Voyager-DE ™ STR Biospectrometry™ Workstation

Manufactured by PerSeptive Biosystems

General Description

The Voyager-DE ™ STR Biospectrometry™ Workstation includes upgraded electronics, a 3-meter flight path, and improved ion optics. It delivers high sensitivity and resolution, as well as, unparalleled mass accuracy, giving you the tools you need to characterize complex mixtures and determine molecular weight on sub-picomole samples. The Voyager-DE STR Biospectrometry Workstation continues to lead the industry as the ultimate research machine for biomolecule characterization.

Application Areas

- Peptide Confirmation
- Oligonucleotide Analysis
- Peptide Sequencing by MS/MS
- Protein Analysis
- C-terminal sequencing
- Oligonucleotide sequencing
- Post transnational Modifications
  Glycolysation, Phosphorylation, Sulfation
- Carbohydrate Analysis
- Small Molecules

Sample Preparation

MALDI is the mechanism used to ionize the sample. The sample is embedded in a low molecular weight, UV – absorbing matrix that enhances sample ionization. When a laser beam hits the matrix, it appears to transfer enough energy to the sample to form molecular ions. The matrix is present in vast excess of sample and therefore isolates individual sample molecules. It is important that contaminants such as buffers, salts and

Selecting a matrix

- The matrix must be capable of forming a fine crystalline solid during codeposition with the analyte.
- The matrix molecules must have a high absorptivity for the laser radiation

The following table lists the concentrations of some matrices that can be used in certain applications.

Matrix	Application	Matrix Solution
2,5-dihydroxybenzoic acid (2,5-DHB) MW 154.03 Da	Peptides, neutral or basic carbohydrates, glycolipids, polar and nonpolar synthetic polymers, small molecules	10 mg/ml in a solvent in which sample and matrix are soluble
Sinapinic Acid MW 224.07 Da	Peptides and Proteins > 10,000 Da	10 mg/ml in 70:30 water/acetonitrile (0.1% TFA) 10 mg/ml in 50:50 water/acetonitrile (0.1% TFA) if sample contaminated
α-cyano-4-hydroxy cinnamic acid (αCHCA) MW 189.04 Da	Peptides, proteins and PNAs < 10,000 Da	10 mg/ml in 50:50 water/acetonitrile (0.1% TFA final conc)
3-hydroxy-picolinic acid (3-HPA) MW 139.03 Da	Large oligonucleotides > 3,500 Da	50 mg/ml in 50:50 water/acetonitrile, plus 50 mg/ml diammonium citrate in water
2,4,6-Trihydroxy acetophenone (THAP) MW 168.04 Da	Small oligonucleotides < 3,500 Acidic carbohydrates, acidic glycopeptides	10 mg/ml in 50:50 water/acetonitrile, plus 50 mg/ml diammonium citrate in water
Dithranol MW 226.06 Da	Nonpolar synthetic polymers	10 mg/ml in tetrahydrofuran
Trans-3-indoleacrylic acid (IAA) MW 123.03 Da	Nonpolar polymers	10^{-1} M in solvent appropriate for sample
2-(4-hydroxyphenylazo)-benzoic acid (HABA) MW 242.07 Da	Proteins Polar and nonpolar synthetic polymers	~1.3 mg/ml in 50:50 water/acetonitrile or in 40:40:20 water/acetonitrile/methanol
2-aminobenzoic (anthranilic) acid MW 137.05 Da	Oligonucleotides (negative ions)	~10 mg/ml in 80:20 water/acetonitrile (20% w/w nicotinic acid can be added)

Preparing Samples

For successful sample preparation, prepare fresh matrix as needed. Some matrices require daily preparation, while other matrices can be stored for up to one week at 4^0C. It is important to determine the proper dilution of sample and to use clean sample plates. Properly apply sample and dry to allow for good crystallization. Good crystallization is obtained when you have small, equally sized crystals that are evenly distributed on the plate. Clumping is not desirable because this will make it difficult to analyze the sample.

The following table provides concentrations of samples that contain certain compounds.

Compound	Concentration
Peptides and proteins*	0.1 to 10 pmol/µl
Oligonucleotides	10 to 100 pmol/µl
Polymers	10^{-4} M

*Some proteins, particularly glycoproteins, yield better results at concentrations up to 10 pmol/µl

Sample Introduction

There are two types of sample plates available:

Flat sample plates where the liquid is held in place by surface tension of the sample/matrix mixture. The sample plate allows you to observe crystallization pattern and the actual sample spot is visible.

Welled sample plates have the liquid held in place by an indentation in the plate. These sample plates are used in running an autosampler because the sample position is well defined.

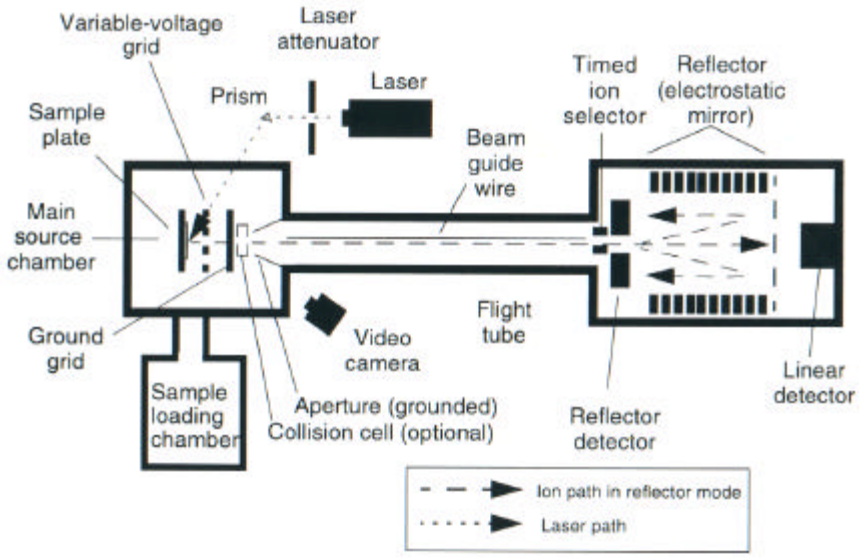
Once the sample/matrix have crystallized on the sample plate, the plate can be loaded into the mass spectrometer.

- Eject the sample holder
- 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.

- Slide the sample plate into the holder from the front until it snaps into place. The ball bearings on the holder snap into the plate.
- Click Load to retract the sample plate and insert it into the main source chamber. It takes a minute or two for the sample plate to reach the correct position.

On the video monitor you will observe a real-time sample image (30 times magnification). Use the control stick to move over the sample plate until you find your sample. To start acquiring data press the left button on the control stick. Find a "hot spot" (areas of high ion intensity) by moving around the sample spot. Once you find the "hot spot", stop acquiring data and restart. You will not want to move around when acquiring actual data.

Mass Spectrometer



Ion Sources

To ionize the sample a nitrogen laser that operates at 337 nm is used. It produces a 3-nanosecond-wide pulse at up to 20 pulses per second. In delayed extraction mode, acquisition does not start until the extraction pulse is applied to the sample. (Delayed extraction is a technique where a time delay is used between the end of the ionization pulse and the start of the extraction pulse. This technique allows for all ions of the same mass to acquire the same kinetic energy. Ions of the same mass will hit the detector at the same time giving rise to sharper peaks.) The laser attenuator varies the intensity of the laser beam reaching the sample and the prism deflects the laser beam into the ion source.

The ion source is a high voltage region used to accelerate ions down the time of flight tube. The sample plate is supplied with 0 to 25,000 V to accelerate the ions into the flight tube. The variable-voltage grid supplies additional voltage to fine-tune ion acceleration. The ground grid is the ground surface for formation of the potential gradient. The grounded aperture is the entrance to the flight tube.

Mass Analyzer

A time-of-flight (TOF) mass analyzer is used to separate the ions formed from MALDI. The light ions formed during each pulse reach the detector first. This is true because all the ions are given the same kinetic energy ($\frac{1}{2}mv^2$) from the ion acceleration region. The lightest ions, therefore, have the highest velocity while the heavier ions have a slower velocity.

Detector

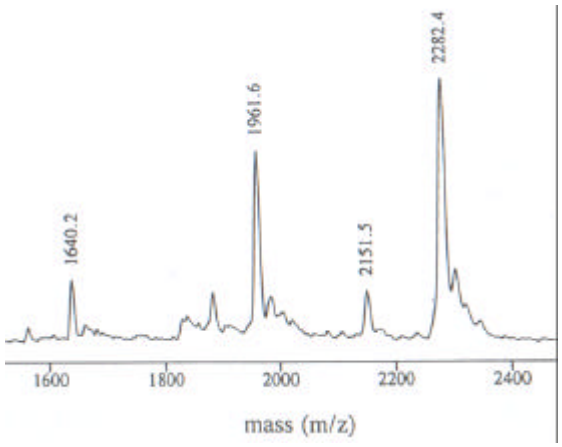
There are two detectors present in the Voyager-DE ™ STR Biospectrometry™ Workstation. A linear detector measures ion abundance over time and sends a signal to the digitizer for conversion. A reflector detector measures ions reflected by a mirror and then sends a signal to the digitizer for conversion. The reflector filters out neutral molecules, corrects for time dispersion due to initial kinetic energy variations and provides greater mass accuracy and resolution.

Sensitivity Specifications

A MALDI/TOF mass spectrometer analyzes for protein molecular weights. To determine the protein molecular weight there is a tradeoff between resolution and sensitivity, for higher sensitivity there is lower resolution.

For increased sensitivity analysis of smaller amounts of sample can yield a better signal. Serial dilutions of the sample before mixing with the matrix could be done to obtain a good sample to matrix ratio, as well as, decreasing any contamination present in the sample. An optimum concentration ratio between sample and matrix is important for larger molecules (>10 kDa).

Example Mass Spectrum



MALDI mass spectrum of 15 pmol of reduced somatostatin after reaction with a five-fold molar mass excess of pHMB over total cysteine. (From Watson. Introduction to Mass Spectrometry, 3rd Edition. Lippincott-Raven Publishers, 1997.)

Interviews

Here are four questions asked to and answered by a biochemistry graduate student and a lab technician who use the instrument.

Question: What do you use the MALDI/TOF instrument for?
Student: Determination of nucleic acids by their molecular weights
Lab Tech: Protein identification by determining peptide masses

Question: Is it easy to use?
Student: Yes, very easy to use.
Lab Tech: Very user friendly.

Question: What is good about the instrument:

Student: Has a large mass window.
Lab Tech: Easy sample prep and the data are easily analyzed.

Question: What is bad about the instrument?
Student: Can't change a lot of the variables. Also you need to make sure the sample is very well desalted or else the peaks become very large.
Lab Tech: Outdated oscilloscope and there are software bugs.

References

PerSeptive Biosystems. Voyager™ Biospectrometry™ Workstation with Delayed Extraction™ Technology User's Guide. PerSeptive Biosystems, Inc., 1996.

Watson. Introduction to Mass Spectrometry, 3rd Edition. Lippincott-Raven Publishers, 1997.

Interviews: Date performed 10/28/02
Student: Christina Ymata
Lab Tech: Lauren Wolfe