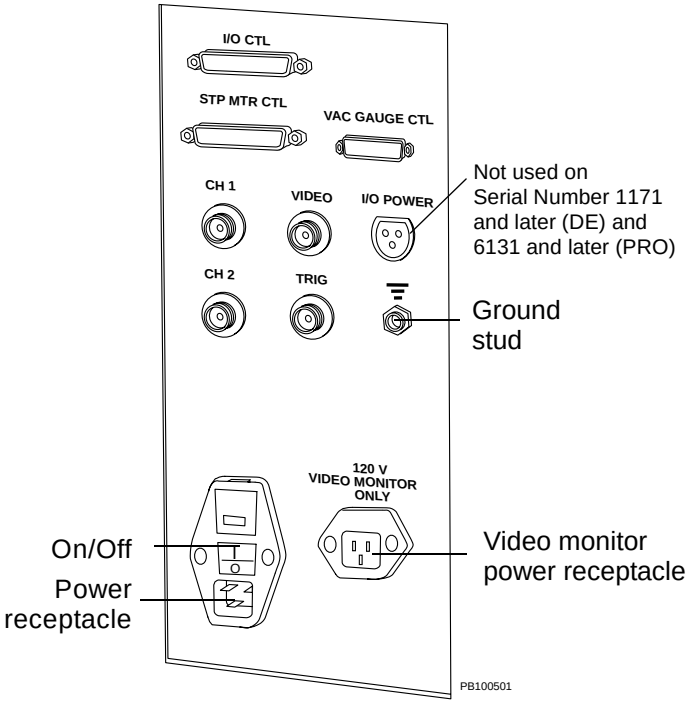


Figure 2-2 Right Side Panel of Voyager-DE and Voyager-DE PRO Mass Spectrometer

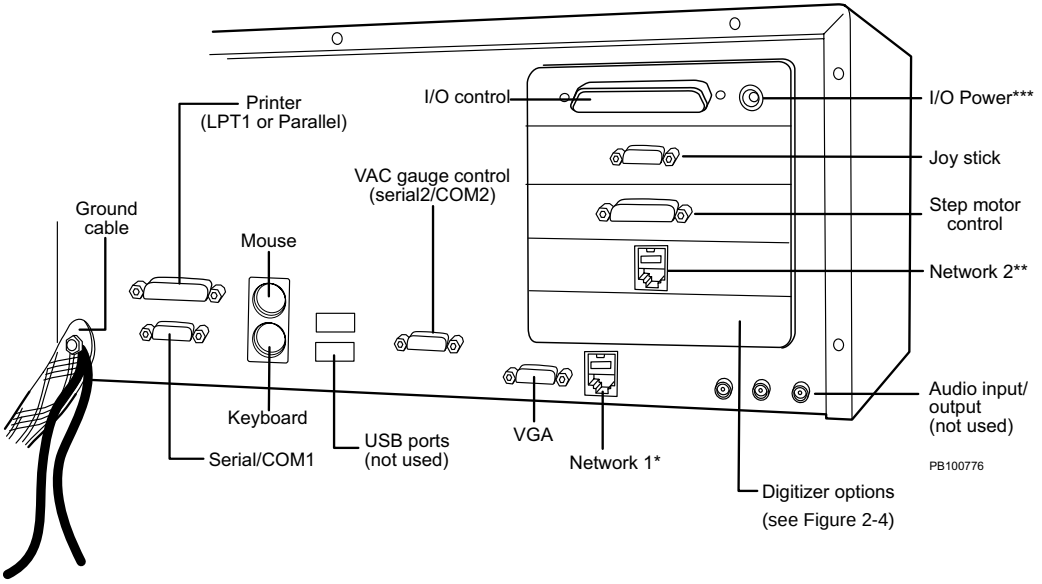
Computer

Depending on your digitizer option, the computer has one of the following installed when you receive it:

- Signatec 500 MHz digitizer board
- Dedicated ethernet for LeCroy LSA 1000 digitizer
- Acqiris 500 MHz and 2 GHz digitizer boards
- GPIB board for oscilloscope option

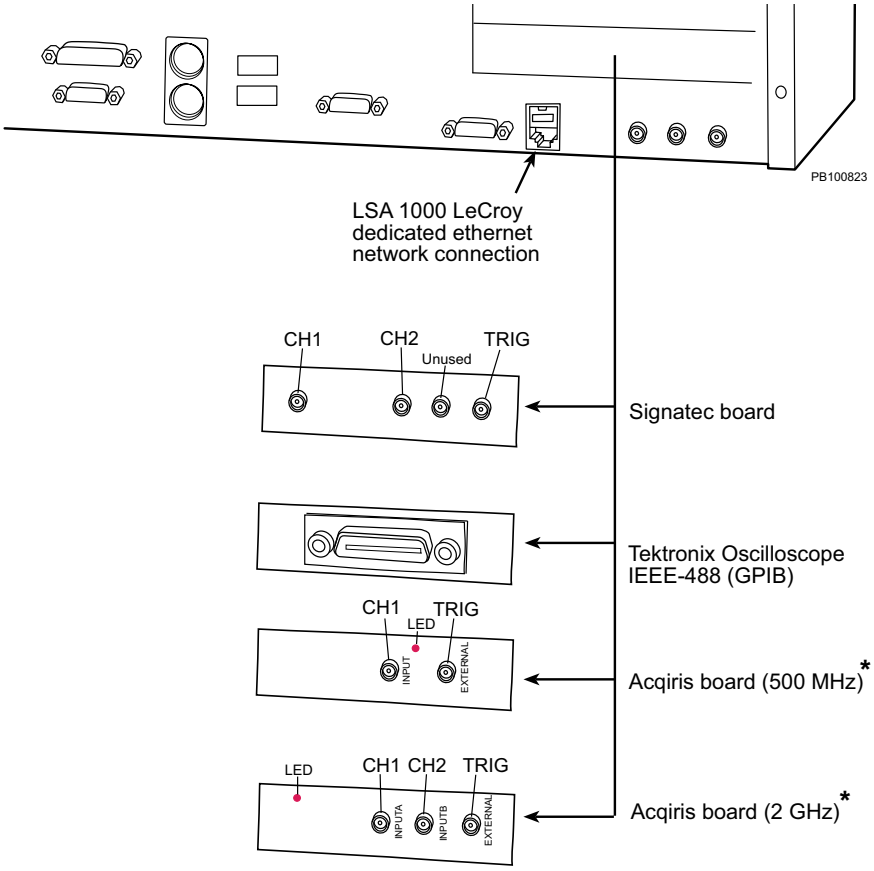
Figure 2-3 shows the rear panel of the computer. Figure 2-4 shows the boards that can be installed in your computer, depending on the digitizer option selected for your system.

NOTE: *The computer layout may change without notice. Boards may be located in slots that differ from those shown in Figure 2-3 and Figure 2-4. The braided ground cable connection may be located in a different position.*



* Network 1—Connection for LAN if your system includes a Signatec digitizer.
** Network 2—Connection for LAN if your system includes a LeCroy, Tektronix, or Acqiris digitizer.
*** I/O power not used on Serial Number 1171 and later (DE) and 6131 and later (PRO).

Figure 2-3 Rear Panel of Computer



* Acqiris boards require a 90V BNC spark gap and 3dB BNC attenuator between the cable and the board.

Figure 2-4 Digitizer Options

NOTE: *The network connection to which the LeCroy digitizer is connected requires a specific IP address. On some systems, the LeCroy digitizer may be connected to a network connector other than the one shown in Figure 2-4. Do not change this connection without consulting an Applied Biosystems technical representative.*

2.3.2 Connecting the Mass Spectrometer to the Computer

Refer to the following table when you connect the mass spectrometer to the computer.

Connection on side panel of mass spectrometer (see Figure 2-2)	Connection on rear panel of computer (see Figure 2-3)	Cable
I/O CTL	I/O CTL	I/O Ctl
STP MTR CTL	STP MTR CTL (25-pin)	25-pin mesh cable
Ground stud	Nut below LPT1 or the tab extension on top of the computer cover	Braided screw-on cable
VAC GAUGE CTL	Serial 2 or COM 2 (9-pin)	Vac Gg Ctl
I/O POWER, 3-pin round connector (Not used on serial number 1171 and later (DE) and 6131 and later (PRO))	I/O power +5 V, 1-pin round connector	I/O power

2.3.3 Connecting the Signatec 500 MHz Digitizer Board

If you have an oscilloscope or an external digitizer on your system, disregard this section.

CAUTION

Do not use the Signatec 500 MHz digitizer board without digitizer signal protection circuits (blue boxes on cables). Operation without digitizer signal protection circuits will result in damage to the internal digitizer.

Refer to the following table when you connect the Signatec 500 MHz digitizer board to the mass spectrometer:

Connection on right side panel of mass spectrometer (see Figure 2-2)	**Connection on rear panel of computer (see Figure 2-4)**	**Cable**
TRIG	Trig	Trig SMA to Trig BNC
CH 1	CH 1	Ch 1 SMA to Ch 1 BNC between blue box and computer. Ch1 BNC to Ch1 BNC between blue box and mass spectrometer. Ground wire to computer chassis ground screw.

Connection on right side panel of mass spectrometer (see Figure 2-2)	Connection on rear panel of computer (see Figure 2-4)	Cable
CH 2 (Voyager-DE PRO only)	CH 2	Ch 2 SMA to Ch 2 BNC between blue box and computer. Ch 2 BNC to Ch 2 BNC between blue box and mass spectrometer. Ground wire to computer chassis ground screw.

2.3.4 Connecting the LSA1000 LeCroy Digitizer

If you have an oscilloscope or an internal digitizer on your system, disregard this section.

This section describes the connections for the LSA1000 LeCroy digitizer that has been previously installed by an Applied Biosystems Technical Representative.

NOTE: *If your LeCroy digitizer has not been installed by an Applied Biosystems Technical Representative, contact Applied Biosystems before installing or connecting it to your system.*

Figure 2-5 shows the connections on the LSA1000 LeCroy digitizer.

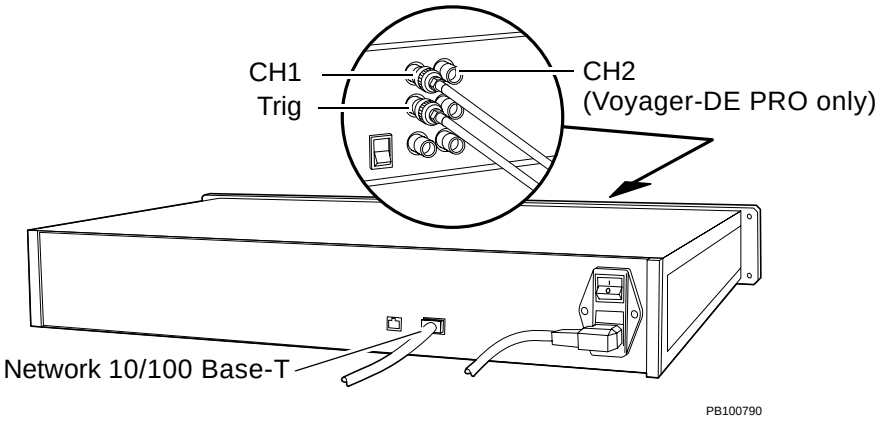


Figure 2-5 LeCroy Digitizer Connections

Refer to the following table when you connect the LSA1000 LeCroy digitizer board to the mass spectrometer:

Connection on digitizer (see Figure 2-5)	Connection on right side panel of mass spectrometer (see Figure 2-2)	Connection on rear panel of computer (see Figure 2-4)	Cable
10/100 Base-T	None	Integrated network connection (not add-in network card)	Cross-over network cable— 100 Base-T (supplied)
TRIG1	TRIG	None	Trig BNC
CH1	CH 1	None	Ch 1 BNC
CH2 (Voyager-DE PRO only)	CH 2	None	Ch 2 BNC

2.3.5 Connecting the Acqiris Digitizers

This section describes how to connect the Acqiris 500 MHz and 2 GHz digitizer boards to the Voyager-DE and Voyager-DE PRO mass spectrometers.

If you have an oscilloscope or an external digitizer on your system, disregard this section.

Refer to the following table when you connect the Acqiris 500 MHz digitizer board to the mass spectrometer:

Connection on right side panel of mass spectrometer (see Figure 2-2)	**Connection on rear panel of computer (see Figure 2-4)**	**Cable**
TRIG	EXTERNAL	Trig BNC on mass spectrometer to EXTERNAL BNC on board.
CH 1	INPUT	Ch1 BNC on mass spectrometer to 90V BNC Spark Gap to 3d BNC Attenuator to INPUT BNC on board. Ground wire to computer chassis ground screw.

Refer to the following table when you connect the Acqiris 2 GHz digitizer board to the mass spectrometer:

Connection on right side panel of mass spectrometer (see Figure 2-2)	Connection on rear panel of computer (see Figure 2-4)	Cable
TRIG	EXTERNAL	Trig BNC on mass spectrometer to EXTERNAL BNC on board.
CH 1	INPUTA	Ch1 BNC on mass spectrometer to 90V BNC Spark Gap to 3d BNC Attenuator to INPUTA BNC on board. Ground wire to computer chassis ground screw.
CH 2 (DE-PRO only)	INPUTB	Ch2 BNC on mass spectrometer to 90V BNC Spark Gap to 3d BNC Attenuator to INPUTB BNC on board. Ground wire to computer chassis ground screw.

2.3.6 Connecting the Tektronix Oscilloscope

If you have an internal digitizer board or external digitizer in your computer, disregard this section.

Figure 2-6 shows the front panel of the oscilloscope.

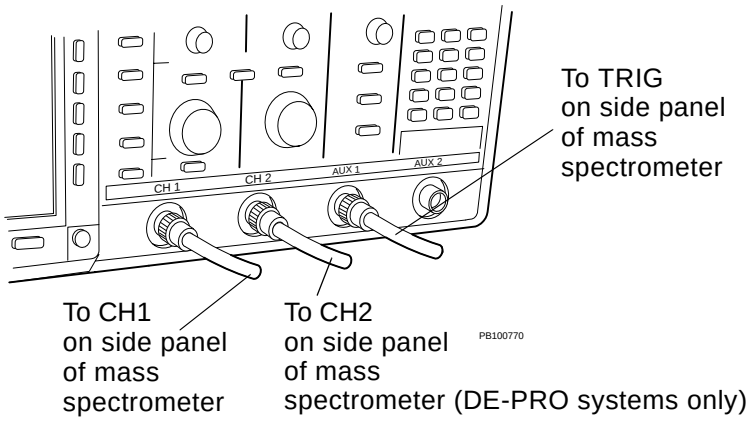


Figure 2-6 Oscilloscope Connections

Refer to the following table when you connect the oscilloscope to the mass spectrometer and to the computer.

Connection on oscilloscope (see Figure 2-6)	Connection on right side panel of mass spectrometer (see Figure 2-2)	Connection on rear panel of computer (see Figure 2-3)	Cable
AUX1 or CH3	TRIG	None	Trig BNC
CH1	CH 1	None	Ch 1 BNC with oscilloscope end
CH2 (Voyager-DE PRO only)	CH 2	None	Ch 2 BNC with oscilloscope end
IEEE-488 (GPIB) (on rear of oscilloscope, not shown)	None	IEEE-488 (GPIB)	IEEE-488

2.3.7 Connecting the Video Monitor

Figure 2-7 shows the rear panel of the video monitor.

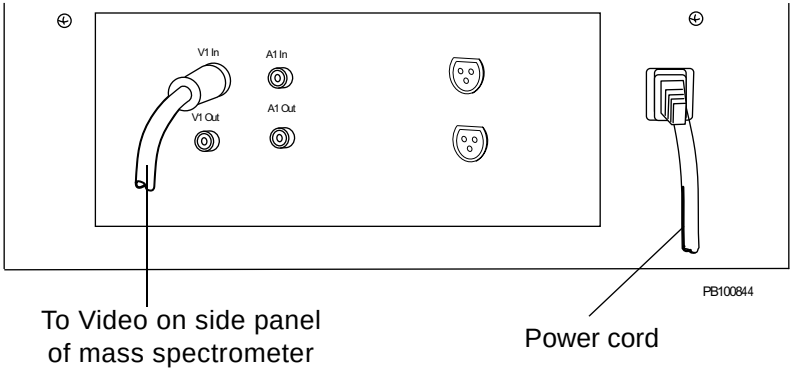


Figure 2-7 Rear Panel of Video Monitor

Refer to the following table when connecting the video monitor to the Voyager-DE and Voyager-DE PRO workstations.

Connection on rear panel of video monitor (see Figure 2-7)	Connection on side panel of Mass Spectrometer (see Figure 2-2)	Cable
Video In	VIDEO	BNC with video adapter
Power receptacle	VIDEO MONITOR ONLY	Power

2.3.8 Connecting Devices to the Computer

Refer to the following table when connecting devices to the Voyager-DE and Voyager-DE PRO computers.

Device	Connection on rear panel of computer (see Figure 2-3 on page 2-10)
Keyboard	5-pin round connector
VGA monitor	12-pin connector (3 rows of pins)
Control stick	Top 15-pin connector ***NOTE:*** *There are two 15-pin connectors. You must connect the control stick to the top connector.*
Mouse	5-pin round connector
Printer	LPT1 or Parallel

2.4 Connecting the Voyager-DE STR Workstation

This section includes:

- Rear panel connections
- Keyboard, mouse, and control stick
- Digitizer
- Video monitor
- Oscilloscope, video monitor, and computer monitor

Rear panel connections

Figure 2-8 shows the connections and the on/off switch (main power circuit breaker) on the rear panel of the mass spectrometer cabinet.

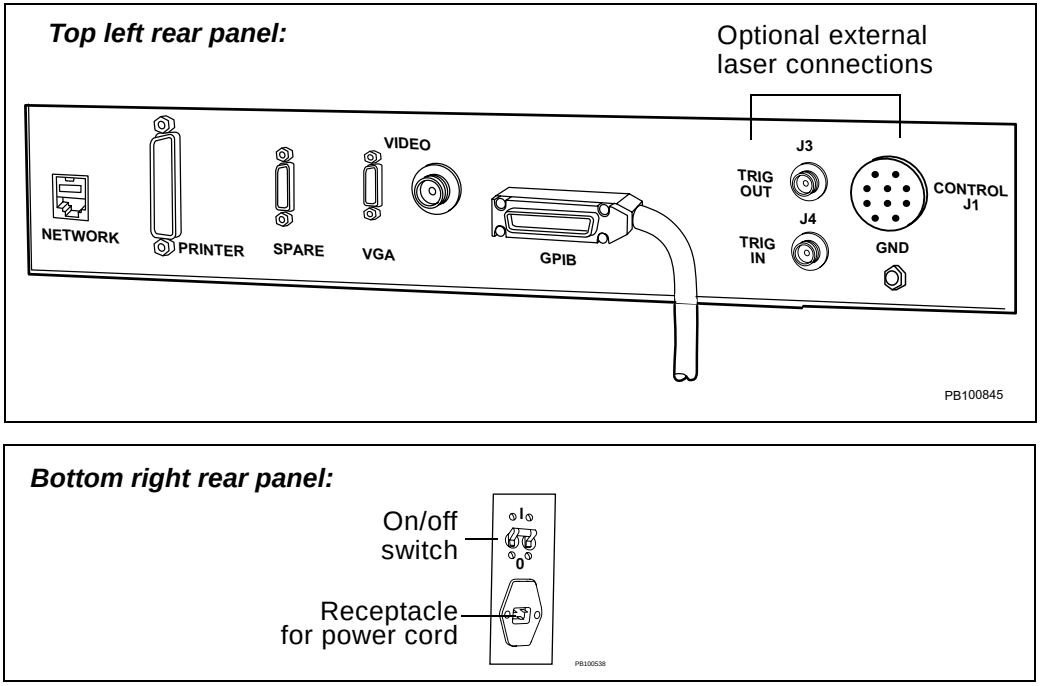


Figure 2-8 Rear Panel of Voyager-DE STR Mass Spectrometer, Component Connections, and On/Off Switch

Keyboard, mouse, and control stick

Thread cables for the computer keyboard, mouse, and control stick through the circular hole in the front panel of the workstation.

CAUTION

Power down before making connections.

Open the right side cabinet and slide out the computer shelf. Connect devices as shown in Figure 2-9.

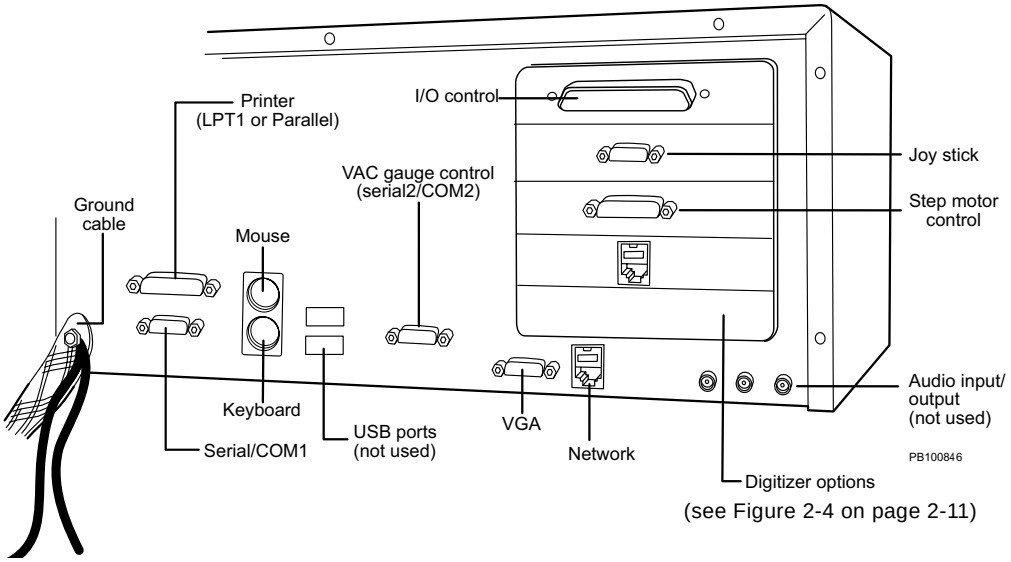


Figure 2-9 Computer Connections for Voyager-DE STR Mass Spectrometer

NOTE: *The computer layout may change without notice. Boards may be located in slots that differ from those shown in Figure 2-9. The braided ground cable connection may be located in a different position.*

Connections are listed below.

Device	Connection
Keyboard	5-pin round connector
VGA monitor	12-pin connector (3 rows of pins)
Control stick	15-pin connector
Mouse	COM1 or 5-pin round connector
Printer	LPT1 or Parallel

Digitizer

Depending on your digitizer option, the computer will have one of the following installed when you receive it:

- GPIB board for the oscilloscope
- Acqiris 2 GHz digitizer board

Figure 2-9 on page 2-24 shows the rear panel of the computer. Figure 2-4 on page 2-11 shows the location of the two boards that can be installed in your computer, depending on the digitizer option selected for your system.

NOTE: *The computer layout may change without notice. Boards may be located in slots that differ from those shown in Figure 2-9 and Figure 2-4.*

Oscilloscope

Figure 2-10 shows the front panel of the oscilloscope. The CH1, CH2 and CH3 (or Aux 1) input cables thread through the center hole in the front panel of the Voyager-STR workstation. These cables are connected internally to the detector and control board.

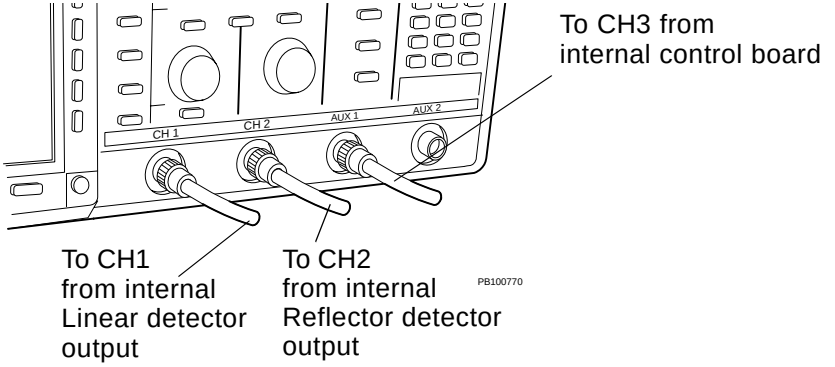


Figure 2-10 Oscilloscope Connections

Acqiris 2 GHz digitizer

Figure 2-11 shows the connectors on the Acqiris 2 GHz digitizer board. The input cables from these connectors (INPUTA, INPUTB, and EXTERNAL) connect internally to the mass spectrometer linear detector, reflector detector, and control board, respectively.

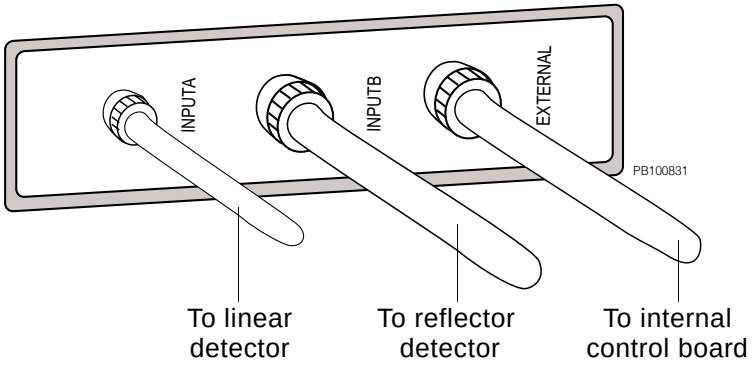


Figure 2-11 Acqiris 2 GHz Digitizer Board Connections

Video monitor Figure 2-12 shows the rear panel of the video monitor.

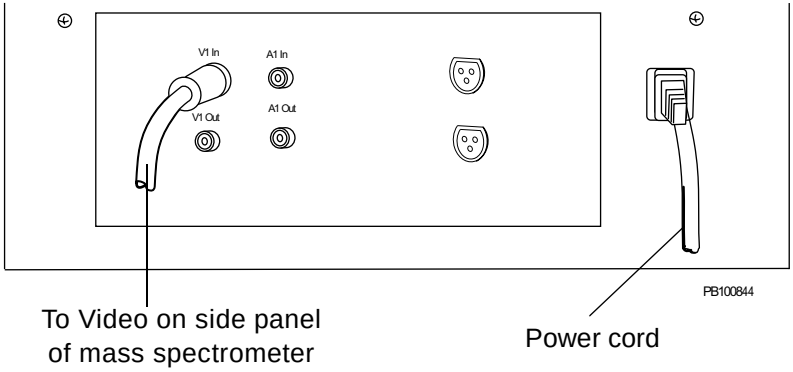


Figure 2-12 Rear Panel of Video Monitor

NOTE: *In the US only, you can plug the video monitor into a grounded wall outlet or into the receptacle on the mass spectrometer.*

Oscilloscope, video monitor, and computer monitor

Make the following connections on the rear panel of the Voyager-DE STR system:

Voyager-DE STR (Figure 2-8 on page 2-23)	Connect to	Cable
Video	Video input of video monitor (Figure 2-12)	BNC with video adapter
VGA	Computer monitor	9-pin connector
GPIB	Oscilloscope (Figure 2-10 on page 2-26)	IEEE-488
NOTE: *Thread cables for CH1, CH2, and CH3 (Aux1) input through the circular hole in the front panel of the workstation.*		

2.5 Installing Software

All necessary software is installed on your Voyager workstation when it is shipped to you. Use these instructions to reinstall software or install a new version of software.

The Voyager software requires a total of 100 MB of free disk space plus additional space for data files.

Before installing the Voyager software, make sure the following software is installed on your computer:

- Microsoft Windows NT-based software
- All display, network, and printer drivers

Follow the instructions provided with the software listed above to install it.

This section describes:

- Installing the Voyager software
- Starting the software
- Exiting the software

2.5.1 Installing the Voyager Software

Installing To install the Voyager software:

1. Insert the **Voyager CD** into the CD drive in the computer.

   The installation routine automatically starts and the Welcome dialog box appears.

   NOTE: *If the installation routine does not automatically start, click* ***Start*** *on the Windows NT desktop, click* ***Run****, type* ***D:\VOYAGER\SETUP*** *(or the drive designation for your CD drive), and click* ***OK****.*

   ---

2. Click **Next**.

   The Select Components dialog box appears with all options selected by default:

   - **Voyager software**—Includes the Instrument Control Panel and Sequence Control Panel, and all standard calibration reference (.REF), plate type (.PLT), default settings (.SET) files, and search pattern (.SP) files provided.
   - **Data Explorer software**—Includes the Data Explorer software and default settings (.SET) files.
   - **Example files**—Includes standard instrument settings (.BIC) files, and installs them in C:\VOYAGER\DATA\INSTALLATION.

   NOTE: *The standard .BIC files are installed in a different directory than the .BIC files that have been optimized for your system. For more information, see* *“Standard instrument settings files” on page 5-3.*

- **User Guides**—Includes PDF (portable document format) versions of the Voyager documentation that you can view online using Adobe® Acrobat® Reader.

3. Click **Next**.
4. A series of dialog boxes in which you specify the hardware options and serial number for your system are displayed. Leave settings at the defaults, or change the settings if needed. Click **Next**.

   The Select Program Folder dialog box appears.
5. Leave the Program Folder set to Voyager. Click **Next**.

   The installation proceeds.
6. A message is displayed asking if you want to install Data Access Packs. Click **Yes**.
7. A message is displayed asking if you want to install Adobe Acrobat Reader. Click **Yes**.

   When the software is installed, a message is displayed. The following icons are created and displayed on the Windows desktop:

   - Voyager Control Panel
   - Data Explorer
   - Voyager Sequence Control Panel

Accessing the online User's Guides

To access the PDF versions of the Voyager documentation:

1. From the Windows Start menu, select **Programs**.
2. Select **Voyager**, then select **User Guides**, and select the document to open.

2.5.2 Starting the Software

Starting Instrument Control Panel

To start the Voyager Instrument Control Panel from the Windows NT desktop, double-click the **Voyager Control Panel** icon on the desktop. The Instrument Control Panel is displayed (Figure 2-13).

NOTE: *If the Instrument Control Panel is not displayed as shown in Figure 2-13, select* ***Instrument Page Control*** *from the View menu, then select* ***Default Layout*** *for control mode.*

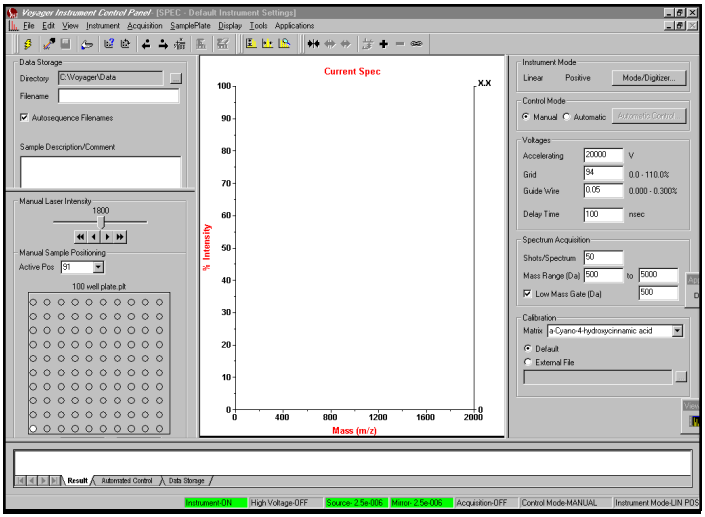


Figure 2-13 Instrument Control Panel

Starting Sequence Control Panel

To start the Sequence Control Panel from the Windows NT desktop:

1. Make sure the Instrument Control Panel is running.

NOTE: *If you start the Instrument Control Panel using the Sequence Control Panel icon, it functions the same way as if you started it using the Instrument Control Panel icon, with two exceptions. Warning and error messages are not displayed during operation, and the Instrument Control Panel will close when you close the Sequence Control Panel.*

---

2. Double-click the **Sequence Control Panel icon** on the desktop.

NOTE: *You can also click a toolbar button in the Instrument Control Panel to start the Sequence Control Panel.*

The Sequence Control Panel opens (Figure 2-14).

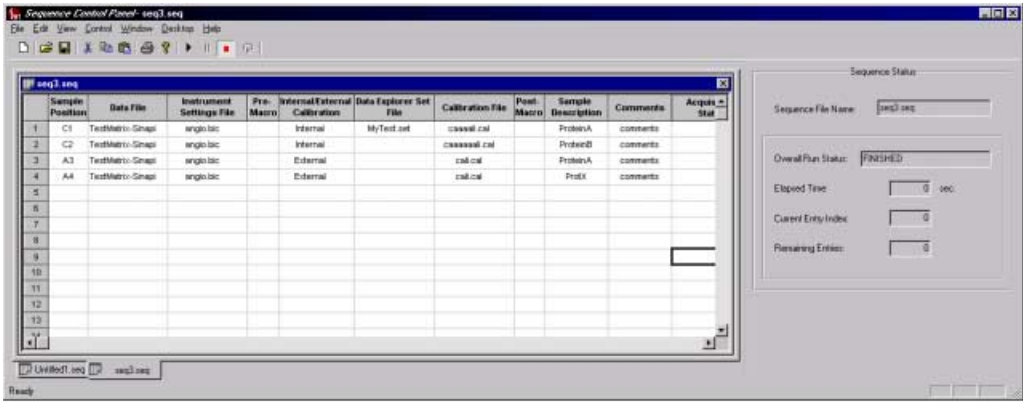


Figure 2-14 Sequence Control Panel

2.5.3 Exiting the Software

CAUTION

If you are using the Voyager Workstation and you exit the Voyager Instrument Control software, you can no longer control the workstation. Do not exit the Voyager software until you have finished using the workstation.

Sequence Control Panel

To exit the Sequence Control software:

1. In the Sequence Control window, select **Exit** from the File menu.

   A message is displayed. Click **Yes**.

   The Sequence Control software closes.

   If you started the Instrument Control Panel by double-clicking the Sequence Control Panel icon, the Instrument Control software closes.

Instrument Control Panel

2. If you started the Instrument Control Panel by double-clicking the Instrument Control Panel icon, select **Exit** from the File menu in the Instrument Control Panel window.

   The Instrument Control software closes.

2.6 Hardware Configuration

CAUTION

Do not alter the Hardware Configuration unless instructed to do so by an Applied Biosystems Technical Representative. Altering these settings may cause your Voyager Biospectrometry Workstation to function improperly.

In this section

This section describes:

- Vacuum configuration
- High voltage configuration
- Timed (Precursor) ion selector configuration
- Instrument configuration
- Laser configuration
- Digitizer configuration

2.6.1 Vacuum Configuration

To check the vacuum configuration:

1. In the Instrument Control Panel, select **Hardware Configuration** from the Instrument menu.
2. Click the **Vacuum** tab to display the Vacuum page (Figure 2-15 on page 2-36).

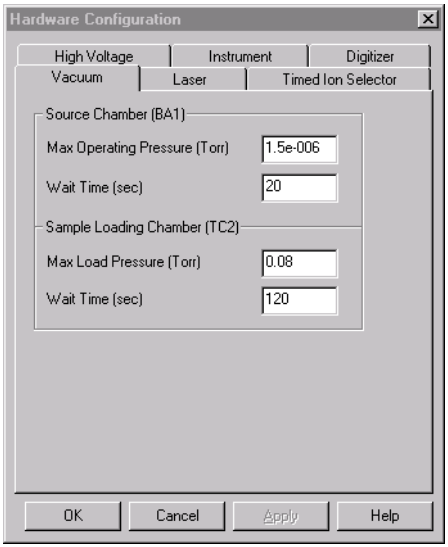


Figure 2-15 Vacuum Configuration

3. Check the following values as needed:

   - **Source Chamber (BA1) Max Operating Pressure**—Pressure (Torr) above which the high voltage power supplies are automatically turned off to prevent damage to the instrument. Valid range is 10^{-5} to 10^{-9}. Default is 9×10^{-6}. If the Source Chamber pressure is above the Max Operating Pressure, an error message is displayed and the high voltage cannot be turned on. The software writes an error in the Windows NT Event log. For more information, see “Checking the Windows NT Event Log” on page 9-22.

- **Source Chamber (BA1) Wait Time**—Time (seconds) that the software waits for the instrument to reach the Source Chamber Maximum Operating Pressure, after the sample plate is loaded. If the wait time is exceeded, an error message is displayed which gives you the option of an additional wait time or ejecting the plate. Valid range is 0 to 300 seconds. Default is 120 seconds.
- **Sample Loading Chamber (TC2) Max Load Pressure**—Pressure (Torr) which must be reached before the system moves the sample plate from the load chamber to the source chamber during the load cycle. Valid range is 8×10^{-2} to 10^{-3}. Default is 8×10^{-2}. If Max Load Pressure is not reached, sample plate will not load.
- **Sample Loading Chamber (TC2) Wait Time**—Time (seconds) that the software waits for the instrument to reach the Sample Loading Chamber Maximum Load Pressure after the sample plate is loaded. If the wait time is exceeded, an error message is displayed which gives you the option of an additional wait time or ejecting the plate. Valid range is 0 to 300 seconds. Default is 120 seconds.

4. Click **OK** to exit vacuum configuration.

2.6.2 High Voltage Configuration

To check high voltage configuration:

1. In the Instrument Control Panel, select **Hardware Configuration** from the Instrument menu.
2. Click the **High Voltage** tab to display the High Voltage page (Figure 2-16).

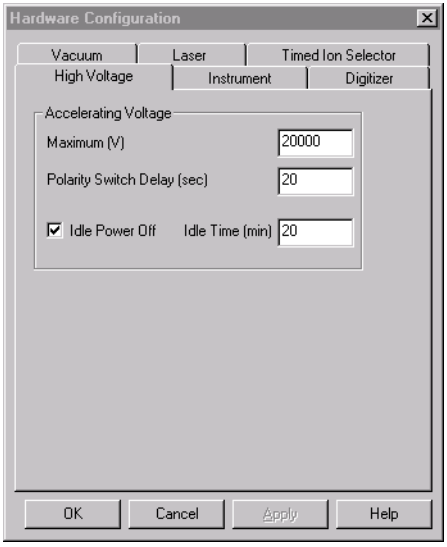


Figure 2-16 High Voltage Configuration

3. Check the following values as needed:
   - **Maximum Accelerating Voltage**—Maximum value in volts that the Accelerating Voltage is configured (25,000 V).
   - **Polarity Switch Delay (Read-only)**—Time (seconds) that the high voltage power supply waits between switching from positive to negative polarity.

- **Idle Power Off/Idle Time**—When enabled, the number of minutes after which the high voltage power supplies automatically turn off, if the instrument is not used. Default is 60 minutes. If this value is zero, the high voltage remains on until any of the following occur: you select Instrument/Turn off High Voltage, Source Pressure exceeds Maximum Operating Pressure, you click **Load** or **Eject**, you align a sample plate, or you exit the software.

4. Click **OK** to exit.

2.6.3 Timed Ion Selector Configuration

To check the Timed Ion Selector (called Precursor Ion Selector in PSD mode) configuration:

1. In the Instrument Control Panel, select **Hardware Configuration** from the Instrument menu.
2. Click the **Timed Ion Selector** tab to display the Timed Ion Selector page (Figure 2-17).

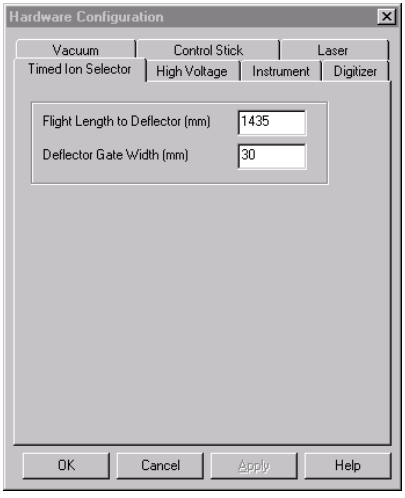


Figure 2-17 Timed Ion Selector Configuration

3. Check the following values as needed:

   - **Flight Length to Deflector**—Distance in millimeters from the grid to the deflector.
   - **Deflector Gate Width**—Distance in millimeters that the Timed Ion Selector is on.

CAUTION

Do not alter the Timed Ion Selector parameters unless instructed to do so by an Applied Biosystems Technical Representative. Altering these settings may cause your Voyager Biospectrometry Workstation to function improperly.

4. Click **OK** to exit.

2.6.4 Instrument Configuration

To check the instrument configuration:

1. In the Instrument Control Panel, select **Hardware Configuration** from the Instrument menu.
2. Click the **Instrument** tab to display the Instrument page (Figure 2-18).

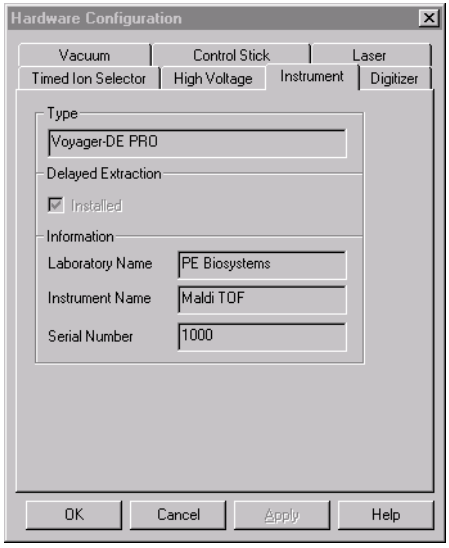


Figure 2-18 Instrument Configuration

3. Check the following fields as needed:

   - **Instrument type (Read-only)**—Displays your instrument type.
   - **Delayed Extraction (Read-only)**—Reflects whether your system has delayed extraction hardware installed.
   - **Laboratory Name**—You can edit this field to display your laboratory name. Names listed in this field are included in .DAT files and on printouts.
   - **Instrument Name**—You can edit this field to display your instrument name. Names listed in this field are included in .DAT files and on printouts.
   - **Serial Number**—Displays the serial number for the connected instrument.

4. Click **OK** to exit.

2.6.5 Laser Configuration

To check the laser configuration:

1. In the Instrument Control Panel, select **Hardware Configuration** from the Instrument menu.
2. Click the **Laser** tab to display the Laser page (Figure 2-19).

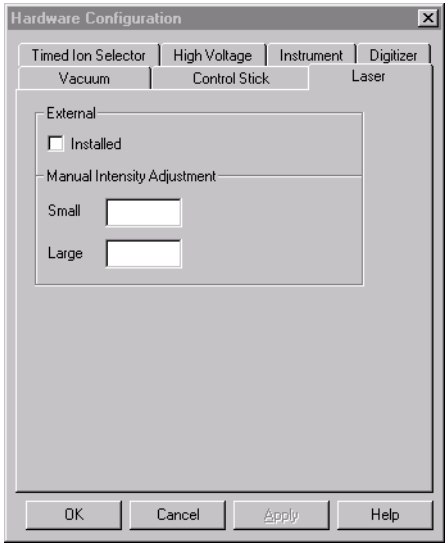


Figure 2-19 Laser Configuration

3. Check the following fields as needed:

   - **External laser (Read-only)**—Checked if an external laser is installed.
   - **Manual Intensity Adjustment**—Determines the increments in which the laser attenuator moves when using the Fine and Coarse laser controls on the Manual Laser/Sample Positioning control page:

     Small—Determines laser adjustment increments when you click the Fine laser controls  or press **Ctrl+PgUp**/**Ctrl+PgDn**.

     Large—Determines laser adjustment increments when you click the Coarse laser controls  .

NOTE: *You set laser firing rate in the Mode/Digitizer dialog box described on page 5-26.*

---

4. Click **OK** to exit.

2.6.6 Digitizer Configuration

To check the configuration of the digitizer:

1. In the Instrument Control Panel, select **Hardware Configuration** from the Instrument menu.
2. Click the **Digitizer** tab to display the Digitizer page (Figure 2-20).

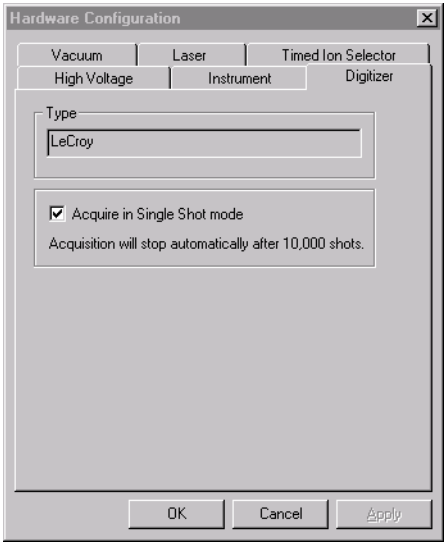


Figure 2-20 Digitizer Configuration

Check the Digitizer type field. This is a read-only value that displays the digitizer type installed.

3. Select Acquire in **Single Shot Mode** to set the system to save only the last acquired spectrum when you save data, instead of averaging spectra.

You can use single-shot mode:

- As a diagnostic to determine if there is scan-to-scan variation in signal
- To acquire data

When single-shot mode is enabled, a maximum of 10,000 shots are acquired, but are not averaged. When acquisition is stopped, the last single shot acquired is displayed in the Spectrum window. A data file contains data for a single scan.

When single-shot mode is disabled, the Total Shots specified in the instrument settings are acquired and are averaged as they are acquired.

4. Click **OK** to exit.

2.7 Aligning the Sample Plate

In this section This section describes:

- Overview
- When to align
- .PLT files and multiple alignments
- How the system aligns a plate
- Overview of video monitor display
- What you need
- Corner positions in .PLT files
- Using the control stick
- Before aligning
- Aligning
- Alignment information saved for future use

Overview The Sample Plate Alignment function allows you to align the center of sample positions under the laser beam.

When to align For most standard welled plates and .PLT files provided, alignment is not frequently required.

Alignment is more typically required:

- When you are using a custom .PLT file and are spotting sample plates using an automated device.
- When you are using Teflon™ plates, because there can be some variation in the laser-etched indentations on the plate.

Align the Sample Plate if the laser beam is not centered on a sample position:

- When you advance to a sample position using the Manual Sample Position control page
- Before you acquire data from the Instrument Control Panel and use automated sample positioning
- Before you acquire data from the Sequence Control Panel

.PLT files and multiple alignments

A .PLT file defines the sample positions on a sample plate. For example, 100.PLT may define a 100-well plate. If you have more than one 100-well plate, you may need to align each plate. The Voyager software allows you to assign a unique Plate ID to each plate that allows customized alignment of more than one plate that uses the sample .PLT file (Figure 2-21).

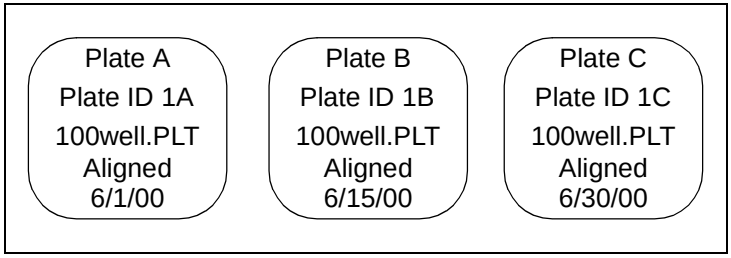


Figure 2-21 Multiple Alignments Using Sample .PLT File

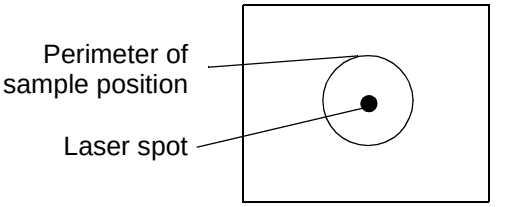
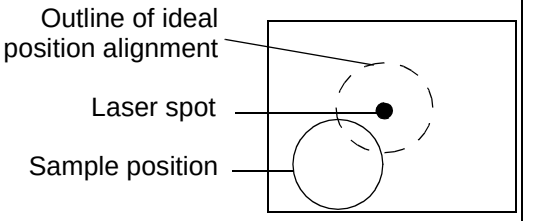
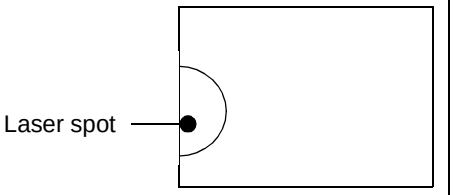
How the system aligns a plate

When you load a sample plate, the system aligns the plate as described below:

- The software looks for alignment information associated with the Plate ID you load and adjusts accordingly.
- If there is no alignment information available for the Plate ID (sample plate has not been aligned), the software uses the default alignment.

Overview of video monitor display

The following examples show how the sample positions and laser spot may be displayed on the video monitor during sample plate alignment.

Example	Description
 ***Figure 2-22 Ideal Sample Position Alignment***	**Ideal sample position alignment**—The center of the sample position is aligned with respect to the laser spot. You mark the outline of this alignment on a transparency to use to calibrate other sample positions during alignment.
 ***Figure 2-23 Sample Position Misalignment***	**Sample position misalignment**—The center of the sample position is not aligned with respect to the laser spot. You adjust misaligned sample positions to ideal alignment.
 ***Figure 2-24 Camera Misalignment***	**Camera misalignment**—The laser spot is not centered on the video monitor. The camera requires adjustment. Do not perform the sample plate alignment procedure. Call Applied Biosystems Technical Support.

What you need

To align the sample plate you need the following materials:

- Sample plate with α-cyano-4-hydroxycinnamic acid (CHCA) matrix spotted in four corner sample positions, as described in Table 2-1. For more information, see Section 3.5.5, Adjusting the Laser Position for a Custom .PLT File.

WARNING

CHEMICAL HAZARD. Alpha-cyano-4-hydroxycinnamic acid (CHCA) *matrix may cause eye, skin, and respiratory tract irritation. Read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

NOTE: *If your monitor screen is marked with the position at which the laser strikes the sample plate and you are aligning plates with wells or etched sample positions, you do not need to spot the sample plate with matrix before aligning. You can align the boundaries of the sample positions with the laser position mark on your monitor.*

- Transparency (mylar overhead or acetate)
- Marking pen

NOTE: *View the video monitor from a consistent angle when determining laser positions.*

Corner positions in .PLT files

Different sample position numbers are associated with different .PLT files. To determine which numbers correspond to the four corners of each .PLT file, see Table 2-1.

Table 2-1 Four-Corner Positions on Sample Plates

.PLT file	Four Corner Position Numbers
64 well disposable plate.PLT	82, 12, 19, 89
100 well plate.PLT	91, 11, 19, 99
384 well plate.PLT	P1, C1, C21, P21
400 well plate.PLT	T1, A1, A20, T20
96 well × 2 plate.PLT	H1_b, A1_a, A12_a, H12_b

Using the control stick

To align a sample plate, you must use the control stick. For details on using the control stick, see Appendix I, Using the Oscilloscope and Control Stick.

Before aligning

Before aligning the sample plate:

1. Spot the sample plate with matrix as described in “What you need” on page 2-49.
2. Load the sample plate into the system and select the Plate ID that corresponds to the plate:
   - From the Sample Plate menu, select **Select Sample Plate** to display the Select Sample Plate Type dialog box.
   - Select a **Plate ID**. The .PLT file and alignment information associated with the Plate ID are automatically loaded.

     Alternatively you can specify a new Plate ID and select a .PLT file. For more information, see “Assigning Plate IDs” on page 3-50.
   - Click **OK**.

Aligning

To align the loaded plate:

1. Tape the transparency to the video monitor.
2. Select **Align Sample Plate** from the Sample Plate menu.

   The Sample Plate Alignment wizard (Figure 2-25) is displayed.

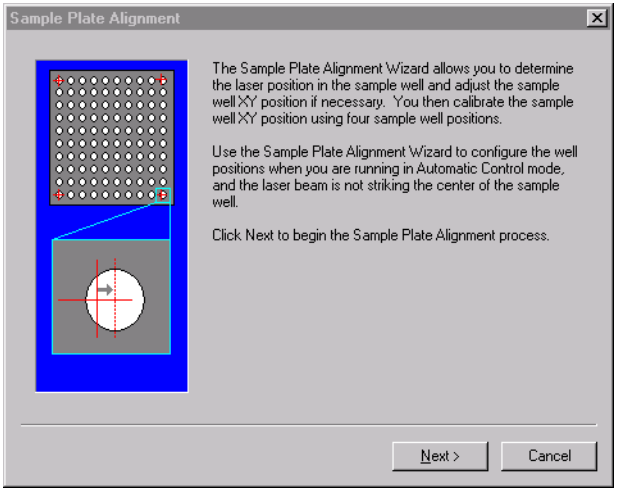


Figure 2-25 Sample Plate Alignment Wizard

3. Click **Next**.

   The sample plate moves to the first alignment position on the sample plate. See Table 2-1, “Four-Corner Positions on Sample Plates,” on page 2-50, to determine your plate positions.

4. Start the laser using the control stick.

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

---

5. Mark the laser position on the transparency. Do not mark the sample position. Be sure the laser is centered on the video monitor and does not appear as shown in Figure 2-24 on page 2-48.
6. Center the sample position under the laser using the control stick.
7. When the position is centered, click **Next**. The system advances to the next corner position for the .PLT type.
8. Repeat step 3 through step 7 for each of the four corners of the sample plate.

   The software calculates the alignment and uses the settings to ensure all sample positions are centered under the laser. If the alignment is successful, a message is displayed.
9. Do one of the following:

Click	To
Finish	Save the alignment for the specified Plate ID
Cancel	End the Sample Plate procedure without saving the alignment for the plate
Back	Repeat the alignment procedure

NOTE: *A message is displayed if the alignment is outside the preferred tolerance. Repeat the alignment procedure by clicking* ***Back****. If the alignment is still outside of tolerance, call Applied Biosystems Technical Support.*

Alignment information saved for future use

The alignment information is saved with the Plate ID, and applied each time you load this Plate ID into the system. For more information, see “How the system aligns a plate” on page 2-47.

2.8 Running OptiPlate to Optimize Mass Accuracy

This section includes:

- Overview
- Requirements
- Preparing to optimize
- Running OptiPlate
- Evaluating and saving results

2.8.1 Overview

What OptiPlate does

The OptiPlate software compensates for differences in calibration across a sample plate caused by variations in:

- Sample plate well depth
- Sample plate surface (flatness)
- Electric field in the source chamber of the mass spectrometer

When you run the OptiPlate software, it:

- Calculates a correction factor (called Extraction Correction) that minimizes the differences in calibration across a sample plate caused by the factors listed above.
- Creates a mass accuracy optimization file that contains the Extraction Correction for each optimized position on the plate.

Using the optimization created by OptiPlate

To use the optimization created by OptiPlate during sample analysis, select the **Use Mass Accuracy Optimization** option when you load a sample plate in the Instrument Control Panel. The Mass Accuracy Optimization option allows you to use fewer standards and still obtain optimum mass accuracy.

For more information, see:

- Section 3.2.2, Locating Standards for Optimum Mass Accuracy
- Section 3.4.2, Using the Mass Accuracy Optimization Option
- Section 3.4.4, Loading Sample Plates

2.8.2 Requirements

Standard and matrix

To run the OptiPlate software, you need:

- α-cyano-4-hydroxycinnamic acid (CHCA) matrix
- Standard mixture containing the components in Table 2-2

Table 2-2 Standard Requirements

Component	Final concentration after mixed with matrix
Angiotensin I	2.0 pmol/µl
ACTH (1–17 clip)	2.0 pmol/µl
ACTH (18–39 clip)	1.5 pmol/µl
ACTH (7–38 clip)	3.0 pmol/µl
Insulin, bovine	3.5 pmol/µl

WARNING

CHEMICAL HAZARD. Alpha-cyano-4-hydroxycinnamic acid (CHCA) *matrix may cause eye, skin, and respiratory tract irritation. Read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

WARNING

CHEMICAL HAZARD. Angiotensin, ACTH, and Insulin *may cause an allergic skin and respiratory reaction. Exposure may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

NOTE: *You can use Calibration Mixture 2 from the Sequazyme™ Peptide Mass Standards Kit. See Appendix B.3, Spare Parts, for the Sequazyme Kit part number.*

Prepare standards and matrix in 50:50 acetonitrile, 0.1% TFA in deionized water. For more information, see Section 3.1, Preparing Samples.

WARNING

CHEMICAL HAZARD. ***Acetonitrile*** *is a flammable liquid and vapor. It may cause eye, skin, and respiratory tract irritation, central nervous system depression, and heart, liver, and kidney damage. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

High-voltage warmup

At least 30 minutes before you perform plate optimization, turn on high voltage by clicking  in the Instrument Control Panel toolbar. Allow the hign voltage power supplies to warm up.

NOTE: *By default, high voltage turns off after 60 minutes if the instrument is not used. You can change the default in Hardware Configuration. See* *“High Voltage Configuration” on page 2-38.*

The warm-up period allows maximum mass accuracy during plate optimization by reducing variability in accelerating voltages, and yields more reproducible ion flight times.

.PLT file

To correctly display results in OptiPlate, use a .PLT file that specifies a maximum of 900 positions with either of the following patterns (Figure 2-26):

- A grid with a maximum of 30 rows and 30 columns
- An offset grid with a maximum 15 rows and 60 columns

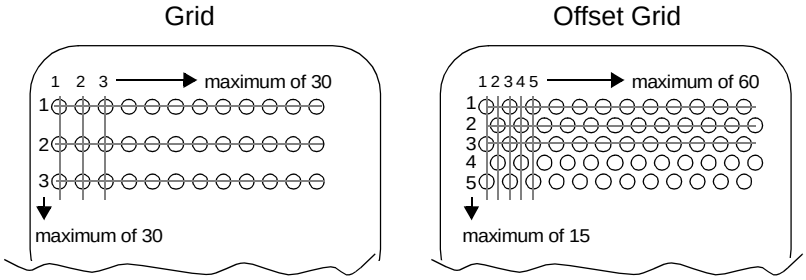


Figure 2-26 Grid or Offset Grid Pattern in .PLT File

NOTE: *All .PLT files provided with the system contain fewer than 900 positions and specify a grid (see ".PLT files provided" on page 3-67). New .PLT files created using Create PLT File specify a grid (see "Creating a .PLT file using Create PLT File" on page 3-79).*

If you use a .PLT file that does not meet these requirements, the position location and spacing displayed in the OptiPlate dialog box do not accurately reflect the position location and spacing specified by the .PLT file. However, you can still obtain valid Extraction Corrections for the positions.

If you use a .PLT file that contains more than 900 positions, an error message is displayed, and you cannot run OptiPlate.

2.8.3 Preparing to Optimize

This section includes:

- Preparing the plate to optimize
- Preparing the system
- Verifying laser intensity

Preparing the plate to optimize

WARNING

CHEMICAL HAZARD. Angiotensin, ACTH, and Insulin *may cause an allergic skin and respiratory reaction. Exposure may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

WARNING

CHEMICAL HAZARD. Alpha-cyano-4-hydroxycinnamic acid (CHCA) *may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

To prepare the plate:

1. Spot the sample plate with the standard mixture and matrix prepared in the previous section. Follow the procedure in Section 3.2.3, Loading Samples (Dried Droplet Application).

   Spot a minimum of 15 positions on a plate. For best results, spot the number of positions indicated below for the plate type you are optimizing.

Plate	Number of Positions to Spot
100-well	100 positions
400-well	100 positions (every fourth row) and all perimeter positions
96 × 2-well	96—Spot either A or B rows and all perimeter positions

2. Spot one position as the reference position with the standard mixture and matrix. For best results, select a position in the center of the plate, for example, position 55 or 56 on a 100-well plate.

   NOTE: *During the optimization procedure, the system automatically reanalyzes this position to maintain an accurate calibration. A position in the middle of the plate minimizes the distance the plate must travel to reanalyze the reference position.*

3. Allow the sample plate to dry.

Preparing the system

To prepare the system:

1. Assign a Plate ID if you have not already assigned one for the plate. For information, see “Assigning Plate IDs” on page 3-50.
2. Load the plate into the mass spectrometer as described in Section 3.4, Loading Sample Plates in the Mass Spectrometer.

Verifying laser intensity

Two instrument settings (.BIC) files are provided for use with OptiPlate:

- OptiPlate_Linear.BIC
- OptiPlate_Reflector.BIC

These files are set with values that have been optimized for your system and contain the following acceptance criteria to ensure best results when you run OptiPlate:

Parameter	Setting
Minimum Signal Intensity	20,000
Maximum Signal Intensity	60,000
Stop Conditions	Stop after 8 consecutive failing acquisitions
Criteria Evaluation Mass Range	• **Linear mode**—1,000 to 6,000 Da • **Reflector mode**—1,000 to 3,000 Da

Before running OptiPlate, you need to:

- Determine the laser intensity that yields a signal intensity between 20,000 and 60,000 counts (see below).
- Set the Minimum and Maximum Laser Intensity settings in Automatic Control to ±50 of the laser intensity that yields signal intensity between 20,000 and 60,000 counts (see below).

Determining the laser intensity

Due to variations in sample preparation and crystallization, it may be necessary to determine the laser intensity each time you run OptiPlate.

To determine the laser intensity that yields a signal intensity between 20,000 and 60,000 counts:

1. In the Instrument Control Panel, load the **OptiPlate_Linear.BIC** or **OptiPlate_Reflector.BIC** file provided on your system.
2. In the Instrument Settings page, change Control Mode from Automatic to **Manual**.
3. Select a position spotted with standard mixture and matrix from the sample plate you spotted in “Preparing the plate to optimize” on page 2-58.
4. Start acquisition, move around on the sample position until you observe an acceptable signal, then adjust the laser intensity until signal intensity is between 20,000 and 60,000 counts.
5. Move around to several other locations on the sample position to verify that the selected laser intensity yields signal in the correct range.
6. Stop acquisition.

Setting Minimum and Maximum Laser Intensity

To set the Minimum and Maximum Laser Intensity settings in Automatic Control:

1. In the Instrument Settings page, change Control Mode from Manual to **Automatic**.
2. Click **Automatic Control**.

3. In the Automatic Control dialog box, set the Minimum and Maximum Laser Intensity settings to ±50 of the laser intensity that yields signal intensity between 20,000 and 60,000 counts.

   The recommended ±50 setting is intended to keep the laser intensity range as narrow as possible, to allow OptiPlate to run as quickly as possible. However, if the laser intensity range is too narrow, it may not yield signal intensity in the specified range, which will cause OptiPlate to fail. If you observe failures during the OptiPlate run, you may need to increase the laser intensity range.

4. Click **OK**.

5. From the File menu, select **Save Instrument Setting As**, then name the file **OptiPlate_Linear1.BIC** or **OptiPlate_Reflector1.BIC**.

NOTE: *Save the .BIC file before selecting it in OptiPlate. If you make changes to the .BIC file after you select it in OptiPlate, OptiPlate does not recognize the changes.*

2.8.4 Running OptiPlate

This section includes:

- Running OptiPlate
- During an OptiPlate run

Running OptiPlate

To run OptiPlate:

1. In the Instrument Control Panel, select **OptiPlate** from the Tools menu to display the OptiPlate dialog box (Figure 2-27 on page 2-64).

   By default, the Plate ID for the plate loaded in the mass spectrometer is specified in the OptiPlate software. If there are OptiPlate results associated with the selected Plate ID, color-coded well positions are displayed. For more information, see “Color coding” on page 2-69.

   If Use Mass Accuracy Optimizations was selected when you loaded the specified Plate ID, a message box indicates that this function is disabled for the OptiPlate run.

NOTE: *For optimum display of results, make sure the color palette on your computer is set to more than 256 colors. To check, select* ***Settings*** *from the Start menu, select* ***Control Panel****, select* ***Display****, then click* ***Settings****.*

If you change the Plate ID in the OptiPlate dialog box, make sure the correct physical plate is loaded in the mass spectrometer before you continue.

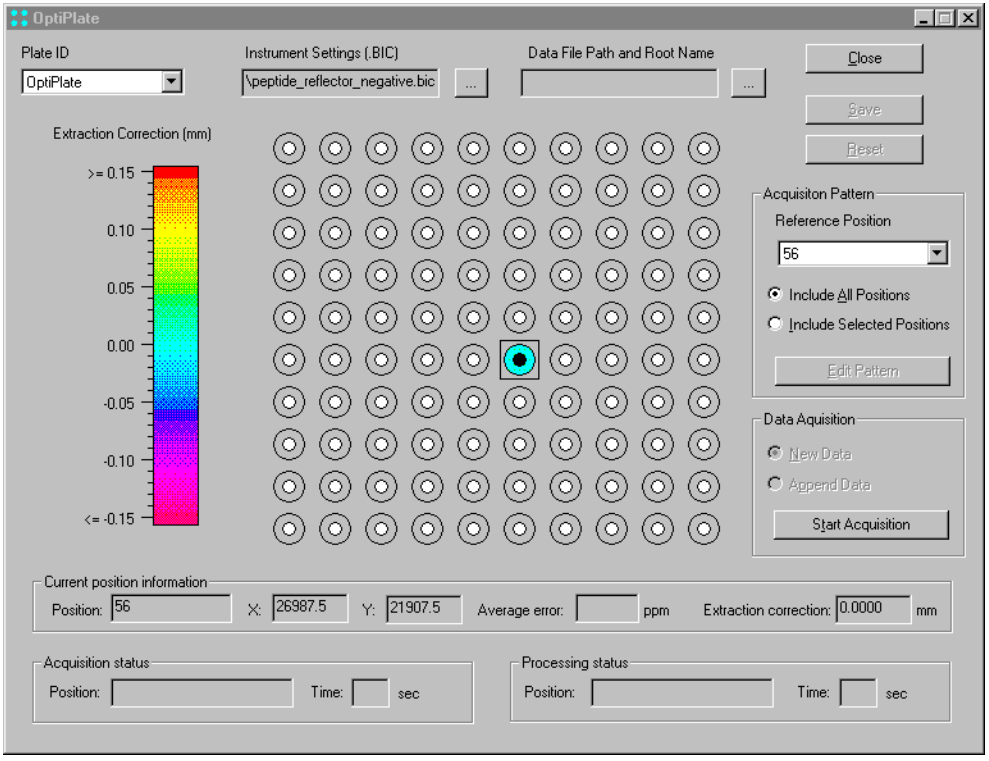


Figure 2-27 OptiPlate Dialog Box

2. In the Instrument Settings (.BIC) field, select the **OptiPlate_Linear1.BIC** or **OptiPlate_Reflector1.BIC** in which you set laser intensities that yield signal intensity between 20,000 and 60,000 counts (described in “Verifying laser intensity” on page 2-60).

NOTE: *It is good practice to determine the laser intensity that yields acceptable signal intensity before running OptiPlate. Due to variations in sample preparation and crystallization, it may be necessary to determine the laser intensity each time you run OptiPlate.*

Setting data storage

3. Click [...] next to the Data File Path and Root Name field. Specify the directory in which to store the data files collected by this procedure, then type the base name to use for the data files. Click **Open**.

   Data file names are automatically numbered (autosequenced) when they are acquired.

Setting reference position

4. Under Acquisition Pattern, select the position of the reference position you spotted in step 2 in "Preparing the plate to optimize" on page 2-58.

   The reference position is indicated with a black dot in the center of the position.

Selecting positions to optimize

5. Under Acquisition Pattern, select **Include All Positions** or **Include Selected Positions** to specify the positions to optimize.

6. If you select the Include Selected Positions option, click **Select Pattern**, then specify the sample positions to optimize:

To specify	Do the following
Individual positions	Click on a single position.
Multiple positions	• Click-drag positions or • Press **Shift** and click a position to turn on multi select mode, then drag positions (click again to turn off multi select mode)
Evenly distributed number of positions	Select a Fill Increment, then click **Fill**. For example, select **2** as the fill increment to fill every other position in every other row.

NOTE: *If you change the reference position after you specify the Acquisition Pattern using Select Pattern, the original reference position is no longer selected for acquisition. You must manually select the position if you want to acquire it. For example, if you select position 44 as the reference position and select positions 43 and 45 for acquisition, then change the reference position to position 42, position 44 is not automatically selected for acquisition.*

Setting acquisition mode

7. Under Data Acquisition, select:

   - **New Data**—To create a new optimization file for a plate that has not been optimized, or to reoptimize a plate.
   - **Append Data**—To do either of the following:

     Optimize additional positions on a plate that has been optimized, and add the correction information to the existing optimization file.

     Replace existing correction factors for positions you select.

Positions selected for optimization are indicated with a white dot in the center of the position.

Starting acquisition

8. Click **Start Acquisition**.

Acquisition starts in the Instrument Control Panel. Acquisition and processing status are displayed at the bottom of the OptiPlate screen for each position as it is acquired.

The Data Acquisition mode is automatically set to Append Data when you start acquisition.

During an OptiPlate run

During an OptiPlate run, the software:

- Acquires a spectrum at the Reference position.
- Internally calibrates the reference spectrum using monoisotopic masses in Reflector mode or average masses in Linear mode.
- Acquires a spectrum at each specified position and applies the reference calibration to the data.
- Calculates and displays the Average Error (ppm) for the position (compares the observed masses to the observed reference masses, then determines the average error across all expected peak masses for the components listed in Table 2-2, “Standard Requirements,” on page 2-55).
- Calculates and displays the Extraction Correction (mm) for the position by performing a least-squares fit of the data.
- Displays a color code for each position (see “Color coding” on page 2-69), and saves the data file for the position.
- Reacquires the reference spectrum every 3 minutes and updates the calibration to adjust for instrument drift over time.

If acquisition fails or peaks are not found

The OptiPlate software may analyze a position more than one time as described below.

If	The software
Acquisition fails in Reference or non-reference position	Continues acquiring until the number of consecutive failing acquisitions set in Stop Conditions is met (see page 2-60).
Expected masses not found in reference position	Reacquires the reference position until a second successful acquisition occurs, then checks for presence of expected peaks. If no peaks are found, the OptiPlate run stops.
Expected masses not found in non-reference position	Does not calculate Extraction Correction. Marks position with  and continues the OptiPlate run.

Pausing and resuming the plate optimization

To stop the OptiPlate run for any reason (for example, you notice that many positions display a black edge indicating they have failed optimization, or you realize that you did not save changes to the .BIC file):

1. Click **Stop Acquisition**.
2. Wait for acquisition to stop in the Instrument Control Panel.
3. If necessary, eject the sample plate and spot or respot positions, or modify then save instrument settings.
4. Allow the sample plate to dry, if necessary.
5. Load the sample plate, if necessary.

6. Select **Append Data** for Data Acquisition mode.

   Each failed position and each position previously selected for optimization (but not yet acquired) is indicated with a white dot in the center of the position.

   If you want to overwrite all previously acquired positions, click **Reset**, then select **New Data** for Data Acquisition mode.

7. Click **Start Acquisition**.

2.8.5 Evaluating and Saving Results

Color coding Positions are marked with the following colors:

Color	Meaning
	Position selected for optimization
	Reference position
	Extraction Correction value exceeded the allowed 1 mm maximum, or the Extraction Correction could not be calculated, for any of the following reasons: • Data was not acquired. • Laser range specified in instrument settings does not yield the minimum/maximum laser intensity specified in acceptance criteria. See “Verifying laser intensity” on page 2-60. • Required peaks were not detected in the reference position or the acquired position during processing.
	Position not selected for optimization
Other colors	Colors correspond to the legend for Extraction Correction.

NOTE: *If the reference position is located in the center of the plate, most corrections tend to be negative values. Do not expect to see all colors in the color legend represented in position results.*

Evaluating results

Examine colors to ensure:

- No positions display a black edge, indicating a problem with optimization
- The reference position corresponds to the color legend at position 0.00
- Colors of positions change evenly and gradually with increasing distance from the reference position

Saving

When acquisition of all positions is complete and you have examined results, click **Save**.

The optimization file is assigned the same name as the Plate ID, given an OPT_ prefix and an .XML extension, and stored in the same directory as the .PLT file.

For example, if you run OptiPlate for a “Lab1” Plate ID, an optimization file called OPT_Lab1.XML is created.

Do not delete or move the .XML files. These files are automatically accessed by the software when you select the Optimize Mass Accuracy option when loading a sample plate.

Deleting data files

After performing plate optimization and saving the optimization file, you can delete the data files created during this procedure. OptiPlate results are associated with the .XML file that was created (described above), not with data files. Data files are no longer needed, even if you append to an existing optimization file.

2.9 Resetting the Optional External Laser

When to reset Reset the external laser when you switch from the internal to the external laser (when you save or load a .BIC file with the external laser specified, or run a sequence that contains .BIC files with the external laser specified.)

Resetting To reset:

1. Click **OK** in the message dialog box instructing you to reset the external laser.
2. On the left side of the external laser cabinet (Figure 2-28), turn the key (located next to the Keyswitch Reset LED) from the On position (right) to the Off position (left), then to the On position (right).

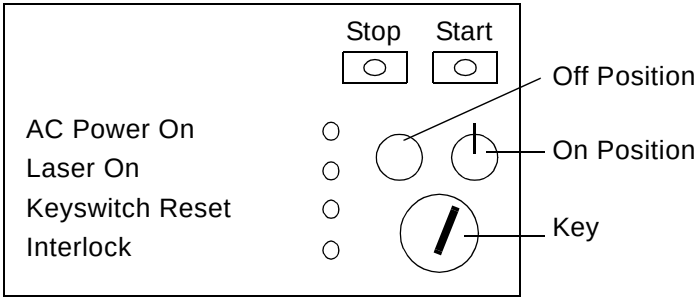


Figure 2-28 Left Side of External Laser Cabinet

The Laser On LED flashes then remains on.

The light on the Stop button is illuminated.

3. Press the **Start** button. The light on the Stop button goes off, and the light on the Start button flashes then goes off.

The laser is now powered on and ready for acquisition.

Problem with external laser positioning over time

If the external laser is left on for more than 15 minutes, the position at which the laser strikes the sample may drift. If this occurs, press the **Stop** button on the left side of the external laser cabinet to reset the laser. Then press the **Start** button to reset the laser.

CAUTION

If you do not press the Start button after pressing the Stop button, no power is supplied to the laser. The software will allow you to start acquisition, but the laser will not fire. If the laser is left on for long periods of time, there may be a problem with laser positioning on the sample. If this occurs, press the Stop button then the Start button to reset the laser.

2.10 Startup and Shutdown

In this section This section describes:

- Powering up
- Initializing
- Reinitializing
- Powering down system components
- Powering down the mass spectrometer

Powering up To power up the Voyager Workstation:

1. Turn on the main power switch. The power switch is located:

   - On the right side panel of the mass spectrometer cabinet on Voyager-DE and Voyager-DE PRO systems.
   - On the back panel of the mass spectrometer cabinet on the Voyager-DE STR system.

   The vacuum pumping system begins running.

   After two minutes, the turbo pump starts. You will hear a whine as the vacuum system spins up to speed.

2. Turn on remaining system devices in this order:

   - Video monitor
   - External digitizer, if your system includes one
   - Oscilloscope, if your system includes one
   - Computer
   - Printer

3. If your system includes an oscilloscope, wait approximately one minute for its initialization cycle to finish. A message indicates that the power on self check passed, then the oscilloscope screen is displayed. See the Appendix I, Using the Oscilloscope and Control Stick, for more information.

If your system includes a LeCroy digitizer, wait approximately one minute until the digitizer completes its internal calibration before starting the Instrument Control Panel.

4. Log on to the Voyager Workstation using your User Name and Password. See your system administrator for your User name and Password.

5. Start the Voyager Instrument Control Panel software by double-clicking the **Voyager Instrument Control** icon on the Windows NT desktop.

Initializing

The hardware is automatically initialized when you start the software.

During initialization, the video image on the sample stage is displayed. The sample stage moves to the home position, and then to the load position. If problems occur, an error message is displayed when you log on to the workstation. Further details on any problem can be obtained by viewing the Windows NT Event Log. For more information, see “Checking the Windows NT Event Log” on page 9-22.

Reinitializing

To reinitialize the hardware:

1. Open the Instrument Control Panel.

2. From the Instrument menu, select **Reinitialize**. The software resets all system components and sets up communication between the computer and workstation.

Powering down system components

Leave the Voyager mass spectrometer powered up unless:

- You need to perform maintenance on internal parts
- You need to move the system

To power down:

Software and computer shutdown

1. Close the Instrument Control Panel by selecting **Exit** from the File menu.
2. On the Windows desktop start task bar, click **Start**, then click **Shut down**. In the Shut Down Windows dialog box, click Shut down the computer and click **Yes**. Hardware is reinitialized.

NOTE: *If you select* ***Close all programs and log on as a different user****, hardware is not reinitialized.*

---

3. Power down all other system components (oscilloscope or digitizer, monitor, and so on).

   Wait until a message is displayed indicating that you can shut down your computer. Power down the computer and computer monitor.

Powering down the mass spectrometer

If you need to perform maintenance on internal parts or move the system, power down the spectrometer:

1. Power down the system components.
2. Turn off the main power switch. The power switch is located:
   - On the right side panel of the mass spectrometer cabinet on Voyager-DE and Voyager-DE PRO systems.
   - On the back panel of the mass spectrometer cabinet on the Voyager-DE STR system.

2.11 Checking System Status and Pressures

Display the System Status page for complete status information.

In the Instrument Control Panel, select **System Status** from the View menu to display the System Status page (Figure 2-29).

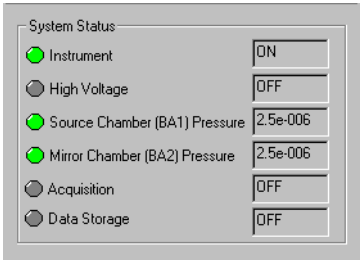


Figure 2-29 System Status Control Page

System and acquisition status are represented by colored indicators and read-only text fields described in Table 2-3. Colors represent:

- **Green**—OK
- **Yellow**—Fault
- **Gray**—Off

System pressures

Before acquiring data, allow the pressure in the Source Chamber (BA1) and Mirror Chamber (BA2) to reach:

Model	Source Chamber (BA1) Pressure (Torr)	Mirror Chamber (BA2) Pressure (Torr)
Voyager-DE and Voyager-DE PRO	Less than 10^{-6}	Less than 10^{-6}
Voyager-DE STR	Less than 5×10^{-7}	Less than 5×10^{-8}

NOTE: *At initial startup or after venting the system, it will require one to two days after startup to reach these vacuum level.*

Status parameters

Status parameters are described in Table 2-3.

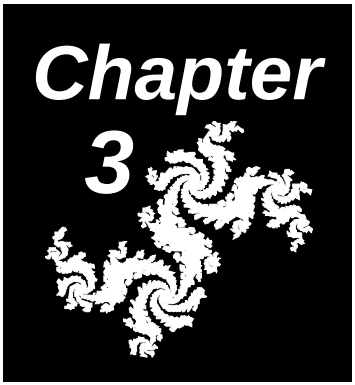
Table 2-3 System Status Parameters

Parameter	Description
System Status	
Instrument State	Possible states are: • ON—Instrument is initialized and high voltage is on. • OFF—Instrument is not initialized and high voltage is off. • FAULT—Indicates a fault condition. Follow the instructions displayed to correct the fault.
High Voltage	Possible states are: • RAMPING—Voltage is turning on. • ON—High voltage is on. • OFF—High voltage is off. • FAULT—Indicates a fault condition. Follow the instructions displayed to correct the fault.
Source Chamber (BA1) Pressure	Displays the source chamber pressure. Operating pressure ranges are: • Voyager-DE, Voyager-DE PRO—Less than 10^{-6} • Voyager-DE STR—Less than 5×10^{-7}

continued

Table 2-3 System Status Parameters (Continued)

Parameter	Description
Mirror Chamber (BA2) Pressure	Displays the mirror chamber pressure. Mirror chamber pressure ranges are: • Voyager-DE PRO—Less than 5×10^{-7} • Voyager-DE STR—Less than 5×10^{-8} ***NOTE:*** *This status field is not displayed on Voyager-DE systems.*
Acquisition	Displays acquisition status: • ON—Instrument is currently acquiring a spectrum. • OFF—Instrument is not acquiring.
Data Storage	Displays data storage status: • ON—Software is currently storing a data file. • OFF—Software is currently not storing a data file.



3 Preparing Samples

This chapter contains the following sections:

3.1 Preparing Samples 3-2

3.1.1 Selecting a Matrix 3-3

3.1.2 Preparing Matrix 3-4

3.1.3 Matrix Information 3-6

3.1.4 Preparing Sample 3-22

3.1.5 Sample Cleanup 3-25

3.1.6 Mixing Sample and Matrix (Dried Droplet Application) 3-33

3.2 Loading Samples on Sample Plates..... 3-35

3.3 Cleaning Sample Plates..... 3-47

3.4 Loading Sample Plates in the Mass Spectrometer..... 3-50

3.5 Sample Plate Types..... 3-61

3.1 Preparing Samples

NOTE: *Sample preparation technique has a direct impact on the quality of the data you obtain in MALDI-TOF applications.*

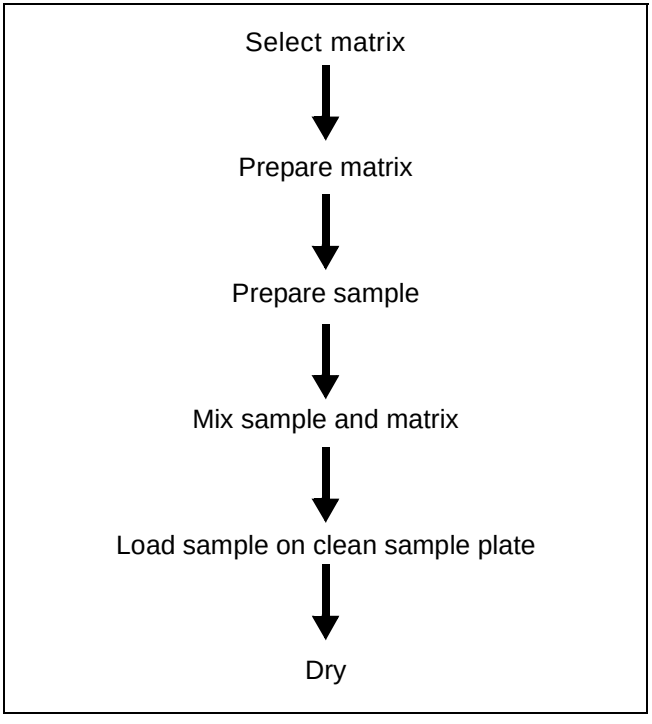
In this section

This section describes:

- Selecting matrix
- Preparing matrix
- Matrix information
- Preparing sample
- Sample cleanup
- Mixing sample and matrix (dried droplet application)

Overview

To prepare samples, you:



3.1.1 Selecting a Matrix

Purpose of matrix In MALDI-TOF, the matrix plays a key role in the ionization process. The well-developed crystals of matrix material assist in ionizing the molecules you are analyzing.

Common matrixes For most applications, you can use one of the following matrixes:

Matrix	Application
Sinapinic acid (3,5-Dimethoxy-4-hydroxy cinnamic acid)	Peptides and proteins >10,000 Da
α-cyano-4-hydroxycinnamic acid (CHCA)	• **Dried drop application**—Peptides/proteins <10,000 Da • **Thin layer application**—Peptides <~3,000 Da
2,4,6-Trihydroxy acetophenone (THAP)	Small oligonucleotides <3,500 Da, acidic carbohydrates, acidic glycopeptides, acid sensitive compounds
3-hydroxypicolinic acid (3-HPA) in ammonium citrate	Large oligonucleotides >3,500 Da
2,5-dihydroxybenzoic acid (2,5-DHB)	Peptides, neutral or basic carbohydrates, glycolipids (negative ions), polar and non-polar synthetic polymers, small molecules
2,5-dihydroxybenzoicacid and 5-methoxysalicylic acid (DHBs)	Peptides and proteins >10,000 Da, glycosylated proteins
Dithranol and Ag TFA	Aromatic polymers
trans-3-indoleacrylic acid (IAA)	Non-polar polymers

Procedures for preparing matrix are included in Section 3.1.2, Preparing Matrix.

Mixtures If you are examining a sample mixture, it may be necessary to prepare the mixture with several different matrixes.

Specialized applications For specialized applications, you may need to investigate other matrixes, for example:

Matrix	Application
1-(4-hydroxyphenylazo)-benzoic acid (HABA)	Proteins, polar and nonpolar synthetic polymers

Additional information Appendix C, Matrixes, contains additional information on matrix structure, appearance, and matrix solutions. For additional information on other matrixes and their applications, refer to the bibliography.

3.1.2 Preparing Matrix

Matrix stability Prepare fresh matrix as needed. Some matrixes degrade upon exposure to light or humidity. Some matrixes require daily preparation. Other matrixes can be stored for up to one week at room temperature. Follow the matrix manufacturer's instructions for storage conditions of chemicals, and refer to individual matrix descriptions in the following section for additional stability information.

What you need Materials required:

- Balance
- 1.5 ml microcentrifuge tubes
- Micropipettor and disposable tips
- Centrifuge
- Vortex mixer
- Matrix
- Deionized water
- Solvents

NOTE: *HPLC-grade water may vary in salt concentration and may produce adduct ions in mass spectra. A high salt concentration may interfere with some applications, particularly oligonucleotide analysis. Use double-deionized water, such as Milli-Q™ grade 18* mΩ*, which is appropriate for most applications.*

WARNING

CHEMICAL HAZARD. *Refer to the Material Safety Data Sheet (MSDS) provided by the chemical manufacturer before handling solvents or matrixes.*

Preparing matrix

To prepare matrix:

1. Label a 1.5 ml microcentrifuge tube with the name of the matrix, the final concentration, and the date prepared.
2. Before weighing out the matrix, zero the balance with the labeled tube.
3. Weigh out the matrix into the tube. See “Matrix Information” on page 3-6 to determine the proper concentration for your matrix.

   NOTE: *It is not necessary to weigh out the exact amount of matrix. You can record the weight of the matrix and adjust the final concentration accordingly.*

   NOTE: *Use a fresh tip each time you pipette a different substance.*

4. Add a volume of appropriate solvent to achieve the needed concentration for your matrix. See “Matrix Information” on page 3-6. The solvent should be miscible with the sample.

5. Cap the tube and vortex thoroughly for approximately 1 minute or until dissolved. You can shake the tube by hand if you do not have a vortex mixer.
6. Microcentrifuge the tube for 30 seconds at 2,000 to 5,000 rpm. Alternatively, allow the solution to settle for about 10 minutes. You may see a precipitate at the bottom of the tube.

   When applying matrix, use the supernatant, not the precipitate.

3.1.3 Matrix Information

Refer to the tables on the following pages to determine the requirements for the matrix you are using. This section includes information for:

- Sinapinic acid
- α-cyano-4-hydroxycinnamic acid (CHCA)
- THAP
- 3-HPA
- DHB
- DHBs
- Synthetic polymer matrixes

WARNING

CHEMICAL HAZARD. *Refer to the Material Safety Data Sheet (MSDS) provided by the chemical manufacturer before handling solvents or matrixes.*

Sinapinic acid

Use sinapinic acid for peptides and proteins >10,000 Da.

WARNING

CHEMICAL HAZARD. *Please read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*
Sinapinic acid *may cause eye, skin, and respiratory tract irritation.*
Acetonitrile *is a flammable liquid and vapor. It may cause eye, skin, and respiratory tract irritation, central nervous system depression, and heart, liver, and kidney damage.*
Trifluoroacetic acid *causes severe burns to the eyes, skin, and respiratory tract.*

Table 3-1 Sinapinic Acid Matrix Information

Matrix concentration	10 mg/ml
Final sample concentration	0.1–5 pmol/µl
Solvents	Acetonitrile, 0.1% TFA in deionized water
Preparation	Follow the procedure in “Preparing matrix” on page 3-5 and combine 700 µl of 0.1% TFA solution in water, and 300 µl acetonitrile to 10 mg of solid matrix. If sample is contaminated with buffer, salt, or detergent, instead of the proportions listed above, combine 500 µl 0.1% TFA and 500 µl acetonitrile. Air dry sample plate after loading sample and matrix.

continued

Table 3-1 Sinapinic Acid Matrix Information (Continued)

NOTE: *The organic concentration above assumes you are premixing sample solution:matrix solution in a 1:10 dilution. If you are mixing sample solution:matrix solution on the plate in a 1:1 dilution, increase the proportion of acetonitrile to 50 percent to prevent the matrix from precipitating. Adjust the other components accordingly.* ***NOTE:*** *A higher concentration of TFA (up to 3%) may improve sample solubility.*	
Crystals	Uniform rhomboid shape (see Figure 3-1 on page 3-45)
Stability	Prepare daily.

CHCA

Use α-cyano-4-hydroxycinnamic acid (CHCA) for:

- **Dried drop application**—Peptides/proteins <10,000 Da
- **Thin layer application**—Peptides <~3,000 Da[1]

CHCA for dried droplet application

Use for peptides/proteins <10,000 Da.

WARNING

CHEMICAL HAZARD. *Please read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*
Alpha-cyano-4-hydroxycinnamic acid (CHCA) *may cause eye, skin, and respiratory tract irritation.*
Acetonitrile *is a flammable liquid and vapor. It may cause eye, skin, and respiratory tract irritation, central nervous system depression, and heart, liver, and kidney damage.*
Trifluoroacetic acid *causes severe burns to the eyes, skin, and respiratory tract.*

Table 3-2 CHCA Matrix Information for Dried Droplet Application

Matrix concentration	10 mg/ml
Final sample concentration	0.1–5 pmol/µl
Solvents	Acetonitrile, 0.1% TFA in deionized water
Preparation	Follow the procedure in “Preparing matrix” on page 3-5 and combine one part 0.1% TFA solution with one part acetonitrile. Dissolve the matrix at a concentration of 10 mg/ml (for example, add 500 µl 0.1% TFA solution and 500 µl acetonitrile to 10 mg of solid matrix). Air dry sample plate after loading sample and matrix, or use gentle air flow to speed drying.

continued

Table 3-2 CHCA Matrix Information for Dried Droplet Application (Continued)

NOTE: *If the dry matrix is a mustard-yellow color instead of bright yellow, it may contain impurities. To recrystallize (purify), dissolve the α-cyano matrix in warm ethanol. Filter and add about two volumes of deionized water. Let stand in the refrigerator overnight. Filter, then wash the precipitate with cold water. This procedure is not optimized for yield.*	
NOTE: *The organic concentration above assumes you are premixing sample solution:matrix solution in a 1:10 dilution. If you are mixing sample solution:matrix solution on the plate in a 1:1 dilution, increase the proportion of acetonitrile to 60 percent to prevent the matrix from precipitating. Adjust the other components accordingly.* ***NOTE:*** *A higher concentration of TFA (up to 3%) may improve sample solubility.*	
Crystals	Rounded (see Figure 3-1 on page 3-45)
Stability	Prepare weekly.

1. Shevchenko, A., M. Wilm, O. Vorm, M. Mann, *Anal. Chem.,* **1996**, *68*, 850–858.

CHCA for thin layer application

Use for peptides <3,000 Da.

WARNING

CHEMICAL HAZARD. *Please read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

Alpha-cyano-4-hydroxycinnamic acid (CHCA) *may cause eye, skin, and respiratory tract irritation.*

Acetone *is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin. It may cause central nervous system effects such as drowsiness, dizziness, and headache, and so on.*

Isopropanol *is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin and cause irritation. It may cause central nervous system effects such as drowsiness, dizziness, and headache, and so on.*

Ethanol *is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin. Exposure may cause central nervous system depression and liver damage.*

Table 3-3 Sinapinic Acid Matrix Information for Thin Layer Application

Matrix concentration	20 mg/ml
Final sample concentration	Up to 0.1 pmol/µl
Materials and solvents	• Pure nitrocellulose (for example, Bio-Rad Laboratories Trans-Blot® 162-0146) • Acetone • Isopropanol

continued

Table 3-3 Sinapinic Acid Matrix Information for Thin Layer Application (Continued)

Preparation[1]	1. Dissolve nitrocellulose to a concentration of 20 mg/ml in acetone by vortexing for 15 minutes. 2. Add isopropanol at a ratio of 1:1. 3. Add α-cyano-4-hydroxycinnamic acid to a final concentration of 20 mg/ml. Air dry sample plate after loading matrix. Dry with gentle air flow after adding sample.
NOTE: *If the dry matrix is a mustard-yellow color instead of bright yellow, it may contain impurities. To recrystallize (purify), dissolve the α-cyano matrix in warm ethanol. Filter and add about two volumes of deionized water. Let stand in the refrigerator overnight. Filter, then wash the precipitate with cold water. This procedure is not optimized for yield.*	
Crystals	Rounded (see Figure 3-1 on page 3-45)
Stability	Prepare weekly.

1. Other special sample preparation techniques are described in Shevchenko, A., M. Wilm, O. Vorm, M. Mann, *Anal. Chem.,* **1996**, *68*, 850–858.

THAP

Use THAP for oligonucleotides <3,500 Da, acidic carbohydrates, acidic glycopeptides, acid sensitive compounds. THAP provides a more even response than 3-HPA.

WARNING

***CHEMICAL HAZARD**. Please read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*
***THAP** may cause eye, skin, and respiratory tract irritation.*
***Acetonitrile** is a flammable liquid and vapor. It may cause eye, skin, and respiratory tract irritation, central nervous system depression, and heart, liver, and kidney damage.*

Table 3-4 THAP Matrix Information

Matrix concentration	• **Oligonucleotides**—10 mg/ml • **Acidic carbohydrates**—2 mg/ml
Additive concentration	50 mg/ml diammonium citrate in deionized water
Final sample concentration	1–10 pmol/µl
Solvents	50 percent acetonitrile and deionized water ***NOTE:** HPLC-grade water may vary in salt concentration. Do not use for oligonucleotide analysis.*

continued

Table 3-4 THAP Matrix Information (Continued)

Matrix concentration	• **Oligonucleotides**—10 mg/ml • **Acidic carbohydrates**—2 mg/ml
Additive concentration	50 mg/ml diammonium citrate in deionized water
Preparation[1]	Follow the procedure in "Preparing matrix" on page 3-5, the for: • **Small oligonucleotides**—Combine 8:1 THAP:diammonium citrate. Air dry sample plate after loading sample and matrix. • **Acidic carbohydrates and glycopeptides**—Combine 20:1 THAP:diammonium citrate. Dry sample plate under vacuum after loading sample and matrix. Wait a few minutes after drying until the sample absorbs ambient humidity and turns faint white.
Crystals	Larger than sinapinic, overlapping shingles, non-uniform shape (see Figure 3-2 on page 3-46)
Stability	Prepare weekly.

1. Other special sample preparation techniques are described in Papac, D.I., A. Wong, A.J.S. Jones, *Anal. Chem.,* **1996**, *68*, 3215–3223.

3-HPA

Use 3-HPA for oligonucleotides >3,500 Da.

WARNING

CHEMICAL HAZARD. *Please read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

3-Hydroxypicolinic acid (3-HPA) *may cause eye, skin, and respiratory tract irritation.*

Acetonitrile *is a flammable liquid and vapor. It may cause eye, skin, and respiratory tract irritation, central nervous system depression, and heart, liver, and kidney damage.*

Table 3-5 3-HPA Matrix Information

Matrix concentration	50 mg/ml
Additive concentration	50 mg/ml diammonium citrate in deionized water
Final sample concentration	1–10 pmol/µl
Solvents	50 percent acetonitrile and deionized water ***NOTE:*** *HPLC-grade water may vary in salt concentration. Do not use for oligonucleotide analysis.*
Preparation	Follow the procedure in “Preparing matrix” on page 3-5 and combine 8:1 3-HPA:diammonium citrate. Air dry sample plate after loading sample and matrix.
Crystals	Needle-like crystals inside a ring (see Figure 3-2 on page 3-46)
Stability	Prepare weekly.

DHB

Use DHB for:

- Neutral carbohydrates
- Small molecules

DHB for neutral carbohydrates

WARNING

CHEMICAL HAZARD. *Please read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

Table 3-6 DHB Matrix Information for Neutral Carbohydrates

Matrix concentration	10 mg/ml
Final sample concentration	10 pmol/µl
Solvents	Deionized water
Preparation	Follow the procedure in “Preparing matrix” on page 3-5. Dry down quickly under vacuum for even response. If you allow to air dry, you will see uneven response during analysis.
Crystals	Milky amorphous appearance for promoting cationization (see Figure 3-3 on page 3-46). Difficult to see crystals when vacuum dried.
Stability	Prepare weekly.

DHB for small molecules

WARNING

CHEMICAL HAZARD. *Please read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

Methanol *is a flammable liquid and vapor. Exposure may cause eye, skin, and respiratory tract irritation, and central nervous system depression and blindness.*

Acetone *is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin. It may cause central nervous system effects such as drowsiness, dizziness, and headache, and so on.*

Table 3-7 DHB Matrix Information for Small Molecules

Matrix concentration	10 mg/ml
Final sample concentration	Highly sample-dependent. Ideally a minimum of 10–200 pmol/µl (10–20 ng/µl). With lower concentrations, sample signal may be difficult to distinguish from matrix signal.
Solvents	Any solvent in which molecules are soluble (deionized water to 100% methanol or acetone).
Preparation	Follow the procedure in “Preparing matrix” on page 3-5. Dry down quickly under vacuum for even response. If you allow to air dry, you will see a less even response during analysis.
Crystals	Needle-like crystals arranged in a ring if air-dried (see Figure 3-3 on page 3-46).
Stability	Prepare weekly.

DHBs Use DHBs for peptides and proteins >10,000 Da, and glycosylated proteins.

WARNING

CHEMICAL HAZARD. *Please read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*
Acetonitrile *is a flammable liquid and vapor. It may cause eye, skin, and respiratory tract irritation, central nervous system depression, and heart, liver, and kidney damage.*

Table 3-8 DHBs Matrix Information

Matrix concentration	10 mg/ml
Additive concentration	10 mg/ml 5-methoxysalicylic acid
Final sample concentration	10 pmol/µl to 100 fmol/µl
Solvents	80% 0.1% TFA in deionized water:20% acetonitrile (for DHB) + 50% acetonitrile:50% deionized water (for 5-methoxysalicylic acid)
Preparation	Follow the procedure in “Preparing matrix” on page 3-5 and combine 9:1 DHB:5-methoxysalicylic acid solutions. Air dry sample plate after loading sample and matrix.
Crystals	Needle-like crystals arranged in a ring if air-dried (see Figure 3-3 on page 3-46). No obvious crystals if vacuum-dried, or if solvent is a fast-drying organic.
Stability	Prepare weekly.

Synthetic polymer matrixes

WARNING

CHEMICAL HAZARD. *Please read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

Tetrahydrofuran *is a flammable liquid and vapor. It may be harmful if swallowed. Exposure may cause eye and respiratory tract irritation, central nervous system depression, and liver and kidney damage.*

N,N-Dimethylformamide (DMF) *is harmful if inhaled. It is a combustible liquid and vapor. Exposure may cause eye, skin, and respiratory tract irritation, and damage to the liver, kidneys, and heart. It is a suspect cancer hazard.*

Acetone *is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin. It may cause central nervous system effects such as drowsiness, dizziness, and headache, and so on.*

Choice of matrix depends on the sample you are analyzing:

- **Aromatic (for example, polystyrene)**—Dithranol (25 mg/ml) and 1 mg/ml silver trifluoroacetate (Ag TFA) dissolved in tetrahydrofuran (THF)
- **Polar**—DHB (10 mg/ml) in deionized water
- **Non-polar**—Indole acetic acid (10 mg/ml) or DHB in THF, dimethylformamide (DMF), or acetone

Table 3-9 Synthetic Polymer Matrix Information

Matrix concentration	~0.1 M (10^{-1} M)
Final sample concentration	~0.1 mM (10^{-4} M)
Solvents	Sample and matrix dependent. Choose solvents in which polymer and matrix are soluble.
Preparation	Follow the procedure in “Preparing matrix” on page 3-5 and combine 1:1 sample:matrix. See page 3-21 for application techniques. Apply vacuum drying for non-volatile solvents (water, DMF). Acetone and THF dry instantaneously.
Crystals	No crystals visible. If sample position looks glassy or shiny, it may indicate sample concentration is too high. Analyze polymer samples within one hour of loading on the sample plate. Many polymer/matrix mixtures are not stable once they are loaded. Areas in which you see hot and cold spots are much smaller with polar compounds than with non-polar compounds.
Stability	Prepare weekly. Store THF and acetone solutions in tightly sealed vials.

You can load polymer samples on sample plates in two ways:

- **Thin layer polymer method (yields even response, but provides adequate sample response for only 10 laser shots)**—Load 0.3 µl sample/matrix solution in one sample position.
- **Thick layer polymer method (yields less even response, but provides adequate sample response for 50 to 100 laser shots)**—Load 2 to 3 µl sample/matrix solution in one sample position by placing the pipette tip in the sample position and slowly expelling while keeping the tip in contact with the sample plate.

NOTE: *In general, do not touch the pipette tip to the sample plate surface. However, when you use THF as the solvent in the thick layer method, it is necessary to touch the tip to the sample plate to slowly expel the sample onto the sample plate.*

3.1.4 Preparing Sample

In this section

This section includes:

- Sample concentration
- Preparing samples for dried droplet application
- Preparing samples for thin layer application
- Internal standards

Sample concentration

Prepare sample just before loading the sample plate. Refer to Section 3.1.5, Sample Cleanup, to determine if your samples should be cleaned up before preparing.

The ideal sample amount for analysis is:

Compound	Typical Concentration	
	Dried Droplet Application	Thin Layer Application
Peptides	0.1 to 10 pmol/µl	<0.1 pmol/µl
Proteins*	0.1 to 10 pmol/µl	_____
Oligonucleotides	10 to 100 pmol/µl	____
Polymers	100 pmol/µl	_____
* Some proteins, particularly glycoproteins, yield better results at concentrations up to 10 pmol/µl.		

Preparing samples for dried droplet application

For peptides and proteins, dilute sample at this phase of sample preparation to a concentration of 1 to 10 pmol/µl. For other compounds, refer to Section 3.1.3, Matrix Information, to determine the appropriate concentration for this phase of sample preparation.

Note the following:

- When diluting sample, keep in mind that you will be further diluting when you mix sample with matrix.
- If you are unsure of the starting concentration of sample, make a serial dilution to prepare various dilutions of the same sample.
- Different samples are soluble in different liquids. Try water first, then add acetonitrile, and then add 0.1% TFA to increase solubility if required.

WARNING

***CHEMICAL HAZARD**. **Acetonitrile** is a flammable liquid and vapor. It may cause eye, skin, and respiratory tract irritation, central nervous system depression, and heart, liver, and kidney damage. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

- A higher concentration of TFA may enhance sample ionization and improve sensitivity in samples prepared in buffers.

WARNING

***CHEMICAL HAZARD**. **Trifluoroacetic acid** causes severe burns to the eyes, skin, and respiratory tract. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

- Many samples adhere strongly to plastic tubes and pipet tips. You can minimize sample loss by preparing samples in 30 percent acetonitrile with 5 to 10 percent TFA.
- Do not dilute sample with phosphate buffered saline (PBS) or other buffer solutions. A high salt concentration can interfere with sample ionization and may cause increased sodium and potassium adduct peaks.

Preparing samples for thin layer application

Prepare samples in water. If samples contain organic solvents, they will dissolve the matrix applied to the sample plate.

Internal standards

If you require mass accuracy greater than the accuracy provided by external calibration, (for example, >0.05% on a Voyager-DE system), use an internal standard. See Appendix A, Specifications, for the mass accuracy on other systems.

Add two standards of known molecular weight to the sample. Standards should:

- Closely bracket the molecular weight of your unknown
- Be easily distinguished from the unknown

As a starting point, use an internal standard concentration in the same range as your sample concentration. Because an internal standard may affect the intensity of the sample signal, it is a good idea to prepare sample with several different internal standard concentrations.

3.1.5 Sample Cleanup

Cleaning techniques

Use the following techniques to clean samples:

- Washing
- Drop dialysis (floating membrane)
- Cation exchange
- ZipTips®

When to clean samples

Sample cleanup is needed if samples:

- Are prepared in phosphate buffers. Ammonium salts or derivatives of organic amines (ammonium bicarbonate, TRIS HCl) do not usually cause a problem in low concentrations (less than 50 mM).
- Contain salt, for example, from cation or anion exchange purification.
- Are contaminated with detergent.

Symptoms that indicate the need for sample cleanup include:

- Poor crystallization on the sample plate.
- Large tail on the high mass side of peaks, which may be unresolved salt or buffer adducts.
- Poor sensitivity with a sample concentration that should yield a strong signal. To detect this condition, mix the sample with a standard that you know yields a strong signal. If the standard no longer exhibits the expected signal, a contaminant in the sample is affecting sensitivity.

3.1.5.1 Washing

When to use Use this technique when you know the solubility of the contaminant. You can wash a dried sample directly on the sample plate.

What you need Select a solvent in which the contaminant is more soluble than the matrix and the sample. For example, use:

- Cold water (to prevent sample dissolving) with 0.1% TFA for a polar contaminant, such as a salt or buffer
- 5% isopropanol in water for a non-ionic detergent such as octyl glucoside

WARNING

***CHEMICAL HAZARD**. **Isopropanol** is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin and cause irritation. It may cause central nervous system effects such as drowsiness, dizziness, and headache, and so on. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

Procedure To wash:

1. Apply sample and matrix to the sample plate and allow to dry.
2. Add 1 to 3 µl of solvent to the dry sample/matrix.
3. Wait 10 seconds, then remove the liquid.

***NOTE:** Try not to touch the sample plate with the pipet tip. If you do touch the spot, do not analyze the area you touched.*

4. Repeat step 2 and step 3 one more time.
5. Allow the sample plate to dry before analyzing.

   If one wash does not improve results, you can wash again.

3.1.5.2 Drop Dialysis (Floating Membrane)

When to use Use this technique on polar compounds when you know contaminants are of low molecular weight. This technique works well for DNA and polar proteins such as glycoproteins.

What you need For drop dialysis, you need a membrane:

- With a pore size of 0.025 µm or smaller
- That does not adhere to your sample

Procedure To perform drop dialysis:

1. Fill a small container (for example, a pipet tip box) with about an inch of deionized water.
2. Place the container on a stable surface.
3. Use forceps to place the membrane in the water with the appropriate side up (refer to manufacturer's information). Do not use your fingers because you can contaminate the membrane with oil and salt.
4. Place about 10 parts of sample (for example, 10 µl) in the center of the membrane.

5. Place 1 part of pure acetonitrile (for example, 1 µl) on top of the sample spot to increase surface area. Do not exceed a 10 percent concentration of organic. It may dissolve the membrane.

WARNING

***CHEMICAL HAZARD**. **Acetonitrile** is a flammable liquid and vapor. It may cause eye, skin, and respiratory tract irritation, central nervous system depression, and heart, liver, and kidney damage. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

6. Cover the container to prevent drying and allow it to sit for 30 minutes.

7. Note the size of the sample/organic spot, then let it sit for 30 more minutes.

   The size of the sample spot increases as dialysis is occurring. This can be used to check for completion.

8. Observe the size of the sample/organic spot. If the size of the spot is larger than the first time you checked it, allow the sample to sit for 15 more minutes. Observe the size of the sample/organic spot again.

***NOTE:** Sample also passes through the membrane during dialysis, particularly low mass samples. Dialyze small molecules for a shorter time than larger molecules. In general, do not dialyze for more than two hours.*

9. When the size of the sample spot stabilizes, remove the sample and place it in a microcentrifuge tube.

***NOTE:** The size of the sample drop can increase by a factor of 10 when salt concentration is high.*

3.1.5.3 Cation Exchange

When to use

Use this technique for non-polar proteins or DNA when you know the sample contains only a salt contaminant. This technique is faster than dialysis but does not remove other contaminants.

What you need

Use 200-mesh cation exchange beads. Cation exchange beads in the ammonium form work best for MALDI applications.

Preparation of beads in ammonium form

To prepare cation exchange beads in the ammonium form:

1. Place beads in twice the bead volume of 1M ammonium acetate.
2. Leave beads in ammonium acetate overnight.

IMPORTANT. Ammonium acetate. *Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

3. Remove beads, and wash with water, acetone, and then hexane through a Buchner funnel under vacuum.

WARNING

CHEMICAL HAZARD*.* ***Acetone*** *is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin. It may cause central nervous system effects such as drowsiness, dizziness, and headache, and so on. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

WARNING

***CHEMICAL HAZARD**. **Hexane** is a flammable liquid and vapor. It may cause eye, skin, and respiratory tract irritation. Prolonged or repeated contact may dry skin. It may cause central nervous system effects such as drowsiness, dizziness, and headache, and so on, and damage the peripheral nervous system (numbness of the extremities). Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

After washing, beads can be stored indefinitely and used as needed.

Procedure

To perform cation exchange:

1. Place about 0.1 mg (a small spatula-tip-full) of cation exchange beads on a piece of laboratory film.
2. Add 5 to 10 µl of sample for 200-mesh cation exchange beads. If sample quantity is limited, you can make up the difference with deionized water.
3. Add 5 to 10 µl of matrix on top of the sample, as matrix may also contain salt.
4. Mix by withdrawing and expelling about 20 times with a pipet.

***NOTE:** The volume of sample decreases as the beads absorb water.*

5. Allow the beads to settle for 30 seconds.
6. Remove the supernatant with a clean pipet tip and spot on the sample plate.

3.1.5.4 ZipTips®

When to use

Use this technique for peptides, proteins, and oligonucleotides when you know the sample contains salt, buffer, or glycerol contaminants. This technique is faster, easier, and more effective than dialysis for removing contaminants.

NOTE: *This technique introduces organic solvent into the sample, which is not compatible with thin layer sample application. If you will apply sample using the thin layer application technique, remove the organic solvent from the sample before loading sample on the plate. For more information, see Section 3.2.4, Loading Samples (Thin Layer Application).*

Millipore ZipTips® can be used for a wide range of applications and are compatible with digestion procedures. You can expect 50 to 70 percent recovery using the following procedure.

NOTE: *ZipTips can also be used for sample concentration and fractionation of complex mixtures.*

What you need

To clean samples with ZipTips you need:

- Micro-adsorptive C_{18} pipette ZipTips
- Acetonitrile (ACN)
- TFA

WARNING

CHEMICAL HAZARD. ***Acetonitrile*** *is a flammable liquid and vapor. It may cause eye, skin, and respiratory tract irritation, central nervous system depression, and heart, liver, and kidney damage. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

Procedure

To clean samples with ZipTips:

1. Wash a C_{18} ZipTip in the following order with:

   - 10 µl of ACN
   - 10 µl of 50:50 ACN:0.1%TFA
   - 10 µl of 0.1% TFA
   - Repeat wash of 10 µl of 0.1% TFA

   To wash, draw a few microliters of a wash solution up into the ZipTip and expel to waste.

2. Draw a few microliters of the sample up and down in the ZipTip several times.
3. Discard the liquid. The sample is bound to the C_{18} surface in the ZipTip.
4. Wash the ZipTip again with 10 µl of 0.1% TFA by drawing up into the ZipTip and expel to waste.
5. Repeat step 4 two times.
6. Elute the sample with 2 to 3 µl of organic solvent or matrix containing approximately 50 percent ACN by drawing solvent or matrix/solvent up and down in the ZipTip several times.

   NOTE: *A 50 percent organic concentration should give 50 to 70 percent sample recovery. You can vary the organic concentration to optimize recovery.*

   ---

7. Elute samples directly on the plate as described in Section 3.2.3, Loading Samples (Dried Droplet Application), or into a microcentrifuge tube for storage.

3.1.6 Mixing Sample and Matrix (Dried Droplet Application)

When to use

Use dried droplet application if you are analyzing samples with a concentration >0.01 pmol/µl.

If you are analyzing samples with a concentration <0.01 pmol/µl, use the thin layer application technique described in Section 3.2.4, Loading Samples (Thin Layer Application).

Premixing sample and matrix

Mix sample and matrix in microcentrifuge tubes before applying sample to the sample plate when you are:

- Working with concentrated samples with a high salt concentration and need to make dilutions
- Preparing many samples
- Analyzing non-polar samples and matrix prepared in high organic concentration that evaporates rapidly

For peptides and proteins, mix 1 µl of sample (concentration of 0.1 to 100 pmol/µl) and 9 µl of matrix in a microcentrifuge tube, for a final concentration of 0.1 to 10 pmol/µl. Mix well on a vortex mixer or shake by hand.

For other compounds, refer to “Matrix Information” on page 3-6 to determine the volume of sample and matrix to mix to yield the necessary final sample concentration.

NOTE: *If sample concentration is too high, the sample signal may be suppressed. If sample concentration is too low, sample signal may not be present.*

Mixing sample and matrix on the sample plate

Mix sample and matrix directly on the sample plate when you are:

- Working with dilute samples and can make a 1:1 preparation of sample:matrix
- Analyzing only a few samples
- Concerned about sample adhering to plastic tubes

When mixing directly on the plate, you may need to use a higher concentration of organic and matrix for matrix solutions. With the 1:1 dilution used in this strategy, the typical concentration of organic may not be high enough to keep the matrix in solution.

Load 0.5 to 1 µl of sample on the plate of the appropriate concentration to yield the necessary final sample concentration, followed by 0.5 to 1 µl of matrix. See “Matrix Information” on page 3-6 for final sample concentrations.

A homogeneous mixture is critical for good crystallization. Applying sample before matrix prevents matrix from drying before it mixes with sample.

3.2 Loading Samples on Sample Plates

In this section This section describes:

- Overview
- Locating standards for optimum mass accuracy
- Loading samples (dried droplet application)
- Loading samples (thin layer application)
- Examining crystals on sample plates

3.2.1 Overview

In this section This section describes:

- Types of applications
- Types of sample plates
- Handling sample plates
- Guidelines for good crystallization
- Recommended pipette tips

Types of applications Two techniques are available for sample application:

- **Dried droplet**—Simple application technique suitable for most applications. Can use any type of sample plate.
- **Thin layer**—Use for increased sensitivity when analyzing peptides with a concentration <0.1 pmol/µl. Requires polished blank sample plate (no etched positions).

Types of sample plates

Three types of 100-position reusable sample plates are available:

- **Polished blank sample plates (with or without sample numbers)**—Liquid is held in place by surface tension of sample/matrix mixture. Advantage of this type of plate is that you can observe crystallization pattern, and the actual sample spot is visible.
- **Laser-etched sample plates**—Liquid is held in place by laser-etched indentation in plate. Advantage of this type of plate is that the sample position on the plate is well-defined. Use laser-etched sample plates when running in Automatic Control mode.
- **Welled sample plates**—Liquid is held in place by indentation in plate. Advantage of this type of plate is that the sample position on the plate is well-defined. Use welled sample plates:
  - When running in Automatic Control mode
  - To contain larger sample/matrix volumes that will spread beyond the sample position boundaries, due to volatile solvent content

In addition to 100-position reusable sample plates, the Voyager software supports other reusable and disposable plates. For more information, see:

- Section 3.5, Sample Plate Types
- Section 3.5.1, Sample Plate Types and Applications

Handling sample plates

To prevent contamination of your analysis:

- Start with a clean sample plate. See Section 3.3, Cleaning Sample Plates, for more information.
- Handle the sample plate by the edges.
- Use powder-free gloves if you wear gloves.

Guidelines for good crystallization

To ensure good crystallization:

- Mix sample and matrix before applying. If you are mixing sample and matrix directly on the sample plate, apply sample before matrix to prevent matrix from drying.
- If you are using a welled plate, fill the entire well when loading the sample plate, if possible, especially if acquiring in Automatic control mode. Surface tension and sample availability may determine whether you fill the well completely.
- Do not touch the surface of the sample well with the pipette tip (may cause uneven crystallization).

Recommended pipette tips

Use finely-tapered pipette tips to dispense sample/matrix solution on the sample plate. Tips with blunt ends do not easily dispense the small volumes used for sample loading.

3.2.2 Locating Standards for Optimum Mass Accuracy

In a typical analysis run that requires optimum mass accuracy, you include samples interspersed with many calibration standards, and externally calibrate the samples using the closest calibration standard.

The number and location of standards you need depends on whether or not you use the Mass Accuracy Optimization option when you analyze samples (described in Section 3.4.2, Using the Mass Accuracy Optimization Option).

Without Mass Accuracy Optimization

To obtain optimum mass accuracy without using the Mass Accuracy Optimization option, you must:

- Spot standards in every other position relative to samples
- Locate the standards as close as possible to samples (as on the 96 × 2 sample plate)

Do not use the outer wells on the sample plate when using flat or polished blank plates.

With Mass Accuracy Optimization

If you use the Mass Accuracy Optimization option, the number and location of standards you need to obtain optimum mass accuracy depends on:

- Whether or not you use an optimized sample plate
- The type of sample plate you use

With an optimized plate

To obtain optimum mass accuracy using the Mass Accuracy Optimization option and a plate you have optimized with OptiPlate you can:

- Spot one or a few standards on the plate. The number of standards required depends on the plate you use and your mass accuracy needs.

Plate	Number of Standards Needed
96 × 2	1 (center)
64, 100, 384, 400 (plates with positions located near the physical edge of the plate)	5 (center and 4 corners)

- Locate standards anywhere on the plate, for example, in a center position (on a 96 × 2 plate) and in four corner positions (on other plates that have positions located near the physical edge of the plate).
- When you analyze, set the run to update the calibration approximately every 3 minutes (calibration update may be required less frequently or not at all, depending on your mass accuracy needs).

  To set the run to update the calibration, list the standard position in the sequence run list every fifth line or so (the precise line on which to list the standard depends on the time required for each line in the sequence to run) and specify **Internal-Update** calibration. For more information, see Section 7, Acquiring Spectra from the Sequence Control Panel.

With an unoptimized plate

You can use an unoptimized sample plate and still obtain better mass accuracy than if you do not use Mass Accuracy Optimization. However, you must run the OptiPlate software and optimize at least one sample plate on your system before using an unoptimized plate. For more information, see Section 2.8, Running OptiPlate to Optimize Mass Accuracy.

To obtain optimum mass accuracy during analysis using the Mass Accuracy Optimizations option and a plate you have not optimized with OptiPlate you can:

- Spot more standards on the plate than if you are using an optimized plate (but fewer than if you are not using the Mass Accuracy Optimization option).
- Locate standards in regular positions on the plate, for example, every fifth position across the plate.
- When you analyze, set the run to update the calibration approximately every 3 minutes (calibration update may be required less frequently or not at all, depending on your mass accuracy needs).

  To set the run to update the calibration, list the standard position in the sequence run list every fifth line or so (the precise line on which to list the standard depends on the time required for each line in the sequence to run) and specify **Internal-Update** calibration. For more information, see Section 7, Acquiring Spectra from the Sequence Control Panel.

3.2.3 Loading Samples (Dried Droplet Application)

NOTE: *If you are loading a plate for acquisition or automated sample positioning in Automatic Control mode, use a laser-etched or welded sample plate to provide a reference point for sample application.*

When to use

Use this technique for most applications, when sample concentration is >0.1 pmol/ µl.

Before loading samples

Note the following:

- Some organic solvents such as methanol, acetone, and THF spread very easily on metal surfaces. If the sample contains these solvents, try to load smaller volumes (0.5 µl or less).
- Try to place the sample in the center of the sample position. If the sample is not in the center, the laser position shown on the Sample View (see Figure 4-9 on page 4-31) may not be centered on the sample.

WARNING

CHEMICAL HAZARD*.* ***Methanol*** *is a flammable liquid and vapor. Exposure may cause eye, skin, and respiratory tract irritation, and central nervous system depression and blindness. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

WARNING

***CHEMICAL HAZARD**. **Acetone** is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin. It may cause central nervous system effects such as drowsiness, dizziness, and headache, and so on. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

WARNING

***CHEMICAL HAZARD**. **Tetrahydrofuran** is a flammable liquid and vapor. It may be harmful if swallowed. Exposure may cause eye and respiratory tract irritation, central nervous system depression, and liver and kidney damage.*

Loading samples

1. Create a log sheet listing sample position and sample name. Appendix D, Log Sheets, contains a master sheet that you can copy and use.

***NOTE:** For optimum mass accuracy, do not use the outer rows of the sample plate, unless you are using internal standards.*

2. Using a clean pipette tip for each new sample, load the following:

   - **If you premixed samples**—Load 0.5 to 2 µl of sample/matrix solution on the appropriate position.
   - **If you are mixing sample and matrix on the sample plate**—See “Mixing sample and matrix on the sample plate” on page 3-34.
   - **If you prepared samples with ZipTips**—Load 0.5 to 2 µl of sample/matrix solution on the appropriate position.

   Do not touch the tip of the pipette to the sample spot. Make sure the sample is evenly applied to the spot.

3. If you are using DHB matrix for neutral carbohydrates, dry the sample plate quickly under vacuum.

   If you are using other matrixes, allow the sample plate to dry for at least five minutes. Preparations with high water or salt content may require longer to dry.

4. Visually examine the sample spots to make sure they are dry.

NOTE: *If you load the sample plate into the Voyager Biospectrometry Workstation before the plate is dry, the pressure in the sample chamber rises, and a “TC2 pressure too high” error code may be displayed in the Control window. Wait a few minutes for the chamber to reach pressure.*

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5. Place a protective cover over the plate until you are ready to load the plate. Do not allow the cover to touch the surface of the plate.

3.2.4 Loading Samples (Thin Layer Application)

When to use

Use the thin layer application technique[1] for increased sensitivity when analyzing peptides with a concentration <0.1 pmol/ µl.

Loading matrix and samples

To load matrix and samples:

1. Dispense 0.5 µl of matrix prepared for thin layer application on the sample plate to form a thin layer. It will dry in seconds.

   For information, see “CHCA for thin layer application” on page 3-11.

2. Load 0.5 to 1 µl of the sample in each sample position onto the thin matrix layer applied in step 1.
3. Dry the plate with a heat gun.
4. After drying, load 2 to 3 µl of double-distilled water on top of each dried sample to wash the sample.
5. Wait 5 seconds, and blow off the water droplet with compressed air.
6. Repeat step 4 and step 5 two more times.

NOTE: *During acquisition, use the “surfing method” (moving the sample plate after every 5 to 10 shots, to a new position on the sample).*

1. Shevchenko, A., M. Wilm, O. Vorm, M. Mann, *Anal. Chem.,* **1996**, *68*, 850–858.

3.2.5 Examining Crystals on Sample Plates

Overview

If you are using polished blank or laser etched sample plates (see “Types of sample plates” on page 3-36), you can look at the crystallization of sample and matrix under a microscope. A magnification of 30X is sufficient to see crystals.

You can also view crystallization on the video monitor on the instrument.

The morphology of crystals is a critical element for successful analysis. Ideally, you want small crystals that are evenly distributed in the sample well.

α-cyano or sinapinic acid

Figure 3-1 illustrates good patterns of crystallization for α-cyano and sinapinic acid matrixes.

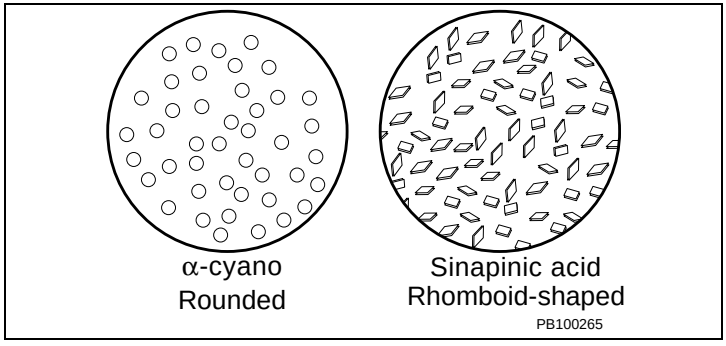

Figure 3-1 Microscopic View of Sample Plate with α-Cyano or Sinapinic Acid Matrixes

With good crystallization, you see small, equally-sized crystals that are evenly distributed on the plate. Clumping is not desirable. If you see clumped crystals with α-cyano or sinapinic acid matrixes, it may indicate:

- Matrix concentration is too high
- Organic concentration is too high
- Sample plate was dried too quickly
- Sample contains contaminants

You can acquire data from a well that does not have an ideal crystallization pattern. However, when sample contains unevenly distributed crystalllization, it may be difficult to analyze. This can cause a problem in Automatic mode.

Other matrixes

Typical appearance of other matrixes under magnification are shown in Figure 3-2 and Figure 3-3.

When analyzing 3-HPA crystals, aim the laser at the base of the fan-like crystals for best response.

Each matrix/sample class may require different laser intensities for analysis.

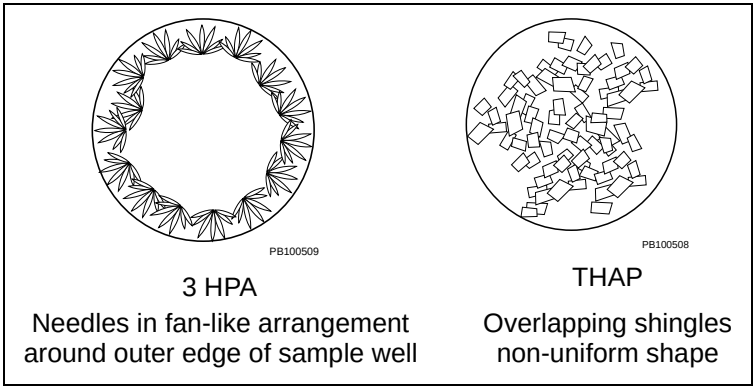

Figure 3-2 Microscopic View of Sample Plate with 3-HPA and THAP Matrixes

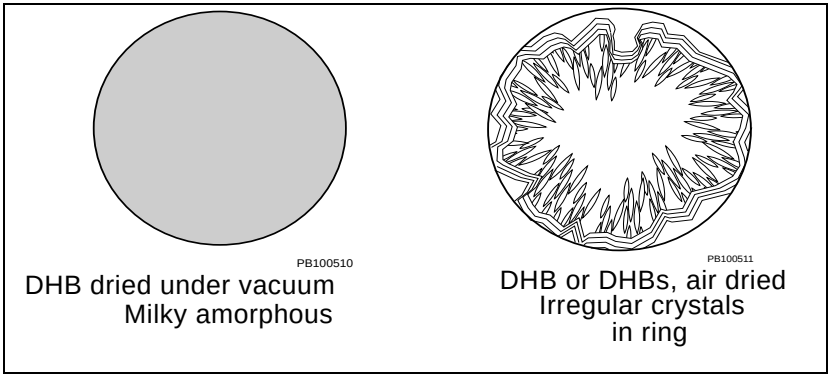

Figure 3-3 Microscopic View of Sample Plate with DHB or DHBs Matrix

3.3 Cleaning Sample Plates

In this section

This section describes procedures for cleaning:

- Teflon® plates
- Gold and stainless steel plates

Cleaning Teflon plates

NOTE: *Avoid using strong organic solvents such as acetone. Use acetonitrile if a solvent is necessary. Avoid ultrasonic cleaning devices.*

WARNING

CHEMICAL HAZARD. Acetonitrile *is a flammable liquid and vapor. It may cause eye, skin, and respiratory tract irritation, central nervous system depression, and heart, liver, and kidney damage. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

NOTE: *Do not use metal brushes, abrasive surfaces, or solid detergents to clean Teflon sample plates; they are fragile. Avoid rubbing the surface of the plate. If necessary, wipe gently with lint-free lab tissues.*

To clean Teflon plates:

1. Rinse the plate with luke warm water.
2. If necessary, wipe gently with a lint-free lab tissue to remove matrix.
3. Wash the plate with a 1 percent solution of a liquid detergent.
4. Rinse with deionized water.

5. If an organic solvent is necessary, rinse with acetonitrile at the lowest percentage that will dissolve the matrix.

6. Examine the plate. If you see any sample or matrix residue, oil, or fingerprints on the plate, soak the sample plate in a working solution of laboratory detergent in water (for example, RBS 35 detergent from Pierce or LIQUI-NOX™ from VWR) for the minimum time required to remove samples.

IMPORTANT. RBS, LIQUI-NOX. *Please read the manufacturer's MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

CAUTION

Do not leave the sample plate in detergent for longer than 10 minutes. Longer exposure can cause the bottom holders on the sample plate to corrode. Do not sonicate sample plates or use acid to clean sample plates. Both can alter the surface of the sample plate, and reduce the quality of the data obtained.

7. Rinse the plate thoroughly in deionized water.

8. Allow the plate to dry at room temperature in an area where it will not be exposed to contaminants. Allow the plate to dry completely before use to obtain the maximum hydrophobic effect.

Cleaning gold and stainless steel plates

NOTE: *Use a cleaning solvent similar to the sample solvent.*

To clean gold and stainless steel sample plate surfaces:

1. Rinse the plate with a squeeze bottle of solvent.

2. Rub with a lint-free lab tissue to clean.

3. Rinse with deionized water.

4. Examine the plate. If you see any sample or matrix residue, oil, or fingerprints on the plate, soak the sample plate in a working solution of laboratory detergent in water (for example, RBS 35 detergent from Pierce or LIQUI-NOX™ from VWR) for 5 to 10 minutes.

IMPORTANT. RBS, LIQUI-NOX. *Please read the manufacturer's MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

CAUTION

Do not leave the sample plate in detergent for longer than 10 minutes. Longer exposure can cause the bottom holders on the sample plate to corrode. Do not sonicate sample plates or use acid to clean sample plates. Both can alter the surface of the sample plate, and reduce the quality of the data obtained.

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5. If residue remains, wipe the plate with a lint-free tissue or cotton swab. A soft toothbrush also works well.
6. Rinse the plate thoroughly in deionized water.
7. To speed drying, rinse the plate in acetone.

WARNING

CHEMICAL HAZARD. ***Acetone*** *is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin. It may cause central nervous system effects such as drowsiness, dizziness, and headache, and so on. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

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8. Allow the plate to dry in an area where it will not be exposed to contaminants.

3.4 Loading Sample Plates in the Mass Spectrometer

This section describes:

- Assigning Plate IDs
- Using the Mass Accuracy Optimization option
- Ejecting the sample holder
- Loading sample plates

NOTE: *If you load the sample plate into the Voyager Biospectrometry Workstation before the plate is dry, the pressure in the sample chamber rises, and a "TC2 pressure too high" error code may be displayed in the Instrument Control Panel. Wait a few minutes for the chamber to reach pressure.*

3.4.1 Assigning Plate IDs

You assign a unique Plate ID to each physical plate you use. When you load a plate, you select the Plate ID. The following information you previously assigned is automatically loaded:

- .PLT file
- Alignment information (if the plate has been aligned)
- Mass accuracy optimization information (if the plate has been optimized)

To assign a Plate ID, select **Select Sample Plate** from the Sample Plate menu and enter the information described in Table 3-10 in the Select Sample Plate dialog box.

NOTE: *You can also assign a Plate ID when you load a sample plate.*

Table 3-10 Sample Plate Information

Parameter	Description
Plate ID	Unique identifier (up to 32 alphanumeric characters) that you assign to a physical plate. Allows customized alignment and mass accuracy optimization of more than one physical plate using the same .PLT file. For more information, see “.PLT files and multiple alignments” on page 2-47.
Plate Type	.PLT file which contains plate configuration information. All .PLT files are located in the C:\VOYAGER directory. For more information on .PLT files, see “.PLT file format” on page 3-68.
Last Aligned	Read-only field that displays the date of the last alignment performed. For information, see Section 2.7, Aligning the Sample Plate.
Optimization Created	Read-only field that displays the date and time that the plate associated with the Plate ID selected above was optimized for mass accuracy. For information, see Section 2.8, Running OptiPlate to Optimize Mass Accuracy.
Use Mass Accuracy Optimizations	Applies correction factors that optimize mass accuracy to the data as it is acquired. For information, see Section 3.4.2, Using the Mass Accuracy Optimization Option.

3.4.2 Using the Mass Accuracy Optimization Option

In a typical analysis run that requires optimum mass accuracy, you include samples interspersed with many calibration standards, and externally calibrate the samples using the closest calibration standard.

Benefits of using Mass Accuracy Optimization during analysis

The Mass Accuracy Optimization option compensates for differences in calibration across a sample plate and increases the throughput of sample analysis by:

- Reducing the number of standard positions needed to obtain the optimum mass accuracy. For more information, see Section 3.2.2, Locating Standards for Optimum Mass Accuracy.
- Allowing larger distances between samples and standards, which makes more positions available for sample analysis.

Before using Mass Accuracy Optimization, optimize sample plates using the OptiPlate software. For more information, see Section 2.8, Running OptiPlate to Optimize Mass Accuracy.

How mass accuracy optimization is applied

To use the Mass Accuracy Optimization option, select the **Use Mass Accuracy Optimizations** option when you load a sample plate in the Instrument Control Panel (described in Section 3.4.4, Loading Sample Plates).

The software automatically retrieves the optimization information (created by the OptiPlate software) for the Plate ID associated with the plate. To compensate for differences in calibration across a sample plate, the software applies the Extraction Correction (calculated by the OptiPlate software) for each position on the sample plate as the data is acquired.

If positions have not been optimized

If you select the Use Mass Accuracy Optimization option for a Plate ID, and have not optimized all positions on the plate, the software interpolates an Extraction Correction for the coordinates that correspond to an unoptimized position using the correction distances from the closest optimized positions.

If no optimization found

If you select the Use Mass Accuracy Optimization option for a Plate ID, and no optimization file exists (either the plate has not been optimized, or the optimization file has been moved or deleted), the most recently created optimization file is used, regardless of plate type. Although using an optimization from another plate does not yield the same degree of mass accuracy as using an optimization specific for a plate, it still yields better mass accuracy than using no optimization.

Optimization strategy

Depending on your mass accuracy needs, you may or may not choose to optimize all sample plates.

For best mass accuracy, use an optimized sample plate (select a PlateID/.PLT associated with a specific physical plate you have optimized, then load the optimized physical plate in the mass spectrometer).

However, you can also do the following (items are listed in order of decreasing mass accuracy):

- Use the same type of plate as a plate you have optimized (select a PlateID/.PLT associated with a plate you have optimized, then load a different physical plate of the same type in the mass spectrometer).
- Use a different type of plate than a plate you have optimized (select the PlateID/.PLT associated with the plate you are using, and the last optimization created will be used).

Note that all items listed above yield mass accuracy better than the accuracy you obtain if you do not use the Mass Accuracy Optimization option.

3.4.3 Ejecting the Sample Holder

To eject the sample holder:

1. In the Voyager Instrument Control Panel, select **Eject** from the Sample Plate menu. The Load/Eject dialog box is displayed.

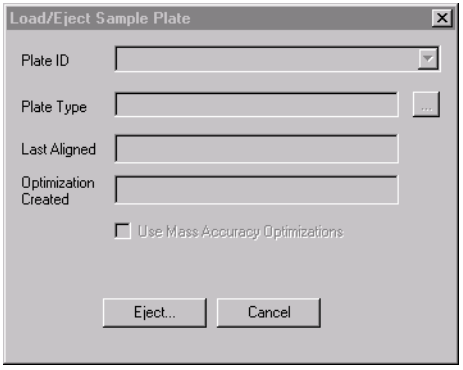


Figure 3-4 Load/Eject Sample Plate Dialog Box

2. Click **Eject**.

   The following occurs:

   - A Load Status dialog box displays hardware status during the ejection sequence.
   - High voltage is turned off.
   - The mass spectrometer video monitor displays the sample plate moving.
   - The sample holder moves out of the main source chamber, out of the sample loading chamber, then is ejected from the instrument.

NOTE: *Do not leave the sample holder in the Eject position for more than a few minutes. Minimize the time the instrument is exposed to the atmosphere to reduce the time needed to reach high vacuum after you insert a new plate.*

3.4.4 Loading Sample Plates

This section describes loading sample plates in:

- Voyager-DE and Voyager-DE PRO
- Voyager-DE STR

Voyager-DE and Voyager-DE PRO

To load sample plates:

1. Eject the sample holder as described in Section 3.4.3, Ejecting the Sample Holder.
2. Hold the sample plate with the bottom of the numbers facing toward the analyzer (for standard 100-well plate) and with the slanted underside of the plate facing to the left.
3. Slide the sample plate into the holder from the right side until it snaps into place (Figure 3-5). The ball bearings on the holder snap into the plate.

CAUTION

If the sample plate does not snap into place, it may be inserted into the holder the wrong way, and it may jam inside the instrument. Remove the plate, slide it into the holder with the slanted underside of the plate facing to the left and toward the back of the instrument, and snap it into place.

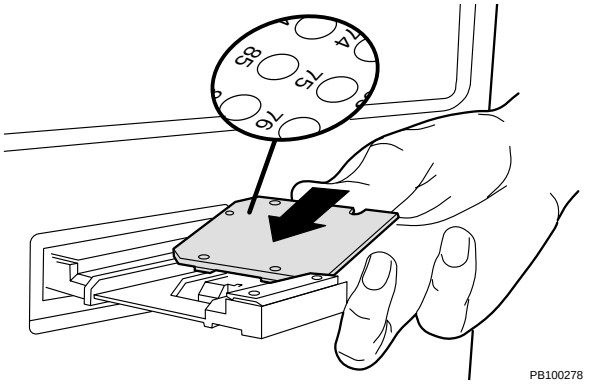


Figure 3-5 Loading the Sample Plate in a Voyager-DE or Voyager-DE PRO

WARNING

PHYSICAL INJURY HAZARD. *Fingers can get caught in the sample holder. To avoid injury, do not click* ***Load*** *to retract the sample holder when your fingers are near the sample holder.*

4. From the Sample Plate menu, select **Load** to retract the sample plate and insert it into the main source chamber. The Load/Eject Sample Plate dialog box (Figure 3-6) is displayed.

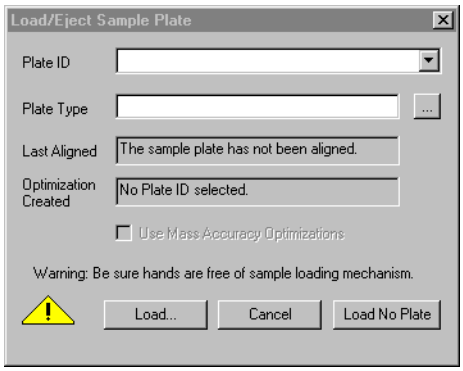


Figure 3-6 Load/Eject Sample Plate Dialog Box

If you are removing a plate but not loading a new plate, click **Load No Plate** and skip the remaining steps.

5. Select a Plate ID. The .PLT file, plate alignment information, and plate optimization information associated with the Plate ID are automatically loaded. For more information, see Section 2.7, Aligning the Sample Plate, and Section 2.8, Running OptiPlate to Optimize Mass Accuracy.

   Alternatively you can specify a new Plate ID, then select a .PLT file. For more information, see “Assigning Plate IDs” on page 3-50.

6. Select **Use Mass Accuracy Optimizations** to apply calibration corrections to the data acquired from the plate. Before selecting this option, read Section 3.4.2, Using the Mass Accuracy Optimization Option, to understand how mass accuracy optimization is applied. See “Optimization strategy” on page 3-53 to understand the options you have when using Mass Accuracy Optimization.

7. Click **Load**.

   The sample plate is loaded and aligned as needed. For more information, see “How the system aligns a plate” on page 2-47.

It takes 1 or 2 minutes for the sample plate to reach the correct position. While the sample plate is moving, the Load/Eject Status dialog box displays messages about the status of the hardware.

Voyager-DE STR

To load sample plates:

1. Eject the sample holder as described in Section 3.4.3, Ejecting the Sample Holder.
2. Hold the sample plate vertically, with the sample surface facing to the right, and with the slanted underside of the plate facing toward the back of the instrument.
3. Slide the sample plate into the holder from the front until it snaps into place (Figure 3-7). The ball bearings on the holder snap into the plate.

CAUTION

If the sample plate does not snap into place, it may be inserted into the holder the wrong way, and it may jam inside the instrument. Remove the plate, slide it into the holder with the slanted underside of the plate facing the left and toward the back of the instrument, then snap it into place.

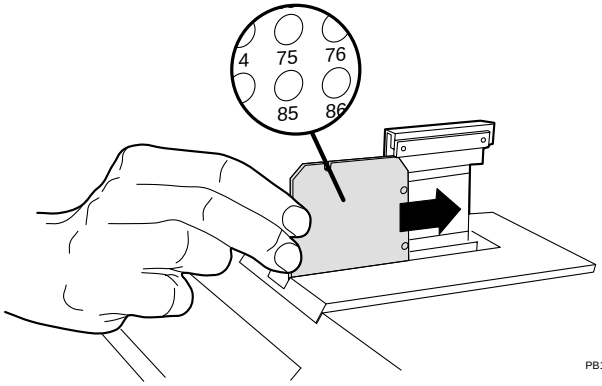


Figure 3-7 Loading the Sample Plate in a Voyager-DE STR

WARNING

PHYSICAL INJURY HAZARD. *Fingers can get caught in the sample holder. To avoid injury, do not click* ***Load*** *to retract the sample holder when your fingers are near the sample holder.*

4. From the Sample Plate menu, select **Load** to retract the sample plate and insert it into the main source chamber. The Load/Eject Sample Plate dialog box (Figure 3-8) is displayed.

   If you are removing a plate but not loading a new plate, click **Load No Plate** and skip the remaining steps.

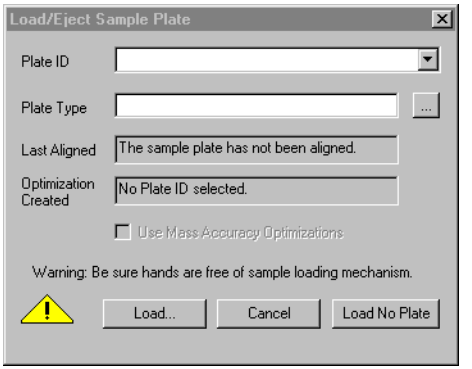


Figure 3-8 Load/Eject Sample Plate Dialog Box

5. Select a **Plate ID**. The .PLT file, plate alignment information, and plate optimization information associated with the Plate ID are automatically loaded. For more information, see Section 2.7, Aligning the Sample Plate, and Section 2.8, Running OptiPlate to Optimize Mass Accuracy.

   Alternatively you can specify a new Plate ID, then select a .PLT file. For more information, see “Assigning Plate IDs” on page 3-50.

6. Select **Use Mass Accuracy Optimizations** to apply calibration corrections to the data acquired from the plate. Before selecting this option, read Section 3.4.2, Using the Mass Accuracy Optimization Option, to understand how mass accuracy optimization is applied. See “Optimization strategy” on page 3-53 to understand the options you have when using Mass Accuracy Optimization.

7. Click **OK**.

   The sample plate is loaded and aligned as needed. For more information, see “How the system aligns a plate” on page 2-47.

   It takes 1 or 2 minutes for the sample plate to reach the correct position. While the sample plate is moving, the Load/Eject Status dialog box displays messages about the status of the hardware.

3.5 Sample Plate Types

Overview

The Voyager Instrument Control software supports a variety of reusable and disposable plates. Each type of physical plate has a corresponding plate (.PLT) file that defines the location and dimensions of the positions on the plate. Standard .PLT files for all standard plates are provided with the software.

You can:

- Customize the standard plate types by copying and editing their .PLT files. See Section 3.5.3, Guidelines for Defining Custom Plate Types.

  These additional plate types are custom options. Contact Applied Biosystems for more information.

- Define your own plate types of unlimited positions by editing existing or creating new .PLT files. For information, see Section 3.5.2, Editable-Configuration Plate (.PLT) Types Provided with the System, and Section 3.5.4, Creating and Editing .PLT Files.

In this section

This section describes:

- Sample plate types and applications
- Editable-configuration plate types provided with the system
- Guidelines for defining custom plate types
- Creating and editing .PLT files
- Adjusting the laser position for a custom .PLT file

3.5.1 Sample Plate Types and Applications

The Voyager Instrument Control software supports several types of reusable and disposable plates. See Table 3-11 for the applications of different sample plates.

NOTE: *For additional information on available sample plates and ordering information, see Appendix B.3, Spare Parts.*

Table 3-11 Sample Plate Types, Applications, and Benefits

Sample Plate Type	Applications/Benefits
Welled Sample Plates	
Gold (100-position)	• GPC-MALDI or HPLC fractions when high concentration of organic solvent provides no surface tension. • Polymer analysis using highly volatile solvents. • Easier automated acquisition, specifically for search pattern (.SP) file creation. The wells ensure correct sample placement for running in Automatic Control mode.
Flat Sample Plates with laser etched circles indicating sample position	
Stainless steel (100-position and 400-position)	• Increased mass accuracy, allows close external calibration procedures. • Ensures correct sample placement for running in Automatic Control mode. • Crystallization, particularly with DHB, DHBs, and 3-HPA matrixes, is more defined and easier to observe on the video monitor.

Table 3-11 Sample Plate Types, Applications, and Benefits (Continued)

Sample Plate Type	Applications/Benefits
Special Sample Plates	
Stainless steel, polished blank	• Allows customized sample positioning and preparation using an automated sample preparation device.
Disposable (gold-coated) (100-position)	• Can derivatize the surface of the sample plate for protein or enzyme immobilization.[1, 2] • Long-term storage of samples. • Eliminates cross-contamination.
Biacore® Chip	• Direct analysis of affinity-isolated species. • Confirmation of binding constant determined by surface plasmon resonance.[3]
Membrane, Gels	The base surface of the plate is recessed to accommodate the depth of gels or membranes and can be used with: • PVDF or nitrocellulose membranes • PAGE gels (for example, PhastGel™)
Teflon surface (384-position or 400-position)	• Increases sensitivity because hydrophobic walls concentrate the sample into a small area. • Accurate sample positioning for high throughput.

Table 3-11 Sample Plate Types, Applications, and Benefits (Continued)

Sample Plate Type	Applications/Benefits
96 × 2 (192-position), flat, hydrophobic plastic surface plate	• Increases sensitivity because hydrophobic walls concentrate the sample into a small area. • Useful for close external calibration due to the proximity of positions. • Ensures correct sample placement for running in Automatic Control mode. • Number of sample positions corresponds to number of microtiter plate wells. • Sample positions are inset from the plate perimeter, which minimizes variation in mass accuracy that can be caused by variation in electric field at plate edges.

1. Dogruel D., P. Williams and R.W. Nelson. *Anal. Chem.* 1995, **67**, 4343–4348.
2. Brockman A.H. and R. Orlando. *Anal. Chem.* 1995, **67**, 4581–4585.
3. Krone, *et al., Anal. Biochem*. 1997, **244**, 124–132.

3.5.2 Editable-Configuration Plate (.PLT) Types Provided with the System

Selecting plate types (.PLT files)

You can select plate types (.PLT files) in two ways:

- When loading the plates in the mass spectrometer
- From the Sample Plate menu in the Instrument Control Panel

For an example of how to select plate types, see Section 3.4.4, Loading Sample Plates.

Customizing .PLT files

You can select and use the plate types described below. You can also customize these plate types by editing .PLT files provided with the system.

Each .PLT file describes a plate configuration available for selection when a plate is loaded into the Voyager system. For more information, see:

- Section 3.5.3, Guidelines for Defining Custom Plate Types
- “Creating or editing a .PLT file using Notepad editor” on page 3-83

NOTE: *You cannot edit the original .PLT files provided with the system. These are read-only files. Open the .PLT file that describes the plate type you are customizing, edit the file to describe the plate type you need, then save the file with a new file name.*

384-position Teflon plate staggered locations

Sample locations on 384-position Teflon plates are staggered, as shown in Figure 3-9.

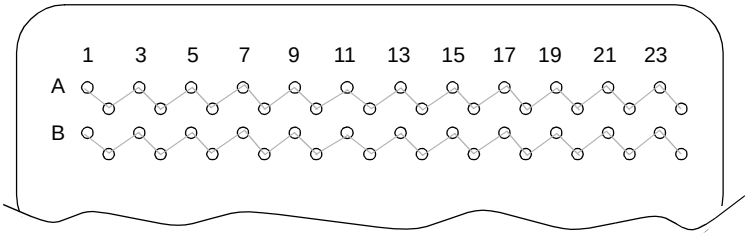


Figure 3-9 384-Position Staggered Sample Locations

96 × 2-position plate staggered “a” and “b” locations

Sample locations on 96 × 2-position plates are staggered, as shown in Figure 3-10.

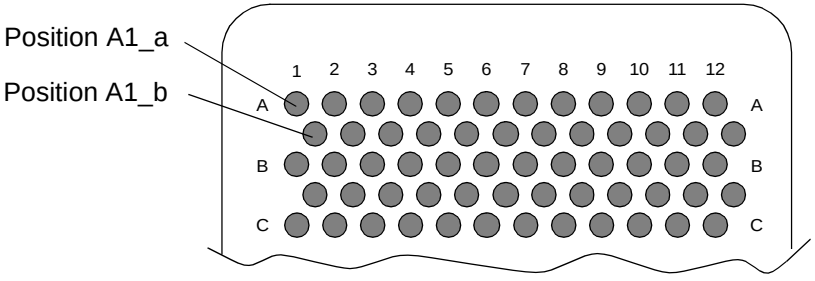


Figure 3-10 96 × 2-Position Plate Staggered “a” and “b” Sample Locations

.PLT files provided

The editable-configuration plate types provided with the system are described in Table 3-12.

Table 3-12 Editable-Configuration Plate Types

Plate Type (name of .PLT file)	Plate Description	Number of Positions	Position Arrangement	Position Diameter (µm)	Position Center-to-Center Distance (µm)
64 well disposable plate.PLT	Disposable with wells	64	8 × 8 (subset of 10 × 10)	2,540	5,080 × 5,080
100 well plate.PLT	Reusable with wells	100	10 × 10	2,540	5,080 × 5,080
384 well plate.PLT	Reusable with staggered wells	384	16 × 24 (A–P × 1–24)	990.60	1,803.40 × 2,639.06
400 well plate.PLT	Reusable with wells	400	20 × 20 (A–T × 1–20)	990.60	1,760.22 × 1,709.42
96 well x 2 plate.PLT	Reusable with staggered wells	192	8 × 24 A–H × 1_a–12_a and 1_b–12_b	1,500	1,100 × 1,900

3.5.3 Guidelines for Defining Custom Plate Types

You can define custom plate types of unlimited positions by creating your own .PLT files.

This section describes:

- .PLT file format
- .PLT file parameters
- Guidelines for creating .PLT files
- Guidelines for creating search pattern (.SP) files

For information on creating new and editing existing .PLT files, see Section 3.5.4, Creating and Editing .PLT Files.

.PLT file format

A .PLT file is an ASCII text file in which each line of text represents an individual position on the plate (Figure 3-11). Table 3-13 describes the parameters in a .PLT file.

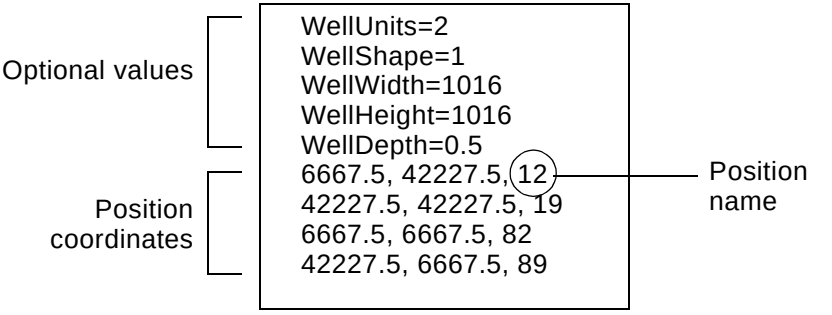


Figure 3-11 Example .PLT File

.PLT file parameters

The parameters in a .PLT file are described in Table 3-13.

Table 3-13 .PLT File Parameters

Parameter	Description
WellUnits (optional)	Defines the units of the sample positions that are displayed in the Manual Laser/Sample Positioning control page (see Figure 4-9 on page 4-31). If you do not include the WellUnits parameter, the software uses the default. Allowable values: • 1 = Steps (default) • 2 = Microns Automatically set to microns if you create the .PLT file using the Create PLT File function (described in "Creating a .PLT file using Create PLT File" on page 3-79).
WellShape (optional)	Defines the shape of the sample positions that are displayed in the Manual Laser/Sample Positioning control page. If you do not include the WellShape parameter, the software uses the default. Allowable values: • 1 = Elliptical (default) • 2 = Rectangular Corresponds to Position Shape parameter in the Create PLT File dialog box (see Table 3-14, "Create PLT File Parameters," on page 3-81).
WellWidth (optional)	Defines the width of the sample positions that are displayed in the Manual Laser/Sample Positioning control page. If you do not include the WellWidth parameter, the software uses the default (based on the value selected for WellUnits). Default values: • Default Step value = 800 • Default Micron value = 2,540 Corresponds to Position Width parameter in the Create PLT File dialog box (see Table 3-14, "Create PLT File Parameters," on page 3-81). Automatically set to microns if you create the .PLT file using Create PLT File.

continued

Table 3-13 .PLT File Parameters (Continued)

Parameter	Description
WellHeight (optional)	Defines the height of the sample positions that are displayed in the Manual Laser/Sample Positioning control page. If you do not include the WellHeight parameter, the software uses the default (based on the value selected for WellUnits). Default values: • Step value = 800 • Micron value = 2,540 Corresponds to Position Height parameter in the Create PLT File dialog box (see Table 3-14, “Create PLT File Parameters,” on page 3-81). Automatically set to microns if you create the .PLT file using Create PLT File.
WellDepth (optional)	Depth (mm) of indentation of a non-flat plate (for example, a welled plate) from the surface of the plate to the bottom of the indentation. If not present, the system assumes a depth of 0 (equal to a flat plate). Corresponds to Position Depth (mm) parameter in the Create PLT File dialog box (see Table 3-14, “Create PLT File Parameters,” on page 3-81).
X and Y	The coordinates of the position center in reference to the lower left corner of the sample plate. To determine the coordinates to enter in the .PLT file, display the Sample View (see Figure 4-9 on page 4-31) and record the Absolute X, Absolute Y (logical coordinates) pairs for each specified position. See “Logical Coordinates in Plate View in Manual Laser/Sample Positioning Control Page” on page 3-78 for information on displaying logical coordinates. ***NOTE:*** *On a standard 100-well plate, position 91 is located at X=1587.5, Y=1587.5, and corresponds to the lower left of the sample plate.* For optimum mass accuracy, do not specify coordinates on the outer edges of a plate.

continued

Table 3-13 .PLT File Parameters (Continued)

Parameter	Description
X and Y *(continued)*	The following coordinates correspond to the corner positions that define the area of the sample plate that provides optimum mass accuracy: • Absolute X=6667.5, Absolute Y=42227.5—Top left (corresponds to position 1) • Absolute X=42227.5, Absolute Y=42227.5—Top right • Absolute X=6667.5, Absolute Y=6667.5—Bottom left • Absolute X=42227.5, Absolute Y=6667.5—Bottom right ***NOTE:*** *Sample plate alignment for custom plate types (.PLT files that you create) requires sample spots on any four corner positions. You can use any four corner positions, but for maximum mass accuracy, do not use positions outside the area defined by the coordinates above.*
Position name (optional)	Displayed as the Active Position name in the Sample View (see Figure 4-8 on page 4-27). ***NOTE:*** *If you do not specify a position name in the file, the position number is displayed. The first sample in the .PLT file is considered sample 1. The Nth sample in the .PLT file is considered sample N.* Corresponds to Position Numbering parameter in the Create PLT File (see Table 3-14, “Create PLT File Parameters,” on page 3-81).
Comment	Text used to describe the file. Optional. Start with a semicolon.

Guidelines for creating .PLT files

Note the following:

- The diameter of a position on a plate is determined by the plate used:
  - If you are using welled or laser-etched plates, the position diameter is determined by the well or laser-etched position size (2,540 µm on a 100-well plate).
  - If you are using plates with polished blank surfaces, the position diameter is determined by the size of the physical sample spot delivered by manual application or automated sample preparation device.
- When creating a .PLT file, specify absolute X,Y coordinates that accommodate the sample diameter, but do not allow samples to overlap.
- After you create a .PLT file, create an .SP file to accommodate the sample diameters defined in the .PLT file. The DEFAULT.SP file is set to analyze 2,540-µm diameter positions, corresponding to standard sample positions on a 100-well plate.

NOTE: *For optimum mass accuracy (if you do not use the Optimize Mass Accuracy option), do not spot sample on outer rows of the plate.*

Guidelines for creating .SP files

When creating search pattern (.SP) files to analyze sample positions defined in custom .PLT files, note that the area available for analysis around the absolute X,Y coordinate of the position specified in the .PLT file is determined by three factors:

- Plate being used
- Tolerances and non-systematic errors
- Search pattern radius

Search pattern files are described in detail in Section 6.6.4, Search Patterns.

NOTE: *You can correct for systematic errors introduced by a sample preparation device by aligning the sample plate in the Voyager Workstation. See Section 2.7, Aligning the Sample Plate.*
If you are using different sample preparation devices, you can compensate for different systematic errors by creating different .PLT files and sample plate alignments for each system.

Effect of plate type on area available for analysis

When creating search pattern files, consider the plate types used:

- If you are using laser-etched position plates, the area available for analysis is limited to the well or laser-etched position size (2,540 µm on a 100-well plate).
- If you are using plates with polished blank surfaces, the area available for analysis is determined by the proximity of other positions specified. Approximately half the distance between the X,Y coordinates of two adjacent positions is available for analysis of each position. For example, if the center X,Y coordinates of the two positions are 5,080 µm apart (plus positional tolerance, see below), approximately 2,540 µm around the X,Y coordinates is available for analysis of each position (Figure 3-12 on page 3-75).

Tolerances and non-systematic errors

When creating search pattern files, make sure to compensate for:

- Positional tolerance (related to variability in the position of the plate)

Positional Tolerance	
Plate type	**µm**
Reusable plates	76.2
Disposable plates	381

NOTE: *Due to limited surface area and variability in the position of the disposable inserts, do not specify more than 384 positions for a disposable plate.*

- Total allowable tolerance (includes the positional tolerance plus any non-systematic error introduced by sample application)

Search pattern radius

Note the following:

- To ensure complete analysis, the radius of the search pattern file used must include the radius of the sample spot.
- To prevent analysis of the wrong sample, the radius of the search pattern used must not exceed half of the center-to-center spacing minus the total allowable tolerance (Figure 3-12).

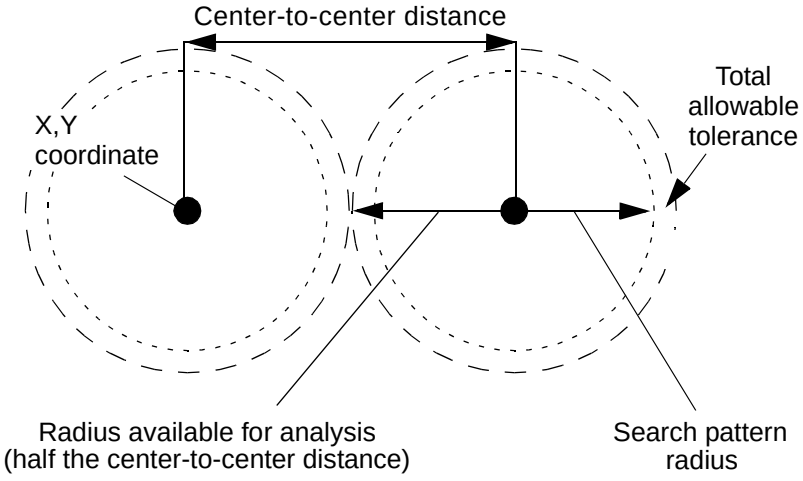


Figure 3-12 Area Available for Analysis

3.5.4 Creating and Editing .PLT Files

This section includes:

- Preparing the sample plate
- Creating a .PLT file using Create PLT File
- Creating or editing a .PLT file using Notepad editor
- Creating .PLT files for SymBiot® plates

Preparing the sample plate

Preparing the sample plate involves:

- Spotting a sample plate with matrix in specific positions to allow you to see the positions on the video monitor and correctly locate the center of the sample position under the laser.
- Loading a BLANK.PLT file containing one sample position that occupies the entire plate, to allow you to navigate anywhere on the plate and determine x,y coordinates.

WARNING

CHEMICAL HAZARD. Alpha-cyano-4-hydroxycinnamic acid (CHCA) *may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

Preparing

To prepare the sample plate before creating a .PLT file:

1. Spot a sample plate with α-cyano-4-hydroxycinnamic acid:
   - If you are using the Create PLT File function to create the .PLT file, spot the first and last positions on the plate (Figure 3-13).
   - If you are using Notepad editor to create the .PLT file, spot the complete sample position layout for which you are creating the .PLT file.

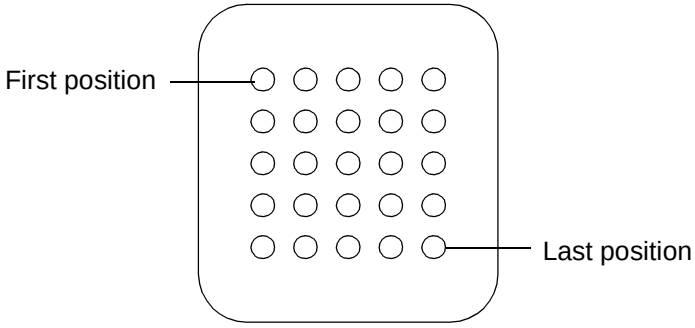


Figure 3-13 First and Last Positions for a .PLT File

For more information see, “Loading samples” on page 3-42.

2. Load the sample plate into the system, then select **BLANK.PLT** as the Plate Type. For information, see Section 3.4, Loading Sample Plates in the Mass Spectrometer.
3. From the View menu in the Instrument Control Panel, select **Manual Laser/Sample Positioning**.

Figure 3-14 shows the Plate View displaying the logical coordinates for the BLANK.PLT file.

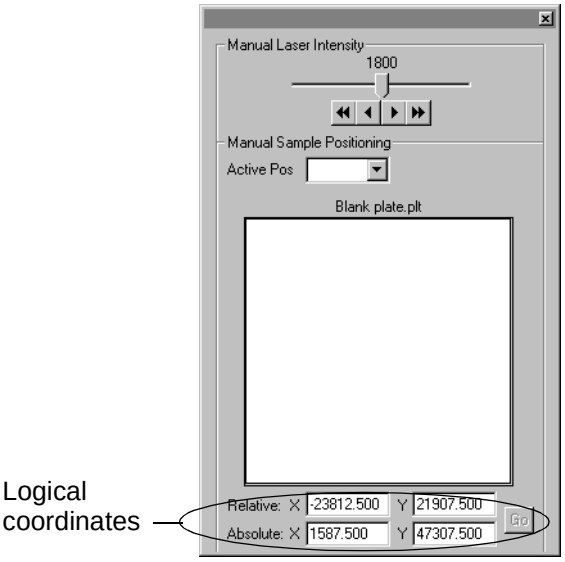


Figure 3-14 Logical Coordinates in Plate View in Manual Laser/Sample Positioning Control Page

Creating a .PLT file using Create PLT File

The Create PLT File function creates .PLT files that specify positions:

- In evenly spaced rows and columns (identical Y coordinates for all positions in a row, and identical X coordinates for all positions in a column).
- Sequentially from left to right and top to bottom across the plate.
- With units in microns.

To create .PLT files that specify staggered positions (for example, the 384-well plate shown in Figure 3-9 on page 3-66) or positions that are not evenly spaced, or to edit an existing .PLT file, see “Creating or editing a .PLT file using Notepad editor” on page 3-83.

Using Create PLT File

To create a .PLT file using Create PLT File:

1. Perform the procedure in “Preparing the sample plate” on page 3-76.
2. Use the Manual Laser/Sample Positioning control page (see Figure 3-14 on page 3-78) or the control stick to determine the center Absolute X and Y coordinates for the first and last positions on the plate for which you are creating the .PLT file (see Figure 3-13 on page 3-77).
3. In the Instrument Control Panel, select **Create PLT File** from the Tools menu to display the Create PLT File dialog box (Figure 3-15).

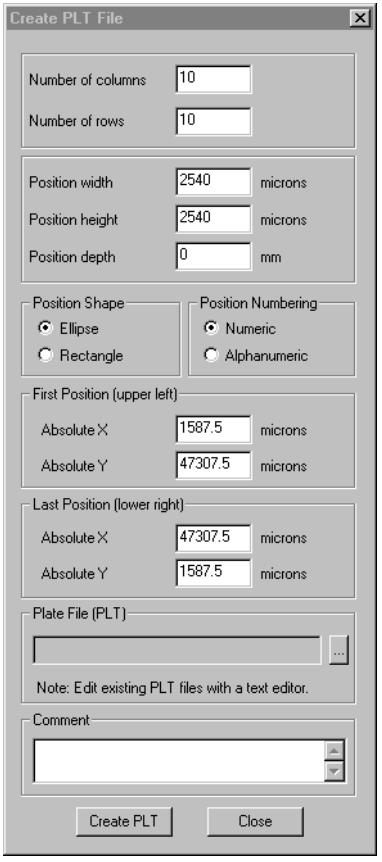


Figure 3-15 Create PLT File Dialog Box

4. Set the parameters described below:

Table 3-14 Create PLT File Parameters

Parameter	Description
Number of columns	Limited by plate size. Total number of positions (columns × rows) cannot exceed 32,766. ***NOTE:*** *The OptiPlate application can use .PLT files with a maximum of 30 columns in a grid pattern, or 60 columns in an offset grid pattern.*
Number of rows	Maximum of 52. Total number of positions (columns × rows) cannot exceed 32,766. ***NOTE:*** *The OptiPlate application can use .PLT files with a maximum of 30 rows.* ***NOTE:*** *The OptiPlate application can use .PLT files with a maximum of 30 rows in a grid pattern, or 15 rows in an offset grid pattern.*
Position Width and Height (µm)	Width and height of positions. Corresponds to WellWidth and WellHeight parameters in the .PLT file (see Table 3-13, ".PLT File Parameters," on page 3-69).
Position Depth (mm)	Depth of indentation of a non-flat plate (for example, a welled plate) from the plate surface to the bottom of the indentation. A setting of 0 is equal to a flat plate. Measure the depth of a position on the plate with a micrometer to determine the value to enter. This parameter corrects for the distance between the sample in a welled plate and the variable-voltage grid in the ion source, which affects calibration. An accurate well depth increases the accuracy of the default calibration, external calibration, and mass optimization with OptiPlate. Corresponds to WellDepth parameter in the .PLT file (see Table 3-13, ".PLT File Parameters," on page 3-69).

continued

Table 3-14 Create PLT File Parameters (Continued)

Parameter	Description
Position Shape	Ellipse or Rectangle. Corresponds to WellShape parameter in the .PLT file (see Table 3-13, ".PLT File Parameters," on page 3-69).
Position Numbering	If you select Numeric, positions are numbered sequentially from 1 to 32,766. If you select Alphanumeric, rows are assigned letters, and positions are numbered sequentially within the row (for example, A1 through A*n* for the first row, B1 through B*n* for the second row, and so on.) If the number of rows exceeds 26, double letters are used (for example AA1 through AA*n*.) Corresponds to PositionName parameter in the .PLT file (see Table 3-13, ".PLT File Parameters," on page 3-69).
First position (upper left) X and Y and Last position (lower right) X and Y	Absolute coordinates of starting and ending position centers relative to the lower left corner of the sample plate. The software extrapolates to determine coordinates of all other positions. To determine the coordinates to enter in the .PLT file, display the Sample View (see Figure 4-9 on page 4-31) and record the Absolute X, Absolute Y (logical coordinates) pairs for the first and last position. See "Logical Coordinates in Plate View in Manual Laser/Sample Positioning Control Page" on page 3-78 for information on displaying logical coordinates.
Comment	Comment stored with .PLT file. Optional.

5. Click ... next to the Plate File (PLT) field. Specify the directory in which to store the .PLT file, type the name for the file, then click **Save**.
6. Click **Create PLT**.
7. Check the sample plate alignment. See Section 3.5.5, Adjusting the Laser Position for a Custom .PLT File.

Creating or editing a .PLT file using Notepad editor

NOTE: *Display the Sample View when you create or edit a .PLT file with Notepad editor. Move the sample plate to the positions you want analyzed and note the Absolute X, Absolute Y coordinates to enter in the .PLT file.*

To create or edit a .PLT file using Notepad editor:

1. Perform the procedure in “Preparing the sample plate” on page 3-76.
2. Open the Microsoft Windows NT® Notepad text editor. See the *Microsoft Windows NT User’s Guide* if you need help using Notepad.
3. Open the .PLT file to edit.
4. To enter a comment on the first line, type a semicolon (;), then type a description of the sample plate, for example:

   ```
   ;96-position,reusable,no wells,.PLT file
   ```

5. Enter or edit WellUnits, WellShape, WellWidth, WellHeight, and WellDepth if necessary. For more information, see Table 3-13, “.PLT File Parameters,” on page 3-69.
6. On the second line, type or edit the absolute coordinates for the first position. Separate absolute x and y coordinates with a comma, and include one X,Y pair per line (see Figure 3-11 on page 3-68 for X,Y pair example). Type in the position name. Blank lines are allowed.
7. Use the Manual Laser/Sample Positioning control page or the Control Stick to move to the next position, then note the absolute coordinates for that position.
8. Repeat step 5 through step 7 for remaining positions.
9. Save the file with a unique name and a .PLT extension (for example, Lab01.PLT).

Hint: *Some applications automatically append a .TXT extension to file names. To name the file with a .PLT extension, include the file name and extension in double-quotes in the Save File dialog box, for example "100Well.PLT".*

10. Check the sample plate alignment. See Section 3.5.5, Adjusting the Laser Position for a Custom .PLT File.

Creating .PLT files for SymBiot plates

If you spot sample plates in evenly spaced rows and columns using the SymBiot® Sample Workstation, you can use the Create PLT File function to extrapolate the X,Y coordinates of the sample positions and quickly and easily create a .PLT file. Follow the procedure in "Creating a .PLT file using Create PLT File" on page 3-79.

If you spot sample plates in more complex patterns, you must create the. PLT file manually. Follow the procedure in "Creating or editing a .PLT file using Notepad editor" on page 3-83.

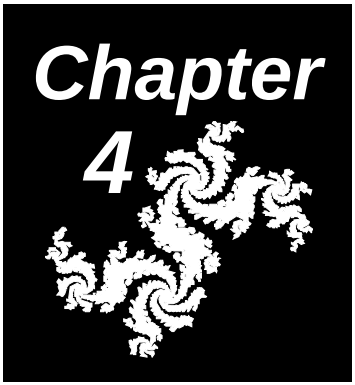
3.5.5 Adjusting the Laser Position for a Custom .PLT File

After you have defined your own sample plate format:

1. Spot sample on the four corner positions defined in the .PLT file. For optimum mass accuracy, do not spot sample on the outer edges of the plate.
2. Load the sample plate and .PLT file as described in Section 3.4.4, Loading Sample Plates.
3. Check the laser beam position on these sample positions as described in Figure 3-14 on page 3-78.

   If the beam does not strike the center of the sample position, align the sample plate as described in Section 2.7, Aligning the Sample Plate.

4 Voyager Instrument Control Panel Basics



This chapter contains the following sections:

4.1 Instrument Control Panel 4-2

4.2 Using the Control Pages 4-8

4.3 Using the Spectrum Window 4-10

4.4 Customizing the Instrument Control Panel 4-21

4.5 Controlling the Workstation 4-24

4.6 Sequence Control Panel 4-32

4.7 How the Instrument and Sequence Control Panels Interact 4-33

4.1 Instrument Control Panel

The Voyager Instrument Control Panel allows you to directly control the Voyager mass spectrometer to acquire and inspect mass spectra in Manual or Automatic Control mode.

NOTE: *The Voyager Sequence Control Panel allows you to collect data for multiple samples using different conditions. For more information, see Section 4.6, Sequence Control Panel, and Section 4.7, How the Instrument and Sequence Control Panels Interact.*

This section includes:

- Parts of the Instrument Control Panel
- Manual and Automatic Control modes
- Accessing the Sequence Control Panel and the Data Explorer Software

4.1.1 Parts of the Instrument Control Panel

The Instrument Control Panel includes:

- Toolbar
- Control pages
- Spectrum window
- Status bar
- Output window

When you start the Voyager Instrument Control software, the Instrument Control Panel (Figure 4-1) is displayed.

NOTE: *If the Instrument Control Panel is not displayed as shown in Figure 4-1, select* ***Default Layout*** *from the View menu.*

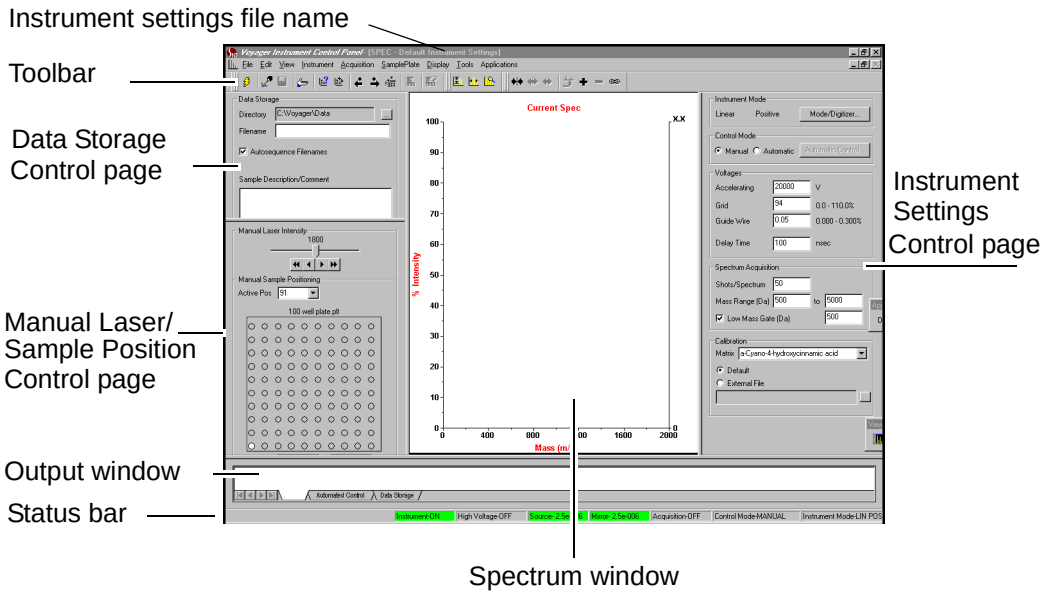


Figure 4-1 Instrument Control Panel

Toolbar

The toolbar contains buttons that control the software and the instrument.

For a description of a toolbar button, place the cursor on it. A brief description of the button (Tooltip) is displayed below the button.

For more information, see Section 4.5.1, Using Toolbar Buttons and Instrument Menu Commands.

4

Control pages

The Instrument Control Panel contains five control pages:

- **Instrument Settings**—Controls settings for instrument mode, voltages, spectrum acquisition, and calibration. For more information, see Chapter 5, Optimizing Instrument Settings.
- **Data Storage**—Controls data storage information such as file location and file name. For more information, see “Setting Data Storage parameters” on page 6-14.
- **System Status**—Contains information regarding the status of the instrument. For more information, see Section 2.11, Checking System Status and Pressures.
- **Manual Laser/Sample Positioning**—Allows you to manually control the laser intensity and sample plate position. For more information, see Section 4.5.2, Adjusting Laser Intensity and Selecting Sample Position.
- **PSD Acquisition Settings**—Allows you to control PSD acquisition. For more information, see Chapter 8, PSD Analysis.

NOTE: *The PSD Acquisition control page is displayed only if you are in PSD mode on a Voyager-DE PRO or Voyager-DE STR system.*

You can access a control page by selecting it from the View menu or clicking the corresponding toolbar button.

You can display any combination of control pages. For information, see Section 4.2, Using the Control Pages.

For a description of parameters contained on the control pages, see Section 5.2, Instrument Settings Parameter Descriptions, and Chapter 8, PSD Analysis.

Spectrum window

The Spectrum window provides a display of data. The data displayed depends on your digitizer option:

- **Signatec, LeCroy, or Acqiris digitizers**—Displays a live (real-time) spectrum trace as data is acquired. Trace changes from Live to Current when acquisition ends.
- **Tektronix oscilloscope**—No trace displayed during acquisition. Displays a Current spectrum trace when acquisition is complete.

When acquisition is complete, peaks can be detected and labeled.

For more information, see Section 4.3, Using the Spectrum Window.

Status bar

The status bar (Figure 4-2) at the bottom of the Instrument Control Panel displays status for Instrument State, High Voltage, Source (BA1) and Mirror (BA2) Chamber Pressure, Acquisition status, Control Mode set in the loaded .BIC, Instrument Mode and Ion Polarity set in the loaded .BIC, the Active Position selected for analysis, and the current laser intensity.

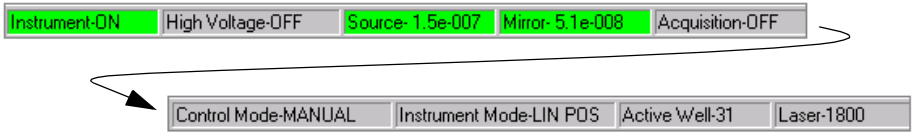


Figure 4-2 Status Bar

Output window

The Output window includes two tabs at the bottom that display information:

- **Data Storage**—Displays the file name of the .DAT file where the spectrum is stored when you save a spectrum. This tab displays a running list of .DAT files. You can clear the window by right-clicking on the window and selecting **Clear**.
- **Automatic Control**—If you are operating in Automatic Control mode, the Automatic Control tab displays current laser setting, search pattern position, and spectrum evaluation results. For information on Automatic Control mode, see Section 6.6, Acquiring in Automatic Mode from the Instrument Control Panel.

Displaying

The Output window is automatically displayed when you:

- Acquire data
- Store data

To display the Output window manually, select **Output Window** from the View menu.

To close the Output window, deselect **Output Window** from the View menu, or right-click in the Output window and select **Hide**.

Maximum number of entries

The Output window can contain a maximum of 1,000 lines. If the total number of lines exceeds 1,000, the oldest 500 lines are automatically deleted. The most recent 500 entries are retained.

4.1.2 Manual and Automatic Control Modes

Overview

The Instrument Control Panel allows two modes of operation:

- **Manual Control mode**—Enables manual control of the laser, sample positioning, and data storage.
- **Automatic Control mode**—Enables automated control of the laser, sample positioning, data storage after you set spectrum accumulation parameters, and spectrum acceptance criteria.

Manual Control mode

To select Manual Control mode:

1. Open the Instrument Control Panel.
2. Select **Instrument Settings** from the View menu.
3. Click the **Manual** button on the Instrument Settings control page.

   Laser controls, sample positioning, and data storage are available for manual adjustment and control.

   For additional information on manual control mode, see Section 5.2.1, Instrument Settings Page.

Automatic Control mode

To select Automatic Control mode:

1. Open the Instrument Control Panel.
2. Select **Instrument Settings** from the View menu.
3. Click the **Automatic** button on the Instrument Settings control page.
4. Click the **Automatic Control** button.
5. Set laser controls, sample positioning, and data storage for automatic adjustment and control as described in Section 6.6.2, Setting Instrument Settings for Automatic Control Mode.

4.1.3 Accessing the Sequence Control Panel and the Data Explorer Software

You can access the Sequence Control Panel and Data Explorer software from the Instrument Control Panel by:

- Selecting commands from the Applications menu
- Clicking toolbar buttons

Accessing Data Explorer

If you access the Data Explorer software from the Instrument Control Panel, the following happens:

- The last data file you saved in the Instrument Control Panel is automatically opened in Data Explorer.
- If Data Explorer is already running and displaying a data file, the data file is automatically closed when you access Data Explorer from the Instrument Control Panel.

4.2 Using the Control Pages

The Instrument Control Panel allows you to display, organize, and rearrange one or more control pages.

This section describes:

- Displaying control pages
- Types of page control

Displaying control pages

You can display control pages in several ways:

- Select individual control pages from the View menu.
- Select the View menu, then select **Default Layout** to display the Spectrum window, Data Storage, and Manual Laser/Sample Positioning control pages.

NOTE: *Depending on the size of your computer screen, some control pages may be only partially displayed. To display the entire control page, click-drag the page borders to resize.*

Hint: *A toolbar button is available for selecting Default Layout. See the Data Explorer Software User's Guide, "Customizing toolbars" on page 1-21, for information on customizing the toolbar. The*  *button is located in the* ***Window*** *category.*

Types of page control

You can select between two types of page control for the control pages:

- **Docked**—Pages are attached, or “anchored” to other pages or the edge of the Instrument Control Panel.

  You can do the following with a docked page:

  - Deselect it from the View menu to close it.
  - Double-click it to change it to a floating page (described below). This automatically maximizes the page. Double-click it again to dock the page.
  - Right-click on it and deselect **Allow Docking** to change it to a floating page. This automatically maximizes the page. Double-click on the page to dock the page.
  - Click-drag the page borders to resize it.

- **Floating**—Pages are not docked to other pages or the edge of the Instrument Control Panel.

  You can do the following with a floating page:

  - Deselect it from the View menu, or click  in the top-right corner of the page to close it.
  - Click-drag the page borders to resize it.
  - Click-drag it to a different location on the screen.
  - Double-click to change it to docked page.

4.3 Using the Spectrum Window

This section includes:

- Adjusting the display range
- Zooming on traces
- Adding traces to a window
- Annotating traces
- Previewing and printing traces

4.3.1 Adjusting the Display Range

To set the display range in Spectrum window:

1. Click the Spectrum window to activate it.
2. From the Display menu, select **Range**.

X range

3. Select **X Range** to set the x-axis range.

   The X Axis Setup dialog box (Figure 4-3) is displayed.

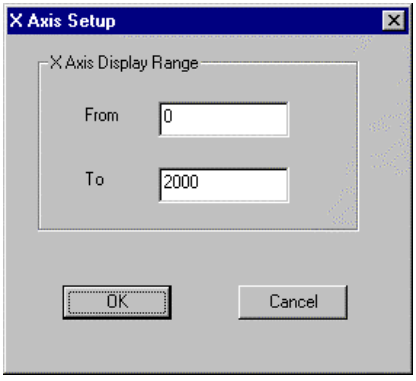


Figure 4-3 X Axis Setup Dialog

4. Set From and To values for the display range (m/z units), then click **OK**.

Y range

5. From the Display menu, select **Range**.
6. Select **Y Range** to scale the y-axis.

   The Y Axis Setup dialog box (Figure 4-4) is displayed.

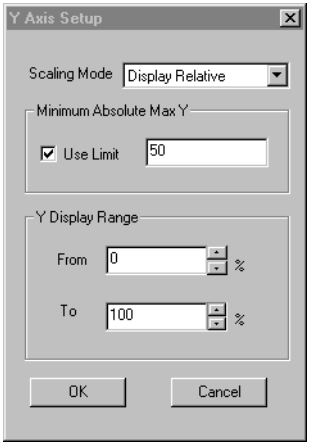


Figure 4-4 Y Axis Setup Dialog Box

7. Set the parameters described below:

Parameter	Description
Scaling Mode	
Display Relative	Autoscales the trace to the largest peak in the selected range.
Base Peak Relative	Autoscales the trace to the base peak in the entire range, not the selected range. Displays a right-axis label with the base peak intensity. ***NOTE:*** *To turn off the right axis, select* ***Graphic Options*** *from the Display menu, click the* ***Graph #1 Setup*** *tab, then deselect the Show Right Y-Axis check box.*

continued

Parameter	Description
Scaling Mode *(continued)*	
Absolute Value	Sets the trace to the Y Display Range you enter in the Y Display Range From/To boxes.
Display Min/Max	Sets the trace display to the minimum and maximum Y values.
Minimum Absolute Max Y	
Use Limit	Sets the minimum value for the maximum Y-axis setting. Useful to maintain relative scaling of peaks and to prevent autoscaling noise to full scale.
Y Display Range	
From/To	Sets the range for scaling. If Display Relative or Base Peak Relative is selected, range is in percent. If Absolute Value is selected, range is in counts.

8. Click **OK**.

4.3.2 Zooming on Traces

Zooming and unzooming

You can expand (zoom) an area of a trace by click-dragging a box around the area of interest.

You can also click buttons in the toolbar to:

- Zoom in 
- Zoom out to the previous zoom 
- Full Unzoom 

Expanding and linking traces

When you have more than one trace displayed in the same data file in a window, you can:

- Select (click on) a trace, then click  in the toolbar to expand the selected trace for closer examination. To display all traces, click the button again.
- Click  in the toolbar to link all traces. Any zooming actions you perform on one trace affect all traces linked to it. To unlink traces, click the button again.

4.3.3 Adding Traces to a Window

This section describes:

- Types of traces
- Adding traces
- Removing traces

Types of traces

The Spectrum window can contain two types of traces (see Figure 4-5 on page 4-15):

- **Live/Current**—A live, real-time trace of data. The display updates as you view or acquire data. When acquisition is complete, the trace name changes from Live to Current.

NOTE: *On systems with Signatec, LeCroy, or Acqiris digitizers, the Live Trace dynamically updates as data is acquired. On systems with Tektronix oscilloscopes, a complete Current trace is displayed only after acquisition is complete.*

- **Accumulated Spectrum**—A trace that is made up of one or more spectra that you have manually or automatically accepted.

Adding traces

The Spectrum window can display a total of four traces at one time. To add a new trace to a window:

1. Activate (click on) the Spectrum window.
2. Click  in the toolbar.

NOTE: *You can add traces by selecting Add/Remove Traces from the Display menu.*

If the trace position has not previously been used for the current acquisition, the software adds a Not Used trace to the window.

If the trace position has previously been used for the current acquisition, the software adds a trace that contains the data originally displayed. For example, if you add a second trace, then remove the trace, then add the second trace again, the original trace is displayed again, instead of a Not Used trace.

Hint: *Resize the window to view all added traces.*

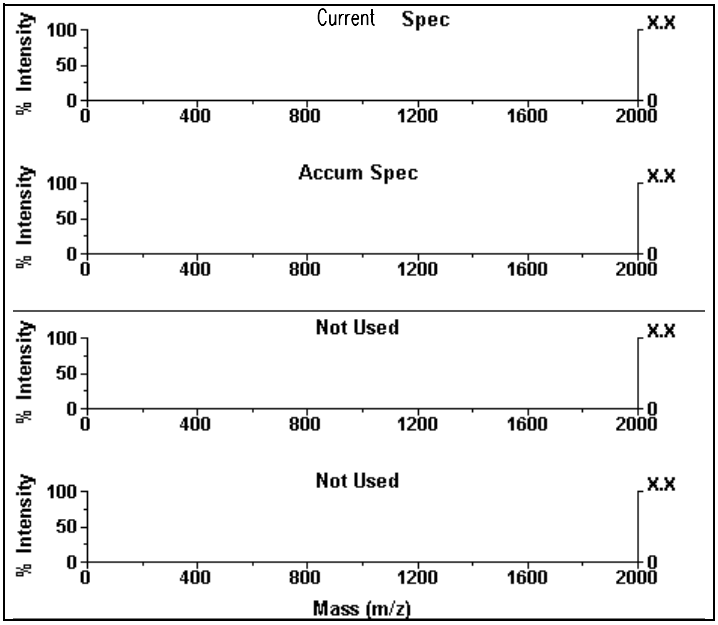


Figure 4-5 Adding Traces

When you add a specific type of trace, the label of the trace changes from Not Used to the label for the type of trace created.

Removing traces

To remove a trace from the Spectrum window:

1. Select (click on) the trace.
2. Click  in the toolbar.

   The trace is removed.

4.3.4 Annotating Traces

Two ways to annotate

You can add text annotation to traces by:

- Copying ASCII text from any source, then pasting on the trace
- Typing text on the trace

Copying text from ASCII source

To copy ASCII text:

1. Open the ASCII text file.
2. Select the line of text to copy, then right-click and select **Copy** from the menu displayed.

NOTE: *If you select more than one line of text, only the first line is pasted when you annotate the trace.*

Annotating the trace

To annotate the trace:

1. Click on the trace at the location at which you want to insert text.
2. Right-click, then select **Paste text** if you copied results, or **Add text annotation** if you want to type in text. Type in text as needed.

   The text is added to the trace.

NOTE: *The text is associated with the x-coordinate. If you zoom on a different region of the trace and the x-coordinate moves out of view, the annotated text also moves out of view.*

Hint: *To move the text, left-click and hold on the text, then drag to the desired position.*

---

3. To customize the appearance of the annotated text, see Section 4.4, Customizing the Instrument Control Panel.

NOTE: *Text annotations are associated with the Spectrum window, not the trace. Text annotations remain in the window after the trace is overwritten by a new trace. Text annotations are not saved in the data file.*

---

4. To delete the annotation, do either of the following:

   - Select the **text** and right-click. Select the appropriate delete or cut option.
   - Right-click on the trace and select **Delete All Text**.

4.3.5 Previewing and Printing Traces

This section includes:

- Previewing and printing traces
- Dedicating a printer to landscape orientation
- Print Setup

Previewing and printing

To preview and print traces:

Setting trace colors manually

1. Display the traces as desired.

   For a clear printout, you can set the Trace Color and other attributes to black before printing traces:

   - Select **Graphic Options** from the Display menu.
   - Click **Use the same settings for all graphs** in View Setup (to change all traces if desired).
   - Click **Graph #1 Setup**, and set colors as needed.

   When manually setting colors, note the following:

   - Selections set to white (or line widths set to 0) may not print on certain printers.
   - If you select different trace colors for multiple traces, only the color for the active trace is saved.

Printing

2. Click on the Spectrum window to print.
3. To print with the x-axis along the longest length of the paper, select **Print Setup** from the File menu and select **Landscape** orientation.

NOTE: *If you set Landscape printing orientation within Instrument Control Panel, this setting is lost when you close Instrument Control Panel. To permanently set the printer, see "Dedicating a printer to landscape orientation" on page 4-20.*

---

4. From the File menu, select **Print Preview** to view the traces before printing.

NOTE: *To print without previewing, select* ***Print Spectrum*** *from the File menu.*

---

5. Click **Print**.

NOTE: *To print a trace that is displayed as Vertical Bars, change the Line Width to 1. If Line Width is set to 0, Vertical Bar traces may not print. See Section 4.4, Customizing the Instrument Control Panel, for information.*

NOTE: *Line Widths of 0 or 1 (or lines set to the color white) may not print on certain printers. If traces do not print, change the line width (or color).*

Dedicating a printer to landscape orientation

To dedicate the printer to landscape orientation, set the orientation from the Windows desktop:

1. Click **Start**, then select **Settings**.
2. Click **Printers**.
3. Select the printer name in the list displayed.
4. Click on **File** and select **Document Defaults**.
5. In the Page Setup Tab, select **Landscape** orientation.

NOTE: *This printer setting will affect all applications that use the printer, not just the Voyager software.*

NOTE: *If you cannot select Landscape orientation, you do not have access permission. See your administrator.*

Print Setup

Print Setup allows you to select a printer and set other printer options. For more information on Print Setup and connecting printers to your computer, refer to the documentation provided with your computer.

4.4 Customizing the Instrument Control Panel

Undocking toolbars

The toolbar at the top of the Instrument Control Panel is divided into sections. A section is preceded by a double vertical bar.

You can “undock” each section of the toolbar and move it anywhere within the Instrument Control Panel by click-dragging the double bar at the left of the toolbar section.

To move the toolbar section back to the top of the window, click-drag the toolbar back to the original position.

Customizing the toolbar

See the *Data Explorer Software User’s Guide*, “Customizing toolbars” on page 1-21, for information on customizing the toolbar.

Customizing the display

To customize the display, use the Graph and Plot Options dialog box (Figure 4-6) to:

- Change colors of the trace and trace background
- Change color of peak labels
- Change the appearance of annotation text
- Turn axes on and off, and change axis appearance
- Turn cursor and grids on and off

Hint: *You can use the same settings for all graphs. Before making individual graph selections, select the* ***View Setup*** *Tab. Select* ***Use same settings for all graphs****. Select any Graph Setup tab and make selections. The selections will be applied to the remaining Graphs Setup tabs.*

Accessing graphic options

To access the graphic options:

1. Display the trace of interest.
2. From the Display menu, select **Graphic Options**, then click a **Graph Setup** tab in the Graph and Plot Options dialog box (see Figure 4-6 on page 4-23).
3. Set Setup parameters.
4. Set colors, line widths, data cursors, and graphic compression. See the *Data Explorer Software User's Guide*, Section 1.5, Setting Graphic Options, for more information.

NOTE: *Line Widths of 0 or 1 (or lines set to the color white) may not print on certain printers. If traces do not print, change the line width (or color).*

---

5. Click **OK**.

Changing line type of trace

You can change between two line types for the trace:

- **Lines**—Displays a continuous trace of connected data points.
- **Vertical Bars**—Displays a vertical line for each data point, where the height of the bar is equal to the intensity of the data point.

NOTE: *To print a trace that is displayed as Vertical Bars, change the Line Width to 1. If Line Width is set to 0, Vertical Bar traces may not print.*

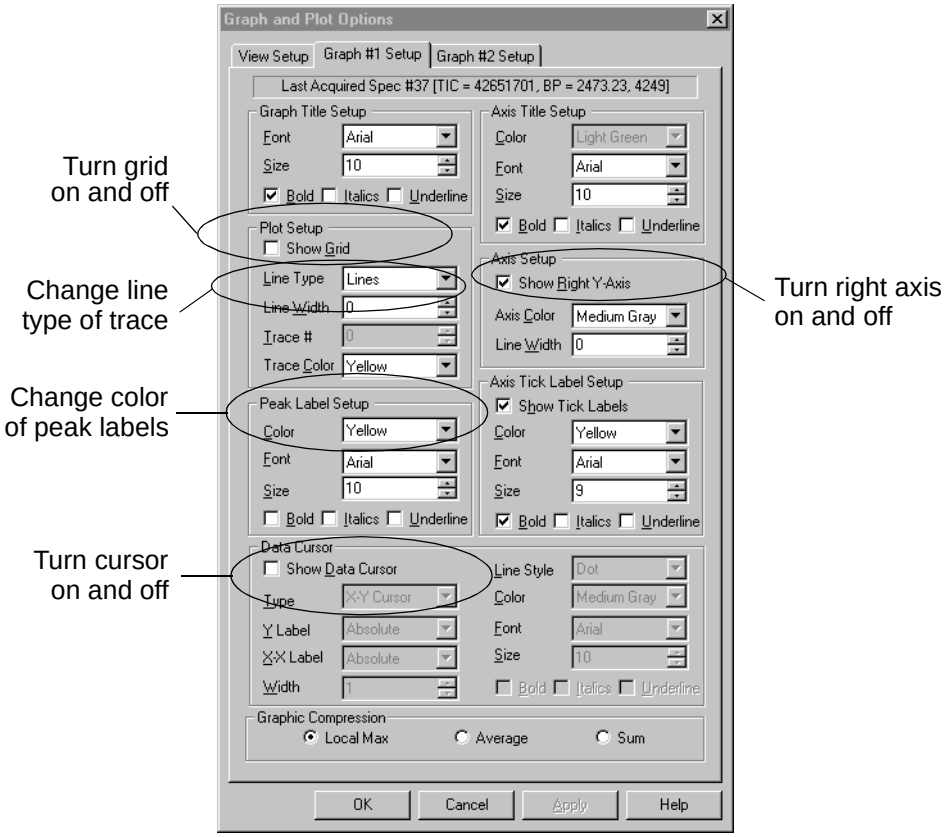


Figure 4-6 Graph and Plot Options Dialog Box

For additional graphic and plot option descriptions, see the *Data Explorer Software User's Guide*, Section 1.5, Setting Graphic Options.

4.5 Controlling the Workstation

This section includes:

- Using toolbar buttons and Instrument menu commands
- Adjusting laser intensity and selecting sample position

4.5.1 Using Toolbar Buttons and Instrument Menu Commands

Instrument buttons in the toolbar (Figure 4-7) and Instrument menu commands allow you to control the software and the Voyager mass spectrometer.

Figure 4-7 Instrument Toolbar

Use toolbar buttons and Instrument menu commands in the Instrument Control Panel to:

- Turn high voltage on and off
- Load/eject the sample plate
- Select the active sample position
- Turn acquisition (laser) on and off
- Save data
- Accumulate and clear spectra

Turning high voltage on and off

Click  in the toolbar to turn the high voltage on and off. You can also control high voltage by selecting **Turn On/Off High Voltage** from the Instrument menu.

NOTE: *High voltage is automatically turned on when an acquisition is started. High voltage is automatically turned off when exiting the Instrument Control Panel or ejecting a sample plate.*

Loading and ejecting the sample plate

Click  in the toolbar to load or eject the sample plate. You can also load or eject the sample plate by selecting **Load** or **Eject** from the Sample Plate menu.

Selecting the active sample position

Click  in the toolbar to select the active sample position. You can also select from the Manual Laser/Sample Position control page described on page 4-27.

Turning acquisition (laser) on and off

Click  in the toolbar to turn acquisition (laser) on and off. You can also control acquisition by selecting commands from the Instrument menu.

When the system is acquiring:

- The button is depressed
- The Acquisition field in the status bar at the bottom of the Instrument Control Panel is green and displays “Acquisition-ON”
- High voltage is on

Saving data

Click  in the toolbar to manually save the selected current or accumulated trace. You can also save data by selecting **Save Spectrum** from the Acquisition menu. For additional information, see “Saving data” on page 6-18.

Accumulating spectra

Click  in the toolbar to manually accumulate spectra. You can also accumulate spectra by selecting **Accumulate Spectrum** from the Acquisition menu. For more information, see Section 6.2.2, Manually Accumulating Spectra from Multiple Acquisitions.

Clearing an accumulated spectrum

Click  in the toolbar to manually clear an accumulated spectrum. You can also clear an accumulated spectrum by selecting **Clear Accumulated Spectrum** from the Acquisition menu. For more information, see Section 6.2.2, Manually Accumulating Spectra from Multiple Acquisitions.

4.5.2 Adjusting Laser Intensity and Selecting Sample Position

This section describes:

- Displaying the Manual Laser/Sample Position page
- Manually adjusting laser intensity
- Selecting the active sample position in Plate view
- Displaying coordinates of active position
- Switching between Plate view and Sample view
- Adjusting sample position in Sample view

For information on automatically controlling the laser and sample position see Section 5.2.3, Automatic Control Dialog Box.

Displaying the Manual Laser/ Sample Position control page

Select **Manual Laser/Sample Position** from the View menu to display the Manual Laser/Sample Position control page (Figure 4-8 and Figure 4-9 on page 4-31).

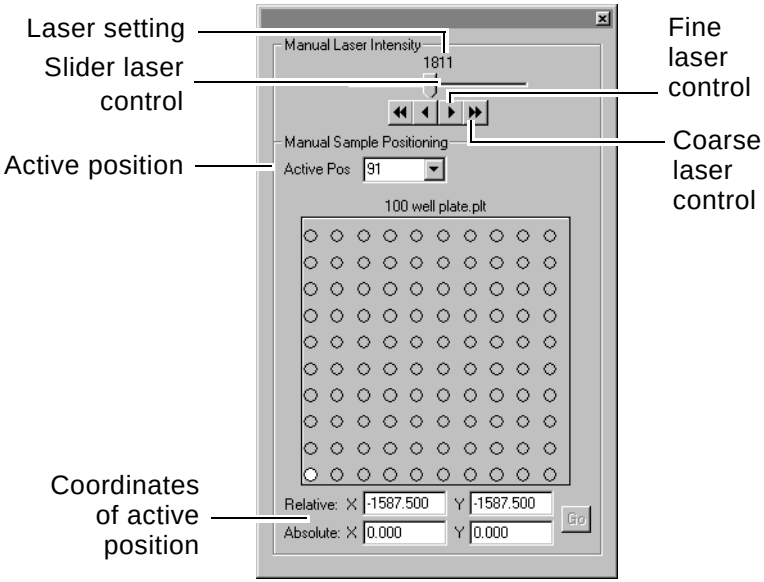


Figure 4-8 Manual Laser/Sample Position Control Page—Plate View

Manually adjusting laser intensity

You can adjust the laser intensity using any of the following:

- **Slider control**—Use to set laser intensity to an exact setting. To use the slider control, click-drag the slider 

to adjust laser intensity.

- **Fine laser controls**  —Use to adjust the intensity in small increments.

- **Coarse laser controls**  —Use to adjust the intensity in large increments.

You can set the small and large increments in the Laser Configuration dialog box. For more information, see “Laser Configuration” on page 2-42.

NOTE: *After adjusting the laser intensity, the slider and fine and coarse adjusting buttons are disabled while the system adjusts the laser to the intensity specified.*

Hint: *You can also set laser intensity by pressing* ***Ctrl+PgUp*** *and* ***Ctrl+PgDn*** *on the keyboard. Pressing these keys adjusts intensity in the same increments as the fine laser control buttons.*

Selecting the active sample position in Plate view

The active sample position is the sample position from which data is acquired. Select the active sample position (from the Plate view) by doing any of the following:

- Type a position name or number in the Active Position field.
- Select a number from the drop-down list in the Active Position field.
- Click on a sample position (ToolTip displays the position number).
- Use the control stick to move to a sample position. For more information on using the control stick, see Section I.3, Using the Control Stick.

NOTE: *When you use the control stick to select a sample position, if the software determines that you have gone beyond the boundary of a position, it will automatically change to the closest Active Position. Position boundaries are determined/calculated from the Sample Plate Template (.PLT) file. For more information, see "Effect of plate type on area available for analysis" on page 3-73.*

Displaying coordinates of active position

The location (X,Y) coordinates of the active position are displayed at the bottom of the Manual Laser/Sample Position control page:

- **Relative**—Displays the X,Y coordinates (microns) relative to the center of the Active Position.
- **Absolute**—Displays the X,Y coordinates (microns) relative to the home position (position in the lower left corner of the sample plate).

NOTE: *If the coordinates are not displayed, you can click-drag the bottom border of the control page to make them visible.*

You can use these coordinates when you create a search pattern file. For information on creating an .SP file, see “Creating and Editing .SP Files Using the Search Pattern Editor” on page 6-50.

Switching between Plate and Sample view

You can change the view of the sample plate between the whole sample plate and a single sample position. Refer to the following table for plate view choices:

In this view...	If you...	The view...
Plate (Figure 4-8 on page 4-27)	Single-click on a position	• Moves to the selected position • Displays position number in the Active Position field • Turns the Active Position white
	Double-click on the active position ***NOTE:*** *If you double-click between positions, the software zooms on the location on which you clicked, and selects the closest defined position as the Active Sample Position.*	• Moves to the selected position • Displays position number in the Active Position field • Switches to Sample View • Turns the Active Position white
	Double-click on a non-active position	• Moves to the selected position • Makes it the active position • Displays position number in the Active Position field
Sample (Figure 4-9 on page 4-31)	Single-click or double-click on a position	• Moves to selected location of the sample position

Hint: *If you right-click on a position, you can change between Sample View and Plate View.*

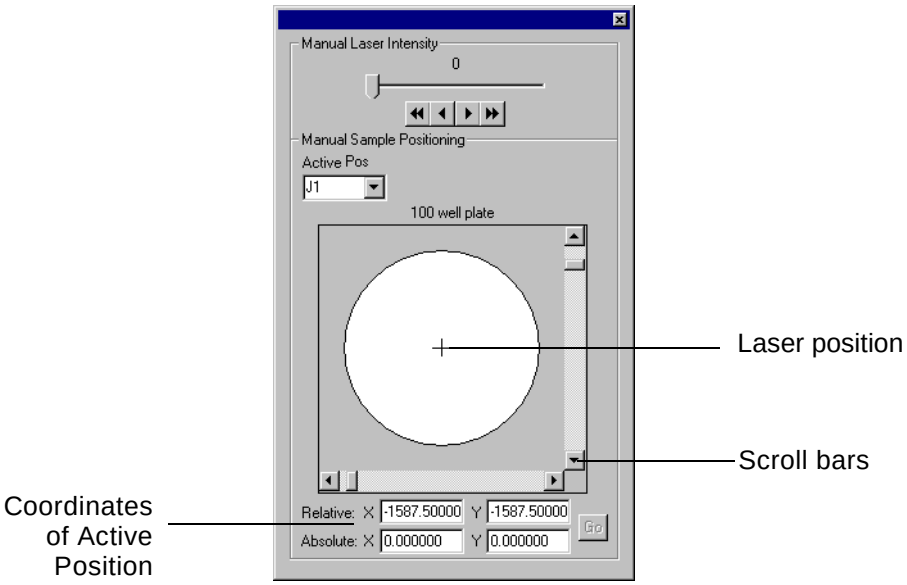


Figure 4-9 Manual Laser/Sample Position Control Page—Sample View

Adjusting sample position in the Sample view

Adjust the sample position (in the Sample view display) by doing any of the following:

- Click the up/down and left/right scroll bars.
- Click on the sample position to move to that X,Y position.
- Type coordinates (microns) in X and Y fields and click **GO**.

NOTE: *The motor controlling plate movement moves in step units. Coordinate values entered are rounded to the nearest step value (1 step=3.175 microns).*

- Use the control stick to move to a sample position. For more information, see Section I.3, Using the Control Stick.

4.6 Sequence Control Panel

Sequence Control Panel

The Voyager Sequence Control Panel (Figure 4-10) allows you to collect data for multiple samples using different conditions.

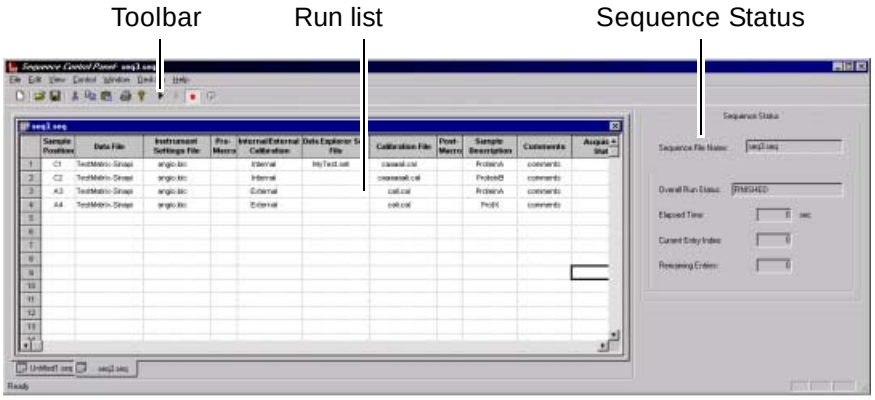


Figure 4-10 Sequence Control Panel

The Sequence Control Panel includes:

- **Toolbar**—Contains buttons that control the software and the instrument. For a description of a toolbar button, place the cursor on it. A brief description of the button (ToolTip) is displayed below the button.
- **Sequence Status**—Displays information about the currently running entry in the sequence.
- **Run list**—Allows you to define sample position, data file name, instrument settings file, Data Explorer .SET file, processing macros, calibration type and .CAL file used, sample description, comments for each sample, acquisition status, and a run checklist column to track completed samples.

When you start the Sequence Control Panel, the Instrument Control Panel is automatically started and tiled horizontally at the bottom of the screen. You can hide the Instrument Control Panel by deselecting **Instrument Control Panel** from the View menu.

For more information, see Section 4.7, How the Instrument and Sequence Control Panels Interact, and Chapter 7, Acquiring Spectra from the Sequence Control Panel.

4.7 How the Instrument and Sequence Control Panels Interact

This section includes:

- Overview
- Two ways to start the Instrument Control Panel
- During acquisition
- Keeping both control panels open
- Organizing the desktop and windows

Overview

The Sequence Control Panel works in conjunction with the Instrument Control Panel to allow you to acquire multiple samples using different instrument settings (.BIC) files.

Two ways to start the Instrument Control Panel

You can start the Instrument Control Panel in either of two ways:

- Double-click the **Instrument Control Panel icon**
- Double-click the **Sequence Control Panel icon**

If you start the Instrument Control Panel using the Sequence Control Panel icon, it functions the same way as if you started it using the Instrument Control Panel icon, with the following exceptions:

- Warning and error messages are not displayed during operation.
- The Instrument Control Panel will close when you close the Sequence Control Panel.

The preferred method is to start the Instrument Control Panel using the Instrument Control Panel icon, create instrument settings files, then open the Sequence Control Panel using the Sequence Control Panel icon, or to start the Sequence Control Panel by clicking on the toolbar button in the Instrument Control Panel.

During acquisition

If you are acquiring data from one Control Panel, the acquisition functions in the other control panel are inactive:

- If you are acquiring data in the Instrument Control Panel, Sequence Control Panel functions are disabled until acquisition is complete.
- If you are acquiring data in the Sequence Control Panel, Instrument Control Panel acquisition functions are disabled until acquisition is complete.

NOTE: *Other Instrument Control Panel functions are available while acquisition is running in the Sequence Control Panel. However, prompts and error messages are suppressed to prevent the sequence from being interrupted.*

Keeping both control panels open

You can keep the Sequence Control Panel and the Instrument Control Panel open at the same time. However, if you do not need Sequence Control Panel functions, close the Sequence Control Panel to improve system performance.

CAUTION

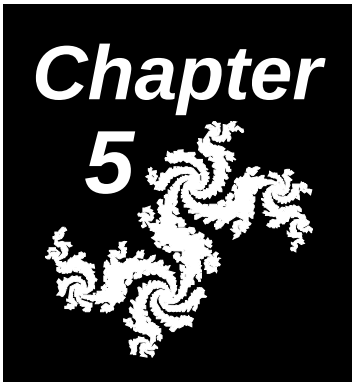
If you started the Instrument Control Panel by double-clicking the Sequence Control Panel icon, the Instrument Control Panel closes when you close the Sequence Control Panel.

Organizing the desktop and windows

You can organize the display to suit your needs.

To rearrange the Sequence Control Panel and the Instrument Control Panel, select commands from the Desktop menu:

- **Tile Horizontal**—Places the Sequence Control Panel at the top or bottom of the desktop, and the Instrument Control Panel in the opposite location.
- **Tile Vertical**—Places the Sequence Control Panel at the left or right of the desktop, and the Instrument Control Panel in the opposite location.



5 Optimizing Instrument Settings

This chapter contains the following sections:

5.1 Loading, Modifying, and Saving Instrument Settings 5-2
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5.2.1 Instrument Settings Page 5-15
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5.1 Loading, Modifying, and Saving Instrument Settings

This section includes:

- Using instrument settings (.BIC) files
- Standard instrument settings (.BIC) files provided
- Opening and viewing instrument settings
- Modifying an instrument settings (.BIC) file
- Saving and printing instrument settings
- Setting instrument settings files to “read-only” status

5.1.1 Using Instrument Settings (.BIC) Files

NOTE: *Instrument settings and .BIC files replace methods and .MNU files used in previous versions of Voyager systems.*

Instrument settings file definition

Instrument settings control operation of the Voyager mass spectrometer and are stored in .BIC files. Instrument settings include:

- Mode/digitizer settings
- Control mode (manual or automatic)
- Voltages
- Spectrum acquisition settings
- Laser intensity settings
- Calibration settings
- PSD acquisition settings

For more information, see Section 5.2, Instrument Settings Parameter Descriptions, and Chapter 8, PSD Analysis.

You can create instrument settings files with different settings and save each file for future use. When you analyze samples, you can select an instrument settings file with the appropriate settings instead of entering new settings.

NOTE: *Data storage parameters are not stored in .BIC files. See "Setting Data Storage parameters" on page 6-14, for more information.*

Converting version 4 methods and search pattern files

Before using methods (.MNU) or search pattern files (.SP) created using version 4.0 software, convert to instrument settings (.BIC) and version 5-compatible search pattern (.SP) files. For information, see Section 5.5, Converting Version 4 Methods and Search Pattern Files.

5.1.2 Standard Instrument Settings (.BIC) Files Provided

This section includes:

- Standard instrument settings files
- Location of .BIC files
- .BIC files and control mode
- List of .BIC files

Standard instrument settings files

Standard read-only instrument settings files are provided on your system. These instrument settings files have been optimized for your instrument.

CAUTION

These instrument settings files have been used to test and optimize your system. Do not delete or change these instrument settings files.

Standard instrument settings (.BIC) files are initially located in the C:\VOYAGER\DATA\FACTORY directory.

NOTE: *You can also export .BIC files from an existing data (.DAT) file in the Data Explorer software.*

Location of .BIC files

The standard .BIC files provided are initially located in the C:\VOYAGER\FACTORY directory. They can be saved to any directory on your system.

.BIC files and control mode

The default control mode for all .BIC files is Manual. You can modify any .BIC file for use in Automatic Control mode, and then save it as a new .BIC file. For information on modifying instrument settings (.BIC) files, see Section 5.1.4, Modifying an Instrument Settings File (.BIC).

List of .BIC files

Table 5-1 through Table 5-3 list the standard .BIC files provided on your system for the following modes:

- Linear mode
- Reflector mode
- PSD mode

Table 5-1 Linear Mode .BIC Files

.BIC File	Sample	Test	Mass Range in .BIC Optimized at (Da)*
Angiotensin_Linear.BIC	Low mass peptide mix1	Calibration and Resolution (angiotensin I)	500–2,000
ACTH_Linear.BIC	Peptide mix2	Calibration and Resolution (ACTH 18–39)	2,000–3,000
Insulin_Linear.BIC	Peptide mix2	Resolution (insulin)	5,000–6,000
Myoglobin_Linear.BIC	Low mass protein mix3	Resolution (myoglobin)	15,000–20,000
BSA_Linear.BIC	BSA	Resolution	60,000–70,000
IgG_Linear.BIC	IgG	Resolution	100,000–200,000
Peptide_Sensitivity_Linear.BIC	Neurotensin in mix1	Sensitivity	1,000–2,000
Peptide_Linear_Negative.BIC	Low mass peptide mix1	Negative ion mode	500–2,000

* Mass Range specified for acquisition may be wider.

Table 5-2 Reflector Mode .BIC Files

.BIC File	Sample	Test	Mass Range in .BIC Optimized at (Da)*
Angiotensin_Reflector.BIC	Low mass peptide mix1	Calibration and Resolution (angiotensin I)	500–2,000
ACTH_Reflector.BIC	Peptide mix2	Resolution across mass range	1,000–4,000 (optimized at 2,500)
Insulin_Reflector.BIC	Peptide mix2	Resolution (insulin)	5,000–7,000
Peptide_Sensitivity_Reflector.BIC	Neurotensin in dilute mix1	Sensitivity	1,000–2,000
Thioredoxin_Reflector.BIC	Low mass protein mix3	Resolution (thioredoxin)	10,000–15,000
Peptide_Reflector_Negative.BIC	Low mass peptide mix1	Negative ion mode	500–2,000
TIS_Test.BIC	Substance P mix	Timed Ion Selector	1,300–1,400

* Mass Range specified for acquisition may be wider.

Table 5-3 PSD Mode .BIC Files

.BIC File	Sample	Test	Mass Range in .BIC Optimized at (Da)*
PSD_Precursor.BIC	Angiotensin	Mirror ratio 1 for Precursor ion in Reflector mode	1,000–1,400
Angiotensin_PSD.BIC	Angiotensin	Mirror ratio varies for PSD analysis	PSD ions for precursor mass 1296.69
Angiotensin_PSD_Auto.BIC	Angiotensin	Mirror ratio varies for PSD analysis	PSD ions for precursor mass 1296.69
Substance_P_PSD.BIC	Substance P	Mirror ratio varies for PSD analysis	PSD ions for precursor mass 1347.74

* Mass Range specified for acquisition may be wider.

NOTE: *For CID applications, use the Substance_P_Precursor.BIC with the appropriate precursor mass, 1347.74 Da.*

5.1.3 Opening and Viewing Instrument Settings

Overview There are two ways to open an instrument settings file:

- Directly open a .BIC file
- Select a .DAT file that contains the instrument settings of interest, and the software loads the .BIC

Opening From the Instrument Control Panel:

1. Select **Open Instrument Settings** from the File menu. The Open dialog box is displayed (Figure 5-1).

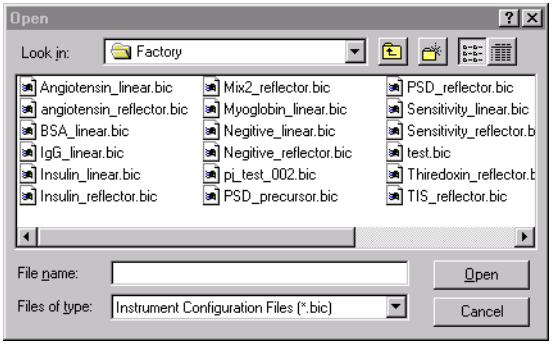


Figure 5-1 Open Dialog Box

Standard instrument settings files provided are located in the C:\VOYAGER\FACTORY directory.

Opening from a .BIC file

2. To open an instrument settings file directly, double-click the .BIC file, or select a file and click **Open**.

Hint: *If the .BIC file you need is not visible, click the scroll bar to view more instrument settings files.*

5

Opening from a .DAT file

To open an instrument settings file from a .DAT file, select **.DAT** from the Files of Type drop-down list, select the .DAT file that contains the .BIC of interest, and click **OK**.

The instrument settings file is loaded. The currently loaded instrument settings file name is displayed in the title bar of the Instrument Control Panel.

Viewing

To view all of the instrument settings in a .BIC file, print the instrument settings as described in "Printing" on page 5-12.

5.1.4 Modifying an Instrument Settings File (.BIC)

In this section

This section includes:

- Selecting a .BIC file
- Modifying for Manual Control mode
- Optimizing
- Modifying for Automatic Control mode

Selecting a .BIC file

We suggest that you use standard instruments settings (.BIC) files provided, and modify as needed. However, you can open and modify any .BIC file as needed.

To select a standard instrument settings (.BIC) file:

1. Open a .BIC file for the mass range you are analyzing. For information on mass ranges in .BIC files, see Section 5.1.2, Standard Instrument Settings (.BIC) Files Provided.

   If a .BIC file for the mass range you are analyzing does not exist, open a standard .BIC file with the closest higher mass.

   Hint: *To optimize a wide mass range, select a .BIC file with the mass of the highest component in the range.*

   ---

2. Save the .BIC file under a new name. Standard .BIC files are read-only and cannot be saved.

Modifying for Manual Control mode

To modify the instrument settings file for Manual Control mode:

1. If the Instrument Settings control page (Figure 5-2) is not displayed, select **Instrument Settings** from the View menu.

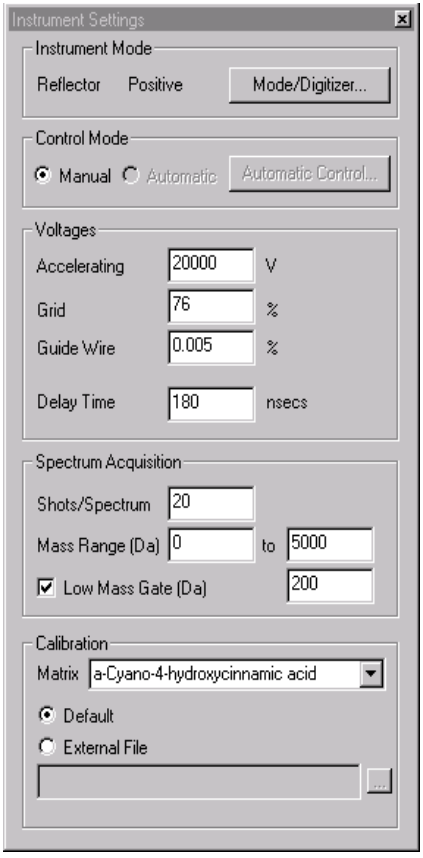


Figure 5-2 Instrument Settings Control Page

2. Click **Mode/Digitizer** to select settings. For parameter descriptions, see “Linear/Reflector Digitizer parameters” on page 5-28.

3. Select **Manual** Control mode. For parameter descriptions, see Section 5.2.1, Instrument Settings Page.
4. Adjust the mass range if needed.
5. To include matrix peaks in the spectrum for calibration, deselect **Low Mass Gate** and set the mass to a mass below the matrix peak mass. For matrix masses, see, Appendix C, Matrixes.
6. Select a calibration file (.CAL), or if you are screening samples, use the default calibration. For better mass accuracy, select a .CAL file you recently (same day) generated in the Data Explorer software. For more information, see the *Data Explorer Software User's Guide*, Section 5.3.2, Manually Calibrating.
7. Save the .BIC file. For more information, see Section 5.1.5, Saving and Printing Instrument Settings.

Optimizing

For suggested strategies for optimizing parameters, see:

- Section 5.3, Impact of Changing Instrument Settings Parameters
- Section 5.4, Optimizing Instrument Settings Parameters

Modifying for Automatic Control mode

After you optimize the instrument settings file for Manual Control mode and ensure that parameters are adjusted to yield optimum performance, you can adjust the instrument settings for Automatic Control mode, if desired. For more information, see Section 6.6.2, Setting Instrument Settings for Automatic Control Mode.

5.1.5 Saving and Printing Instrument Settings

Saving

To save instrument settings:

1. Set all parameters as needed.
2. To save the changes under the current instrument settings file name, select **Save Instrument Settings** from the File menu.

   The name of the current instrument settings (.BIC) file is displayed in the title bar of the Instrument Control Panel.

NOTE: *If you try to save a "read-only" instrument settings file under the current instrument settings file name, a message is displayed indicating that the file cannot be saved. The current settings can be used for analysis, but cannot be permanently saved to the instrument settings file. You can save the changes by saving the file with a new name by selecting* ***Save Instrument Settings As*** *from the File menu. Changes to the instrument settings file are lost if you do not save them before you open a different instrument settings file or exit the Voyager Instrument Control Panel.*

---

3. To save the settings under a different instrument settings file name, select **Save Instrument Settings As**.

   Enter a new name and click **Save**.

Saving .BIC files for different matrixes

The laser intensity required to yield optimum resolution and signal-to-noise ratio for a sample can vary substantially for different matrixes. Other instrument settings do not vary for the same sample in different matrixes.

Because the laser setting is saved in the .BIC file, you can create and save different .BIC files for different matrixes.

For more information on laser intensity/settings, see Section 5.4.2, Determining the Laser Setting.

Saving .BIC files for different modes

When saving .BIC files for use in different operating modes, make sure to create an identifier so that you will know which instrument settings (.BIC) files are optimized for which experiments. For example:

- **Linear mode**—Use _LIN.BIC
- **Reflector mode**—Use _REF.BIC
- **PSD mode**—Use _PSD.BIC

Printing

To print instrument settings:

1. Open the instrument settings file in the Instrument Control Panel.
2. Select **Print Instrument Settings** from the File menu.

   The instrument settings file prints.

5.1.6 Setting Instrument Settings Files to “Read-Only” Status

Standard instrument settings files are “read-only” files. Read-only files cannot be changed and saved. However, they can be changed temporarily and used without saving them, or saved with a new name.

You can set any instrument settings file to “read-only” status.

To set an instrument settings file to read-only status:

1. Display the Windows NT Explorer.
2. Select the instrument settings (.BIC) file you want to set to read-only status.
3. Select **Properties** from the File menu. The Properties dialog box is displayed.
4. Select **Read-only** from the Attributes section.
5. Click **OK**.

   The file is listed with “r” next to the file name in the Attributes column.

NOTE: *If attributes are not displayed, select Details from the View menu.*

Saving “read-only” instrument settings files

To save changes to a “read-only” instrument settings file, you must save the file with a new name. Select **Save Instrument Settings As** from the File menu, enter a new name, and save the file.

5.2 Instrument Settings Parameter Descriptions

This section describes the parameters on the Instrument Settings control page and associated dialog boxes that are stored in a .BIC file. The following control pages or dialog boxes are available from the View menu or Instrument menu in the Instrument Control Panel:

- Instrument Settings Page
- Mode/Digitizer dialog box
- Automatic Control dialog box

NOTE: *For a description of PSD acquisition settings, see Chapter 8, PSD Analysis.*

5.2.1 Instrument Settings Page

Select **Instrument Settings** from the View menu to display the Instrument Settings page (Figure 5-3).

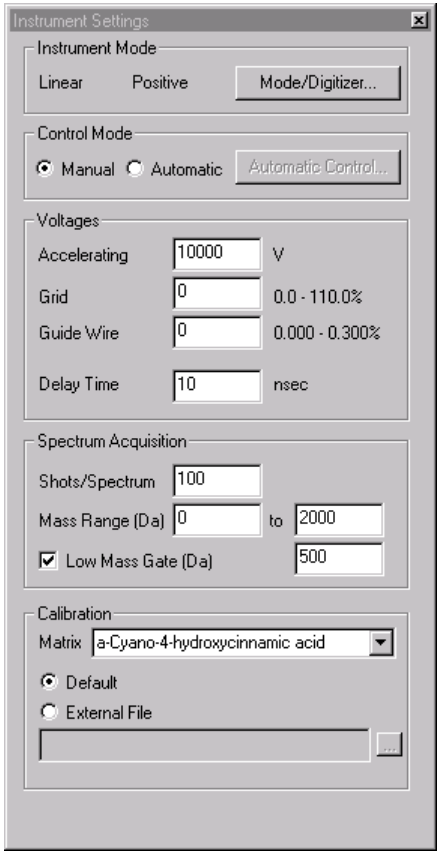


Figure 5-3 Instrument Settings Page

5

Instrument settings parameters are described in Table 5-4.

Table 5-4 Instrument Settings Parameters

Parameter	Description
Instrument Mode	Displays: • Reflector, Linear, or PSD operating mode • Positive or Negative polarity For more information, see Section 1.4, Voyager-DE™ (Delayed Extraction®) Technology.
Mode/Digitizer settings	Click to display Instrument Mode/Digitizer dialog box. See Section 5.2.2, Mode/Digitizer Dialog Box.
Control Mode	Select control mode: • **Manual**—Enables manual control of laser, sample positioning, data storage, and spectrum accumulation. • **Automatic**—Enables automated control of laser, sample positioning, data storage, spectrum accumulation, and spectrum acceptance. See Section 5.2.3, Automatic Control Dialog Box.
Automatic Control settings	Click to display automated laser, sample positioning, data storage, spectrum accumulation parameters, and spectrum acceptance criteria. See Section 5.2.3, Automatic Control Dialog Box. ***NOTE:*** *This button is disabled until you select Automatic Control mode.*

continued

Table 5-4 Instrument Settings Parameters (Continued)

Parameter	Description
Voltages	
Accelerating Voltage	Voltage applied to the first stage ion source. Valid range is 0 to 25,000 V. For information on settings for different mass ranges, see Section 5.4.4.2, Setting Accelerating Voltage. ***NOTE:*** *The calibration of the mass scale changes significantly when you change the Accelerating Voltage. Default calibration adjusts for these changes. However, you will observe more accurate calibration if you use an external calibration (.CAL) file generated with the same Accelerating Voltage. See the Data Explorer Software User's Guide, Section 5.3.2, Manually Calibrating, for more information.*
Grid Voltage%	Voltage applied to the grid above the sample plate. Valid range is determined by the Accelerating Voltage. Optimize in conjunction with Delay Time (described on page 5-18). See Section 5.3.2, Understanding Grid Voltage%, and Section 5.4.3.5, Optimizing Grid Voltage%, for more information.

continued

Table 5-4 Instrument Settings Parameters (Continued)

Parameter	Description
Guide Wire Voltage% ***NOTE:*** *Guide Wire Voltage% is not available on Voyager-DE STR models with serial number 4154 and later.*	Voltage applied to the beam guide wire. Overcomes the dispersion effect from the source and refocuses ions on the detector. The valid range for Guide Wire Voltage% is 0.000 to 0.300% of the Accelerating Voltage: • **Linear mode**—Use 0.05 to 0.3% as suggested by the standard instrument settings, and increase the Grid Voltage% with increasing mass. • **Reflector mode**—Use 0.00 to 0.050% as suggested by the standard methods. • **In PSD mode**—Use settings ≤0.02%. Higher settings may compromise the selectivity of the Precursor Ion Selector. See Section 5.3.4, Understanding Guide Wire Voltage%, and Section 5.4.3.3, Optimizing Guide Wire Voltage%, for more information.
Delay Time (nsec)	Works in conjunction with the Delayed Extraction parameter. Time in nanoseconds (after the laser ionizes the sample) at which full Accelerating Voltage is applied, creating the potential gradient that accelerates ions. Recommended range is 0 to 3,000 nsec. Optimize in conjunction with Grid Voltage% (described on page 5-17). See Section 5.3.3, Understanding Delay Time, and Section 5.4.3.4, Optimizing Delay Time, for information.

continued

Table 5-4 Instrument Settings Parameters (Continued)

Parameter	Description
Shots/Spectrum	Determines the number of laser shots that each spectrum will contain. For more information, see Section 5.4.4.4, Setting Shots/Spectrum. The maximum number of Shots per Spectrum for each digitizer are as follows: • **Signatec**—10,000 • **LeCroy**—1,000 • **Acqiris**—10,000 • **Tektronix**—10,000 ***NOTE:*** *The actual number of times the laser fires may be greater than the number of Shots/Spectrum specified, due to the limited averaging speed of the digitizer. For example, if 250,000 data points are recorded, every other laser shot is skipped by the digitizer. In any case, the specified number of Shots/Spectrum will be stored in the data file.* ***NOTE:*** *This parameter is dimmed if the system is set to Single Shot mode. For information, see page 2-44.*
Spectrum Acquisition	
Mass Range (Da)	Determines the mass range collected. Valid ranges are: • **Start Mass**—0 to End Mass. • **End Mass**—Start Mass to End Mass. Mass Range can affect optimized laser rate (described on page 5-26).

continued

Table 5-4 Instrument Settings Parameters (Continued)

Parameter	Description
Spectrum Acquisition *(continued)*	
Low Mass Gate (Da)	Turns on the detector voltage after the ions of the Mass specified have passed the detector. Ion masses below this entry are not considered during analysis. Suppresses matrix peaks that can interfere with ion detection, and saturate the detector as laser intensity increases. Turn on Low Mass Gate when: • Analyzing masses greater than 2,000 to 3,000 Da • Matrix signal is much higher than the sample signal ***NOTE:*** *Low mass gate is automatically disabled when you change the instrument mode to PSD.* See Section 5.4.4.5, Setting Low Mass Gate, for more information.
Calibration	
Matrix	Displays the matrix used for the analysis. Available matrixes in this field are: • α-cyano-4-hydroxycinnamic acid • Sinapinic acid • 2,5-Dihydroxybenzoic acid • 3-Hydroxypicolinic acid If you do not specify a matrix, a default value of 300 m/sec is used. Corrects for the initial velocity contributed by the matrix, for a more accurate calibration[1, 2]. Correction factors for each matrix are listed in “Matrix influence” on page 5-22. If your matrix is not listed, you can add other matrixes to this list by editing the Matrix reference file. For information, see “Modifying the matrix reference file” on page 5-23.

continued

Table 5-4 Instrument Settings Parameters (Continued)

Parameter	Description
Calibration *(continued)*	
Default	Enables default calibration. For more information, see “Default calibration” on page 6-9.
External file	Specifies calibration using a specified external (.CAL) file. Click ... to select a .CAL file previously generated in the Data Explorer software. For more information, see the *Data Explorer Software User’s Guide*, Section 5.3.2, Manually Calibrating.

1. Juhasz, P., M. Vestal, and S.A. Martin, “On the Initial Velocity of Ions Generated by Matrix-Assisted Laser Desorption Ionization and Its Effect on the Calibration of Delayed Extraction Time-Of-Flight Mass Spectra”, *J. Am. Soc. Mass. Spectrom.*, **1997**, *8*, 209—217.
2. Juhasz, P., M.L. Vestal, and S.A. Martin, “Novel Method for the Measurement of the Initial Velocity of Ions Generated by MALDI”, *Proceedings of the 44th ASMS Conference on Mass Spectrometry and Allied Topics*, May 12–16, **1996**, Portland, OR, p.730.

Matrix influence

The initial velocity is the average speed at which matrix ions desorb. The initial velocity of matrix contributes to the higher order terms in the calibration equation (see Figure 6-1 on page 6-9). The software allows you to correct the calibration equation for matrix initial velocity by selecting a matrix in instrument settings (see page 5-20).

Table 5-5 Matrix Initial Velocity Settings

Matrix	Initial Velocity (m/sec)[1,2]
α-cyano-4-hydroxycinnamic acid	300
Sinapinic acid	350
DHB	500
3-hydroxypicolinic acid	550

1. Juhasz, P., M. Vestal, and S.A. Martin, "On the Initial Velocity of Ions Generated by Matrix-Assisted Laser Desorption Ionization and Its Effect on the Calibration of Delayed Extraction Time-Of-Flight Mass Spectra", *J. Am. Soc. Mass. Spectrom.,* **1996**, *8*, 209–217.
2. Juhasz, P., M. Vestal, and S.A. Martin, "Novel Method for the Measurement of the Initial Velocity of Ions Generated by MALDI", presented at the 44th ASMS Conference on Mass Spectrometry and Allied Topics, May 12–16, **1996**, Portland, OR, p.730.

Modifying the matrix reference file

Matrix options are located in the Matrix field in the Instrument Settings control page. You can add or delete information in the matrix reference file using Microsoft Notepad Editor.

You can add information to the matrix reference file by doing the following:

1. Open the Microsoft Windows NT **Notepad** text editor. See the *Microsoft Windows NT User's Guide* if you need help using Notepad.
2. Navigate to the C:\VOYAGER directory.
3. Open the file named **MATRIX.TXT**.

   The following text file appears:

```
Matrix\Type1=a-Cyano-4-hydroxycinnamic acid
Matrix\Velocity1=300
Matrix\Type2=Sinapinic acid
Matrix\Velocity2=350
Matrix\Type3=2,5-Dihydroxybenzoic acid
Matrix\Velocity3=500
Matrix\Type4=3-Hydroxypicolinic acid
Matrix\Velocity4=550
```

4. Type new matrix and velocity information by using the format above.

   If you do not have a velocity value for a matrix, use 300 m/sec (α-cyano-4-hydroxycinnamic acid value).

5. Save the modified .TXT file.

5.2.2 Mode/Digitizer Dialog Box

Click **Mode/Digitizer** in the Instrument Settings control page (see Figure 5-3 on page 5-15) to display the Mode/Digitizer dialog box (Figure 5-4).

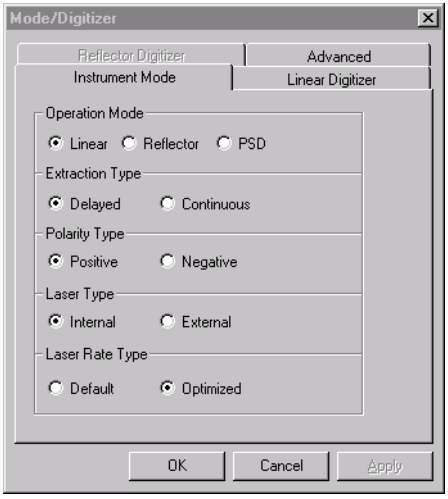


Figure 5-4 Mode/Digitizer Dialog Box with Instrument Mode Tab Displayed

The Mode/Digitizer settings dialog box includes tabs for the the following instrument settings parameters:

- Instrument Mode
- Linear/Reflector Digitizer
- Advanced

Instrument Mode parameters

Click the **Instrument Mode** tab to display the Instrument Mode page (Figure 5-4).

Instrument Mode parameters are described in Table 5-6.

Table 5-6 Instrument Mode Parameters

Parameter	Description
Operation Mode	Specifies the Operation Mode: • **Linear**—Uses Linear detector only. Most sensitive mode due to shorter flight path. Also more sensitive because fragments, neutrals, and molecular ions arrive at the detector at the same time. Less sample fragmentation is observed. • **Reflector**—Uses Reflector detector only. Higher resolution, greater mass accuracy due to longer flight path and action of reflector. • **PSD**—For PSD Analysis, allows you to create composite spectra from fragment ion spectra. Uses the reflector detector. See Chapter 8, PSD Analysis, for more information. ***NOTE:*** *Reflector and PSD modes are not available on the Voyager-DE system. If you have a Voyager-DE system, buttons for these two modes are not displayed.*
Extraction Type	Specifies the type of extraction to use: • **Delayed**—Use for normal operation. Described in Section 1.4, Voyager-DE™ (Delayed Extraction®) Technology. • **Continuous**—Use for diagnostic purposes. For more information, see Appendix H, Continuous Extraction Mode.
Polarity Type	Specifies the polarity of spectra to acquire: • Positive • Negative

continued

Table 5-6 Instrument Mode Parameters (Continued)

Parameter	Description
Laser Type	Specifies the type of installed laser: • Internal • External (optional) ***NOTE:*** *When you switch from the internal laser to the external laser, you must manually reset the external laser before acquiring data. See Section 2.9, Resetting the Optional External Laser.* ***NOTE:*** *When you save or load a .BIC file with the external laser specified, a message prompts you to reset the external laser, the Manual Laser/Sample Position control page (see Figure 4-8 on page 4-27) displays "External", and the laser intensity range is reset to the 0 to 4,000 range for the external laser.*

Laser Rate
NOTE: *This parameter is available only on systems with the appropriate hardware. For more information, contact Applied Biosystems.*
	Specifies the laser firing rate:
	• **Default**—The default firing rate (3 Hz).
	• **Optimized**—Fastest possible firing rate for:
	  • Digitizer used on the system
	  • Mass Range (described on page 5-19)
	  • Bin Size setting (described on page 5-29)
	Acqiris digitizers allow a laser firing rate of 20 Hz and are independent of Mass Range and Bin Size settings.
	To operate at the fastest laser firing rate when using other digitizers, increase the Bin Size or decrease the Mass Range. These parameters affect the Number of Data Points Digitized, which in turn affects the laser firing rate.
	If you switch to faster optimized laser firing rates, you may need to increase or decrease the laser intensity. Laser firing rate does not affect resolution. For optimum mass accuracy, use the same laser setting (optimized or default) for calibrants and unknowns.

continued

Table 5-6 Instrument Mode Parameters (Continued)

Parameter	Description
Laser Rate *(continued)*	***NOTE**: Laser firing rate does not have a linear relationship to Bin Size. For example, if an acquisition of 100 Shots/Spectrum takes 5 seconds with a Bin Size of 2 nsec, an acquisition of 100 Shots/Spectrum may not necessarily take 10 seconds with a Bin Size of 1 nsec. Changes in mass in the low mass range has a greater impact on laser firing rate than changes in mass in the high mass range.* For additional information on laser firing rates and digitizers, see Figure A-1 on page A-12.

Linear/Reflector Digitizer parameters

Click the **Linear** or **Reflector Digitizer** tab to display the Linear Digitizer or Reflector Digitizer page (Figure 5-5).

NOTE: *The Reflector Digitizer tab is not displayed on the Voyager-DE system.*

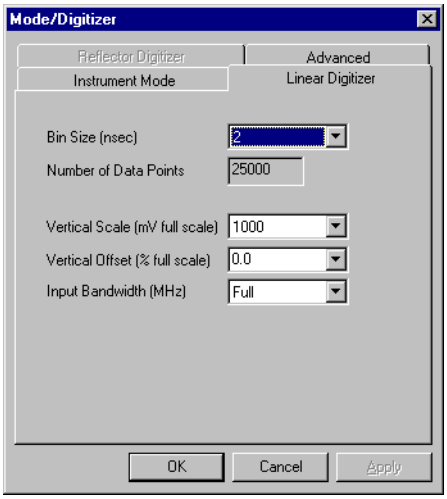


Figure 5-5 Mode/Digitizer Settings Dialog Box with Linear Digitizer Tab Displayed

Linear and Reflector Digitizer parameters are described in Table 5-7.

Table 5-7 Linear and Reflector Digitizer Parameters

Parameter	Description
Bin Size (nsec)	Determines the time interval (nanoseconds) between subsequent acquired data points (see Figure 5-17 on page 5-59). Use this setting to optimize resolution. Bin Size and Number of Data Points Digitized are dependent values (larger Bin Size settings typically yield a lower Number of Data Points Digitized). The Bin Size settings available on your system depend on the sampling rate of your digitizer. Default is 2 nsec. For more information, see Section 5.3.5, Understanding Digitizer Settings.
Number of Data Points Digitized	Displays the number of data points that the digitizer will sample (based on Bin Size setting and Mass Range), which has a relative impact on laser firing rate. A higher number of data points results in a lower laser firing rate. Number of Data Points Digitized is dependent on Bin Size Setting (larger Bin Size settings typically yield a lower Number of Data Points Digitized). ***NOTE:*** *On LeCroy digitizers, a larger Bin Size setting can yield a larger Number of Data Points Digitized (caused by hardware control restrictions). However, the actual number of data points saved in the data file will be smaller in proportion to the larger Bin Size setting, as described in "Effects of adjusting Bin Size" on page 5-59.* For more information, see Section 5.3.5, Understanding Digitizer Settings. ***NOTE:*** *The relationship between Number of Data Digitized and the laser firing rate is not linear.* *The actual number of data points saved in the data file may be less than the Number of Data Points Digitized (depends on your digitizer).*

continued

Table 5-7 Linear and Reflector Digitizer Parameters (Continued)

Parameter	Description
Vertical Scale	Specifies the input range of the digitizer in millivolts. To take full advantage of the dynamic range of the digitizer, set the Vertical Scale high enough to allow full vertical resolution of the signal intensity. Choices depend on the digitizer model. For more information, see Section 5.3.5, Understanding Digitizer Settings.
Vertical Offset	Applies a y-offset to the signal to bring the baseline closer to zero counts on the y-axis. This makes data processing easier. Vertical Offset is a percentage of the selected Vertical Scale. For example, with a 200 mV Vertical Scale, a 1% offset is 2 mV. Enter or select an offset. Valid entries depend on digitizer model. For more information, see Section 5.3.5, Understanding Digitizer Settings.
Input Bandwidth (not available with Signatec digitizers)	Reduces higher-frequency noise when analyzing wide (high-mass) peaks. Choices depend on the digitizer model. In general, use: • **Full**—For no filtering • **Higher settings**—For high-resolution data such as small and large peptides • **Lower settings**—For high mass proteins, for noisy peaks, or to apply more filtering For more information, see Section 5.3.5, Understanding Digitizer Settings.

Advanced parameters

Click the **Advanced** tab to display the Advanced page (Figure 5-6).

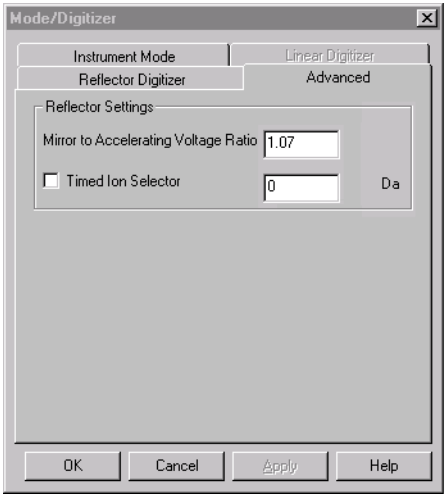


Figure 5-6 Mode/Digitizer Settings Dialog Box with Advanced Tab Displayed

Advanced parameters are described in Table 5-8.

Table 5-8 Advanced Parameters

Parameter	Description
Mirror to Accelerating Voltage Ratio (not available in Linear mode)	Specifies the ratio between the Mirror Voltage and the Accelerating Voltage in Reflector mode, to adjust the voltage of the mirror so that it is slightly higher than the Accelerating Voltage. A higher voltage is needed at the mirror to reflect ions. If the voltage at the mirror is equal to the Accelerating Voltage, ions will pass the mirror. To reflect intact ions, the Mirror to Accelerating Voltage Ratio must be close to or greater than 1. Maximum value is: 30,000V / Accelerating Voltage ***NOTE:*** *This parameter influences the tuning characteristics and default calibration of the instrument. Do not change this setting unless instructed to do so by an Applied Biosystems Technical Representative. Changing this value significantly alters the optimum settings of Grid Voltage% and Delay Time.* For information on the voltage applied to the mirror in PSD mode, See Section 8.2, Overview of PSD Analysis.
Timed Ion Selector (with mass)	Allows analysis of the mass of interest by deflecting other ions. This parameter is typically only used in PSD mode, but is available in Reflector mode for advanced applications. See Section 8.2, Overview of PSD Analysis, and Section 8.6.2, Observing the Effects of Precursor Ion Selector, for more information. ***NOTE:*** *The Timed Ion Selector in Reflector mode is the same parameter as the Precursor Ion Selector in PSD mode. If you change the mass for Timed Ion Selector in Reflector mode, the Precursor mass used by the Precursor Ion Selector in PSD mode also changes.*

5.2.3 Automatic Control Dialog Box

On the Instrument Settings control page (see Figure 5-3 on page 5-15), select **Automatic Control** mode, then click the **Automatic Control** button to display the Automatic Control dialog box (Figure 5-7).

NOTE: *The Automatic Control button is dimmed if Automatic Control mode is not selected.*

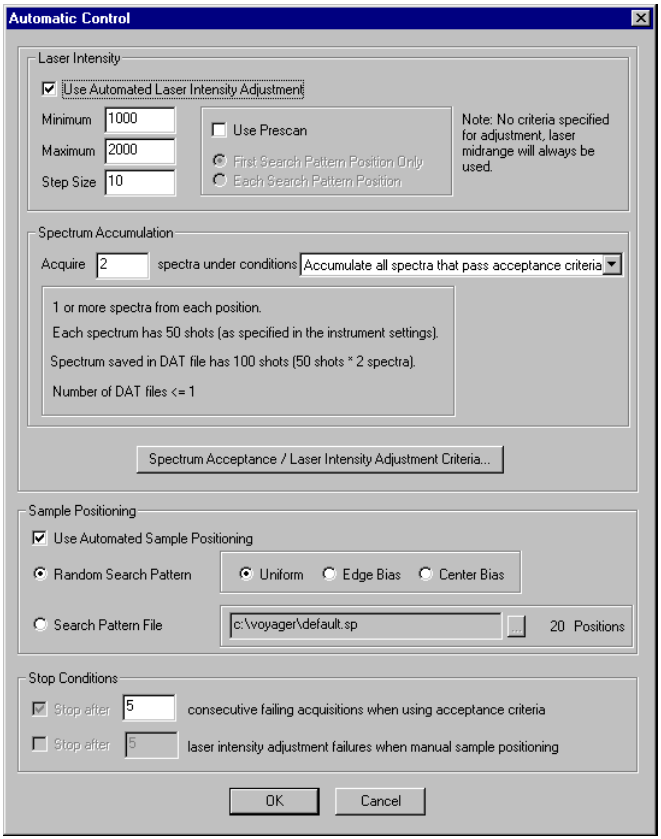


Figure 5-7 Automatic Control Dialog Box

Automatic Control parameters are described in Table 5-9 through Table 5-11. For information on setting Instrument Settings for Automatic Control mode, see Section 6.6.2, Setting Instrument Settings for Automatic Control Mode.

Table 5-9 Automatic Control Parameters—Laser

Parameter	Description
Laser Intensity Adjustment	
Use Automated Laser Intensity Adjustment	Enables or disables automated laser intensity adjustment. For more information, see “Laser” on page 6-39.
Minimum and Maximum	Controls the minimum and maximum laser intensity when Automated Laser Intensity Adjustment is enabled. To specify a fixed setting, use the same values for Min and Max. ***NOTE:*** *To automate laser adjustment, you must specify adjustment criteria as described in “Setting spectrum acceptance and laser adjustment criteria” on page 6-42. If you do not specify adjustment criteria, the laser is not adjusted. The mid-range setting between the minimum and maximum is used.*
Step Size	Sets the size of the increments the system uses when automatically adjusting the laser intensity. Maximum step size cannot exceed the difference between the minimum and maximum laser intensity settings.
Use Prescan	Enables or disables Prescan mode. Prescan mode determines the laser setting (within the specified range) needed to meet the Signal Intensity criteria. There are two Prescan options: • First Search Pattern Position Only • Each Search Pattern Position For more information, see “Prescan mode” on page 6-58.

Table 5-10 Automatic Control Parameters—Spectrum Accumulation

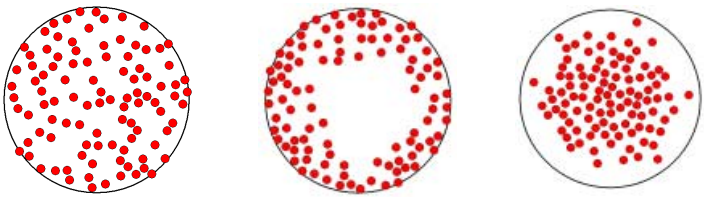
Parameter	Description
Spectrum Accumulation	
Number to Acquire	Specifies the number of spectra to save or accumulate. This field is dependent on the selected Spectrum Accumulation option (described on page 5-39). If you select Save First Spectrum to Pass Acceptance Criteria, the number to acquire is restricted to 1. If you select Save All Spectra or Save All Spectra that Pass Acceptance Criteria Save Conditions (which save individual multiple spectra in one data file as described below), the recommended maximum Number to Acquire is 500. Acquiring more than 500 individual spectra in one file generates a very large data file that can be slow to open and process.
Spectrum Accumulation	The following Spectrum Accumulation conditions create one data file that contains **multiple** spectra: • Save all spectra • Save all spectra that pass acceptance criteria If you select either of these conditions, the recommended maximum Number of spectra to acquire is 500. Acquiring more than 500 individual spectra in one file generates a very large data file that can be slow to open and process. You can display the Chromatogram window in the Data Explorer software for .DAT files containing multiple spectra. For more information, see Section 5.2.4, Description of Spectrum Accumulation Options.

continued

Table 5-10 Automatic Control Parameters—Spectrum Accumulation (Continued)

Parameter	Description
Spectrum Accumulation *(continued)*	The following Spectrum Accumulation conditions create one data file that contains **one** spectrum: • Save the first spectrum to pass acceptance criteria • Save the best spectrum • Accumulate all • Accumulate all passing For more information, see Section 5.2.4, Description of Spectrum Accumulation Options.
Spectrum Acceptance Criteria	Click to display Spectrum Acceptance Criteria parameters. See “Setting spectrum acceptance and laser adjustment criteria” on page 6-42.

Table 5-11 Automatic Control Parameters—Sample Positioning

Parameter	Description
Sample Positioning	
Use Automated Sample Positioning	Enables or disables automated sample positioning. For more information, see “Sample positioning” on page 6-41.
Random Search Pattern	Sets the software to randomly determine search pattern positions as data is acquired. Options include: • **Uniform**—Samples from positions evenly distributed across a sample position. • **Edge Bias**—Samples from the perimeter of a sample position. • **Center Bias**—Samples from the center of a sample position.  Uniform Edge Bias Center Bias If you specify Random Search Pattern and specify Acceptance Criteria, you must specify a Stop Condition. Each time you acquire data using the same .BIC file with Random Search Pattern selected, the software starts random sampling at an x,y position different from the last acquisition.

continued

Table 5-11 Automatic Control Parameters—Sample Positioning (Continued)

Parameter	Description
Search Pattern File	Determines the search pattern used when Use Automated Sample Positioning is enabled. See Section 6.6.4, Search Patterns, for more information. ***NOTE:*** *Before using search pattern files created in version 4 software, convert to version 5 format. See Section 5.5, Converting Version 4 Methods and Search Pattern Files.*
Number of Positions	Displays the number of positions to be collected in the currently selected search pattern file.
Stop Conditions	Enable Stop Conditions if you want acquisition to stop after a specified consecutive number of: • Acquisitions fail acceptance criteria • Laser adjustments do not yield acceptable data ***NOTE:*** *You must specify a Stop Condition if you have selected Resolution in Spectrum Acceptance Criteria parameters (described on page 6-42).*

5.2.4 Description of Spectrum Accumulation Options

This section describes the following Spectrum Accumulation options available in the Automatic Control dialog box (described on page 5-33):

- Save all spectra
- Save all spectra that pass acceptance criteria
- Save the first spectrum to pass acceptance criteria
- Save the best spectrum
- Accumulate all spectra
- Accumulate all spectra that pass acceptance criteria

Save all spectra

In Save All Spectra mode (Figure 5-8):

- All spectra are saved.
- One spectrum per search pattern position is acquired (if the Use Automated Sample Positioning option is disabled, acquisition is performed on the positions you manually select instead of search pattern positions).
- All search pattern positions may not be analyzed.
- Acceptance criteria are not used.
- One .DAT file containing multiple spectra is created.

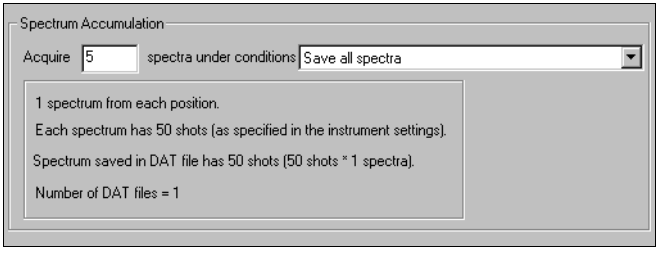


Figure 5-8 Save All Spectra Mode— Automatic Control Dialog Box

Examples

If Random search pattern is selected and Number to Acquire is 5, five positions are analyzed, and one .DAT file containing five spectra is created.

If an .SP file with 20 positions is selected and Number to Acquire is 5, five positions are analyzed, and one .DAT file containing five spectra is created.

If an .SP file with 20 positions is selected and Number to Acquire is 25, 20 positions are analyzed, and one .DAT file containing 20 spectra is created.

Save all spectra that pass acceptance criteria

In Save All Spectra That Pass Acceptance Criteria mode (Figure 5-9):

- Each spectrum that meets the specified acceptance criteria is saved.
- Acquisition is performed on the same search pattern position until Acceptance Criteria fail (if the Use Automated Sample Positioning option is disabled, acquisition is performed on the positions you manually select instead of search pattern positions).
- Acquisition continues until the Number of Spectra to Acquire have been acquired, or the end of the search pattern is reached.
- All search pattern positions may not be analyzed.
- One .DAT file containing one or more spectra is created. If no spectra pass acceptance criteria, no data file is saved.

NOTE: *In this mode, acquisition moves to the next search pattern position only if acceptance criteria fail.*

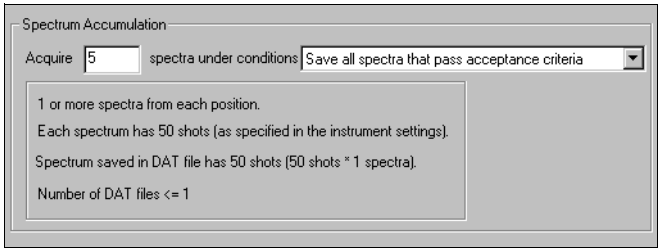


Figure 5-9 Save All Spectra Mode That Pass Acceptance Criteria Mode— Automatic Control Dialog Box

Examples If Random search pattern is selected and Number to Acquire is 5, the number of positions analyzed depends on whether or not Acceptance Criteria fail (acquires from same search pattern position until Acceptance Criteria fail) and on how many times the system must acquire until it obtains five spectra that pass acceptance criteria. One .DAT file containing five spectra is created.

NOTE: *If you set the Stop After X Consecutive Failing Acquisitions stop condition, acquisition stops when the specified number of failing acquisitions is reached, regardless of the specified number of spectra to acquire or the number of search pattern positions in the .SP file.*

If an .SP file with 7 positions is selected and the Number to Acquire is 5, the number of positions analyzed is determined by whether or not acceptance criteria fail:

- If all fail, seven positions are analyzed (total number of positions in the .SP file) and no .DAT file is created.
- If the first position fails, but all remaining pass, the first two positions are analyzed, and one .DAT file containing five spectra is created.
- If the first and second positions fail, but all remaining pass, the first three positions are analyzed, one .DAT file containing five spectra is created, and so on.
- If acceptance criteria never fail, only the first position is analyzed, and one .DAT file containing five spectra is created.

NOTE: *If you set the Stop After X Consecutive Failing Acquisitions stop condition, acquisition stops when the specified number of failing acquisitions is reached, regardless of the specified number of spectra to acquire or the number of search pattern positions in the .SP file.*

Save the first spectrum to pass acceptance criteria

In Save the First Spectrum To Pass Acceptance Criteria mode (Figure 5-10):

- The first spectrum that meets the selected acceptance criteria is saved.
- Acquisition is performed on each search pattern position until a spectrum passes or until the end of the search pattern is reached (if the Use Automated Sample Positioning option is disabled, acquisition is performed on the positions you manually select instead of search pattern positions).
- All search pattern positions may not be analyzed.
- One .DAT file containing one spectrum is created. If no spectra pass acceptance criteria, no data file is saved.

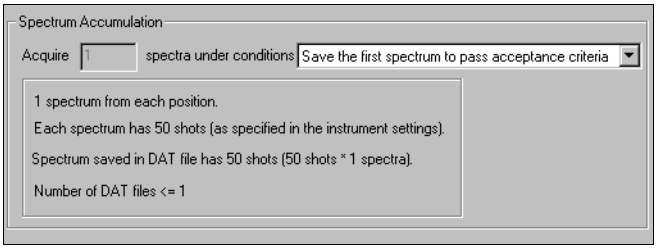


Figure 5-10 Save the First Spectrum To Pass Acceptance Criteria Mode— Automatic Control Dialog Box

Example If Random search pattern or an .SP file is selected, the Number to Acquire is automatically set to 1, and the number of positions analyzed is equal to the position number in which the first passing spectrum is found. One .DAT file containing one spectrum is created. If no spectra meet criteria, no .DAT file is created.

NOTE: *If you set the Stop After X Consecutive Failing Acquisitions stop condition, acquisition stops when the specified number of failing acquisitions is reached, regardless of the specified number of spectra to acquire or the number of search pattern positions in the .SP file.*

Save the best spectrum

In Save the Best Spectrum mode (Figure 5-11):

- Automatic Sample Positioning is required.
- Acquisition is performed on each search pattern position until the specified number of spectra to acquire is reached.

NOTE: *In Save the Best Spectrum mode, acquisition moves to the next search pattern position for every acquisition, even if acceptance criteria pass.*

- All Acceptance Criteria are evaluated.
- If the first spectrum passes all acceptance criteria, it is displayed in the Spectrum window as an accumulated trace and considered the current “best” spectrum.
- When a subsequent spectrum that passes all acceptance criteria is acquired, its Signal-to-Noise result is compared to the Signal-to-Noise result of the current “best” spectrum. If the result is higher, the new spectrum replaces the current “best” spectrum in the accumulated trace. If the result is lower, the new spectrum is discarded.
- After all positions have been analyzed, one .DAT file containing the current “best” spectrum displayed in the accumulated trace is created. If no spectra pass acceptance criteria, no .DAT file is created.

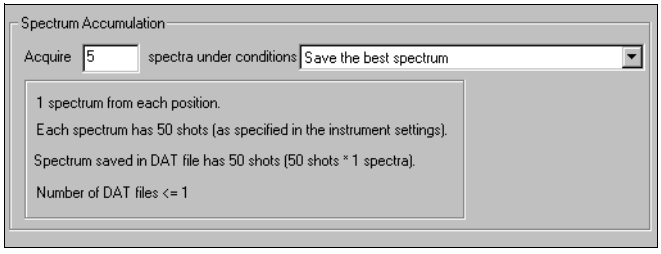


Figure 5-11 Save the Best Spectrum Mode— Automatic Control Dialog Box

Example If Random search pattern or an .SP file is selected and Number to Acquire is 5, the number of positions analyzed is five. One .DAT file containing the spectrum with the highest Signal-to-Noise result is created. If no spectra meet criteria, no .DAT file is created.

NOTE: *If you set the Stop After X Consecutive Failing Acquisitions stop condition, acquisition stops when the specified number of failing acquisitions is reached, regardless of the specified number of spectra to acquire or the number of search pattern positions in the .SP file.*

Accumulate all spectra

In Accumulate All Spectra mode (Figure 5-12):

- One spectrum is acquired from a search pattern position (if the Use Automated Sample Positioning option is disabled, acquisition is performed on the positions you manually select instead of search pattern positions).
- The number of search pattern positions analyzed (and therefore the number of spectra accumulated) is equal to the Number of Spectra to Acquire, even if the number of positions in the search pattern is larger.
- All search pattern positions may not be analyzed.
- Acceptance criteria are not used.
- All spectra acquired are accumulated into one .DAT file that contains one spectrum.

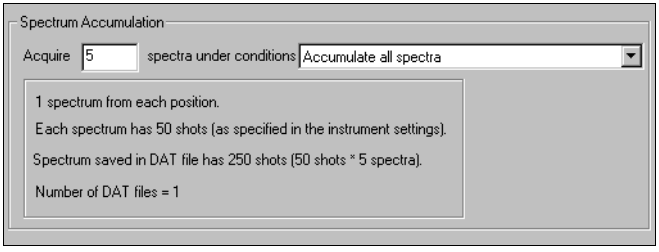


Figure 5-12 Accumulate All Spectra Mode—Automatic Control Dialog Box

Examples

If Random search pattern is selected and the Number to Acquire is 5, five positions are analyzed, and one .DAT file containing one accumulated spectrum is created.

If an .SP file with 20 positions is selected and Number to Acquire is 25, 20 positions are analyzed, and one .DAT file containing one accumulated spectrum is created.

For more information, see Section 6.6.7, Process that Occurs when Accumulating Spectra from Multiple Search Pattern Positions.

Accumulate all spectra that pass acceptance criteria

In Accumulate All Spectra That Pass Acceptance Criteria mode (Figure 5-13):

- Acquisition is performed on the same search pattern position until Acceptance Criteria fail (if the Use Automated Sample Positioning option is disabled, acquisition is performed on the positions you manually select instead of search pattern positions).
- Acquisition continues until the Number of Spectra to Acquire have been acquired.
- All search pattern positions may not be analyzed.
- Each spectrum that meets the specified acceptance criteria is accumulated into one .DAT file that contains one spectrum. If no spectra pass acceptance criteria, no .DAT file is created.

NOTE: *In this mode, acquisition moves to the next search pattern position only if acceptance criteria fail.*

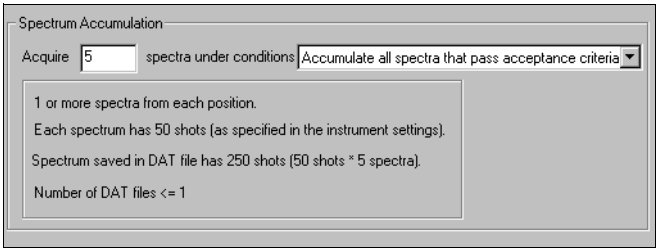


Figure 5-13 Accumulate All Spectra That Pass Acceptance Criteria Mode— Automatic Control Dialog Box

Example If Random search pattern is selected and Number to Acquire is 5, the number of positions analyzed depends on whether or not Acceptance Criteria fail (acquires from same search pattern position until Acceptance Criteria fail) and on how many times the system must acquire until it obtains five spectra that pass acceptance criteria. One .DAT file containing one accumulated spectrum is created.

NOTE: *If you set the Stop After X Consecutive Failing Acquisitions stop condition, acquisition stops when the specified number of failing acquisitions is reached, regardless of the specified number of spectra to acquire or the number of search pattern positions in the .SP file.*

If an .SP file with 7 positions is selected and Number to Acquire is 5, the number of positions analyzed is determined by whether or not acceptance criteria fail:

- If all fail, seven positions are analyzed (total number of positions in .SP), no .DAT file is created.
- If the first position fails, but all remaining pass, the first two positions are analyzed, one .DAT file containing one accumulated spectrum is created.
- If the first and second positions fail, but all remaining pass, the first three positions are analyzed, one .DAT file containing one accumulated spectrum is created, and so on.
- If acceptance criteria never fail, the first search pattern position is analyzed and one .DAT file containing one accumulated spectrum is created.

NOTE: *If you set the Stop After X Consecutive Failing Acquisitions stop condition, acquisition stops when the specified number of failing acquisitions is reached, regardless of the specified number of spectra to acquire or the number of search pattern positions in the .SP file.*

For more information, see Section 6.6.7, Process that Occurs when Accumulating Spectra from Multiple Search Pattern Positions.

5.3 Impact of Changing Instrument Settings Parameters

This section includes:

- Summary of parameters
- Understanding Grid Voltage%
- Understanding Delay Time
- Understanding Guide Wire Voltage%
- Understanding Digitizer settings

5.3.1 Summary of Parameters

Optimizing parameters in a specific order

Changing instrument settings parameters can impact the sensitivity, resolution, or signal-to noise ratio in different ways. For optimum performance, optimize parameters in the order listed in:

- Section 5.4.3, Optimizing Resolution
- Section 5.4.4, Optimizing Signal-to-Noise Ratio

List of parameters

The following table lists the impact of changing these parameters.

Parameter	Mode	Impact
Delay Time (nanoseconds)	Linear/ Reflector	Impact dependent on Grid Voltage%. Performance drops off if set too high or too low for corresponding Grid Voltage%. May vary with matrix. Critical parameter with optimum values for maximum resolution.

continued

Parameter	Mode	Impact
Guide Wire Voltage% ***NOTE:*** *Guide Wire Voltage% is not available on Voyager-DE STR models with serial number 4154 and later.*	Linear/ Reflector	Decreasing improves resolution. Increasing improves sensitivity for higher mass.
Grid Voltage%	Linear/ Reflector	Critical parameter with optimum value for maximum resolution.
Digitizer Bin Size (nanoseconds)	Linear/ Reflector	Decreasing improves resolution. ***NOTE:*** *Decreasing Bin Size increases file size.*
Digitizer Input Bandwidth (not available with Signatec digitizers)	Linear/ Reflector	Decreasing may improve signal-to-noise ratio. Increasing may improve the resolution.
Accelerating Voltage	Linear	Increasing improves sensitivity and resolution, but is limited by other factors such as the digitization rate.
	Reflector	Decreasing to 10,000 to 15,000 V can improve resolution for compounds <2,000 Da.
Laser intensity	Linear/ Reflector	Increasing improves signal-to-noise ratio. Increasing too high increases fragmentation, and resolution is sacrificed due to saturation. Decreasing too low decreases sensitivity.
Shots/ Spectrum	Linear/ Reflector	Increasing improves signal-to-noise ratio.

5.3.2 Understanding Grid Voltage%

NOTE: *You must calibrate the mass scale for each Grid Voltage% you use. See Data Explorer Software User's Guide, Section 5.3.2, Manually Calibrating, for more information.*

Grid Voltage% works in conjunction with Accelerating Voltage (described in Section 5.4.4.2, Setting Accelerating Voltage) to define an adjustable potential gradient or electric field in the ionization region of the ion source. This adjustable potential gradient can be fine-tuned to obtain optimum performance:

- In Delayed Extraction mode, you must fine-tune Grid Voltage% in conjunction with Delay Time to optimize resolution. See Section 5.4.3, Optimizing Resolution.
- In PSD mode, you can use higher Grid Voltage% settings than the optimum Reflector mode settings. Higher Grid Voltage% improves fragment ion resolution and the resolution at the position of the Precursor Ion Selector (see Figure 8-20 on page 8-74). The standard .BIC files provided on your system for PSD mode include Grid Voltage% settings that are approximately 5 percent higher than the Grid Voltage% settings in the .BIC files provided for Reflector mode. For more information, see Chapter 8, PSD Analysis.

  For more information on Grid Voltage%, see:

  - Section 5.4.3.5, Optimizing Grid Voltage%
  - "Grid Voltage%" on page 5-17

Potential gradient

The potential gradient in the ionization region (Figure 5-14) is determined by the voltages applied to the sample plate (Accelerating Voltage) and the variable-voltage grid (Grid Voltage%).

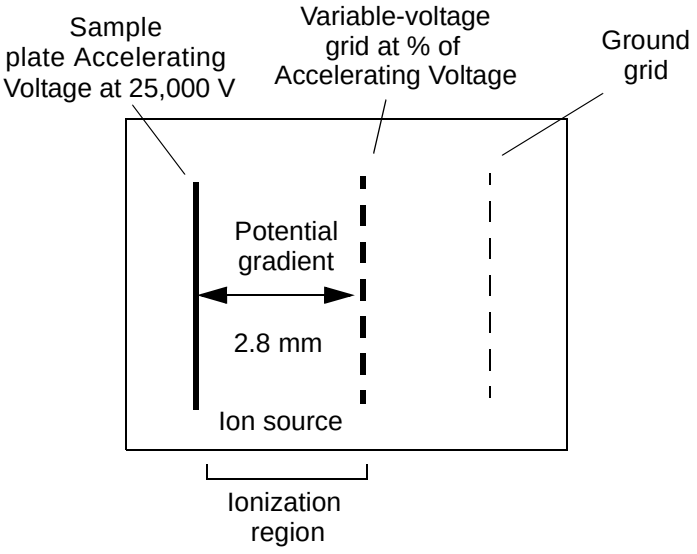


Figure 5-14 Sample Plate and Grids

$$\text{Potential gradient} = \frac{\text{Accelerating Voltage} - \text{Grid Voltage}}{\text{Distance between plate and grid}}$$

$$\text{Grid voltage} = (\text{Grid Voltage\%})(\text{Accelerating Voltage})$$

For example (Figure 5-14), with a 25,000 V Accelerating Voltage and a Grid Voltage of 56%, the potential gradient is:

$$\text{Potential gradient} = \frac{25{,}000 - 14{,}000\ \text{V}}{2.8\ \text{mm}}$$

$$= 11{,}000\ \text{V} / 2.8\ \text{mm}$$

$$= 3{,}928\ \text{V} / \text{mm}$$

Vary the potential gradient by varying the Grid Voltage% and use the recommended Accelerating Voltage for the mass range. For more information, see Section 5.4.4.2, Setting Accelerating Voltage.

Maximum allowed potential difference

The Voyager Instrument Control Panel and electronics allow a maximum absolute potential difference of 10,000 V between the Accelerating Voltage and the voltage applied to the grid (Grid Voltage%).

You can calculate the potential difference using the following equation:

Accelerating Voltage × (100 – Grid Voltage% / 100)

Grid Voltage%, in conjunction with Delay Time, has a direct impact on resolution. See Section 5.1, Loading, Modifying, and Saving Instrument Settings, for more information.

Adjusting

In general:

- A higher Grid Voltage with a lower potential difference, decreases ion fragmentation.
- Grid Voltage% has a large impact on the quality of data obtained. For each Grid Voltage% setting, there is an optimum Delay Time.

5.3.3 Understanding Delay Time

Delay Time is the time in nanoseconds after the laser ionizes the sample at which full Accelerating Voltage is applied. This creates the potential gradient that accelerates ions. Delay Time corrects the dependence of ion flight time on initial velocity. Observed mass resolution increases in proportion to the effective length of the ion flight path.

NOTE: *There is an inherent 180 ±5 nsec delay between the time the laser fires and the time the voltage is applied. The actual time that voltage is applied is equal to the Delay Time you enter plus the inherent delay on your system. Changing the Delay Time in increments smaller than 20 nsec may have no significant impact on resolution.*

Figure 5-15 illustrates Delay Time.

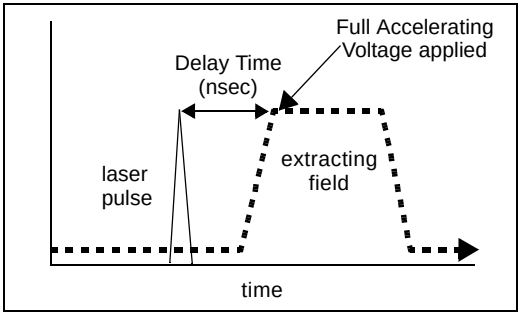


Figure 5-15 Delay Time

Relationship to Grid Voltage%

Delay Time and Grid Voltage% are interactive parameters. For each Grid Voltage% there is an optimum Delay Time, and for each Delay Time there is an optimum Grid Voltage%.

The best approach for optimizing Delay Time is to leave the Grid Voltage% at a fixed value, and optimize Delay Time until you obtain optimum resolution. For more information, see Section 5.4.3.4, Optimizing Delay Time.

Adjusting

In general:

- Higher masses require a longer Delay Time
- Delay Time is affected by matrix

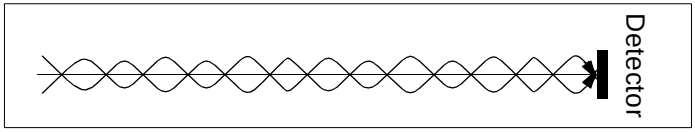
For more information on Delay Time, see:

- Section 5.4.3.4, Optimizing Delay Time
- “Delay Time (nsec)” on page 5-18

5.3.4 Understanding Guide Wire Voltage%

NOTE: *Guide Wire Voltage% is not available on Voyager-DE STR models with serial number 4154 and later.*

By applying voltage to the beam guide wire (Figure 5-16), you can overcome the dispersion effect from the source and refocus ions on the detector.



Guide Wire Voltage applied,
ions focused on detector

Figure 5-16 Beam Guide Wire

All models In general:

- Increase Guide Wire Voltage to increase sensitivity.
- Decrease Guide Wire Voltage to increase resolution. A higher Beam Guide Wire voltage can negatively impact resolution, because it is focusing ions that would not otherwise be reaching the detector.

  For more information, see “Guide Wire Voltage%” on page 5-18.

Linear mode

- For Linear mode analysis of peptides in the 1,000 to 2,000 Da range, a setting of 0.05 to 0.1 percent is adequate.
- For Linear mode analysis of ions over 20,000 Da range, start with a setting of 0.3 percent and decrease as needed.
- Settings below 0.02 percent may not give adequate sensitivity.

Reflector mode

- To obtain maximum resolution in Reflector mode for isotopically resolved species, set the Guide Wire% to 0.
- To increase sensitivity in Reflector mode, increase the Guide Wire Voltage% to:
  - Up to 0.02% for <5,000 Da
  - Up to 0.05% for >10,000 Da

PSD mode

In PSD mode, use settings between 0.005 and 0.02 percent. For more information, see Chapter 8, PSD Analysis.

5.3.5 Understanding Digitizer Settings

This section describes:

- Bin Size
- Vertical digitizer settings

For more information on default digitizer settings, see “Mode/Digitizer Dialog Box” on page 5-24.

5.3.5.1 Bin Size

The Bin Size (nanoseconds) parameter is the sampling rate of the digitizer, which is equal to the time interval between subsequent data points. For information, see “Effects of adjusting Bin Size” on page 5-59.

The default Bin Size is 2 nsec.

Decreasing for improved resolution

To decrease the Bin Size:

1. In the Instrument Settings control page, click **Mode/Digitizer**.
2. Click **Linear** or **Reflector Digitizer**.

3. Change the Bin Size to improve resolution as needed:

Mode	Digitizer Bin Size
Linear	1 nsec ***NOTE:*** *A Bin Size setting below 1 nsec does not typically improve observed mass resolution in Linear mode*
Reflector	0.5 or 1 nsec

NOTE: *Setting the Bin Size lower than the default calculated by the system may result in a larger data file size.*

Effects of adjusting Bin Size

A smaller Bin Size (lower number of nanoseconds):

- Increases resolution between peaks when peaks are narrow in time width (see Figure 5-17).
- Increases the size of the data file because the mass range is recorded with finer sampling and therefore increases the number of data points collected.

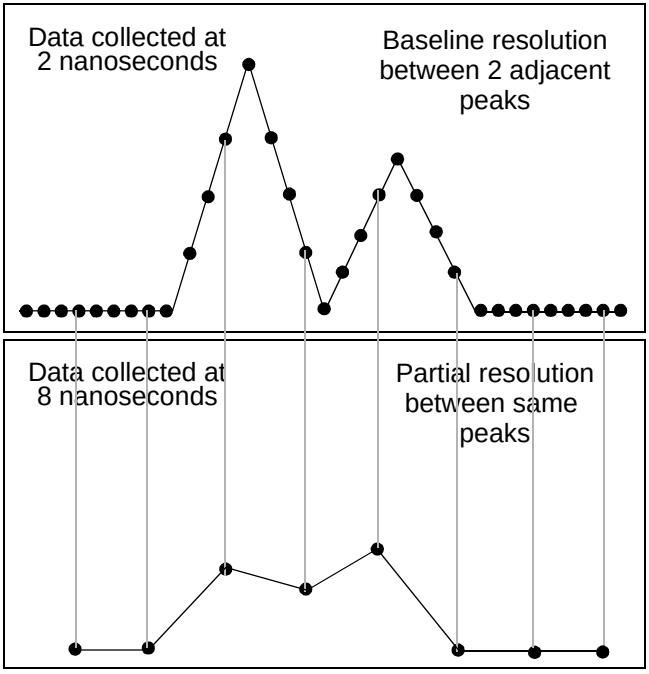


Figure 5-17 Effect of Bin Size (nsec) on Peak Resolution and Data Quality

A larger Bin Size (higher number of nanoseconds):

- Decreases resolution between peaks when peaks are narrow in time width (see Figure 5-17).
- Decreases the size of the data file because the mass range is recorded with coarser sampling (decreases the number of data points collected).

5.3.5.2 Vertical Digitizer Settings

This section describes:

- Vertical settings
- Vertical Scale
- Vertical Offset
- Input Bandwidth

Vertical settings

The digitizer settings include three vertical parameters that affect the signal acquired:

- **Vertical Scale**—Adjusts the dynamic range of the digitizer scale to accommodate the signal you are acquiring. For more information, see:
  - “Vertical Scale” on page 5-30
  - “Vertical Scale” on page 5-60
- **Vertical Offset**—Applies a Y-Offset to the signal. For more information, see:
  - “Vertical Offset” on page 5-30
  - “Vertical Offset” on page 5-62
- **Input Bandwidth** (not available with Signatec digitizers)—Reduces higher frequency noise. For more information, see:
  - “Input Bandwidth (not available with Signatec digitizers)” on page 5-30
  - “Input Bandwidth (not available with Signatec digitizers)” on page 5-63

Vertical Scale

A correct Vertical Scale setting ensures optimum vertical dynamic range for the acquired data (Figure 5-18). If the Vertical Scale is set incorrectly, the following occurs:

- **Too high**—Decreases the dynamic range of the data.
- **Too low**—Truncates the tops of peaks, decreases observed mass resolution, and distorts the relative distribution of ion abundances in multicomponent mixtures.

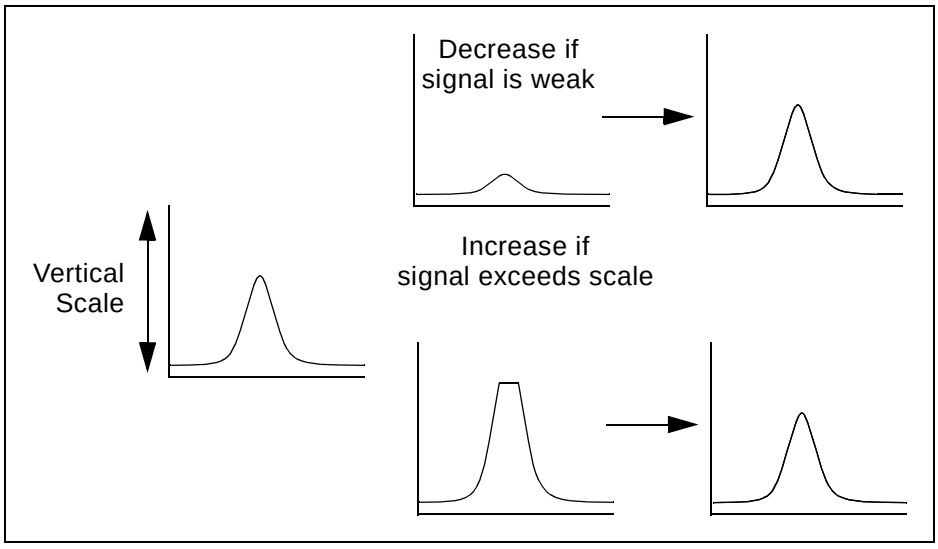


Figure 5-18 Effect of Vertical Scale in Digitizer Settings

Suggested settings

Use the settings listed below as a starting point for Vertical Scale. Adjust as needed to bring the signal into the proper range.

Mass Range (Da)	Vertical Scale (mV)
0–10,000	1,000
10,000–50,000	200
>50,000	50*

* Not available on the Signatec digitizer.

When to increase

Increase the Vertical Scale if signal goes offscale (Figure 5-18).The Vertical Scale setting is typically between 50 mV and 1,000 mV. If you set it at 1,000 mV (the maximum Vertical Scale setting) and signal is offscale, decrease the laser intensity to decrease the signal intensity. The offscale signal exceeds 64,000 counts (see the right hand axis).

When to decrease

Decrease the Vertical Scale if signal intensity of tallest peak is not greater than approximately 20 percent of the vertical maximum (Figure 5-18). If you are at the minimum Vertical Scale setting and signal is not greater than approximately 20 percent of the vertical maximum, follow the guidelines in Section 5.4.4, Optimizing Signal-to-Noise Ratio, to increase signal intensity.

Vertical Offset

The Vertical Offset allows fine adjustment of the spectrum baseline position. It is specified as a percentage of the Vertical Scale.

For most applications, leave the Vertical Offset at 0%.

Figure 5-19 illustrates the effect of setting a positive or negative offset. A positive offset shifts the baseline up. A negative offset shifts the baseline down.

NOTE: *If you set a Negative Vertical Offset, some low-intensity noise signal may not be collected in the data file. Some processing functions, for example Baseline Correction or Noise Filter, require complete collection of noise signal to generate valid results.*

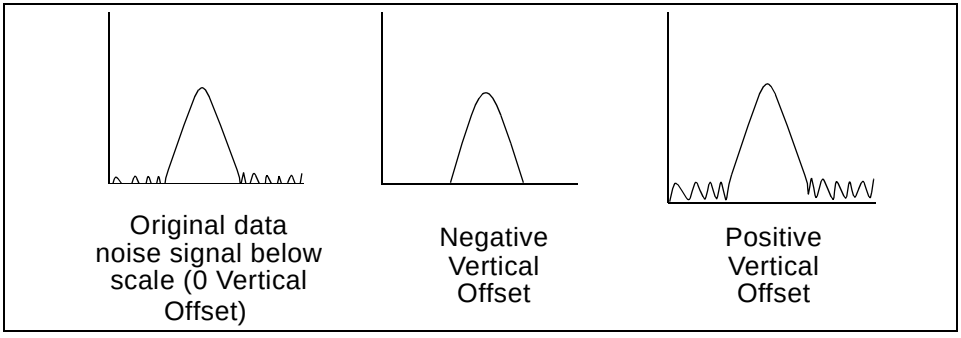


Figure 5-19 Effect of Vertical Offset in Digitizer Settings

Input Bandwidth (not available with Signatec digitizers)

Decreasing the Input Bandwidth decreases the response time of the detector, and can reduce higher frequency noise. A lower setting can improve resolution and smooth out a baseline, but may also decrease signal-to-noise ratio.

Because the Input Bandwidth is an electronic analog filter, it may slightly shift peak centroids toward higher masses relative to peaks recorded with Full Bandwidth.

Suggested settings

Use the settings listed below as a starting point for Input Bandwidth. Adjust as needed to eliminate noise.

NOTE: *To maintain signal-to-noise ratio, use a lower Input Bandwidth setting.*

Mass Range (Da)	Input Bandwidth		
	Linear Mode	**Reflector Mode**	**PSD Mode**
0–500	Full	Full	200–250 MHz or 20–25 MHz on PSD segments below 0.3 Mirror Ratio
500–6,000	200–250 MHz	Full	
6,000–50,000	200–250 MHz	200–250 MHz	
>50,000	20–25 MHz	20–25 MHz	

NOTE: *Settings below 200 in Linear mode may not allow resolution of isotopes.*

5.4 Optimizing Instrument Settings Parameters

In this section

This section describes:

- Optimization strategy
- Determining the laser setting
- Optimizing resolution
- Optimizing signal-to-noise ratio

Before you begin

Before optimizing instrument settings parameters, be familiar with the information in:

- *Data Explorer Software User's Guide,* Appendix B, Overview of Isotopes
- Section 5.1.2, Standard Instrument Settings (.BIC) Files Provided
- Section 5.1.3, Opening and Viewing Instrument Settings
- Section 5.1.5, Saving and Printing Instrument Settings
- Section 6.1, Before You Begin
- Section 6.3, Obtaining Good Spectra in Delayed Extraction Mode

5.4.1 Optimization Strategy

This section gives a suggested approach for optimizing instrument settings. For details on changing instrument settings, see Section 5.1.4, Modifying an Instrument Settings File (.BIC).

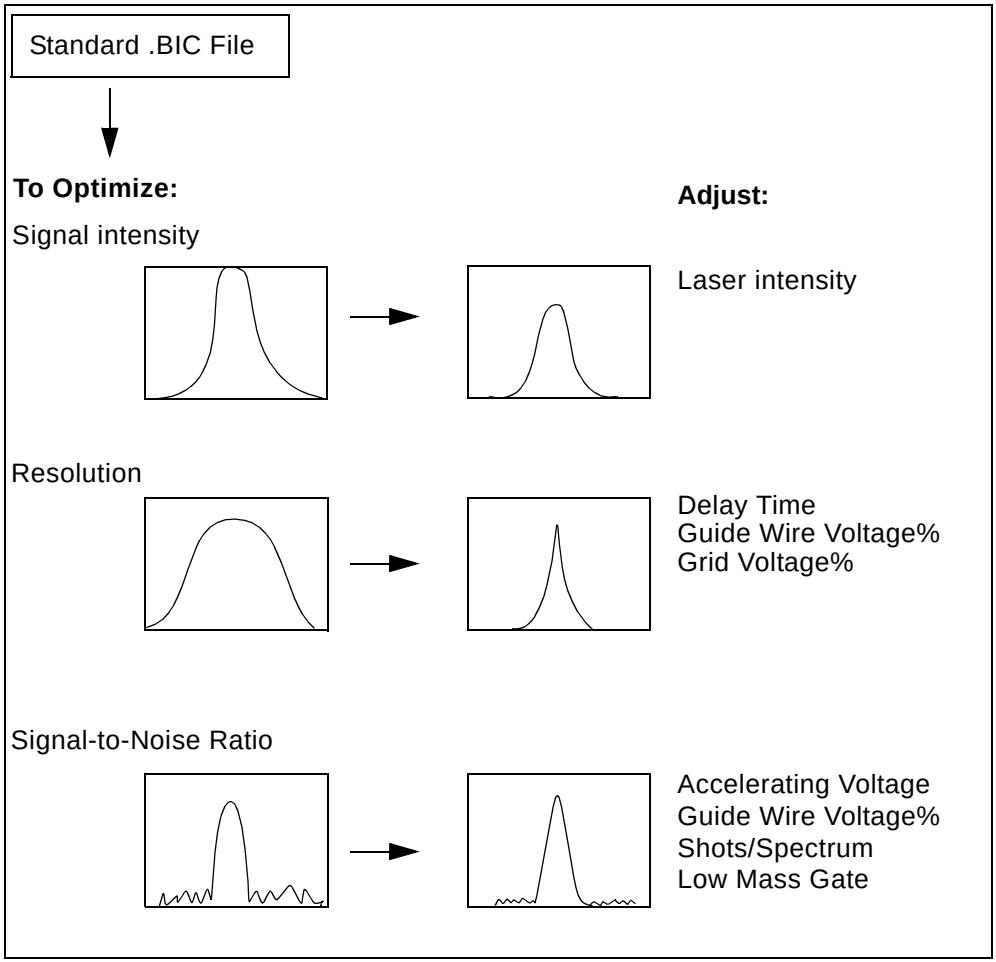


Figure 5-20 Strategy for Optimizing Instrument Settings

Strategy To optimize instrument settings, do the following:

1. Open a .BIC file for the mass range you are analyzing. For information on mass ranges in .BIC files, see Section 5.1.2, Standard Instrument Settings (.BIC) Files Provided.

   If a .BIC file for the mass range you are analyzing does not exist, open a standard .BIC file with the closest higher mass.

   Hint: *To optimize a wide mass range, select a .BIC file with the mass of the highest component in the range.*

   ---

2. Find an adequate laser setting. See Section 5.4.2, Determining the Laser Setting.
3. Optimize resolution. See Section 5.4.3, Optimizing Resolution.
4. Optimize signal-to-noise ratio. See Section 5.4.4, Optimizing Signal-to-Noise Ratio.

Remaining parameters in the standard instrument settings provided are optimized for your workstation and should not require adjustment.

For more information on the remaining instrument settings parameters, see Section 5.2, Instrument Settings Parameter Descriptions.

5.4.2 Determining the Laser Setting

This section includes:

- Overview
- Laser intensity and matrix
- Adjusting laser intensity
- Signal saturation

Overview

Adjust laser intensity as described below to obtain a setting that allows you to optimize resolution and signal-to-noise ratio, as described in the following sections. See Section 6.3.2, Laser Intensity, for more information.

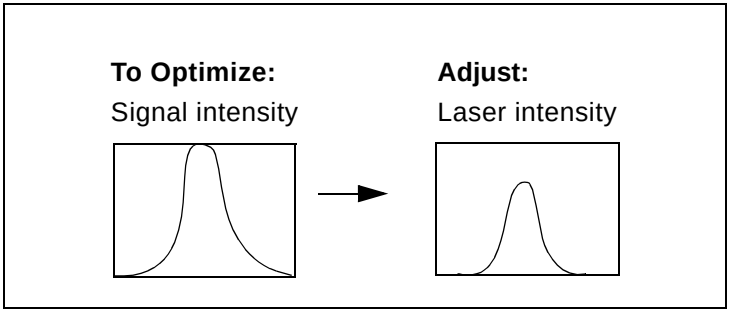


Figure 5-21 Optimizing Signal Intensity

Laser intensity and matrix

Optimum laser intensity is sample- and matrix-dependent. You can obtain an estimate of the starting intensity for an unknown sample by analyzing a standard in the same mass range using the same matrix.

The order of needed laser intensity (from low to high) for each matrix is as follows:

- α-cyano-4-hydroxycinnamic acid
- Sinapinic acid
- 2,5-Dihydroxybenzoic acid
- 3-Hydroxypicolinic acid

Adjusting laser intensity

To efficiently optimize the laser setting, increase or decrease the laser settings to the mid-setting of a continuously decreasing laser window. To adjust the laser settings, click-drag the slider bar on the Manual Laser Intensity/Sample Positioning control page. For more information, see “Manually adjusting laser intensity” on page 4-28.

Adjusting laser intensity depends on the following:

If you are using...	Then...
A .BIC file for the same mass range and matrix.	Laser intensity may require only minor adjustments.
A .BIC file for the same mass range, but different matrix.	Increase or decrease the laser in 50 to 100-step increments (or decrements) following the strategy described below. For more information, see “Selecting a .BIC file” on page 5-8, and “Laser intensity and matrix” on page 5-67.
Default Instrument Control Panel settings. The mid-range default laser setting (if a .BIC file is not loaded) is approximately 1,800.	This setting is typically too high for many samples and matrix. Decrease the laser in 50 to 100-step decrements, following the strategy described below. For more information, see “Laser intensity and matrix” on page 5-67.

General strategy

As a general strategy for adjusting laser intensity:

- Start at the default setting for your system (approximately 1,800), or the setting in the loaded .BIC file.
- If the spectrum you obtain is not acceptable, increase or decrease the laser in 50 to 100-step increments or decrements and reacquire. For more information on acceptable laser signal, see “Signal saturation” on page 5-69.

- If the spectrum you obtain is not acceptable, increase or decrease the laser again in 50 to 100-step increments or decrements and reacquire.

  If you pass the optimum laser setting, increase or decrease using a setting that is midway between the previous two step increments or decrements.

Signal saturation

If the laser intensity is too high, the signal may be saturated (Figure 5-22). A saturated peak can be determined by:

Display	Signal is saturated if it exceeds
Spectrum window	64,000 counts
Oscilloscope (in 1 V full scale range)	5 screen-grid-line divisions in the 200 mV mode
	10 screen-grid-line divisions in the 100 mV mode

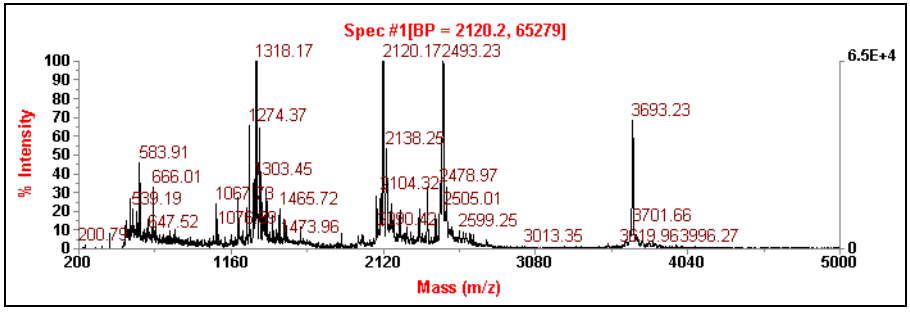


Figure 5-22 Signal Saturated at High Laser Intensity

NOTE: *Saturated signal in any region of the spectrum may suppress peaks in the Mass Range of interest.*

Decreasing the laser should optimize the signal (Figure 5-23).

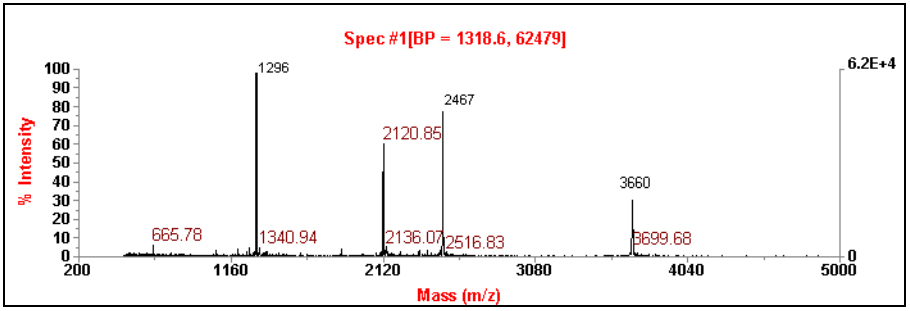


Figure 5-23 Signal Acceptable at Lower Laser Intensity

To optimize laser intensity, continue acquiring and adjusting the laser intensity (if necessary) until you observe:

- Acceptable peaks (no saturation)
- Moderate resolution
- Signal-to-noise ratio of approximately 50:1

NOTE: *By default, the right axis is set to Display Relative mode, which means it updates to reflect the intensity of the most intense peak in the currently displayed region of the trace. You can set the right axis to display an absolute value if desired. For more information, see "Accessing graphic options" on page 4-22.*

5.4.3 Optimizing Resolution

This section includes:

- Overview
- Acceptable resolution in Delayed Extraction Mode
- Optimizing Guide Wire Voltage%
- Optimizing Delay Time
- Optimizing Grid Voltage%

For information on measuring resolution, see Section 6.5.2, Calculating Mass Resolution, and *Data Explorer Software User's Guide*, Section 6.3, Using the Mass Resolution Calculator.

Manually accumulating spectra to improve resolution

The manual accumulation feature of the Voyager system allows you to acquire a spectrum, examine the spectrum for signal quality (visually, or using the Resolution or Signal-to-Noise Ratio calculator), and then accumulate the spectrum with other previously examined spectra, or discard the spectrum. This process of accumulating only acceptable spectra allows you to improve the resolution of the final spectrum you save. For more information on manually accumulating spectra, see Section 6.2.2, Manually Accumulating Spectra from Multiple Acquisitions.

5.4.3.1 Overview

This section includes:

- Parameters to adjust
- DE parameters
- Relationship between DE parameters
- Readjusting the laser after optimization

Parameters to adjust

You can set the following parameters to optimize resolution (Figure 5-24):

- Guide Wire Voltage%
- Delay Time
- Grid Voltage%

The following sections give guidelines for setting these parameters.

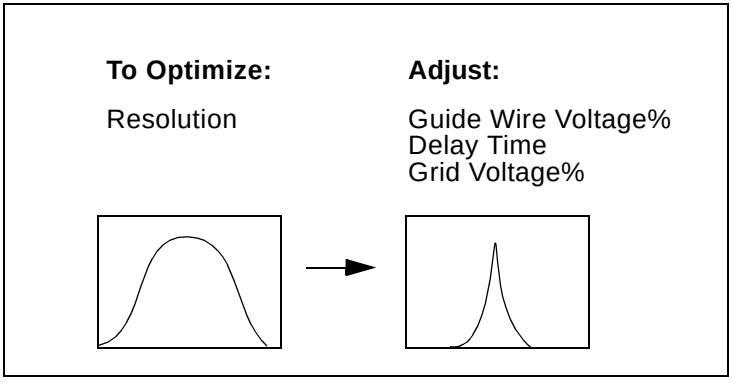


Figure 5-24 Optimizing Resolution

5

DE parameters

Improved resolution in Delayed Extraction technology is achieved by velocity-focusing ions. See “Velocity focusing” on page 1-15, for more information.

Two instrument settings parameters affect the velocity-focusing of ions in Delayed Extraction:

- **Delay Time**—Time in nanoseconds (after the laser ionizes the sample) at which full Accelerating Voltage is applied, creating the potential gradient that accelerates ions.
- **Grid Voltage%**—Voltage applied to the variable-voltage grid located above the sample plate. Grid Voltage is a percentage of Accelerating Voltage.

For a list of Delay Time and Grid Voltage% pairs, see Appendix E, Grid Voltage% and Delay Time Settings. Optimum resolution for a given mass range is obtained by finding the ideal Delay Time and Grid Voltage% combination for the mass range.

NOTE: *Although Guide Wire Voltage% does not affect velocity focusing of ions, it has a strong impact on resolution in Reflector mode and sensitivity in all modes. See Section 5.4.3.3, Optimizing Guide Wire Voltage%.*

Relationship between DE parameters

Delay Time and Grid Voltage% are interactive parameters. For each Delay Time there is an optimum Grid Voltage%, and for each Grid Voltage% there is an optimum Delay Time.

Linear mode

For each optimum Delay Time and Grid Voltage% combination, there is a sharp optimum in Linear mode performance. Over a wider mass range, performance is less optimized.

Reflector mode

For each optimum Delay Time and Grid Voltage% combination, the mass range across which resolution is optimized is wider in Reflector mode.

Effects of mass and matrix

Note the following:

- At a fixed Delay Time, higher masses require a lower Grid Voltage%. See Appendix E, Grid Voltage% and Delay Time Settings, for a graphic representation of the relationship between mass, Grid Voltage%, and Delay Time.
- At a fixed Grid Voltage%, higher masses require a higher Delay Time. See Appendix E, Grid Voltage% and Delay Time Settings, for a graphic representation of the relationship between mass, Grid Voltage%, and Delay Time.
- Optimum tuning of Delay Time and Grid Voltage% is slightly dependent on matrix due to the different initial velocities associated with different matrixes. The optimum Delay Time may be different, within 100 nsec at the same Grid Voltage%, for the same sample in different matrixes.

NOTE: *The Grid Voltage% setting in Reflector mode is typically significantly lower than the setting in Linear mode for the same mass range. For example, if Grid Voltage% in Linear mode is 94%, Grid Voltage% in Reflector mode may be 70%. (These values are used as an example they may not be optimal for your system.)*

Readjusting the laser after optimization

After you have optimized Guide Wire Voltage%, Delay Time, and Grid Voltage for optimum resolution, you can further fine-tune by adjusting the laser intensity. For information on adjusting the laser intensity, see “Adjusting laser intensity” on page 5-68.

5.4.3.2 Acceptable Resolution in Delayed Extraction Mode

Isotope resolution

You should be able to partially resolve isotopes up to the following masses:

- **Linear mode**—Approximately 2,000 Da
- **Reflector mode**—Approximately 6,000 Da

Guidelines for acceptable resolution

Acceptable resolution is determined by the mass range you are analyzing. Refer to the following tables for the typical resolution at a given signal-to-noise ratio for different mass ranges:

Mass Range	Typical Resolution* in Linear Mode	Signal-to-Noise Ratio**
1,000–2,000	2,000 or greater	50:1 or greater
2,000–20,000	Determined by width of isotopic envelope	50:1 or greater
>20,000	Sample-dependent	20:1 or greater

* On Voyager-DE STR models, resolution is approximately 30 percent higher than the values listed.

** Required signal-to-noise ratio to obtain the resolution stated.

Mass Range	Typical Resolution* in Reflector Mode	Signal-to-Noise Ratio**
500–1,500	5,000 or greater	50:1 or greater
1,500–6,000	7,000 or greater	50:1 or greater
6,000–15,000	Determined by width of isotopic envelope	20:1 or greater
>15,000	Sample-dependent	20:1 or greater

* On Voyager-DE STR models, resolution is approximately 30 percent higher than the values listed.

** Required signal-to-noise ratio to obtain the resolution stated.

If you cannot obtain the resolution listed for the mass range of interest, consider the following possible causes:

- Laser intensity is too high
- Sample oxidized, fresh sample needed
- Sample does not ionize well in the matrix
- Sample is too dilute or too concentrated
- Impurities are suppressing ionization of sample
- There are heterogeneous components in the peak

5.4.3.3 Optimizing Guide Wire Voltage%

NOTE: *Guide Wire Voltage% is not available on Voyager-DE STR models with serial number 4154 and later.*

Start with the .BIC file in which you optimized Delay Time or Grid Voltage%.

Use the following Guide Wire% settings to optimize resolution:

Mode	Guide Wire Voltage% Setting
Linear	Peptides (<10,000 Da)—0.05% Proteins—0.1% or higher for best sensitivity
Reflector	0–0.005%, or as low as possible

NOTE: *Decreasing Guide Wire% to these suggested values may decrease signal-to-noise ratio.*

For more information

For more information, see:

- Section 5.4.4.3, Setting Guide Wire Voltage%
- Section 5.3.4, Understanding Guide Wire Voltage%

5.4.3.4 Optimizing Delay Time

This section includes:

- Overview
- Inherent Delay Time offset
- Optimizing Delay Time
- Setting Delay Time to resolve isotopes across a broad mass range

Overview

Use this procedure when operating in Linear mode or Reflector mode. Start with a standard .BIC file, optimize the Delay Time, and leave the Grid Voltage% unchanged.

Before optimizing, read “Setting Delay Time to resolve isotopes across a broad mass range” on page 5-81.

NOTE: *You can alternatively follow the procedure in Section 5.4.3.5, Optimizing Grid Voltage%. However, optimizing Delay Time is a simpler procedure.*

For more information

For more information, see Section 5.3.3, Understanding Delay Time.

Inherent Delay Time offset

There is an inherent 180±5 nsec delay between the time the laser fires and the time the voltage is applied. The actual time that voltage is applied is equal to the Delay Time you enter plus the inherent delay on your system. Changing the Delay Time in increments smaller than 20 nsec may have no significant impact on resolution.

Optimizing Delay Time

NOTE: *If you are analyzing a broad mass range and need optimum resolution in all mass regions, you may need to acquire smaller portions of the mass range and set the Delay Time to optimize resolution for each mass range.*

To optimize Delay Time:

1. Open a standard .BIC file for the mass range you are acquiring. For more information, see “Selecting a .BIC file” on page 5-8.
2. Acquire a spectrum and observe the resolution. For information, see Section 6.5.2, Calculating Mass Resolution.

   If the resolution is not acceptable, increase the Delay Time by 100 nsec (Linear mode) or 50 nsec (Reflector mode), acquire a spectrum and observe. Leave the Grid Voltage% setting unchanged.

   If the signal is saturated at the higher Delay Time setting, decrease the laser intensity and acquire a new spectrum. If the signal decreases at the higher Delay Time setting, increase the laser and acquire a new spectrum.

Hint: *If you are analyzing a mixture and the resolution of the peak of interest is not acceptable, observe the resolution of a higher mass peak and a lower mass peak. If resolution is better on the higher mass peak, decrease the Delay Time (by 100 nsec for Linear mode or 50 nsec for Reflector mode). If resolution is better on the lower mass peak, increase the Delay Time (by 100 nsec for Linear mode or 50 nsec for Reflector mode). Collect a spectrum and observe. Leave the Grid Voltage% setting unchanged.*

3. If the resolution improves by at least 20 percent (±10 percent), continue increasing the Delay Time in 100 nsec for Linear mode, or 50 nsec for Reflector mode increments. Table 5-12 through Table 5-14 list valid Delay Time settings for different systems and mass ranges.

   If the resolution does not improve, decrease the Delay Time (by 100 nsec for Linear mode, or 50 nsec for Reflector mode), acquire a new spectrum and observe.

Table 5-12 Voyager-DE, Voyager-DE PRO, and Voyager-DE STR Linear Mode—Delay Time and Grid Voltage% Values

Mass Range (Da)	Linear Mode	
	Delay Time (nsec)	Grid Voltage%
500–2,000	50–150	90–95
2,000–10,000	50–400	90–95
10,000–20,000	200–500	90–95
20,000–100,000	400–1,000	90–95
>100,000	1,000–2,000	90–95

Table 5-13 Voyager-DE PRO Reflector Mode—Delay Time and Grid Voltage% Values

Mass Range (Da)	Reflector Mode	
	Delay Time (nsec)	Grid Voltage%
500–2,000	50–200	72–78

Table 5-13 Voyager-DE PRO Reflector Mode—Delay Time and Grid Voltage% Values (Continued)

Mass Range (Da)	Reflector Mode	
	Delay Time (nsec)	Grid Voltage%
2,000–10,000	100–500	72–78
10,000–100,000	300–600	72–78
>100,000	No data available	No data available

Table 5-14 Voyager-DE STR Reflector Mode—Delay Time and Grid Voltage% Values

Mass Range (Da)	Reflector Mode	
	Delay Time (nsec)	Grid Voltage%
500–2,000	50–100	62–72
2,000–10,000	50–500	62–72
10,000–20,000	200–700	62–72
20,000–100,000	500–1,000	62–72
>100,000	No data available	No data available

NOTE: *Changing the Delay Time in increments smaller than 20 nsec may have no significant impact on resolution. For information, see “Inherent Delay Time offset” on page 5-77.*

4. Continue increasing (or decreasing) the Delay Time in 100 nsec for Linear mode, or 50 nsec for Reflector mode increments (or decrements) until optimum resolution is obtained.

Hint: *If you obtain close to acceptable resolution at one setting, but less acceptable resolution at the next setting, you may have adjusted the Delay Time too far. Increase or decrease in smaller increments until you obtain optimum resolution.*

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5. If resolution is still not optimized after you adjust the Delay Time setting, adjust the Grid Voltage% as described in Section 5.4.3.5, Optimizing Grid Voltage%. Reload the standard .BIC file you started with, and use the Delay Time value provided in the .BIC file.
6. If you are analyzing samples below 2,000 Da, you may be able to improve resolution by adjusting the Accelerating Voltage:
   - **Linear mode**—Decrease from 20,000 V to 15,000 V to increase flight times.
   - **Reflector mode**—Decrease to 15,000 V, 10,000 V, or 5,000 V. However, these lower settings will compromise sensitivity.
7. Save the .BIC file.

Setting Delay Time to resolve isotopes across a broad mass range

To resolve isotopes across a broad mass range, select a Delay Time setting that yields optimum resolution at the higher end of the mass range. Isotopes at lower masses can be resolved at lower resolution. Selecting a Delay Time that maximizes resolution at lower masses may not resolve isotopes at higher masses.

When selecting a Delay Time to resolve isotopes across a broad mass range, resolution and signal intensity at the lower end of the mass range may be compromised, but you should observe isotopic resolution across the entire mass range.

5.4.3.5 Optimizing Grid Voltage%

Overview

You can use this procedure as an alternative to the procedure in Section 5.4.3.4, Optimizing Delay Time. However, optimizing Delay Time is the recommended procedure. Optimizing the Delay Time ensures maximum stability of the high voltage power supplies, and therefore results in better mass accuracy.

You can use this procedure when operating in Linear mode or Reflector mode. Start with a standard .BIC file, optimize the Grid Voltage%, and leave the Delay Time unchanged.

NOTE: *The Grid Voltage% setting in Reflector mode is typically significantly lower than the setting in Linear mode for the same mass range. For example, if Grid Voltage% in Linear mode is 94%, Grid Voltage% in Reflector mode may be 70%. (These values are used as an example they may not be optimal for your system.)*

For more information

For more information, see Section 5.3.2, Understanding Grid Voltage%.

Optimizing Grid Voltage%

To optimize Grid Voltage%:

1. Open a standard .BIC file for the mass range you are acquiring. For more information, see “Selecting a .BIC file” on page 5-8.

2. Acquire a spectrum and observe the resolution. For more information, see Section 6.5.2, Calculating Mass Resolution.

   If the resolution is not acceptable, increase the Grid Voltage% (by 0.25 percent for Linear mode, or 0.5 percent for Reflector mode), acquire a spectrum and observe. Leave the Delay Time setting unchanged.

   If the signal is saturated at the higher Grid Voltage% setting, decrease the laser and acquire a new spectrum. If the signal decreases at the higher Grid Voltage% setting, increase the laser and acquire a new spectrum.

   Hint: *If you are analyzing a mixture and the resolution on the peak of interest is not acceptable, observe the resolution of a higher mass peak and a lower mass peak. If resolution is better on the higher mass peak, increase the Grid Voltage% setting (by 0.25 percent for Linear mode, or 0.5 percent for Reflector mode). If resolution is better on the lower mass peak, decrease the Grid Voltage% setting (by 0.25 percent for Linear mode, or 0.5 percent for Reflector mode). Collect a spectrum and observe. Leave the Delay Time setting unchanged.*

   ---

3. If the resolution improves by at least 20 percent (you can see fluctuations in resolution of up to 10 percent with the same settings), continue increasing the Grid Voltage% (in 0.25 percent for Linear mode, or 0.5 percent for Reflector mode) increments. Table 5-12 on page 5-79 through Table 5-14 on page 5-80 list valid Grid Voltage% settings for different systems and mass ranges.

If the resolution does not improve, decrease the Grid Voltage% (by 0.25 percent for Linear mode, or 0.5 percent for Reflector mode), acquire a new spectrum and observe.

4. Continue increasing (or decreasing) the Grid Voltage% in increments (or decrements) of 0.25 percent (Linear mode) or 0.5 percent (Reflector mode) until optimum resolution is obtained.

Hint: *If you obtain close to acceptable resolution at one setting, but less acceptable resolution at the next setting, you may have adjusted the Grid Voltage% too far. Increase or decrease in small increments until you obtain optimum resolution.*

---

5. Adjust the Guide Wire Voltage as needed:

   - Increase the setting to increase sensitivity for higher masses.
   - For Voyager-DE PRO, and Voyager-DE STR models in Reflector mode, adjust the Guide Wire between 0 and 0.04 percent.

   NOTE: *Guide Wire Voltage% is not available on Voyager-DE STR models with serial number 4154 and later.*

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6. If you are analyzing peptides below 2,000 Da, you may be able to improve resolution by adjusting the Accelerating Voltage:

   - **Linear mode**—Decrease from 20,000 V to 15,000 V to increase flight times.
   - **Reflector mode**—Decrease to 15,000 V, 10,000 V, or 5,000 V. However, these lower settings will compromise sensitivity.

7. Save the .BIC file.

5.4.4 Optimizing Signal-to-Noise Ratio

This section includes:

- Overview
- Setting Accelerating Voltage
- Setting Guide Wire Voltage%
- Setting Shots/Spectrum
- Setting Low Mass Gate

For more information on measuring Signal-to-Noise, see Section 6.5.3, Calculating Signal-to-Noise Ratio, and the *Data Explorer Software User's Guide*, Section 6.4, Using the Signal-to-Noise Ratio Calculator.

Manually accumulating spectra to improve signal-to-noise ratio

The manual accumulation feature of the Voyager system allows you to acquire a spectrum, examine the spectrum for signal quality (visually, or using the Resolution or Signal-to-Noise Ratio calculator), and then accumulate the spectrum with other previously examined spectra, or discard the spectrum. This process of accumulating only acceptable spectra allows you to improve the signal-to noise ratio of the final spectrum you save. For more information on manually accumulating spectra, see Section 6.2.2, Manually Accumulating Spectra from Multiple Acquisitions.

5.4.4.1 Overview

You can set the following parameters to optimize signal-to-noise ratio (Figure 5-25):

- Accelerating Voltage
- Guide Wire Voltage%
- Shots per Spectrum
- Low Mass Gate

The following sections give guidelines for setting these parameters.

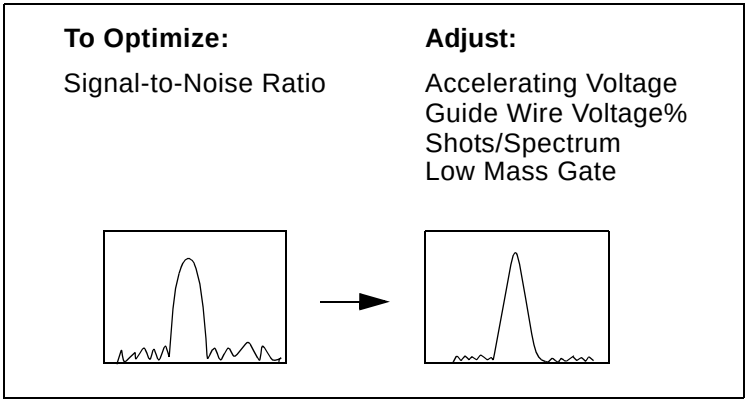


Figure 5-25 Optimizing Signal-to-Noise Ratio

Readjusting the laser after optimization

After you have optimized Accelerating Voltage, Guide Wire Voltage%, and Shots/Spectrum for optimum signal-to-noise, you can further fine-tune by adjusting the laser intensity. For information on adjusting the laser intensity, see “Adjusting laser intensity” on page 5-68.

5.4.4.2 Setting Accelerating Voltage

Overview

Accelerating Voltage defines the energy of ions as they travel in the flight tube and reach the detector. Efficiency of detection, particularly for high mass ions, increases with higher ion energy. Therefore, the maximum Accelerating Voltage typically yields optimum performance when analyzing masses above 10,000 Da. However, a lower Accelerating Voltage can increase flight times, and can improve resolution in spectra.

NOTE: *The calibration of the mass scale changes significantly when you change the Accelerating Voltage. Default calibration adjusts for these changes. However, you will observe more accurate calibration if you use an external calibration (.CAL) file generated with the same Accelerating Voltage. See the Data Explorer Software User's Guide, Section 5.3.2, Manually Calibrating, for more information.*

Recommended setting

Higher masses decrease detector sensitivity. To overcome this effect, set the Accelerating Voltage appropriate for the mass range you are analyzing:

Mass Range (Da)	Accelerating Voltage (V)
<1,000	15,000
1,000 to 10,000	20,000
>10,000	25,000

If you are analyzing very dilute samples, or a sample that ionizes poorly, use a setting of 25,000 V.

A lower Accelerating Voltage setting does the following:

- Provides more data points across a peak for better peak definition when analyzing low mass ions (Linear mode only).
- Improves resolution when analyzing compounds below 2,000 Da, and the resolution is limited by the digitization rate of the system (Reflector mode).

5.4.4.3 Setting Guide Wire Voltage%

NOTE: *Guide Wire Voltage% is not available on Voyager-DE STR models with serial number 4154 and later.*

To optimize sensitivity, you can adjust the Guide Wire Voltage%:

- **Linear mode**—Increase in increments of 0.01 percent until you see signal-to-noise ratio start to decrease.
- **Reflector mode**—Increase in increments of 0.002 percent. Settings above 0.05 percent do not typically improve signal-to-noise ratio.

NOTE: *In Reflector mode, Guide Wire Voltage% also affects mass resolution. To fine tune signal-to-noise ratio and resolution at the same time, adjust the Guide Wire Voltage% in increments smaller than 0.01 percent.*

For more information

For more information, see:

- Section 5.4.3.3, Optimizing Guide Wire Voltage%
- Section 5.3.4, Understanding Guide Wire Voltage%

5.4.4.4 Setting Shots/Spectrum

NOTE: *This parameter is dimmed if the system is set to Single Shot mode. For information, see page 2-44.*

Overview

A higher number of Shots/Spectrum can improve signal-to-noise ratio, and also improve the dynamic range of the acquisition. However, acquiring a higher number of Shots/Spectrum increases acquisition time.

When random noise is present in a spectrum, the improvement in signal-to-noise ratio is approximately proportional to the square root of the number of Shots/Spectrum taken.

Manually accumulating to override limit

The maximum number of Shots/Spectrum is determined by the digitizer on your system (see “Shots/Spectrum” on page 5-19). You can acquire a greater number of Shots/Spectrum than the maximum number of Shots/Spectrum noted above by manually accumulating spectra. For more information, see Section 6.2.2, Manually Accumulating Spectra from Multiple Acquisitions.

5.4.4.5 Setting Low Mass Gate

Description

Low Mass Gate suppresses matrix peaks that can interfere with ion detection which can saturate the detector as laser intensity increases. This saturation effect is less pronounced on systems with Linear High Current Detectors. For serial numbers of systems that include High Current Detectors, see page 1-23 and page 1-35.

The detector is not turned on until ions greater in mass than the mass you enter pass the detector.

NOTE: *When Low Mass Gate is enabled, the mass range below the Low Mass Gate starting mass is still detected, but detector sensitivity below this mass is decreased by 100 to 1,000 fold. If strong matrix signal is present below the Low Mass Gate, you can use it for calibration if desired.*

When to use

Turn on Low Mass Gate when:

- Analyzing masses greater than 2,000–3,000 Da
- Matrix signal is more intense than the sample signal

Optimum setting for starting mass

You will need to experiment with the optimum setting for the starting mass (threshold) for Low Mass Gate. As a general guideline:

- If you are analyzing peptides in the 400 to 2,000 Da range, set the Low Mass Gate ~350 Da
- If you are analyzing peptides in the 2,000 to 8,000 Da range, set the Low Mass Gate ~500 Da
- If you are analyzing samples in the 10,000 to 30,000 Da range, set the Low Mass Gate ~3,000 Da

When not to use

Do not use Low Mass Gate if:

- You are analyzing peaks in the same mass range as matrix peaks
- You are using a matrix peak to calibrate

NOTE: *Low mass gate is automatically disabled when you change the instrument mode to PSD.*

Low Mass Gate spike

When Low Mass Gate is turned on, a characteristic spike may occur in the spectrum (Figure 5-26). This spike is typically only seen in Reflector mode.

NOTE: *Systems with high current detectors (Voyager-DE systems with serial number 1128 and later; Voyager-DE PRO systems with serial number 6007 and later; Voyager-DE STR systems with serial numbers 4112, 4113, and 4116 and later) do not typically exhibit this spike. Older systems (with a dual channel plate detector in Linear mode) may exhibit a 50 to 1,000 mV spike.*

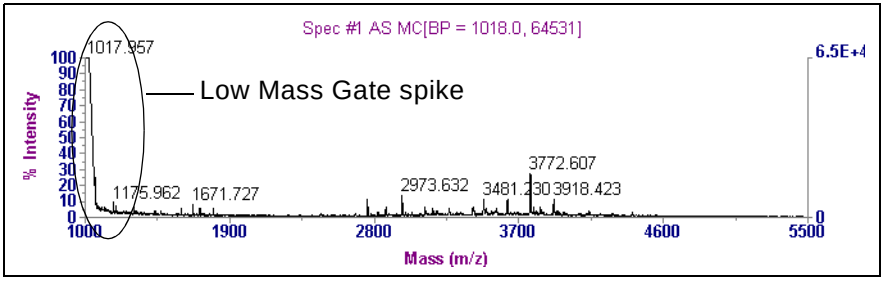


Figure 5-26 Low Mass Gate Spike

The spike occurs at a time that corresponds to just below the Mass for the Low Mass Gate entered in the .BIC file. For example, if the Mass is set to 400 Da, you would see the spike at approximately the time that corresponds to 370 Da.

You can identify the Low Mass Gate spike by its sharp lift-off, its narrow width, and the noise as it returns to baseline.

Eliminating Low Mass Gate spike when acquiring

You can eliminate the Low Mass Gate spike from the data by setting the Mass for acquisition slightly higher than Low Mass Gate Mass.

Eliminating Low Mass Gate spike after acquiring

You can eliminate the Low Mass Gate spike after you acquire by using the Truncate Spectrum function in the Data Explorer software. For information, see the *Data Explorer Software User's Guide*, Section 5.9, Truncating a Spectrum.

5.5 Converting Version 4 Methods and Search Pattern Files

To use method (.MNU) files and search pattern (.SP) files created in version 4.x of the Voyager software, convert them for use with the current version 5.x software using the Voyager 4.x File Converter. This utility:

- Converts .MNU files to instrument settings (.BIC) files.
- Inserts a Units=1 line in search pattern (.SP) files to indicate that units are Steps.

NOTE: *The convert function does not change values in the .SP file from step values to micron values. It inserts a line in the .SP file that identifies the values as step values.*

When the system runs a search pattern file, it checks for the presence of the Units=1 line:

- If the line is present, the software converts the step values to micron values, and displays position information in microns.
- If the line is not present, the software assumes the values are micron values, and uses the values as is.

NOTE: *If you use a search pattern file that contains step values, but has not been converted (that is, it does not include the Units=1 line) the software assumes the values in the file are microns, and a smaller area than expected in the sample position will be analyzed (1 step=3.175 microns).*

Method information that is converted

Autosampler parameters, Data Acquisition parameters, Digitizer/Laser parameters, and PSD parameters are converted from .MNU files to .BIC files. However, some version 4.x parameters have been renamed or relocated, and some parameters are not supported in Version 5.x.

Table 5-15 Method Information That Is Not Converted

Version 4.x Method Parameter Location	Parameter Changes in Version 5.x	Version 5.x Instrument Settings Parameter Location	Described in
Autosampler tab	Scans per position is replaced by Number of Spectra To Acquire.	Automatic Control Mode dialog box	Section 5.2.3, Automatic Control Dialog Box
	Total Scans is replaced by Shots/Spectrum.	Instrument Settings page	Section 5.2.1, Instrument Settings Page
	Acceptance Criteria Mass Range is replaced by Criteria Evaluation Mass Range.	Spectrum Acceptance Criteria dialog box	"Setting spectrum acceptance and laser adjustment criteria" on page 6-42
Data Acquisition tab	Instrument Range is replaced by Mass Range. Data File Range is replaced by Mass Range. Total Scans is replaced by Shots/Spectrum. Method Description is not supported.	Instrument Settings page	Section 5.2.1, Instrument Settings Page
	Instrument Mode is replaced by Operation Mode. Timed Ion Selector moved (same function as Precursor Ion Selector in PSD Acquisition Settings). Polarity moved. Extraction mode moved.	Mode/Digitizer Settings dialog box	Section 5.2.2, Mode/Digitizer Dialog Box
	Precursor Ion Selector now present (same function as Timed Ion Selector in Mode/Digitizer Settings). Precursor Ion Mass moved.	PSD Acquisition Settings page	Section 8.4.3, Setting PSD Acquisition Parameters for Manual Mode

continued

Table 5-15 Method Information That Is Not Converted (Continued)

Version 4.x Method Parameter Location	Parameter Changes in Version 5.x	Version 5.x Instrument Settings Parameter Location	Described in
Digitizer/ Laser tab	All parameters moved.	Mode/Digitizer Settings dialog box	Section 5.2.2, Mode/Digitizer Dialog Box
	Step size is replaced by Small Manual Intensity Adjustment. This parameter is not part of Instrument Settings.	Hardware Configuration	Section 2.6, Hardware Configuration
Calibration tab	Automatically set to Default. If a matrix is specified in the .MNU, checks the matrix file during conversion, and if it is present in the matrix file, retains the matrix in the BIC. If the matrix specified in the .MNU is not present in the matrix file, the matrix in the .BIC is set to alpha-cyano.	Instrument Settings page	Section 5.2.1, Instrument Settings Page
	Automatic Calibration not supported within .BIC.	Sequence Control Panel, not in .BIC	Chapter 7, Acquiring Spectra from the Sequence Control Panel
Data Processing tab	Parameters not supported in instrument settings. Peak detection parameters set in Data Explorer software and stored in .SET file.	--------	--------
PSD tab	C1, C3, C4 Calibration Constants not supported. Values are calculated using the PSD calibration specified in PSD Acquisition Settings.	PSD Acquisition Settings page	Section 8.4.3, Setting PSD Acquisition Parameters for Manual Mode

NOTE: *All methods are converted to Manual Control mode. If you are converting an autosampler method, you must set the control mode to automatic, and respecify the .SP file after you convert the.MNU file to a .BIC file.*

Information that is not converted

Control mode is set to Manual for all files. The path for search pattern files is not maintained. Automatic Calibration parameters are not converted from .MNU files to .BIC files.

Calibration mode is automatically set to Default.

Data Processing parameters are not supported.

New parameter

A new parameter called Mirror To Accelerating Voltage Ratio is included on the Advanced tab of the Mode/Digitizer Settings dialog box in the .BIC file. This parameter was previously called HV Tune Ratio, and was not part of the .MNU file. You accessed this parameter in Tune Mode from the version 4.x Control Panel.

The following default values are set for Mirror To Accelerating Voltage Ratio:

- DE RP—1.06
- Elite—1.06
- DE STR—1.12
- DE PRO—1.2

NOTE: *This parameter influences the tuning characteristics and default calibration of the instrument. Do not change this setting unless instructed to do so by an Applied Biosystems Technical Representative. Changing this value significantly alters the optimum settings of Grid Voltage% and Delay Time.*

Before converting

Before converting, determine the methods and search pattern files that you want to convert. It is good practice to move the files you want to convert to a new directory (for example, a directory called “Converted”) for easy access. All converted files are placed in the same directory as the original files. Because converted search pattern files are given the same name as the original search pattern files, it is important to keep track of which search pattern files you have converted.

Converting

1. From the Tools menu, select **Voyager 4.x File Converter**. The Voyager 4.x File Converter dialog box is displayed (Figure 5-27).
2. Click **Add** and select the .MNU files to convert.
3. Click **Add** and select the .SP files to convert.
4. Click **Convert**.
5. If you are converting an autosampler .MNU file, open the converted .BIC file and:
   - On the Instrument Settings page, set the Control mode to **Automatic**.
   - In the Automatic Control mode dialog box, respecify the **.SP file** to ensure that the correct path is used for the .SP file.

NOTE: *No path is retained for the .SP file during conversion. If the .SP file specified is located in the Voyager program directory, the software can use the file during acquisition. If the .SP file is not in the Voyager program directory, an error message is displayed when the .BIC file is run.*

It is good practice to move .SP files to a specific directory before converting, and to respecify the .SP file in the .BIC after converting.

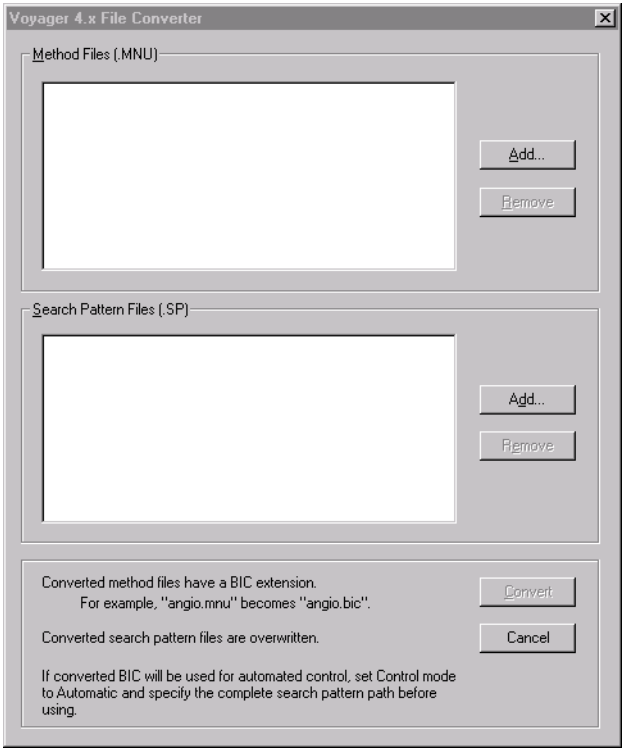
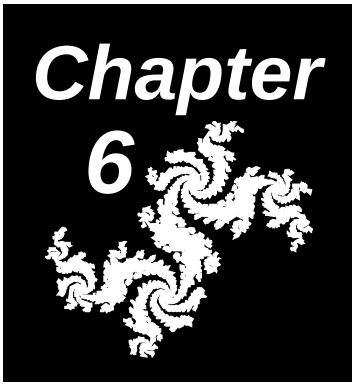


Figure 5-27 Voyager 4.x File Converter Dialog Box

6. If you are converting an .MNU file that specifies an external calibration, calibration mode is reset to Default in the .BIC file. To specify an external calibration for the converted .BIC file, create an appropriate .CAL file in the Data Explorer software, then select the .CAL file on the Instrument Settings page of the .BIC file.

6 Acquiring Spectra from the Instrument Control Panel



This chapter contains the following sections:

6.1 Before You Begin 6-2

6.2 Acquiring in Manual Mode from the Instrument Control Panel 6-11

6.3 Obtaining Good Spectra in Delayed Extraction Mode 6-21

6.4 Making Accurate Mass Measurements 6-26

6.5 Evaluating Data in the Instrument Control Panel 6-28

6.6 Acquiring in Automatic Mode from the Instrument Control Panel 6-35

6

6.1 Before You Begin

This section includes:

- Overview of acquisition options
- Guidelines for acquiring
- Calibrating the mass scale

6.1.1 Overview of Acquisition Options

NOTE: *You cannot operate the mass spectrometer with the front or side panels off. Safety interlocks prevent operation when panels are not in place.*

The Voyager system provides the following options for acquiring data:

Table 6-1 Voyager Acquisition Options

Acquisition Option	Description	For more information, see
Manual Control mode in Instrument Control Panel	• Single sample • Manual control of laser intensity, sample position, data accumulation, data storage • Manual data evaluation • Default or external calibration	Section 6.2, Acquiring in Manual Mode from the Instrument Control Panel

continued

Table 6-1 Voyager Acquisition Options (Continued)

Acquisition Option	Description	For more information, see
Automatic Control mode in Instrument Control Panel	• Single sample • Automatic/manual control of laser intensity or sample position • Automatic control of data accumulation and data storage • Automatic data evaluation based on acceptance criteria you specify • Default or external calibration	Section 6.6, Acquiring in Automatic Mode from the Instrument Control Panel
Batch mode in Sequence Control Panel	• Multiple samples • Uses instrument settings previously optimized for Automatic Control in Instrument Control Panel • Default, external, internal, and internal/update calibration • Optional macro functions that can be applied before and after calibration	Chapter 7, Acquiring Spectra from the Sequence Control Panel

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6.1.2 Guidelines for Acquiring

This section includes:

- High voltage warmup for improved mass accuracy
- Initial laser setting
- Using optimized instrument settings
- When acquiring a spectrum
- Obtaining acceptable data
- Obtaining maximum mass accuracy

High voltage warmup for improved mass accuracy

For maximum mass accuracy, allow the high voltage power supplies to warm up for a short period of time before acquisition. This reduces variability in accelerating voltages, and yields more reproducible ion flight times.

To turn on the high voltage power supplies, click  in the toolbar.

NOTE: *The high voltage power supplies are automatically turned off after 60 minutes if the mass spectrometer is not used. To change the Idle Time, see "High Voltage Configuration" on page 2-38.*

Initial laser setting

After starting the software, the laser intensity is set to the mid-range default laser setting (approximately 1,800). When you load a .BIC file, the laser intensity that was stored in the .BIC file is loaded in the Instrument Control Panel. See "Adjusting laser intensity" on page 5-68, for more information.

Using optimized instrument settings

Before analyzing samples in the Instrument Control Panel, open and optimize instrument settings in a .BIC file. You can optimize more than one .BIC file to cover mass ranges or compounds of interest.

For more information, see Section 5.4, Optimizing Instrument Settings Parameters.

When acquiring a spectrum

When acquiring a spectrum, note the following:

- For the first spectrum you acquire, the laser setting saved in the .BIC file selected is applied. If the laser intensity in the .BIC is not optimized for the mass range, you may not see a signal.
- Make subsequent laser adjustments as described in “Adjusting laser intensity” on page 5-68.
- To locate an acceptable signal, click-drag the scroll bars on the Manual Laser/Sample Position control page to aim the laser at different parts of the sample position. Samples often contain “hot spots” (areas of high ion intensity) and “cold spots” (areas of low ion intensity). When you locate a hot spot, stop acquiring and restart.
- When acquiring actual data, do not move the sample position. You observe the best resolution and mass accuracy when you collect ions produced from one location.

NOTE: *When analyzing high masses or samples of low concentration, or when using thin-film matrices (for example, in polymer analysis or in PSD mode), you may run out of sample if you acquire from the same area in the sample position. You may need to move around on the sample position when acquiring to build up adequate signal-to-noise ratio.*

- Before adjusting laser intensity for subsequent spectra, stop acquisition. The laser setting recorded with the data file is the last laser setting used.

NOTE: *It is good practice to use one laser setting to acquire a spectrum, but you may need to adjust the laser intensity to maintain adequate signal intensity. However, varying the laser intensity during acquisition may affect mass accuracy.*

- Observe the Spectrum window or Oscilloscope screen to determine if data is acceptable. See Section 4.3, Using the Spectrum Window, or Appendix I, Using the Oscilloscope and Control Stick, for more information.

Obtaining acceptable data

The quality of the data you acquire is directly affected by:

- Ionization properties of the sample
- Sample preparation, discussed in Section 3.1, Preparing Samples
- Laser intensity
- Laser position on the sample
- Acquisition instrument settings

It may take some practice before you observe spectra that are acceptable for your analyses.

For examples of acceptable data and guidelines for obtaining acceptable data, refer to Section 6.3, Obtaining Good Spectra in Delayed Extraction Mode.

Obtaining maximum mass accuracy

For maximum mass accuracy, note the following:

- Use the Mass Accuracy Optimization option when acquiring. For information, see Section 3.4.2, Using the Mass Accuracy Optimization Option.
- Acquire at least six replicates of each sample for a statistically significant sampling of data.
- Collect at least 50 shots of data (determined by the Shots/Spectrum parameter in the Instrument Settings control page).
- Set %Centroid at 50% for most applications. Centroid% is described in the *Data Explorer Software User's Guide.*

6.1.3 Calibrating the Mass Scale

This section describes:

- Types of calibration
- When to calibrate
- Calibration equations
- Default calibration
- Generated calibration
- Acquiring calibration standards

Types of calibration

The Voyager software includes a default calibration routine that provides adequate mass accuracy for many applications.

If you require optimum mass accuracy, you can generate a calibration based on the observed time of flight of the known masses of calibration standards. The Calibration function in the Voyager system allows you to generate four types of calibration:

- **Default calibration**—Provides typically 0.1% accuracy in Linear mode, and typically 0.01% or better accuracy in Reflector mode.
- **External calibration**—Provides 0.05% accuracy in Linear mode, and 0.01% or better accuracy in Reflector mode. To perform external calibration, you create a calibration (.CAL) file in the Data Explorer software using standards of known mass. You then specify the file in the Instrument Control Panel when acquiring data, or apply the file to existing data in the Data Explorer software.
- **Internal calibration**—Provides accuracy of 0.02% or better in Linear mode, and 0.002% (20 ppm) in Reflector mode. See Appendix A, Specifications, for the internal calibration mass accuracy specification for your system type. To perform internal calibration, you include an internal standard in your sample. You then display the data file in the Data Explorer software and calibrate using the internal standard.

- **Sequence Control calibration**–Provides external, internal, and internal with automatic updating calibration options during acquisition from the Sequence Control Panel. For more information, see Section 7.7.1, Calibration Options in a Sequence.

For the mass accuracy specifications for your instrument, refer to Appendix A, Specifications.

NOTE: *Mass accuracy for sample positions in the outer rows of a sample plate may be poorer than the mass accuracy of the sample positions in the inner rows.*

When to calibrate

Calibrate:

- At least once a day. You may want to include calibration standards on each sample plate.
- If the ambient temperature in your lab is fluctuating more than 5°C, check calibration, and recalibrate if necessary.

The calibration software compensates for changes in instrument settings. However, if you require optimum mass accuracy, generate a calibration file with identical instrument settings or use internal calibration if possible.

Calibration equations

The general equation that the Voyager software uses for calibration with a .CAL file is described in Figure 6-1.

$$t \text{ (nsec)} = t_0 + A\sqrt{m/z} + \text{(higher order terms)}$$

where:

t = Flight time of the ion

t_0 = Difference in time between the start time of the analysis and the time of ion extraction in Delayed Extraction, or the time of ionization in Continuous Extraction. Also called Effective Delay.

$$A \approx \frac{\textit{Effective Length (mm)}}{\sqrt{\textit{Accelerating Voltage (V)}}} \times \sqrt{\frac{m_0}{2e}} \times 10^6$$

where:

m_0 = 1 dalton mass in SI units (1.66054×10^{-27} kg)

e = charge of electron in SI units (1.602177×10^{-19} Coulomb)

Effective Length = Length of flight tube corrected for ion acceleration through the stages of the source and flight tube, and for the impact of the guide wire.

m/z = mass-to-charge ratio

Figure 6-1 Calibration Equation

Default calibration

The default theoretical calibration is a multi-parameter equation that uses calculated values for t_0 and A. Calculated values are based on the configuration of the system, such as flight tube length and accelerating voltage.

Generated calibration

If you are performing an internal standard calibration, the software determines the constants as listed below:

Internal Standard Calibration	Constant	Value Used
One-point	A	Calculates from standard mass
	t_0	Uses value from default calibration
Two-point	t_0 and A	Calculates from standard mass
Three-point	t_0 and A	Calculates by linear least-squares fit of the data points from standard mass

Acquiring calibration standards

Use standards that are above and below the mass range of interest.

To ensure that sample and standard peaks are similar in size and shape, use the same acquisition conditions for sample and standard, in particular:

- Instrument mode (linear or reflector)
- Signal intensity
- Laser intensity
- Voltages (Accelerating, Grid, and Guide Wire)
- Low Mass Gate

To obtain accurate mass values in the calibration, you need well-shaped, symmetrical peaks. You may need to smooth or noise filter peaks before calibrating. See the *Data Explorer Software User's Guide* for more information.

6.2 Acquiring in Manual Mode from the Instrument Control Panel

This section includes:

- Manually acquiring, evaluating, and saving spectra
- Manually accumulating spectra from multiple acquisitions

Before acquiring spectra, become familiar with the information in Section 6.1, Before You Begin.

6.2.1 Manually Acquiring, Evaluating, and Saving Spectra

This section describes the following manual mode procedures:

- Selecting instrument settings
- Selecting sample position and laser intensity
- Setting Data Storage
- Starting acquisition
- During acquisition
- Evaluating data
- Saving data
- Saving the instrument settings (.BIC) file

Selecting instrument settings

To select instrument settings to manually acquire data:

1. In the Instrument Control Panel, open or create an instrument settings (.BIC) file with the appropriate parameters. See Section 5.4, Optimizing Instrument Settings Parameters, for information.

6

The parameters from the selected instrument settings (.BIC) file are displayed in the Instrument Settings Control page (Figure 6-2).

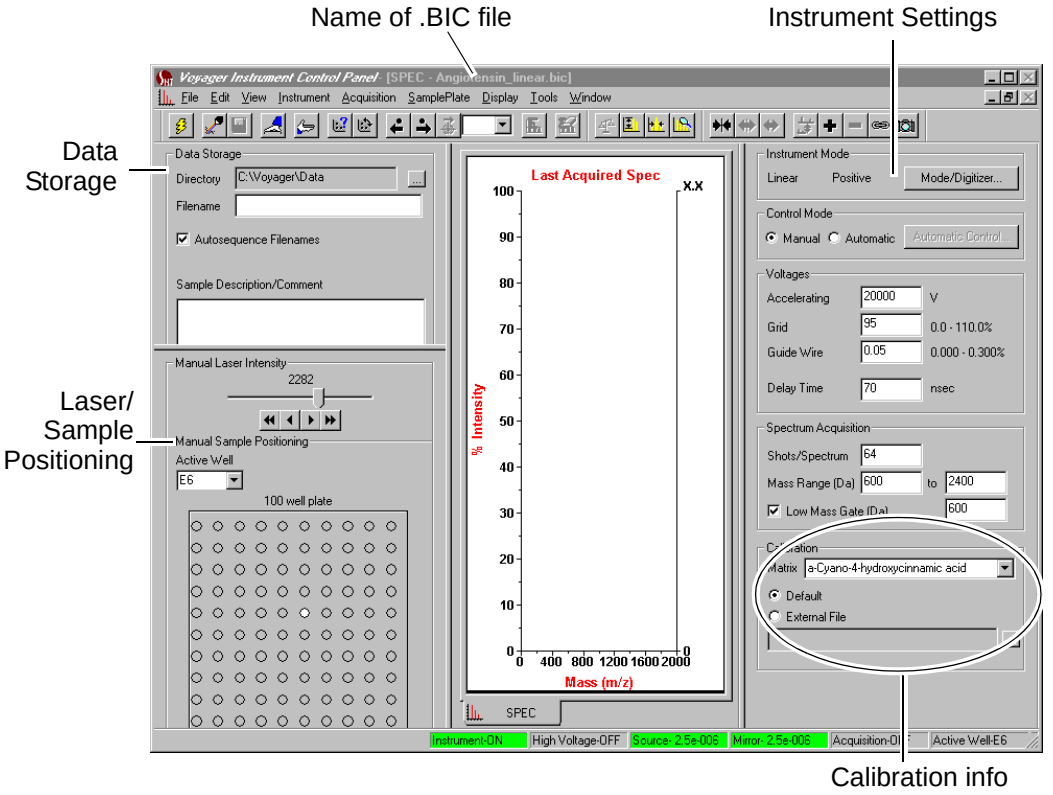


Figure 6-2 Instrument Control Panel Before Acquiring

NOTE: *If the Instrument Settings control page is not displayed, select* ***Instrument Settings*** *from the View menu.*

---

2. In the Instrument Settings control page, click **Manual**.

3. Specify calibration information:

   - **Calibration Matrix**—Select the matrix used for your application. For more information, see “Matrix influence” on page 5-22.
   - **Default or External**—Select Default, or select an external .CAL file. For more information, see “Types of calibration” on page 6-7.

Selecting sample position and laser intensity

To select sample position and laser intensity, do the following in the Manual Laser/Sample Positioning control page (see Figure 6-2 on page 6-12):

1. If the Manual Laser/Sample Positioning control page is not displayed, select **Manual Laser/Sample Positioning** from the View menu.
2. Select the sample to analyze by doing any of the following in the **Active Position** box:

   - Type a position number
   - Select a position number from the drop-down list
   - Single-click on a sample position

Hint: *You can also move to a sample position using the control stick, by selecting* ***Move to Sample Position*** *from the Sample Plate menu, or by clicking*  .

To zoom on the Active Sample Position, you can do any of the following:

- Double-click on the sample position in the Plate view.
- Select **Sample View** from the Sample Plate menu.
- Right-click the mouse and select **Toggle Sample/Plate View**.

Hint: *You can double-click on the control page to "undock" it, and click-drag it to any location on the screen. Double-click again to re-dock the page.*

3. Set the laser intensity by click-dragging the slider or clicking the arrows. For more information, see Section 4.5.2, Adjusting Laser Intensity and Selecting Sample Position, and Section 5.4.2, Determining the Laser Setting.

Hint: *You can also set laser intensity by pressing* ***Ctrl+PgUp*** *and* ***Ctrl+PgDn*** *on the keyboard.*

Setting Data Storage parameters

You can specify Data Storage parameters before or after acquiring in Manual Control mode. To specify Data Storage location and file name:

1. If the Data Storage control page (Figure 6-3) is not displayed, select **Data Storage** from the View menu.

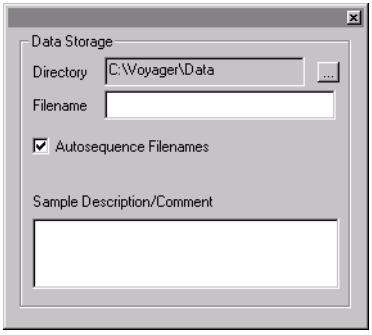


Figure 6-3 Data Storage Control Page

2. Click ... to select a directory in which all data files will be stored.

3. Type a filename.
4. Select **Autosequence Filenames** if you want the software to determine the next available sequential filename.

NOTE: *If you deselect Autosequence Filenames, the software uses the name in the Filename field and will overwrite an existing data file.*

If Autosequence is enabled, a 4-digit suffix starting at 0001 is automatically appended to the filename specified. For example, SAMPLE_0001.

Before acquiring, the software checks the directory specified in the Data Storage page for root file name, then appends the next available suffix. The software does not overwrite existing file names or reset the suffix to 0001.

If you want to reset the suffix to 0001, delete or move existing files from the directory, or use a different file name.

5. Enter comments that will be saved with the data file. This step is optional.

Starting acquisition

To start acquiring, select **Start Acquisition** from the Acquisition menu, or click  .

CAUTION

If you do not save data before starting a new acquisition, the data in the Current trace is lost.

Acquisition starts and continues until the number of Shots/Spectrum specified in Spectrum Acquisition on the Instrument Settings control page is collected, or until you select **Stop Acquisition** from the Acquisition menu or click  again.

During acquisition

During acquisition:

- The Live/Current Spectrum trace in the Spectrum window updates to display the spectrum that results from each laser shot, with the following exceptions:
  - If you are acquiring a large number of data points, the Spectrum window is not updated with every laser shot. The exact update rate depends on the Mass Range setting and the Bin Size setting you are using.
  - If your system includes an oscilloscope, the Current Spectrum does not display a trace until acquisition is complete. The spectra that result from each laser shot are displayed on the oscilloscope screen.
- The system averages all spectra acquired after you start acquisition.

NOTE: *If the system is set to acquire in single-shot mode, spectra are not averaged.* *See page 2-44*

When acquisition is complete, the software displays the averaged spectrum in a Current trace in the Spectrum window.

Evaluating data

As data is acquired, observe the trace in the Spectrum window:

1. Examine the number at the top of the right axis in the Current Spectrum trace to determine if signal is saturated (Figure 6-4). Signal with counts greater than 64,000 may be saturated. Adjust the laser intensity as needed.

   For information, see “Signal saturation” on page 5-69.

2. Use the Resolution calculator or the Signal-To-Noise calculator on the Current trace to determine if data is acceptable. For more information see Section 6.5, Evaluating Data in the Instrument Control Panel.

Hint: *You can accumulate traces to improve resolution and signal-to-noise ratio. For more information, see Section 6.2.2, Manually Accumulating Spectra from Multiple Acquisitions.*

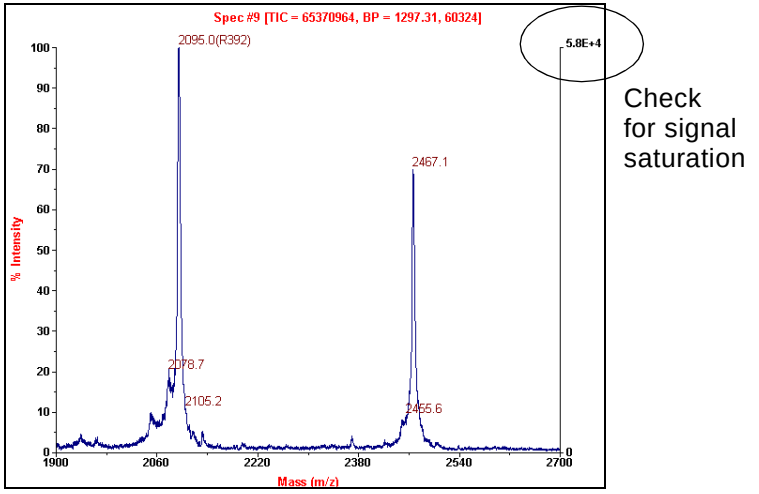


Figure 6-4 Checking for Signal Saturation

3. At this point, you can:
   - Save the data, described in “Saving data” on page 6-18.
   - Acquire additional spectra and create an accumulated spectrum, described in Section 6.2.2, Manually Accumulating Spectra from Multiple Acquisitions.

CAUTION

If you do not save data before starting a new acquisition, the data in the Current trace is lost.

CAUTION

If you change the laser setting after acquisition is complete, but before you save the data, the last laser setting is saved with the data, not the laser setting used to acquire the data.

Saving data

To save the data when you have more than one trace displayed in the Spectrum window:

1. Select (click on) the Current trace.
2. Select **Save Spectrum** from the Acquisition menu.

   The data is saved using the file name specified in the Data Storage control page. Information about the data file is displayed in the Data Storage tab in the Output window (Figure 6-5) at the bottom of the Instrument Control Panel.

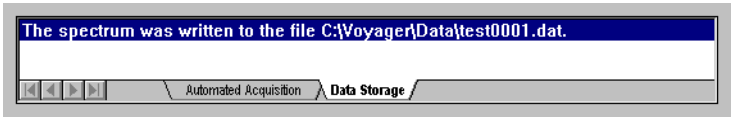


Figure 6-5 Data Storage Tab in Output Window

Saving the instrument settings (.BIC) file

When acquisition is complete, save the instrument settings (.BIC) file used to acquire the data if you want to store the optimized laser intensity used during this acquisition. For information on saving a .BIC file, see Section 5.1.5, Saving and Printing Instrument Settings.

NOTE: *You can export instrument settings from a .DAT file that has been saved in Data Explorer. For more information, see the Data Explorer User's Guide.*

6.2.2 Manually Accumulating Spectra from Multiple Acquisitions

You can manually accumulate spectra from different acquisitions to improve resolution and signal-to-noise ratio of your data.

Accumulating

To manually accumulate spectra from multiple acquisitions:

1. Acquire a spectrum and evaluate the data as described in Section 6.2.1, Manually Acquiring, Evaluating, and Saving Spectra.
2. When acquisition is complete, do not save the data.
3. If the spectrum is acceptable, select **Accumulate Spectrum** from the Acquisition menu or click  to accumulate the spectrum.

   The software adds an Accumulated trace to the Spectrum window and copies the Current trace in the Accumulated trace.

   If the spectrum is not acceptable, start a new acquisition. The Current trace will be overwritten by the new acquisition.

CAUTION

If you change any instrument settings, with the exception of Shots/Spectrum and laser intensity, the Accumulated trace is automatically cleared before the next acquisition.

4. Repeat step 1 though step 3 to acquire and accumulate additional spectra. Each spectrum you accumulate is averaged with the existing Accumulated trace.

NOTE: *If needed, select* ***Clear Accumulated*** *trace from the Acquisition menu or click*  *.*

5. When the data is acceptable, select (click on) the Accumulated trace.
6. Select **Save Spectrum** from the Acquisition menu.

6.3 Obtaining Good Spectra in Delayed Extraction Mode

This section describes:

- What is a good spectrum?
- Laser intensity
- Parameters affecting resolution and signal-to-noise ratio

6.3.1 What Is a Good Spectrum?

A good spectrum is one that is acceptable for your analysis. In general, it:

- Contains sharp, symmetrical, well-defined peaks
- Has acceptable resolution
- Has acceptable signal-to-noise ratio

For some applications (for example, when you are looking for detailed structural information), you may require very well-separated isotope peaks and the maximum resolution possible. For other applications (for example, when looking for an estimate of molecular weight), your requirements may be less strict.

Figure 6-6 through Figure 6-9 are examples of poor and good spectra.

NOTE: *Masses are typically accurate in Delayed Extraction mode, even at high laser power.*

NOTE: *Dimers in a spectrum may indicate that the molecular ion is saturated, or that sample is too concentrated. Decrease laser intensity or sample concentration to minimize the dimer.*

6

High laser power causing the following:

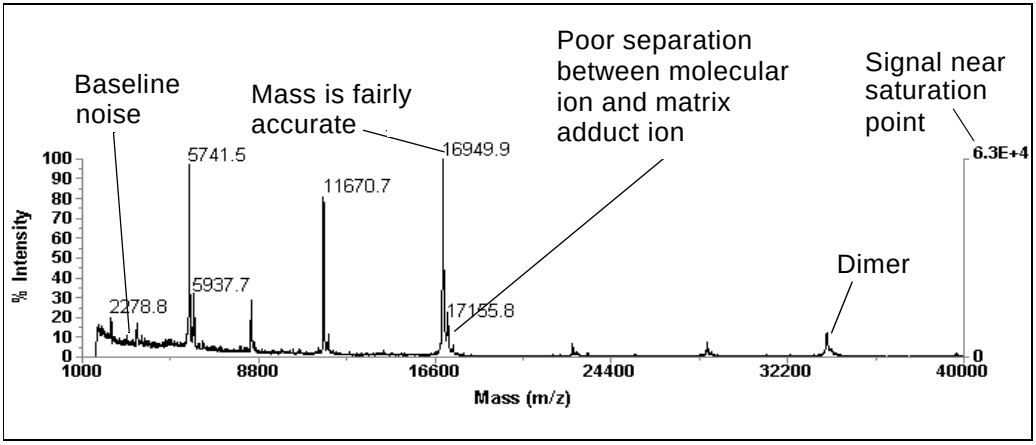


Figure 6-6 Example of Poor Mass Spectrum for Myoglobin Using Sinapinic Acid

Lower laser power producing:

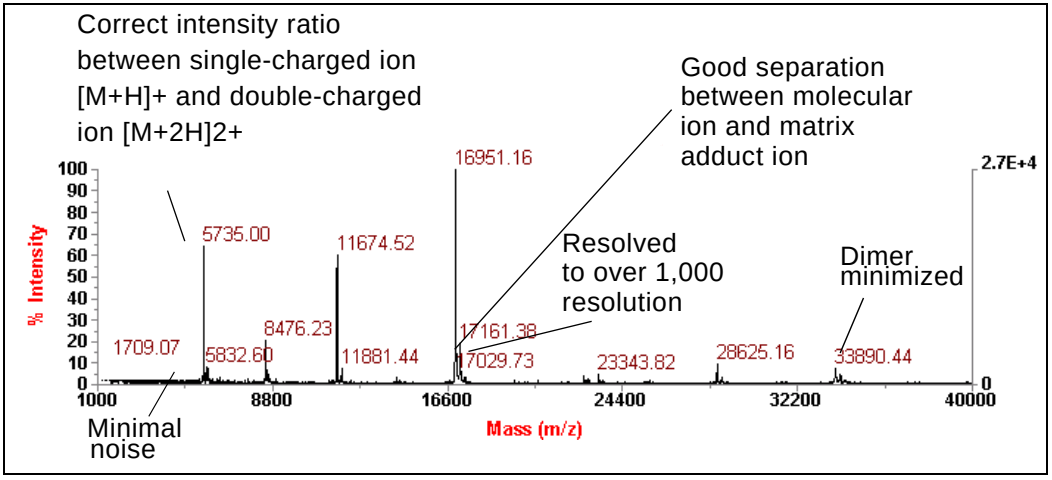


Figure 6-7 Example of Good Mass Spectrum for Myoglobin Using Sinapinic Acid

High laser power causing the following:

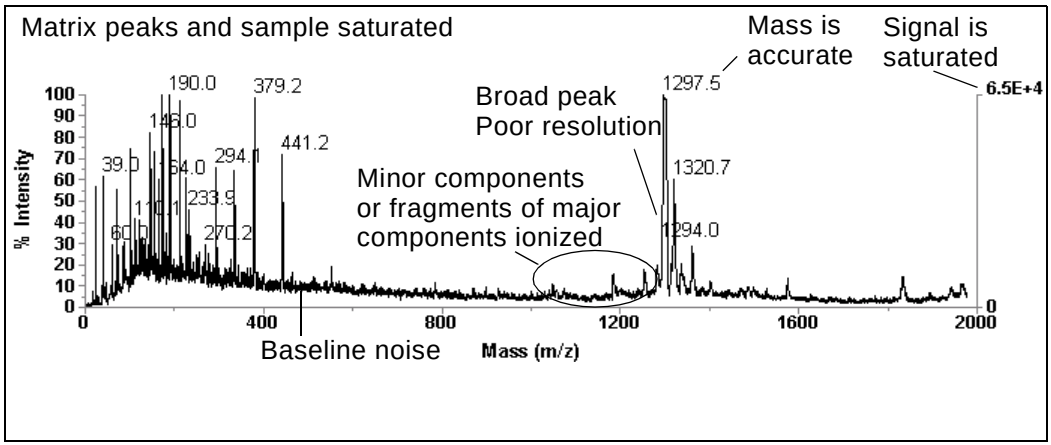


Figure 6-8 Example of Poor Mass Spectrum for Angiotensin

Lower laser power producing:

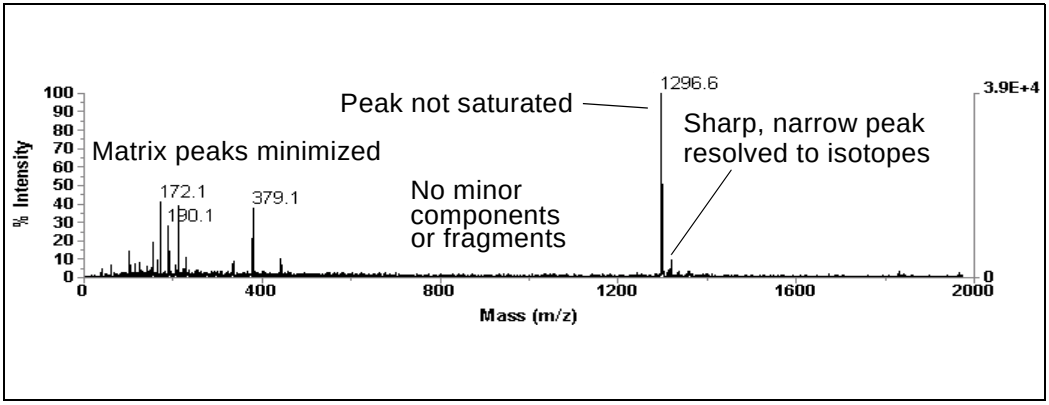


Figure 6-9 Example of Good Mass Spectrum for Angiotensin

6

6.3.2 Laser Intensity

Overview

Laser intensity does not have a major impact on resolution or signal-to-noise ratio. You need to find the laser setting that gives you an acceptable signal-to-noise ratio and acceptable resolution (optimum is not necessary), and then fine-tune. If adjusting Grid Voltage% and Delay Time causes the signal to saturate, you may need to make additional laser adjustments. For more information on adjusting the laser, see Section 5.4.2, Determining the Laser Setting.

Continue acquiring and decreasing the laser intensity until you observed a moderate resolution and a signal-to-noise ratio of approximately 50:1.

Fragment ions generated at higher laser intensity

Higher laser power can cause two types of fragment ions to form:

- **Prompt (fast) fragments**—Form in the ion source before ion acceleration, detected in Linear and Reflector mode.
- **PSD (slow) fragments**—Form in the flight tube after ion acceleration, detected in Reflector mode.

Figure 6-10 shows a partial spectrum of Angiotensin I obtained at high laser intensity in Reflector mode. The high laser intensity has generated fragments.

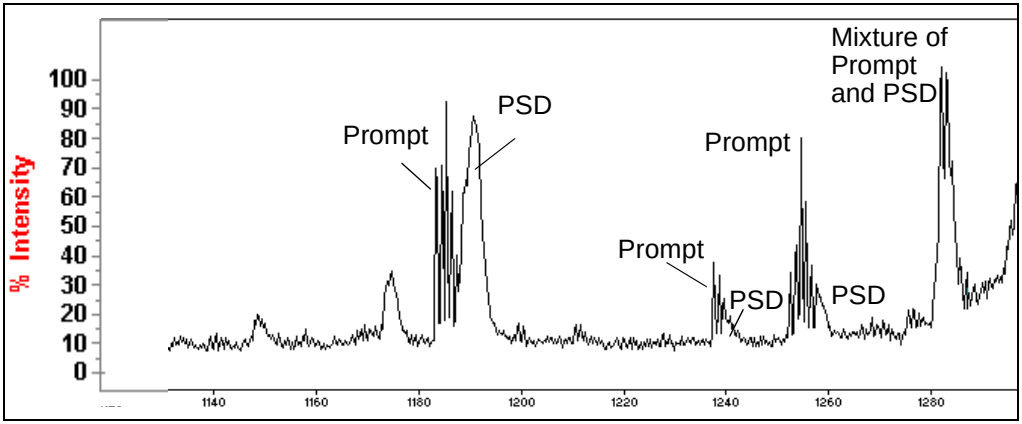


Figure 6-10 Fragment Ions in Reflector Mode

Prompt fragments Prompt fragments appear in the spectrum at masses that correspond to the theoretical masses of the fragments, because they are formed in the source.

PSD fragments PSD fragments appear in the spectrum at masses slightly higher than the theoretical masses of the fragments because they are formed after the source, but travel at the same speed as the precursor until they reach the reflector. Because they have lower kinetic energy than the precursor, they separate in mass from the precursor in the reflector.

6.3.3 Parameters Affecting Resolution and Signal-to-Noise Ratio

Parameters affecting resolution

These parameters have a primary impact on resolution:

- Delay Time
- Guide Wire Voltage%
- Grid Voltage%
- Digitizer Bin size and Input Bandwidth

NOTE: *Guide Wire Voltage% is not available on Voyager-DE STR models with serial number 4154 and later.*

For more information on optimizing resolution, Section 5.4.3, Optimizing Resolution.

Parameters affecting signal-to-noise ratio

These parameters have a primary impact on signal-to-noise ratio:

- Accelerating Voltage
- Guide Wire Voltage%
- Shots/Spectrum
- Digitizer Input Bandwidth
- Low Mass Gate

NOTE: *Guide Wire Voltage% is not available on Voyager-DE STR models with serial number 4154 and later.*

For more information on optimizing signal-to-noise, see Section 5.4.4, Optimizing Signal-to-Noise Ratio.

6

6.4 Making Accurate Mass Measurements

Overview

Accurate mass measurements are typically made by including reference compounds (internal standards) in the same spectrum as the analyte, and then recalibrating the spectrum.

In Reflector mode (Voyager-DE PRO and Voyager-DE STR), internal calibration provides mass spectra with a mass accuracy of 10 to 20 ppm routinely.

This section describes the factors that influence mass accuracy at this level.

During acquisition

When acquiring data, the following factors contribute to the accuracy of mass measurements:

- **Symmetrical peak shape**—Depending on the m/z in question, the spacing between data points can represent a change in mass from 10 or 20 ppm to more than 100 ppm. When determining the position of an ion in terms of m/z, the software interpolates between data points to make a mass assignment at the 10 ppm level. Therefore, a skewed peak shape can have a strong impact on the observed mass accuracy.
- **Signal-to-noise ratio as high as possible without skewing peak shape**—Peak position can be affected by noise. On Voyager instruments, avoid full scale signals (64,000 counts) which may be saturated. To avoid the saturation problem, accumulate spectra with appropriate evaluation criteria. See “Setting spectrum acceptance and laser adjustment criteria” on page 6-42 for more information.
- **Signal intensity of reference and analyte peaks above 1,000 counts**—Signal intensity above 1,000 counts with a Shots/Spectrum setting of 50 or greater ensures adequate peak statistics needed for accurate assignment of peak position.

- **Signal intensity of reference and analyte peaks is in the same range**—Signal intensity of reference and analyte peaks should be of similar magnitude. If they are not in the same range, you may see weak analyte peaks with poor signal-to-noise ratio while the internal standard is adequate, or saturated internal standard peaks with adequate analyte peaks.
- **No contaminants present**—Unresolved contaminants can affect peak shape.
- **Two internal reference masses that bracket the mass range of interest**—Best results are obtained when masses above and below the mass of interest are correctly identified.

NOTE: *Although not required, using more than two reference masses can help to minimize the effects of an asymmetrical reference peak.*

- **Internal reference masses are within the same range as analyte masses**—Best results are obtained when masses are in the same range. For example, reference masses of 904 and 1,570 are adequate when evaluating an analyte mass of 1,296 Da.

During mass calibration and peak detection

When calibrating mass (described in the *Data Explorer Software User's Guide*) and detecting peaks (described in the *Data Explorer Software User's Guide*), the following factors contribute to the accuracy of mass measurements:

- **Use Centroid mass instead of Apex mass**—Before calibrating, set peak labels to Centroid. Centroid mass is the best representation of peak position. Apex mass represents the highest data point.
- **Set Centroid Percent to 50%**—Lower settings can include noise or contaminant peaks. Higher settings may not include adequate data to ensure accurate mass assignment.

6

6.5 Evaluating Data in the Instrument Control Panel

This section describes:

- Detecting, integrating, and labeling peaks
- Calculating mass resolution
- Calculating signal-to-noise ratio

6.5.1 Detecting, Integrating, and Labeling Peaks

Detecting peaks

Peaks are not detected in the Spectrum window until acquisition is complete.

To adjust peak detection when acquisition is complete

1. From the Tools menu, select **Peak Detection**.

   The Peak Detection dialog box is displayed (Figure 6-11).

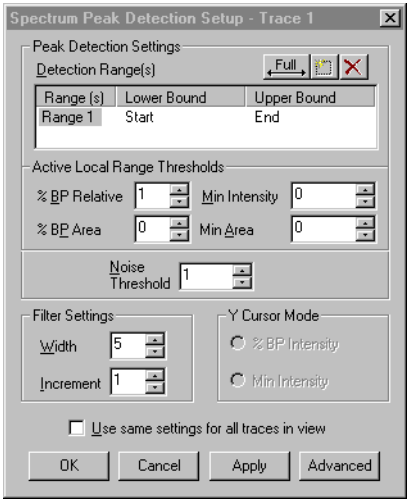


Figure 6-11 Peak Detection Dialog Box

NOTE: *The parameters in this dialog box correspond to the Advanced Settings tab in the Peak Detection dialog box in the Data Explorer software.*

2. Select a detection range and set parameters as described in the *Data Explorer Software User's Guide*.
3. Click **Apply** to accept the parameters and leave the dialog box open, or click **OK** to accept the parameters and close the dialog box. If peaks are not labeled, enable labels as described in the next section.

NOTE: *The Instrument Control Panel uses many of the same parameters, detection, and integration algorithms as the Data Explorer software. For information, see the Data Explorer Software User's Guide.*

Labeling spectrum peaks

Labeling spectrum peaks in the Instrument Control Panel is similar to labeling spectrum peaks in the Data Explorer software.

Activate the trace to label, select **Peak Label** from the Display menu, enable and select labels, and set the number of decimal points to display. For information, see the *Data Explorer Software User's Guide*.

Disabling spectrum peak labels

To disable, select **Peak Label** from the Display menu, and disable labels as needed.

6

6.5.2 Calculating Mass Resolution

You can calculate mass resolution for up to four peaks in the spectrum currently being acquired. The resolution values are displayed in the trace next to the mass value for the peak.

Calculating resolution for live data

To calculate mass resolution:

1. When acquisition is complete, select the Current spectrum.
2. From the Tools menu, select **Resolution Calculator**.
3. In the Resolution Calculator dialog box (Figure 6-12), set the percentage of peak height at which to calculate resolution. The default is 50%, which calculates the resolution at the full width/half maximum of the peak height (FWHM).

NOTE: *Measuring resolution at the full width/half maximum is the industry standard. Use 50% Peak Height for most applications.*

---

4. Set Minimum Peak Intensity. Signals below this intensity (absolute counts) are not included in the calculation.

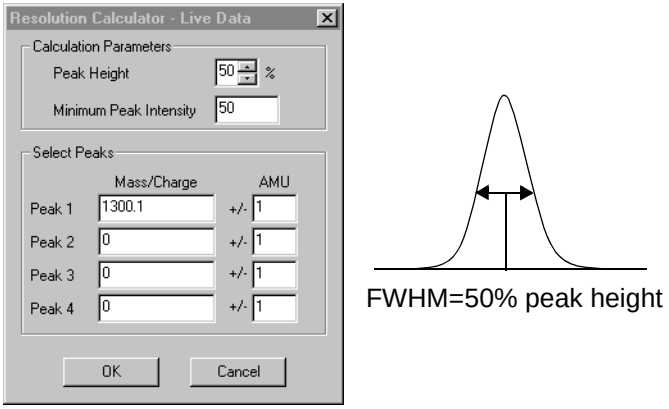


Figure 6-12 Resolution Calculator

5. Type in up to four **Mass/Charge** values for which to calculate resolution.

6. For each Mass/Charge, enter the window for calculation (± AMU).

NOTE: *To label isotopes, set the ± AMU value low enough to prevent the calculation windows for each isotope peak from overlapping. If the calculation windows overlap, only the highest peak is labeled. If you set too low, the peak of interest may not be labeled. However, if you set ± AMU too low, the peak of interest may not appear in the window, and resolution will not be calculated.*

---

7. Click **OK**.

   The peaks are labeled with (R*XXXX*) next to the peak mass, where *XXXX* is the resolution (Figure 6-13). To turn off resolution labels, see “Labeling spectrum peaks” on page 6-29.

NOTE: *If you do not apply peak detection parameters after acquisition is complete, resolution labels are not displayed.*

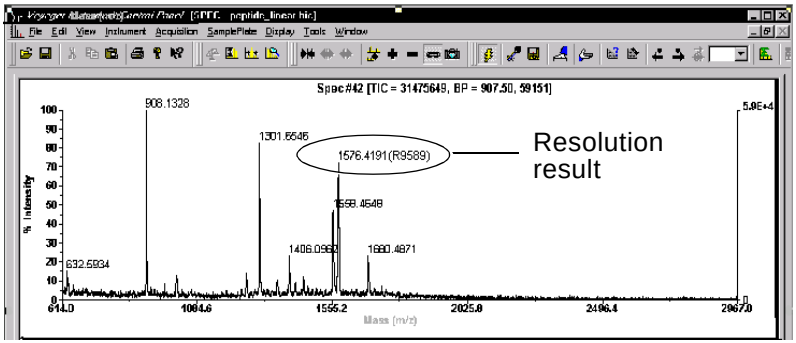


Figure 6-13 Resolution Calculator Results

The table below lists a general rating scale for resolution and molecular weight ranges for compounds acquired in Linear mode.

Table 6-2 Resolution Rating Scale

Compounds <2,000 Da		Compounds 2,000 to 5,000 Da		Compounds 5,000 to 25,000 Da		Compounds >25,000 Da	
Resolution	**Rating**	**Resolution**	**Rating**	**Resolution**	**Rating**	**Resolution**	**Rating**
500	Not acceptable	250	Not acceptable	500	Not acceptable	50	Acceptable
1,500	Acceptable	400	Acceptable	700	Acceptable	100	Good
2,000	Good	500	Good	900	Good	>200	Very good
2,500	Very good	>600	Very good	1,000	Very good	——	——

6.5.3 Calculating Signal-to-Noise Ratio

Overview

A signal-to-noise ratio is typically used to describe how well a mass of interest in a spectrum is distinguished from background chemical and electronic noise.

The Control Panel software measures the signal-to-RMS noise ratio in a user-defined region of a mass spectrum.

Calculating signal-to-RMS noise ratio

To calculate a signal-to-RMS noise ratio:

1. When acquisition is complete, select the Current spectrum of interest.
2. From the Tools menu, select **Signal-to-Noise Calculator**.
3. In the Signal to Noise Calculator dialog box (Figure 6-14), set the Baseline Region by doing one of the following:
   - Type in From and To values
   - In the Spectrum window, right-click-drag over the baseline area you want to use in calculating signal-to-noise ratio. For accurate results, specify a flat (non-rising) region of baseline that does not include peaks.

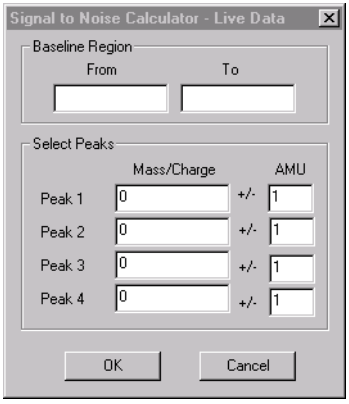


Figure 6-14 Signal to Noise Calculator

4. For each Mass/Charge, enter the window for calculation (± AMU).

NOTE: *To label peaks, set the ± AMU value low enough to prevent the calculation windows for each peak from overlapping. If the calculation windows overlap, only the first peak is labeled. However, if you set ± AMU too low, the peak of interest may not appear in the window, and signal-to-noise will not be calculated.*

---

5. Click **OK**.

   The peaks are labeled with (S*XXX*) next to the peak mass, where *XXX* is the signal-to-noise ratio.

NOTE: *If you do not apply peak detection parameters after acquisition is complete, signal-to-noise labels are not displayed.*

6.6 Acquiring in Automatic Mode from the Instrument Control Panel

This section includes:

- Before acquiring in Automatic Control mode
- Setting Instrument Settings for Automatic Control mode
- Automatically acquiring, evaluating, and saving spectra
- Search patterns
- Process that occurs during acquisition in Automatic Control mode
- Process that occurs when accumulating spectra from multiple search pattern positions

PSD analysis in Automatic mode

For information on performing PSD analysis in automatic mode, see Section 8.5, Acquiring PSD Data with Standard .BIC Files in Automatic Control Mode.

6

6.6.1 Before Acquiring in Automatic Control Mode

Background information

Before acquiring spectra in Automatic Control mode, become familiar with the information in:

- Section 6.1, Before You Begin
- Section 6.2, Acquiring in Manual Mode from the Instrument Control Panel

Aligning the sample plate

Sample plate alignment is necessary for automated sample positioning in Automatic Control mode if the laser is not striking the center position of the sample position.

Sample plate alignment may not be necessary on your system, particularly if you use the SPIRAL.SP search pattern file provided with the system.

See Section 2.7, Aligning the Sample Plate, to determine if sample plate alignment is necessary.

Using Mass Accuracy Optimization

In a typical analysis run that requires optimum mass accuracy, you include samples interspersed with many calibration standards, and externally calibrate the samples using the closest calibration standard.

By using the Mass Accuracy Optimization feature in the Voyager software, you can obtain optimum mass accuracy using fewer calibration standards. For information, see Section 3.4.2, Using the Mass Accuracy Optimization Option, and Section 2.8, Running OptiPlate to Optimize Mass Accuracy.

6.6.2 Setting Instrument Settings for Automatic Control Mode

This section includes:

- Specifying Automatic Control settings
- Setting spectrum acceptance and laser adjustment criteria
- Saving the instrument settings (.BIC) file

Specifying Automatic Control settings

After you optimize an instrument settings file for Manual Control mode and ensure that parameters are adjusted for optimum performance, you can adjust the instrument settings for Automatic Control mode, if desired. For information on optimizing for Manual Control mode, see Chapter 5, Optimizing Instrument Settings.

You can set parameters to control the following in Automatic Control mode:

- Laser intensity adjustment
- Spectrum accumulation and saving
- Sample positioning
- Stop conditions

To set instrument settings for Automatic Control mode:

1. In the Instrument Control Panel, open or create an instrument settings (.BIC) file with the appropriate parameters. For more information, see Section 5.4, Optimizing Instrument Settings Parameters.

   NOTE: *Make sure the instrument settings yield acceptable results in Manual Control mode before setting to Automatic Control mode.*

2. In the Instrument Settings control page, select **Automatic Control** mode.

3. Click **Automatic Control** to display the Automatic Control dialog box (Figure 6-15).

NOTE: *The Automatic Control button is dimmed if Automatic Control mode is not selected.*

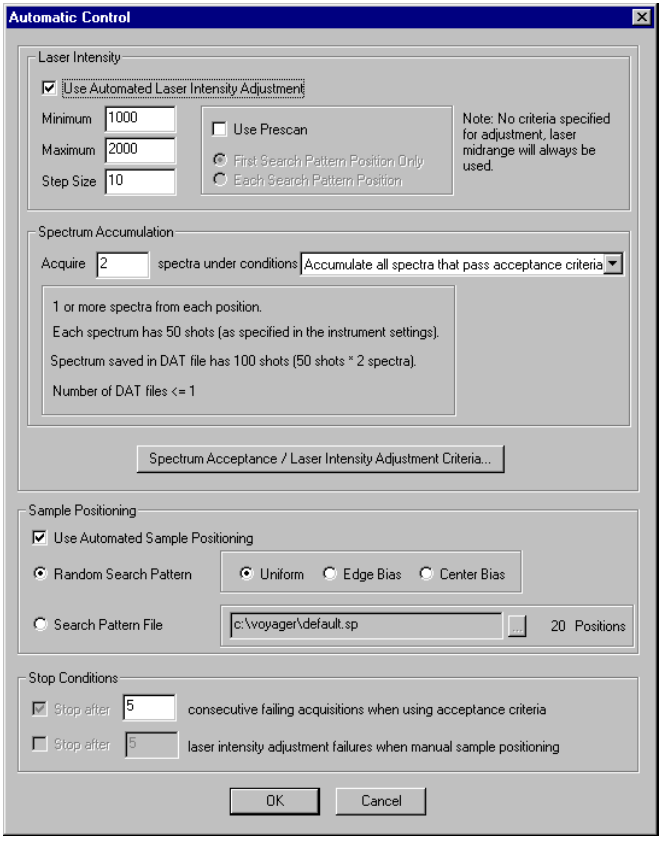


Figure 6-15 Automatic Control Dialog Box

Laser

4. Select **Use Automated Laser Intensity Adjustment**.

NOTE: *To manually control the laser intensity when acquiring in Automatic Control mode, deselect Use Automated Laser Intensity Adjustment. For more information, see Section 4.5.2, Adjusting Laser Intensity and Selecting Sample Position.*

---

5. If you enable Use Automated Laser Intensity Adjustment, specify the Minimum and Maximum Laser Intensity and the Step Size to use. For more information, see “Laser Intensity Adjustment” on page 5-34.

6. If you enable Use Automated Laser Intensity Adjustment and Use Automated Sample Positioning (described in step 10 on page 6-41), select **Use PreScan** if desired, then select an option:

   - **First Search Pattern Position Only**
   - **Each Search Pattern Position**

NOTE: *Prescan options are not available unless you select Use Automated Laser Intensity Adjustment and Use Automated Sample Positioning.*

For more information on the Prescan process, see “Prescan mode” on page 6-58.

7. If you enable Use Automated Laser Intensity Adjustment, set adjustment criteria as described in “Setting spectrum acceptance and laser adjustment criteria” on page 6-42.

NOTE: *If you do not specify laser adjustment criteria, the laser is not adjusted. The mid-range laser setting between the minimum and maximum specified in step 5 is used.*

Spectrum accumulation and saving

8. Specify Spectrum Accumulation parameters:

   - **Acquire X Spectra**—Specifies the number of spectra to acquire. The software compares the value you enter with the possible number of positions in the selected search pattern and displays a message if you enter a value greater than the number of positions.
   - **Under Conditions**—Determines if spectra are saved individually or accumulated, and if spectra are evaluated against Acceptance Criteria.

   NOTE: *If you select Save All Spectra or Save All Spectra that Pass Acceptance Criteria Save Conditions (which save individual multiple spectra in one data file), the recommended maximum number of spectra to acquire is 500. Acquiring more than 500 individual spectra in one file generates a very large data file that can be slow to open and process.*

   When you select conditions, the software displays the information about the number of data files and spectra that will be acquired and saved.

   For more information, see “Spectrum Accumulation” on page 5-35.

9. If you selected saving or accumulation conditions in step 8 that specify acceptance criteria, set Spectrum Acceptance Criteria as described in “Setting spectrum acceptance and laser adjustment criteria” on page 6-42. Saving or accumulation conditions that specify acceptance criteria are:

   - Save all spectra that pass acceptance criteria
   - Save first spectrum to pass acceptance criteria
   - Save best spectrum
   - Accumulate all spectra that pass acceptance criteria

Sample positioning

10. Select **Use Automated Sample Positioning**.

NOTE: *To manually adjust sample positioning when acquiring in Automatic Control mode, deselect Use Automated Sample Positioning. For more information, see Section 4.5.2, Adjusting Laser Intensity and Selecting Sample Position.*

---

11. If you enabled Use Automated Sample Positioning, select **Random Search Pattern** or **Search pattern file**, then select an .SP file.

    For information on search pattern files, see Section 6.6.4, Search Patterns.

NOTE: *Before using search pattern files created in version 4 software, convert to version 5 format. See Section 5.5, Converting Version 4 Methods and Search Pattern Files.*

Stop conditions

12. Select Stop Conditions if you want to stop the acquisition when a specified number of:

    - Consecutive acquisitions fail acceptance criteria
    - Laser adjustments within a search pattern position do not yield acceptable data when using manual sample positioning

    For more information, see “Stop Conditions” on page 5-38.

13. Click **OK**.

    For more information on Automatic Control parameters, see Section 5.2.3, Automatic Control Dialog Box.

6

Setting spectrum acceptance and laser adjustment criteria

If you selected an accumulation condition that uses acceptance criteria (see step 9 on page 6-40):

1. In the Automatic Control dialog box (see Figure 6-15 on page 6-38), click the **Spectrum Acceptance Criteria** button.

   The Spectrum Acceptance Criteria dialog box (Figure 6-16) is displayed.

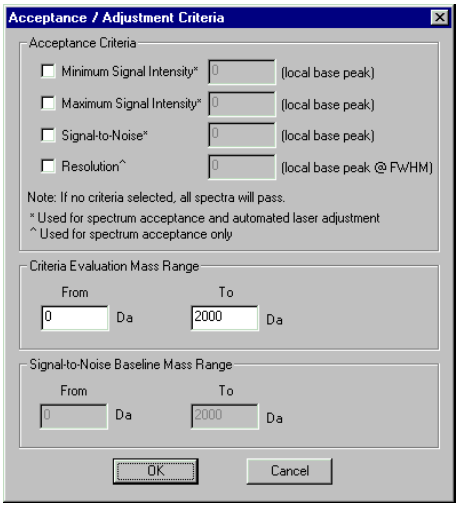


Figure 6-16 Spectrum Acceptance Criteria Dialog Box

2. Set Spectrum acceptance and Laser adjustment criteria parameters as described in Table 6-3.

Table 6-3 Spectrum Acceptance Criteria Parameters

Parameter	Description
Acceptance Criteria	
Minimum Signal Intensity (Used for spectrum acceptance and laser adjustment)	Sets the minimum signal intensity accepted for the most abundant peak (local base peak) within the mass range of interest. Also used to determine laser adjustment. When the system adjusts the laser, it checks that the signal intensity is above the minimum specified. If signal intensity is not above the minimum, the system increases the laser intensity. If signal intensity is not above the minimum when the laser is at the highest setting specified in Automatic Control, the system moves to the next search pattern position.
Maximum Signal Intensity (Used for spectrum acceptance and laser adjustment)	Sets the maximum signal intensity for the most abundant peak (local base peak) within the mass range of interest. Also used to determine laser adjustment. When the system adjusts the laser, it checks that the signal intensity is below the maximum specified. If signal intensity is not below the maximum, the system decreases the laser intensity. If signal intensity is not below the maximum when the laser is at the lowest setting specified in Automatic Control, the system moves to the next search pattern position.
Signal-to-Noise (Used for spectrum acceptance and laser adjustment)	Enables or disables the signal-to-noise filtering. Type the signal-to-noise ratio threshold. Spectrum is rejected if the base peak has a ratio below this value. The base peak in the specified Mass Range is used to calculate signal. The noise is calculated over the Baseline Range specified (see below).

continued

Table 6-3 Spectrum Acceptance Criteria Parameters (Continued)

Parameter	Description
Signal-to-Noise (Used for spectrum acceptance and laser adjustment) *(continued)*	Also used to determine laser adjustment. When the system adjusts the laser, it checks that the signal-to-noise ratio is above the value entered. If it is not, the system increases the laser intensity. If signal-to-noise ratio is not above the specified value when the laser is at the highest setting specified in Automatic Control, the system moves to the next search pattern position.
Resolution	Enables or disables the resolution filtering. Type the resolution threshold. Spectrum is rejected if the base peak has a resolution below this value. Resolution is measured at 50 percent of the most intense peak in the specified mass range.
Criteria Evaluation Mass Range	Specifies the mass range to evaluate.
Signal-to-Noise Baseline Range	Mass range in which to calculate noise, if Signal-to-Noise is enabled above.

3. Click **OK** to close the Spectrum Acceptance Criteria dialog box.
4. Click **OK** to close the Automatic Control dialog box.

Saving the instrument settings (.BIC) file

From the File menu in the Instrument Control Panel, select **Save Instrument Settings**.

All instrument settings, including Automatic Control mode setting, Spectrum Acceptance Criteria, and Laser Intensity Adjustment Criteria are saved.

6.6.3 Automatically Acquiring, Evaluating, and Saving Spectra

To automatically acquire a spectrum:

1. In the Instrument Control Panel, open or create an instrument settings file with the appropriate parameters. See Section 6.6.2, Setting Instrument Settings for Automatic Control Mode, for information.
2. Set Data Storage parameters as described in “Setting Data Storage parameters” on page 6-14.

NOTE: *You must specify a file name in the Data Storage control page before acquiring in Automatic Control mode.*

---

3. In the Manual Laser/Sample Positioning control page, select the Active Position to analyze.
4. To start acquiring, select **Start Acquisition** from the Acquisition menu, or click  .

During acquisition

During acquisition:

- Laser is adjusted, sample position is adjusted, and Spectrum Acceptance Criteria are applied to each search pattern position in a spectrum. For more information, see “Spectrum Accumulation” on page 5-35, and Section 6.6.6, Process that Occurs During Acquisition in Automatic Mode.
- Each spectrum is saved or accumulated as determined by the conditions selected in Spectrum Accumulation in the Automatic Control dialog box. For more information, see, Section 5.2.4, Description of Spectrum Accumulation Options.
- Information about the acquisition is displayed in the Automated Control tab of the Output window.

6.6.4 Search Patterns

This section describes:

- Search patterns
- Search pattern (.SP) files
- Units in search pattern files
- Default search pattern file
- Spiral search pattern file
- Search pattern for custom plates

Search patterns

A search pattern determines the path that the laser follows when scanning the selected Active Position using Automatic Control Mode. You have two options for search patterns:

- **Random**—The software randomly determines search pattern positions as data is acquired (see page 5-37).
- **Search pattern (.SP) file**—The software analyzes positions that correspond to specific x,y coordinates defined in the .SP file. For more information, see "Search pattern (.SP) files" below.

You specify the search pattern option in the Automatic Control Settings of the Instrument Settings control page. See Section 5.2.3, Automatic Control Dialog Box, for more information.

NOTE: *Before using search pattern files created in version 4 software, convert to version 5 format. See Section 5.5, Converting Version 4 Methods and Search Pattern Files.*

Search pattern (.SP) files

You can create customized search pattern files or use the DEFAULT.SP or SPIRAL.SP files provided (for 100-position plates). For details, see "Creating and Editing .SP Files Using the Search Pattern Editor" on page 6-50.

A search pattern file is an ASCII text file that contains a list of relative X,Y position pairs with respect to the center of the current sample position measured in microns, that represent points in the sample position.

On a 100-position plate, a sample position is 2,540 µm in diameter with the origin (0,0) at the center of the position. The centers of the sample positions are 5,080 µm apart. Figure 6-17 illustrates the location of different coordinates.

The system sequentially reads each entry in the search pattern file as it analyzes a sample. The first entry in the file is the first position analyzed, the second entry is the second position analyzed, and so on.

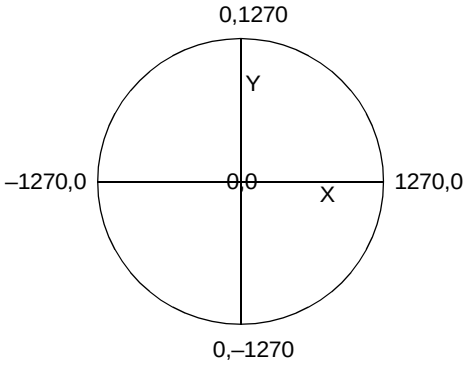


Figure 6-17 Location of Coordinates in a Sample Position on a 100-Position Plate

Units in search pattern files

For Voyager software version 5.0 and later, the default units for X,Y coordinates in search pattern files is microns. For pre-5.0 Voyager software, the default units for X, Y coordinates is steps.

Before using search pattern files created in version 4 software, convert to version 5 format. See Section 5.5, Converting Version 4 Methods and Search Pattern Files.

Default search pattern file

The default search pattern file for a 100-position plate (DEFAULT.SP) causes a serpentine crossing of the sample position determined by the following 20 X,Y coordinates:

	X coordinate (µm)	Y coordinate (µm)		X coordinate (µm)	Y coordinate (µm)
1.	–952.5	158.75	11.	–635	–635
2.	–635	317.5	12.	–238.125	–396.875
3.	–317.5	476.25	13.	158.75	–238.125
4.	0	635	14.	476.25	0
5.	317.5	793.75	15.	873.125	238.125
6.	635	635	16.	1031.875	–158.75
7.	317.5	396.875	17.	635	–396.875
8.	0	158.75	18.	317.5	–555.625
9.	–396.875	–79.375	19.	0	–714.375
10.	–873.125	–317.5	20.	–317.5	–952.5

Spiral search pattern file

The SPIRAL.SP file provided is a 20-point search pattern that begins searching at the center of the sample position and spirals outward. This is the best search pattern for uneven matrix crystals.

Search pattern for custom plates

If you create a custom plate type for a plate without laser-etched sample position or wells, with position diameter larger or smaller than 2,540 microns, create an .SP file for analysis of the plate. The DEFAULT.SP file is set to analyze 2,540-micron diameter positions, corresponding to standard sample positions.

For more information, see “Guidelines for creating .SP files” on page 3-73.

6

6.6.5 Creating and Editing .SP Files Using the Search Pattern Editor

This section includes:

- Creating or editing an .SP file
- Using the Search Pattern Generator
- Drawing a search pattern
- Setting x, y coordinates

Creating or editing an .SP file

To create or edit an .SP file:

1. In the Instrument Control Panel:
   - Select **Select Sample Plate** from the Sample Plate menu.
   - Select the Plate ID associated with the .PLT file for which you are creating the .SP file, then click **OK**.
2. From the Tools menu, select **Search Pattern Editor**. The Search Pattern Editor opens (Figure 6-18) displaying:
   - Pathname of the .PLT file (selected in the Instrument Control Panel) in the title bar.
   - Sample position area (size and shape defined by the .PLT file).
   - Coordinates of the cursor position.

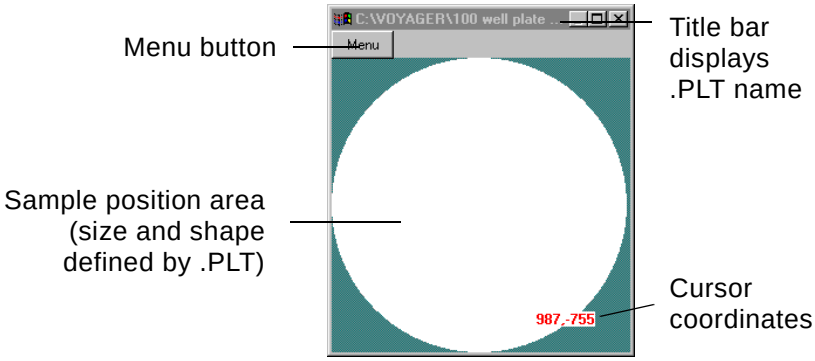


Figure 6-18 Search Pattern Editor

3. If you are creating a new .SP file, skip to step 4.

   If you are editing an .SP file, click **Menu**, select **Open**, then select an .SP file.

   NOTE: *Instead of clicking Menu, you can right-click to display the list of available commands.*

   ---

4. Create or edit the search pattern in any of the following ways:

   - Use the Search Pattern Generator to automatically generate a search pattern. See “Using the Search Pattern Generator” on page 6-52.
   - Draw a new search pattern using the mouse. See “Drawing a search pattern” on page 6-56.
   - Edit or add spots by setting x,y coordinates. See “Setting x, y coordinates” on page 6-57.

5. To add and store descriptive comments in the new .SP file, click **Menu**, then select **Properties**. Type text that describes or identifies this particular search pattern.

6. To display or suppress numbers and lines displayed in the area, click **Menu**, then select **Numbers** or **Lines**.

NOTE: *If the search pattern contains a large number of spots, turn off lines to more easily see the spot positions.*

---

7. Click **Menu**, then select **Save** or **Save As**.

Using the Search Pattern Generator

To automatically generate a search pattern:

1. Click **Menu**, then select **Generate Search Pattern** to open the Generate Search Pattern dialog box (Figure 6-19).

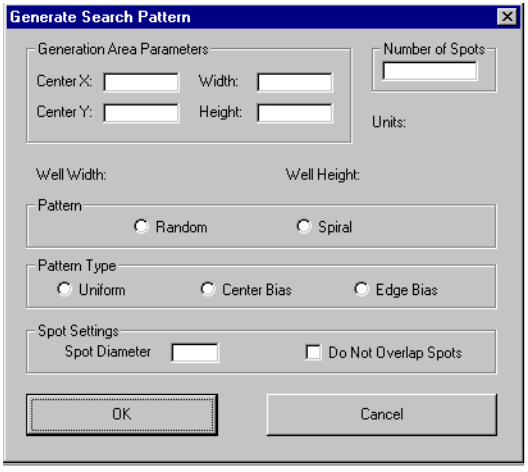


Figure 6-19 Generate Search Pattern Dialog Box

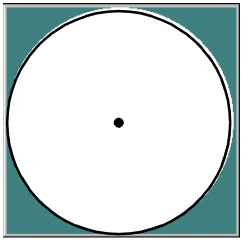
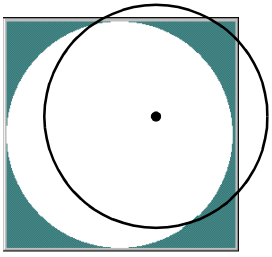
2. Enter the following parameters:

Table 6-4 Search Pattern Generator Parameters

Parameter	Description
Generation Area Parameters	Type values for the parameters that define the center coordinate of the search pattern and the area in which the search pattern is generated (generation area): • **Center X** and **Center Y**—Coordinates that define the center of the search pattern. The default values of 0 set the center of the generation area to the center of the sample position area. • **Width** and **Height**—Set the width and height of the generation area. If you are creating a new .SP file, units are in microns. If you are editing an .SP file, units are based on the units in the existing .SP file. Default values are the width and height of the sample position as defined in the .PLT file. If you set Center X, Center Y, Width, and Height values so that any part of the generation area falls outside the sample position area (defined in the .PLT file), an error message is displayed when you generate the pattern, and spots that fall outside the sample position area are not created (see Figure 6-20). ***NOTE:*** *Center X,Y and generation area are not shown on the screen.*

continued

Table 6-4 Search Pattern Generator Parameters (Continued)

Parameter	Description
Generation Area Parameters *(continued)*	Generation area overlaps sample position area  Center XY=0,0 Width, Height=2540, 2540 Generation area falls outside sample position area  No spots created outside sample position area Center XY=100,100 Width, Height=2540, 2540 ***Figure 6-20 Impact of Changing Center X,Y***
Number of Spots	Type the number of spots (up to 10,000) you want the search pattern to generate automatically. You can add or insert more spots in a search pattern manually as described in “Drawing a search pattern” on page 6-56 and “Setting x, y coordinates” on page 6-57.
Units (display only)	Displays the units for the .SP file. Default is microns. New .SP files you create with the Search Pattern Editor default to micron units. Existing .SP files you edit retain their original units (microns or steps).
Well Width Well Height (display only)	Displays the width and height dimensions of the sample position retrieved from the loaded .PLT file. Default units are steps if you display this dialog box when no .SP file is open. If you display this dialog box when an .SP file is open, Well Width and Well Height values are displayed in the units that correspond to the open .SP file.

continued

Table 6-4 Search Pattern Generator Parameters (Continued)

Parameter	Description
Pattern	Select a generation method: • **Random**—Generates randomly placed spots within the generation area. • **Spiral**—Generates spots within the generation area that spiral out from the Center X, Y coordinates specified.
Pattern Type	Select a distribution method: • **Uniform**—Evenly distributes spots in the generation area. • **Center Bias**—Clusters spots around the center of the generation area. • **Edge Bias**—Clusters spots around the edge of the generation area.
	continued
Spot Settings	Determines whether or not spots overlap: • **Spot Diameter**—Defines the size of the spots. Default value is 50 microns (30 steps). • **Do Not Overlap Spots**—When enabled, prevents spots from overlapping. If the Number of Spots specified of the size specified by the Laser Spot Diameter cannot fit in the generation area when this option is enabled, an error message is displayed when you generate the pattern, and only the spots that fit in the generation area without overlapping are created.

3. Click **OK**. The search pattern spots are displayed in the sample position area.
4. Use the drawing tools to adjust and refine the search pattern if necessary. For details, see “Drawing a search pattern” on page 6-56.

Drawing a search pattern

Use the drawing tools in the sample position area to add, insert, edit, or delete a spot.

When you select a drawing tool, it remains selected and active until you select another tool or another menu item.

To	Click Menu, then select	Cursor changes to	Then
Add a spot	Add spot		Click on the sample position at the location where you want to add a spot. If you click and hold when you add, you can adjust the position of the spot before it is added.
Insert a spot	Insert spot		Move the cursor to the location within the existing chain of spots where you want to insert another spot, then right-click.
Move a spot	Edit spot		Place the cursor on the spot you want to move, then click and drag the spot to its new location within the sample position area.
Delete a spot	Delete spot		Right-click the mouse button on the spot you want to delete.
Delete all spots	Delete all spots	———	All spots are deleted.

Setting x, y coordinates

To set x, y coordinates for a spot:

1. Click **Menu**, then select **X,Y**. The Set X,Y dialog box is displayed (Figure 6-21).

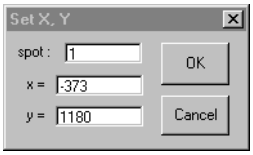


Figure 6-21 Set X,Y Dialog Box

The x,y values displayed correspond to the location of the cursor in the sample position area when you select the X,Y command, not to the spot number displayed in the Spot field.

2. In the Spot field, type a number:
   - If you type a spot number that exists, the spot is edited.
   - If you type a new spot number, the spot is added.
3. Type x and y coordinates for the spot.

   Valid x and y coordinate values correspond to approximately one-half the dimensions of the sample position of the currently loaded or selected plate. For example, the maximum x or y value for a 2540H by 2540W sample position is approximately ±1270.
4. Click **OK**.

6

6.6.6 Process that Occurs During Acquisition in Automatic Mode

NOTE: *This process occurs when you acquire using a .BIC file that has Use Automated Laser Intensity Adjustment enabled.*

Checking disk space

Before starting a run that uses automated data evaluation (described on “Setting spectrum acceptance and laser adjustment criteria” on page 6-42), the system checks that at least 100 MB of disk space is available.

NOTE: *If the run requires more than 100 MB for data file storage, a message is displayed during the run when free disk space is no longer available.*

Prescan mode

Prescan mode allows the system to determine the appropriate laser setting for a sample position before acquiring spectra. This feature minimizes sample loss. Prescan mode does not consider signal-to-noise ratio or resolution when determining laser setting.

You can select one of the following Prescan mode choices:

- At First Search Pattern Position Only
- At Each Search Pattern Position

NOTE: *If Prescan is not selected, the system operates in Acquisition mode. See “Acquisition mode” on page 6-61.*

Shots/Spectrum in Prescan mode

Each time the system begins acquiring in Prescan mode, it acquires five Shots/Spectrum, or the number of Shots/Spectrum specified in the Instrument Settings control page, if less than five.

During Prescan

In Prescan mode, the system does the following:

1. The system sets the laser to the maximum setting specified in the Automatic Control dialog box and acquires a spectrum (see “Shots/Spectrum in Prescan mode” on page 6-58) at the first point specified in the search pattern.

   If the signal intensity is:

   - **Too low** (lower than the minimum signal intensity set in the Spectrum Acceptance Criteria dialog box) with the laser at maximum—The system moves to the next position specified in the search pattern and acquires again.
   - **Too high** (higher than the maximum signal intensity set in the Spectrum Acceptance Criteria dialog box) with the laser at maximum—The system continues with step 2.
   - **Within range**—The system begins acquiring in Acquisition mode. See “Acquisition mode” on page 6-61.

NOTE: *If Use Automated Sample Positioning is disabled, the system always uses the center of the sample position.*

NOTE: *If no minimum or maximum signal intensity criteria is specified, the laser is not adjusted. The mid-range laser setting specified in the Automatic Control dialog box is used.*

---

2. The system sets the laser to the minimum setting specified in the Automatic Control dialog box and acquires a spectrum (see “Shots/Spectrum in Prescan mode” on page 6-58).

   If the signal intensity is:

   - **Too low with the laser at minimum**—The system continues with step 3.
   - **Too high with the laser at minimum**—The system begins acquiring in Acquisition mode.
   - **Within range**—The system begins acquiring in Acquisition mode.

3. The system sets the laser halfway between the minimum and maximum and acquires a spectrum (see “Shots/Spectrum in Prescan mode” on page 6-58).

   For the first spectrum, minimum and maximum laser settings are taken from the Automatic Control dialog box.

   For subsequent spectra, minimum and maximum laser settings are determined as described below:

   - If the signal is too low, the current setting is used as the new minimum setting.
   - If the signal is too high, the current setting is used as the new maximum setting.

4. The system repeats step 3 until either:

   - The signal falls between the specified maximum and minimum values.
   - The difference between the maximum and minimum laser intensity in step 3 is smaller than 2 times the laser step size specified in the Automatic Control dialog box.

Acquisition mode

Acquisition mode starts after Prescan mode determines the laser setting, or immediately if Prescan mode is disabled.

NOTE: *Each time the system begins acquiring in Acquisition mode, it acquires the number of Shots/Spectrum specified in the Instrument Settings control page.*

In Acquisition mode, the system does the following:

1. The system sets the laser to one of the following and acquires spectra:

   - **If Prescan At First Search Pattern Position Only is enabled**—Uses the laser setting and search pattern position determined in “Prescan mode” on page 6-58 and acquires spectra.
   - **If Prescan At Each Search Pattern Position is enabled**—Uses the laser setting and search pattern position determined in “Prescan mode” on page 6-58 and acquires spectra.
   - **If Prescan mode is not enabled**—Sets the laser halfway between the minimum and maximum specified in the Automatic Control Settings dialog box. Starts acquiring data at the first position specified in the search pattern.

   If the signal intensity is:

   - **Too high**—The system decreases the laser setting one laser step as specified in Automatic Control dialog box, and acquires a new spectrum.
   - **Too low**—The system increases the laser setting one laser step as specified in Automatic Control dialog box, and acquires a new spectrum.

NOTE: *If no minimum or maximum signal intensity criteria is specified, the laser is not adjusted. The mid-range laser setting specified in the Automatic Control dialog box is used.*

- **Within range**—The system saves the data file if Signal-to-Noise is not enabled, or continues with step 2 if Signal-to-Noise is enabled.

2. If Signal-to-Noise is enabled in the Spectrum Acceptance Criteria dialog box, the system evaluates the signal-to-noise ratio for each spectrum.

   If the signal-to-noise ratio is:

   - **Equal to or greater than the ratio set in the Spectrum Acceptance Criteria dialog box**—The system checks resolution as described in step 3, if it is enabled. If resolution is not enabled, the system saves the data file.
   - **Less than the ratio set in the Spectrum Acceptance Criteria dialog box**—The system increases the laser setting one laser step as specified in instrument settings, and acquires a new spectrum.

3. If Resolution is enabled in the Spectrum Acceptance Criteria dialog box, the system evaluates the resolution for each spectrum.

   If the resolution is:

   - **Equal to or greater than the resolution set in the Spectrum Acceptance Criteria dialog box**—The system saves the data file.
   - **Less than the resolution set in the Spectrum Acceptance Criteria dialog box**—The system acquires a new spectrum.

4. If Save All or Accumulate All is selected for accumulation, the system moves to the next search pattern position.

   For all other accumulation conditions, the system repeats step 1 through step 3 until one of the following is true:

   - Acceptance criteria selected are met (the signal is in range, the minimum Signal-to-Noise ratio and resolution are achieved).
   - The specified Number of Spectra to Acquire are acquired.
   - The difference between the maximum and minimum laser intensity is smaller than the laser step size specified in Automatic Control dialog box, and the laser can no longer be adjusted.
   - Any stop conditions are met.
   - The end of the search pattern file is reached.

   NOTE: *If the number of positions in the search pattern file is lower than the specified number of spectra to acquire, acquisition stops before the total number of spectra specified are acquired. For example, if you specify 5 spectra to acquire, but the search pattern file specifies 4 positions, acquisition stops after the fourth position is sampled, even if 5 spectra have not yet been acquired.*

   ---

5. The system saves data files based on the Spectrum Accumulation conditions selected. Acceptance criteria must be met for all collection modes except Save All and Accumulate All. See Section 5.2.4, Description of Spectrum Accumulation Options, for a description of the files saved in each Spectrum Accumulation condition.

6. If an accumulation condition that uses Acceptance Criteria is selected, and Acceptance Criteria are met, the system begins a new acquisition from the current search pattern position.

   If Acceptance Criteria are not met, if an accumulation condition that does not use Acceptance Criteria is selected, or if the laser can no longer be adjusted, the system begins a new acquisition from the next search pattern position, depending on the Spectrum Accumulation conditions and Spectrum Acceptance Criteria. Acceptance criteria are defined in “Setting spectrum acceptance and laser adjustment criteria” on page 6-42.

   - If collection mode is set to Save First Passing and an acceptable spectrum is collected, the system saves the data file, and the run is complete.
   - If acceptance criteria are not met, or collection mode is set to Save All**,** Save All Passing, or Save Best, the acquisition sequence is repeated at the next position specified in the random search pattern or the search pattern file.

7. If the system moves across an entire sample position and does not find an acceptable signal, it:

   - Logs the error in the Automatic Control tab in the Output window of the Instrument Control Panel.
   - Does not create a data file.

6.6.7 Process that Occurs when Accumulating Spectra from Multiple Search Pattern Positions

NOTE: *These processes occur when you acquire using a .BIC file that has Use Automated Sample Positioning enabled.*

You can obtain a single spectrum from multiple positions within a single sample position by specifying a search pattern. To do so, enable the Automatic Control mode in Instrument Settings control page and select one of the Spectrum Accumulation conditions described below.

For information on the Automatic Laser/Sample Positioning/Spectrum Accumulation function, see:

- Section 5.2.3, Automatic Control Dialog Box
- Section 6.6, Acquiring in Automatic Mode from the Instrument Control Panel

For a description of a search pattern file, see Section 6.6.4, Search Patterns.

Two options are available for accumulating spectra from multiple search pattern positions:

- **Accumulate all**—Averages the acquired spectra regardless of their quality. A single spectrum is created. See Section 5.2.3, Automatic Control Dialog Box, for more information.
- **Accumulate all passing**—Evaluates the acquired spectra against the Acceptance Criteria specified, then averages only passing spectra. See Section 5.2.3, Automatic Control Dialog Box, for more information.

NOTE: *If no Acceptance Criteria are specified, all spectra are averaged*.

6.6.7.1 Process that Occurs when Accumulating All Spectra

When accumulating all spectra (Accumulation mode is determined by the Spectrum Accumulation conditions described in Section 5.2.3, Automatic Control Dialog Box), the system does the following during acquisition:

1. Sets the laser intensity as described in Section 6.6.6, Process that Occurs During Acquisition in Automatic Mode.
2. Starts firing the laser.
3. Acquires the specified number of Shots/Spectrum at the first search pattern position.
4. Stops firing the laser.
5. Holds the data in memory, and averages current data with previous data.
6. Moves to the next search pattern position.
7. Repeats step 2 through step 6 in subsequent search pattern positions until any of the following is true:
   - Number of spectra to acquire that you select in the Automatic Control settings is reached
   - All search pattern positions have been scanned
   - Stop conditions are met
8. Saves the averaged spectrum to disk in the directory designated in the Data Storage control page, described on page 6-14.

6.6.7.2 Process that Occurs when Accumulating Passing Spectra

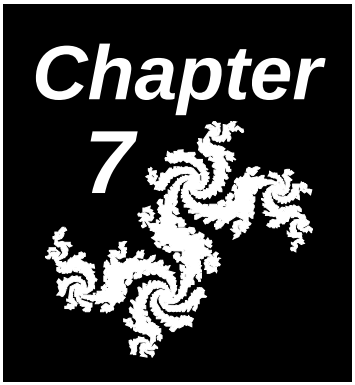
When accumulating only the spectra that meet the Acceptance Criteria (Accumulation mode is determined by the Spectrum Accumulation conditions described in Section 5.2.3, Automatic Control Dialog Box), the system does the following during acquisition:

1. Sets the laser intensity as described in Section 6.6.6, Process that Occurs During Acquisition in Automatic Mode.
2. Starts firing the laser.
3. Acquires the number of Shots/Spectrum at the first search pattern position.
4. Stops firing the laser.
5. Evaluates the data against the Acceptance Criteria in Section 5.2.3, Automatic Control Dialog Box.
6. If the data meets the Acceptance Criteria, holds the data in memory and averages current data with previous data. If it fails, the system displays an error message in the Output window describing which spectrum failed, and which acceptance criteria specified have failed. The data is discarded.
7. If the data meets the Acceptance Criteria, stays on the same search pattern position.

   If the data fails the Acceptance Criteria, moves to the next search pattern position.

8. Repeats step 2 through step 7 in subsequent search pattern positions until any of the following is true:
   - Number of spectra to acquire that you select in the Automatic Control dialog box is reached
   - All search pattern positions have been scanned
   - Stop conditions are met
9. Saves the averaged spectrum to disk in the directory designated in the Data Storage control page, described in “Setting Data Storage parameters” on page 6-14.

7 Acquiring Spectra from the Sequence Control Panel



This chapter contains the following sections:

7.1 Overview 7-2

7.2 Understanding Settings, Macros, and Calibration 7-3

7.3 Before Creating a Sequence 7-7

7.4 Creating a Sequence 7-13

7.5 Preparing to Run a Sequence 7-24

7.6 Running a Sequence 7-25

7.7 Automatic Calibration During a Sequence Run 7-32

7.8 Customizing the Sequence Control Panel 7-43

7.1 Overview

The Voyager Sequence Control Panel (Figure 7-1) allows:

- Acquisition of multiple samples using different instrument settings (.BIC) files
- Selection of macros for advanced processing to apply before or after calibration. You can use the macros supplied or create your own in the Data Explorer software.
- External, internal, and internal-update calibration options, described in Section 7.7, Automatic Calibration During a Sequence Run.

Before you begin

Before you begin, read:

- "Starting Sequence Control Panel" on page 2-33
- Section 4.6, Sequence Control Panel
- Section 4.7, How the Instrument and Sequence Control Panels Interact

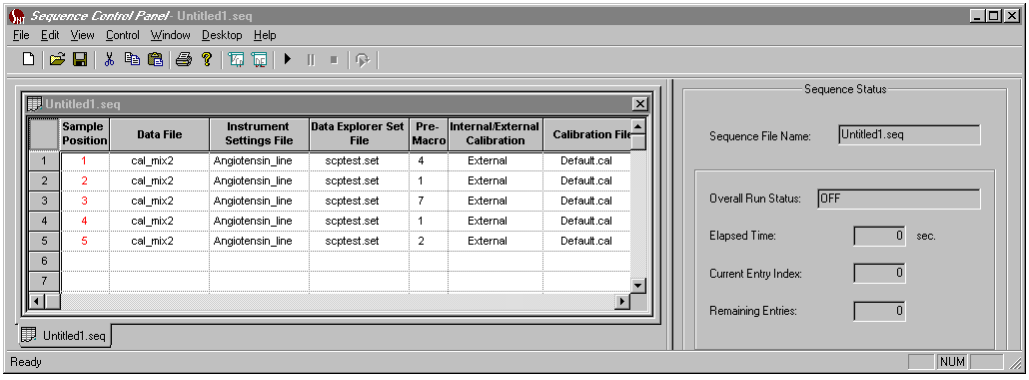


Figure 7-1 Sequence Control Panel

7.2 Understanding Settings, Macros, and Calibration

This section includes:

- Types of files and calibration you specify in the Sequence Control Panel
- How files and calibration you specify affect the data during a sequence run

Types of files and calibration

You select the following types of files and the Calibration Type in the Sequence Control Panel to specify how data is detected, calibrated, and processed:

File Type	Description	For information see
Data Explorer .SET	Contains peak detection parameters, monoisotopic peak filtering, and the reference masses needed for Internal or Internal-update calibration. Optional if you are not calibrating, or are performing External calibration.	• "Creating Processing Settings (.SET) Files" on page 7-12 • "Data Explorer .SET File" on page 7-17
Macro	Optional selections that allow you to specify processing before and after calibration.	• "Creating Macros" on page 7-8 • "Pre-Macro" on page 7-17 • "Post-Macro" on page 7-20
Calibration Type	Specifies External, Internal, or Internal-Update calibration.	"Internal/External Calibration" on page 7-18

Continued

File Type	Description	For information see
.CAL	Contain calibration constants used according to specified Calibration Type: • **External calibration**—Constants are applied and saved in a .DAT file.* • **Internal calibration (.CAL optional)**—If a .CAL file is specified (and it exists), constants are applied to the .DAT file* before the reference masses in the .SET file are matched. New calibration constants are generated using the reference masses specified in the .SET file, and applied to the data. Constants are then updated within the .DAT file after calibration. • **Internal-Update calibration**—If a .CAL file is specified (and it exists), constants are applied to the .DAT file* before the reference masses in the .SET file are matched. New calibration constants are generated using the reference masses specified in the .SET file, and applied to the data. Constants are then updated within the .DAT file after calibration. The specified .CAL file is updated or created and contains new calibration constants after calibration.	• "Creating Calibration (.CAL) Files" on page 7-11 • "Calibration File" on page 7-19

**** NOTE:** If the .DAT file contains multiple spectra generated using the Save All or the Save All Passing option in Automatic Control, the .CAL file is applied to all spectra in the .DAT file.*

How file types and calibration you specify affect the data

Figure 7-2 and Figure 7-3 illustrate how the type of calibration and the files you specify affect the data that is acquired in a sequence run.

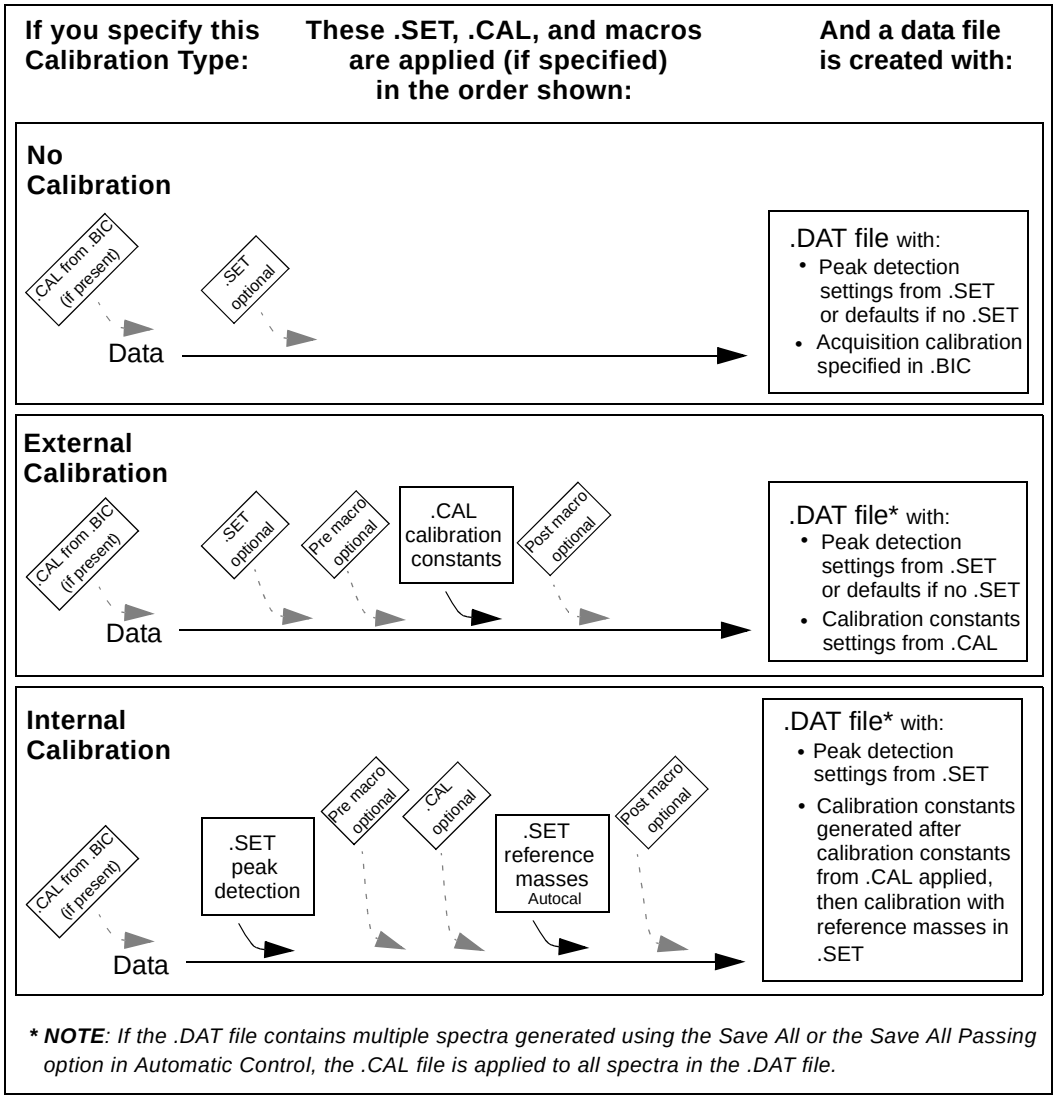
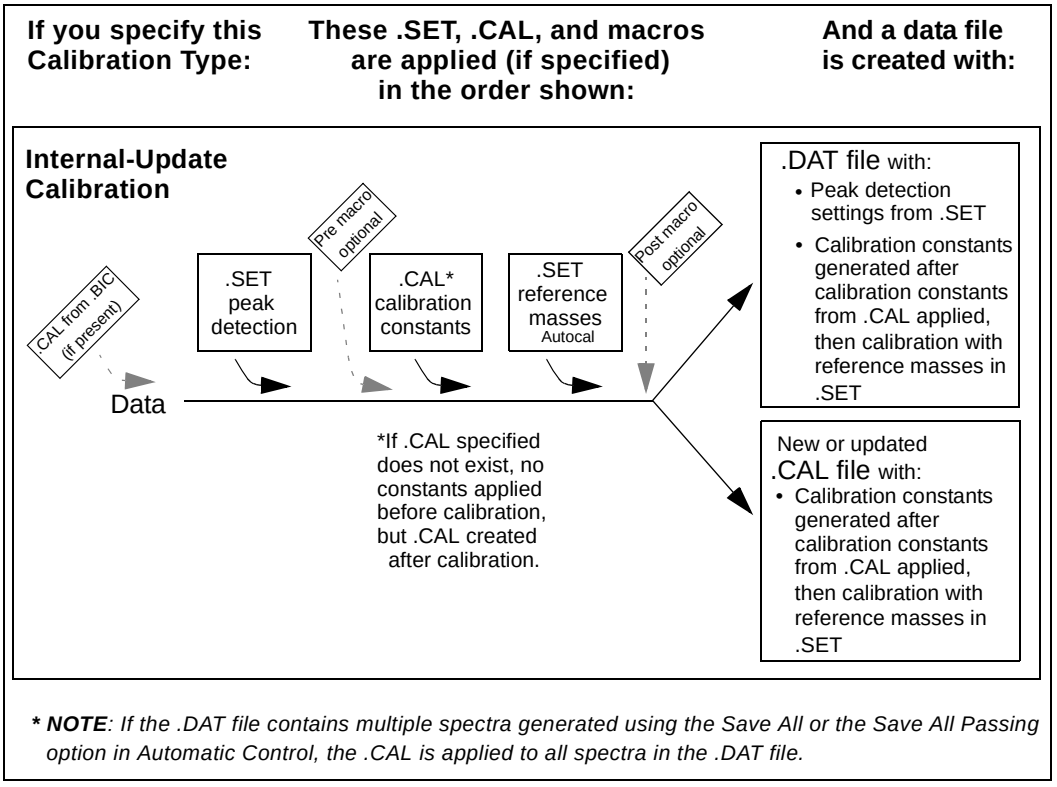


Figure 7-2 How Settings, Macros, and Calibration Are Applied During a Sequence Run—Part 1



**** NOTE**: If the .DAT file contains multiple spectra generated using the Save All or the Save All Passing option in Automatic Control, the .CAL is applied to all spectra in the .DAT file.*

Figure 7-3 How Settings, Macros, and Calibration are Applied During a Sequence Run—Part 2

7.3 Before Creating a Sequence

This section describes:

- Optimizing instrument settings (.BIC) files for a sequence run
- Creating macros
- Creating calibration (.CAL) files
- Creating settings (.SET) files

7.3.1 Optimizing Instrument Settings (.BIC) Files for a Sequence Run

Select .BIC files based on the compound type and mass range you are analyzing. See Section 5.1.2, Standard Instrument Settings (.BIC) Files Provided.

You can use more than one .BIC file in your sequence run if you are analyzing a wide mass range, for example, when analyzing a mixture of protein and peptide samples. As a general guideline, you may need separate .BIC files for different mass ranges, for example:

- 1,000 to 6,000 Da
- 5,000 to 12,000 Da
- 10,000 to 30,000 Da

.BIC requirements

Optimize the instrument settings for Automatic Control mode. All .BIC files used in the Sequence Control Panel must specify:

- Automatic Control mode
- Linear or reflector Operation mode (PSD mode is not allowed)
- The same laser type (internal or external). You can not switch between internal and external lasers when running a sequence.

For more information see Section 6.6.2, Setting Instrument Settings for Automatic Control Mode.

Note the following:

- If Use Automated Laser Intensity Adjustment is disabled in the .BIC file, the laser intensity is not automatically adjusted. Instead, the intensity set in the Instrument Control Panel is used for the analysis. You can manually adjust the laser intensity by pressing **Ctrl**+**PageUp** or **Ctrl**+**PageDn**.
- If Use Automated Sample Positioning is disabled in the .BIC file, the sample does not automatically move and the analysis is performed on the center of the sample position unless you manually adjust the position using the control stick.

7.3.2 Creating Macros

You can specify macros that execute before and after calibration. You can use the macros supplied with the system or create your own.

For information on using the Visual Basic Editor, refer to the online help available within the Visual Basic Editor.

Creating a macro

To create a macro for the Sequence Control Panel:

1. Open the Data Explorer software.
2. Create a macro as described in the *Data Explorer Software User's Guide*, Section 6.7.2, Recording a Macro.

NOTE: *If you create a macro to apply a SET file, there are two variables you can use:*
RestorePreferences2*, which applies* ***either*** *processing or graphic settings (syntax: ActiveDocument.RestorePreferences2 "path and name of SET file", deProcessingSettings (or deGraphicsSettings).*
RestorePreferences*, which applies* ***both*** *processing and graphic settings (syntax: ActiveDocument.RestorePreferences2 "path and name of SET file".*
If you use RestorePreferences, make sure the SET file you apply contains both processing and graphic settings. If it does not, the system applies default graphic settings (black background with yellow traces) when the macro runs.

---

3. If desired, assign the macro to a button as described in the *Data Explorer Software User's Guide*, Section 6.7.3, Assigning Macros to Buttons.

   The button number to which you assign the macro is the number that you select in the Sequence Control Panel.

NOTE: *Assigning a macro to a button is not required. You can select a macro by name or number in the sample list.*

Selecting a macro in a sequence

NOTE: *Do not select in the Sequence Control Panel macros that require user action (macros in which you must enter or select values, then click OK to execute). If you select macros that require user action, the sequence stops when the macro is activated by the Sequence Control Panel, and waits for you to enter values and click OK.*

To select a macro in the sample list:

1. Create a sequence as described in Section 7.4, Creating a Sequence.
2. In the macro field for each row, select a macro by doing either of the following:
   - Click the field and select the macro name from the list. All macros and scripts you have created in the Data Explorer software are listed.
   - Type the button number to which you assigned the macro.

   Hint: *To determine the macro assigned to the button number, display the Data Explorer software. Place the cursor over the macro button to display the macro name. Macro buttons are numbered sequentially from left to right. If the Macro toolbar is not displayed in the Data Explorer software, select* ***Toolbar*** *from the View menu, select* ***Macros****, then click* ***Close****.*
3. Save the sequence file by selecting **Save Sequence** from the File menu.

Recommended Pre-Macro

To ensure that the correct peak is identified as the monoisotopic peak before peak matching/calibration, create a macro that performs peak deisotoping. Specify this macro in the Pre-Macro column in the Sequence. For more information, see the *Data Explorer Software User's Guide*, Section 3.4, Deisotoping a Spectrum.

Macros for multispectrum data files

To perform processing on data files that contain multiple Voyager spectra, you must create advanced macros. Figure 7-4 is an example macro that deisotopes and prints all spectra in a multispectrum Voyager data file. Standard macros created with the macro recorder process only the first spectrum in a data file.


```
Sub MultiSpecProcessing()
    Dim NumSpectra As Long
    NumSpectra = ActiveDocument.SpecView.TotalSpectrum
    Dim J As Long
    For J = 1 To NumSpectra
        ActiveDocument.SpecView.ActiveSpectrum = J
        ActiveDocument.SpecSetup.DeisotopeAdductFormula = "H"
        ActiveDocument.SpecSetup.DeisotopeBaseFormula = "C6H5NO"
        ActiveDocument.SpecView.DeisotopeSpectrum
        ActiveDocument.SpecView.PrintTrace
    Next J
End Sub
```

Insert per spectrum processing here

Figure 7-4 Example Macro to Deisotope and Print a Data File Containing Multiple Voyager Spectra

7.3.3 Creating Calibration (.CAL) Files

Create calibration (.CAL) files in the Data Explorer software if you plan to specify calibration in the Sequence Control Panel:

- **External calibration**—.CAL file required.
- **Internal calibration**—.CAL file optional.
- **Internal-Update calibration**—.CAL file name required, but .CAL file does not have to exist. If the specified .CAL file exists, constants are applied before the reference masses in the .SET file are matched. If the specified .CAL file does not exist, no constants are applied. New calibration constants are generated using the reference masses specified in the .SET file and applied to the data file. Constants are updated within the .DAT file. The specified .CAL file is updated or created and contains new calibration constants after calibration.

For information, see the *Data Explorer Software User's Guide,* Section 5.3.2, Manually Calibrating.

7.3.4 Creating Processing Settings (.SET) Files

Overview

Create processing settings (.SET) files in the Data Explorer software if you plan to specify Internal or Internal-Update calibration, or if you want peak detection settings other than default settings stored with the data file.

The .SET file can also apply monoisotopic peak filtering.

NOTE: *If you do not specify a Pre-Macro that performs peak deisotoping, or you do not set monoisotopic peak filtering, set the Mass Tolerance for Reference Matching in Autocalibration settings narrow enough to ensure that the monoisotopic peak is correctly identified before calibration.*

Processing settings only

When you create the .SET file, save processing settings only. Graphic settings are not applied in the Sequence Control Panel, even if they are present in the .SET file.

For more information

For more information on .SET files, see the *Data Explorer Software User's Guide*:

- Section 1.4.2, Customizing Processing and Graphic Settings (.SET)
- Section 3.2.3, Setting Peak Detection Parameters
- Section 3.3.4, Sorting, Filtering, and Printing the Peak List
- Section 5.4.2, Importing and Specifying Automatic Calibration Settings

7.4 Creating a Sequence

This section includes:

- Setting General Sequence parameters
- Creating a run list

Parts of a sequence

A sequence includes:

- **General sequence parameters**—Apply to all entries in the run list, described in Section 7.4.1, Setting General Sequence Parameters.
- **Run list parameters**—Apply to individual entries in the run list, described in Section 7.4.2, Creating a Run List.

7.4.1 Setting General Sequence Parameters

Set parameters as needed:

1. From the View menu, select **General Sequence Parameters**.

   The General Sequence Parameters dialog box is displayed (Figure 7-5).

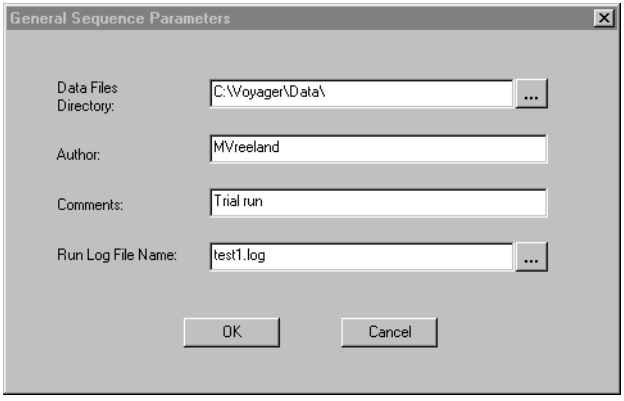


Figure 7-5 General Sequence Parameters Dialog Box

2. Type or select the Directory name in which to store the data files.
3. Enter text as needed for Author and Comments. This information is stored with the sequence (.SEQ) file.
4. Specify the Run Log file name (the default name is taken from the sequence name). If you want to store the run log in a directory other that the Directory specified above, click ... and select a directory for the file.

   The run log file contains the list of lines from the run list that were executed during the sequence, and any errors that occurred. If a line in the run list generated more than one data file, the log lines are duplicated and file names are incremented accordingly.
5. Click **OK**.

7.4.2 Creating a Run List

This section includes:

- Sample order in the run list
- Creating a run list
- Modifying and customizing the run list
- Filling down the sample position
- Importing and exporting the run list

Sample order in the run list

The type of calibration you perform may require a specific sample order in the run list:

- **Internal or external calibration**—List samples in any order.
- **Close external calibration (internal-update)**—List samples and standards as described in Section 7.7.3, Performing Close External Calibration.

Creating a run list

To create a new run list:

1. Select **New Sequence** from the File menu.

   A new sequence run list grid opens (Figure 7-6).

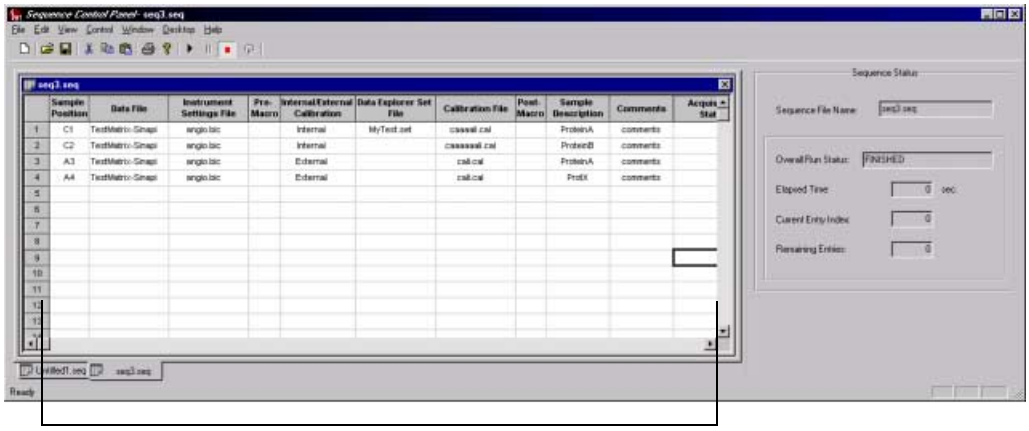


Figure 7-6 Sequence Run List

> ***Hint:*** *You can show and hide columns by selecting a column, then selecting* ***Show/Hide*** *from the View menu.*

2. Click the scroll bar at the bottom of the grid to display columns in the grid that are not in view.
3. Click a cell to activate it, then enter Run List parameters as described in Table 7-1.

   You can also import parameters into the run list. See page 7-22.

Table 7-1 Run List Parameters

Field	Description
Sample Position *(required entry)*	Position from which to acquire data.
Data File *(required entry)*	Base name of the data file to acquire. During acquisition, the software appends to the base name a 4-digit suffix starting at 0001. For example, if you specify a base name of SAMPLE, the final data file name is SAMPLE_0001.DAT. By default, the position name/number is also appended and precedes the 4-digit suffix. For example, if you specify a base name of SAMPLE, the final data file name is SAMPLE_A1_0001.DAT for the data file acquired from position A1. To suppress the position name/number in the file name, deselect **Append Sample Position to File Name** in Preferences (described on page 7-45).
Instrument Settings File *(required entry)*	.BIC file to use for the current row. Click the down arrow and select an instrument settings (.BIC) file that you have optimized for Automatic Control mode. For more information, see Section 6.6.2, Setting Instrument Settings for Automatic Control Mode. ***NOTE**: If you are editing a .BIC file, save it before selecting it in the Sequence Control Panel. If you make changes to the .BIC file after you select it in the Sequence Control Panel, the Sequence Control Panel does not recognize the changes.* ***NOTE:** All .BIC files specified in a sequence must specify either the internal laser or the external laser. You cannot switch between internal and external lasers when running a sequence. If you specify an internal laser .BIC file, then specify an external laser .BIC, the second .BIC is flagged as invalid when you try to run the sequence.*

continued

Table 7-1 Run List Parameters (Continued)

Field	Description
Data Explorer .SET File *(required entry for Internal and Internal-Update calibration)*	.SET file to use to detect peaks, apply monoisotopic peak filtering (if specified), and calibrate (if specified) the data file acquired in the row. The .SET file contains peak detection parameters and the reference masses needed for calibration. Click the down arrow and select a .SET file. If you do not specify a .SET file for rows that specify External or no calibration, the peak detection settings from the default .SET file (VOYAGERLINEAR.SET or VOYAGERREFLECTOR.SET) are stored in the data file. For information, see: • Section 7.2, Understanding Settings, Macros, and Calibration • “Creating Processing Settings (.SET) Files” on page 7-12
Pre-Macro	Macro to execute before calibration. Select a macro by doing either of the following: • Click the field and select the macro name from the list. All macros and scripts you created in the Data Explorer software are listed. • Type the button number to which you assigned the macro. For more information, see “Creating Macros” on page 7-8. ***Hint:*** *To display the macro name assigned to the button, display the Data Explorer software, then place the cursor over a macro button. Macro buttons are numbered sequentially from left to right. If the Macro toolbar is not displayed in the Data Explorer software, select* ***Toolbar*** *from the View menu, select* ***Macros****, then click* ***Close****.*

continued

Table 7-1 Run List Parameters (Continued)

Field	Description
Internal/External Calibration	Calibration type to use for the row. Click the down arrow and select one of the following: • **Blank**—No calibration is applied during processing. Acquisition calibration (calibration specified in the .BIC file used to acquire) is maintained. • **External**—Applies the constants in the specified .CAL file to the data file acquired in the row. • **Internal**—If a .CAL file is specified (and it exists), constants are applied to the .DAT file before the reference masses in the .SET file are matched. New calibration constants are generated using the reference masses specified in the .SET file, and applied to the data. Constants are then updated within the .DAT file after calibration. For more information, see Section 7.7.4, Internal Standard Calibration Considerations. • **Internal-Update**—If a .CAL file is specified (and it exists), constants are applied to the .DAT file before the reference masses in the .SET file are matched. New calibration constants are generated using the reference masses specified in the .SET file, and applied to the data. Constants are then updated within the .DAT file after calibration. The specified .CAL file is updated or created and contains new calibration constants after calibration. For more information, see Section 7.7.3, Performing Close External Calibration. For information on .CAL files, see: • Section 7.2, Understanding Settings, Macros, and Calibration • “Creating Calibration (.CAL) Files” on page 7-11 ***NOTE:*** *The calibration specified in the Sequence Control Panel is performed after the calibration performed by the .BIC file used to acquire the data file, and the data file is updated with the calibration performed in the Sequence Control Panel.*

continued

Table 7-1 Run List Parameters (Continued)

Field	Description
Internal/External Calibration *(continued)*	***NOTE:*** *If the .DAT file contains multiple spectra generated using the Save All or the Save All Passing option in Automatic Control, the calibration is applied to all spectra in the .DAT file.*
Calibration File *(required entry for External and Internal-Update calibration; optional entry for Internal calibration)*	.CAL file to use to calibrate the data file acquired in the row. If you specify External or Internal-Update, a .CAL file name is required, even if a corresponding .CAL file with constants does not exist (see below for further explanation). You can specify an existing .CAL file or the name of a .CAL file that will be created by a previous line in the Sequence. The way the .CAL file is used depends on the calibration type: • **External**—Constants are applied. • **Internal**—If a .CAL file is specified, constants are applied. • **Internal-update**—If the specified .CAL file exists, constants are applied before calibration, then the .CAL file is *updated* with new constants after calibration. If the specified .CAL file does not exist, no constants are applied before calibration, and the .CAL file is *created* containing new constants after calibration. ***NOTE:*** *If the specified.CAL file for Internal-Update does not exist, the software creates the .CAL file during processing in the path you specify. If you do not specify a path, the software creates the .CAL file in the C:\VOYAGER directory.* ***NOTE:*** *If the .BIC file specifies a .CAL file, the .CAL file in the .BIC file is not updated, unless it is the same .CAL file specified in the Sequence Control Panel.* For information on how .CAL files are used in the Sequence Control Panel, see Section 7.2, Understanding Settings, Macros, and Calibration.

continued

Table 7-1 Run List Parameters (Continued)

Field	Description
Post-Macro	Macro to execute after calibration. Select a macro by doing either of the following: • Click the field and select the macro name from the list. All macros and scripts you created in the Data Explorer software are listed. • Type the button number to which you assigned the macro. For more information, see “Creating Macros” on page 7-8. ***Hint:*** *Display the Data Explorer software, then place the cursor over a macro button to display the macro name assigned to the button. Macro buttons are numbered sequentially from left to right. If the Macro toolbar is not displayed, select* ***Toolbar*** *from the View menu, select* ***Macros****, then click* ***Close****.*
Sample Description *(optional)*	Text that describes the sample.
Comments *(optional)*	Additional descriptive text.
Acquisition Status	Displays the status of the current acquisition in the run list. For information, see “Checking the Acquisition Status field” on page 7-29.
Run	If checked, the row is acquired when the sequence runs. If it is not checked, the row is skipped.

4. When all necessary rows are filled in, save the sequence by selecting **Save Sequence** from the File menu.

   The run list is saved as part of the .SEQ file.

Modifying and customizing the run list

You can modify and customize the run list using the following commands on the Edit menu:

- **Cut**, **Copy**, **Paste**—Use to cut, copy, and paste information.
- **Insert Row**, **Insert Multiple Rows**, **Delete Row**—Use to insert and delete rows.
- **Fill Down**—Use to automatically fill in run list grid entries. Click-drag to select the rows to fill then select **Fill Down** from the Edit menu. The first entry selected is copied to all selected rows below.

Filling down the sample position

You can set the Sample Position field to fill down in two ways:

- Copy the first sample position to all selected rows below, by click-dragging to select the rows to fill, then selecting **Fill Down** from the Edit menu.
- Increment sample positions in all selected rows below based on the type of sample plate loaded in the Instrument Control Panel, as described below.

Incrementing sample positions

To increment sample positions when filling down:

1. In the Instrument Control Panel, select **Select Sample Plate** from the Sample Plate menu, then select the Sample Plate you will use for the sequence. For more information, see Section 3.4.4, Loading Sample Plates.
2. In the Sequence Control Panel, select **Preferences** from the View menu. In the Preference dialog box:
   - Select **Fill Down Sample Position Sequentially Based on PLT File**.
   - Type the number of positions contained on the loaded sample plate in the Default Number of Rows in the Grid field.
   - Click **OK**.

   For more information, see Section 7.8.2, Setting Sequence Control Panel Preferences.

3. In the Run list, type the starting sample position to fill down.

NOTE: *If you want to fill down* ***all*** *positions on the plate, you must start at row 1.*

---

4. Select the rows to fill down.

NOTE: *To fill down all positions on the plate, click on the Sample Position column header to select all rows.*

---

5. From the Edit menu, select **Fill Down**.

   Selected rows are filled with the incremented sample position number.

Importing and exporting the run list

You can import and export the following file types for use in the Sequence Control Panel:

- Text files (.TXT), tab- or comma-delimited
- Excel Worksheet files (.XLS)

File requirements for importing

A file that you import into the run list must meet the following conditions:

- Must contain 11 columns of information (to correspond to all columns in the grid except the Acquisition Status column). If you do not have information for a column, leave a blank in that column.
- Columns must be in the same order as the columns in the run list (see Table 7-1, “Run List Parameters,” on page 16).
- The Run column must include a 1 (equal to the Run field being checked) or 0 (equal to the Run field being unchecked)
- .BIC, .SET, or .CAL file columns must specify a full path for the file.

NOTE: *If you do not specify a path, the software assumes a path of C:\VOYAGER.*

- Does not contain blank lines. Any lines following a blank line are ignored and not imported.

Importing

To import a .TXT or .XLS file:

1. Select **Import** from the File menu.
2. Select a file or type in a file name.
3. Click **Import**.

   Information is imported into all columns, even if columns are hidden.

Exporting

To export a .TXT or .XLS file:

1. Select **Export** from the File menu.
2. Type in a file name.
3. Select **.TXT** or **.XLS** from the Save As Type drop-down list.
4. Click **Save**.

NOTE: *The Acquisition Status column is not exported. The exported Run column contains 0 if Run is unchecked and 1 if Run is checked.*

7.5 Preparing to Run a Sequence

This section describes:

- Aligning the sample plate
- High voltage warmup for improved mass accuracy
- Before acquiring

Aligning the sample plate

Sample plate alignment is necessary for a sequence run if the laser is not striking the center of the sample position.

Sample plate alignment may not be necessary on your system, particularly if you use 100-well plates and the SPIRAL.SP search pattern file provided with the system.

See Section 2.7, Aligning the Sample Plate, to determine if sample plate alignment is necessary. If you do need to align the sample plate, you must do so for each sample plate you use.

High voltage warmup for improved mass accuracy

For maximum mass accuracy, allow the high voltage power supplies to warm up for a short period of time before acquisition. This reduces variability in accelerating voltages, and yields more reproducible ion flight times.

To turn on the high voltage power supplies, click  in the toolbar.

NOTE: *The high voltage power supplies are automatically turned off after 60 minutes if the mass spectrometer is not used. To change the Idle Time, see "High Voltage Configuration" on page 2-38.*

Before acquiring

Before acquiring a sequence:

1. Load a sequence by doing one of the following:
   - Create a new sequence. See Section 7.3, Before Creating a Sequence.
   - Open an existing sequence by clicking  in the toolbar and selecting an .SEQ file.
2. Check system status. See Section 2.11, Checking System Status and Pressures.

7.6 Running a Sequence

This section includes:

- Starting a sequence
- What the system checks when you start a sequence
- During and after acquiring a sequence
- Stopping a sequence
- Checking sequence status

7.6.1 Starting a Sequence

CAUTION

After you start a sequence, do not change instrument settings or any other parameters in the sequence. Changing settings while a sequence is running may cause the sequence to stop.

Checking disk space

Before starting a sequence run, the system checks that at least 150 MB of disk space is available if you have enabled the Low Disk space message in Preferences (described in Section 7.8.2, Setting Sequence Control Panel Preferences).

NOTE: *If the run requires more than 150 MB for data file storage, a message is displayed during the run when free disk space is no longer available.*

Acquiring sequence data

To acquire sequence data:

1. Perform the steps in Section 7.3, Before Creating a Sequence.
2. Create a sequence as described in Section 7.4, Creating a Sequence, or open an existing sequence.

NOTE: *You can have more than one sequence open at the same time. However, only one sequence can be active. For example, you cannot edit one sequence while another sequence is running.*

3. Select the rows to run by clicking the check box in the Run column. Only rows with a check in the Run column are acquired when the sequence runs.

Hint: *You can select* ***Run Column State*** *from the Edit menu then select* ***Set All*** *or* ***Clear All*** *to quickly check or uncheck all rows.*

4. To start the sequence, click  in the toolbar, or select **Start Sequence** from the Control menu.

7.6.2 What the System Checks When You Start a Sequence

What the system checks

When you start a sequence, the software examines the contents of all selected rows (checked in the Run column) to make sure specified files exist and entries are valid.

If the system finds invalid parameters

If the system finds invalid parameters, it:

- Unchecks all rows containing invalid parameters
- Changes the color of the invalid parameter to red
- Displays an error message indicating that invalid parameters have been found, and allows you to run rows that do not contain errors

If error is displayed

If an error is displayed, check the following and correct as needed:

- Sample positions correspond to the type of .PLT file loaded in the Instrument Control Panel
- All directories specified for data files exist
- All specified .BIC, .SET, and .CAL files exist

NOTE: *If a .CAL file specified for an External calibration line does not exist, the software checks preceding rows in the sequence to see if an Internal-Update calibration row will create the .CAL file. For more information, see* *"Types of files and calibration" on page 7-3.*

- All .BIC files specify Automatic Control mode
- All .BIC files specify Linear or Reflector Operation mode (PSD mode is not allowed)
- All .BIC files specify the same laser type (internal or external). You cannot switch between internal and external lasers when running a sequence. If you specify an internal laser .BIC file, then an external laser .BIC file, the second .BIC file is flagged as invalid when you try to run the sequence.

7.6.3 During and After Acquiring a Sequence

Pausing and resuming a sequence

To pause a sequence, click  .

The sequence pauses after the current entry is acquired and processed, and Sequence Acquisition Status is Paused.

To resume the sequence, click  again. The sequence resumes with the next row selected to run.

Skipping a line

To skip a row in the sequence, select **Skip Line** from the Control menu.

NOTE: *The log file does not contain any information for rows you skip.*

Modifying the run list during acquisition

To modify the run list after you start the sequence, click  to pause the sequence. You can modify lines below the line at which the sequence has paused.

Process that occurs

During the sequence run:

- After each data file is acquired, it is processed.
- Sequence status is displayed in the locations listed in “Checking Sequence Status” on page 7-29.

After the sequence run:

- The run log is created in the location specified in Section 7.4.1, Setting General Sequence Parameters. The run log contains all errors that are logged and a list of files that are acquired.
- The last .BIC file that was executed remains loaded in the Instrument Control Panel.

7.6.4 Stopping a Sequence

To stop a sequence, click  .

The sequence stops as soon as the current processing function can safely stop. You cannot resume a stopped sequence. You must restart at the beginning.

7.6.5 Checking Sequence Status

You can check sequence status in three places:

- Acquisition Status field in the run list
- Sequence Status control page (general status of run)
- Instrument Control Panel

Checking the Acquisition Status field

The Acquisition Status field in the run list displays the status of each spectrum as it is acquired. Possible states are:

- **Acquiring**—Acquiring a sample.
- **Processing**—Applying macros or calibrating the data file.
- **Acq Error**—Error occurred during acquisition.
- **Proc Error**—Error occurred during processing.
- **Success**—Sample processing successful.

NOTE: *The Acquisition Status field is updated each time a spectrum is acquired and processed for a line in the sequence. When acquisition and processing is complete for the line, the status reflects the status for the last spectrum acquired and processed. It does not reflect the overall status of all acquisitions performed by the line.*

Checking the Sequence Status panel

Select **Status Panel** from the View menu to check the status of a sequence. The Sequence Status panel (Figure 7-7) is displayed.

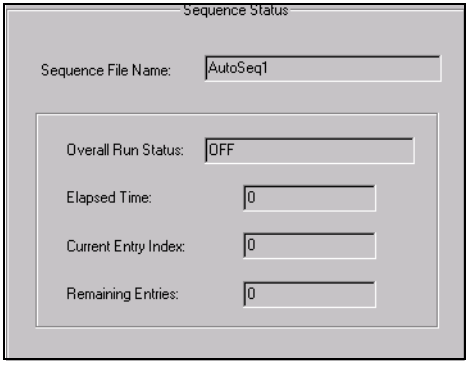


Figure 7-7 Sequence Status Panel

Sequence status parameters include:

Field	Description
Sequence File Name	Displays the name of the .SEQ file currently running.
Overall Run Status	Displays overall run status. Possible states are: • **Off**—Not running. • **Running**—Acquiring a sample. • **Pausing/Paused**—Pause button clicked. • **Stopping/Stopped**—Stop button clicked. • **Finished**—Sample acquired and processed. • **Error**—Error occurred during acquisition that terminated the sequence.
Elapsed Time	Displays the time that has elapsed during sequence acquisition.
Current Entry Index	Displays the row number that is being acquired.
Remaining Entries	Displays the remaining number of rows selected for acquisition.

Checking status in the Instrument Control Panel

You can monitor the following parameters in the Instrument Control Panel during a sequence run:

Parameter	Where to check
Laser intensity	Status bar of Instrument Control Panel
Search Pattern Position	Automatic Control tab in Output window
Data file name	Data Storage tab in Output window
Current Peak Intensity	Spectrum window
Signal-to-Noise of previous peak	Automatic Control tab in Output window

7.7 Automatic Calibration During a Sequence Run

This section includes:

- Calibration options in a sequence
- External calibration standard requirements
- Performing close external calibration
- Internal standard calibration considerations

7.7.1 Calibration Options in a Sequence

The Sequence Control Panel allows three types of automatic calibration:

Type	Function
External	• Applies calibration constants in a specified .CAL file.
Internal	• Applies calibration constants if a .CAL file is specified • Generates new calibration constants using standard reference masses specified by a .SET file. • Applies the constants.
Internal-Update	• Applies calibration constants if a .CAL file is specified • Generates new calibration constants using standard reference masses specified by a .SET file. • Applies the constants. • Updates the specified .CAL file, or creates a new .CAL file if the specified file does not exist. Use this option to perform a close external calibration, described in Section 7.7.3, Performing Close External Calibration.

NOTE: *If the .DAT file contains multiple spectra generated using the Save All or the Save All Passing option in Automatic Control, the calibration is applied to all spectra in the .DAT file.*

For the mass accuracy provided by each calibration type, see "Types of calibration" on page 6-7.

7.7.2 External Calibration Standard Requirements

Mass calibration standards

The requirements for mass calibration standards are determined by your application. The following are general guidelines:

- To improve external calibration mass accuracy (see Appendix A, Specifications, for external calibration specifications for your system), perform plate optimization before acquiring data. For more information, see Section 3.4.2, Using the Mass Accuracy Optimization Option.
- Mass calibrate on the same sample plate you will use to analyze samples.

If you do not use the Optimize Mass Accuracy option, note the following:

- For rapid screening in which high mass accuracy is not needed, one calibration standard located in the center of the plate is adequate.
- For applications requiring higher mass accuracy, more calibration standards are needed. You must experiment to determine the number and location of standards required to provide the mass accuracy you require.
- For optimum mass accuracy, place samples in sample positions adjacent to standards.

- For maximum mass accuracy, spot sample and standard as close to each other as possible (within 1 mm). This is referred to as close external calibration. For more information, see Section 7.7.3, Performing Close External Calibration.

7.7.3 Performing Close External Calibration

NOTE: *The Mass Accuracy Optimization feature provides mass accuracy comparable to close external calibration, and requires fewer calibration standards. For information, see Section 3.4.2, Using the Mass Accuracy Optimization Option.*

Overview

You have two options for close external calibration:

- Sample and standard in separate sample positions
- Sample and standard in the same sample position

NOTE: *The closer the locations of sample and standard, the better the mass accuracy.*

Sample and standard in separate sample positions

If you are acquiring samples and standards from different sample positions, enter the standards in the run list preceding the unknowns that use the calibration.

For optimum mass accuracy, place samples in sample positions adjacent to standards. See the example in Figure 7-8.

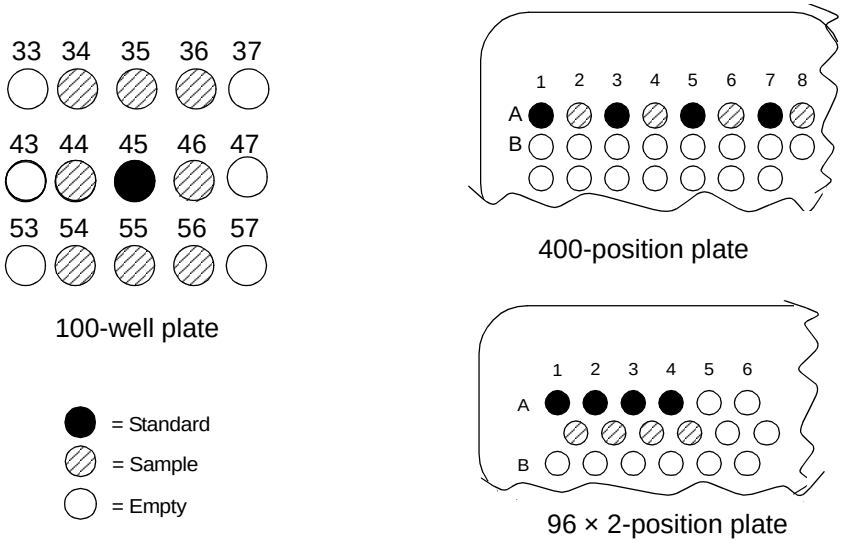


Figure 7-8 Sample and Standard Locations for Optimum Mass Accuracy (Without Using the Optimize Mass Accuracy Option)

To run samples spotted on a 100-well plate as illustrated in Figure 7-8, set up the run list as shown in the following table.

100-well Position	Base File Name	Instrument Settings File	Calibration Type	Calibration File
45	STD1	STD.BIC	INTERNAL-UPDATE	STD.CAL
34	SAMP1	SAMP.BIC	EXTERNAL	STD.CAL
35	SAMP2	STD.BIC	EXTERNAL	STD.CAL
36	SAMP3	SAMP.BIC	EXTERNAL	STD.CAL
44	SAMP4	STD.BIC	EXTERNAL	STD.CAL
46	SAMP5	SAMP.BIC	EXTERNAL	STD.CAL
54...56	SAMP6...8	STD.BIC	EXTERNAL	STD.CAL

To run samples spotted on a 400-position or 96 × 2-position plate as illustrated in Figure 7-8, set up the run list as shown in the following table.

400-well Position	96×2-well Position	Base File Name	Instrument Settings File	Calibration Type	Calibration File
A1	A1_a	STD1	STD.BIC	INTERNAL-UPDATE	STD.CAL
A2	A1_b	SAMP1	SAMP.BIC	EXTERNAL	STD.CAL
A3	A2_a	STD2	STD.BIC	INTERNAL-UPDATE	STD.CAL
A4	A2_b	SAMP2	SAMP.BIC	EXTERNAL	STD.CAL
A5	A3_a	STD3	STD.BIC	INTERNAL-UPDATE	STD.CAL
A6	A3_b	SAMP3	SAMP.BIC	EXTERNAL	STD.CAL
A7	A4_a	STD4	STD.BIC	INTERNAL-UPDATE	STD.CAL
A8	A4_b	SAMP4	SAMP.BIC	EXTERNAL	STD.CAL

The number and placement of standards needed depend on your application. See “Mass calibration standards” on page 7-33.

Hint: *You can use the same calibration file more than once in a sequence run.*

Sample and standard in the same sample position

You may see improved mass accuracy by spotting sample and standard in as close together as possible within a sample position (Figure 7-9). A standard spot within 1 mm of a sample spot can yield mass accuracy close to that expected for internal calibration.

NOTE: *This method is useful for plates with larger sample positions. If you are using a 400-position or 92×2-position plate, follow the procedure in "Sample and standard in separate sample positions" on page 7-34.*

NOTE: *Use plates without laser-etched sample positions or wells if you spot standard and sample as shown below.*

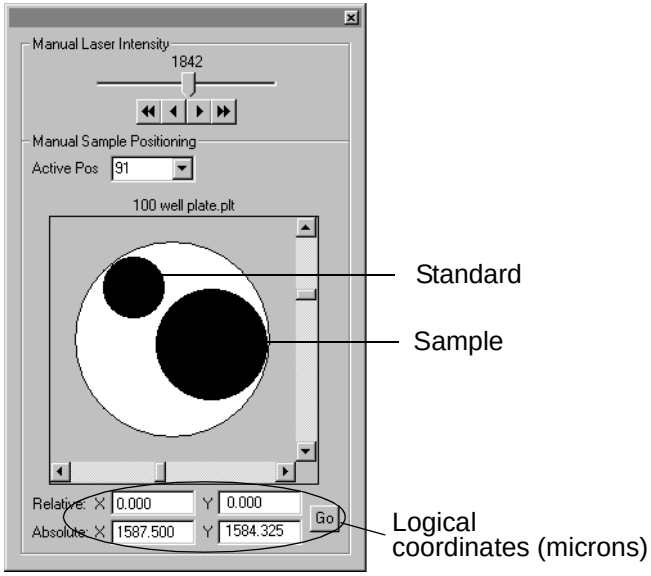


Figure 7-9 Location of Sample and External Standard for Maximum Mass Accuracy (Without Using the Optimize Mass Accuracy Option)

To acquire sample and standard, create two search pattern files to analyze the sample and standard spots. See “Creating search pattern files for close external calibration on separate spots” on page 7-38, for more information. Create two instruments settings (.BIC) files that contain the same settings, but specify the sample search pattern file and the standard search pattern file.

To run samples spotted as illustrated in Figure 7-9 (assuming that subsequent positions are spotted in the same way), set up the run list as shown in the following table.

Position	Base File Name	Instrument Settings File	Calibration Type	Calibration File
91	STD1	STD.BIC (specifies STD.SP)	INTERNAL-UPDATE	STD.CAL
91	SAMP1	SAMP.BIC (specifies SAMP.SP)	EXTERNAL	STD.CAL
92	STD2	STD.BIC (specifies STD.SP)	INTERNAL-UPDATE	STD.CAL
92	SAMP2	SAMP.BIC (specifies SAMP.SP)	EXTERNAL	STD.CAL
93	STD3	STD.BIC (specifies STD.SP)	INTERNAL-UPDATE	STD.CAL
93...	SAMP3	SAMP.BIC (specifies SAMP.SP)	EXTERNAL	STD.CAL

Creating search pattern files for close external calibration on separate spots

To create search pattern files for close external calibration on separate spots:

1. Spot sample and standard as close to each other as possible (within 1 mm) (see Figure 7-9 on page 7-37).

NOTE: *For optimum mass accuracy, calibration standards must be as close to unknowns as possible. An internal standard spot within 1 mm of a sample spot can yield mass accuracy close to the expected mass accuracy for internal standard calibration on your system.*

NOTE: *Use plates without laser-etched sample positions if you spot standard and sample as shown in Figure 7-9.*

2. Load the sample plate, select the position containing sample and standard, and display the Sample View.
3. Note the logical x and y coordinates for a minimum of three positions on the standard spot and a minimum of six positions on the sample spot.
4. Create the two search pattern files using Windows Notepad as described in “Creating and Editing .SP Files Using the Search Pattern Editor” on page 6-50.

7.7.4 Internal Standard Calibration Considerations

This section includes:

- Optimizing unknown and standard concentration
- Determining if standard suppresses signal from unknown
- Creating a search pattern file for internal calibration on separate spots
- Performing internal standard calibration with an external calibration “backup”

Optimizing unknown and standard concentration

Before combining sample and internal standard, determine the concentration of each that yields optimum signal intensity without saturation.

To optimize relative peak intensities, determine the peak intensity of the sample, and adjust the concentration of the standard to bring the standard peak intensity into the same range.

Determining if standard suppresses signal from unknown

In some cases, standard signal can suppress unknown signal. Mix optimized concentrations of standard and unknown and analyze to determine if you see adequate signal for all compounds.

If standard signal suppresses unknown signal, you have two options for analysis:

- **Perform internal calibration using separate spots**—Spot standard and sample as close to each other as possible. Create a search pattern file that analyzes both spots. Create an instrument settings file that generates a single spectrum (Accumulate All data collection mode) and specify internal calibration in Sequence Control. See "Creating a search pattern file for internal calibration on separate spots" on page 7-40.

NOTE: *To use this strategy, make sure standard and sample are compatible with the laser intensity and acceptance criteria in the instrument settings, and that the standard and sample peaks do not overlap.*

- **Perform close external calibration**—Spot standard and sample as close to each other as possible and perform close external calibration as described in Section 7.7.3, Performing Close External Calibration.

Creating a search pattern file for internal calibration on separate spots

To create a search pattern file for internal calibration on separate spots:

1. Spot sample and standard as close to each other as possible (within 1 mm) (see Figure 7-9 on page 7-37).

NOTE: *For optimum mass accuracy, calibration standards must be as close to unknowns as possible. An internal standard spot within 1 mm of a sample spot can yield mass accuracy close to the expected mass accuracy for internal standard calibration on your system.*

NOTE: *Use plates without laser-etched sample positions if you spot standard and sample as shown in Figure 7-9 on page 7-37.*

2. Load the sample plate, select the position containing sample and standard, and display the Sample View.
3. Note the relative x and y coordinates for a minimum of three positions on the standard spot and a minimum of six positions on the sample spot.
4. Create the search pattern file using Windows Notepad as described “Creating and Editing .SP Files Using the Search Pattern Editor” on page 6-50.

   Figure 7-10 shows an example search pattern (.SP) file for internal calibration using separate spots.

```
;internal calibration using two spots, 5/25/00
;std spots
-1048,635
-857,635
-762,635
;sample spots
635,-635
730,-635
825,-635
635,-730
730,-730
825,-730
```

Figure 7-10 Example .SP File for Internal Calibration Using Separate Spots

Performing internal standard calibration with an external calibration "backup"

For applications in which the concentration of internal standard to sample varies, you can set the sequence to perform an external and an internal calibration. This provides a certain level of mass accuracy, even if the internal standard peak is suppressed, and the internal calibration fails. An example of this application is analysis of proteolytic digests which use autolytic enzyme peaks as internal standards.

Set up the run list as shown in the following table.

Position	Base File Name	Instrument Settings File	Calibration Type	Calibration File
91	STD1	STD.BIC	INTERNAL-UPDATE	STD.CAL
92	SAMP1	SAMP.BIC	INTERNAL	STD.CAL
93	STD2	STD.BIC	INTERNAL-UPDATE	STD.CAL
94	SAMP2	SAMP.BIC	INTERNAL	STD.CAL
95	STD3	STD.BIC	INTERNAL-UPDATE	STD.CAL
96...	SAMP3	SAMP.BIC	INTERNAL	STD.CAL

During the run, the external calibration (.CAL) file is applied, then the internal calibration is performed.

7.8 Customizing the Sequence Control Panel

This section includes:

- Customizing the sequence display
- Setting Sequence Control Preferences

7.8.1 Customizing the Sequence Display

Using Workbook mode

Workbook mode displays the run list (contains sample information and conditions for acquisition and processing) in tabbed, framed format.

To use Workbook mode:

1. Open or create a sequence run list.
2. Open or create another sequence run list.
3. Select **Workbook mode** from the View menu.

   Figure 7-11 displays the Sequence Control Panel in Workbook mode (two tabs at bottom of run list).

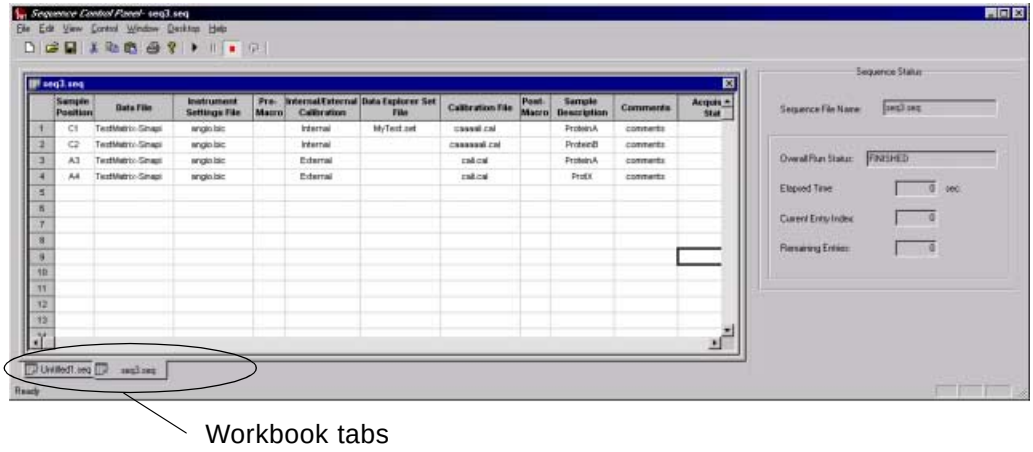


Figure 7-11 Sequence Control Panel in Workbook Mode

4. Switch between the run lists by clicking the appropriate tab.

Showing or hiding columns

You can select **Grid Columns** from the View menu, then select the columns that are displayed. Checked columns are displayed. Hidden columns that require an entry are automatically displayed if you select a row to run and it does not contain the necessary entry.

If you import information into the run list, entries are imported into hidden columns.

Positioning windows

To arrange the Sequence Control Panel and Instrument Control Panel on the desktop, select a command from the Desktop menu:

- **Tile Horizontal**—Places the Sequence Control Panel at the top or bottom of the desktop, and the Instrument Control Panel in the opposite location.
- **Tile Vertical**—Places the Sequence Control Panel at the left or right of the desktop, and the Instrument Control Panel in the opposite location.

7.8.2 Setting Sequence Control Panel Preferences

To set preferences:

1. Select **Preferences** from the File menu to display the Preferences dialog box (Figure 7-12).

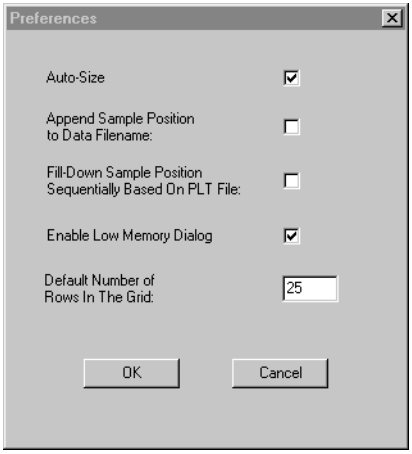


Figure 7-12 Preferences Dialog Box

2. Specify preferences as needed:

Table 7-2 Sequence Control Panel Preferences

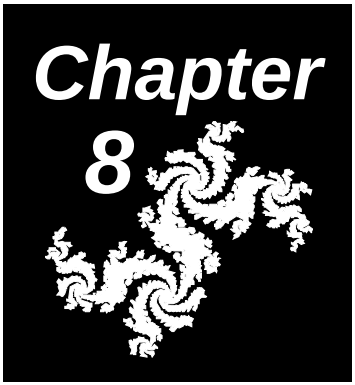
Preference	Description
Autosize	Automatically enabled when you are in Workbook mode. The run list is automatically resized and displayed appropriately in tabbed frames when you resize the window. When Workbook mode is disabled, Autosize is not available. You can manually resize and move the run list window to any dimensions.

continued

Table 7-2 Sequence Control Panel Preferences (Continued)

Preference	Description
Append Sample Position to Data File Name	Includes position number in the data file name suffix. For example, if you specify a base name of SAMPLE, the final data file name is SAMPLE_A1_0001.DAT for the data file acquired from position A1.
Fill Down Sample Position Based on PLT File	When you use the fill down function, increments sample positions in all selected rows below based on the type of sample plate loaded in the Instrument Control Panel. See “Incrementing sample positions” on page 7-21.
Enable Low Memory Dialog	Verifies before each acquisition step that at least 150 MB of disk space are available on the destination drive.
Default number of rows	Determines the number of rows displayed when you create a new sequence. You can override the default and add or delete rows as needed.

3. Click **OK**.



8 PSD Analysis

This chapter contains the following sections:

8.1 PSD Quick Start 8-2

8.2 Overview of PSD Analysis 8-20

8.3 Enhancing Fragmentation with CID 8-31

8.4 Acquiring PSD Data with Standard .BIC Files in Manual Control Mode 8-37

8.5 Acquiring PSD Data with Standard .BIC Files in Automatic Control Mode 8-57

8.6 Exploring PSD Mode 8-65

8.7 Viewing PSD Data 8-77

8.1 PSD Quick Start

This section gives a quick overview of how to perform a PSD acquisition of angiotensin and an unknown in manual mode.

For detailed PSD information, refer to the sections that follow this PSD Quick Start.

Before you begin

Before starting this PDS Quick Start, be familiar with the information in:

- Section 8.2.1, Post-Source Decay Analysis
- Section 8.6.1, Observing the Effects of Laser Intensity
- Section 5.1, Loading, Modifying, and Saving Instrument Settings
- Section 6.2, Acquiring in Manual Mode from the Instrument Control Panel
- Section 6.4, Making Accurate Mass Measurements
- *Data Explorer User's Guide*, the sections that describe examining spectra and manual calibration

Two types of calibration in PSD analysis

When you perform PSD analysis, you generate two types of mass calibration:

- **External calibration applied to precursor ion mass**—Normal mass calibration applied to a reflector mode spectrum, to ensure accurate mass of the precursor ion (described in Section 6.1.3, Calibrating the Mass Scale).
- **PSD calibration applied to fragment ion masses**—Special PSD calibration that optimizes fragment ion mass based on precursor ion mass and Mirror Ratio setting (described in Section 8.2.5, Mass Calculation for Fragment Ions).

Steps to perform PSD analysis

The steps to perform PSD analysis on angiotensin and on an unknown are summarized in Table 8-1 and Table 8-2.

Table 8-1 Steps to Perform PSD Analysis of Angiotensin

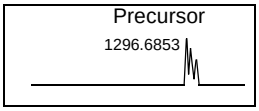
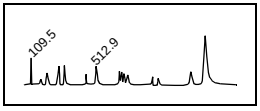
Step		Result	See page
1.	Generate a Reflector mode precursor spectrum (use PSD_Precursor.BIC provided).	Angiotensin Reflector spectrum 	8-7
2.	Generate a normal single-point external calibration using the spectrum acquired in step 1. You use this single-point external calibration in PSD analysis to obtain maximum mass accuracy for the precursor ion.	Normal external calibration for precursor ion mass accuracy Angio.CAL	8-7
3.	Set PSD Acquisition/Instrument Settings parameters—Open **Angiotensin_PSD.BIC** provided, and: • **In Instrument Settings**—Type the angiotensin precursor mass and select **Angio.CAL** generated above (for precursor). • **In PSD Acquisition Settings**—Select default PSD calibration (for fragments).	Angiotensin_PSD.BIC with: Angio.CAL Default PSD calibration	8-7
4.	Acquire PSD segments (precursor and fragment spectra).	Angiotensin PSD Composite spectrum 	8-9
5.	Generate a PSD multi-point external calibration using the spectrum acquired in step 4. You use this multi-point external calibration in PSD analysis to obtain maximum mass accuracy for the fragment ions.	PSD calibration for fragment ion mass accuracy Angio_PSD.CAL	8-13

Table 8-1 Steps to Perform PSD Analysis of Angiotensin (Continued)

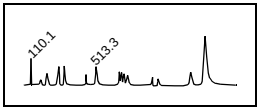
Step		Result	See page
6.	To verify that the Angio_PSD.CAL yields acceptable mass accuracy: • Add the PSD calibration generated in step 5 to PSD Acquisition parameters—Open **Angiotensin_PSD.BIC**, select **External PSD Calibration File**, then select **Angio_PSD.CAL** (for fragments)	Angiotensin_PSD.BIC with: Angio.CAL Angio_PSD.CAL	8-14
	• Acquire PSD segments (precursor and fragment spectra) with PSD calibration	Recalibrated angiotensin PSD Composite spectrum with optimum fragment ion mass accuracy 	8-14

Table 8-2 Steps to Perform PSD Analysis of an Unknown

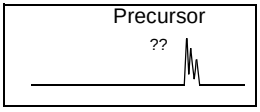
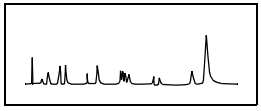
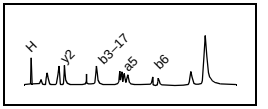
Step		Result	See page
1.	Generate a PSD calibration on angiotensin by performing step 1 through step 6 in "Steps to Perform PSD Analysis of Angiotensin" on page 8-3.	PSD calibration for fragment ion mass accuracy Angio_PSD.CAL	8-3
2.	Generate a precursor spectrum on the unknown in Reflector mode (use PSD_Precursor.BIC provided). ***NOTE:*** *This step assumes that you have already determined an accurate mass for the precursor ion using reflector mode high-resolution analysis with internal or external calibration.*	Unknown Reflector spectrum 	8-7
3.	Generate a normal single-point external calibration using the spectrum acquired in step 2. You use this single-point external calibration in PSD analysis to obtain maximum mass accuracy for the unknown precursor ion.	Normal external calibration for precursor ion mass accuracy Precursor.CAL	8-7
4.	Set PSD Acquisition/Instrument Settings parameters—Open **Angiotensin_PSD.BIC** provided, and: • **In Instrument Settings**—Type the precursor mass and select **Precursor.CAL** generated in step 3 (for precursor). • **In PSD Acquisition Settings**—Select **Angio_PSD.CAL** generated in step 1 (for fragments).	Angiotensin_PSD.BIC with: Precursor.CAL Angio_PSD.CAL	8-7
5.	Acquire unknown PSD segments (precursor and fragment spectra) with Angio_PSD calibration.	Unknown PSD Composite spectrum with optimum fragment ion mass accuracy 	8-14

Table 8-2 Steps to Perform PSD Analysis of an Unknown (Continued)

Step		Result	See page
6.	Optionally, confirm or investigate fragment ion identity using the Ion Fragment calculator or the Peptide Fragmentation macro.	Unknown PSD Composite spectrum with fragment ion labels 	8-19

8.1.1 PSD Analysis of Angiotensin

Generating the Reflector mode precursor spectrum

1. Open the **PSD_Precursor.BIC** file provided with the software. This is a reflector mode instrument settings (.BIC) file. All other instrument settings should be identical to the settings in the PSD mode Angiotensin_PSD.BIC you use later to acquire segments.
2. Acquire using a laser intensity that does not saturate the reflector spectrum.
3. Save the precursor ion .DAT file by clicking  in the toolbar.

Generating an external calibration for the precursor ion

To obtain maximum mass accuracy for the precursor ion, follow the steps below to generate a single-point external calibration file to use when you perform the PSD acquisition.

NOTE: *This is not a PSD calibration that affects fragment ion masses. It ensures accurate mass of the precursor.*

1. Click  in the Instrument Control Panel toolbar to open the precursor ion data file in the Data Explorer software.
2. Create a single-point calibration using the precursor ion mass, then save the calibration file as **ANGIO.CAL** by exporting the calibration constants from the data file. For more information, see the *Data Explorer Software User's Guide*, Section 5.3.2, Manually Calibrating.

Setting PSD acquisition parameters

1. Open the **Angiotensin_PSD.BIC** file provided with the software. This is a PSD mode .BIC file.

   The PSD Acquisition Settings control page (Figure 8-1) is automatically displayed in the Instrument Control Panel if you open a .BIC file set to PSD mode.

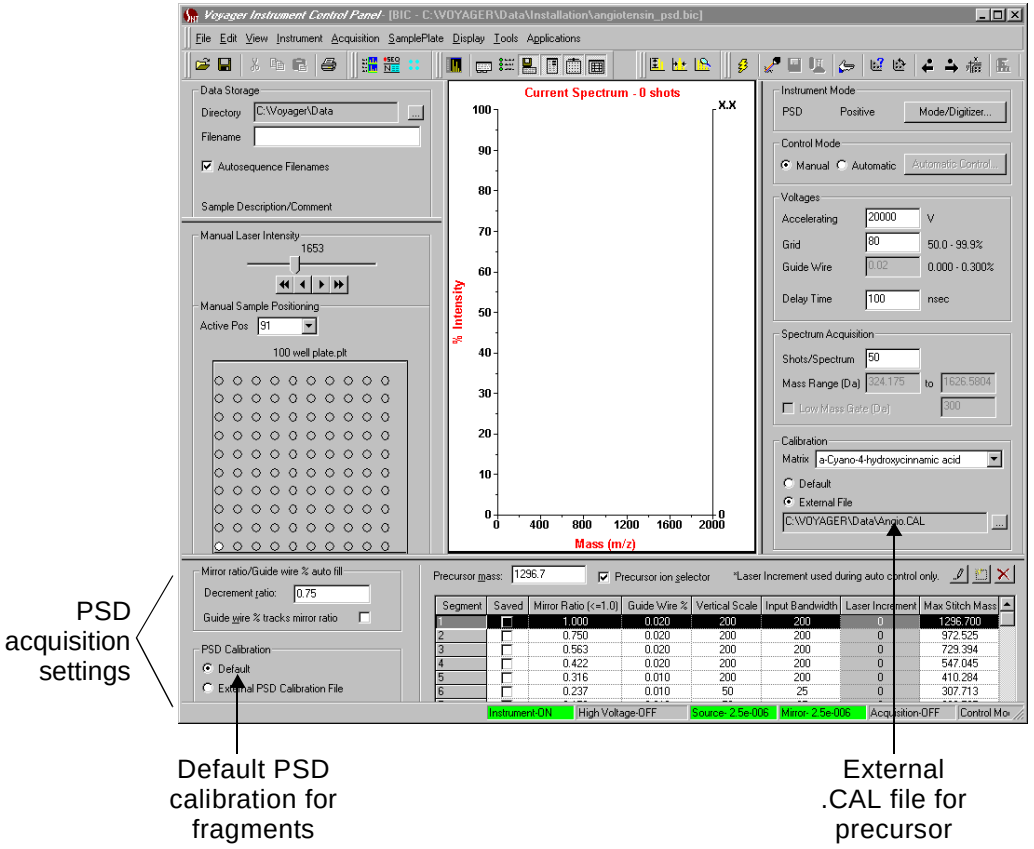


Figure 8-1 PSD Instrument Control Panel with Acquisition Settings Control Page

2. In the Calibration section of the Instrument Settings control page, select:

   - The matrix you are using.
   - **External File**, then select the **ANGIO.CAL** file you created in “Generating an external calibration for the precursor ion” on page 8-7.

3. In the PSD Acquisition Settings control page, type the angiotensin Precursor mass.

NOTE: *Precursor mass is used for PSD calibration. Type in an accurate value with appropriate precision (for example, type 1296.68, not 1297).*

---

4. Ensure that PSD calibration is set to **Default**.

5. Select **Save Instrument Settings As** from the File menu, then save the instrument settings file with a new name.

NOTE: *The instrument settings files provided with your system (PSD_Precursor.BIC and Angiotensin_PSD.BIC) are read-only. You cannot save changes to these files unless you assign a new name.*

Acquiring PSD segments

1. In the Manual Laser Intensity/Sample Positioning control page, select the same sample position from which you acquired the precursor spectrum.

2. From the View menu, select **Data Storage**. Set parameters as needed. See “Setting Data Storage parameters” on page 6-14 for information.

Hint: *Include a _PSD suffix when you name PSD data files to help you distinguish them from non-PSD data files. For example, if you type in Experiment1_PSD as the file name, the complete data file name will be Experiment1_PSD.DAT or Experiment1_PSD_0001.DAT (if Autosequence File Names is enabled).*

Selecting and acquiring a segment

3. In the PSD Acquisition Settings control page, select the first row (click the number box in the Segment column) that corresponds to the segment you want to acquire.
4. To start acquiring, select **Start Acquisition** from the Acquisition menu, or click  .
5. Adjust laser intensity to optimize signal intensity. You typically need a higher laser intensity to optimize signal intensity for segments with lower Mirror Ratio settings.

   The laser intensity needed for the first segment (the segment with the highest Mirror Ratio setting) is typically 150 to 200 counts higher than the laser intensity used to acquire the reflector mode precursor spectrum, and increases with each subsequent segment.

Examining and saving the segment

6. Examine the spectrum to ensure that fragments are produced (Figure 8-2).

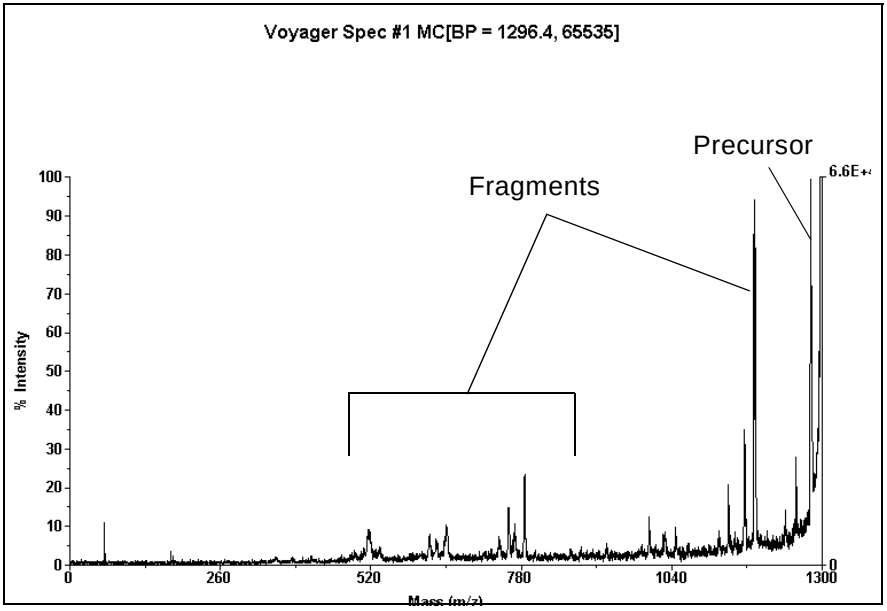


Figure 8-2 Segment Spectrum

7. If fragments are present and data is acceptable (Figure 8-2), click  in the toolbar to save the segment to the .DAT file. After you save the segment, the Saved check box in the segment list is checked.

   If the current spectrum does not contain significant fragment ion signal and you do not want to save the spectrum, reselect the row and reacquire the spectrum, or select a new row.

CAUTION

Save the current segment (if the data is acceptable) before starting to acquire the next segment. If you do not, you will lose the data for the current segment.

Selecting and acquiring remaining segments

8. Repeat step 3 through step 7 to collect remaining segments.

NOTE: *Segments are listed in the Data Explorer software in the order in which they are acquired. If segments with duplicate Mirror Ratios are contained in the file, the software uses the last acquired segment when it generates the composite spectrum.*

Stopping the experiment

9. After you acquire all necessary segments, select **Stop Experiment** from the Acquisition menu. The PSD data file is closed.

   You cannot view the PSD data file in the Data Explorer software until you stop the experiment.

CAUTION

If you stop an experiment without saving any segments, no .DAT file is created.

Determining if PSD calibration is needed

1. Click  in the Instrument Control Panel toolbar to open the PSD data file in the Data Explorer. The software “stitches” together portions of the fragment spectra and displays a composite spectrum (see Figure 8-6 on page 8-26 for an explanation of how the software generates the composite spectrum).

   Your angiotensin composite spectrum should be similar to the spectrum shown in Figure 8-3.

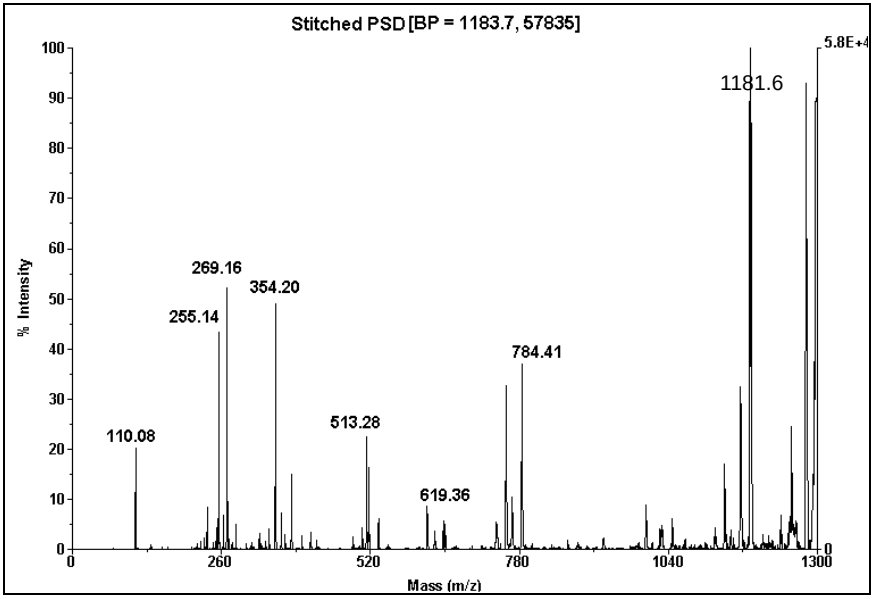


Figure 8-3 Angiotensin Spectrum

2. Examine the masses in the stitched spectrum and compare them to the expected masses listed in Table 8-3.

Table 8-3 Expected Masses in Angiotensin Spectrum

Expected Mass	Ion Type
110.08	His
255.16	b2–17
269.16	y2
354.20	b3–17
513.08	y4
619.36	a5
784.41	b6
1,181.6	y9

If masses are not within ±0.2 Da (STR models) or ±0.3 Da (PRO models) of the expected masses, generate a PSD calibration, and reacquire the PSD segments with the PSD calibration.

Generating a PSD calibration

This section gives a brief description of how to generate a PSD calibration. For more information, see the *Data Explorer User's Guide*, Section 8.3.3, Creating PSD Calibration (.CAL) Files and Applying to Other Data Files.

NOTE: *This is a PSD calibration that affects fragment ion masses.*

---

1. In the Data Explorer software, open the PSD data file acquired in the previous section, if it is not already open.
2. From the Process menu, select **Mass Calibration**, then select **PSD Calibration**.
3. Select the **Angiotensin_Fragments.REF** file provided with the software.

4. Select the peaks listed in Table 8-3 as reference masses for calibration by right-click-dragging on a peak, then select the correct mass for the peak in the Reference Peak dialog box.

   For best mass accuracy:

   - Select a minimum of seven reference masses.
   - Select peaks from different regions of the spectrum to ensure that high and low Mirror Ratios are represented.

5. Click **Solve and Plot** to generate the calibration, then click **Apply Calibration** to save the calibration constants in the data file. Click **Close**.

6. Save the calibration file as **ANGIO_PSD.CAL** by selecting **Export** from the File menu, then selecting **Calibration**.

Adding PSD calibration

1. Open the Angiotensin_PSD.BIC file that you renamed in “Setting PSD acquisition parameters” on page 8-7.
2. In the PSD calibration section of the PSD Acquisition settings control page (see Figure 8-1 on page 8-8), select the **ANGIO_PSD.CAL** file you created in the previous section.
3. Select **Save Instrument Settings** from the File menu.

Acquiring PSD segments with PSD calibration

Reacquire PSD segments with PSD calibration as described in “Acquiring PSD segments” on page 8-9. Check the fragment ion masses to make sure they are within acceptable error.

8.1.2 PSD Analysis of an Unknown

Generating the Reflector mode precursor spectrum

This step assumes that you have already determined an accurate mass for the precursor ion using reflector mode high-resolution analysis with internal or external calibration (this analysis requires conditions that differ from PSD analysis conditions). You acquire the precursor ion again (using the same conditions you will use for PSD analysis) to generate a spectrum from which you can generate an external calibration. The external calibration you generate from the precursor ion is used to obtain maximum mass accuracy for the precursor ion during the PSD analysis, which helps ensure maximum mass accuracy for the fragment ions.

1. Open the **PSD_Precursor.BIC** file provided with the software. This is a reflector mode instrument settings (.BIC) file. All other instrument settings are identical to the settings in the PSD mode Angiotensin_PSD.BIC file you use to acquire segments.
2. Acquire using a laser intensity that does not saturate the reflector spectrum.
3. Save the precursor ion .DAT file by clicking  in the toolbar.

Generating an external calibration for the unknown precursor ion

To obtain maximum mass accuracy for the precursor ion, generate an external calibration file using the spectrum acquired in the previous section. You will use this external calibration when you perform the PSD acquisition. If you do not obtain maximum mass accuracy for the precursor ion during the PSD analysis, you will not obtain maximum mass accuracy for the fragment ions.

NOTE: *This is not a PSD calibration that affects fragment ion masses. It is an external calibration that is applied before PSD analysis to ensure accurate mass of the precursor.*

---

1. Click  in the Instrument Control Panel toolbar to open the precursor ion data file in the Data Explorer software.

2. Create a single-point calibration using the precursor ion mass and save the calibration file as **PRECURSOR_UNKNOWN.CAL** by exporting the calibration constants from the data file.

   You may need to add the mass for the unknown to your calibration reference file before creating the single-point calibration.

   For more information, see the *Data Explorer Software User's Guide*, Section 5.3.2, Manually Calibrating.

Setting PSD Acquisition parameters

1. Open the **Angiotensin_PSD.BIC** file provided with the software. This is a PSD mode .BIC file.

2. In the Calibration section of the Instrument Settings control page, select:

   - The matrix you are using.
   - **External File**, then select the **PRECURSOR_UNKNOWN.CAL** file you created for the unknown in "Generating an external calibration for the unknown precursor ion" on page 8-15.

3. In the PSD Acquisition Settings control page:

   - Type the accurate **Precursor mass**.
   - In the Calibration section, select **External File**, then select the he **ANGIO_PSD.CAL** file you created in "Generating a PSD calibration" on page 8-13.

4. Select **Save Instrument Settings As** from the File menu, then save the instrument setting file with a new name.

Acquiring PSD segments

1. In the Manual Laser Intensity/Sample Positioning control page, select the same sample position from which you acquired the precursor spectrum.
2. From the View menu, select **Data Storage**. Set parameters as needed. See “Setting Data Storage parameters” on page 6-14, for information.

Hint: *Include a _PSD suffix when you name PSD data files to help you distinguish them from non-PSD data files. For example, if you type in Experiment1_PSD as the file name, the complete data file name will be Experiment1_PSD.DAT or Experiment1_PSD_0001.DAT (if Autosequence File Names is enabled).*

Selecting and acquiring a segment

3. In the PSD Acquisition Settings control page, select the row (click the number box in the Segment column) that corresponds to the segment you want to acquire.
4. To start acquiring, select **Start Acquisition** from the Acquisition menu, or click  .
5. Adjust laser intensity to optimize signal intensity. You typically need a higher laser intensity to optimize signal intensity for segments with lower Mirror Ratio settings.

   The laser intensity needed for the first segment (the segment with the highest Mirror Ratio setting) is typically 150 to 200 counts higher than the laser intensity used to acquire the reflector mode precursor spectrum, and increases with each segment.

Examining and saving the segment

6. Examine the spectrum to ensure that fragments are produced.
7. If fragments are present, click  in the toolbar to add the segment to the .DAT file. After you save the segment, the Saved check box in the segment list is checked.

   If the current spectrum does not contain significant fragment ion signal and you do not want to save the spectrum, reselect the row and reacquire the spectrum, or select a new row.

CAUTION

Save the current segment (if the data is acceptable) before starting to acquire the next segment. If you do not, you will lose the data for the current segment.

Selecting and acquiring remaining segments

8. Repeat step 3 through step 7 to collect remaining segments.

NOTE: *Segments are listed in the Data Explorer software in the order in which they are acquired. If segments with duplicate Mirror Ratios are contained in the file, the software uses the last acquired segment when it generates the composite spectrum.*

Stopping the experiment

9. After you acquire all necessary segments, select **Stop Experiment** from the Acquisition menu. The PSD data file is closed.

   You cannot view the PSD data file in the Data Explorer software until you stop the experiment.

CAUTION

If you stop an experiment without saving any segments, no .DAT file is created.

Confirming or investigating fragment ion identity

To confirm or investigate fragment ion identity, you can use the following tools:

- **If the peptide sequence is known**—Use the Ion Fragmentation calculator in the Data Explorer software to apply fragment labels to the unknown composite spectrum.

  For information, see the *Data Explorer Software User's Guide*, Section 8.2, Applying Fragment Labels.

- **If the peptide sequence is not known**—Use the Peptide Fragmentation macro provided with the Data Explorer software to investigate the sequence in the unknown composite spectrum.

  For information, see the *Data Explorer Software User's Guide*, Appendix C, Data Explorer Toolbox (Visual Basic Macros).

8.2 Overview of PSD Analysis

This section includes:

- Post-source decay analysis
- Differences from regular analysis
- Segments and composite spectra
- PSD data files
- Mass calculation for fragment ions
- Optimizing the Precursor Ion Selector

NOTE: *Analysis of post-source decay is available on Voyager-DE PRO and Voyager-DE STR workstations only.*

Before you begin

For successful analysis of post-source decay, you must be familiar with the information in:

- Chapter 5, Optimizing Instrument Settings
- Chapter 6, Acquiring Spectra from the Instrument Control Panel

8.2.1 Post-Source Decay Analysis

What is post-source decay analysis?

You can obtain valuable structural information about a sample by analyzing the fragment ions generated from the original ions in the flight tube. On a MALDI-TOF system, this is known as post-source decay (PSD) analysis.

PSD analysis on the Voyager system is an acquisition method tailored to the analysis of fragment ions. PSD mode allows you to better analyze ions that fragment in the flight tube by optimizing the following settings:

- **Mirror Ratio setting**—Brings different mass ranges of fragment ions into focus.
- **Precursor Ion Selector**—Selectively analyzes the ion of interest, without interference from other compounds. Specificity of ion selection is determined by the resolution of the Precursor Ion Selector (also called Timed Ion Selector) on your system. See Appendix A, Specifications, for more information.

PSD fragment ions

At higher laser intensities, some molecular ions decompose into PSD fragment ions in the flight tube after they leave the ion source (the post-source decay process).

Before fragmentation, the intact molecular ion travels with a kinetic energy of:

$$KE = 1/2\ mv^2$$

where:
KE = kinetic energy (accelerating voltage)
m = mass
v = velocity

After fragmentation, the fragment ions continue travelling with the same velocity as the original ion, but with reduced kinetic energy. Fragment ions travel with the original (precursor) ion until they reach the reflector where they separate from the original ion and behave as though they received less initial acceleration. Therefore, PSD fragment ions are not correctly focused and appear at a mass higher than the expected mass.

After fragmentation, the fragment ion travels with a kinetic energy of:

$$KE_{(\text{fragment ion})} = KE_{(\text{original ion})}\left(\frac{m_{(\text{fragment ion})}}{m_{(\text{original ion})}}\right)$$

where:
KE = kinetic energy (accelerating voltage)
m = mass

Focusing fragment ions with Mirror Ratio setting

Consider an ion ($M+H^+$) fragmenting during flight into two fragments, A and B, of lower mass than the original ion. Both of the following reactions occur:

$$MH^+ \longrightarrow AH^+ + B$$

$$MH^+ \longrightarrow A + BH^+$$

If MH^+ = 1,000 Da, AH^+ = 700 Da, and BH^+ = 300 Da.

Figure 8-4 shows the projected flights of these ions within the reflector portion of the mass spectrometer.

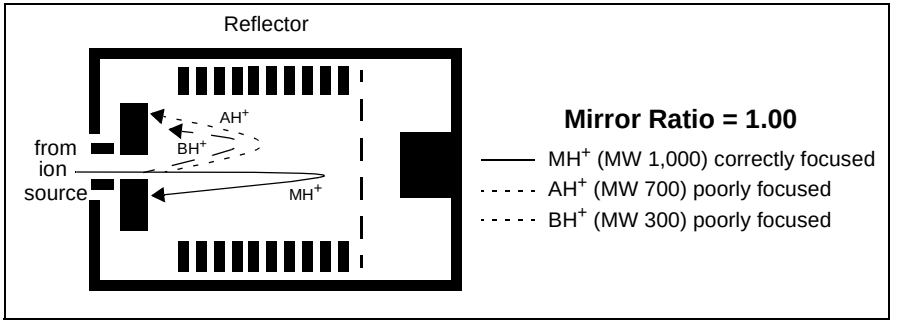


Figure 8-4 Molecular and Fragment Ion Flight in the Reflector

The AH^+ fragment has 70 percent of the kinetic energy of the MH^+ ion and the BH^+ ion has 30 percent. With a Mirror Ratio setting of 1.0000, ions with lower kinetic energy are reflected quickly and are not focused by the mirror.

Fragment ions with a lower energy than that of the precursor ion can be focused by adjusting the voltage applied to the mirror. Mirror Voltage is equal to:

$$\text{Mirror Voltage} = \text{Mirror Ratio} \times \text{Mirror to Accelerating Voltage Ratio} \times \text{Accelerating Voltage}$$

where:

Mirror Ratio is the value you set in PSD Acquisition Settings to focus fragment ions.

Mirror to Accelerating Voltage Ratio is a value set in the Advanced tab of Mode/Digitizer settings in Instrument Settings to adjust the voltage of the mirror to reflect intact ions. Do not change this value unless instructed to do so by Applied Biosystems.

Accelerating Voltage is the value you set in Instrument Settings.

You adjust the Mirror Voltage by decreasing the Mirror Ratio. This adjusts the field strength of the mirror, and allows lower energy ions to travel farther into the reflector portion of the mass spectrometer and be better focused (Figure 8-5).

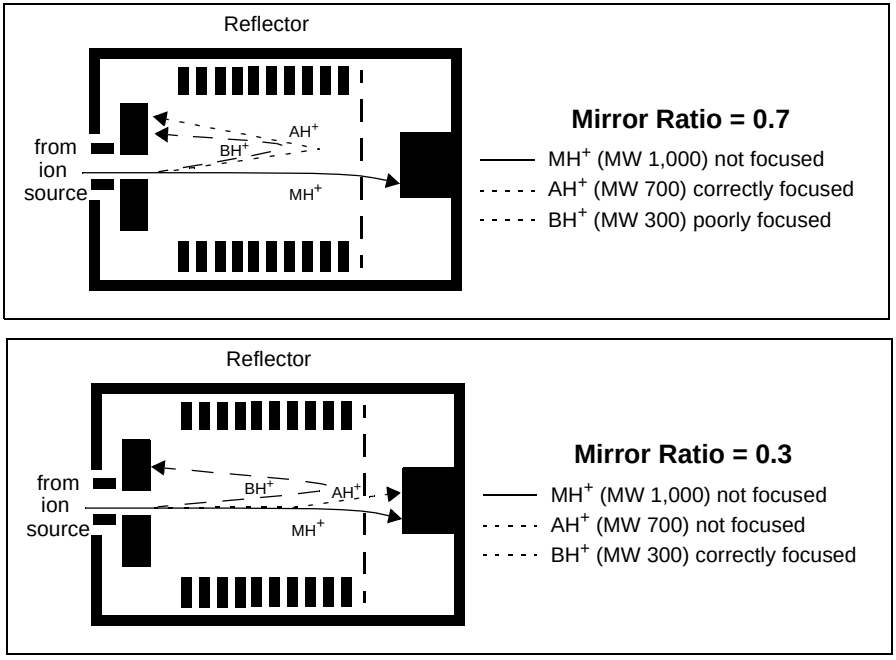


Figure 8-5 Effect of Changing Mirror Ratio

A Mirror Ratio setting of 1.0000 correctly focuses the original ion. Values of 0.7 and 0.3 correctly focus the lower energy fragments.

Note that the best focusing conditions occur when the fragment ion mass is equal to the Mirror Ratio multiplied by the precursor ion mass.

Immonium ions

Peptide PSD spectra usually include some immonium and other low mass fragment ions that are useful in determining peptide composition. For a list of immonium ions for the 20 standard amino acids and for selected modified amino acids, see Falick, A.M., W.M. Hines, K.F. Medzihradszky, M.A. Baldwin, and B.W. Gibson, “Low-Mass Ions Produced from Peptides by High-Energy Collision-Induced Dissociation in Tandem Mass Spectrometry”, *J. Am. Soc. Mass Spectrom.,* **1993**, *4*, 882–893.

8.2.2 Differences From Regular Analysis

When operating in PSD mode, note the following differences from analysis in non-PSD mode:

- **Higher laser intensity**—In non-PSD mode, you use a laser intensity that yields acceptable performance without fragmentation. You need a higher laser intensity to generate PSD fragments.
- **Higher Grid Voltage% and shorter Delay Time**—A higher Grid Voltage% and shorter Delay Time generates a sharper ion packet at the position of the Precursor Ion Selector, and improves fragment ion resolution.
- **Sample consumed more quickly**—Higher laser intensities ionize and consume sample more quickly than lower laser intensities used in non-PSD mode.
- **Different calibration used**—PSD analysis uses a different calibration function to correctly determine the masses of PSD fragment ions. For information, see Section 8.2.5, Mass Calculation for Fragment Ions.
- **Acquisition of multiple spectra at different Mirror Ratio settings to create a single composite spectrum**—To correctly focus fragments with different mass ranges, collect PSD segment spectra with different Mirror Ratio settings. The Data Explorer software "stitches" the segments together to create a composite spectrum. For information, see the *Data Explorer Software User's Guide*, Chapter 8, Viewing Voyager PSD Data.

8.2.3 Segments and Composite Spectra

Overview

To obtain the most information about an ion, collect fragment ion spectra across a molecular weight range from the mass of the original precursor ion down to 50 Da (determined by the desire to see immonium ions that indicate the presence of individual amino acids).

Each fragment ion spectrum is referred to as a segment, and is collected with a discrete focusing region (controlled by the Mirror Ratio setting). Segments are combined to generate a composite spectrum.

Number of segments

The number of PSD segments you must collect depends on the quality of data needed. The PSD software allows you to collect any number of segments. A larger number of fragment spectra generally yield better resolution in the composite spectrum. For more information, see Section 8.4.2, Determining the Number of Segments to Acquire for a Complete Composite Spectrum.

Composite spectrum

When you view the PSD data in the Data Explorer software, the software automatically assembles or “stitches” together the best portions of fragment ion spectra (referred to as PSD segments) to generate the full composite PSD spectrum.

Region of segments included in composite spectrum

The composite spectrum is generated from portions of the segment traces. The upper mass limit of the composite region in each segment is determined by the PSD Mirror Ratio (R_n) with which the segment was acquired and the mass of the precursor ion (m_p) (Figure 8-6).

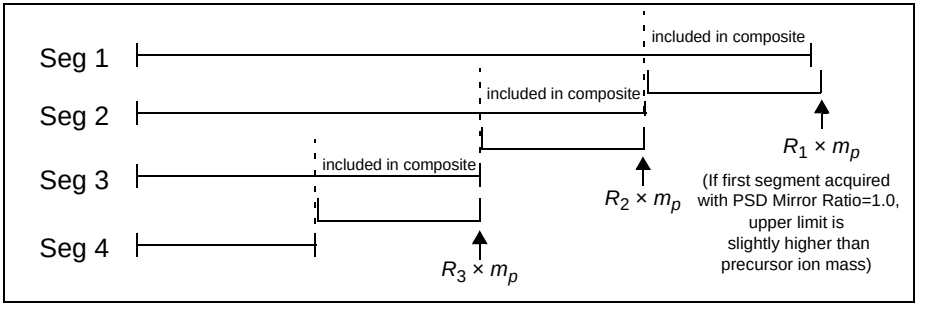


Figure 8-6 Portions of Segment Traces Included in the Composite Spectrum

For more information, see the *Data Explorer Software User's Guide*, Chapter 8, Viewing Voyager PSD Data.

8.2.4 PSD Data Files

PSD data (.DAT) files (Figure 8-7) include:

- Precursor ion mass
- All segments acquired during a PSD experiment
- Composite spectrum

Segments are stored in the data file in the order in which they are collected.

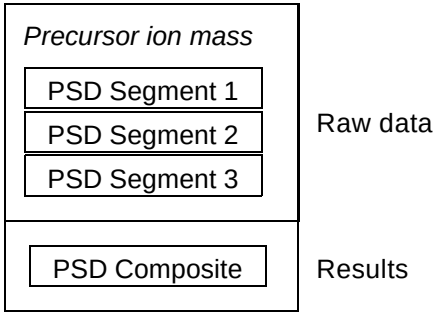


Figure 8-7 PSD .DAT File Structure

8.2.5 Mass Calculation for Fragment Ions

PSD calibration equation

PSD calibration specifies the mass of a fragment ion as a function of:

- Mass and flight time of the precursor ion
- Mirror Ratio setting
- α, β, and γ calibration constants

The equation that the Voyager software uses to calculate the mass for fragment ions is shown below:

$$m_f = (\alpha R_i + \beta)\,[(t_f - t_p) + \gamma\,(t_f - t_p)^2] + \frac{\alpha R_i + \beta}{\alpha + \beta}\, m_p$$

where:

m_f	=	Fragment ion mass
α, β, γ	=	Calibration constants
R_i	=	Mirror Ratio
t_f	=	Fragment ion flight time
t_p	=	Precursor ion flight time (at $R_i = 1$), calculated using the standard calibration equation (described on page 6-9)
m_p	=	Precursor mass entered in Instrument Settings (see page 8-45)

Default PSD calibration

If you select default PSD calibration, the Voyager software uses the calibration equation above and substitutes the following values for calibration constants:

- α —Calculates from instrument geometry
- β and γ—Uses zero (0)

8.2.6 Optimizing the Precursor Ion Selector

The Precursor Ion Selector (called Timed Ion Selector in Reflector mode) allows you to analyze the ion of interest by deflecting ions until the time that corresponds to the mass of the ion of interest. At the time that corresponds to the mass of the ion of interest, the Precursor Ion Selector voltage is turned off, and the ion of interest passes to the reflector. After the ion passes through, voltage is turned on again in the ion selector.

***NOTE**: Leave the Precursor Ion Selector enabled when performing PSD analysis. The only time you may want to disable the Precursor Ion Selector is to determine if it is working.*

The width of the mass window in which the Precursor Ion Selector voltage is turned off is determined by the Deflector Gate Width parameter. The Deflector Gate Width is optimized for your system when your system is installed. However, you can increase or decrease the selectivity of the Precursor Ion Selector by decreasing or increasing the Deflector Gate Width setting.

CAUTION

Do not alter the Timed Ion Selector parameters unless instructed to do so by an Applied Biosystems Technical Representative. Altering these settings may cause your Voyager Biospectrometry Workstation to function improperly. Before changing the Deflector Gate Width setting, write down the current setting. This setting has been optimized for your system and you may want to reset to the optimized value.

Smaller width values provide narrower Precursor Ion Selector resolution. If you set the width too low, fragment ion yield is compromised due to product ion dispersion that occurs as ions travel down the flight tube.

To change the width setting, select **Hardware Configuration** from the Instrument menu in the Instrument Control Panel, then click the **Timed Ion Selector** tab (Figure 8-8).

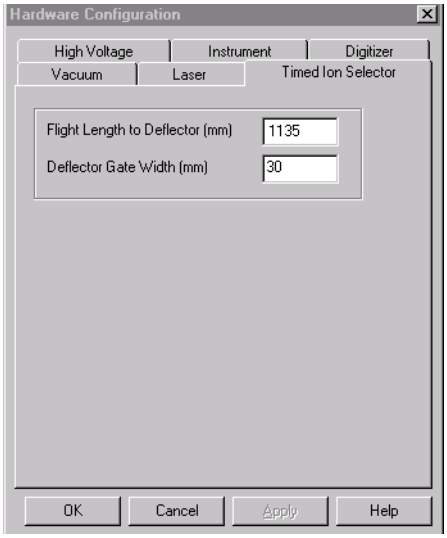


Figure 8-8 Timed Ion Selector Tab in Hardware Configuration Dialog Box

Set the Deflector Gate Width as needed. A lower setting increases specificity, but may decrease sensitivity.

CAUTION

Do not change the Flight Length to Deflector parameter. This parameter is optimized for your system.

8.3 Enhancing Fragmentation with CID

Overview

Collision-induced dissociation (CID) is a technology that enhances fragmentation in Post-Source Decay (PSD) analysis. CID is available as an option on the Voyager-DE PRO and Voyager-DE STR workstations.

The CID option includes:

- A 0.5 cc cylindrical cell (collision cell) in the ion source region connected to the grounded aperture
- External CID box with valves to control CID gas flow

Figure 8-9 shows the plumbing of the CID option.

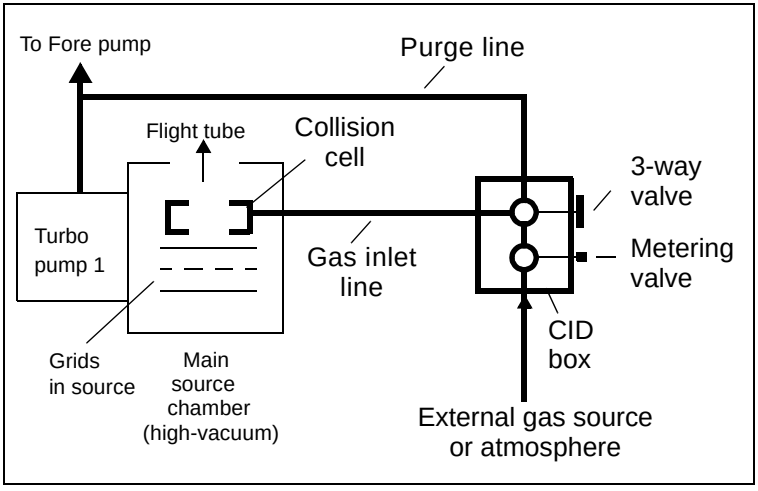


Figure 8-9 CID Option Plumbing

When supplied with gas, the collision cell has a higher pressure than the source. As ions leave the source, they pass through the collision cell and interact with the collision gas molecules. Energy is transferred to the ions and fragmentation is enhanced.

Benefits

The benefits provided by CID include:

- Fragmentation of ions that does not occur under normal PSD conditions
- Side chain fragmentation that may allow you to distinguish between Leucine and Isoleucine isomers
- Greater number of immonium ions generated for peptide analysis

Figure 8-10 and Figure 8-11 are sample spectra from low and mid mass ranges that illustrate the impact of CID gas.

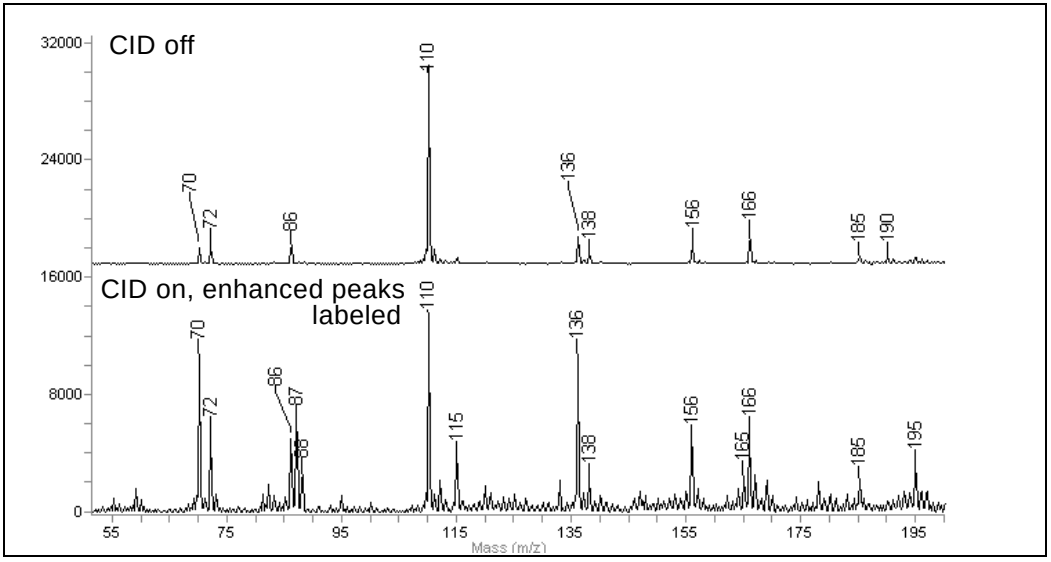


Figure 8-10 Low Masses—Impact of CID (Angiotensin I)

In the top trace (CID off), typical fragments are seen and labeled.

In the bottom trace (CID on), fragments not seen without CID, or fragments that are significantly enhanced with CID, are labeled.

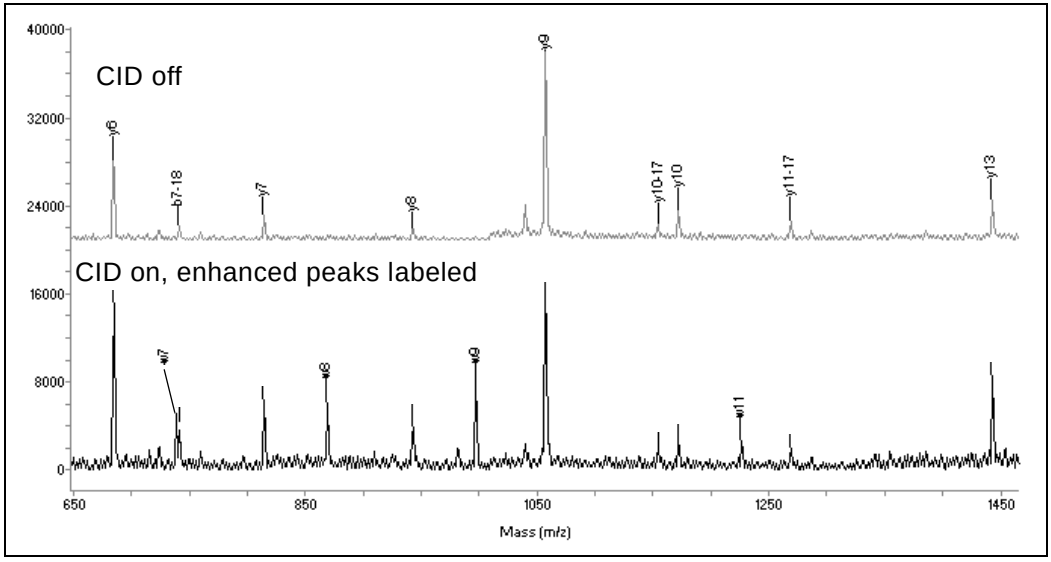


Figure 8-11 Mid Masses—Impact of CID (Glu-1-Fibrinopeptide)

In the top trace (CID off), typical fragments are seen and labeled.

In the bottom trace (CID on), w fragments[1] not seen without CID are labeled.

Gas requirements

You can use room air, helium, argon, or xenon as the collision gas.

If you are using a compressed gas source, regulate the gas source between 2 and 5 psi.

1. *Meth. Enzymol.*, McCloskey, J.A, ed., 1990, **193**, 886.

Purging collision gas lines

Before turning on the collision gas, purge the lines to prevent disruption of the vacuum.

CAUTION

If you do not purge the lines, the CID gas introduction may increase the pressure in the vacuum and cause an Interlock error.

Perform the following procedure if the CID gas has been off for more than 15 minutes:

1. Observe the vacuum gauge, and allow the pressure on BA1 (Source Chamber pressure) to reach the typical pressure listed below:

Model	Pressure
Voyager-DE PRO	~8×10^{-7} Torr
Voyager-DE STR	~2×10^{-7} Torr

2. Turn the top 3-way valve on the CID box to the Purge Cell position for 1 to 2 seconds.
3. Turn the top 3-way valve to the Gas Inlet position for 1 second, then turn immediately to the Purge Cell position.
4. Observe BA1 on the vacuum gauge panel.
5. When BA1 reads approximately 1×10^{-6} Torr, turn the top 3-way valve to the Gas Inlet position for 2 seconds, then turn immediately to the Purge Cell position.
6. Observe BA1 on the vacuum gauge panel.
7. When BA1 reads approximately 1×10^{-6} Torr, turn the top 3-way valve to the Gas Inlet position for 3 seconds, then turn immediately to the Purge Cell position.

   At this point, the lines should be purged. If pressure is greater than 2×10^{-6} Torr, repeat step 7.
8. Turn the top 3-way valve to the Gas Inlet position, then turn on the collision gas as described on page 8-35.

Turning on collision gas

To turn on the collision gas after purging:

1. If the metering valve on the side of the CID box is set at zero, turn the metering valve approximately 1/4 turn.

   If the metering valve is not set at zero, do not turn the valve.

2. Wait 1 to 2 minutes for BA1 on the vacuum gauge panel to stabilize at 3×10^{-6} Torr, or at the optimum pressure for CID operation that you have determined and recorded for your system.

3. If the pressure is lower than the optimum, turn the metering valve one notch clockwise to a higher setting. Wait for BA1 to stabilize at the optimum pressure.

   If the pressure is higher than the optimum, turn the metering valve one notch counterclockwise to a lower setting. Turn the top 3-way valve to the Purge Cell position, then to the Gas Inlet position. Wait for BA1 to stabilize at the optimum pressure.

4. Continue adjusting the metering valve until BA1 is stable at the optimum pressure.

NOTE: *If BA1 is below the optimum pressure when using a compressed gas supply, and you cannot increase the pressure by turning the metering valve clockwise to a higher setting, make sure the gas supply is regulated to 2 to 5 psi.*

Turning off collision gas

To turn off the collision gas:

1. Turn the top 3-way valve on the CID box to the Purge Cell position.
2. Wait approximately 20 seconds for the gas to evacuate.
3. Turn the top 3-way valve on the CID box to the Off position.

Adjusting collision gas

To optimize fragmentation, adjust the flow of the collision gas. Turn the metering valve on the CID box until you observe the desired fragmentation.

If gas pressure is too high, signal is suppressed. If gas pressure is too low, you will not obtain the desired fragmentation.

Adjusting Guide Wire Voltage%

When using the CID option, you may observe enhanced performance by increasing the Guide Wire Voltage% above the setting used for PSD analysis without CID.

NOTE: *Guide Wire Voltage% is not available on Voyager-DE STR models with serial number 4154 and later.*

8.4 Acquiring PSD Data with Standard .BIC Files in Manual Control Mode

Steps to acquire PSD data

The steps to acquire PSD data in Manual Control mode are:

Step	Described on page
Determine the precursor ion mass	8-38
Determine the number of segments to acquire for a complete composite spectrum	8-40
Set the PSD Acquisition parameters for Manual Control mode	8-43
Fill in the segment list and save the .BIC file	8-47
Acquire and save PSD segments	8-52

Practicing in PSD mode

This section tells you how to use the software to collect spectra in PSD mode. However, to be successful in PSD analysis, you need to understand how spectra behave in PSD mode and how to optimize acquisition conditions.

Follow the steps in this section to understand how to use the software. Then follow the steps in Section 8.6, Exploring PSD Mode, to learn how to optimize the parameters that affect PSD analysis.

NOTE: *Before you run unknowns, we recommend that you perform the steps above using a standard.*

8.4.1 Determining the Precursor Ion Mass

Overview

Before beginning an analysis in PSD mode:

- Generate a precursor spectrum in Reflector mode to determine the mass of the precursor ion. A Reflector mode analysis provides optimum resolution and mass accuracy.
- Generate an external calibration for the precursor ion to use during the PSD acquisition.

Generating the precursor spectrum

To generate the precursor spectrum:

1. Open the **PSD_Precursor.BIC** file provided with the software. This is a reflector mode instrument settings (.BIC) file. All other settings should be identical to the settings in the PSD mode .BIC you will use to acquire segments.
2. Modify the mass range and optimize other parameters as needed. See Chapter 5, Optimizing Instrument Settings.
3. In the Calibration section of the Instrument Settings control page, select the matrix you are using. See “Calibration” on page 5-20.
4. From the View menu, select **Data Storage**. Set parameters as needed. See “Setting Data Storage parameters” on page 6-14.
5. In the Manual Laser Intensity/Sample Positioning Control page, select the sample to acquire. See “Selecting sample position and laser intensity” on page 6-13.
6. To start acquiring, select **Start Acquisition** from the Acquisition menu, or click  .
7. Acquire the spectrum using a laser intensity that does not generate fragments.
8. Save the data file by clicking  .

The precursor spectrum is displayed in the Current trace in the Spectrum window, and the .DAT file is saved in the directory specified.

Generating an external calibration for the precursor ion

To obtain maximum mass accuracy for the precursor ion, generate an external calibration file that you will use when you perform the PSD acquisition. This external calibration is used to determine the t_p value (precursor ion flight time) needed for the PSD calibration equation (described on page 8-28). The t_p value is determined using the standard calibration equation ("t" in the standard equation described on page 6-9).

NOTE: *The calibration you specify on the Instrument Settings Control page is used to determine the t_p value (precursor ion flight time) needed for the PSD calibration equation (described on page 8-28). The t_p value is determined using the standard calibration equation ("t" in the standard equation described on page 6-9). You will also specify an external PSD calibration below, which is used to determine the values for α , β , and γ needed for the PSD calibration equation.*

To generate the calibration:

1. Open the precursor spectrum data file in the Data Explorer software.
2. Create a single-point calibration using the precursor ion mass. For more information, see the *Data Explorer Software User's Guide*, Chapter 5.3.2, Manually Calibrating.
3. Save the calibration file (.CAL) by exporting the calibration constants from the data file. For more information, see the *Data Explorer Software User's Guide*, "Exporting .BIC, .MSM, and .CAL files" on page 1-36.

8.4.2 Determining the Number of Segments to Acquire for a Complete Composite Spectrum

This section includes:

- Resolution and the number of segments
- Decrement ratio
- Default Mirror Ratio settings
- Size of segments
- Default number of segments
- Acquiring only select segments

Resolution and the number of segments

If you are interested in a complete composite spectrum, the number of segments you need to acquire is determined by:

- Resolution you require
- Mass range of interest

You see better resolution in the composite spectrum with more segments.

NOTE: *If you are interested in selected fragment masses only (instead of a complete composite spectrum), see* *"Acquiring only select segments" on page 8-42.*

Use the following equation to determine the number of segments to acquire:

$$n \approx \frac{\ln (m_f / m_p)}{\ln D}$$

where:

m_f	=	Fragment ion mass of the lowest mass of interest
D	=	Decrement Ratio, which determines the size of the segment
n	=	Number of segments
m_p	=	Precursor ion mass

Hint: *You can use the Windows calculator to determine natural log values. To open the calculator, select* ***Programs*** *from the Windows Start menu, select* ***Accessories****, then select* ***Calculator****. If the ln function is not displayed when you open the Windows calculator, select* ***Scientific*** *from the View menu in the calculator to access advanced functions.*

Hint: *The Angiotensin_PSD.BIC file provided with the software includes 10 segments, which is suitable for many applications. If the mass you are analyzing differs by more than 300 Da from the mass in the Angiotensin_PSD.BIC file (1296 Da), you may need to acquire more or fewer than 10 segments to observe the fragment ions of interest.*

Decrement ratio

The software can automatically calculate the Mirror Ratio settings needed for each segment. To do so, it uses a Mirror Ratio setting of 1.000 and a Decrement Ratio parameter that you enter.

The correlation between the Mirror Ratio and the Decrement Ratio is the inverse percentage. That is, if you want to obtain 20 percent segments, set the Decrement Ratio to 0.8000. If you want to obtain 80 percent segments, set the Decrement Ratio to 0.2000.

Default Mirror Ratio settings

The default Mirror Ratio is 1.0000. The default Decrement Ratio is 0.7500. Therefore, the default Mirror Ratios yield the following 25 percent segments:

1.000	0.237
0.750	0.178
0.563	0.133
0.422	0.100
0.316	0.075

Increase the Decrement Ratio to collect smaller segments. Decrease the Decrement Ratio to collect larger segments.

Size of segments

You can acquire segments of different sizes by varying the Mirror Ratio setting. For example, you can set the first Mirror Ratio to collect a 20 percent segment, then set the next Mirror Ratio to collect a 10 percent segment. If you change the default Decrement Ratio, fill-down subsequent existing rows, and the Mirror Ratio values are automatically recalculated. Fill-down is described in “Using the Fill Down function” on page 8-50.

Default number of segments

The standard instrument settings (.BIC) file provided with the software, Angiotensin_PSD.BIC, is set to acquire 10 segments with the Mirror Ratios listed in “Default Mirror Ratio settings” on page 8-41. These settings are adequate for many applications.

You can add or delete segments, or change the Mirror Ratio for a segment as needed.

Acquiring only select segments

The PSD software allows complete flexibility in the way you acquire and process PSD data. You are not required to acquire a complete set of PSD segments. If you are interested in specific fragment masses only, you can acquire only the number of segments you are interested in by using Mirror Ratio settings that focus the fragment ion masses of interest.

Hint: *The best focusing conditions occur when the fragment ion mass is equal to the Mirror Ratio multiplied by the precursor ion mass (equal to the Max Stitch Mass).*

When you type in a Mirror Ratio (described in Section 8.4.4, Filling in the Segment List and Saving the .BIC File), observe the Max Stitch Mass displayed. This value reflects the maximum mass of the segment that will be included in the composite spectrum. (The mass range included in the segment is approximately 15 percent higher than the Max Stitch Mass.)

8.4.3 Setting PSD Acquisition Parameters for Manual Mode

To set PSD Acquisition parameters:

- Display the PSD Acquisition control page
- Set voltages and external calibration for the precursor spectrum
- Set precursor mass and PSD calibration for fragment spectra

Displaying the PSD Acquisition control page

To display PSD Acquisition control page:

1. Open the **Angiotensin_PSD.BIC** file provided with the software. This is a PSD mode .BIC file.

   The PSD Acquisition Settings control page is automatically displayed if you open a .BIC file that is set to PSD mode.

   To manually display the PSD Acquisition Settings control page, change the operation mode to PSD by:

   - Clicking **Mode/Digitizer** in the Instrument Settings control page
   - Clicking the **Instrument Mode** tab
   - Selecting **PSD**

NOTE: *Low Mass Gate is automatically disabled if PSD is specified for Instrument Mode. The Precursor Ion Selector provides the selectivity needed to screen out unwanted masses.*

2. If the PSD Acquisition Settings control page (Figure 8-12) is not displayed, select **PSD Acquisition** from the View menu.

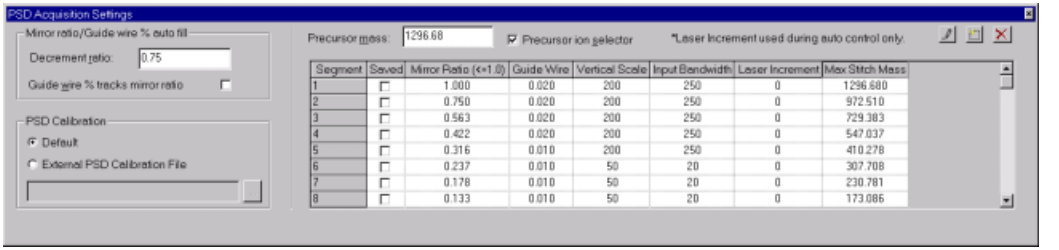


Figure 8-12 PSD Acquisition Settings Control Page—Manual Mode

8

NOTE: *The Precursor Ion Selector in PSD mode is the same parameter as the Timed Ion Selector in Reflector mode. If you change the Precursor mass used by the Precursor Ion Selector in PSD mode, the mass specified for the Timed Ion Selector in Reflector mode also changes.*

Setting voltages and external calibration for the precursor spectrum

3. For optimum mass accuracy, change the Accelerating Voltage and Grid Voltage% (on the Instrument Settings control page) to the values you used to acquire the precursor spectrum and to generate the calibration.

NOTE: *If you use different voltage settings (Accelerating Voltage, Grid Voltage, or Delay Time) to acquire the precursor spectrum, generate the calibration, and acquire the fragment spectra, the software compensates for the different settings. However, using different voltage settings may not yield optimum mass accuracy.*

4. In the Calibration section of the Instrument Settings control page, select the matrix you are using and the .CAL file you created in “Generating an external calibration for the precursor ion” on page 8-39.

NOTE: *The calibration you specify on the Instrument Settings control page is used to determine the t_p value (precursor ion flight time) needed for the PSD calibration equation (described on page 8-28).The t_p value is determined using the standard calibration equation (“t” in the standard equation described on page 6-9). You will also specify an external PSD calibration below, which is used to determine the values for α , β , and γ needed for the PSD calibration equation.*

NOTE: *If default calibration yields acceptable mass accuracy for your application, an external calibration is not required.*

Setting precursor mass and PSD calibration for fragment spectra

5. In the PSD Acquisition Settings control page, type the Precursor mass.

NOTE: *Precursor mass is used for PSD calibration. Type in an accurate value with appropriate precision (for example, type 1296.68, not 1297).*

---

6. Select **Precursor Ion Selector** if it is not already selected.

NOTE*: Leave the Precursor Ion Selector enabled when performing PSD analysis. The only time you may want to disable the Precursor Ion Selector is to determine if it is working.*

---

7. To set PSD calibration, select the default calibration or select a previously generated external PSD .CAL file. For more information, see the *Data Explorer User's Guide*, Chapter 8.3.3, Creating PSD Calibration (.CAL) Files and Applying to Other Data Files.

NOTE: *The calibration you specify on the PSD Acquisition Settings control page is used to determine the values for* α *,* β *, and* γ *needed for the PSD calibration equation (described on page 8-28). The calibration you specified on the Instrument Settings control page in step 4 is used to determine the value for* t_p *(precursor ion flight time) needed for the PSD calibration equation.*

NOTE: *If default PSD calibration yields acceptable mass accuracy for your application, an external calibration is not required.*

NOTE: *PSD .CAL files are named with the same extension as reflector or linear .CAL files. If you select a reflector or linear .CAL file when in PSD mode, an error message is generated.*

8.4.4 Filling in the Segment List and Saving the .BIC File

This section describes:

- Filling in the list
- Typing or selecting new values
- Using the Fill Down command
- Adding or deleting rows
- Saving the .BIC file

Filling in the list

To fill in the segment list:

If you are using Angiotensin_PSD.BIC

1. If you are using the standard instrument settings (.BIC) file provided with the software, Angiotensin_PSD.BIC, the segment list contains 10 segments with the Mirror Ratio settings listed in “Default Mirror Ratio settings” on page 8-41.

   If the precursor ion mass you are analyzing differs by more than 300 Da from the mass in the Angiotensin_PSD.BIC (1296 Da), you may need to acquire more or fewer than 10 segments to observe the fragment ions of interest. You can add or delete segments, or change the Mirror Ratio for a segment as needed.

If you are creating a new .BIC file

If you are creating a new .BIC file, click  to add the required number of rows for the number or segments to acquire. For more information, see Section 8.4.2, Determining the Number of Segments to Acquire for a Complete Composite Spectrum.

Rows are added with the default values listed in Table 8-4 for all columns.

Table 8-4 Default Values for the PSD Segment List

Parameter	Default Value
Segment	Sequential number starting at 1.
Saved check box	Blank until the segment is saved (the software automatically places a check mark in this field when you save a segment).
Mirror Ratio	1.000
Guide Wire%	0.020 ***NOTE:*** *Guide Wire Voltage% is not available on Voyager-DE STR models with serial number 4154 and later.*
Vertical Scale	Lowest setting for your digitizer type.
Input Bandwidth	Lowest setting for your digitizer type.
Laser Increment	Disabled in Manual Control mode. For information, see Appendix 8.5, Acquiring PSD Data with Standard .BIC Files in Automatic Control Mode.
Max Stitch Mass	Calculated value equal to (Precursor Mass)×(Mirror Ratio) Reflects the maximum mass of the segment that will be included in the composite spectrum. The mass range included in the segment is approximately 15 percent higher than the Max Stitch Mass.

2. Edit the values as needed. See:

   - “Typing or selecting new values” on page 8-49
   - “Using the Fill Down function” on page 8-50

Guidelines for Guide Wire Voltage%, Vertical Scale, and Input Bandwidth

As a general guideline when setting Guide Wire Voltage%, Vertical Scale, and Input Bandwidth, use decreasing values to compensate for decreasing ion energy seen at lower Mirror Ratios. You need to experiment to determine the optimum values for these settings at lower Mirror Ratios.

NOTE: *Guide Wire Voltage% is not available on Voyager-DE STR models with serial number 4154 and later.*

Typing or selecting new values

To type or select new values in the segment list:

1. Type in new values for Mirror Ratio or Guide Wire% in any row in the table. You can specify Mirror Ratios in any order in the table.

NOTE: *When you click the Mirror Ratio field, the entry is displayed with more than 3-digit precision, which is the precision used to calculate the mass range for the segment during the analysis.*

You can specify the same Mirror Ratio for multiple segments if needed. When the composite spectrum is generated, the software uses the most recently acquired segment if duplicate Mirror Ratios are present.

Observe the Max Stitch Mass when you type a Mirror Ratio. Optimum focus and resolution is achieved for fragment ions close to this mass.

2. To select new values for Vertical Scale or Input Bandwidth, click the cell.

   A drop-down list of values appropriate for your system is displayed. Select a value.

Using the Fill Down function

To use the Fill Down function:

1. If you want the Fill Down function to calculate values for Mirror Ratio and Guide Wire% (instead of copying the value from the first row), set the following in the Mirror Ratio/Guide Wire% Fill Down section:

   - **Decrement Ratio**—To calculate the needed Mirror Ratios. For more information, see “Decrement ratio” on page 8-41. Increase this value to create smaller segments. Decrease this value to create larger segments.
   - **Guide Wire% Tracks Mirror Ratio** (not available on Voyager-DE STR models with serial number 4154 and later)—If you want to automatically adjust the Guide Wire% settings according to the Mirror Ratio setting.

   NOTE: *Do not select Guide Wire% Tracks Mirror Ratio option on Voyager-DE PRO or Voyager-DE STR systems. This parameter is for use with older systems only.*

2. Type in values in any row that you want to fill down into selected rows.
3. Click on the **Segment number** to select the row containing the values to fill down.
4. Click  . The following occurs:

   - The Mirror Ratio is calculated and entered in all rows below. If no Decrement Ratio is specified, the value from the first cell is copied to selected cells below.

   NOTE: *If you selected Guide Wire% Tracks Mirror Ratio, Guide Wire% is also calculated and filled in.*

- The information in the first row of remaining columns is copied to all rows below.
- The Max Stitch Mass is calculated and displayed for each row.
- Any rows for segments that have already been saved are skipped.

Adding or deleting rows

To add rows, select any existing row, then click  . A row is added to the end of the list, and the settings from the selected row (or the first row if no row is selected) are copied to the added row. Change settings as needed.

To delete rows, select the rows to delete and click  . You cannot delete a row if the segment has been saved.

Saving the .BIC file

To save the instrument settings (.BIC) file, select **Save Instrument Settings As** from the File menu, then save the .BIC file with a new name.

8.4.5 Acquiring and Saving PSD Segments in Manual Mode

This section includes:

- Overview
- Acquiring PSD segments
- Selecting and acquiring a segment
- During acquisition
- Changing settings
- Accumulating or saving the segment
- Selecting and acquiring remaining segments
- Reacquring a segment
- Stopping the experiment

Overview

When you start acquiring in PSD mode, the software automatically opens a PSD experiment. When a PSD experiment is open:

- You cannot change instrument settings. This ensures that all PSD segments are acquired under the same conditions.
- All spectra that you save are placed in one .DAT file (no .DAT file is created until you save a segment).

When you save the first PSD segment, the software creates a PSD .DAT file. During a PSD acquisition, the PSD .DAT file remains open and you can acquire multiple segment traces, accumulate or discard traces, and save traces. Each time you save a trace, it is appended to the currently open PSD .DAT file. At the end of the acquisition, you stop the experiment, which closes the PSD .DAT file and makes it available for viewing in the Data Explorer software.

After an experiment is closed and the .DAT file is closed, you cannot reopen the .DAT file to append additional segments.

NOTE: *In the Data Explorer software, you cannot view the PSD .DAT file you are currently acquiring until you stop the experiment. The PSD .DAT file is open and not available for viewing while the experiment is running.*

NOTE: *If you stop an experiment without saving any segments, no .DAT file is created.*

NOTE: *All instrument settings except Shots/Spectrum are disabled as soon as you start acquisition in PSD mode, until you stop the experiment. Make sure instrument settings are correct before starting acquisition.*

Acquiring PSD segments

To acquire PSD segments:

1. In the Manual Laser Intensity/Sample Positioning control page, select the same sample position from which you acquired the precursor spectrum.
2. From the View menu, select **Data Storage**. Set parameters as needed. See “Setting Data Storage parameters” on page 6-14, for information.

Hint: *Include a _PSD suffix when you name PSD data files to help you distinguish them from non-PSD data files. For example, if you type in Experiment1_PSD as the file name, the complete data file name will be Experiment1_PSD.DAT or Experiment1_PSD_0001.DAT (if Autosequence Filenames is enabled).*

Selecting and acquiring a segment

3. In the PSD Acquisition Settings control page, select (click on) the row that corresponds to the segment you want to acquire.
4. To start acquiring, select **Start Acquisition** from the Acquisition menu, or click  .
5. Adjust laser intensity to optimize signal intensity. You typically need a higher laser intensity to optimize signal intensity for segments with lower Mirror Ratio settings.

During acquisition

The following occurs:

- Mass range for the segment is set to:

Mass	Equivalent to
Start	(Precursor mass/4) which is equal to (Precursor flight time/2)
End	(Mirror to Accelerating Voltage Ratio^2 × Precursor mass) which is equal to (Mirror to Accelerating Voltage Ratio × Precursor flight time)

- Acquisition starts.
- All instrument settings except Shots/Spectrum are disabled. Settings remain inactive until you stop the PSD experiment after you acquire all segments of interest.
- Acquisition of the segment continues until the number of Shots/Spectrum specified in Spectrum Acquisition on the Instrument Settings control page is collected, or until you select **Stop Acquisition** from the Acquisition menu.
- The spectrum is displayed in the Current Trace in the Spectrum window.

Changing settings

During acquisition, you can vary laser intensity, parameters in the segment list, and Shots/Spectrum.

Accumulating or saving the segment

6. Evaluate the spectrum, then do one of the following:

   - Click  in the toolbar to accumulate the spectrum. You can accumulate as many spectra as needed. When the accumulated spectrum is acceptable, click on the Accumulated trace, then click  .
   - Click  in the toolbar to add the segment to the .DAT file. After you save the segment, the Saved check box in the segment list is checked.
   - If the current spectrum does not contain significant fragment ion signal and you do not want to accumulate or save the spectrum, acquire remaining segments as described below.

CAUTION

Save the current segment (if the data is acceptable) before starting to acquire the next segment. If you do not, you will lose the data for the current segment.

Selecting and acquiring remaining segments

7. Repeat step 3 through step 6 to collect remaining segments. You can collect segments in any order, and do not have to acquire all segments in the list. You can also acquire multiple segments with the same Mirror Ratio.

NOTE: *Segments are listed in the Data Explorer software in the order in which they are acquired. If segments with duplicate Mirror Ratios are contained in the file, the software uses the last acquired segment when it generates the composite spectrum.*

Reacquring a segment

8. To reacquire a segment:
   - Select the segment to reacquire.
   - Click  . A new row is added to the end of the segment list and contains the settings from the selected segment.
   - Set parameters as needed.
   - Repeat step 3 through step 6.

Stopping the experiment

9. When you have acquired all necessary segments, select **Stop Experiment** from the Acquisition menu. The PSD data file is closed.

CAUTION

If you stop an experiment without saving any segments, no .DAT file is created.

8.5 Acquiring PSD Data with Standard .BIC Files in Automatic Control Mode

During PSD analysis in Automatic Control mode:

- The laser is automatically adjusted for PSD segments with different Mirror Ratios.
- All selected segments are automatically acquired and appended to the currently open PSD .DAT file.
- The experiment is automatically closed when all selected segments are acquired and saved.

Before you begin

Before acquiring PSD spectra in Automatic Control mode, become familiar with the information in Section 8.4, Acquiring PSD Data with Standard .BIC Files in Manual Control Mode.

Steps to acquire PSD data

The steps to acquire PSD data in Automatic Control mode are:

Step	Described on page
Determine the precursor ion mass (same procedure as in Manual Control mode).	8-38
Determine the number of segments to acquire for a complete composite spectrum (same procedure as in Manual Control mode).	8-40
Set the PSD Acquisition parameters for Automatic Control mode.	8-58
Fill in the segment list (same procedure as in Manual Control mode).	8-47
Set laser increment and save the .BIC file.	8-62
Acquire PSD segments in Automatic mode.	8-63

8.5.1 Setting PSD Acquisition Parameters for Automatic Control Mode

To set PSD Acquisition parameters:

- Display the PSD Acquisition control page
- Set voltages and external calibration for the precursor spectrum
- Set precursor mass and PSD calibration for fragment spectra

Displaying the PSD Acquisition control page

To display the PSD Acquisition control page:

1. Open the **Angiotensin_PSD_Auto.BIC** file provided with the software. This is a PSD mode .BIC file set to Automatic Control mode with the following settings:

Parameter	Setting
Automated Laser Intensity	Enabled (Minimum/Maximum Laser, Step, and Prescan settings not used)
Number of Spectra to Acquire	1 (one spectrum saved for each PSD segment, all segments saved in one .DAT file)
Spectrum Accumulation	Save All Spectra, no Acceptance Criteria applied
Automated Sample Positioning	Enabled, with Random Uniform as the default; however, if you change the setting, your change is retained.

NOTE: *If you start with a .BIC file that is not set as indicated in the table above, and you change to Automatic Control mode and PSD Operation mode, the software automatically changes the settings in the .BIC file to those in the table above. These changes are not stored permanently in the .BIC file unless you save it.*

2. If Control mode is not set to Automatic, click **Automatic**.
3. If the PSD Acquisition Settings control page (Figure 8-13) is not displayed, select **PSD Acquisition** from the View menu.

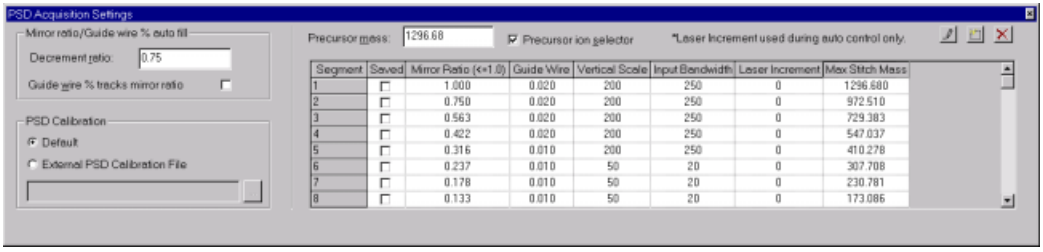


Figure 8-13 PSD Acquisition Settings Control Page—Automatic Mode

Setting voltages and external calibration for the precursor spectrum

4. For optimum mass accuracy, change the Accelerating Voltage and Grid Voltage% (on the Instrument Settings control page) to the values you used to acquire the precursor spectrum and to generate the calibration.

NOTE: *If you use different voltage settings (Accelerating Voltage, Grid Voltage, or Delay Time) to acquire the precursor spectrum, generate the calibration, and acquire the fragment spectra, the software compensates for the different settings. However, using different voltage settings may not yield optimum mass accuracy.*

5. In the Calibration section of the Instrument Settings control page, select the matrix you are using and the .CAL file you created in “Generating an external calibration for the precursor ion” on page 8-39.

NOTE: *The calibration you specify on the Instrument Settings control page is used to determine the t_p value (precursor ion flight time) needed for the PSD calibration equation (described on page 8-28).The t_p value is determined using the standard calibration equation (“t” in the standard equation described on page 6-9). You will also specify an external PSD calibration below, which is used to determine the values for α , β , and γ needed for the PSD calibration equation.*

NOTE: *If default calibration yields acceptable mass accuracy for your application, an external calibration is not required.*

Setting precursor mass and PSD calibration for fragment spectra

6. In the PSD Acquisition Settings control page, type the Precursor mass.

NOTE: *Precursor mass is used for PSD calibration. Type in an accurate value with appropriate precision (for example, type 1296.68, not 1297).*

---

7. Select **Precursor Ion Selector** if it is not already selected.

8. To set PSD calibration, select the default calibration or select a previously generated external PSD .CAL file. For more information, see the *Data Explorer User’s Guide*, Chapter 8.3.3, Creating PSD Calibration (.CAL) Files and Applying to Other Data Files.

NOTE: *The calibration you specify on the PSD Acquisition Settings control page is used to determine the values for α, β, and γ needed for the PSD calibration equation (described on page 8-28). The calibration you specify on the Instrument Settings control page in step 4 is used to determine the value for t_p (precursor ion flight time) needed for the PSD calibration equation.*

NOTE: *If default PSD calibration yields acceptable mass accuracy for your application, an external calibration is not required.*

NOTE: *PSD .CAL files are named with the same extension as reflector or linear .CAL files. If you select a reflector or linear .CAL file when in PSD mode, an error message is generated.*

Filling in the segment list

9. Fill in the segment list as described in Section 8.4.4, Filling in the Segment List and Saving the .BIC File.

8.5.2 Setting Laser Increment and Saving the .BIC File

Setting Laser Increment

Set the Laser Increment for each segment. The Laser Increment value is added to the starting laser intensity set in the Manual Laser/Sample Positioning control page.

For example:

Initial Laser Intensity	Segment	Laser Increment	Laser Intensity for Acquisition
1,800	1	20	1,820
	2	40	1,840
	3	100	1,900

NOTE: *You typically need a higher laser intensity to optimize signal intensity for segments with lower Mirror Ratio settings.*

Saving the .BIC file

To save the instrument settings (.BIC) file, select **Save Instrument Settings As** from the File menu, then save the .BIC file with a new name.

8.5.3 Acquiring PSD Segments in Automatic Control Mode

Acquiring PSD segments

To acquire PSD segments in Automatic mode:

1. In the Manual Laser Intensity/Sample Positioning control page, select the same sample position from which you acquired the precursor spectrum.
2. From the View menu, select **Data Storage**. Set parameters as needed. See “Setting Data Storage parameters” on page 6-14, for information.

Hint: *Include a _PSD suffix when you name PSD data files to help you distinguish them from non-PSD data files. For example, if you type in Experiment1_PSD as the file name, the complete data file name will be Experiment1_PSD.DAT or Experiment1_PSD_0001.DAT (if Autosequence File Names is enabled).*

Selecting and acquiring a segment

3. In the PSD Acquisition Settings control page, press and hold the Control key and click all rows that correspond to the segments you want to acquire.

NOTE: *All instrument settings are disabled as soon as you start acquisition in PSD mode, until you stop the experiment. Make sure instrument settings are correct before starting acquisition.*

CAUTION

Select all necessary segments before continuing. You cannot acquire additional segments into the current data file after you start acquisition. When acquisition is complete, the data file is automatically closed. If you want to acquire additional segments as part of the same data file, you must repeat the acquisition with all necessary segments selected.

4. To start acquiring, select **Start Acquisition** from the Acquisition menu, or click  .

During acquisition

The following occurs for each segment spectrum as it is acquired:

- Mass range for the segment is set to:

Mass	Equivalent to
Start	(Precursor mass/4) which is equal to (Precursor flight time/2)
End	(Mirror to Accelerating Voltage Ratio^2 × Precursor mass) which is equal to (Mirror to Accelerating Voltage Ratio × Precursor flight time)

- All instrument settings are disabled. Settings remain inactive until you stop the PSD experiment or all segments are acquired.
- Acquisition of the segment continues until the number of Shots/Spectrum specified in Spectrum Acquisition on the Instrument Settings control page is collected, or until you select **Stop Acquisition** from the Acquisition menu.
- The segment spectrum is displayed in the Current Trace in the Spectrum window and saved to the data file.

After all segments are acquired, the software automatically stops the experiment, then closes and saves the PSD data file.

8.6 Exploring PSD Mode

To be successful in PSD analysis, you need to understand how ions behave in PSD mode, and how to optimize acquisition conditions. Before running samples, spend some time practicing with standards.

In this section, you will observe the effects of:

- Laser intensity on fragment ion production and signal intensity
- Precursor Ion Selector on prompt fragments
- Grid Voltage% on resolution

This section assumes that you are familiar with the information in Section 8.4, Acquiring PSD Data with Standard .BIC Files in Manual Control Mode.

Preparing the practice standard

WARNING

CHEMICAL HAZARD. *Please read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*
Angiotensin *may cause an allergic skin and respiratory reaction. Exposure may cause eye, skin, and respiratory tract irritation.*
Alpha-cyano-4-hydroxycinnamic acid (CHCA) *may cause eye, skin, and respiratory tract irritation.*

To prepare the practice standard:

1. Prepare a 50 pmol/µl solution of angiotensin I.
2. Prepare α-cyano-4-hydroxycinnamic acid matrix as described in Section 3.1.2, Preparing Matrix.
3. Mix 1 µl of angiotensin I with 24 µl of matrix for a final concentration of 2 pmol/µl.
4. Apply 1 µl of angiotensin/matrix to a sample plate.

8.6.1 Observing the Effects of Laser Intensity

Adjusting laser intensity affects fragment ion production and signal intensity.

In this section

In this section, you will:

- Observe the effects of setting the laser intensity too high and too low
- Determine the laser intensity for your system that yields the best signal for PSD spectra

Observing effects of high and low laser intensity

To observe the effects of laser intensity:

1. Open the **Angiotensin_PSD.BIC** file provided with the software.
2. In the PSD segment list, select the first row with a Mirror Ratio setting of 1.0.
3. Acquire a spectrum.
4. Observe the 1,180 to 1,190 Da mass region for the fragment ion cluster typically seen for angiotensin I (Figure 8-14).

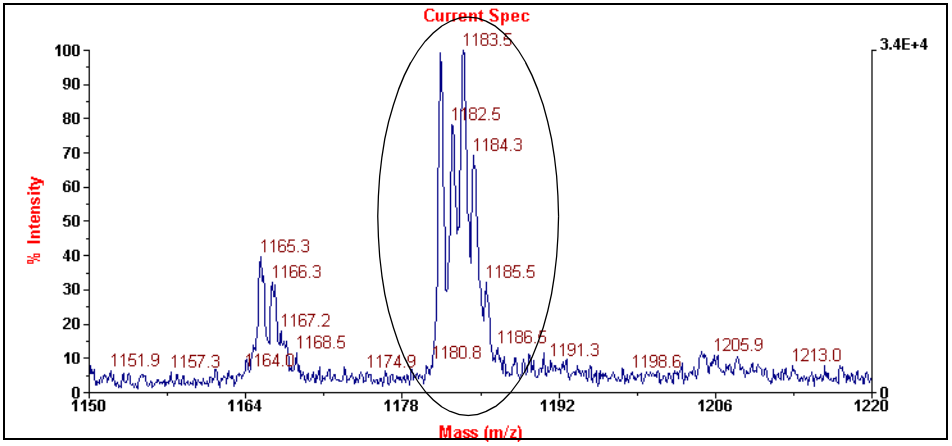


Figure 8-14 Angiotensin I Fragment Ions (Precursor Ion Selector On)

5. Note the behavior of the signal intensity for the first few spectra and subsequent continued samplings at a given laser power.

NOTE: *Sample preparations that contain high salt contamination or other impurities often yield increased signal intensity after an initial period of low intensity as the top layer of sample is consumed.*

You typically see signal intensity reach maximum and decrease more quickly than in non-PSD mode as sample is consumed. You may need to move around on the sample spot to maintain signal intensity.

6. Continue increasing the laser intensity and observing the signal. Figure 8-15 shows the effect of higher laser intensity on the angiotensin I spectrum.

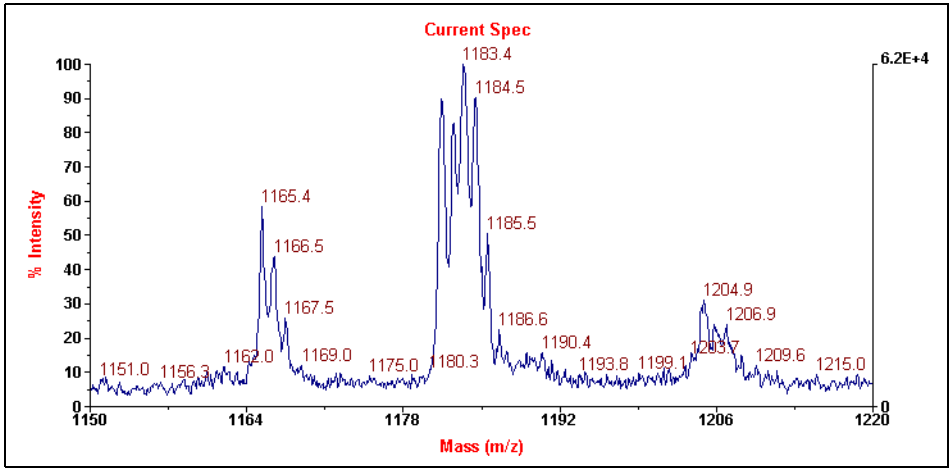


Figure 8-15 Angiotensin I Fragment Ions at High Laser Intensity

Hint: *As you initially experiment, adjust the laser in large steps, for example 100 counts. As you begin to fine-tune the laser, use smaller steps.*

Fragment ion yield initially increases with higher laser intensities, and then decreases at very high settings. To make sure that the signal decrease is not due to sample exhaustion, move around in the sample position.

7. Decrease the laser intensity and observe the signal. Signal intensity decreases with lower laser intensities. When laser intensity is too low, signal intensity is weak.
8. Continue decreasing the laser intensity until signal is no longer visible.
9. Continue experimenting with laser settings until you are familiar with the appearance of a spectrum acquired when the laser intensity is set too high or too low.

Determining optimum laser intensity for fragments

Fine-tune the laser setting until you find a laser intensity that provides a signal strength for the angiotensin I fragment ions observed between 1,180 and 1,190 Da that is around 20,000 to 40,000 counts in the Spectrum window.

Record this laser setting.

NOTE: *The precursor ion peak will be saturated at a laser intensity that yields adequate intensity for fragment ions.*

8.6.2 Observing the Effects of Precursor Ion Selector

Turning on the Precursor Ion Selector eliminates prompt fragments in a spectrum.

In this section

In this section, you will:

- Turn off the Precursor Ion Selector (called Timed Ion Selector in Reflector mode) and observe the spectrum
- Distinguish between the two types of fragments, prompt and PSD
- Understand the impact of the Precursor Ion Selector on prompt fragments

Turning off Precursor Ion Selector

To observe the effects of the Precursor Ion Selector (called Timed Ion Selector in Reflector mode):

1. In the PSD Acquisition Settings control page, deselect **Precursor Ion Selector**.
2. Acquire a spectrum.
3. Observe the 1,180 to 1,190 Da mass region. Note the additional peaks that appear below 1,185 Da when the Precursor Ion Selector is turned off (Figure 8-16).

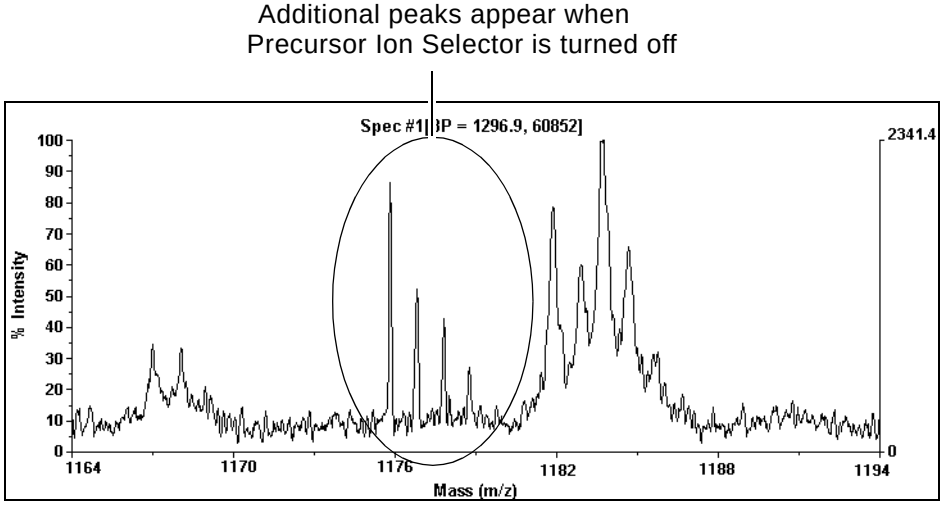


Figure 8-16 Angiotensin I Fragment Ions (Precursor Ion Selector Off)

Figure 8-17 compares the spectrum above with the spectrum acquired in the previous section so you can more easily see the peaks that appear when the Precursor Ion Selector is turned off. Figure 8-17 also identifies the two types of fragments seen when the Precursor Ion Selector is turned off.

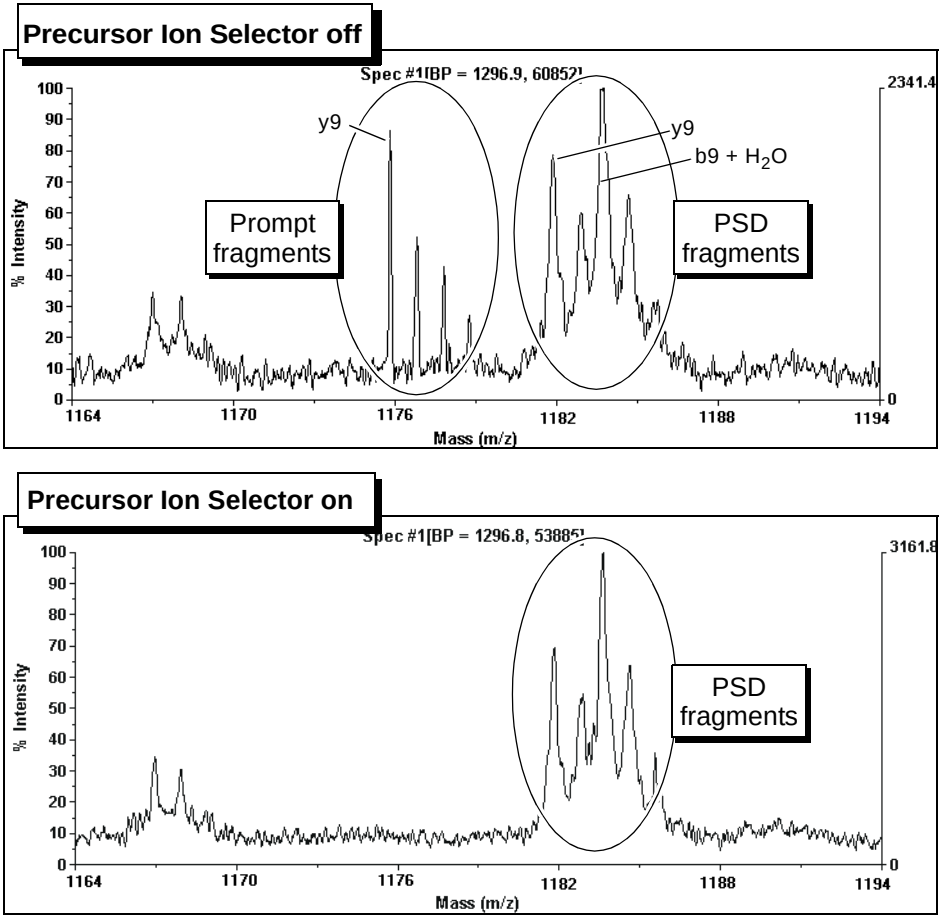


Figure 8-17 Comparison of Spectra with Precursor Ion Selector Off and On

Observing prompt and PSD fragments

When operating under conditions that yield high resolution for angiotensin I, and when the Precursor Ion Selector is turned off, you should observe the following:

- **Prompt fragments**—Well-resolved fragment ions generated in the source before acceleration. The sharp peak at 1,181.7 Da is the y9 ion.

  Prompt fragments appear at the expected mass in reflector mode because their flight time is the same as a molecular ion of the same mass accelerated from the source. They do not appear at the expected mass in PSD mode.

- **PSD fragments**—A poorly resolved peak cluster that is a mixture of the y9 fragment (1,181.7 Da) and the b9+H_2O fragment (1,183.6 Da). PSD fragments are generated in the flight tube after acceleration.

  PSD fragments appear at a higher mass than expected in a reflector-calibrated spectrum. They appear at the expected mass in PSD mode. See “PSD fragment ions” on page 8-21. These higher than expected masses are corrected in PSD calibration when you generate the composite spectrum.

Effect of Precursor Ion Selector on prompt and PSD fragments

Even if prompt and PSD fragments are identical in chemical structure, they will travel down the flight tube at different times (Figure 8-18):

- **Prompt fragments**—Accelerated based on their initial fragment mass, and reach the reflector sooner than their corresponding PSD fragments.

- **PSD fragments**—Accelerated based on the mass of the molecular ion from which they form, and reach the reflector later than the prompt fragments.

Because of this difference in flight times, the Precursor Ion Selector can screen out prompt fragments while allowing PSD fragments to pass into the reflector.

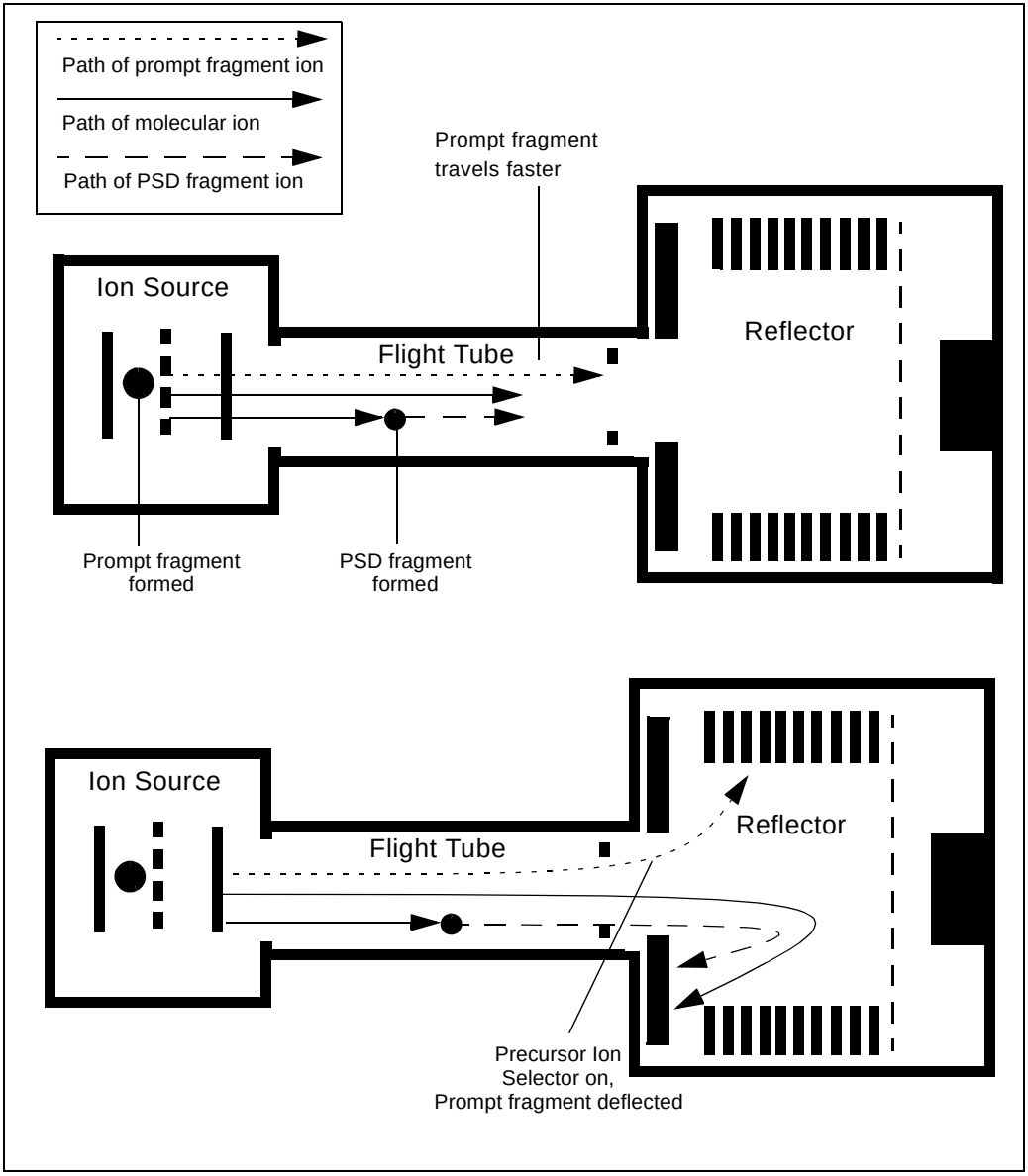


Figure 8-18 Flight Path of Prompt and PSD Fragments

8.6.3 Observing the Effects of Grid Voltage%

Adjusting Grid Voltage% affects resolution.

In this section

In this section, you will:

- Understand the function of Grid Voltage% in focusing ions
- Observe the impact of Grid Voltage% on higher and lower mass ions

NOTE: *For typical applications, use the Grid Voltage% in the standard .BIC file provided with the software. This section is a demonstration of the impact of Grid Voltage%.*

Effect of Grid Voltage% on product ion resolution

The resolution of an ion is affected by the amount of time it spends in the reflector. After an ion is accelerated and is velocity-focused (ions of the same mass align in time), it defocuses as it moves down the flight tube. When an ion spends an equal amount of time refocusing in the reflector, resolution is optimized (Figure 8-19).

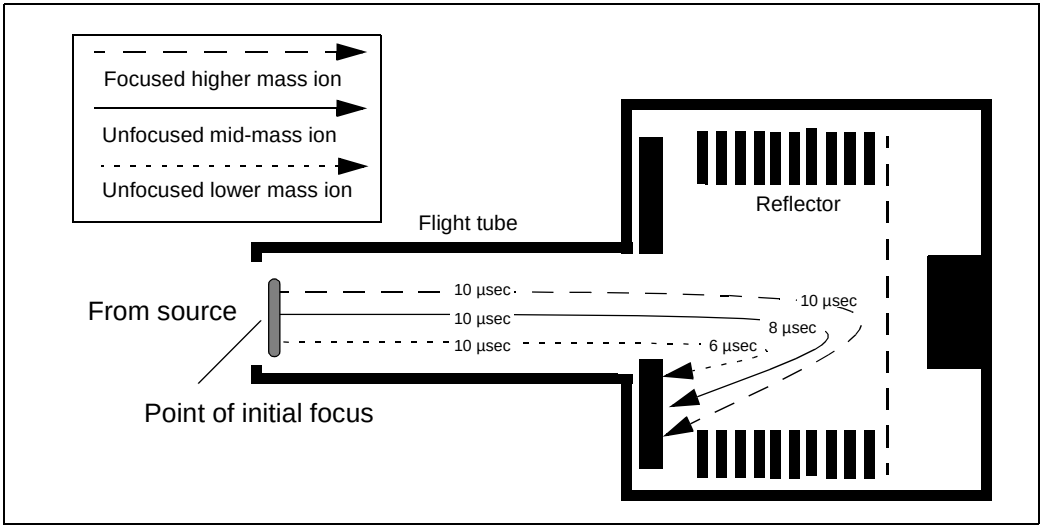


Figure 8-19 Ion Focusing

By fine-tuning the Grid Voltage%, you can alter the point of initial time focus of the ions, which decreases the amount of time the ion spends defocusing. This allows you to balance the defocusing time with the refocusing time in the reflector (Figure 8-20).

The goal in optimizing the Grid Voltage% is to find a suitable setting that optimizes resolution in the middle of the mass range of interest. Notice that as you bring lower mass ions into focus, you compromise the focus of higher mass ions.

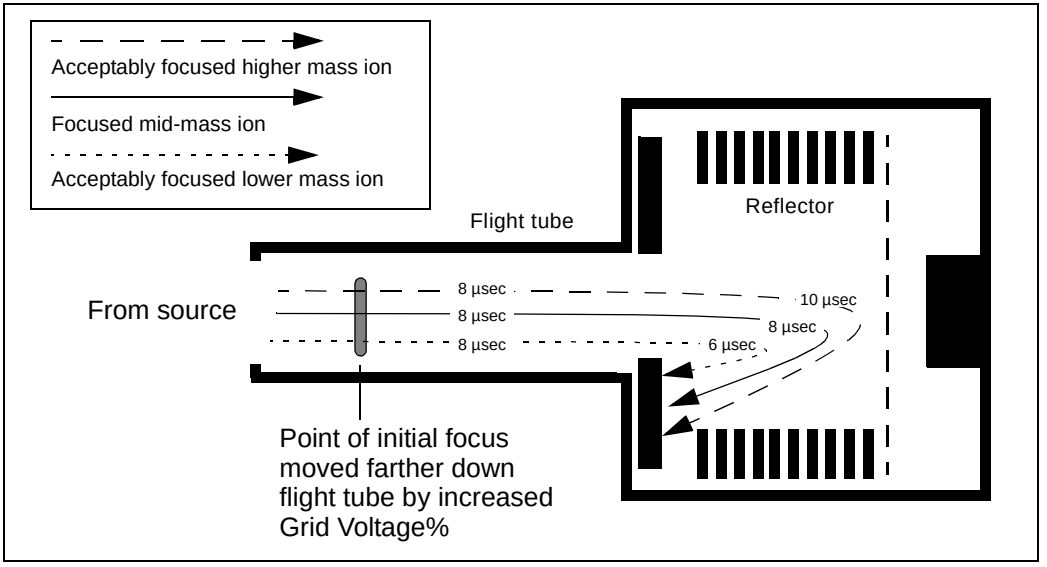


Figure 8-20 Increased Grid Voltage% and Ion Focusing

Acquiring

NOTE: *In this exercise, you will observe the impact of the Grid Voltage% on the precursor ion at 1,297 Da and the fragment ion at 1,181 Da. At the laser intensity you optimized for the fragment ion, the precursor ion will be saturated. To allow you to observe precursor and fragment ions in the spectrum, change the Vertical Scale setting in the .BIC file (Digitizer/Mode dialog box) to 1,000 mV full scale.*

1. Enable the **Precursor Ion Selector** if it is disabled.
2. Acquire a spectrum to make sure the precursor is not saturated.

   Hint: *If the precursor ion peak is still saturated but the peak valleys are noticeable, you can estimate the resolution at the +2 isotopic peak.*

3. Make a note of the resolution of the fragment ions and the precursor ion.

   NOTE: *You may need to switch between Vertical Scale settings of 1,000 mV and 200 mV and reacquire spectra to see the resolution on the precursor and fragment peaks.*

4. From the Acquisition menu, select **Stop Experiment**, to allow you to change the Grid Voltage in the Instrument Settings control page.
5. Decrease the Grid Voltage% value in 5 percent increments and observe the spectrum. For example, if the setting in the .BIC file is 80%, decrease to 75%, then 70%.

   At lower Grid Voltage% settings:

   - Lower mass ion resolution degrades
   - Higher mass ion resolution improves

6. Check the Grid Voltage% setting in the standard .BIC file provided on your system for angiotensin I (Angiotensin_Reflector.BIC). Acquire a spectrum using this setting. At this setting, you should observe optimum resolution on the high mass peak.

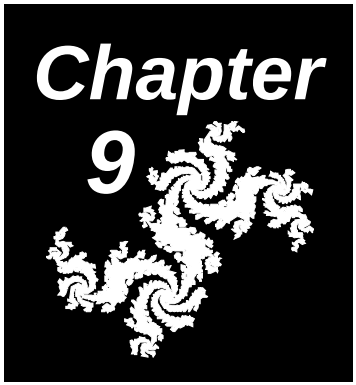
8.6.4 Summary

The table below summarizes the impact of changing PSD acquisition conditions.

Condition	Impact
Laser intensity	• Increase higher than normal to induce fragmentation. • Setting too high increases baseline noise and eventually decreases signal. • Setting too low reduces fragment ion abundance.
Precursor Ion Selector	• Turn on to eliminate fragments that do not travel with the selected precursor mass. • Decrease the Deflector Gate Width (set in Hardware Configuration) to narrow the Precursor Ion Selector mass window. • Extremely small Deflector Gate Widths can weaken fragment ion intensity.
Grid Voltage%	• Increase or decrease to affect the focus of fragment ions. • Higher Grid Voltage% settings shift the optimal focus toward shorter flight times (lower masses within a PSD segment).
Guide Wire Voltage%	• Decreasing the Guide Wire Voltage% with lower Mirror Ratio settings may or may not improve the quality of PSD data. • Avoid using Guide Wire Voltage% settings above 0.02%. Higher settings may compromise the selectivity of the Precursor Ion Selector. ***NOTE:*** *Guide Wire Voltage% is not available on Voyager-DE STR models with serial number 4154 and later.*

8.7 Viewing PSD Data

For information on viewing PSD data, see the *Data Explorer Software User's Guide*, Chapter 8, Viewing Voyager PSD Data.



9 Maintenance and Troubleshooting

This chapter contains the following sections:

9.1 Maintenance 9-2
9.1.1 Maintenance Schedule 9-2
9.1.2 Hardware Maintenance 9-3
9.1.3 Backing Up and Archiving Data 9-6
9.2 Troubleshooting 9-7
9.2.1 Spectrum Troubleshooting 9-7
9.2.2 Software Troubleshooting 9-19
9.2.3 Hardware Troubleshooting 9-23

9.1 Maintenance

This section describes:

- Maintenance schedule
- Hardware maintenance
- Backing up and archiving data

9.1.1 Maintenance Schedule

Maintenance schedule

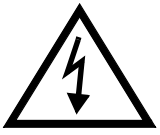
Regular preventative maintenance will help keep your Voyager system functioning properly. Perform the following procedures as indicated:

When to perform	Task	Page
Weekly	Back up or archive data	page 9-6
Yearly	Hardware maintenance performed by Applied Biosystems engineer	page 9-3

Maintenance log

Appendix G, Maintenance Log, includes a log sheet that you can copy and use to keep track of maintenance on your Voyager system.

9.1.2 Hardware Maintenance



WARNING

ELECTRICAL SHOCK HAZARD. *Severe electrical shock can result by operating the instrument without panels in place. Do not remove instrument panels. High voltage contacts are exposed with panels removed. Wear proper eye protection if panels are removed for service.*

WARNING

LASER HAZARD. *The laser emits ultraviolet radiation. Lasers can burn the retina and leave permanent blind spots. Do not remove instrument panels or look directly into the laser beam or allow a reflection of the beam to enter your eyes. Wear proper eye protection if panels are removed for service.*

Yearly preventative maintenance

The Voyager Biospectrometry Workstation requires minimal preventative maintenance. Preventative maintenance procedures should be performed by an Applied Biosystems technical representative.

Please contact Applied Biosystems for information on service contracts for yearly preventative maintenance.

The following preventative maintenance is needed on a yearly basis:

- Replace rotary pump oil
- Inspect molecular sieve, oil mist filter, and vent line dryer filter
- Clean filters and fans
- Lubricate X/Y feedthroughs
- Inspect flap valve 1, flap valve 2, linear actuator o-rings

- Clean optics and air lines
- Inspect grids, compressor, air pressure, turbo pump, and laser power
- Adjust laser flash rate, load offsets, sample offsets, detector gain and voltage, and instrument covers
- Inspect load and eject cycles, high voltage power supplies, and computer
- Calibrate thermocouple gauges
- Check that the instrument meets specifications

Changing fuses

This procedure is required for Voyager-DE and Voyager-DE PRO systems only (the Voyager-DE STR does not require power fuses). Extra fuses for different voltage settings are supplied with the system.

To change the main power fuse:

1. Exit the Voyager software.
2. Power down the mass spectrometer.
3. Remove the power cord from the mass spectrometer.
4. Carefully remove the voltage selector/fuse holder from the system (Figure 9-1) using a small flat-blade screw driver.
5. Carefully remove the voltage selector from the holder and insert the selector with the proper voltage displayed in the window of the holder.

CAUTION

The plastic tabs that hold the voltage selector in place are fragile. Do not exert force when removing this piece.

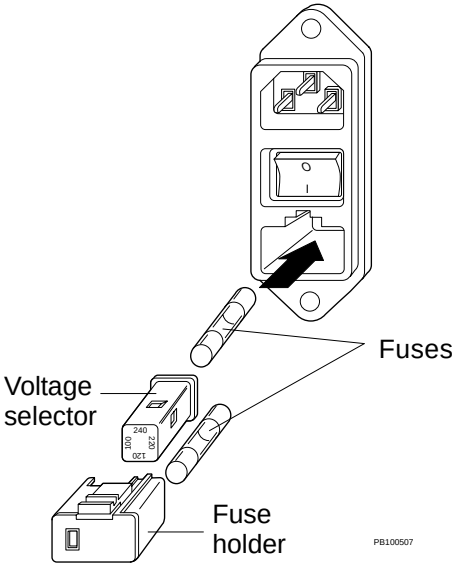


Figure 9-1 Changing Fuses

WARNING

FIRE HAZARD. *Using a fuse of the wrong type or rating can cause a fire. Replace fuses with those of the same type and rating.*

6. Insert two fuses of the proper rating.

Electrical Rating	
Volts/Amps	**Fuse** (5 x 20 mm)
100 V~10A	T10A 250V
120 V~10A	T10A 250V
220 V~6.3A	T6.3A 250V
240 V~5A	T5A 250V

7. Insert the voltage selector/fuse holder into the receptacle.
8. Plug in the mass spectrometer and power up.

9.1.3 Backing Up and Archiving Data

Back up data weekly, or as needed. Archive data as needed.

To operate properly, the Voyager software requires:

- Disk space for data and instrument setting files
- Disk space for printing

If the disk is full, writing data files, printing, or saving instrument settings can fail and result in loss of data. The computer also operates more slowly as free disk space decreases.

To maintain adequate disk space, back up and archive data on a routine basis:

- **Back up**—Temporarily store data in case of a hard disk problem.
- **Archive**—Permanently store important data for retrieval at a later time, and remove the original data.

9.2 Troubleshooting

This section includes:

- Spectrum troubleshooting
- Software troubleshooting
- Hardware troubleshooting

Troubleshooting information is organized according to likelihood of possible cause, from most likely to least likely possible cause. If you are unable to solve your problem using the information in the following tables, call Applied Biosystems Technical Support. To reach Applied Biosystems Technical Support, refer to the list of offices on the back cover of this book.

9.2.1 Spectrum Troubleshooting

Table 9-1 Spectrum Troubleshooting

Symptom	Possible Cause	Action
Flat signal on oscilloscope or in spectrum window for sample region (matrix peaks seen)	Laser intensity too low	Adjust laser by using the slider controls on the Manual Laser/Sample Positioning control page.
	Analyzing “cold” spot	Analyze different position in sample position.
	Sample consumed at location in well where laser is hitting	Analyze different position in sample position.
	Mass range setting incorrect	Adjust. See Section 5.3.5, Understanding Digitizer Settings.

(continued on next page)

continued

Table 9-1 Spectrum Troubleshooting (Continued)

Symptom	Possible Cause	Action
Flat signal on oscilloscope or in spectrum window for sample region (matrix peaks seen) *(continued)*	Accelerating Voltage too low	Adjust. See Section 5.4.4.2, Setting Accelerating Voltage.
	Sample does not ionize	Analyze in negative ion mode.
		Before mixing with matrix, chemically derivatize sample with amino-containing chemical group.
		Use different matrix. See Section 3.1.1, Selecting a Matrix.
	Sample not well-dissolved before applying to sample plate	Prepare new sample, making sure sample is well-dissolved, then apply to sample plate.
	Sample and matrix not well-mixed before applying to sample plate	Prepare new sample and matrix. Mix well before applying to sample plate.
	Sample concentration too high or too low	Prepare sample/matrix with a final sample concentration appropriate for sample and matrix. See Section 3.1.3, Matrix Information.
		Dilute or concentrate sample 5× and run again.

continued

Table 9-1 Spectrum Troubleshooting (Continued)

Symptom	Possible Cause	Action
Flat signal on oscilloscope or in spectrum window for sample and matrix region	Vertical scaling needs adjustment	Adjust. See Section 5.3.5, Understanding Digitizer Settings.
	Spectrum window needs adjustment	See Section 4.3, Using the Spectrum Window.
	Laser set to 0	Adjust laser by using the slider controls on the Manual Laser/Sample Positioning control page.
	Laser beam is not irradiating sample in the sample position	Adjust position of sample position using the Manual Laser/Sample Positioning controls.
	Signal is offscale	Adjust Vertical Offset. See Section 5.3.5, Understanding Digitizer Settings.
	Problem with electronics	Call Applied Biosystems Technical Support.
Saturated signal in sample and matrix region	Laser intensity too high	Adjust laser by using the slider controls on the Manual Laser/Sample Positioning control page.
Poor crystallization on the sample plate	Sample contaminated	Clean up sample. See Section 3.1.5, Sample Cleanup.

continued

Table 9-1 Spectrum Troubleshooting (Continued)

Symptom	Possible Cause	Action
Poor resolution/ sensitivity in Delayed Extraction mode	Delay Time and Grid Voltage% not optimized	Optimize. See Section 5.4, Optimizing Instrument Settings Parameters.
	Guide Wire Voltage% not optimized	Optimize. See Section 5.4, Optimizing Instrument Settings Parameters.
	Wrong Bin size and Vertical Scale selected for components below 15,000 Da	In the Mode/Digitizer dialog box, set Bin size to 2 nsec. In the Mode/Digitizer Options dialog box, select a Vertical Scale of 200 mV.
	Oscilloscope and LeCroy systems only—Wrong Input Bandwidth setting	Select Full for Input Bandwidth in Mode/Digitizer Options dialog box. ***NOTE:*** *Input Bandwidth is not available on Signatec systems.*
	Laser not properly aligned	Call Applied Biosystems Technical Support.
	High voltage control circuit not functioning properly	Call Applied Biosystems Technical Support.
Flat tops on peaks	Signal is saturated, laser power is too high	Decrease laser setting using the slider controls on the Manual Laser control page until peak tops are sharp.

continued

Table 9-1 Spectrum Troubleshooting (Continued)

Symptom	Possible Cause	Action
Round tops on peaks	Saturated (flat top) and unsaturated (sharp top) scans averaged	Decrease laser setting by using the slider controls on the Manual Laser/Sample Positioning control page until peak tops are sharp.
	While acquiring, laser power changed. Averaged scan includes saturated (flat top) and unsaturated (sharp top) scans.	Reacquire using one laser setting that gives sharp peaks. ***NOTE**: Data acquired using different laser settings may have poor mass accuracy.*
Poor mass accuracy in Delayed Extraction mode (external calibration only)	High voltage power supplies not warmed up	Start high voltages by clicking  on the toolbar before calibration.
	Mass Accuracy Optimization option not used	Enable Mass Accuracy Optimization. See page 3-52.
	Standard and sample of interest not in adjacent sample position	Calibrate using standard that is in a sample position adjacent to the sample of interest.
	Correct initial velocity not specified	Specify Initial Velocity in .BIC file before acquisition by selecting matrix. For more information, see “Matrix influence” on page 5-22.

continued

Table 9-1 Spectrum Troubleshooting (Continued)

Symptom	Possible Cause	Action
Poor mass accuracy in Delayed Extraction mode (external calibration only) *(continued)*	Incorrect calibration constants used	Recalibrate with known standards and correct masses.
		Make sure correct external calibration file is selected in the Calibration field on the Instrument Settings control page.

continued

Table 9-1 Spectrum Troubleshooting (Continued)

Symptom	Possible Cause	Action
Poor signal-to-noise ratio or sensitivity	Laser intensity too high	Decrease laser intensity to threshold by using the slider controls in the Manual Laser/Sample Positioning control page (see page 4-27).
	Laser intensity too low	Increase laser intensity by using the slider controls in the Manual Laser/Sample Positioning control page (see page 4-27).
	Sample contaminated	To test, mix the sample with a standard of known sensitivity. If the standard no longer exhibits the expected sensitivity, a contaminant in the sample is affecting sensitivity. Clean up sample. See Section 3.1.5, Sample Cleanup.
	Not averaging enough spectra	Average more spectra: • **Manual Control mode**—Collect more spectra (shots/spectra) before stopping acquisition • **Automatic Control mode**—Increase number of spectra to acquire

continued

Table 9-1 Spectrum Troubleshooting (Continued)

Symptom	Possible Cause	Action
Poor signal-to-noise ratio or sensitivity *(continued)*	Matrix peaks are saturating the detector	Turn on Low Mass Gate to suppress matrix peaks.
	Guide Wire Voltage% too high (lower masses) or too low (higher masses)	Adjust. See Section 5.3.4, Understanding Guide Wire Voltage%.
	Accelerating Voltage too low	Adjust. See Section 5.4.4.2, Setting Accelerating Voltage.
	Too much salt or buffer in sample	Clean up sample. See Section 3.1.5, Sample Cleanup. Decrease salt or buffer.
	Sample:matrix ratio not optimal	Try ratios higher and lower than the recommended 1:10 ratio. May want to prepare a dilution series (factors of 10).
	Accelerating Voltage malfunction	Call Applied Biosystems Technical Support.
	Beam guide wire malfunction	Call Applied Biosystems Technical Support.
	Internal detector power supply setting too high	Call Applied Biosystems Technical Support.
	Grounded grid in ion source damaged	Call Applied Biosystems Technical Support.

continued

Table 9-1 Spectrum Troubleshooting (Continued)

Symptom	Possible Cause	Action
Poor signal-to-noise ratio on sample peaks	Matrix peaks causing excessive noise in detector	Turn on Low Mass Gate in .BIC file to suppress matrix peaks.
Dimer in spectrum	Laser intensity too high, causing signal saturation	Adjust laser by using the slider controls on the Manual Laser/Sample Positioning control page.
Dimers, trimers, and tetramers in spectrum	Sample concentration too high	Prepare sample/matrix with a final sample concentration appropriate for sample and matrix. See Section 3.1.3, Matrix Information.
Mass calibration on Spectrum window not accurate	Wrong Flight Length entered in Hardware Configuration	Call Applied Biosystems Technical Support.

continued

Table 9-1 Spectrum Troubleshooting (Continued)

Symptom	Possible Cause	Action
Peaks not symmetrical	Laser intensity too high	Decrease laser intensity by using the slider controls on the Manual Laser/Sample Positioning control page.
	Sample contains more than one component	Purify sample before analyzing.
	On Voyager-DE PRO and Voyager-DE STR systems, isotopes are only partially separated, because you are using a setting very close to laser threshold	Increase laser intensity by using the slider controls on the Manual Laser control page to improve symmetry, but data is accurate as is. ***NOTE:*** *Increasing the laser intensity may decrease resolution.*
	Signal is saturating detector	Decrease laser intensity by using the slider controls on the Manual Laser control page.

continued

Table 9-1 Spectrum Troubleshooting (Continued)

Symptom	Possible Cause	Action
On Voyager-DE PRO and Voyager-DE STR systems, cannot see high mass ions in Reflector mode	Refer to "Flat signal on oscilloscope or in spectrum window for sample region (matrix peaks seen)" symptom on page 9-7	Refer to "Flat signal on oscilloscope or in spectrum window for sample region (matrix peaks seen)" action on page 9-7.
	Ions not reaching detector (Accelerating Voltage too low)	Verify that you can see ions in Linear mode, then adjust voltages in Reflector mode and rerun. See Section 5.4.4.2, Setting Accelerating Voltage, and Section 5.4.3.5, Optimizing Grid Voltage%.
	Ions fragmenting before reaching the detector (Accelerating Voltage too high or Grid Voltage too low)	
High mass ions fragmenting when you are using α-cyano matrix	Internal energy of ions causing fragmentation	Use Sinapinic acid matrix which yields ions with a lower internal energy than α-cyano, and therefore causes less fragmentation of high mass ions.
Masses off by 22 or 38 Da	Sample ionized to $[M+Na]^+$ or $[M+K]^+$, instead of $[M+H]^+$	No action. Data accurate.

continued

Table 9-1 Spectrum Troubleshooting (Continued)

Symptom	Possible Cause	Action
Observed mass not equal to monoisotopic mass	Observed mass is average mass, except when resolving isotopes	No action. Data accurate.
Large tail on the high mass side of peak	Unresolved salt or buffer adducts due to sample contamination	Clean up sample. See Section 3.1.5, Sample Cleanup.

9.2.2 Software Troubleshooting

This section includes:

- Instrument Control Panel troubleshooting
- Sequence Control Panel troubleshooting
- PSD troubleshooting
- Checking the Windows NT Event Log

Table 9-2 Instrument Control Panel Troubleshooting

Symptom	Possible Cause	Action
Load/Eject button or command dimmed	Communication problem between sample loader and software	Select **Reinitialize** from the Instrument menu.
Acquisition timeout message displayed	Communication problem between digitizer and software	1. Make sure all cables between digitizer and mass spectrometer is securely connected (see Figure 2-4 on page 2-11). If your system includes a LeCroy digitizer, check network connections between the digitizer and the computer. 2. Select Reinitialize from the Instrument menu. 3. If the problem continues, call Applied Biosystems.

continued

Table 9-2 Instrument Control Panel Troubleshooting (Continued)

Symptom	Possible Cause	Action
Slider control does not change laser setting	Voyager Instrument Control Panel is not the active window	Click on the Instrument Control Panel to activate the window before using slider controls.
Active position number does not reflect actual position under laser	Sample plate is not aligned	Align the sample plate. See Section 2.7, Aligning the Sample Plate.
Number of times the laser fires is greater than Shots/Spectrum	Delay in transferring data from the digitizer to the computer.	No action. Normal occurrence.
Negative ion selection not displayed in Instrument Settings control page (and you have the optional hardware)	Instrument not configured for negative ion hardware	Configure for negative ion hardware: 1. From the Mode/Digitizer dialog box, select the **Instrument Mode** tab. 2. Select **Negative** for Polarity type and click **OK**.
Calibration (mass) shifted up or down by 10 Da	Uneven matrix layer causing hot and cold spots	Acquire a number of spectra and accumulate scans.
		Prepare new sample spot.
Resolution labels or signal-to-noise not displayed	Peaks not detected	Apply peak detection when acquisition is complete by clicking  in the toolbar.

Table 9-2 Instrument Control Panel Troubleshooting (Continued)

Symptom	Possible Cause	Action
Spectrum window is not updated with every laser shot	If you are acquiring a large number of data points, window is not updated with every shot. The exact update rate depends on the Mass Range setting and the Bin Size setting you are using.	No action. Normal occurrence.

Table 9-3 Sequence Control Panel Troubleshooting

Symptom	Possible Cause	Action
All lines in .TXT or .XLS file you are importing are not imported	Any lines following a blank line are ignored and not imported	Remove blank lines and import again.

Table 9-4 PSD Troubleshooting

Symptom	Possible Cause	Action
Cannot see low mass ions in PSD mode	Guide Wire Voltage% too high	Adjust. See Section 5.4.3.3, Optimizing Guide Wire Voltage%.

Table 9-4 PSD Troubleshooting

Symptom	Possible Cause	Action
Peak selected with Precursor Ion Selector not appearing, or not appearing at expected mass (single-stage mirror only)	Timed ion selector not working, due to invalid Flight Length value or Gate Width value	Call Applied Biosystems Technical Support.

Checking the Windows NT Event Log

The Windows NT Event Log is a running list of events that automatically starts when you run Windows NT. An event is considered any significant occurrence in the system or application that requires the user to be notified.

You can use Event Viewer to monitor the events that occur in your system. You must be logged in with an account that has administrator rights. To display Event Viewer:

1. Select **Administrative Tools** from the Program folder on the Windows Start taskbar.
2. Click **Event Viewer**.

   The Event Viewer - System Log displays a running log of the events on your system. Critical event messages are displayed on your screen. Events that do not require immediate attention are logged in the Event Log to provide information without disturbing your work.
3. If desired, clear the Event Log:
   - Select **Save As** from the Log menu and save the event log before clearing.
   - Select **Clear All Events** from the Log menu.
4. Select **Exit** to return to the Window NT desktop.

9.2.3 Hardware Troubleshooting

This section includes:

- Mass spectrometer troubleshooting
- Vacuum gauge panel troubleshooting
- External laser troubleshooting

Table 9-5 Mass Spectrometer Troubleshooting

Symptom	Possible Cause	Action
Internal stepper motor making noise when the sample plate is moving	Normal operation of the sample plate stepper motor	No action. Normal occurrence.
High whining sound when you power up the mass spectrometer	Normal startup operation of the turbo pump	No action. Normal occurrence.
Internal stepper motor making grinding noise when the sample plate is not moving or is moving erratically. "Home or Load position not found" may be displayed on startup.	Problem with sample positioning system	Do not restart computer or software. Call Applied Biosystems Technical Support.
In Automatic Control mode, center of sample position is not aligned with laser spot, as observed on the video monitor	Sample plate not aligned	Align. See Section 2.7, Aligning the Sample Plate.

continued

Table 9-5 Mass Spectrometer Troubleshooting (Continued)

Symptom	Possible Cause	Action
Cracking sound in mass spectrometer	Arcing caused by dirty sample plate	Use clean, particulate-free sample plate.
	Arcing caused by negative ion mode	Decrease Accelerating Voltage. See Section 5.4.4.2, Setting Accelerating Voltage.
	Arcing caused by excess matrix in sample preparation (may be required for ionization of certain samples)	Decrease amount of matrix in sample preparation.
		If excess matrix is required to ionize sample, decrease Accelerating Voltage. See Section 5.4.4.2, Setting Accelerating Voltage.
	Arcing caused by faulty grid in ion source	Do not restart computer or software. Call Applied Biosystems Technical Support.
	Arcing caused by faulty electronic components, PCBs, or cables	Do not restart computer or software. Call Applied Biosystems Technical Support.

continued

Table 9-5 Mass Spectrometer Troubleshooting (Continued)

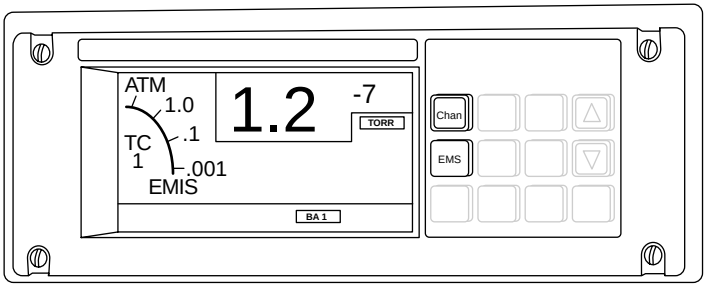
Symptom	Possible Cause	Action
Sample holder empty when you click Eject ***CAUTION:*** *Do not load another plate if the sample holder is empty when you eject.*	Problem with sample ejecting mechanism	Call Applied Biosystems Technical Support.
	Sample plate jammed in system	Call Applied Biosystems Technical Support.

Vacuum gauge panel

The Vacuum Gauge Panel (Figure 9-2) is located on:

- **Voyager-DE and Voyager-DE PRO**—On the right front of the mass spectrometer cabinet.
- **Voyager-DE STR**—On the front lower left of the mass spectrometer cabinet.

NOTE: *The vacuum gauge panel is located behind the front panel of the mass spectrometer cabinet.*



PB100270

Figure 9-2 Vacuum Gauge Panel

CAUTION

Do not press any other buttons on the panel. Pressing buttons other than the Chan and EMIS buttons can recalibrate the pressure scale of the system.

You use two buttons on the pressure gauge panel:

- **Chan**—Toggles through readings for:

Gauge	Measures	Expected Pressure
BA1	Pressure in main source chamber	• **Voyager-DE and Voyager-DE PRO**—Less than 10^{-6} • **Voyager-DE STR**—Less than 5×10^{-7}
BA2	Pressure in mirror chamber (Voyager-DE PRO only)	• **Voyager-DE and Voyager-DE PRO**—Less than $2x10^{-7}$ • **Voyager-DE STR**—Less than 5×10^{-8}
TC2	Pressure in sample loading chamber	Less than 5×10^{-2} during operation. Higher when loading or ejecting sample plate.
TC1, TC3, TC4	Not used, displays E03 (indicates gauge not connected)	——

- **EMIS**—Turns BA1 and BA2 on or off. Used during troubleshooting only.

Table 9-6 Vacuum Gauge Panel Troubleshooting

Symptom	Possible Cause	Action
E02 error message displayed on gauge controller	BA1 or BA2 shut down due to sudden surge of high pressure, for example, when a wet sample plate is inserted	Press the **EMIS** button on Pressure Gauge Control Panel to turn off. Press again to turn on. Call Applied Biosystems Technical Support if error occurs again.
E03 error message displayed on gauge controller	Ion gauge connection broken or disconnected	Call Applied Biosystems Technical Support.
E05 error message displayed on gauge controller		
E09 error message displayed on gauge controller for BA1 or BA2	BA1 or BA2 gauges shut down due to high pressure. May be caused by: • Sudden increase in pressure when sample plate inserted • Vacuum leak	Press the **EMIS** button on Pressure Gauge Control Panel to turn off. Press again to turn on. Call Applied Biosystems Technical Support if error occurs again.
E08 error message displayed on gauge controller	Vacuum gauge board failed	Power down the mass spectrometer and then power up. Call Applied Biosystems Technical Support if error occurs again.

Table 9-7 External Laser Troubleshooting

Symptom	Possible Cause	Action
Laser does not fire when you start acquisition	External laser not set after switching from internal to external laser	Reset. See Section 2.9, Resetting the Optional External Laser.
Laser position on the sample drifts	If the laser is left on for long periods of time, position may drift	Press **Stop** then **Start**, or **Reset**.

A Specifications

This appendix contains the following sections:

A.1 Voyager-DE Specifications A-2
A.2 Voyager-DE PRO Specifications A-5
A.3 Voyager-DE STR Specifications A-8
A.4 Digitizer Specifications A-11

NOTE: *The specifications for this instrument are subject to change without notice.*

A

A.1 Voyager-DE Specifications

This section includes the following specifications for the Voyager-DE Workstation:

- Performance
- Mass Spectrometer
- Miscellaneous

Table A-1 Voyager-DE Performance Specifications for Installation

Condition	Specification
Resolution (FWHM—Full Width at Half Maximum) in Delayed Extraction mode	• ≥800 for Insulin, bovine (3.5 pmol/µl) • ≥1,000 for Myoglobin (4 pmol/µl) • ≥2,000 for Angiotensin I (1.3 pmol/µl) $[M+H]^+$ ion analyzed.
Signal-to-Noise Ratio	• ≥200:1 for IgG (0.6 pmol/µl) • ≥100:1 for BSA (4 pmol/µl)
Mass Accuracy in Delayed Extraction mode (difference between the mean of six measurements and the theoretical mass of the sample)	• External Calibration—0.05% • Internal Calibration—0.02% Mass accuracy is the difference between the measured and the theoretical mass of the sample.
Sensitivity	Routine detection of 5 fmol of neurotensin with signal-to-noise ratio ≥20:1.

Table A-2 Voyager-DE Mass Spectrometer Specifications

Condition	Specification
Mass range	≥300,000 Da Upper limit set by suitably ionized biomolecule.
Flight tube	1.2 m linear

Table A-2 Voyager-DE Mass Spectrometer Specifications (Continued)

Condition	Specification
Ion source	Two-stage
Ion source voltages	Tunable: • Accelerating Voltage—Up to 25,000 V • Grid Voltage—Range determined by Accelerating Voltage
Laser	Nitrogen, 337 nm, 3 ns pulse, 20 Hz maximum firing rate. Actual laser firing rate dependant on digitizer. See Section A.4, Digitizer Specifications.
Digitizer	500 MHz digitization See Section A.4, Digitizer Specifications.
Vacuum system	Automatic, with turbomolecular pumping for high vacuum
Ion detection	Positive and negative
Sample analysis	• Automated single-plate sample-loading system; sample plates of various formats available. See B.3, Spare Parts. • Manual control using control stick or mouse • Sequence control software for automated analysis
Dimensions	• 27 inches (69 cm) deep • 25 inches (64 cm) wide • 65 inches (165 cm) high, includes cabinet and flight tube

Table A-3 Voyager-DE Miscellaneous Specifications

Condition	Specification
Environmental	• Operating temperature—20–25°C • Relative humidity—20–80%, non-condensing • Altitude—≤2,000 m (6,500 ft) above sea level. • Installation category (overvoltage category)—II, classified as portable equipment. • For indoor use only. • Pollution degree rating—2, and may be installed in an environment that has nonconductive pollutants only.
Computer	Minimum configuration: • Pentium® III 500 MHz, with 9 GB hard disk and 128 MB RAM (random access memory) • 17-inch high-resolution color monitor

A

A.2 Voyager-DE PRO Specifications

This section includes the following specifications for the Voyager-DE PRO Workstation:

- Performance
- Mass Spectrometer
- Miscellaneous

Table A-4 Voyager-DE PRO Performance Specifications for Installation

Condition	Specification

Resolution (FWHM—Full Width at Half Maximum) in Delayed Extraction mode	**Linear mode:**
	• ≥1,000 for Myoglobin (4 pmol/µl)
	• ≥2,500 for Angiotensin I (1.3 pmol/µl)
	Reflector mode:
	• ≥10,000 for bovine insulin (3.5 pmol/µl)
	• ≥1,200 for E. coli Thioredoxin (2.75 pmol/µl)
	• ≥7,000 for Angiotensin (2.0 pmol/µl)
	• ≥7,000 for ACTH Clips
	  • ACTH 1-17 (2.0 pmol/µl)
	  • ACTH 18-39 (1.5 pmol/µl)
	  • ACTH 7-38 (3.0 pmol/µl)
	$[M+H]^+$ ion analyzed.

Signal-to-Noise Ratio	• ≥200:1 for IgG (0.6 pmol/µl) • ≥100:1 for BSA (4 pmol/µl)
Mass Accuracy in Delayed Extraction mode (difference between the mean of six measurements and the theoretical mass of the sample)	**Linear mode:** • External Calibration—0.05% • Internal Calibration—0.02% **Reflector mode:** • External Calibration—0.01% • Internal Calibration—0.002%
Sensitivity	Routine detection of 5 fmol of neurotensin with signal-to-noise ratio: • ≥20:1 in Linear mode • ≥10:1 in Reflector mode
Mass Accuracy of Fragment Ions	+/- 1 Da
Resolution of PSD Precursor (Timed) Ion Selection	80

A

Table A-5 Voyager-DE PRO Mass Spectrometer Specifications

Condition	Specification
Mass range	≥300,000 Da Upper limit set by suitably ionized biomolecule.
Reflector	Single-stage with optimized optics for PSD Analysis
Flight tube (horizontal)	• Linear mode—1.3 m • Reflector mode—2.0 m
Ion source	Two-stage
Ion source voltages	Tunable: • Accelerating Voltage—Up to 25,000 V • Grid Voltage—Range determined by Accelerating Voltage
Laser	Nitrogen, 337 nm, 3 ns pulse, 20 Hz maximum firing rate. Actual laser firing rate dependant on digitizer. See Section A.4, Digitizer Specifications.
Digitizer	2 GHz digitization for enhanced resolution See Section A.4, Digitizer Specifications.
Vacuum system	• Dual differential turbomolecular pumping for high vacuum • Multi-vacuum gauge capability for independent monitoring of source and analyzer regions
Ion detection	Positive and negative
Sample analysis	• Automated single-plate sample-loading system; sample plates of various formats currently available. See B.3, Spare Parts. • Manual control using control stick or mouse • Sequence control software for automated analysis
Dimensions	• 27 inches (69 cm) deep • 25 inches (64 cm) wide • 65 inches (165 cm) high, includes cabinet and flight tube

Table A-6 Voyager-DE PRO Miscellaneous Specifications

Condition	Specification
Environmental	• Operating temperature—10–25°C • Relative humidity—10–80%, non-condensing • Altitude—≤2,000 m (6,500 ft) above sea level. • Installation category (overvoltage category)—II, classified as portable equipment. • For indoor use only. • Pollution degree rating—2, and may be installed in an environment that has nonconductive pollutants only.
Computer	Minimum configuration: • Pentium® III 500 MHz, with 9 GB hard disk and 128 MB RAM (random access memory) • 17-inch color monitor

A

A.3 Voyager-DE STR Specifications

This section includes the following specifications for the Voyager-DE STR Workstation with Delayed Extraction technology:

- Performance
- Mass Spectrometer
- Miscellaneous

***NOTE**: For specifications on Voyager-DE STR systems with serial number 4153 and earlier, refer to the original Voyager Workstation User's Guide provided with your system.*

Table A-7 Voyager-DE STR Performance Specifications for Installation

Condition	Specification

Resolution (FWHM—Full Width at Half Maximum) in Delayed Extraction mode	**Linear mode:**
	• ≥3,000 for Angiotensin I (1.3 pmol/µl)
	• ≥3,500 for ACTH (18–39) (1.5 pmol/µl)
	Reflector mode:
	• ≥20,000 for bovine insulin (3.5 pmol/µl)
	• ≥1,200 for E. coli Thioredoxin (2.75 pmol/µl)
	• ≥12,000 for Angiotensin (2.0 pmol/µl)
	• ≥12,000 for ACTH Clips
	  • ACTH 1-17 (2.0 pmol/µl)
	  • ACTH 18-39 (1.5 pmol/µl)
	  • ACTH 7-38 (3.0 pmol/µl)
	$[M+H]^+$ ion analyzed.

Signal-to-Noise Ratio	• ≥200:1 for IgG (0.6 pmol/µl) • ≥100:1 for BSA (4 pmol/µl)
Sensitivity	Routine detection of 5 fmol of neurotensin with a signal-to-noise ratio >80:1

Table A-7 Voyager-DE STR Performance Specifications for Installation

Condition	Specification
Mass Accuracy in Delayed Extraction mode (difference between the mean of six measurements and the theoretical mass of the sample)	**Linear mode:** • External Calibration—0.05% • Internal Calibration—0.02% **Reflector mode:** • External Calibration—0.008% • Internal Calibration—0.001%
Mass Accuracy of Fragment Ions	+/- 1 Da
Resolution of PSD Precursor (Timed) Ion Selection	80

Table A-8 Voyager-DE STR Mass Spectrometer Specifications

Condition	Specification
Mass range	≥300,000 Da Upper limit set by suitably ionized biomolecule.
Reflector	Single-stage with optimized optics for PSD Analysis
Flight tube (horizontal)	• Linear mode—2.0 m • Reflector mode—3.0 m
Ion source	Two-stage
Ion source voltages	Tunable: • Accelerating Voltage—Up to 25,000 V • Grid Voltage—Range determined by Accelerating Voltage
Laser	Nitrogen, 337 nm, 3 ns pulse, 20 Hz maximum firing rate. Actual laser firing rate dependant on digitizer. See Section A.4, Digitizer Specifications.
Digitizer	2 GHz digitization for enhanced resolution See Section A.4, Digitizer Specifications.

Table A-8 Voyager-DE STR Mass Spectrometer Specifications (Continued)

Condition	Specification
Vacuum system	• Dual differential turbomolecular pumping for ultra-high vacuum • Multi-vacuum gauge capability for independent monitoring of source and analyzer regions
Ion detection	Positive and negative
Sample analysis	• Automated single-plate sample-loading system; sample plates of various formats currently available. See B.3, Spare Parts. • Manual control using control stick or mouse • Sequence Control software for automated analysis
Dimensions	• 34 inches (87 cm) deep • 94 inches (239 cm) wide • 46 inches (117 cm) high

Table A-9 Voyager-DE STR Miscellaneous Specifications

Condition	Specification
Environmental	• Operating temperature—10–25°C • Relative humidity—10–80%, non-condensing • Altitude—≤2,000 m (6,500 ft) above sea level. • Installation category (overvoltage category)—II, classified as portable equipment. • For indoor use only. • Pollution degree rating—2, and may be installed in an environment that has nonconductive pollutants only.
Computer	Minimum configuration: • Pentium® III 500 MHz, with 9 GB hard disk and 128 MB RAM (random access memory) • 17-inch color monitor

A.4 Digitizer Specifications

This section includes:

- Digitizer specifications
- Laser firing rates

Digitizer specifications

Table A-10 lists specifications for the digitizer options available on the Voyager system.

Table A-10 Digitizer Specifications

Model	Samples/ Second*	Analog Bandwidth	Available Bin Sizes (ns)	Input Bandwith (Software Selectable)
Acqiris DP105	500 Ms/S (megasample/sec)	150 MHz	2, 4, 10, 20	None
Acqiris DP211	2 Gs/S (gigasample/sec)	500 MHz	0.5, 1, 2, 4, 10, 20	25 MHz
LeCroy LSA1000	2 Gs/S	750 MHz	0.5, 1, 2, 4, 10, 20	25 and 200 MHz
Signatec DA500A	500 Ms/S	500 MHz	2, 4, 10, 20	None
Tektronix Scope**	500 Ms/S	500 MHz	2, 4, 10, 20	20 and 100 MHz
Tektronix Scope**	1 Gs/S	500 MHz	1, 2, 4, 10, 20	20 and 250 MHz
Tektronix Scope**	2 Gs/S	500 MHz	0.5, 1, 2, 4, 10, 20	20 and 250 MHz
Tektronix Scope**	4 Gs/S	1 GHz	0.25, 1, 2, 4, 10, 20	20 and 250 MHz

* Samples/second is equivalent to a sampling rate in hertz.

** Specifications supported by the Voyager software for Tektronix Scopes. Different models of Tektronix Scopes are available. Refer to the Tektronix User's Manual for specifications on your particular model.

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Laser firing rates

Figure A-1 shows the estimated laser firing rates for different digitizer models.

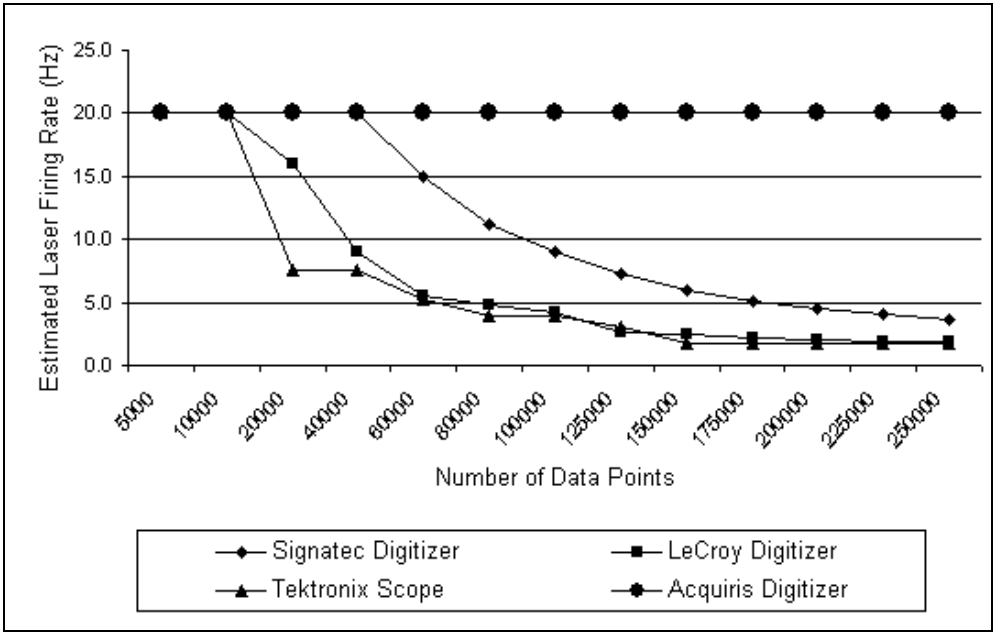

Figure A-1 Estimated Laser Firing Rates Supported by Different Digitizer Models

B Warranty/Service Information

This appendix contains the following sections:

B.1 Limited Product Warranty B-2
B.2 Damages, Claims, Returns B-5
B.3 Spare Parts B-6

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.

B.1 Limited Product Warranty

Limited warranty

Applied Biosystems warrants that all standard components of its Voyager™ Biospectrometry™ Workstations (the "Product") purchased new will be free of defects in materials and workmanship for a period of one (1) year. Applied Biosystems will repair or replace, at its discretion, all defective components during this warranty period. After this warranty period, repairs and replacement components may be purchased from Applied Biosystems at its published rates. Applied Biosystems also provides service agreements for post-warranty coverage. Applied Biosystems reserves the right to use new, repaired, or refurbished instruments or components for warranty and post-warranty replacements. Repair or replacement of products or components under warranty does not extend the original warranty period.

Applied Biosystems warrants that all optional accessories supplied with the Product, such as cameras, peripherals, printers, and special monitors, will be free of defects in materials and workmanship for a period of ninety (90) days. Applied Biosystems will repair or replace, at its discretion, defective accessories during this warranty period. After this warranty period, Applied Biosystems will pass on to the buyer, to the extent that it is permitted to do so, the warranty of the original manufacturer for such accessories.

With the exception of chemicals and other consumable products, replaceable products or components that are obtained from Applied Biosystems and are used on the Product are themselves warranted to be free of defects in materials and workmanship for ninety (90) days. Applied Biosystems warrants that chemicals and other consumable products obtained from Applied Biosystems will be free of defects in materials and workmanship when received by the buyer, but not thereafter, unless otherwise specified in documentation accompanying the product.

Applied Biosystems warrants that for a period of ninety (90) days the software designated for use with the Product will perform substantially in accordance with the function and features described in its accompanying documentation when properly installed on the Product. Applied Biosystems does not warrant that the operation of the instrument or software will be uninterrupted or error free. Applied Biosystems will provide any software corrections or “bug-fixes”, if and when they become available, for a period of ninety (90) days after installation.

Warranty period effective date

Any applicable warranty period under these sections will begin on the date of installation for hardware and software installed by Applied Biosystems personnel, unless that date has been delayed at the buyer's request, but in no event later than thirty (30) days after shipment. In that case, and for all hardware and software installed by the buyer, and for all other products, the applicable warranty period begins the date the product or component is delivered to the buyer.

Warranty claims

Warranty claims must be made within the applicable warranty period or, for chemicals or other consumable products, within thirty (30) days after receipt by the buyer.

Warranty exceptions

The foregoing warranties do not apply to defects resulting from misuse, neglect, or accident, including without limitation: operation with incompatible solvents or samples in the system; operation outside of the environmental or use specifications or not in conformance with the instructions for the product or accessories; performance of improper or inadequate maintenance by the user; installation of software or interfacing not supplied by Applied Biosystems; and modification or repair of the product or the software not authorized by Applied Biosystems.

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THIS WARRANTY IS LIMITED TO THE BUYER OF THE PRODUCT FROM APPLIED BIOSYSTEMS AND IS NOT TRANSFERABLE.

Limited Warranty for refurbished units

The limited warranty period for refurbished units that are less than one (1) year old, is one (1) year for parts, labor and travel. The limited warranty period for refurbished units that are more than one (1) year old is ninety (90) days for parts, labor and travel. All of the foregoing terms, obligations and exclusions regarding the purchase of new products shall apply to refurbished units.

B.2 Damages, Claims, Returns

Damages

Please examine any shipments promptly after receipt to check for damage. Contact Applied Biosystems Service Department if you have questions about checking for damage.

If you discover damage, stop unpacking. Contact the shipping carrier and request inspection by a local agent. Secure a written report of the findings to support any claim. Do not return damaged goods to Applied Biosystems without first securing an inspection report, and contacting Applied Biosystems Technical Support for a Return Authorization (RA) number.

Claims

After a damage inspection report is secured, claims should be processed through Applied Biosystems unless other instructions are provided.

Returns

Please do not return any material without prior notification and authorization.

If, for any reason, it becomes necessary to return material to Applied Biosystems, please contact Applied Biosystems Technical Support, or your nearest Applied Biosystems subsidiary or distributor for:

- Return Authorization (RA) number
- Forwarding address
- Decontamination instructions
- Packing and shipping instructions

Place the RA number in a prominent location on the outside of the shipping container, and return the material to the address designated by the Applied Biosystems representative.

B.3 Spare Parts

Standards and matrixes

The following compounds are available from the listed vendors. Part numbers are listed for your convenience and may change without our knowledge.

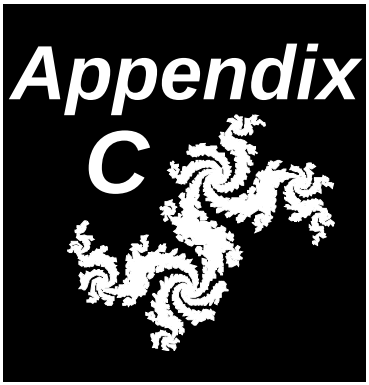
Compound	Vendor	Vendor Part Number
Sequazyme™ Peptide Mass Standards Install Kit includes:	Applied Biosystems	4316866
• Sequazyme Mass Standards Kit		P2-3143-00
• Voyager IgG1 Mass Standard		GEN602151
• Sequazyme BSA Test Standard		2-2158-00
Sequazyme Pinpoint SNP Assay Kit	Applied Biosystems	4315924
Adrenocorticotropic hormone (ACTH) 7–38	Sigma	A-1527
Adrenocorticotropic hormone (ACTH) 18–39	Sigma	A-0673
Angiotensin I (human)	Sigma	A9650
Bradykinin	Sigma	B3259
Insulin	Sigma	I-5500
Myoglobin (horse heart)	Sigma	M-1882
α-cyano-4-hydroxycinnamic acid (CHCA)	Sigma	C-2020
Sinapinic acid (3, 5-dimethoxy-4-hydroxycinnamic acid)	Aldrich	D13,460-0

Sample plates

The following sample plates are available from Applied Biosystems:

Description	Part Number
Welled Sample Plates	
Gold, 100-well	V700401
Gold, 100-well (no pin, for Voyager Workstations manufactured in 1995 or earlier	V700208
Flat Sample Plates (Laser Etched)	
Stainless steel, 100-position (indicated by numbers only)	V700664
Stainless steel, 100-position (indicated by numbers only, no pin, for Voyager Workstations manufactured in 1995 or earlier)	V700665
Stainless steel, 100-position (indicated by numbers and circles)	V700666
Stainless steel, 100-position (indicated by numbers and circles, no pin, for Voyager Workstations manufactured in 1995 or earlier)	V700667
Special Sample Plates	
Stainless steel, polished blank surface	V700668
Stainless steel, polished blank surface (no pin, for Voyager Workstations manufactured in 1995 or earlier)	V700669
Gold, 64-well, disposable, for use with disposable sample plate holder (part number V700314)	V503476
Disposable sample plate holder, for use with gold, 64-well, disposable plate (part number V503476)	V700314
Biacore® Chip (holds 4 biochips)	V700697

Description	Part Number
Membrane, Gels	V700698
Hydrophobic plastic surface, flat, 400-position	V700699
96 × 2-position, flat, hydrophobic plastic surface plate	V700813



C Matrixes

This appendix provides information for commonly used matrixes. The information includes (see Table C-1 on page C-6):

- Matrix mass spectra
- Chemical structure
- Applications
- Description of physical appearance
- Suggested solution concentration
- Characteristic matrix ions

Refer to Figure C-1 through Figure C-9 for characteristic peaks patterns and masses.

For additional matrix information, refer to the bibliography.

WARNING

CHEMICAL HAZARD. *Refer to the Material Safety Data Sheet (MSDS) provided by the chemical manufacturer before handling solvents or matrixes.*

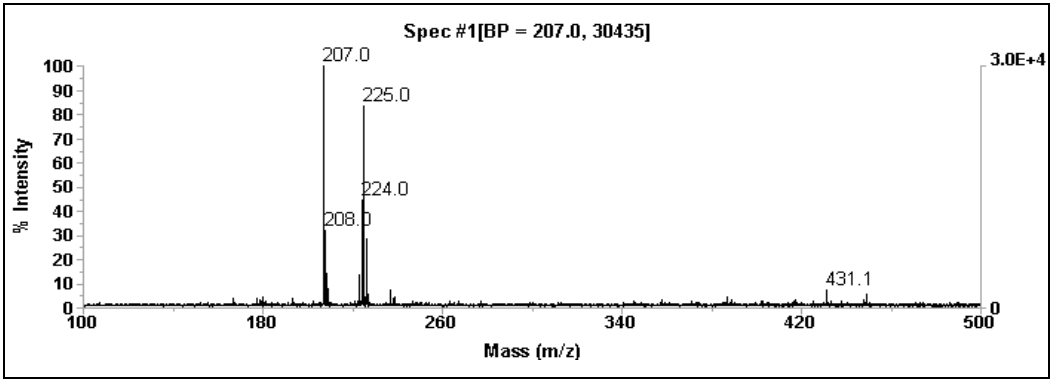


Figure C-1 Sinapinic Acid Matrix Spectrum

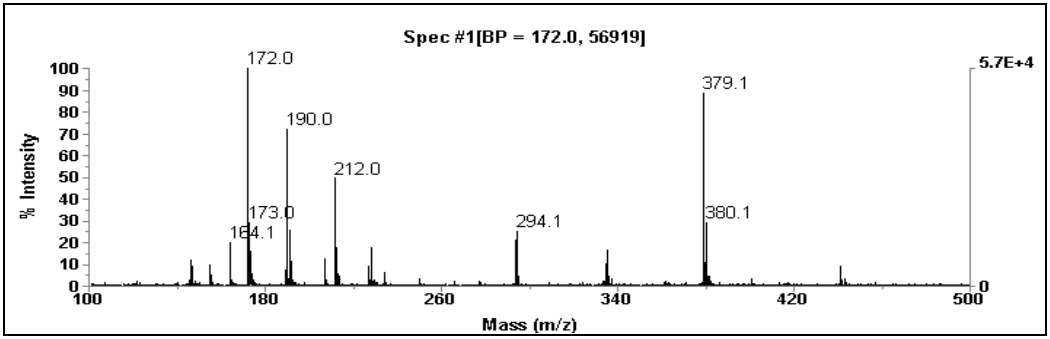


Figure C-2 α-cyano-4-hydroxycinnamic acid (CHCA) Matrix Spectrum

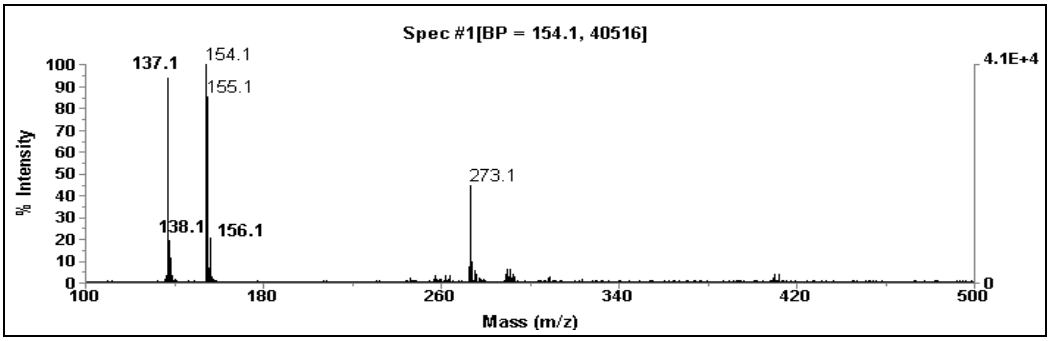


Figure C-3 2,5-dihydroxybenzoic acid (2,5-DHB) Matrix Spectrum

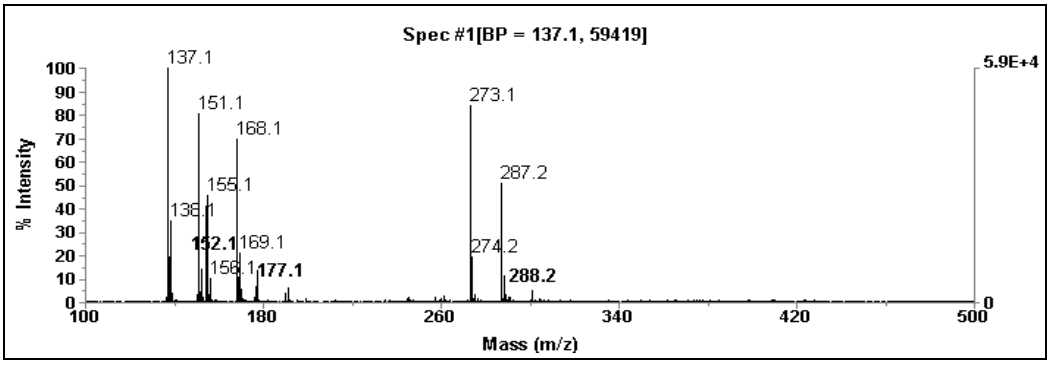


Figure C-4 Mixture of 2,5-dihydroxybenzoic acid and 5-methoxysalicylic acid (DHBs) Matrix Spectrum

C

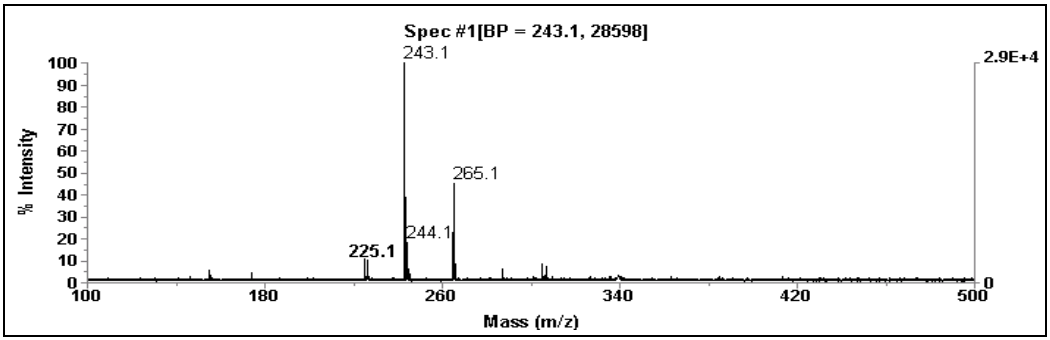


Figure C-5 2-(4-hydroxy-phenylazo)-benzoic acid (HABA) Matrix Spectrum

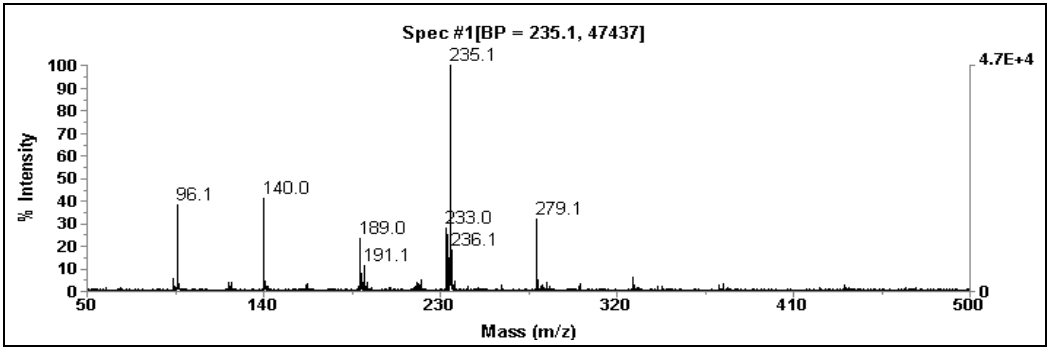


Figure C-6 3-hydroxypicolinic acid (3-HPA) Matrix Spectrum

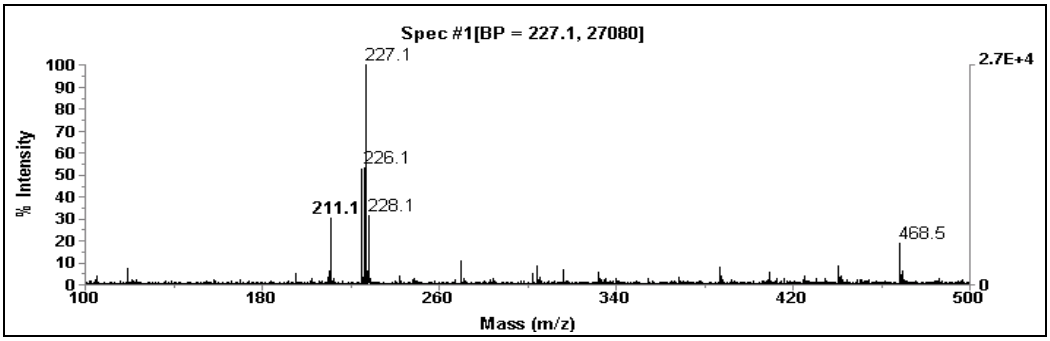


Figure C-7 Dithranol Matrix Spectrum

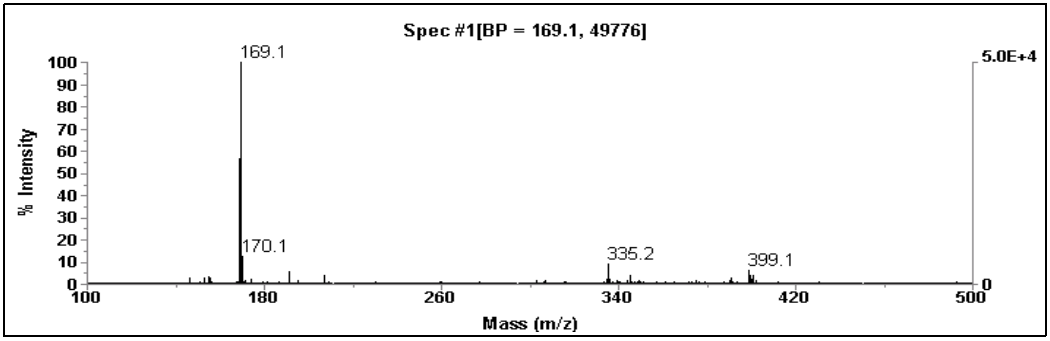


Figure C-8 2,4,6 trihydroxyacetophenone (THAP) Spectrum

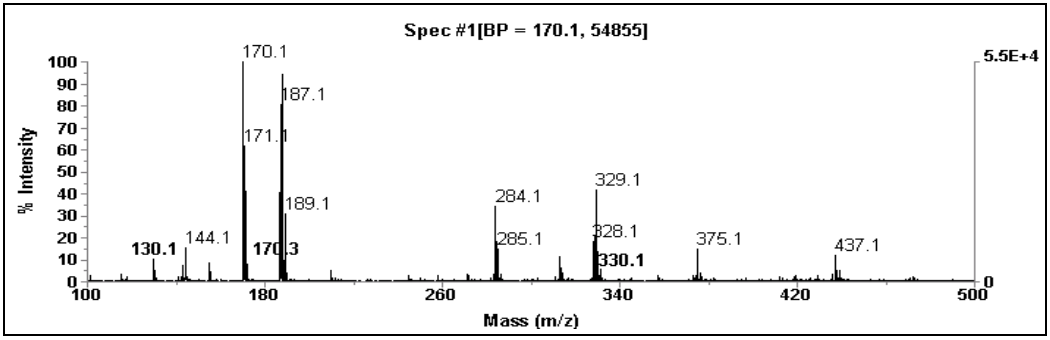


Figure C-9 trans-3-indoleacrylic acid (IAA) Matrix Spectrum

C

Table C-1 Matrix Information

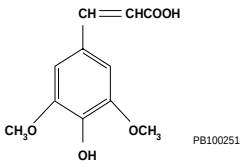
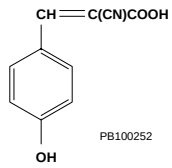
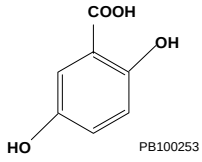
Matrix	Applications/Color	Matrix Solution Concentration	Characteristic Matrix Ions (monoisotopic)
Sinapinic acid (see mass spectrum on page C-2) MW 224.07 Da  	**Applications:** • Peptides • Proteins **Color of crystals/ solution:** White ***NOTE:*** *Matrix powder may also contain orange crystals. Do not use crystals when preparing solutions.*	• 10 mg/ml in 70:30 water/acetonitrile (0.1% TFA final conc.) • 10 mg/ml in 50:50 water/acetonitrile (0.1% TFA final conc.) if sample contaminated	• 225.076 • 224.068 • 207.066 • 431.134

Table C-1 Matrix Information

Matrix	Applications/Color	Matrix Solution Concentration	Characteristic Matrix Ions (monoisotopic)
Alpha-cyano-4-hydroxycinnamic acid (αCHCA) (see mass spectrum on page C-2) MW 189.04 Da 	**Applications:** • Peptides • Proteins **Color of crystals/ solution:** Yellow	10 mg/ml in 50:50 water/acetonitrile (0.1% TFA final conc.)	• 164.047 • 195.050 • 172.040 • 379.093 • 212.032 • 294.076
2,5-dihydroxybenzoic acid (2,5-DHB) (see mass spectrum on page C-3) MW 154.03 Da 	**Applications:** • Peptides (mixtures) • Carbohydrates • Glycolipids (negative ion mode) • Polar synthetic polymers **Color of crystals/ solution:** White	10 mg/ml in water	• 155.034 • 154.027 • 137.024 • 273.040

C

Table C-1 Matrix Information

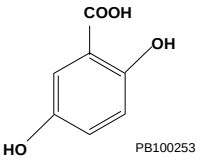
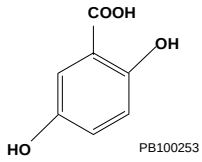
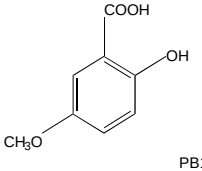
Matrix	Applications/Color	Matrix Solution Concentration	Characteristic Matrix Ions (monoisotopic)
2,5-dihydroxybenzoic acid (2,5-DHB) (see mass spectrum on page C-3) MW 154.03 Da 	**Applications:** Small molecules **Color of crystals/ solution:** White	10 mg/ml in solvent in which sample and matrix are soluble	• 155.034 • 154.027 • 137.024 • 273.040

Table C-1 Matrix Information

Matrix	Applications/Color	Matrix Solution Concentration	Characteristic Matrix Ions (monoisotopic)
Mixture of 2,5-dihydroxybenzoic acid and 5-methoxysalicylic acid (DHBs) (see mass spectrum on page C-3) MW 154.03 Da + MW 168 Da Mixture MW = 322.03 Da  PB100253  PB100775	**Applications:** Large proteins **Color of crystals/ solution:** White	10 mg/ml in solvent in which sample and matrix are soluble	• 155.034 • 154.027 • 137.024 • 273.040 • 151.040 • 168.042 • 169.050

C

Table C-1 Matrix Information

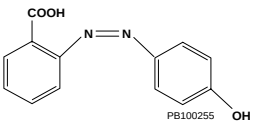
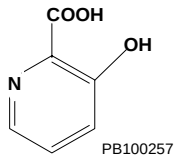
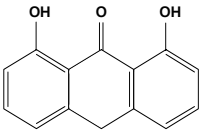
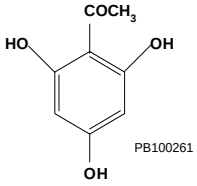
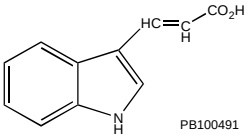
Matrix	Applications/Color	Matrix Solution Concentration	Characteristic Matrix Ions (monoisotopic)
2-(4-hydroxy-phenylazo)-benzoic acid (HABA) (see mass spectrum on page C-4) MW 242.07 Da 	**Applications:** • Proteins • Lipopoly-saccharides • Polar and nonpolar synthetic polymers **Color of crystals/ solution:** Orange	• ~1.3 mg/ml in 50:50 water/ acetonitrile or in 40:40:20 water/ acetonitrile/ methanol • 10 mg/ml in ethanol or methanol	• 243.077 • 265.059
3-hydroxypicolinic acid (3-HPA) (see mass spectrum on page C-4) MW 139.03 Da 	**Applications:** Oligonucleotides **Color of crystals/ solution:** Light brown	Make 9:1 dilution of matrix:diammonium citrate **Matrix**—50 mg/ml in 50:50 water/ acetonitrile **Diammonium citrate**—50 mg/ml in water	• 96.045 • 140.035 • 279.062 • 235.072 • 234.064 • 233.056 • 191.082 • 189.066

Table C-1 Matrix Information

Matrix	Applications/Color	Matrix Solution Concentration	Characteristic Matrix Ions (monoisotopic)
Dithranol (see mass spectrum on page C-5) MW 226.06 Da  PB100258	**Applications:** Nonpolar synthetic polymers **Color of crystals/ solution:** Yellow	10 mg/ml in tetrahydrofuran + silver trifluoroacetate to minimize Na^+ and K^+ adduct formation	• 225.055 • 226.063 • 227.071 • 211.076
2,4,6 trihydroxy acetophenone (THAP) (see mass spectrum on page C-5) MW 168.04 Da  PB100261	**Applications:** Small Oligonucleotides **Color of crystals/ solution:** White	Make 9:1 dilution of matrix:diammonium citrate **Matrix**—10 mg/ml in 50:50 water/ acetonitrile **Diammonium citrate**— 50 mg/ml in water	• 169.050

C

Table C-1 Matrix Information

Matrix	Applications/Color	Matrix Solution Concentration	Characteristic Matrix Ions (monoisotopic)
trans-3-indoleacrylic acid (IAA) (see mass spectrum on page C-6) MW 187.2  	**Applications:** Non-polar polymers **Color of crystals/ solution:** White	10^{-1} M in solvent appropriate for sample	• 187.063 • 188.071 • 170.061 • 144.081 • 130.066 • 375.134 • 329.120 • 284.131
Picolinic acid	Tang, K., N.I. Taranenko, S.L. Allman, C.H. Chen, L.Y. Chang, and K.B. Jacobson, “Picolinic Acid as a Matrix for Laser Mass Spectrometry of Nucleic Acids and Proteins”, *Rapid Commun. Mass Spectrom.*, **1994**, *8*, 673–677.		
Nicotinic acid	Ehring, H.M., M. Karas, F. Hillenkamp, *Org. Mass Spectrom.,* **1992**, *27,* 472–480.		

D Log Sheets

This appendix includes log sheets you can copy and use to log samples before loading.

Voyager™ Biospectrometry™ Sample Log

Plate #:						**Date:**				
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										

Voyager™ Biospectrometry™ Sample Log

Plate:	Date:	Page of	Path and File Name	
Samp #	**Matrix**	**Sample**	**Linear**	**Reflector**
1.				
2.				
3.				
4.				
5.				
6.				
7.				
8.				
9.				
10.				
11.				
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19.				
20.				
21.				
22.				
23.				
24.				
25.				

Voyager™ Biospectrometry™ Sample Log

Plate:	Date:	Page of	Path and File Name	
Samp #	**Matrix**	**Sample**	**Linear**	**Reflector**
26.				
27.				
28.				
29.				
30.				
31.				
32.				
33.				
34.				
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41.				
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43.				
44.				
45.				
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48.				
49.				
50.				

Voyager™ Biospectrometry™ Sample Log

Plate:	Date:	Page of	Path and File Name	
Samp #	**Matrix**	**Sample**	**Linear**	**Reflector**
51.				
52.				
53.				
54.				
55.				
56.				
57.				
58.				
59.				
60.				
61.				
62.				
63.				
64.				
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66.				
67.				
68.				
69.				
70.				
71.				
72.				
73.				
74.				
75.				

Voyager™ Biospectrometry™ Sample Log

Plate:	Date:	Page of	Path and File Name	
Samp #	**Matrix**	**Sample**	**Linear**	**Reflector**
76.				
77.				
78.				
79.				
80.				
81.				
82.				
83.				
84.				
85.				
86.				
87.				
88.				
89.				
90.				
91.				
92.				
93.				
94.				
95.				
96.				
97.				
98.				
99.				
100.				

Voyager™ Biospectrometry™ Sample Log

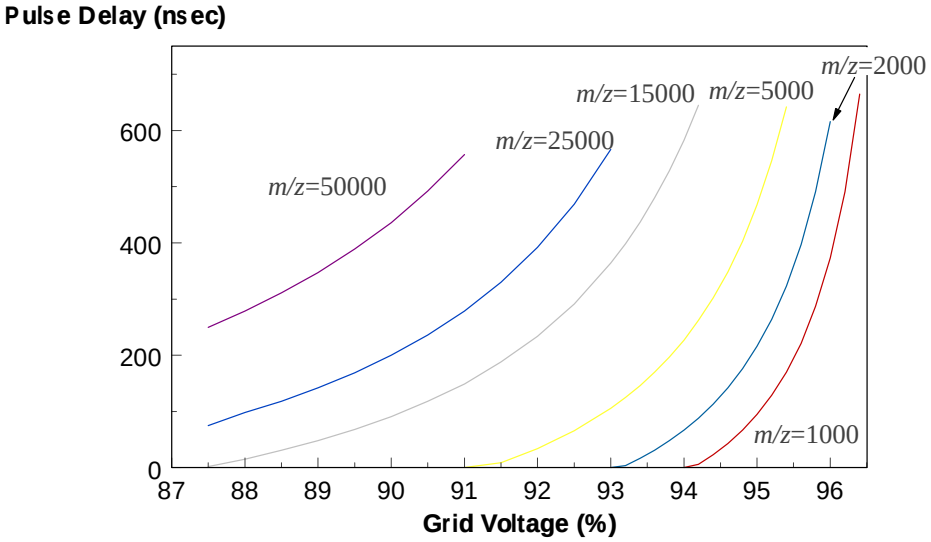
Plate:	Date:	Page of	Path and File Name	
Samp #	**Matrix**	**Sample**	**Linear**	**Reflector**

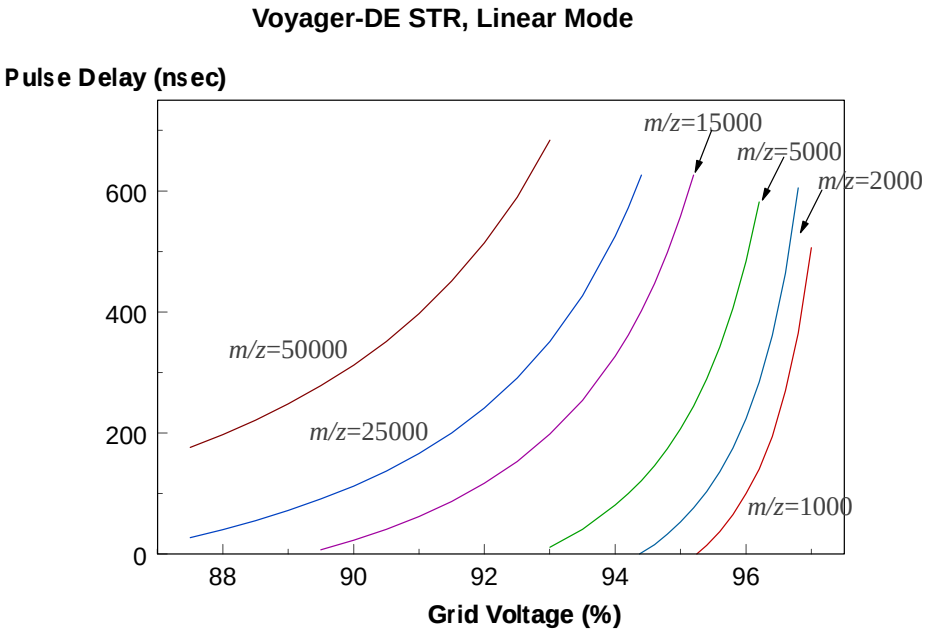
D

E Grid Voltage% and Delay Time Settings

The following figures illustrate the relationship between Grid Voltage% and Delay Time parameters. These values are not absolute values for all systems. Optimum settings may be slightly different for each system.

Voyager-DE and Voyager-DE PRO, Linear Mode




Voyager-DE STR, Linear Mode
Pulse Delay (nsec)
m/z=15000
m/z=5000
m/z=2000
m/z=50000
m/z=25000
m/z=1000
600
400
200
0
88
90
92
94
96
Grid Voltage (%)

F Reference Standard Information

This appendix contains the following sections:

F.1 Calibration Compounds..... F-2

F.2 Conversion of Mass to Time for Typical Standards..... F-4

F.3 Theoretical Cleavages for Angiotensin F-6

F.4 Observed PSD Fragments in Angiotensin F-7

F

F.1 Calibration Compounds

The table below includes masses for common calibration compounds.

NOTE: *You can display reference mass information in the Data Explorer software by viewing the VOYAGER.REF file in the mass calibration function.*

Compound	Molecular Weight		Charge State	Protonated Molecular Ion $[M+H]^+$	
	Monoisotopic	Average		Monoisotopic	Average
Leucine Enkephalin	555.2693	555.63	+1	556.2771	556.64
des-Arg^1 Bradykinin	903.4603	904.04	+1	904.4681	905.05
Bradykinin	1059.5614	1060.23	+1	1060.5692	1061.24
			+2	____	531.1
Angiotensin I	1295.6775	1296.50	+1	1296.6853	1297.51
Substance P-amide	1346.7281	1347.65	+1	1347.7360	1348.66
Glu^1-Fibrino-peptide B	1569.6696	1570.60	+1	1570.6774	1571.61
Neurotensin	1671.9097	1672.95	+1	1672.9175	1673.96
Adrenocorticotropic hormone (ACTH) clip 1–17	2092.0789	2093.45	+1	2093.0867	2094.46
Bovine Trypsin	2162.0492	2163.3413	+1	2163.0574	2164.3413
Porcine Trypsin	2211.0968	2211.4322	+1	2211.1046	2212.4322
Adrenocortico-tropic hormone (ACTH) clip 18–39	2464.1910	2465.71	+1	2465.1989	2466.72

Compound	Molecular Weight		Charge State	Protonated Molecular Ion $[M+H]^+$	
	Monoisotopic	Average		Monoisotopic	Average
Insulin B chain, oxidized	3493.6435	3495.95	+1	3494.6513	3496.96
Adrenocorticotropic hormone (ACTH) clip 7–38	3656.9216	3659.18	+1	3657.9294	3660.19
Insulin, bovine	____	5733.58	+1	5730.6087	5734.59
			+2	2865.8083	2867.80
Thioredoxin (*E. coli*, oxidized)	____	11673.47	+1	____	11674.48
			+2	____	5837.74
Cytochrome *C* (horse heart)	____	12360.5	+1	____	12361.5
			+2	____	6181.25
Myoglobin (horse heart)	____	16951.55	+1	____	16952.56
			+2	____	8476.78
Trypsinogen	____	23980	+1	____	23981
Carbonic anhydrase	____	29023	+1	____	29024
Enolase (Baker's yeast)	____	46671	+1	____	46672
			+2	____	23336
Bovine serum albumin (BSA)	____	66430	+1	____	66431
			+2	____	33216

F

F.2 Conversion of Mass to Time for Typical Standards

The table below includes mass and time values for standards under different acquisition conditions (Accelerating Voltage and flight tube length). You can use this information when observing the signal on the oscilloscope to determine if the peaks you are seeing are at the correct mass.

NOTE: *These values are not exact. Use them as a guide.*

Standards	Z	Average MW $[M+H]^+$	Time (seconds)				
			25,000 V 1.3 m	10,000 V 1.3 m	28,000 V 2.0 m	15,000 V 2.0 m	10,000 V 2.0 m
Leucine Enkephalin	1	556.61	1.40E-05	2.21E-05	2.03E-05	2.77E-05	3.40E-05
Methionine Enkephalin	1	574.65	1.42E-05	2.24E-05	2.06E-05	2.82E-05	3.45E-05
Bradykinin	1	1061.2	1.93E-05	3.05E-05	2.80E-05	3.83E-05	4.69E-05
	2	531.1	1.36E-05	2.16E-05	1.98E-05	2.71E-05	3.32E-05
Angiotensin II (human)	1	1047.18	1.92E-05	3.03E-05	2.78E-05	3.80E-05	4.66E-05
	2	524.09	1.35E-05	2.14E-05	1.97E-05	2.69E-05	3.30E-05
Angiotensin I (human)	1	1297.48	2.13E-05	3.37E-05	3.10E-05	4.23E-05	5.19E-05
	2	649.24	1.51E-05	2.38E-05	2.19E-05	3.00E-05	3.67E-05
Melittin	1	2847.5	3.16E-05	4.99E-05	4.59E-05	6.27E-05	7.68E-05
	2	1424.25	2.23E-05	3.53E-05	3.25E-05	4.44E-05	5.43E-05

Standards	Z	Average MW $[M+H]^+$	Time (seconds)				
			25,000 V 1.3 m	10,000 V 1.3 m	28,000 V 2.0 m	15,000 V 2.0 m	10,000 V 2.0 m
Bovine Insulin B, Oxidized	1	3496.9	3.50E-05	5.53E-05	5.09E-05	6.95E-05	8.51E-05
	2	1748.95	2.48E-05	3.91E-05	3.60E-05	4.92E-05	6.02E-05
Bovine Insulin	1	5734.5	4.48E-05	7.09E-05	6.52E-05	8.90E-05	1.09E-04
	2	2867.75	3.17E-05	5.01E-05	4.61E-05	6.30E-05	7.71E-05
Insulin-like Growth Factor (IgF)	1	7650.76	5.18E-05	8.19E-05	7.53E-05	1.03E-04	1.26E-04
	2	3825.88	3.66E-05	5.79E-05	5.32E-05	7.27E-05	8.91E-05
Cytochrome *C* (horse heart)	1	12361.5	6.58E-05	1.04E-04	9.57E-05	1.31E-04	1.60E-04
	2	6181.25	4.65E-05	7.36E-05	6.76E-05	9.24E-05	1.13E-04
	3	4121.17	3.80E-05	6.01E-05	5.52E-05	7.55E-05	9.24E-05
	4	3091.12	3.29E-05	5.20E-05	4.78E-05	6.54E-05	8.00E-05
Myoglobin (horse heart)	1	16952.5	7.71E-05	1.22E-04	1.12E-04	1.53E-04	1.87E-04
	2	8476.75	5.45E-05	8.62E-05	7.92E-05	1.08E-04	1.33E-04
	3	5651.5	4.45E-05	7.03E-05	6.47E-05	8.84E-05	1.08E-04
	4	4238.76	3.85E-05	6.09E-05	5.60E-05	7.65E-05	9.37E-05
Bovine Serum Albumin (BSA)	1	66431	1.53E-04	2.41E-04	2.22E-04	3.03E-04	3.71E-04
	2	33216	1.08E-04	1.71E-04	1.57E-04	2.14E-04	2.62E-04
	3	22144.3	8.81E-05	1.39E-04	1.28E-04	1.75E-04	2.14E-04
	4	16608.5	7.63E-05	1.21E-04	1.11E-04	1.51E-04	1.86E-04

F

F.3 Theoretical Cleavages for Angiotensin

Monoisotopic masses (Da) for the theoretical cleavages of angiotensin I are listed below as calculated for the positive ion mode. Monoisotopic (M + H)+ for the sequence DRVYIHPFHL is 1296.685.

a	b	c	Fragment
88.040	116.035	133.061	D
244.141	272.136	289.162	DR
343.209	371.204	388.231	DRV
506.273	534.268	551.294	DRVY
619.357	647.352	664.378	DRVYI
756.416	784.411	801.437	DRVYIH
853.468	881.463	898.490	DRVYIHP
1000.537	1028.532	1045.558	DRVYIHPF
1137.596	1165.591	1182.617	DRVYIHPFH
x	**y**	**z**	**Fragment**
158.082	132.1025	112.076	L
295.141	269.1614	249.135	HL
442.209	416.2298	396.204	FHL
539.262	513.2825	493.256	PFHL
676.321	650.3414	630.315	HPFHL
789.405	763.4255	743.399	IHPFHL
952.468	926.4888	906.463	YIHPFHL
1051.537	1025.557	1005.531	VYIHPFHL
1207.638	1181.658	1161.632	RVYIHPFHL

F

F.4 Observed PSD Fragments in Angiotensin

The masses listed below are fragment ions of the $(M+H)^+$ percursor ion.

Fragment Mass		Designation	Fragment Mass		Designation
(monoisotopic)	(average)		(monoisotopic)	(average)	
70.066	70.1	P, R	~426.2	426.5	?
72.081	72.1	V	489.246	489.6	a4-17
86.097	86.1	I, L	~492.2	492.6	?
110.072	110.1	H	506.273	506.6	a4
136.076	136.2	Y	513.283	513.6	y4
156.101	156.2	R?	517.241	517.6	b4-17
166.062	166.2	H	534.268	534.6	b4
207.125	207.3	HP-28	619.357	619.7	a5
217.134	217.3	PF-28	630.325	630.8	b5-17
223.156	223.3	IH-28	632.331	632.8	IHPFH
~230.1	230.3	?	647.352	647.8	b5
235.120, 235.145	235.3	HP, VY-28	650.341	650.8	y5
251.151	251.3	IH	739.389	739.9	a6-17
255.109	255.3	b2-17	756.416	756.9	a6
263.140	263.3	VY	767.384	767.9	b6-17
269.161	269.3	y2	784.411	784.9	b6

Fragment Mass		Designation	Fragment Mass		Designation
(monoisotopic)	**(average)**		**(monoisotopic)**	**(average)**	
272.136	272.3	b2	1000.537	1001.2	a8
285.135	285.3	FH	1046.542	1047.2	b8+H_2O
326.183	326.4	a3-17	~1068.6	1069.2	?
354.178	354.4	b3-17	1137.596	1138.3	a9
~364.2	364.4	?	1165.591	1166.3	b9
371.204	371.4	b3	1181.658	1182.3	y9
382.188	382.4	PFH	1183.601	1184.3	b9+H2O
~400.2	400.5	?	1296.685	1297.5	MH+
416.230	416.5	y3			

G Maintenance Log

The following page includes a log sheet listing maintenance procedures.

Copy this page and keep it by your Voyager system.

Instructions for performing maintenance procedures are listed in Chapter 9, Maintenance and Troubleshooting.

Maintenance Log
for Voyager System Serial Number _____

Record the date and your initials when you perform maintenance procedures.

Weekly	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
Back up and archive data												

Yearly	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
Hardware maintenance performed by Applied Biosystems engineer												

H Continuous Extraction Mode

This appendix contains the following sections:

H.1 Optimizing a Continuous Extraction Standard Instrument (.BIC) Setting H-2

H.2 Obtaining Good Spectra in Continuous Extraction Mode H-8

H.3 Troubleshooting in Continuous Extraction Mode H-19

H.1 Optimizing a Continuous Extraction Standard Instrument (.BIC) Setting

Before you begin

NOTE: *Due to the superior results obtained during Delayed Extraction (DE) mode, use Continuous Extraction mode for diagnostic purposes only.*

Before optimizing a Continuous Extraction .BIC file, be familiar with the following information:

- *The Data Explorer Software User's Guide*, Appendix B, Overview of Isotopes
- "Opening and Viewing Instrument Settings" on page 5-7
- "List of standard instrument settings files" on page H-3
- "Saving and Printing Instrument Settings" on page 5-11
- "Acquiring in Manual Mode from the Instrument Control Panel" on page 6-11
- Section H.2, Obtaining Good Spectra in Continuous Extraction Mode
- "Determining Laser Threshold" on page H-12
- Section H.2.3, Checking Resolution
- Section H.2.4, Fine-Tuning the Laser Setting
- Section 5.1, Loading, Modifying, and Saving Instrument Settings

Instrument settings parameters

Most instrument settings parameters are optimized for your system and should not require adjustment. Change only the parameters listed in the following procedure.

For more information on remaining instrument settings, see Section 5.2, Instrument Settings Parameter Descriptions.

List of standard instrument settings files

This section lists standard instrument settings (.BIC) files for Continuous Extraction in the following modes:

- Linear mode
- Reflector mode
- PSD mode

Standard instrument settings files are located in the C:\VOYAGER\DATA\FACTORY directory.

Table H-1 Continuous Extraction Linear Mode Standard Instrument Settings (.BIC) Files

.BIC File	Sample	Test	Mass Range (Da)
L1000	Low mass peptide mix	Calibration and resolution	100–2,000
L1001	Insulin	Resolution	5,000–7,000
L1002	Myoglobin	Resolution	10,000–20,000
L1003	BSA	Resolution	60,000–70,000
L1004	High mass peptide mix	Calibration (ACTH 7-38)	2,000–6,000
L1005	Neurotensin in mix	Sensitivity	1,000–2,000
L1008	Low mass peptide mix	Negative ion mode	100–2,000

Table H-2 Continuous Extraction Reflector Mode Standard Instrument Settings (.BIC) Files

.BIC File	Sample	Test	Mass Range (Da)
R1000	Angiotensin I	Resolution at 20,000 V	100–2,000
R1001	Angiotensin I	Resolution at 10,000 V	100–2,000
R1002	Insulin	Resolution	5,000–7,000
R1003	E. coli Thioredoxin	Resolution	10,000–14,000
R1004	Low mass peptide mix	Calibration (angiotensin I)	100–2,000
R1005	Neurotensin in mix	Sensitivity	1,000–2,000
R1006	High mass peptide mix	Calibration (ACTH 7-38)	2,000–6,000
R1008	Low mass peptide mix	Negative ion mode	100–2,000
R1012	Substance-P mix	Timed Ion Selector	1,348–1,388

Table H-3 Continuous Extraction PSD Mode Standard Instrument Settings Files

.BIC File	Sample	Test	Mass Range (Da)
P1000 to 1009	Low mass peptide mix or angiotensin I alone	Mirror ratio varies for PSD analysis	PSD ions for precursor mass 1,297

Optimization strategy

When optimizing a Continuous Extraction instrument settings file, you:

- Start with a standard instrument settings file
- Fine-tune laser setting for major improvement in performance
- Optionally adjust Grid Voltage% and Guide Wire Voltage% for slight improvement in performance

Optimizing

1. Open a standard instrument settings file for the mass range you are analyzing.

   If an instrument settings file for the mass range you are analyzing does not exist, open a standard instrument settings file with the closest higher mass.
2. Save the instrument settings file under a new name. Standard instrument settings files are read-only and cannot be saved.
3. Select a calibration (.CAL) file. If you are screening samples, use the default calibration.
4. If the mass range needs adjusting, change the digitizer setting. See Section 5.3.5, Understanding Digitizer Settings.
5. To include matrix peaks in the spectrum for calibration, deselect the Low Mass Gate field, or set the Starting Mass to a mass below the matrix peak mass.

6. Set the Grid Voltage% appropriate for the matrix and mass:

Table H-4 Grid Voltage% Settings for Continuous Extraction Mode

Matrix	Mass (Da)	Grid Voltage%*
α-cyano-4-hydroxycinnamic acid	<5,000	50–70
	>5,000	70–90
Sinapinic acid	<5,000	80–85
	>5,000	85–90
DHB 3-HPA	<5,000	85–90
	>5,000	
* In Reflector mode, lower Grid Voltage% settings may yield greater resolution, but may compromise sensitivity.		

7. Save the instrument settings (.BIC) file.

8. Determine the laser threshold for the sample and adjust the laser setting until you obtain the resolution needed for your application.

   Laser position and laser intensity are the primary factors affecting the quality of spectra. However, you may slightly improve the quality of data by fine-tuning:

   - Grid Voltage% within the ranges listed above
   - Guide Wire Voltage% within the ranges listed below

Table H-5 Guide Wire Voltage% Settings for Continuous Extraction Mode

Mass Range (Da)	Guide Wire Voltage
<1,500	0.05%
1,500–4,00	0.1%
4,000–15,000	0.2%
>15,000	0.3%

H.2 Obtaining Good Spectra in Continuous Extraction Mode

This section describes:

- Spectra, resolution, signal-to-noise ratio, and laser threshold
- Determining laser threshold
- Checking Resolution
- Fine-Tuning the Laser Setting

H.2.1 Spectra, Resolution, Signal-to-Noise Ratio, and Laser Threshold

What is a good spectrum?

A good spectrum is one that is acceptable for your purposes. In general, it:

- Contains sharp, symmetrical, well-defined peaks
- Has acceptable resolution
- Has acceptable signal-to-noise ratio

For some applications (for example, when you are looking for detailed structural information), you may require very well-separated peaks and the maximum resolution possible. For other applications (for example, when looking for an estimate of molecular weight), your requirements may be less strict.

Resolution and signal-to-noise ratio

There is a balance between good resolution and a good signal-to-noise ratio. To achieve maximum resolution, you may a see lower signal-to-noise ratio. Conversely, to maximize the signal-to-noise ratio, you may see less than maximum resolution.

Figure H-1 through Figure H-4 are examples of poor and good spectra.

NOTE: *Dimer in a spectrum may indicate that the molecular ion is saturated, or that sample is too concentrated. Decrease laser intensity or sample concentration to minimize the dimer.*

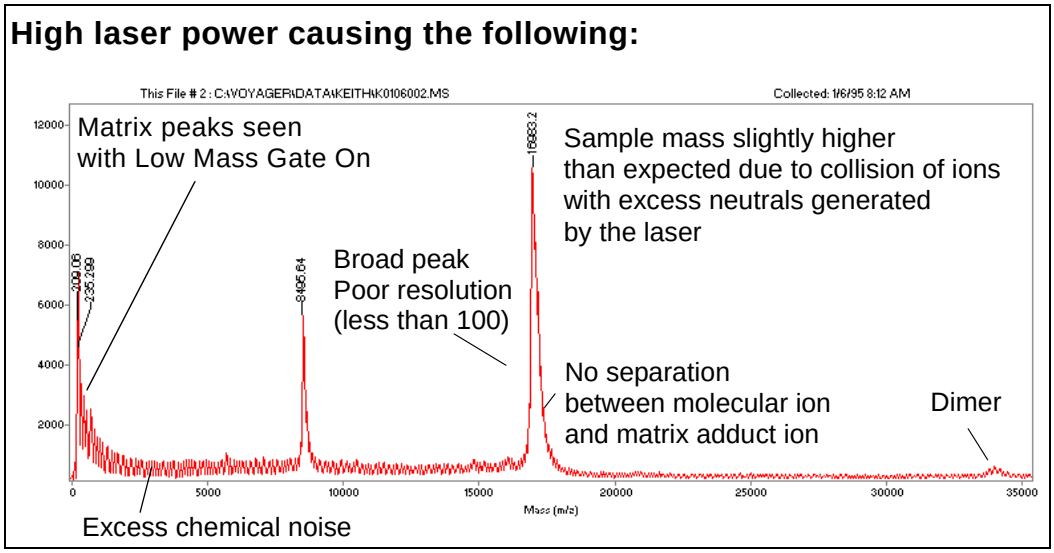


Figure H-1 Example of Poor Mass Spectrum for Myoglobin Using Sinapinic Acid

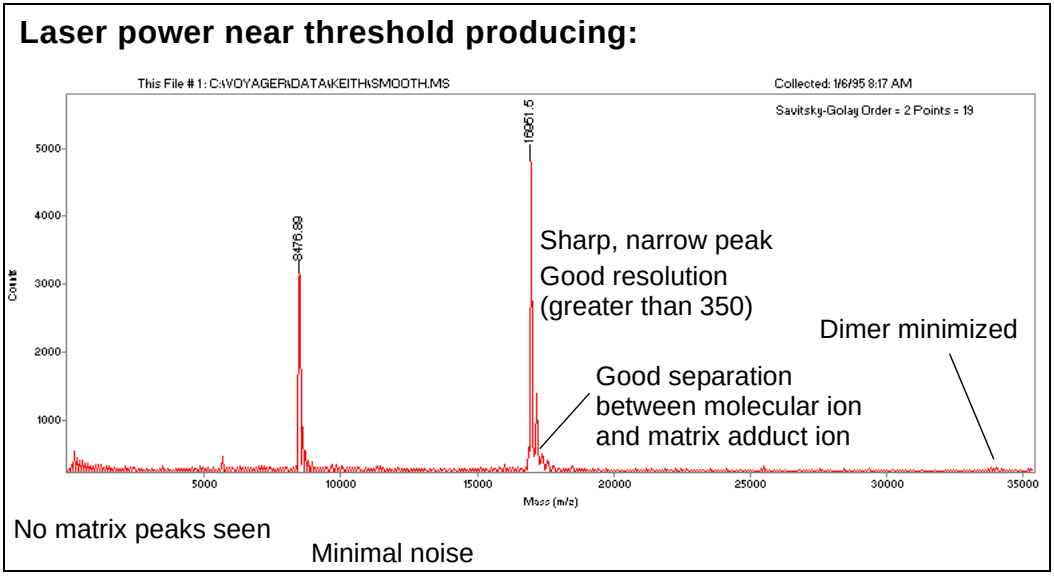


Figure H-2 Example of Good Mass Spectrum for Myoglobin Using Sinapinic Acid

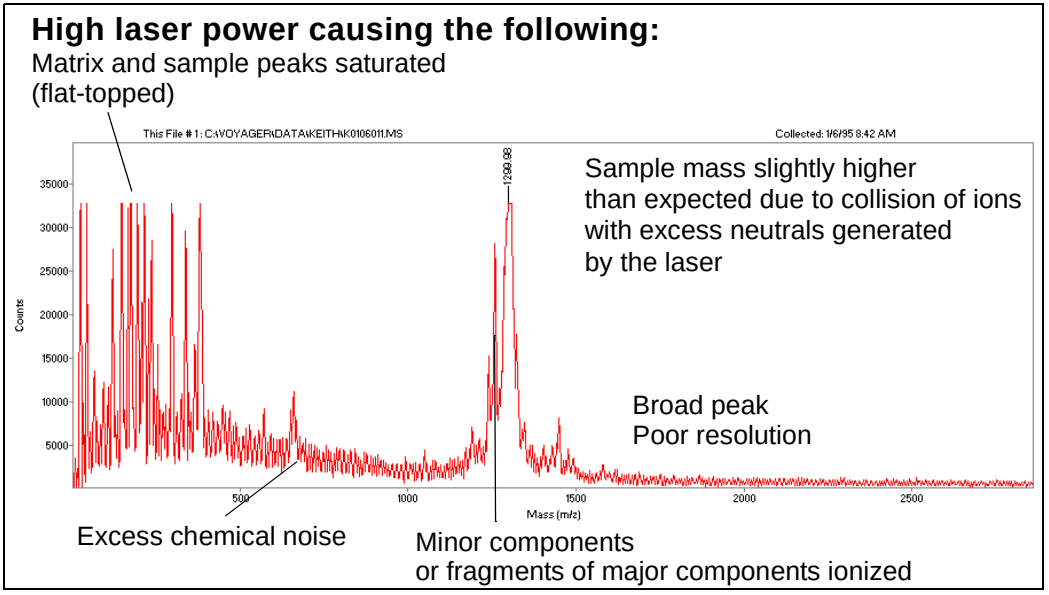


Figure H-3 Example of Poor Mass Spectrum for Angiotensin

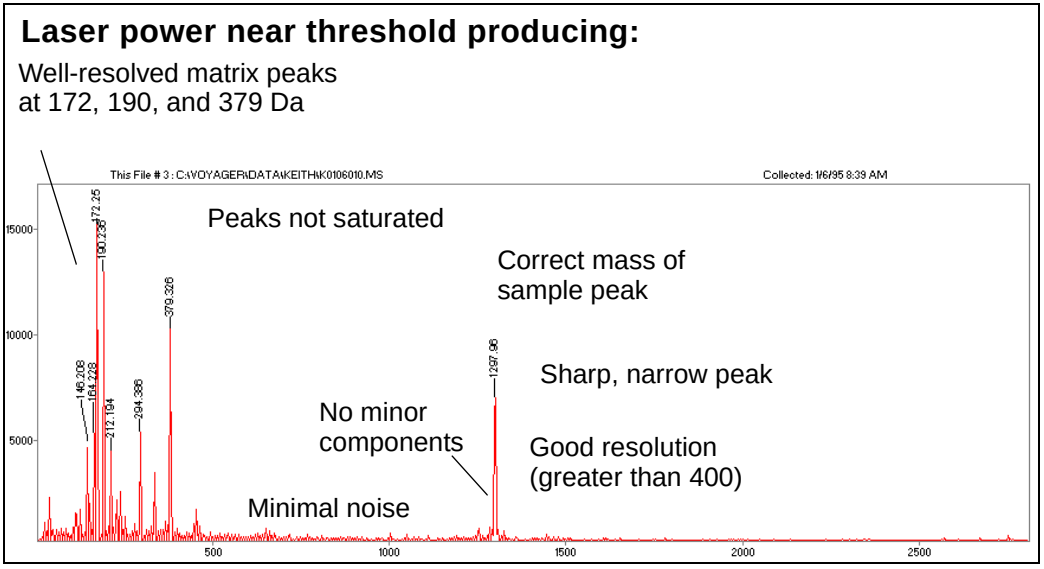


Figure H-4 Example of Good Mass Spectrum for Angiotensin

Parameters affecting resolution and signal-to-noise ratio

These parameters have a primary impact on resolution and signal-to-noise ratio in Continuous Extraction mode:

- Laser position on the sample (hot or cold spot)
- Laser intensity

These parameters have a secondary impact on resolution and signal-to-noise ratio in Continuous Extraction mode:

- Digitizer setting
- Accelerating Voltage
- Grid Voltage%
- Guide Wire Voltage%

To obtain suitable mass spectra, you may need to adjust these parameters in conjunction with each other. See Section H.1, Optimizing a Continuous Extraction Standard Instrument (.BIC) Setting, for more information.

Laser threshold

You see the best resolution at *laser threshold*, the minimum laser intensity required to produce a steady detectable signal.

NOTE: *High mass compounds may require a higher laser intensity to ionize the sample than low mass compounds.*

You may see the best overall spectrum (good resolution and good signal-to-noise ratio) at a setting slightly higher than threshold.

H.2.2 Determining Laser Threshold

This section describes:

- Overview
- Adjusting the laser intensity
- Verifying threshold setting

H.2.2.1 Overview

Definition

Laser threshold is the minimum laser intensity required to produce a steady detectable signal. Laser intensities above threshold generate a dense plume of desorbed neutrals which cause energy loss during acceleration. The observed impact of higher laser intensity is a decrease in resolution and mass accuracy, and a possible increase in fragmentation.

Factors affecting threshold

Laser threshold is affected by sample preparation. The most significant factors affecting threshold are:

- **Matrix**—Absorption coefficient of matrix affects the laser intensity needed to ionize sample.
- **Molecular weight of sample**—Higher masses require a higher laser intensity to generate an acceptable signal.
- **Sample characteristics**—Different classes of compounds in the same matrix may require different laser intensities. For example, carbohydrates in DHB require a higher laser intensity than peptides.
- **Sample contaminants**—Buffers, salts, and detergents in sample can substantially increase the laser intensity needed.
- **Sample exposure**—Fresh sample surfaces ionize better than surfaces that have been exposed to irradiation. The significance of sample exposure varies widely between matrices and changes across the sample surface.

Determining threshold

Determining laser threshold becomes easier with experience. Sample is consumed when exposed to the laser, so minimize the number of spectra you acquire to determine threshold.

H.2.2.2 Adjusting the Laser Intensity

This section describes:

- Adjusting the laser
- Fine-tuning threshold

Adjusting the laser

NOTE: *If settings are not listed for the matrix, set the laser to the lowest "high setting" listed for another matrix and start at step 2.*

1. Set the laser to the high laser setting for your system.
2. Start an acquisition and acquire about 10 spectra. At this high laser setting, you may see a saturated spectrum where peaks are full scale and may be truncated (Figure H-5).

NOTE: *A saturated spectrum has flat-topped peaks. If the spectrum goes off the screen, adjust the position of the spectrum to see if the top is flat.*

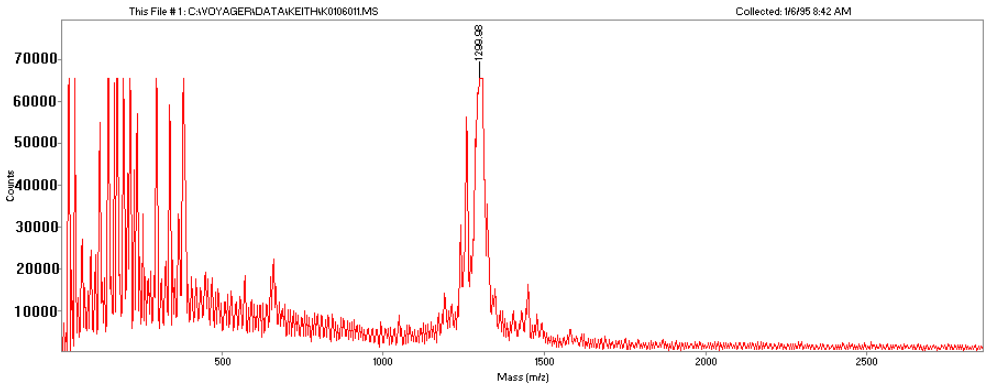


Figure H-5 Saturated Spectrum

3. Check the spectrum for the peak of interest. Zoom in on the spectrum as needed. See Section 4.3, Using the Spectrum Window.

4. Zoom in on the appropriate mass range to check for the peak of interest.

If peak of interest is present

5. If the peak of interest is present, the current laser intensity is the high setting for the sample class/matrix you are analyzing.

   Decrease the laser intensity by 10 percent and acquire another 10 spectra. Continue decreasing the laser intensity by 10 percent until the signal disappears.

   When signal disappears, the current laser intensity is the low setting for the sample class/matrix you are analyzing.

If peak of interest is not present

6. If the peak of interest is not present, the current laser intensity is the low setting for the sample class/matrix you are analyzing.

   Increase the laser intensity by 10 percent and acquire another 10 spectra. Continue increasing the laser intensity by 10 percent until the signal appears.

   When signal appears, the current laser intensity is the high setting for the sample class/matrix you are analyzing.

Fine-tuning threshold

When you determine the high and low setting for the sample class/matrix you are analyzing, you can fine-tune the threshold by setting the laser intensity midway between the high setting and low setting determined above.

If signal is present when you decrease the laser, assume that this is the new high setting. If signal is not present when you decrease the laser, assume that this is the new low setting.

Continue adjusting until the difference between high and low settings is less than 10 laser counts.

NOTE: *If the difference between the high and low setting is equal to the laser step size, decrease the laser step size in the Hardware Configuration by a factor of 2.*

Figure H-6 shows a spectrum generated with an acceptable laser intensity.

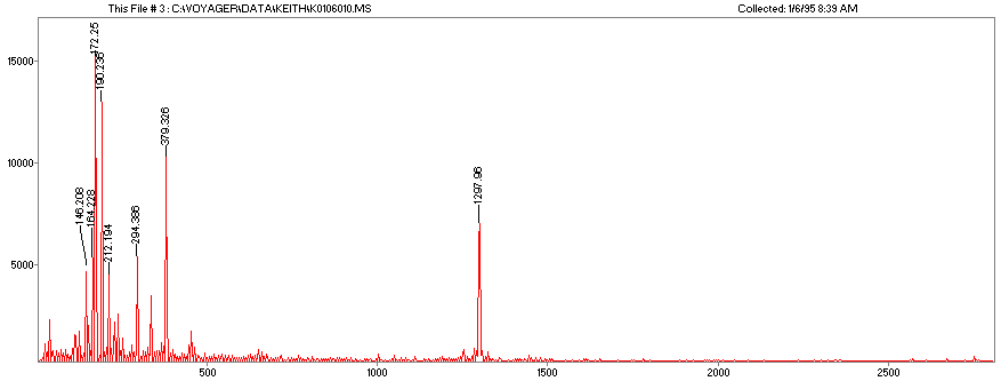


Figure H-6 Spectrum at Lower Laser Setting

H.2.2.3 Verifying Threshold Setting

Move to a new area of the sample well that contains the same sample. Acquire a spectrum to verify that the setting is valid for the laser power selected, and not caused by:

- Sample surface excitement caused by the previous higher laser power setting
- Sample consumption

If the setting is valid, you see a spectrum similar to the one just acquired. You may need to adjust the laser slightly (5 to 10 counts). Examine several spots in the sample well to make sure you have not tuned the laser intensity on a “cold spot”. You can further fine-tune the laser setting by setting a smaller laser step size in Hardware Configuration. For example, instead of a step size of 10, change the step size to 2.

Make sure peak shape and resolution are acceptable. See Section H.2.3, Checking Resolution. If you do not see a signal at the current setting, increase the laser power.

NOTE: *If peak intensity of the final scan is near maximum intensity, it is very possible that some scans averaged into the final scan are saturated. Reacquire using a lower laser power.*

H.2.3 Checking Resolution

After you determine laser threshold, calculate the resolution. Determine if the resolution is acceptable for your application. See Section 6.5.2, Calculating Mass Resolution.

Table H-6 lists a general rating scale for resolution and molecular weight ranges for compounds acquired in Linear mode.

Table H-6 Continuous Extraction Mode Resolution Rating Scale

Compounds <10,000 Da*		Compounds 10,000 to 20,000 Da		Compounds >20,000 Da	
Resolution	**Rating**	**Resolution**	**Rating**	**Resolution**	**Rating**
250	Acceptable	200	Acceptable	60	Acceptable
350	Fair	250	Fair	100	Good
500	Good	400	Good	——	——
600	Very good	600	Very good	——	——

* You may see better resolution than indicated when analyzing compounds below 1,000 Da due to isotope separation.

H.2.4 Fine-Tuning the Laser Setting

When you find the laser threshold, whether you need to fine-tune the setting depends on your needs:

- **If you are looking for an estimate of molecular weight**, a laser setting slightly higher than the laser threshold setting may be sufficient.
- **If you need good peak shapes**, you may need to increase the laser setting to improve signal-to-noise ratio.
- **If you need maximum resolution to differentiate between compounds**, you may need to operate close to the laser threshold setting to improve resolution.

H.3 Troubleshooting in Continuous Extraction Mode

This section includes:

- Laser threshold troubleshooting
- Spectrum troubleshooting

Refer to Table H-7 if you are having trouble determining laser threshold:

Table H-7 Laser Threshold Troubleshooting

Symptom	Action
Signal fades very quickly	Increase the laser intensity by 1 to 2 percent while acquisition is occurring. If signal does not increase, move to a new point in the sample well while acquisition is occurring. If signal does not increase, increase the laser setting further, 2 to 3 percent at a time.
Signal increases quickly then saturates	Stop acquisition. Decrease the laser setting by one step (use the slider controls on the Manual Laser page). Acquire again.
Hysteretic signal behavior (lag in response). For example, you observe signal at a laser intensity of 300, signal disappears when you decrease to 290, signal does not reappear when you increase intensity back to 300.	Move to new spot in sample well if this problem prevents you from generating a signal that is at least 10 percent of full scale. If the signal is below 10 percent of full scale, sample may be consumed.

Table H-7 Laser Threshold Troubleshooting (Continued)

Symptom	Action
Signal is flat	Laser setting may be too low, increase. Sample may be consumed, move to a new position in sample well. Sample may not be present, try new position. Sample may not ionize well, use different matrix.
Cannot see ions in Reflector mode	Check that you can see ions in Linear mode: • If you can see ions in Linear mode, it indicates that voltages or laser power need adjusting. • If you cannot see ions in Linear mode, refer to Section 9.2, Troubleshooting.

Refer to Table H-8 if you are having spectrum trouble:

Table H-8 Spectrum Troubleshooting

Symptom	Possible Cause	Action
Very wide peaks	Refer to “Poor resolution in Continuous Extraction mode” symptom on page H-21	Refer to “Poor resolution in Continuous Extraction mode” action on page H-21.

Table H-8 Spectrum Troubleshooting (Continued)

Symptom	Possible Cause	Action
Poor resolution in Continuous Extraction mode	Laser intensity too high	Adjust laser by using the slider controls on the Manual Laser Control page.
	Accelerating Voltage incorrect	Adjust.
	Guide Wire Voltage% too high	Adjust. See Section 5.3.4, Understanding Guide Wire Voltage%.
	Current calibration not correct for sample (off by a factor of 2 or 3)	Use correct calibration for sample.
	Sample concentration too high	Prepare sample/matrix with a final sample concentration appropriate for sample and matrix. See “Matrix Information” on page 3-6.
	Excess matrix in sample preparation	Increase sample:matrix ratio.
	Too much salt or buffer in sample	Clean up sample. See Section 3.1.5, Sample Cleanup.
	On Voyager-DE PRO and Voyager-DE STR systems, not separating isotopic peaks in reflector mode up to mass 1,000	Adjust Accelerating Voltage to 10,000 V and reacquire spectrum. See Section 5.4.4.2, Setting Accelerating Voltage.

(continued on next page)

Table H-8 Spectrum Troubleshooting (Continued)

Symptom	Possible Cause	Action
Poor resolution in Continuous Extraction mode *(continued)*	Beam guide wire malfunction	Call Applied Biosystems Technical Support.
	Accelerating Voltage malfunction	Call Applied Biosystems Technical Support.
	Microchannel plate detector voltage set incorrectly	Call Applied Biosystems Technical Support.
	Variable-voltage grid contaminated with matrix	Call Applied Biosystems Technical Support.
Poor mass accuracy in Continuous Extraction mode	Mass scale not accurately calibrated	Recalibrate. See the *Data Explorer Software User's Guide*, Chapter 5.3.2, Manually Calibrating.
	Used different voltages when acquiring sample and calibration standard	Use same voltages when acquiring sample and calibration standards.
	In Linear mode, used different laser intensity when acquiring sample and calibration standard	Use same laser intensity when acquiring sample and calibration standards.
	Incorrect masses entered in calibration	Recalibrate. See the *Data Explorer Software User's Guide*, Chapter 5.3, Manual Calibration.

(continued on next page)

Table H-8 Spectrum Troubleshooting (Continued)

Symptom	Possible Cause	Action
Poor mass accuracy in Continuous Extraction mode *(continued)* *(continued on next page)*	Incorrect peaks entered in calibration	Recalibrate. See the *Data Explorer Software User's Guide*, Chapter 5.3, Manual Calibration.
	When calculating peaks, the Centroid% value displayed in the Settings dialog box changed	Use the same Centroid% value for peak calculation and calibration (calibration value is displayed when you open the Settings dialog box). See the *Data Explorer Software User's Guide*, Chapter 5.5, Centroiding, for additional information.

Table H-8 Spectrum Troubleshooting (Continued)

Symptom	Possible Cause	Action
Poor mass accuracy in Continuous Extraction mode *(continued)*	Crystals did not form homogeneously on sample spot	Prepare new sample spot. See “Guidelines for good crystallization” on page 3-37.
		Use mass closest to the mean (for external calibration only): 1. Acquire six averaged scans (six .DAT files) from one sample well. 2. Check masses in Voyager processing software. 3. Use the file with the mass value closest to the mean.
	High voltage power supplies not warmed up	Start high voltages by clicking **[add icon]** 30 minutes before calibration.
	Samples and standards not in adjacent wells	For optimum mass accuracy, place standards in wells adjacent to the samples for which you are calibrating.
	Samples or standards spotted in outer wells on sample plate	For optimum mass accuracy, do not use outer wells of sample plate.

I Using the Oscilloscope and Control Stick

This appendix contains the following sections:

I.1 Guidelines for Acquiring I-3
I.2 Scaling I-4
I.3 Using the Control Stick I-7

NOTE: *If your system includes an internal digitizer board or an external digitizer instead of an external oscilloscope, refer to Section 4.3, Using the Spectrum Window.*

I

Oscilloscope overview

An external oscilloscope (instead of the internal digitizer board or the LeCroy digitizer) is available as an option on Voyager systems.

The oscilloscope converts the signal from the mass spectrometer to a signal that the computer can use. The oscilloscope has its own screen to display the averaged ion signal in real-time. For instrument configurations containing an oscilloscope, the software does not refresh the spectrum view until averaging is finished. Unlike the “real-time” update in the spectrum view, the oscilloscope screen displays the detector signal on a time scale and the signal is inverted on the screen. Following acquisition, the signal is inverted and calibrated onto the mass scale in the spectrum view.

Figure I-1 shows the front panel of the oscilloscope. Only the knobs you use to adjust the signal while acquiring spectra are labeled. Other knobs are not used for general operation.

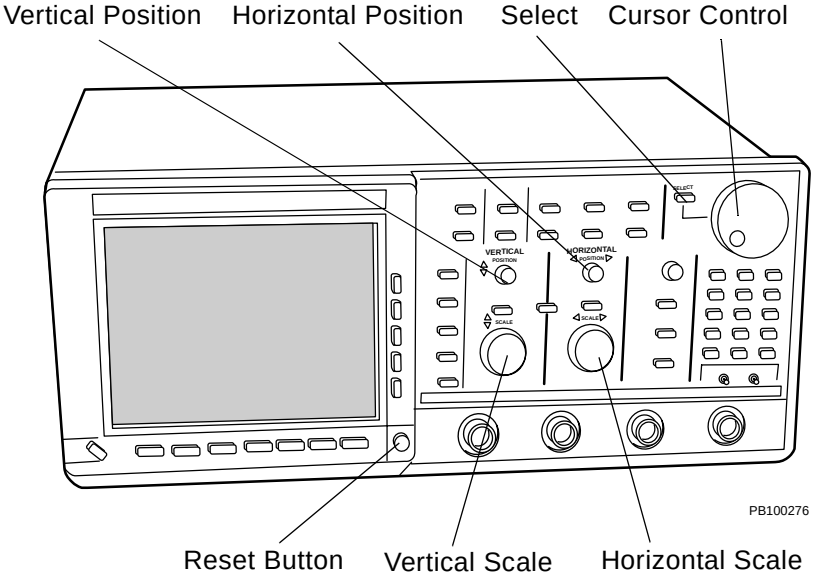


Figure I-1 Front Panel of Oscilloscope

I.1 Guidelines for Acquiring

Consider the following as you acquire a spectrum and use the oscilloscope:

- When you start acquiring, you should see a signal that contains matrix peaks and sample peaks.
- Make sure the full range of the signal is displayed. Brackets must overlap the end range markers (see Figure I-3). If brackets do not overlap, turn the Horizontal Scale and Horizontal Position knobs until the left bracket overlays the left marker.
- Start with a high laser power to saturate the initial signal. This is a good starting point from which you can determine the laser threshold or the appropriate laser setting for your application.

  You may need to move the signal up using the Vertical Position or the Vertical Scale knobs so that you can see the tops of the peaks.
- If peak intensity of the final scan is near maximum intensity (Input range 50, 200, or 1000 mV), it is very possible that some scans averaged in to the final scan are saturated. Reacquire using a lower laser power.

I

I.2 Scaling

Initial scaling

Initial scaling of the oscilloscope is determined by the settings in the Mode/Digitizer dialog box. See Section 5.2.2, Mode/Digitizer Dialog Box for more information.

You can adjust the initial scaling after acquisition starts by using the knobs on the front panel of the oscilloscope (Figure I-2).

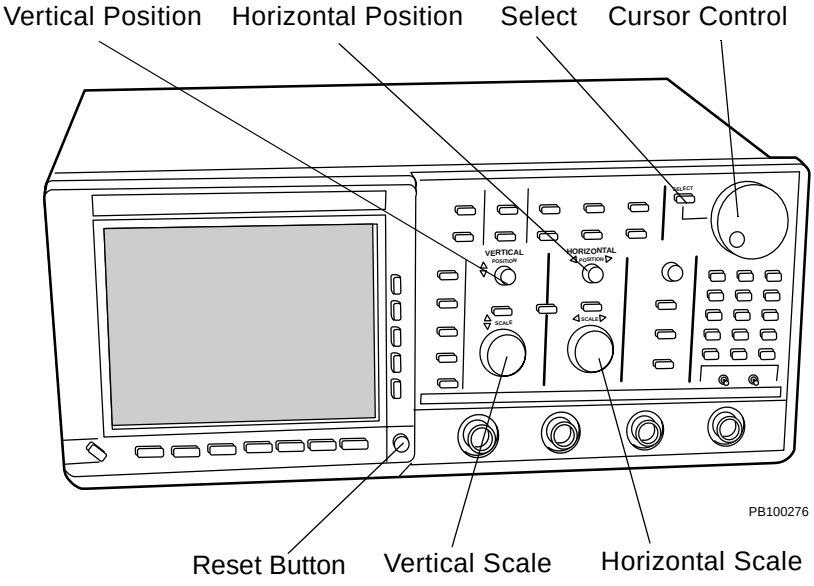


Figure I-2 Front Panel of Oscilloscope

Use these knobs to adjust the signal on the oscilloscope:

- **Vertical position**—Moves the signal up and down
- **Vertical scale**—Adjusts the amplitude of the signal
- **Horizontal scale**—Adjusts the width of the signal
- **Horizontal position**—Moves the signal left and right
- **Select**—Activates the right or left cursor
- **Cursor Control**—Moves the active cursor
- **Reset button**—Resets the oscilloscope screen

For information on using other controls on the oscilloscope, refer to the manual shipped with the oscilloscope.

The spectrum you see on the oscilloscope is inverted (Figure I-3).

NOTE: *The oscilloscope displays an intensity versus time spectrum.*

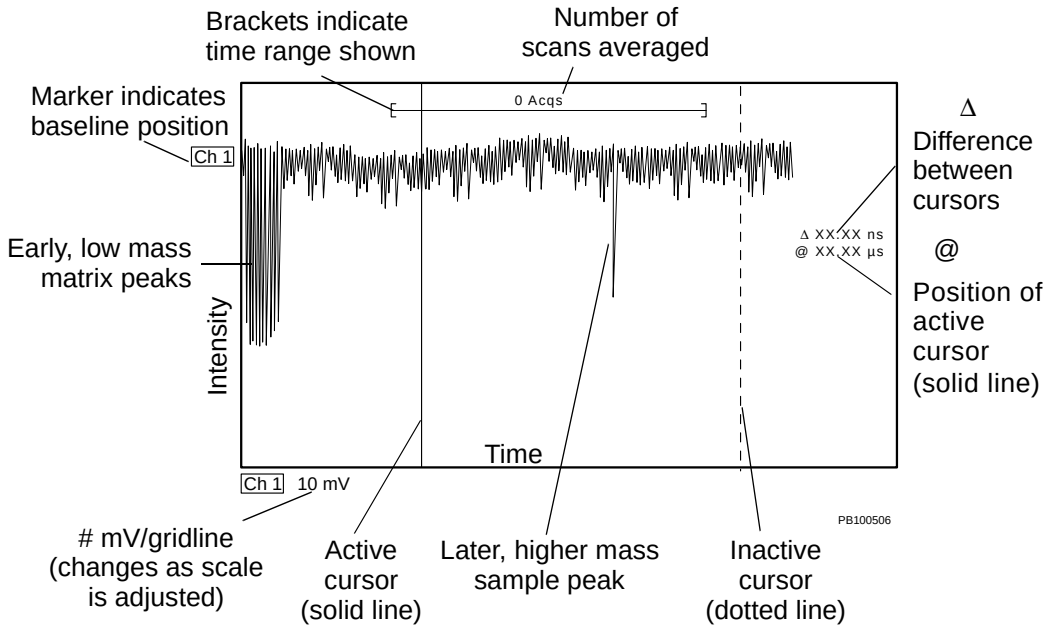


Figure I-3 Example of Signal on Oscilloscope

The Channel indicator (Ch1 in Figure I-3) displays Ch1 in Linear mode. On Voyager-DE PRO and Voyager-DE STR systems, the Channel indicator displays Ch2 in Reflector mode.

The top of the screen also displays:

- **Average**—During acquisition
- **Stop**—When acquisition is complete

CAUTION

The oscilloscope does not save spectra. If you acquire a new spectrum before downloading to the Voyager processing software, you lose the previous spectrum.

Adjusting the display

To adjust the display:

- Center the signal vertically on the screen using the Vertical Position knob.
- Adjust the amplitude of the signal using the Vertical Scale knob. Make sure the peak of interest is on scale.
- Widen peaks using the Horizontal Scale knob.

I.3 Using the Control Stick

Starting acquisition

After you load samples on the sample plate, and load the plate into the system, start acquiring.

To start acquiring, press the **left button** on the base of the control stick (Figure I-4).

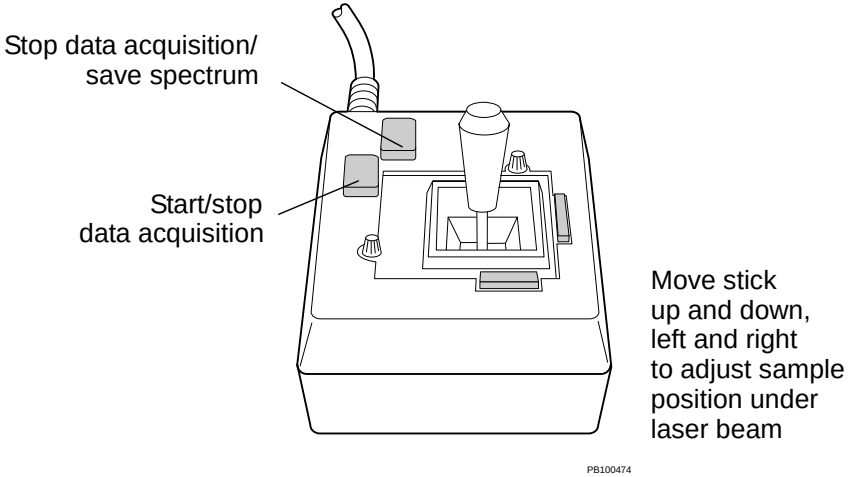


Figure I-4 Control Stick

NOTE: *You can also start acquisition by clicking*  *in the toolbar. Acquisition continues until the number of Shots/Spectrum in the instrument setting is acquired, or until you press the start/stop (left) button on the control stick or the toolbar button again.*

To stop acquiring, press the **left button** on the base of the control stick (Figure I-4).

CAUTION

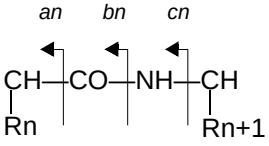
Check to see if acquisition has already stopped automatically before pressing the control stick button. If acquisition has stopped, the Instrument Control Panel status bar is blank (it displays an "Acquiring Data" message during acquisition).

If acquisition has stopped and you press a control stick button, you will begin a new acquisition and overwrite the current spectrum.

NOTE: *To move the sample under the laser beam, deflect the control stick in the appropriate direction. Longer deflection will result in faster movement of the sample plate.*

Glossary

a, b, and c ions —Generic description of potential ions that are formed by fragmentation of a parent peptide/protein. a, b, and c ions are fragments that retain the charge at the amino end (n-terminus) of the molecule. See also x, y, z ions.



ASCII —American Standard Code for Information Interchange. A file format that contains codes that constitute the 128-character ASCII set, and allows exchange of data between information processing systems, communication systems, and associated equipment.

Accelerating voltage — Potential difference between the ion source and ground, used to accelerate ions. Actual acceleration in a TOF instrument usually occurs in the first 1 to 2 cm of the flight tube. Ions then drift the remaining flight tube distance.

Arcing —Electrical discharge between two points. Usually occurs when a high potential difference exists between two points.

BA1 and BA2 (Bayard-Alpert vacuum gauges) — Pressure gauges that operate below 1 millitorr by measuring a positive ion current produced by electrons from a hot filament. Voyager-DE systems include BA1 only.

Beam Guide Wire —See Guide Wire Voltage.

Centroid% —Percentage of the peak height examined during peak identification. For example, with a Centroid% of 10, the software examines the top 10 percent of the signal.

CID —Collision-induced dissociation— A technology that enhances fragmentation for PSD analysis. As ions leave the source, they pass through a collision cell, interact with collision gas molecules, and energy is transferred to the ions. This transfer of energy enhances fragmentation.

Continuous Extraction — Formation of ions in a strong electrical field, with immediate acceleration. Used for diagnostic purposes.

Daughter ion —See Product ion.

Delayed Extraction — Formation of ions in a weak electrical field, with subsequent acceleration by application of a high voltage pulse after a predetermined time delay.

GLOSSARY

Delay Time —Used in Delayed Extraction mode. Time in nanoseconds (after the laser ionizes the sample) at which full Accelerating Voltage is applied, creating the potential gradient that accelerates ions.

Digitizer —A device that converts an analog signal to a digital word and stores the result in memory. This allows the transfer of the digitized signal to a computer for additional processing.

Dimer —A species that results from a association between two identical molecules. Expressed as $[2M+H]^+$. Appears in a mass spectrum at 2 times the protonated molecular ion mass, minus one mass unit.

Edman degradation technique —An approach to amino-end-group determination involving the use of a reagent, phenylisothiocyanate, that can be applied to the liberation of a derivative of the amino-terminal residue without hydrolysis of the remainder of the peptide chain.

Flight tube —Vacuum chamber in which ions drift from the source to the detector.

Fore pump —Rotary pump that maintains vacuum in the sample loading chamber, creates the low-vacuum condition needed by the turbo pump, and provides backing pressure to the turbo pump.

Fragment —Ion dissociated from precursor ion.

FWHM —Full width at half maximum.

GPMAW —General Protein/Mass Analysis for Windows software, a software program used to identify protein sequences.

Grid voltage —Secondary voltage used to fine-tune ion acceleration, on a variable-voltage grid above the sample plate.

Guide Wire voltage —Voltage applied to beam guide wire to focus ions on detector. Particularly useful for enhancing analysis of high mass ions.

Immonium ion —In PSD, low molecular weight fragment ions derived from amino acids.

Initial velocity —The velocity associated with an ion when it is released from the sample plate, before acceleration. Voyager software allows correction for matrix-dependent initial velocities.

Intensity (signal or ion) —Amplitude of signal shown on oscilloscope screen or spectrum window.

Intensity (laser) —Amount of laser-generated light reaching the sample spot. Controlled by adjusting the laser attenuator using laser step setting.

Ion —An isolated electron or proton or an atom or molecule which, by loss or gain of one or more electrons, has acquired a net electric charge.

Ionization —Conversion of sample in solid, gaseous, or liquid phase to ions.

Ion intensity —See Intensity.

Ion source —Device that produces ions. In a TOF instrument, it refers to the surface of the sample plate, the variable-voltage grid above the plate, and the grounded grid and aperture above the variable-voltage grid.

Isomers —Compounds that have the same molecular weight but different structures.

Isotopes —One of two or more atoms with the same atomic number but a different mass. For example, carbon isotopes ^{12}C, ^{13}C, and ^{14}C.

Laser —Energy source for sample ionization in MALDI technology. The Voyager Biospectrometry Workstation uses a Nitrogen laser at 337 nm that provides 3-nanosecond-wide pulses.

Laser attenuator —Device that controls laser intensity. Controlled by laser step setting.

Laser intensity —See Intensity.

Laser step —Unit of movement of laser attenuator. The size of the laser step is set in Laser Configuration. The number of laser steps is controlled by using the slider controls on the Manual Laser Control page.

Low Mass Gate —Mechanism for suppressing low mass ions. The voltage in the detector is not turned on until ions below the specified starting mass have passed.

Ladder sequencing —Peptide or DNA sequencing in which amino acid sequence is read from one of the ends of the peptide chain.

[M+H] $^{+}$—Molecular ion.

[M+2H] $^{2+}$—Doubly-charged ion. Appears in spectrum at approximately half the molecular weight of the ion.

[M+3H] $^{3+}$—Triply-charged ion. Appears in spectrum at approximately one third the molecular weight of the ion.

MALDI —Matrix-assisted laser desorption ionization. Described in Section 1.3, MALDI-TOF MS Technology Overview.

Mass accuracy —The error between the observed mass and the calculated mass from atomic composition. Can be expressed as mass units or the ratio of mass assignment error divided by ion mass in percent terms.

Mass resolution —Describes the separation between adjacent mass peaks. Expressed as the mass (*m)* of the ion signal, divided by the width of the peak at half-height (Δ *m*).

GLOSSARY

Matrix —Low-mass, UV-absorbing substance used in MALDI technology to enable sample ionization. Common matrices include sinapinic acid, dihydroxy benzoic acid, and α-cyano 4-hydroxy cinnamic acid. See Appendix C, Matrixes, for more information.

Metastable ion analysis —See MS/MS analysis.

Mirror —A single plate at high voltage. Commonly used to refer to the reflector in a TOF instrument.

Mirror voltage ratio —Ratio of the mirror high voltage supply to the acceleration high voltage supply. Mirror Voltage = ratio x accelerating voltage.

MS/MS analysis —Selection of a precursor ion for fragmentation, and the collection of the fragment ions. MS/MS analysis can yield sequencing information for a peptide.

By acquiring data using different mirror voltages, you can focus on different segments of the fragment population. After acquisition, you can piece together the segments to examine a composite PSD spectrum.

Multiply charged ions —See $[M+2H]^{2+}$ and $[M+3H]^{3+}$.

m/z —Mass-to-charge ratio.

Parent ion —See Precursor ion.

Post-Source Decay (PSD) —Fragmentation of an ion during flight, after it leaves the ion source region.

Post-translational modification —Non-amino acid modifications made to a peptide or protein by chemical or enzymatic means. Examples include deamidation, acetylation, oxidation, glycosylation, phosphorylation, and sulfation.

Pulsed Source —Ion source in a Delayed Extraction system.

Precursor ion —Used in PSD Analysis software. Original ion from which fragments dissociate.

Precursor Ion Selector —See Timed Ion Selector.

Product ion —Used in PSD Analysis software. Fragment ion that dissociates from precursor ion.

Sensitivity —Measure of the amount of sample required to generate an acceptable mass spectrum. Usually expressed in terms of strength of signal relative to noise.

Signal intensity —See Intensity.

Source —See Ion source.

Stitch —Segment of a PSD composite spectrum.

Tandem mass spectrometry —See MS/MS Analysis.

TC2 (Thermocouple vacuum gauge) —Pressure gauge that operates from 1 millitorr to near atmospheric pressure by measuring the temperature of a heated thermocouple junction. As the pressure rises, more heat is removed, lowering the temperature.

Timed Ion Selector — Mechanism for suppressing all but the ion of interest, used in PSD analysis. Voltage is applied to ion selector plates in the flight tube before and after the ion of interest passes the selector plates.

Time-of-flight (TOF) Mass Analyzer —A mass analyzer that measures mass by measuring drift times. If a packet of ions leaves the ion source at the same time and with the same energy, then their flight time through a field-free drift region will depend on their mass. Time-of-flight mass analyzers have the advantage that they have no upper mass limit and are well suited to the analysis of large molecules.

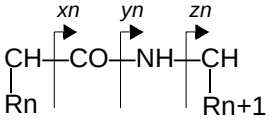
Torr —A unit of pressure equal to one millimeter of mercury.

Turbo pump —A vacuum pump that operates in the pressure range where the mean free path is large compared to the chamber dimensions. Molecules that diffuse into the throat are knocked to the bottom by a fan operating at supersonic speeds. The collected gas is further compressed to atmospheric pressure by a mechanical fore pump. Turbo pumps operate well up to about 10^{-10} Torr.

Velocity focusing —In Delayed Extraction, the tuning of the Grid Voltage% and Delay Time to account for the different initial velocities of ions as they are released from the sample plate. Velocity focusing provides improved resolution. See “Velocity focusing” on page 1-15.

V —Volt.

x, y, and z ions —Generic description of potential ions that are formed by fragmentation of a parent peptide/protein. x, y, and z ions are fragments that retain the charge at the carboxy end (c-terminus) of the molecule. See also a, b, c ions.



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