



BioTOF *Series*

Getting Started



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Preface

This document is an introduction to the BioTOF system. It includes:

- An overview of BioTOF operation
- A brief description of the system indicators and controls
- Instructions on acquiring spectra from a known sample

The purpose of this book is to introduce the system and familiarize you with key concepts. More detailed information on system functions and test sequences is provided in:

- *BioTOF Operator Manual, Volume 1, Theory*
- *BioTOF Operator Manual, Volume 2, Operation*

Three additional manuals in this document set provide information on using the analytical software included with the BioTOF system.

- *DataAnalysis User Manual*
- *QuantAnalysis User Manual*
- *Library Editor User Manual*

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Safety Labels

These labels are placed on the instrument to indicate the respective hazard possibility.



Hazard: Note

Refer to the Operator Manual for details on this hazard.



Warning: High Voltage

A dangerous high voltage or shock hazard exists within this area. Only trained service persons should perform work in this area.



Warning: High Temperature

Hot parts and surfaces are present in this area. Allow the part or surface to cool before performing work in this area. This may require turning off power to selected components or to the entire instrument.



Warning: Biohazard

A biohazard may exist within this instrument or specific area. Handle this biohazard with appropriate caution and protection.

1 BioTOF Operation

This chapter provides an overview of BioTOF system operation. For a more detailed description, refer to the *BioTOF Operator Manual, Volume I Theory*.

The BioTOF system (Figure 1-1) consists of:

- Time-of-flight mass spectrometer
- Electrospray ion (ESI) source
- Computer workstation that controls operation of the system, stores and displays spectra, and provides additional analytical software.

In the basic BioTOF configuration, the sample is introduced to the ESI source by means of a syringe pump (Figure 1-1). The sample can also be delivered to the BioTOF via a liquid chromatography (LC) system.

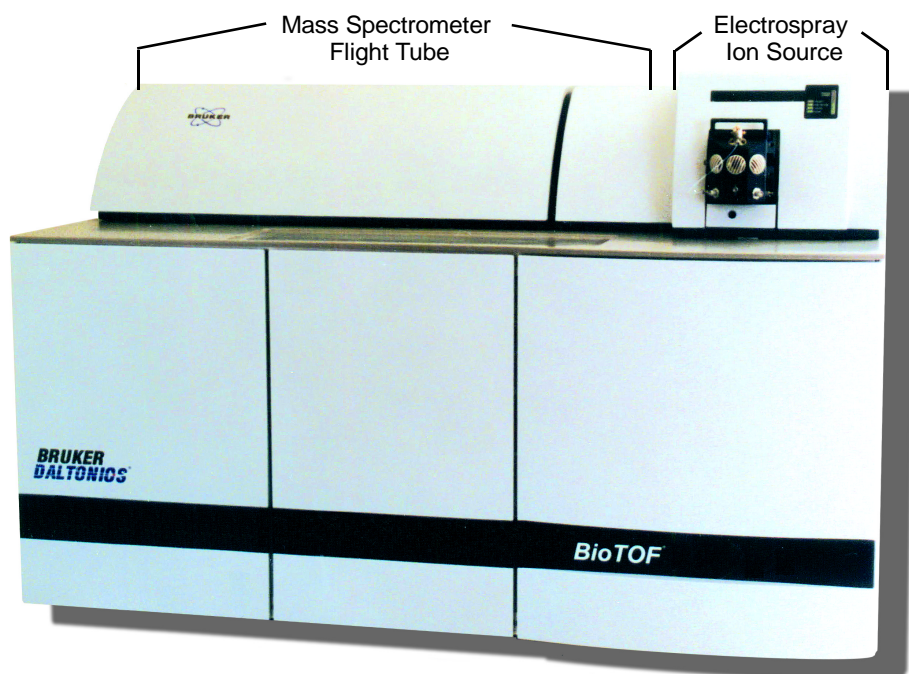


Figure 1-1 Basic BioTOF System Configuration

1.1 Overview

Figure 1-2 shows the relationship of the ESI source to the TOF spectrometer and the orthogonal interface between these two components. The interface contains an accelerator electrode structure that orthogonally deflects the ions (90°) into the TOF ion drift region, and also houses the detector that senses the reflected time-dispersed ions.

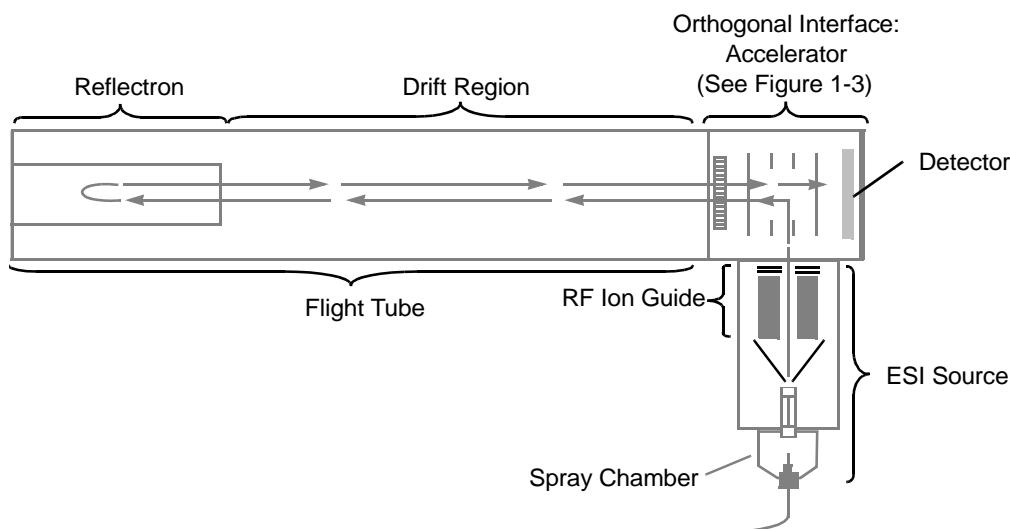


Figure 1-2 ESI and TOF Operation

1.2 ESI Source

In the ESI spray chamber, a fine spray of charged droplets is generated from the sample solution by means of a sprayer under the influence of an electric field only or an electric field with the assistance of a pneumatic nebulizer. When the solvent evaporates from the droplets, their charge per volume increases resulting in electrostatic (Coulomb) forces that break up the droplets into ever smaller particles until, in the final stage, de-solvated sample ions emerge.

The electric field inside the spray chamber is shaped by potentials applied to three electrodes: the cylinder, the end-plate and the metal coated entry of the glass capillary tube.

De-solvation is facilitated by a heated drying gas flowing counter to the stream of droplets and ions as they are electrostatically attracted towards the capillary tube that is the inlet orifice of the vacuum system.

Ions enter the vacuum system of the mass spectrometer through the dielectric glass capillary, stream through a skimmer, and then enter into the RF ion guide, which is biased at an appropriate offset voltage. Neutral gas molecules that enter with the sample ions are pumped away in differential pumping stages at successively lower pressures.

Ions accumulate in the RF ion guide by means of a trapping voltage applied to the guide's exit gate electrode. Then, the voltage on the gate electrode drops for a specified time and ions drift out of the RF ion guide. Thus, the ions form packets of a spatial length determined by the pulse width and velocity distribution. At the end of the gate pulse, the exit electrode potential is raised, starting another cycle of ion accumulation in the RF ion guide. Determination of the pulse width and repetition rate is explained in the next section.

1.3 Time-of-Flight Mass Spectrometer

The packet of ions emerging from the ion guide is collimated by a lens electrode and travels towards the accelerator with a velocity related to its kinetic energy acquired by acceleration due to the offset voltage of the ion guide. After the transfer delay, the ion packet reaches the extraction region of the accelerator. Here a high voltage pulse on the Repeller changes the trajectory by 90° and pushes the ions into the mass spectrometer flight tube. The pulse initiates a clock that measures the time-of-flight of the packet from the accelerator to the Reflectron and ultimately to the ion detector.

When the high voltage pulse is applied to the Repeller to accelerate the ions down the flight tube, the voltage also prevents other ions entering the accelerator. When the first packet of ions has safely entered the field-free drift region of the flight tube, the high voltage in the accelerator is turned off.

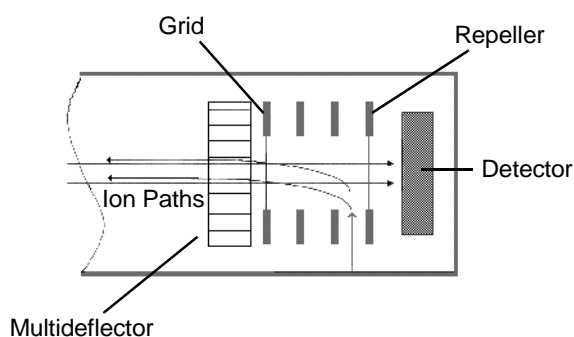


Figure 1-3 Accelerator

Since all ions are accelerated by the same Repeller potential in the accelerator, they reach individual velocities according to their m/z values. All ions of a given m/z ratio take approximately the same amount of time to travel through the flight tube. The smaller m/z ions travel faster than those with higher m/z values; the ion packet begins to separate.

The ions drift through the flight space, pass through the Reflectron lens that provides for ion beam collimation, and enter the Reflectron at the far end of the tube. A potential higher than the Repeller accelerator voltage is applied to the far end of the Reflectron; the ions gradually slow down and eventually reverse direction.

The reflected ions travel back through the flight tube. In order to prevent a second deflection in the multideflector, the main steering voltage is now turned off. The ions penetrate freely through the accelerator and through the Repeller electrode, finally striking the detector and generating an electrical signal. The arrival time and amplitude of this signal constitute the raw output data of the BioTOF, and both are recorded by the data acquisition system.

1.4 Data Acquisition

The ion flight time through the tube is proportional to the square root of the ion's m/z ratio. Thus, during the flight through the mass spectrometer, the ions separate according to their m/z ratio. Lighter ions arrive at the detector before the heavy ions with higher m/z values. Conversely, this ratio can be determined from measurement of the total flight time through the mass spectrometer from the Repeller to the Reflectron and then to the detector. Measurement of the flight time starts synchronously with the Repeller high voltage pulse. Recording of the detector signal starts after a digitizing delay. The digitizer delay determines the lowest m/z value in the recorded spectrum and shifts the recording window along the mass scale.

The electrical signal from the detector is converted from analog to digital for each **scan**. Multiple scans are aggregated to form a single higher density **record**. The records are summed in the digitizer to form a **spectrum** that is transferred to the workstation.

In MS Only mode, the spectra are further accumulated in a single buffer and the buffer contents are displayed in the BioTOF window. At the end of the data acquisition, the contents of the buffer can be saved as a single mass spectrum.

In Chromatography - MS mode, the transferred spectra are stored and displayed consecutively, and can be saved as time-stamped mass spectra.

You specify the width of the recording time window by using one of two methods:

- Selecting a mass range, in which case the BioTOF software automatically determines digitizer delay in keeping with the other timing parameters.
- Specifying the number scans per record and a sampling speed. The recording window is given by the time per data point multiplied by the number of points to be accumulated in each scan. In this case, you must set the appropriate digitizer delay to assure that the mass range includes ion masses of interest.

The methods used for determining the sampling rates for the various BioTOF Series models are described in *BioTOF Operator Manual, Volume I, Theory*.

2 System Controls

The principal means for controlling and monitoring the BioTOF system is a Windows based application configured on the workstation. The software can operate on both Windows NT and Windows XP platforms. By means of a single multi-pane window, you can:


- Start the BioTOF system
- Set the system parameters
- Acquire and display spectra
- Save the data for use with the data processing software included with the system

An array of hardware controls and indicators are located on four panels behind a hinged door on the front of the BioTOF cabinet. These controls include the On/Off switches for the mass spectrometer and ESI source components. Many of the controls and indicators are replicated in the BioTOF software and can be set from the BioTOF window. An additional panel on the upper right of the BioTOF cabinet displays the status of the ESI source.

2.1 BioTOF Window

This section gives a brief description of the main window for the BioTOF application. Refer to Chapter 5 in the *BioTOF Operator Manual, Volume II Operation* for a detailed description of the window features including the menus, tool bars and options.

To view the BioTOF window:

1. Turn the workstation on.
2. Do one of the following when the system is initialized:
 - Double-click the BioTOF desktop or tool bar icon ().
 - Choose **Start > All Programs > BIOTOF > BioTOF**.

The BioTOF window appears (Figure 2-1). Once you have started the application, you can leave it running, minimizing the window when you are not using the BioTOF system.

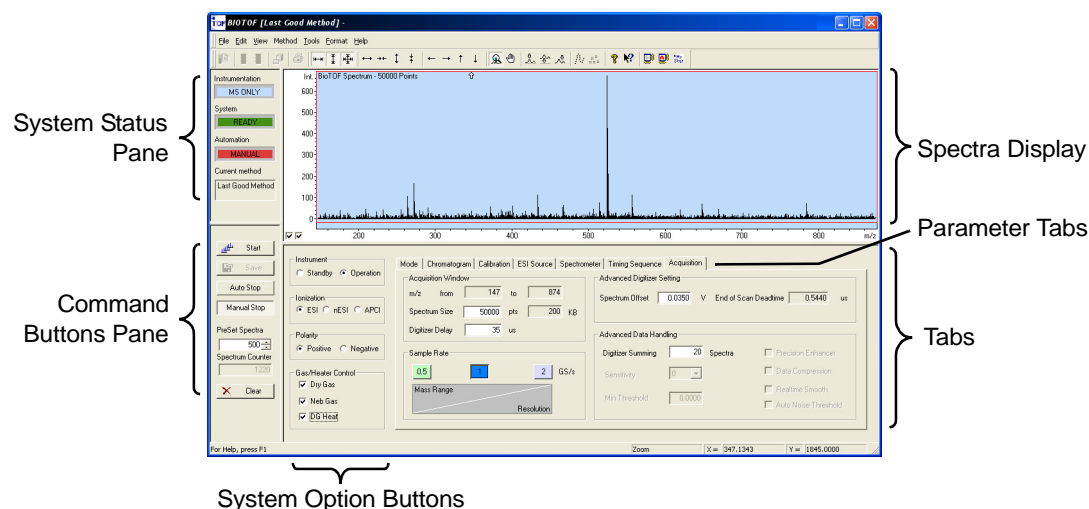


Figure 2-1 Layout of the BioTOF Window

The BioTOF window displays information and controls in five different panes:

- The **Spectra Display** plots the spectra captured by the system. There are a variety of user-selected formats. For example, the graph in Figure 2-2 shows the intensity of ion signals from the detector for each m/z value. The two checkboxes in the bottom left corner of the display control autoscaling for the vertical axis (left) and horizontal axis (right).
- The pane on the upper left side of the window has system status indicators.
- The pane on the lower left side has command buttons for starting and stopping spectra acquisitions.
- The four groups immediately to the right of the command buttons include option button and checkboxes for controlling system operation.
- To the right of these controls are seven tabs for setting system parameters for acquisition and display of the spectra.

Figure 2-2 shows the location of the Autoscaling checkboxes.

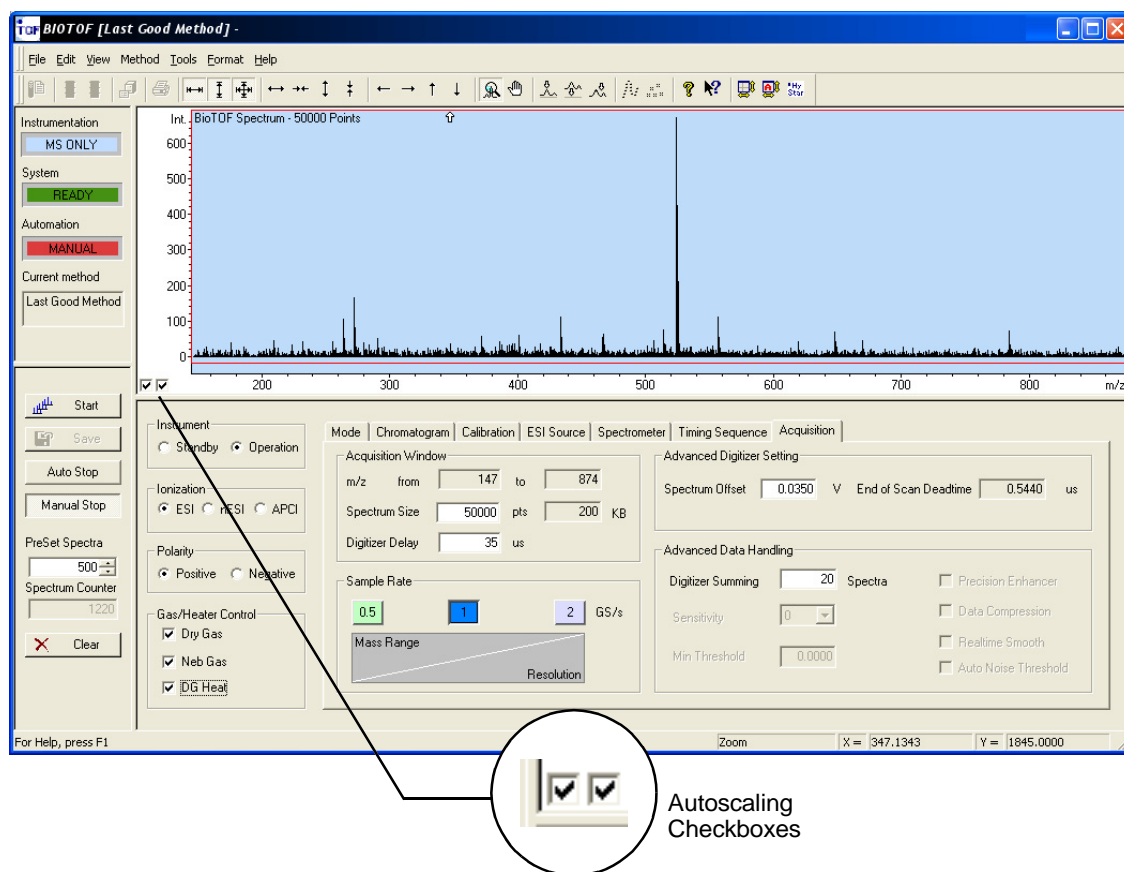


Figure 2-2 BioTOF Window and Autoscaling Checkboxes

2.2 Front Control Panels

The cabinet door on the front of the BioTOF console provides access to four control panels (Figure 2-3):

- The **Main Control Panel** on the top left includes the main power switch and switches for turning on the vacuum pumps, system electronics, and additional electrical outlets on the power supply (labeled Computer). These pushbutton switches light up when they are engaged.
- The **Vacuum Gauge Controller** on the top right includes an LCD display of the vacuum pump gauges. Use the numbered membrane buttons on the right to select the vacuum gauges. Gauges 1 and 3 are used in the basic BioTOF configuration. Below the selection buttons is an array of buttons for selecting units of measure and pressure thresholds.
- The **Source Control Unit** is used to set power for the ESI source and to control drying and nebulizing gas flows. The unit is powered up when the System Electronics are turned on from the Main Control Panel. The unit also has its own power switch in the lower right corner of the unit panel.
- The **Instrument HV Panel** includes two 3-position toggles, one for enabling the supply of high voltage power to the BioTOF electrodes, the other for setting the ion polarity. When a toggle is centered, the HV or polarity is controlled from the BioTOF software.



Figure 2-3 BioTOF Control Panels

2.3 System Status Indicators

Figure 2-4 shows the set of indicators located on the front of the cabinet above the ESI module. These include a source lens current gauge, and indicators for ion polarity, high voltage, vacuum, and power.

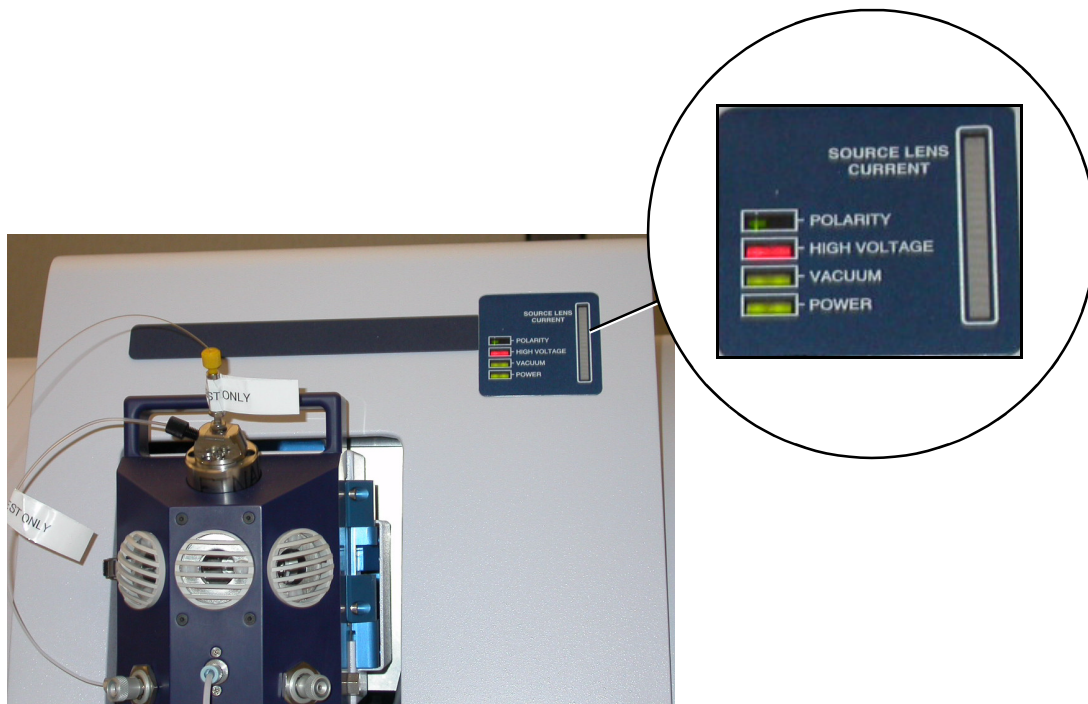


Figure 2-4 System Status Indicators

Polarity	Indicates the ion polarity. Appropriate source and spectrometer voltages are set automatically.
High Voltage	Shows when the instrument high voltage is on (green) and off (red).
Vacuum	Indicates when the flight tube pressure is below threshold.
Power	Indicates that system power is on.
Source Lens Current	Measures current flow at one of the three spray chamber electrodes. The electrode is selected with the Source Control unit.

3 Quick Start

This section lists the essential steps required to start the system and acquire spectra from a known sample.

The sequence description assumes that the BioTOF system has been installed and calibrated, and is configured with the syringe pump. If the configuration includes an LC system, the syringe pump is connected via a tee joint between the LC system and the ESI Source (refer to *BioTOF Operator Manual, Volume II, Operation*).

The sequence includes these activities:

- Start the System
- Check the Spray Chamber and Sprayer
- Turn the Gases On
- Prepare the Sample
- Check BioTOF Parameters
- Start BioTOF Operation
- Acquire Spectra
- Check Calibration
- Place the System on Standby

3.1 Start the System

After installation and calibration of the BioTOF system, the system is normally placed in standby mode rather than being completely powered down, as it can take several hours to pump down the TOF chamber below the pressure threshold. The instructions in this section involve the use of the control panels on the front of the BioTOF cabinet to verify that the system is ready for use.

To start the BioTOF system:

1. Open the front cabinet door to access the BioTOF control panels (Figure 2-3 on page 2-4).
2. On The Main Switch Panel (Figure 3-1), verify:
 - The **MAIN** pushbutton is illuminated (red)
 - The **VACUUM PUMPS** pushbutton is illuminated (green)



The buttons illuminate when pressed to engage the switches.

Figure 3-1 Main Switch Panel

3. Press the **VACUUM PUMPS** button if it is not already lit.
 When this button is pressed, it lights up and the pumps start sequentially. However, the system will not be ready until the pressure in the vacuum system has dropped below threshold. This may take several hours.
4. Press the **ELECTRONICS** button to initialize the system electronics including the vacuum gauge controller (Figure 3-2).

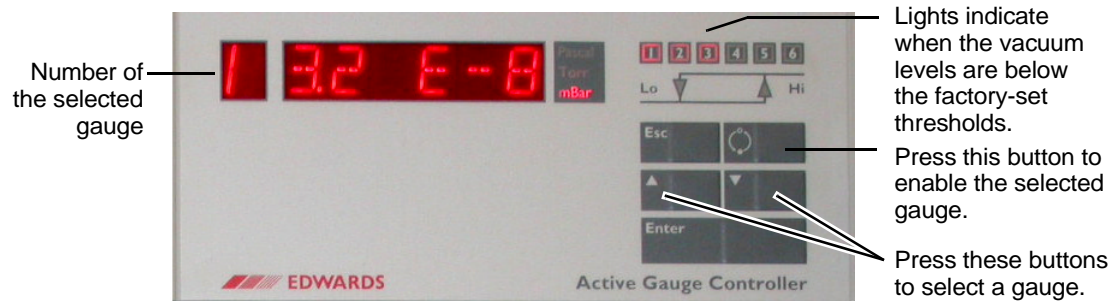


Figure 3-2 Vacuum Gauge Controller

5. Press the ▲ and ▼ buttons on the vacuum gauge controller to cycle through the readouts for the gauges used in the BioTOF system:
 - Gauge 1 reads the flight tube pressure; this gauge is initially off.
 - Gauge 3 reads the pressure in the first pumping stage of the ESI source.

6. Select **Gauge 1** and press the **ON/OFF** button on the gauge controller.

The flight tube pressure must be below 5×10^{-7} mbar to engage the pressure interlock of the high voltage supplies.

7. Select **Gauge 3**.

When the inlet to the vacuum system is open, the pressure should be between 1.5 and 1.9 mbar. When the inlet to the vacuum system is closed, the pressure should be less than 10^{-1} mbar.

The indicators labelled 1 through 6 in the upper right corner of the Vacuum Gauge Controller are lit when the vacuum levels are below the factory-set thresholds. When indicators 1, 2 and 3 are on, you can safely turn the BioTOF system on. Indicators 4, 5 and 6 are not used.

8. Check the **Power Switch** in the lower right corner of the Source Control to make sure that the controller is turned on (Figure 3-3).

The Source Control unit LCD displays the default startup menu.

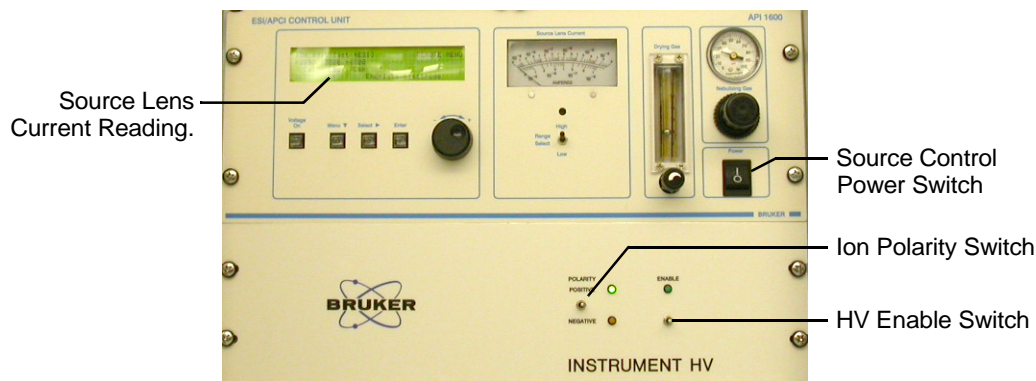


Figure 3-3 Source Control Unit (top) and Instrument HV Panel

9. Use the arrow button below the LCD display to position the display cursor on **End** (Figure 3-4) so that the Source Lens Current gauge on the ESI Source reads the current on the **Endplate Electrode**.

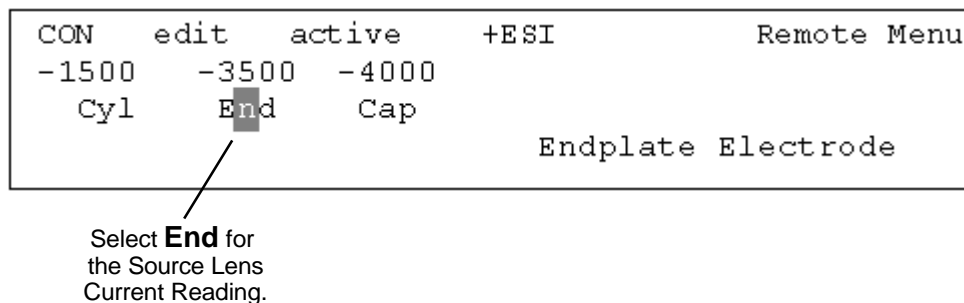


Figure 3-4 Setting the Source Lens Current Reading

10. Set the ion **Polarity** and **Enable** switches on the Instrument HV unit to the middle position.

When the two 3-position switches on the Instrument HV panel (Figure 3-3 on page 3-3) are in the middle position, the unit is remotely controlled from the BioTOF window.
11. Check the nitrogen supply.

3.2 Check the Spray Chamber and Sprayer

The next step is to check the Sprayer Assembly on the front of the ESI Source. The assembly is attached to the ESI Source with hinges on the right and locked with a latch on the left. A sample is introduced to the spray chamber via an off-axis spray needle which is held in position by a bayonet connection. Figure 3-5 shows the sprayer assembly unlatched and open, and the off-axis sprayer removed from the assembly.

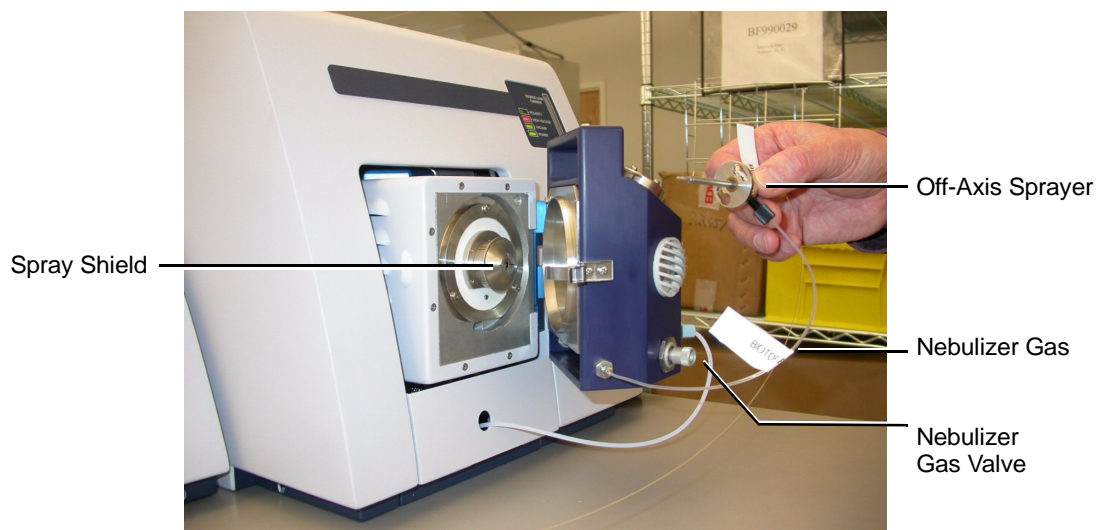



Figure 3-5 Off-axis Sprayer Assembly

To check the Spray Chamber:

1. Release the latch on the left side of the assembly and swing the assembly out so that the spray shield (Figure 3-5) is visible.
2. Remove the spray shield, remove any plug or rubber seal from the end of the capillary tube, and re-install the spray shield.
3. Remove the off-axis sprayer assembly from the top of the spray chamber, verify that the tip of the inner needle protrudes about 0.5 mm from the body of the sprayer, and reinstall the sprayer.
4. Make sure that the sprayer assembly is connected to the nebulizer gas supply, and turn the gas valve counterclockwise until it is fully open.
5. Close the spray chamber and secure the latch on the left side to engage the source voltage interlock.

3.3 Start the BioTOF Software

To start the BioTOF control software:

1. Turn the workstation on.
2. Do one of the following when the system is initialized:
 - Double-click the BioTOF desktop or tool bar icon ().
 - Choose **Start > All Programs > BIOTOF > BioTOF**.

3.4 Turn the Gases On

The next step is to set the nebulizer and drying gas controls.

To turn the gases on:

1. Click the **Dry Gas** and **Neb Gas** checkboxes in the Gas/Heater Control group (Figure 3-6), if they are not already on.
2. Click the **DG Heat** checkbox in the Gas/Heater Control group to turn the heater on.
3. Click the **ESI Source** tab, and set the Dry Gas Temperature to **200 C**.

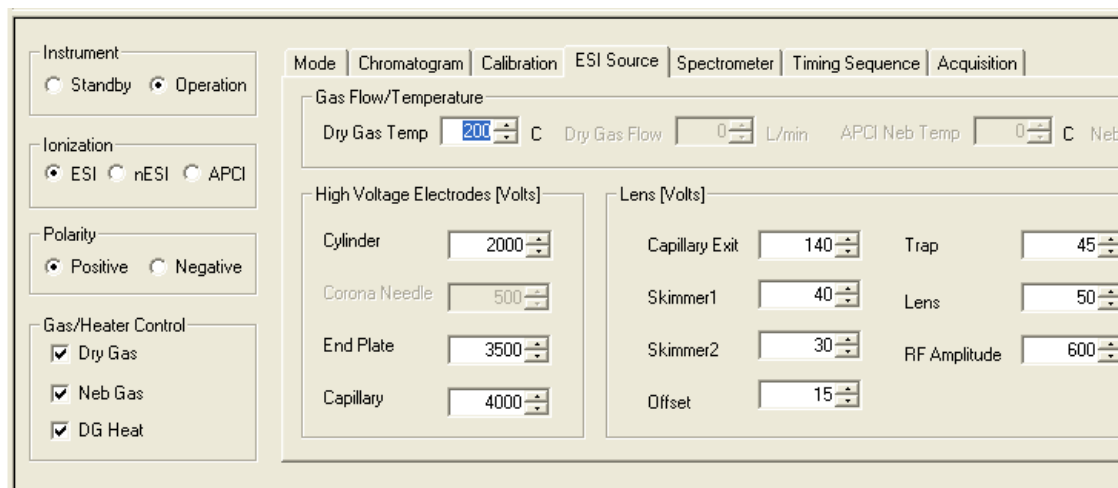


Figure 3-6 Gas/Heater Group and ESI Source Tab

4. At the Source Control panel, set the nebulizer gas pressure to approximately **30 psi**.
5. Set the drying gas flow so that the ball is at about 1/3 scale (see Figure 3-7).



Figure 3-7 Nebulizer and Drying Gas Controls

3.5 Prepare the Sample

Samples of the following compounds are well suited for electrospray ionization.

- Leucine-Enkephalin (556)
- Reserpine (609)
- Angiotensin I (1046/523)
- Angiotensin II (1296/648)
- Arginine-Glycine-Aspartic Acid (347)

Leucine-Enkephaline and Reserpine produce singly charged molecular ions (MH^+). The larger peptides generate doubly charged ions ($M+2H$)²⁺ and triply charged ($M+3H$)³⁺ ions. A typical ESI solvent for these peptides is a 1:1 mixture of methanol-water with 0.1 to 1% acetic acid.

To prepare a standard ESI sample:

1. Prepare an ESI solvent of 1:1 methanol-water mixture with 0.1% Acetic Acid.
2. Prepare a peptide stock solution in ESI solvent at a concentration of 100 μM , which is equal to 100 pmole/ μL .
3. Dilute the stock solution in the ESI solvent to a ratio of 1/100 to 1/20 to obtain a sample solution at an analyte concentration of 1 to 5 μM .

To introduce the sample to the system:

1. Fill a 100 to 250 μL syringe with the ESI solvent and position it in the syringe pump (Figure 3-8).
2. Set the syringe pump to a flow of 120 $\mu\text{L/h}$ (2 $\mu\text{L/min}$).
3. Connect the syringe with the sample inlet of the off-axis sprayer using low volume, clean tubing and fittings (Figure 3-8).

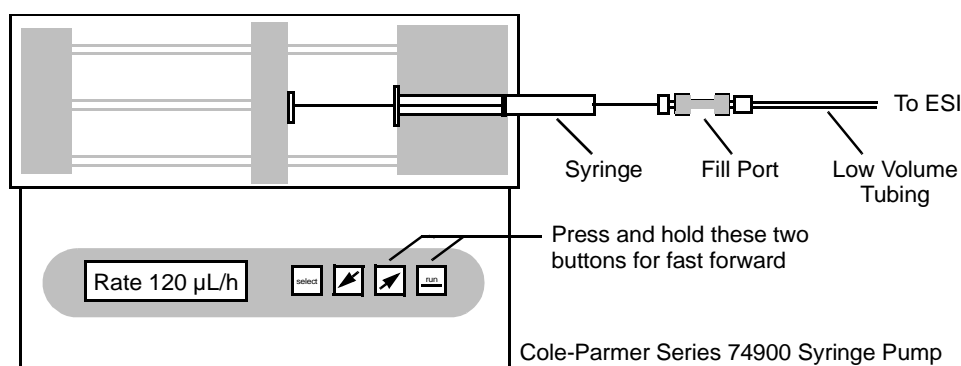


Figure 3-8 Connecting the Syringe Pump to the ESI Source

3.6 Check BioTOF Parameters

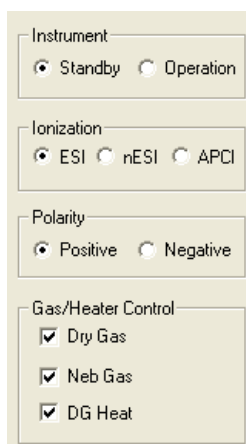
When the BioTOF control software is started, it automatically loads the **Last Good Method** used. If this is the first use of the system since installation, the parameters saved in Last Good Method are the factory defaults applied in the setup process.

You can acquire data using the parameters from the Last Good Method, or you can modify the method by changing one or more parameters. You can also load a previously saved method. In this Quick Start, the Last Good Method is used, but to check the settings and familiarize yourself with the controls, browse through the parameter tabs.

Note: The parameter values shown in the examples that follow are typical values appropriate for the samples used in this Getting Started guide.

To ensure that the Last Good Method is appropriate:

1. Check the option buttons in the Ionization and ion Polarity groups to make sure that the Ionization is set to **ESI** and that the Polarity is set to **Positive** (Figure 3-9).



Instrument

☒ Standby ☐ Operation

Ionization

☒ ESI ☐ nESI ☐ APCI

Polarity

☒ Positive ☐ Negative

Gas/Heater Control

☒ Dry Gas

☒ Neb Gas

☒ DG Heat

Figure 3-9 Ionization and Polarity Groups

- Click the **ESI Source** tab to display the ESI control parameters (Figure 3-10).

The screenshot shows the 'ESI Source' tab selected in a software interface. The interface has a top navigation bar with tabs: Mode, Chromatogram, Calibration, ESI Source, Spectrometer, Timing Sequence, and Acquisition. Below the navigation bar, there are two main sections: 'Gas Flow/Temperature' and 'High Voltage Electrodes [Volts]'. The 'Gas Flow/Temperature' section includes fields for Dry Gas Temp (200 C), Dry Gas Flow (0 L/min), APCI Neb Temp (0 C), and Neb Gas Flow (0 L/min). The 'High Voltage Electrodes [Volts]' section includes fields for Cylinder (2000), Corona Needle (500), End Plate (3500), and Capillary (4000). To the right of this section is a 'Lens [Volts]' section with fields for Capillary Exit (140), Skimmer1 (40), Skimmer2 (30), Offset (15), Trap (45), Lens (50), and RF Amplitude (600). A 'Refresh' button is located at the bottom right of the 'Lens [Volts]' section.

Parameter	Value	Unit
Dry Gas Temp	200	C
Dry Gas Flow	0	L/min
APCI Neb Temp	0	C
Neb Gas Flow	0	L/min
Cylinder	2000	V
Corona Needle	500	V
End Plate	3500	V
Capillary	4000	V
Capillary Exit	140	V
Skimmer1	40	V
Skimmer2	30	V
Offset	15	V
Trap	45	V
Lens	50	V
RF Amplitude	600	V

Figure 3-10 ESI Source Tab

- Set the ESI spray chamber voltages in the **High Voltage Electrodes** group to the values shown in Figure 3-10.

With ion Polarity set to Positive, the actual spray chamber voltages are negative. Only the absolute values are displayed.

Note: The software limits the difference between any two of the spray chamber voltages to a maximum of 2000 V. You may need to increase the voltages sequentially and in steps.

- Verify that the seven voltages in the **Lens** group are in line with the values shown in Figure 3-10.

On the BioTOF, offset must be **15 V**.

- Click the **Timing Sequence** tab to display the timing parameters and verify that the values are those shown in (Figure 3-11).

The screenshot shows the 'Timing Sequence' tab selected in the software interface. The 'Advanced Spectrometer Timing' section contains the following parameters:

Parameter	Value	Unit
Cycle Period	25	ms
Repeller	10	us
Gate	100	us
Transfer	100	us
Deflector off Delay	25	us

A 'Pulse ON/OFF' button is located at the bottom right of the timing parameters section.

Figure 3-11 Timing Sequence Tab

- Click the **Spectrometer** tab to display the spectrometer voltages (Figure 3-12), and verify that the voltage levels are those set during installation.

Note: Changing the parameters in the Spectrometer tab alters the mass calibration. The advanced parameters are set during installation and are specific to each instrument. Modifying them can adversely affect system performance.

The screenshot shows the 'Spectrometer' tab selected in the software interface. The 'High Voltage [Volts]' section contains the following parameters:

Parameter	Value
Repeller	8500
Post Acceleration	2000
Grid	0
Detector	1600
Reflectron	9300
Multi Deflector Low	5.0
Reflectron Lens	100
Multi Deflector High	71.0

Figure 3-12 Spectrometer Tab

7. Click the **Acquisition** tab and ensure that the spectrum acquisition parameters are in line with those shown in Figure 3-13.

The screenshot shows the 'Acquisition' tab selected in the top menu bar. The interface is divided into several sections:

- Acquisition Window:** m/z from 147 to 874, Spectrum Size 50000 pts, 200 KB, Digitizer Delay 35 us.
- Sample Rate:** Three buttons labeled 0.5, 1, and 2 GS/s. The '1' button is highlighted in blue.
- Mass Range:** A graphical representation showing a linear increase in resolution across the mass range.
- Advanced Digitizer Setting:** Spectrum Offset 0.0350 V, End of Scan Deadtime 0.5440 us.
- Advanced Data Handling:** Digitizer Summing 20 Spectra.

Figure 3-13 Acquisition Tab

8. Click the **Mode** tab (Figure 3-14) and set the Instrumentation Mode to **MS Only**.

The screenshot shows the 'Mode' tab selected in the top menu bar. The interface is divided into several sections:

- Instrumentation Mode:** Three radio buttons: **MS Only** (selected), Chromatography - MS, and FIA - MS.
- Data Display:** Three radio buttons: **m/z** (selected), Flight Time, and Points.
- Mass Range [m/z]:** m/z from 147 to 874.
- Chromatographic Setting:** Total Run Time 10.0 mins, Solvent Delay 0.0 mins, MS Acq. Rate 1 < 70 < 70 Spectra / min.
- External Start:** A checkbox labeled 'Enable' is unchecked, and a 'Configure' button is present.

Figure 3-14 Mode Tab

3.7 Start BioTOF Operation

To start BioTOF operation:

1. In the BioTOF window (Figure 3-6 on page 3-6), click the **Operation** option button in the Instrument group (Figure 3-15).

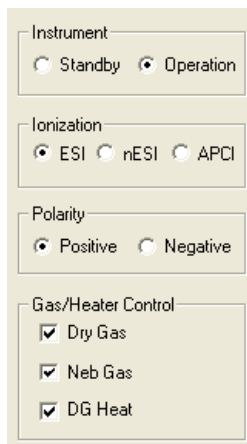


Figure 3-15 Instrument Control Group

The instrument goes from Standby to Operation.

- The Enable LED on the Instrument HV unit lights up (Figure 3-16).
- The high voltages ramp up.
- All ESI source voltages turn on.

You may also be prompted to press the **Voltage On** button on the Source Control unit (Figure 3-16). This switch lights up to indicate that the source voltages have been turned on.

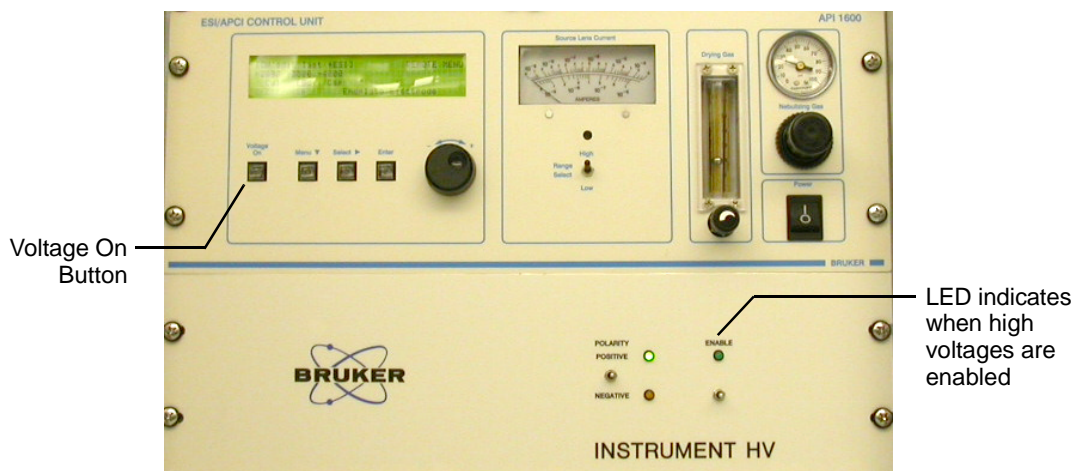


Figure 3-16 Location of the Voltage On Button

Source Control Unit and Instrument HV Panel

2. Set the syringe pump flow rate to **120 $\mu\text{L/h}$** (2 $\mu\text{L/min.}$) and start the pump.
3. Press and hold the two right buttons on the syringe pump to fast-forward the solution to the ESI source.

When sample is flowing through the tip of the spray needle assembly, and nebulizer gas and spray chamber voltages are turned on, the current meter (Figure 3-17) of the ESI Source gauges indicates the spray current. (If there is no reading, verify that the meter is set to read the endplate electrode, as described in “Setting the Source Lens Current Reading” on page 3-4).

4. Return the syringe pump to the **120 $\mu\text{L/h}$** (2 $\mu\text{L/min.}$) rate when the meter indicates current.

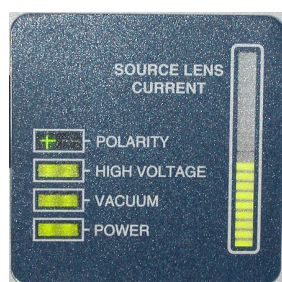


Figure 3-17 Source Lens Current Reading

3.8 Acquire Spectra

Controls and indicators for starting and stopping spectrum acquisition are located in the two panes on the left side of the BioTOF window (Figure 2-1 on page 2-2). You can start acquisition when the status bar is green and displays **Ready** (Figure 3-18).

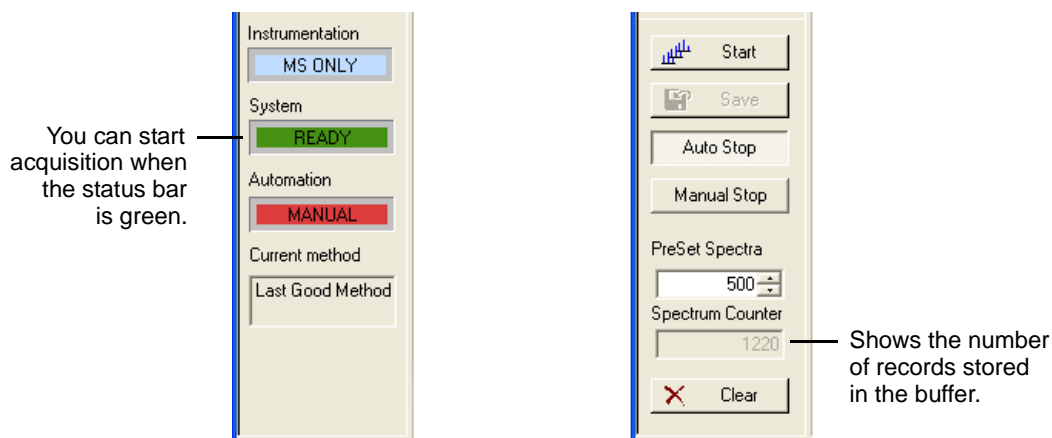


Figure 3-18 Indicators and Controls on the Left Side of the BioTOF Window

To run spectra acquisition:

1. Click the **Start** button.

Data acquisition and transfer begin, and the Spectra Display plots the results. The Spectrum Counter indicates the total number of records accumulated in the buffer. The Start button changes to a Stop button, enabling you to manually stop acquisition when a sufficient number of records have been stored in the buffer.

2. Click the two checkboxes in the lower left corner of the Spectra Display (Figure 2-2 on page 2-3) to enable autoscaling for both axes of the display (Figure 3-19). The BioTOF continues to accumulate records until the buffer contains the number of records specified in PreSet Spectra.
 - If **Manual Stop** is selected, the buffer is cleared and a new acquisition is started.
 - If **AutoStop** is selected, acquisition stops and the data in the buffer is

maintained.

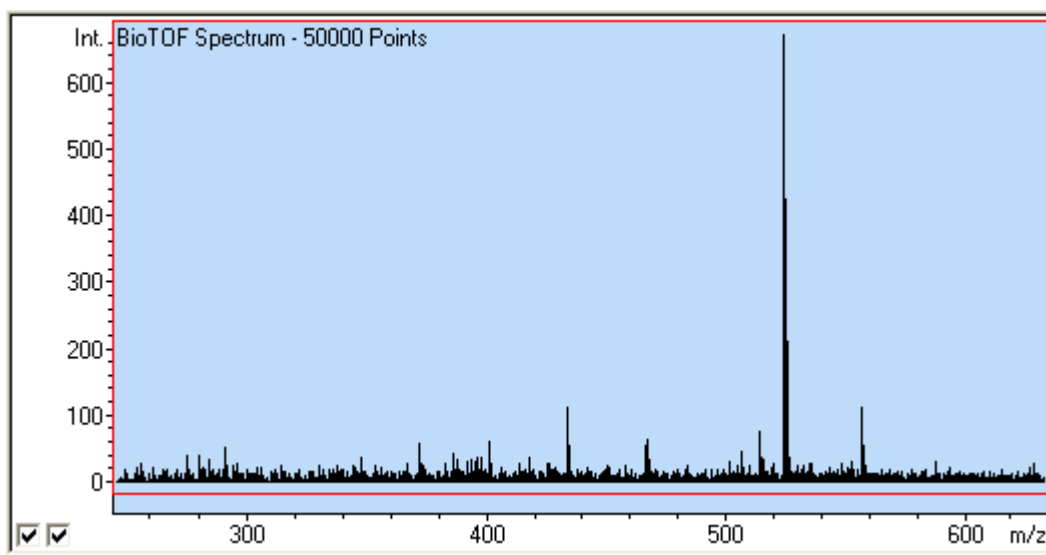


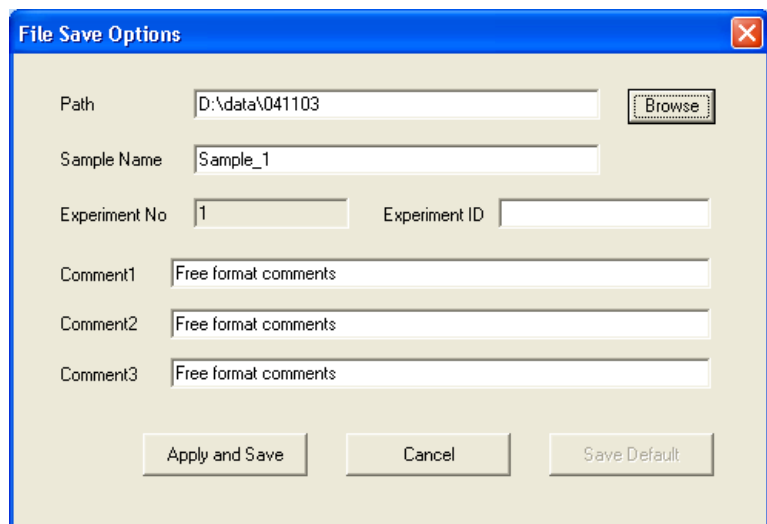
Figure 3-19 Spectra Display

3. Set PreSet Spectra to **500** and click **AutoStop**.

Spectra acquisition automatically stops when 500 spectra have been accumulated.
The Save button becomes active.

4. Click **Save** in the control panel.

The BioTOF software opens a dialog box for setting the file options (Figure 3-20).



The dialog box titled "File Save Options" contains the following fields and buttons:

- Path:** A text box containing "D:\data\041103" and a "Browse" button.
- Sample Name:** A text box containing "Sample_1".
- Experiment No:** A text box containing "1".
- Experiment ID:** An empty text box.
- Comment1:** A text box containing "Free format comments".
- Comment2:** A text box containing "Free format comments".
- Comment3:** A text box containing "Free format comments".
- Buttons:** "Apply and Save", "Cancel", and "Save Default".

Figure 3-20 File Save Options Dialog Box


The BioTOF application creates two files, one formatted for the DataAnalysis application, the other compatible with the XMass program. You can set the application to use either or both formats by choosing **Format > DA format** and/or **Format > Xmass format** from the menu bar.

3.9 Check Calibration

As part of the BioTOF installation, the system was calibrated using a table of reference masses included in the software. The Calibration tab enables you to check the calibration after acquiring spectra from the known sample. See Chapter 8 in the BioTOF Operator Manual, Volume II Operation for detailed instructions on checking and adjusting BioTOF calibration.

To check calibration:

1. Click the **Calibration** tab to display the table of reference masses.
2. Use the pull-down list at the top of the tab to select **Peptides.ref** to display the Reference list.
3. Select the first known sample used in acquiring the spectra (*Angiotensin-II* in Figure 3-21).

A red vertical line appears in the Spectra Display at the calculated position of the selected compound on the m/z scale, and the cursor in the Spectra Display changes to the Max cursor () .

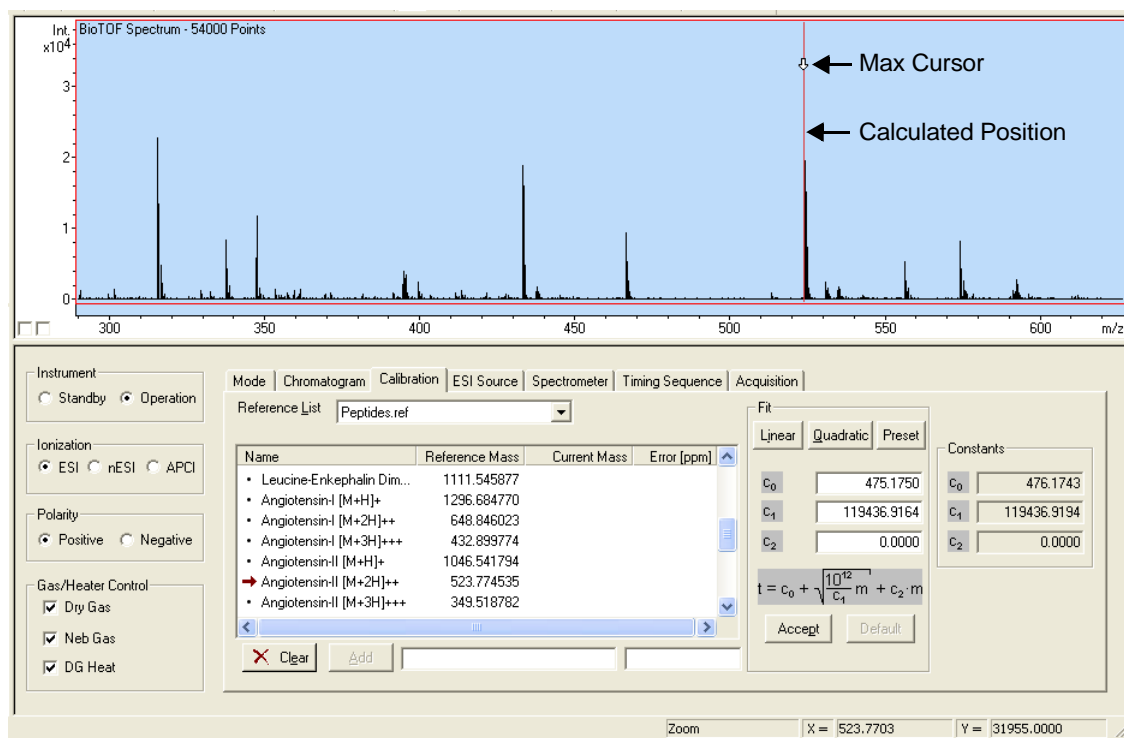


Figure 3-21 Calibrating the BioTOF System

4. Drag the cursor immediately to the right of the most intense peak of the reference compound and click the left mouse button.

The cursor snaps to the peak and the current mass appears in the reference table in the Calibration tab.

5. Use the same technique to snap the line to each calibration peak.
 - A minimum of two peaks is required for linear calibration.
 - A minimum of three peaks is required for quadratic calibration.
6. Click the **Linear** button in the Fit group (Figure 3-21) to display the new calibration constants.
7. Check for obvious errors, such as negative constants.

Errors can occur if the peaks selected are not the proper compounds or the max cursor was snapped to the wrong mass.

8. Click the **Accept** button in the Fit group.

The new values appear in the Constants group to the right, and the Spectrum Display calibration changes accordingly.

With the system calibration verified using the known sample, you can now proceed to acquiring spectra from the material of interest. Refer to Chapter 6 in the *BioTOF Operator Manual, Volume II Operation* for instructions on flushing the syringe and ESI unit and introducing the new sample.

3.10 Place the System on Standby

Take the following steps when you have finished with spectra acquisition:

1. Click **Standby** in the Instrument group to bring the instrument from operation mode to standby mode (Figure 3-22).

The ESI source voltages are turned off (the light in the high voltage indicator on the ESI front panel must be off), the high voltages are turned off, and the Instrument HV unit is disabled (Enable LED off).

2. Double-check that the ESI voltages and the TOF voltages are off.

Note: For this procedure, the high voltages must be off.

3. Uncheck **DG Heat** in the Gas/Heater Control Group (Figure 3-21 on page 3-18) to allow the spray chamber to cool.

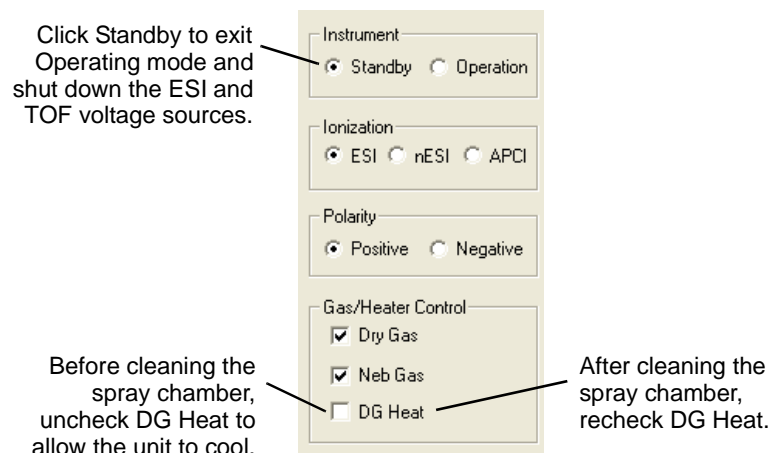


Figure 3-22 System Control Settings for Standby

4. Stop the syringe pump.
5. Fill the syringe with ESI solvent or a plain methanol-water mixture and flush both the transfer line and ESI sprayer several times.
6. Make sure the liquid flows easily through the plumbing.
7. Uncheck **Neb Gas** in the Gas/Heater Control group.
8. After the spray chamber has cooled down, wipe clean the spray shield, endplate, and the face of the inlet capillary.
9. Close the spray chamber.
10. Return to the BioTOF window and recheck **DG Heat**.

Note: You should leave the drying gas flow and drying heat on at all times. However, if is necessary to conserve your source of drying gas, turn the gas off and seal the spray chamber inlet when it has cooled.

You do not need to exit the BioTOF software; simply minimize the application.

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BioTOF *Series*

Operator Manual Volume I: Theory



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been designed and tested
in accordance with IEC
Publication 1010:
Safety Requirements for
Electrical Equipment for
Measurement, Control,
and Laboratory Use.

Class 1 LED Product

WARNING

Connecting an instrument
to a power source that is
not equipped with a
protective earth contact
creates a shock hazard
for the operator and can
damage the instrument.
Likewise, interrupting the
protective conductor inside
or outside the instrument
or disconnecting the
protective earth terminal
creates a shock hazard for
the operator and can
damage the instrument.

WARNING

The instrument must be
disconnected from its power
source before any cover is
removed or the system is
opened.

WARNING

All connections of the
instrument must be used in
correct way. The instrument
should only be used with
the wires and cables
delivered with the system
or otherwise provided by
the manufacturer.

Noise Declaration

70 dB operator position
normal operation per
ISO 7779: 1988

Instrument Identification

Each instrument is
identified by a unique
serial numbers. These
serial number is located on
a label on the lower left side
near the rear of the
instrument.

When corresponding with
Bruker Daltonics Inc. about
your instrument, be sure to
include the model and the
full serial number.

Write the serial number
of the instrument here for
reference.

Model:

Serial Number:

Part Numbers

In this manual,
Bruker Daltonics Inc.
part numbers are generally
listed in parentheses after
the name of the part.

A few tools and supplies
listed have no part numbers
and are not available from
Bruker Daltonics Inc. Most
of these can be obtained
from laboratory supply
companies.

Manual part number: A5001

BioTOF Series Operator Manual, Version 1.0, Volume I: Theory

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OPERATOR MANUAL INDEX		

Preface

This document provides detailed information on using the BioTOF system. It consists of two volumes:

- *BioTOF Operator Manual, Volume 1, Theory*
- *BioTOF Operator Manual, Volume 2, Operation*

Three additional manuals in this document set provide information on using the analytical software included with the BioTOF system.

- *DataAnalysis User Manual*
- *QuantAnalysis User Manual*
- *Library Editor User Manual*

For technical assistance, contact Bruker Daltonics customer service:

In the U.S.: +1 978 663 3660, Ext. 1445

In Germany: +49 (0) 421 2205 430

In Japan: +81 (0) 45 440 0471

Local Service:

Safety Labels

These labels are placed on the instrument to indicate the respective hazard possibility.



Hazard: Note

Refer to the Operator Manual for details on this hazard.



Warning: High Voltage

A dangerous high voltage or shock hazard exists within this area. Only trained service persons should perform work in this area.



Warning: High Temperature

Hot parts and surfaces are present in this area. Allow the part or surface to cool before performing work in this area. This may require turning off power to selected components or to the entire instrument.



Warning: Biohazard

A biohazard may exist within this instrument or specific area. Handle this biohazard with appropriate caution and protection.

1 System Components

The Bruker Daltonics atmospheric pressure ionization time-of-flight systems provide high resolving power, accuracy, and mass range for today's mass spectrometry needs. They all have high performance ESI (Electrospray), APCI (atmospheric pressure chemical ionization) and Nano-Electrospray sample introduction and ionization sources, highly efficient hexapole ion-guide transfer technology, state of the art orthogonal acceleration time-of-flight spectrometers and analog-to-digital (ADC) detection systems for the highest dynamic range and true isotope pattern measurement.

Like all Bruker Daltonics mass spectrometers, these time-of-flight systems share the common COMPASS™ data processing and analysis software tools for viewing, printing, calibration, mass and chromatogram analysis, library search, quantitative analysis, metabolite search, peptide annotation, and database search.

The series includes:

BioTOF™	The highest performance ESI-TOF instrument with unprecedented mass resolution and accuracy. The built-in Multipass feature further enhances mass resolution. Ideal instrument for accurate mass measurement of small molecules. The BioTOF's versatility and mass range make it the best choice for the study of intact proteins, polymers, non-covalent protein-protein or protein-ligand complexes, or proteomics applications.
BioTOF-Q™	New Quadrupole-TOF (Q-q-TOF) mass spectrometer for sensitive and highly accurate MS/MS analysis. It combines state-of-the-art quadrupole and TOF technologies and offers fully automated precursor detection and ms/ms switching and scan functions. The BioTOF-Q is the ideal instrument for peptide analysis and de-novo sequencing. Includes Multipass™ TOF for highest mass resolution.
microTOF™	An easy-to-use bench-top package for powerful and exact mass measurement. It concentrates the performance of a floor-standing instrument in a format that fits on a bench. The microTOF is specifically designed for extreme calibration stability, which makes it the ideal instrument for accurate mass analysis in all LC-MS applications.

This manual describes operation of BioTOF systems.

The BioTOF system (Figure 1-1) consists of:

Electrospray ion (ESI) source	Generates molecular ions from liquid samples and facilitates ion transport from atmospheric pressure into the high vacuum of the mass analyzer. The liquid sample is dispersed into a fine spray of charged droplets using a sprayer under the action of an electric field and pneumatic nebulization. Operation of the source is described in Chapter 2 "Source Operation" along with the various options for sample introduction.
Time-of-Flight (TOF) mass spectrometer	Receives ions from the ESI source, redirects them into the flight tube using high voltage pulses, and records the flight times. The recorded data are delivered to the BioTOF computer workstation for display and analysis. Operation of the TOF mass spectrometer is described in Chapter 3 "TOF Mass Spectrometer Operation".
Computer workstation	Controls the source and mass spectrometer operation, stores and displays mass spectra, and provides additional analytical software.

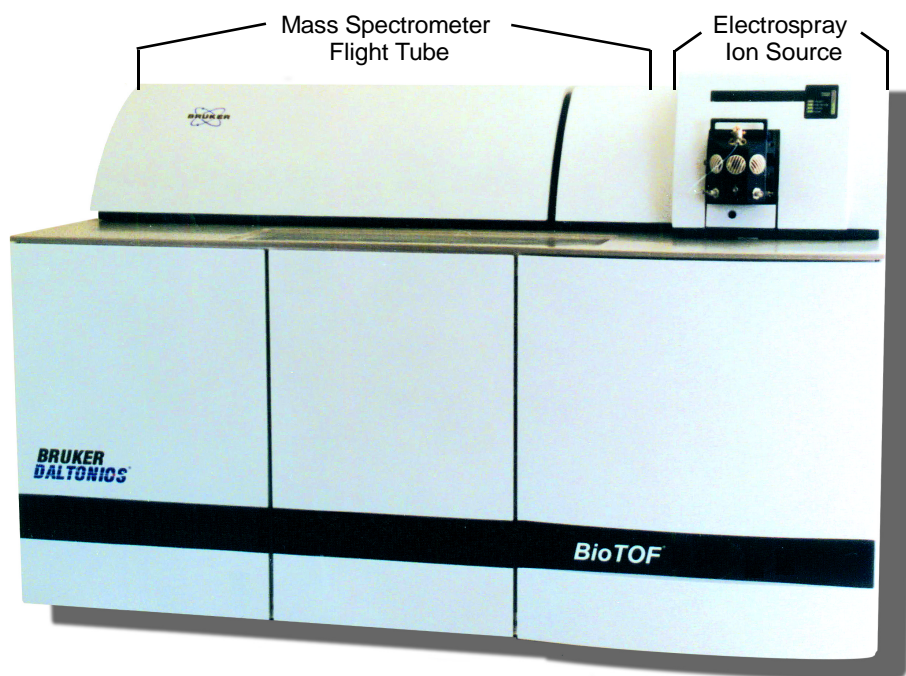


Figure 1-1 Basic BioTOF System Configuration

1.1 Front Control Panels

The cabinet door on the front of the BioTOF console provides access to four control panels (Figure 1-2):

- The **Main Control Panel** on the top left includes the main power switch and switches for turning on the vacuum pumps, system electronics, and additional electrical outlets on the power supply (labeled Computer). These pushbutton switches light up when they are engaged.
- The **Vacuum Gauge Controller** on the top right includes an LCD display of the vacuum pump gauges and lights indicating when pressures are below threshold. Function buttons allow you to select gauges, set units of measurement, and specify threshold settings.
- The **Source Control Unit** is used to set power for the ESI source and to control drying and nebulizing gas flows. The unit is powered up when the System Electronics are turned on from the Main Control Panel. The unit also has its own power switch in the lower right corner of the unit panel.
- The **Instrument HV Panel** includes two 3-position toggles, one for enabling the supply of high voltage (HV) power to the BioTOF electrodes, the other for setting the ion polarity. In normal operation, the toggles are centered and HV and polarity are controlled from the BioTOF software.

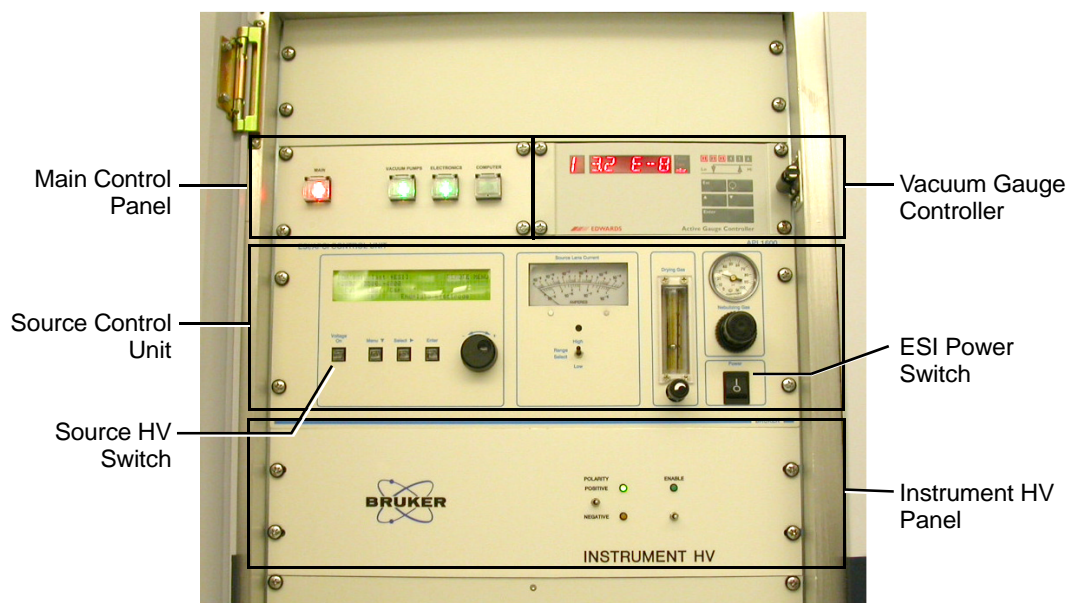


Figure 1-2 BioTOF Control Panels

1.1.1 System Status Indicators

Figure 1-3 shows the set of indicators located on the front of the cabinet above the ion source module. These include a source lens current gauge, and indicators for ion polarity, high voltage, vacuum, and power.

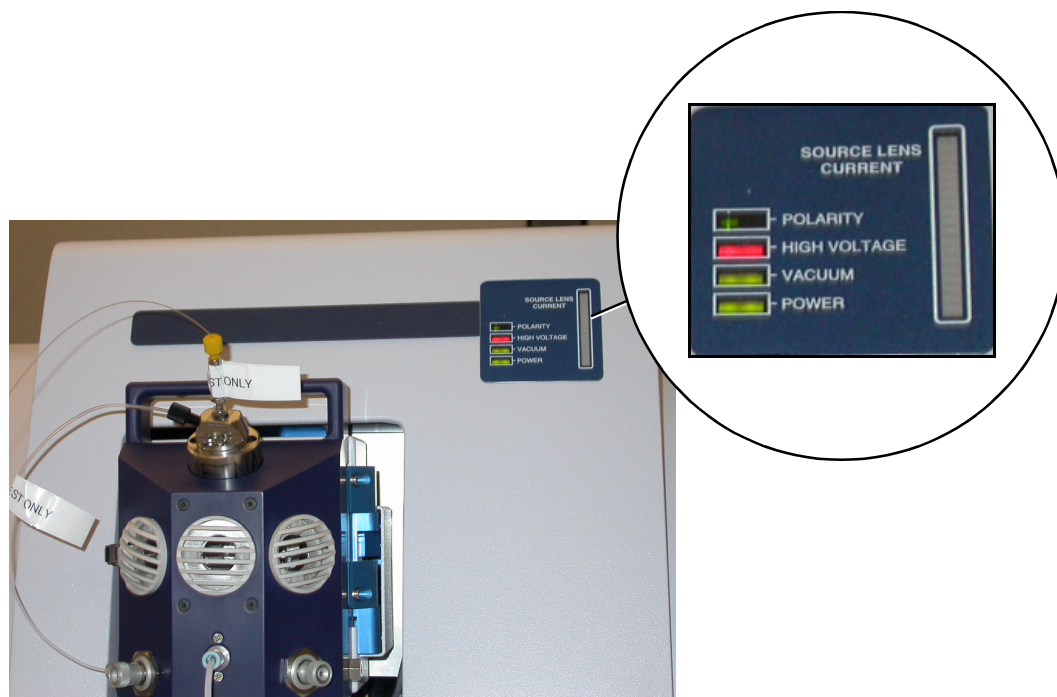


Figure 1-3 System Status Indicators

Polarity	Indicates the ion polarity. Appropriate source and spectrometer voltages are set automatically.
High Voltage	Shows when the instrument high voltage is on (green) and off (red).
Vacuum	Indicates when the flight tube pressure is below threshold (green) or above threshold (red). High voltage can only be switched on when pressure is below threshold.
Power	Indicates that system power is on.
Source Lens Current	Measures current flow at one of the three spray chamber electrodes. The electrode is selected with the Source Control unit.

1.2 Computer Workstation

The principal means for controlling and monitoring the BioTOF system is a Windows® XP based application configured on the workstation. From a single multi-pane window, you can start the BioTOF system, set system parameters, acquire and display spectra, and save the data for use with the DataAnalysis processing software (included with the system) and other optional software tools.

An array of hardware controls and indicators are configured on the front of the BioTOF cabinet. These include On/Off switches for the mass spectrometer and ESI source components. Many of these controls are replicated in the BioTOF software and can be set from the BioTOF window.

1.3 BioTOF Software

This section gives a brief description of the main window for the BioTOF application. Refer to Chapter 5 in the *BioTOF Operator Manual, Volume II Operation* for a detailed description of the window features including the menus, tool bars and options.

The BioTOF window appears (Figure 1-4) when you start the application. Once you have started the application, you can leave it running, minimizing the window when you are not using the BioTOF system.

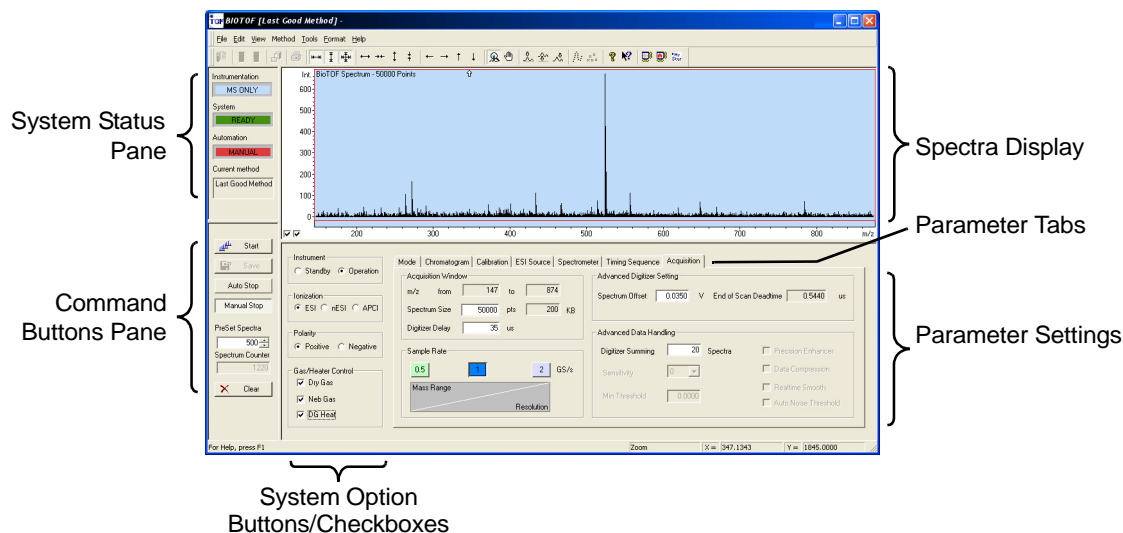


Figure 1-4 Layout of the BioTOF Window

The BioTOF window displays information and controls in five different panes:

Spectra Display	Plots the spectra captured by the system in a variety of user-selected formats. The graph in Figure 1-4 on page 1-5 shows the intensity of ion signals from the detector for each mass value (m/z). The two checkboxes in the bottom left corner of the display control autoscaling for the vertical axis (left) and horizontal axis (right).
System Status Pane	Contains the system status indicators.
Command Buttons Pane	Contains command buttons for starting and stopping data acquisition, and for saving data.
System Option Buttons/Checkboxes	The four vertical groups immediately to the right of the command buttons control system operation.
Parameter Settings	Parameter settings for acquisition and display of the spectra.

2 Source Operation

This chapter describes the various means of introducing sample to the ESI source, the operation of the source using the Electrospray, and the use of the APCI Source.

2.1 Sample Introduction

There are three basic methods of delivering sample to the BioTOF system:

- Syringe pump
- Flow from a liquid chromatography (LC) system
- Off-line Nanospray

2.1.1 Syringe Pump

A syringe pump (Figure 2-1) is included with the instrument system to provide for the introduction of sample directly to the Electrospray, Nanospray, or APCI ion source. Tubing from the syringe can be connected directly to the spray or tee'd to the flow from a LC system. The pump holds syringes from 5 μ L to 5 mL. A 100 μ L syringe is supplied with the pump. Function buttons and a small display on the front of the pump enable you to set flow rate, and stop and start the pump motor.

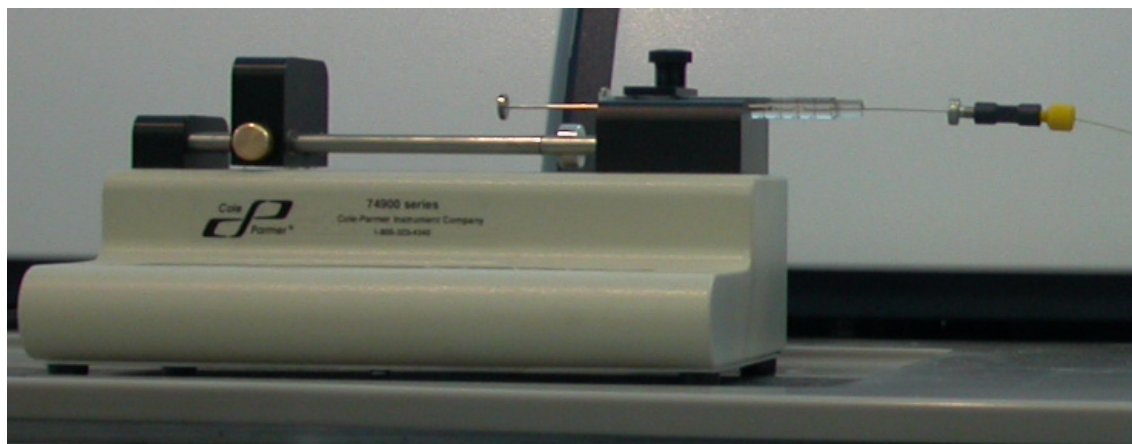


Figure 2-1 Syringe Pump Included with the BioTOF System

The following are typical syringe pump flow rates for various source configurations:

- Electrospray: 1 to 1,000 μL / (60 $\mu\text{L}/\text{h}$ to 60 mL/h)
- On-line Nanospray: 50 to 500 nanoL/min (3 to 30 $\mu\text{L}/\text{h}$)
- APCI: 10 to 1,000 μL / min (600 $\mu\text{L}/\text{h}$ to 60 mL/h)

Note: The units of measure on the syringe pump display are volume per hour.

See “Setup for Low Flow Direct Infusion” on page 6-2 for additional information on setup of the syringe pump.

2.1.2 LC System

Sample can be introduced to the Electrospray, Nanospray or APCI directly from a Liquid Chromatography (LC) system.

In LC applications, the ionization is optimized to accept flow rates up to 1 ml/min with the Electrospray or with the APCI Source. The nebulization process for both of these ion sources is assisted with nebulizing gas and countercurrent drying gas.

The LC system can be operated in several modes in conjunction with the BioTOF system, including standard LC analysis and analysis without LC separation (flow injection analysis, FIA).

The BioTOF system can be quickly adapted to the different instrumentation modes using the BioTOF window Parameters tabs described in “Methods” on page 7-1.

2.1.3 Off-Line Introduction

In off-line Nanospray applications, the sample can be introduced directly into the disposable Nanospray tip itself using a micro-pipet. See “Nanospray” on page 6-10.

2.2 ESI Source

This section describes operation of the source configured with the Electrospray spray chamber. Subsequent sections cover use of the Nanospray and the APCI Source.

Figure 2-2 shows ESI Source with the spray chamber opened to show some of the source components.

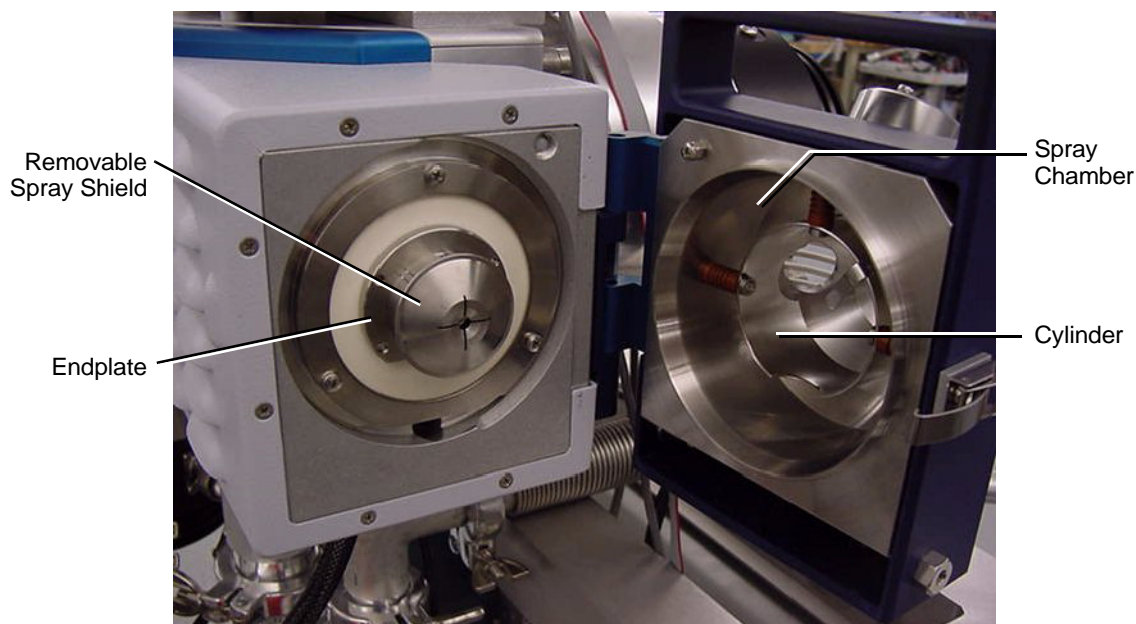


Figure 2-2 ESI Source With Open Spray Chamber

In the ESI spray chamber, a fine spray of charged droplets is generated from the sample solution by means of a sprayer under the influence of an electric field or an electric field assisted by a pneumatic nebulizer. The electric field inside the spray chamber is shaped by potentials applied to three electrodes: the cylinder, the end-plate and the metal coated entry of the glass capillary tube.

When the solvent evaporates from the droplets, the droplets' charge per volume increases resulting in electrostatic (Coulomb) forces that break up the droplets into ever smaller particles until, in the final stage, de-solvated sample ions emerge. The charged droplets contain analyte, solvent, and a surplus of either positive or negative ions. The type of ion formed depends on the composition of the sample liquid sprayed. If, for example, the solution contains the sample in acetic acid with a relative positive potential on the needle, the predominant positive ions are H_3O^+ and positively charged molecular analyte ions of the form $[\text{MH}]^+$.

De-solvation of the charged droplets is facilitated by a heated drying gas flowing counter to the stream of droplets and ions as they are electrostatically attracted towards the capillary tube that is the inlet orifice of the vacuum system.

Ions enter the vacuum system of the mass spectrometer through the dielectric glass capillary, stream through a skimmer, and then enter into the RF ion guide, which is biased at an appropriate offset voltage. Neutral gas molecules that enter with the sample ions are pumped away in differential pumping stages at successively lower pressures.

Ions accumulate in the RF ion guide by means of a trapping voltage applied to the guide's exit gate electrode. Then, the voltage on the gate electrode drops for a specified time and ions drift out of the RF ion guide. Thus, the ions form packets of a spatial length determined by the pulse width and ion velocity. At the end of the gate pulse, the exit electrode potential is raised, starting another cycle of ion accumulation in the RF ion guide. Determination of the pulse width and repetition rate is explained in the next section.

Figure 2-3 shows an ESI source configuration.

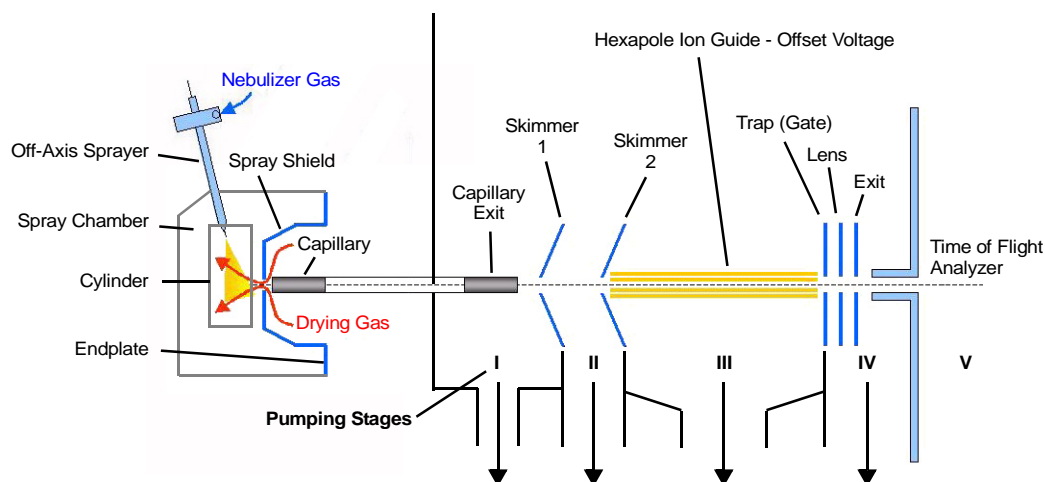


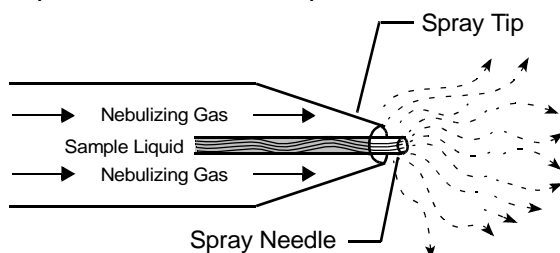
Figure 2-3 ESI Source Operation

The following table lists the system components and how they are used for ESI.

Table 2-1 ESI Source Operation by Component

Component	Function
Off-Axis Sprayer	Receives the solution of sample and solvent from a syringe pump or liquid chromatograph, passes the solution through a very fine needle, and sprays the liquid into the spray chamber.

The needle consists of two concentric tubes, the outer tube carrying the nebulizing gas and the inner tube carrying the sample liquid. The liquid emerges from the needle into the rapid flow of gas and is dispersed into small droplets.



The nebulizing gas pressure is controlled through the software and with a control knob on the ESI Source Control front panel.

The sprayer assembly including the spray needle is electrically grounded.

Nebulizer Gas	Assists in creating an even and steady dispersion of the sample as it is injected into the spray chamber. The nebulizing gas shear forces and the strong electrostatic field at the tip of the sprayer to break the sample solution into droplets. Since the sample solution is not heated when the aerosol is created, ESI ionization does not thermally decompose most analytes.
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In some low flow rate applications, particularly Nanospray, the nebulizer is not used as the electrical fields provide sufficient dispersion of the liquid.

Note: The nebulizer gas pressure is set at the Source Control panel. If a nebulizing gas is in use, ensure that *Neb Gas* is checked in the Gas/Heater Control Group to the left of the tabs.

Table 2-1 ESI Source Operation by Component (Continued)

Component	Function
Cylinder	The first of three electrodes that create an electrical field in the spray chamber.
Endplate	<p>The second of the three spray chamber electrodes. A polished metal disk centered around the entrance of the glass capillary.</p> <p>High voltage is applied to the endplate, which forms the electrostatic gradient in the spray chamber.</p>
Spray Shield	Removable metal cap that is part of the Endplate. An opening in the center of the spray shield, which is immediately in front of the capillary, allows ions to enter the capillary and forces counter-current flow of the drying gas.
Capillary Entrance	<p>The third of the spray chamber electrodes. The metallized entrance to the capillary carries a potential with the following function:</p> <ul style="list-style-type: none">• In positive ion mode, the entrance to the capillary is at -3 kV to -5 kV relative to the needle and approximately -500 V relative to the endcap. The electrostatic gradient created by these voltages supports the production of charged droplets and helps them to migrate towards the capillary entrance. Once near the entrance, the analyte ions are pushed through the capillary by the pressure gradient between the spray chamber (about 1013 mbar, or 760 Torr) and the first pumping stage (approximately 1 to 2 mbar). The ion Polarity is set with option buttons in the BioTOF window, just to the left of the Parameter tabs.

Table 2-1 ESI Source Operation by Component (Continued)

Component	Function
Capillary	<p>The capillary is:</p> <ul style="list-style-type: none"> • A sampling orifice that transfers ions from the spray chamber at atmospheric pressure to the vacuum region of the mass spectrometer • A barrier separating the atmospheric pressure region of the source from the vacuum region • Made of a dielectric material that electrically insulates the spray chamber from the vacuum side.
Drying Gas	<p>A heated gas (typically nitrogen or dry air) flowing counter-current to the spray of charged droplets that evaporates the solvent, thus decreasing the droplet diameter and forcing the charges closer together.</p> <p>While the drying gas assists in the de-solvation process, it does not thermally decompose the analytes.</p> <p>If a drying gas is in use, ensure that <i>Dry Gas</i> is checked in the Gas/Heater Control Group to the left of the tabs. The flow rate is set on the Source Control panel, and the temperature is set from the ESI Source tab in the BioTOF window.</p>
Capillary Exit	<p>The first of the vacuum-side voltages.</p> <p>The metallized exit end of the capillary is typically set in a range from 80 V to 180 V through the Capillary Exit voltage field on the ESI Source tab. As ions exit the capillary, they are electrostatically drawn towards the first skimmer which is at a lower voltage. Due to the dimensions of the capillary and the pressure gradient, the ions emerge from the end of the capillary embedded in a jet flow of neutral gas.</p> <p>High capillary exit voltage can facilitate ion fragmentation.</p>

Table 2-1 ESI Source Operation by Component (Continued)

Component	Function
Skimmers	<p>Transport and focus the ions into a beam, while neutral gas is pumped away in separate, sequential pumping stages.</p> <p>The voltages established for these ion optical components determine the ion transport efficiency.</p> <p>The two skimmers have separate voltage settings, which along with the Lens voltage setting, are found in the Lens group of the ESI Source tab.</p>
RF Ion Guide	<p>Six parallel rods that serve as an ion guide and a trap for the accumulation of ions.</p> <p>As the hexapole RF ion guide transports ions, the neutral gas is pumped away (see pumping stage II and III in Figure 2-3 on page 2-4).</p> <p>Two voltages are applied to the hexapole:</p> <ul style="list-style-type: none">• RF Amplitude, typically set at 600 V, peak to peak, with a frequency of 5 MHz.• Offset, a constant voltage applied for bias. <p>The RF Amplitude and Offset are set in the Lens group in the ESI Source tab.</p>
Trap	<p>Held at a higher voltage than the Offset voltage to prevent ions from emerging from the RF ion guide.</p> <p>The accumulation of ions in the RF ion guide increases the efficiency of the instrument.</p> <p>After a pre-set interval, the Trap voltage drops automatically to 0, and the accumulated ions pass through the Lens and Exit plates and into the TOF spectrometer.</p>
Lens	<p>Concentrates and focuses the analyte ions in the final ESI source pumping stage.</p>
Exit	<p>The final aperture in the ESI Source. The exit is held at ground potential.</p>

2.3 Nanospray

For analyzing small amounts of sample with low concentration, the ESI source can be configured with the Nanospray assembly in place of the standard BioTOF Electrospray.

For on-line Nanospray: The flow rate during operation is less than 200 nL/min

For off-line Nanospray: The flow rate during operation is lower than 20 nL/min

The analyte is delivered to the source via a small inner diameter needle positioned at the center of the source. Typically, nebulizing gas is not used. Small amount of drying gas may be used at lower temperatures. The high voltage is applied to the capillary only.

For Nanospray, the spray chamber with the off-axis Electrospray is removed from the ESI Source assembly and is replaced by a Nanospray spray chamber (Figure 2-4).

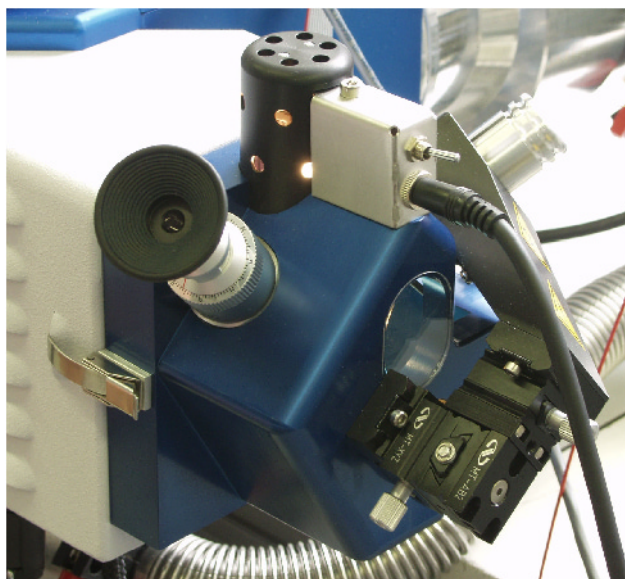


Figure 2-4 Nanospray Spray Chamber Assembly

The assembly includes an XYZ stage mounted on the front of the spray chamber.

For on-line Nanospray: The XYZ stage is connected to a NanoLC or the syringe pump.

For off-line Nanospray: The XYZ stage accepts disposable Nanospray tips.

See "Nanospray" on page 6-10 for information on setup and operation.

2.4 E-Z Nanospray

The E-Z Nanospray enables you to set up off-line nanospray without removing the standard spray chamber assembly. A nanospray needle is filled with 1 to 3 μL of the analyte solution. The needle is then placed inside the E-Z Nanospray assembly and a needle shroud.

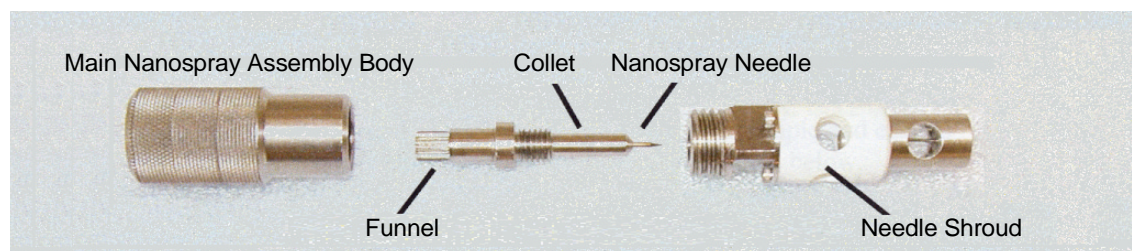


Figure 2-5 E-Z Nanospray

The assembly is inserted into the front of the ESI spray chamber.

See “E-Z Nanospray” on page 6-16 for information on setup and operation.

2.5 APCI Source Operation

Atmospheric Pressure Chemical Ionization (APCI) is a combined liquid chromatography and mass spectrometer technique, closely related to Electrospray ionization.

With the APCI Source, the ESI spray chamber is removed from the front of the source assembly and replaced with the APCI spray chamber (Figure 2-6). See “Configuring the APCI Spray Chamber” on page 6-21 for information on changing the spray chambers.



Figure 2-6 Source Configured with APCI Spray Chamber

The APCI technique involves passing LC eluent through a nebulizing needle, thus creating a fine spray. The spray is then passed through a heated ceramic tube, where the eluent droplets are fully vaporized. The resulting gas/vapor mixture is then passed over a corona discharge needle, where the solvent vapor and carrier gas are ionized to create reagent gas ions. These reagent ions in turn ionize the eluent sample molecules via a chemical ionization process.

APCI is an LC gas phase chemical ionization mechanism. APCI requires the analyte in the gas phase for proper ionization. To bring the mobile phase and analyte into the gas phase, APCI is typically done at vaporizer temperatures of 400 °C as set on the ESI Source tab.

The vaporizer temperature must be carefully controlled. Most compounds work best at higher temperatures while a few compounds work best at lower temperatures. It may be necessary to evaluate a couple of temperatures to determine the optimal APCI vaporizer temperature.

3 TOF Mass Spectrometer Operation

This chapter describes operation of the Time of Flight (TOF) Mass Spectrometer.

The major components of the spectrometer (Figure 3-1) are:

- Orthogonal Interface
- Flight Tube (Drift Region and Reflectron)
- Detector
- Digitizer

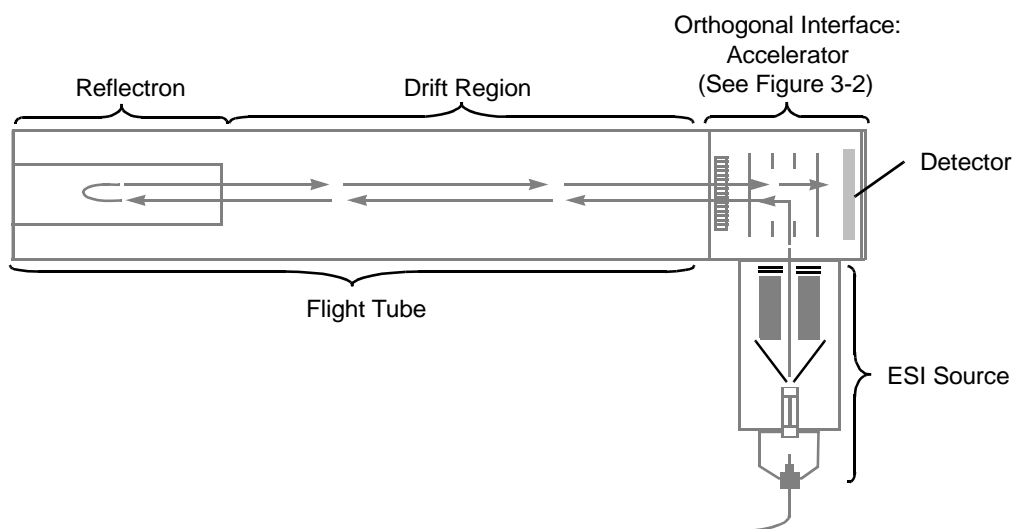


Figure 3-1 BioTOF Components

The spectrometer directs ions on a round-trip through a flight tube, measures their time of flight, and provides the digitized mass spectra data to the BioTOF software.

3.1 Orthogonal Interface

Figure 3-2 shows the orthogonal interface with the accelerator, multideflector, and detector. The interface is located immediately behind the ESI Source.

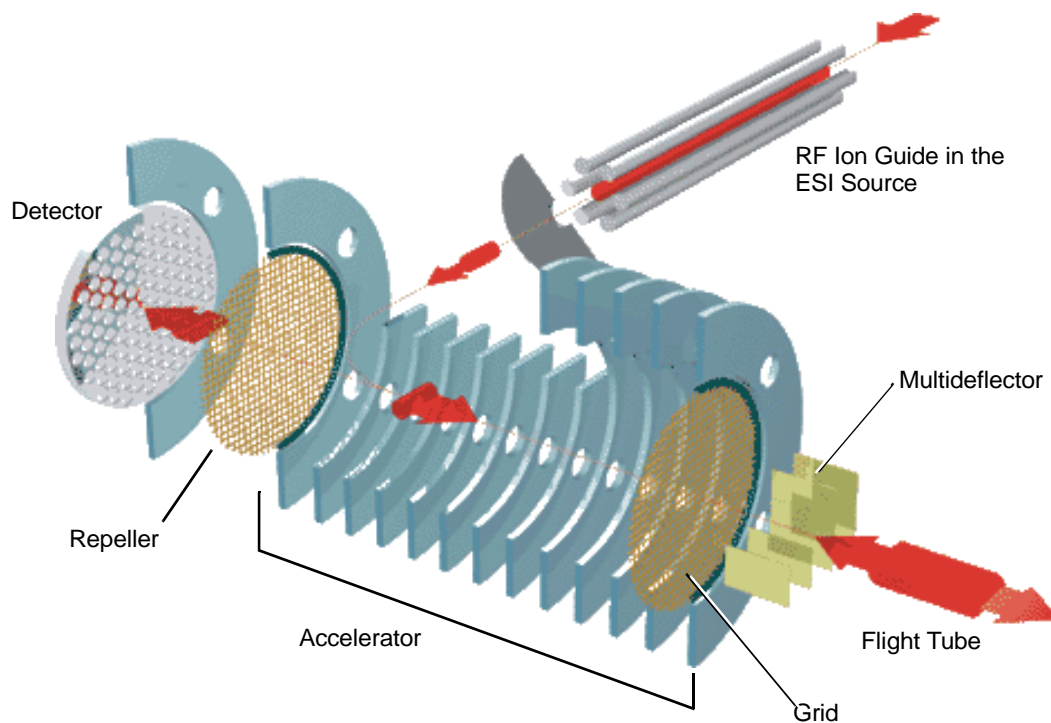


Figure 3-2 Orthogonal Interface

The packet of ions emerging from the ion guide in the ESI source is collimated by a lens electrode and travels into the orthogonal interface with a velocity related to its kinetic energy acquired by acceleration due to the offset voltage of the ion guide.

3.1.1 Accelerator

After the transfer delay, the ion packet reaches the extraction region of the accelerator. Here a high voltage pulse applied to the Repeller (Figure 3-2) changes the trajectory by 90° and pushes the ions into the mass spectrometer flight tube. The pulse coincides with the start of a digital timer that measures the time-of-flight of the packet from the accelerator through the Reflectron, and ultimately to the ion detector.

When the high voltage pulse is applied to the Repeller to accelerate the ions down the flight tube, the voltage also prevents other ions from entering the accelerator. When the packet of ions has safely entered the field-free drift region of the flight tube, the high voltage in the accelerator is turned off.

Figure 3-3 shows the Orthogonal Interface (placed on end). Note that the accelerator includes a series of gold-plated rings separated by resistors that form an homogenous field. The voltages decrease evenly from the Repeller to the grid.

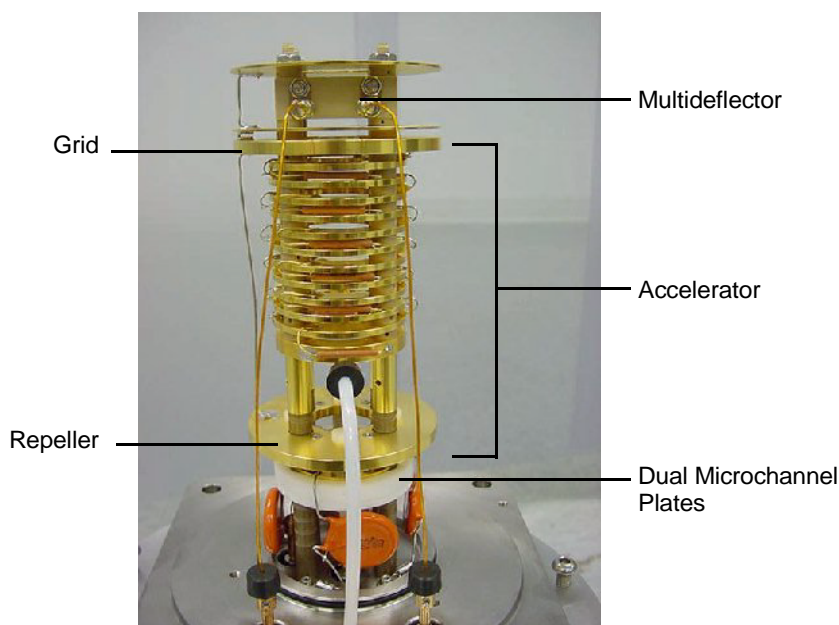


Figure 3-3 Orthogonal Interface (On End)

3.1.2 Multideflector

Before the ions enter the flight tube, they pass through the multideflector, which compensates for the initial orthogonal velocity of the ions and aligns the ion trajectories along the axis of the flight tube. The assembly consists of a series of gates placed parallel to the intended ion flight path (Figure 3-4).

Multiple deflectors are used to minimize distortion. Figure 3-4 illustrates the relative time difference for ions of the same m/z traveling through the narrow multideflector gates compared with ions traveling through a wider single gate.

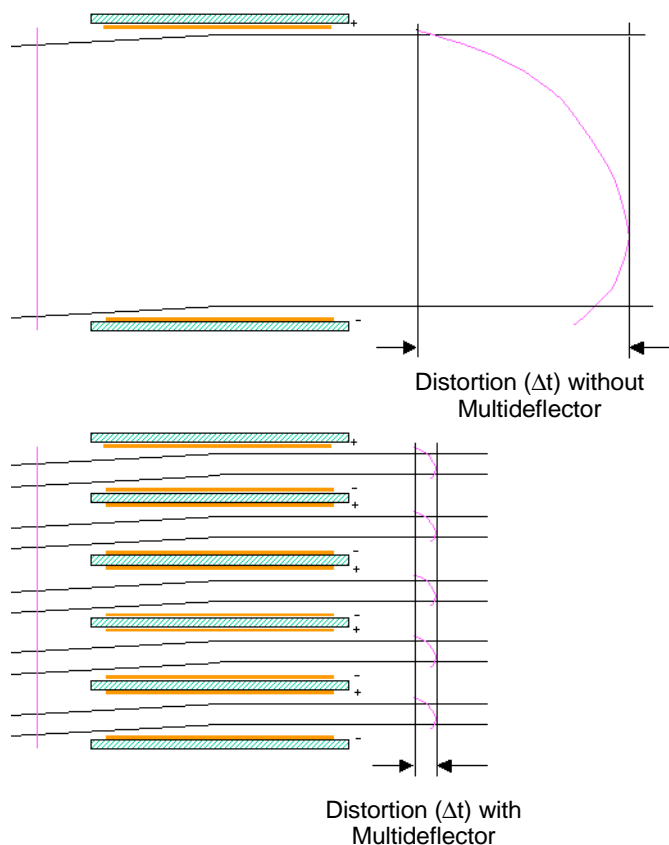


Figure 3-4 Minimized Distortion with the Multideflector

The multideflector uses two steering voltages:

Multideflector High	A pulsed voltage applied across the gates, steering ions as they leave the accelerator.
Multideflector Low	A constant voltage applied across the gates to provide steering as the ions return to the orthogonal interface and, in a multipass application, enter the flight tube again.

Multideflector High and Multideflector Low are set in the Spectrometer tab.

3.2 Flight Tube

The ions pass through the multideflector into the flight tube, which consists of a drift region and the Reflectron assembly.

3.2.1 Drift Region

Since all ions are accelerated by the same Repeller potential in the accelerator, they reach individual velocities according to their m/z values. All ions of a given m/z ratio take approximately the same amount of time to travel through the flight tube. The smaller m/z ions travel faster than those with higher m/z values; the ion packet begins to separate. The ion flight time through the tube is proportional to the square root of the ion's m/z ratio.

3.2.2 Reflectron

At the end of the drift region, the ions enter the Reflectron assembly at the far end of the tube. The assembly (Figure 3-5) consists of the Reflectron lens and a series of evenly spaced gold-plated disks with a hole in the center and separated by resistors, similar to the rings in the accelerator.

The Reflectron Lens assists in collimating the beam.

A potential higher than the Repeller accelerator voltage is applied to the far end of the Reflectron; the ions gradually slow down and eventually reverse direction.

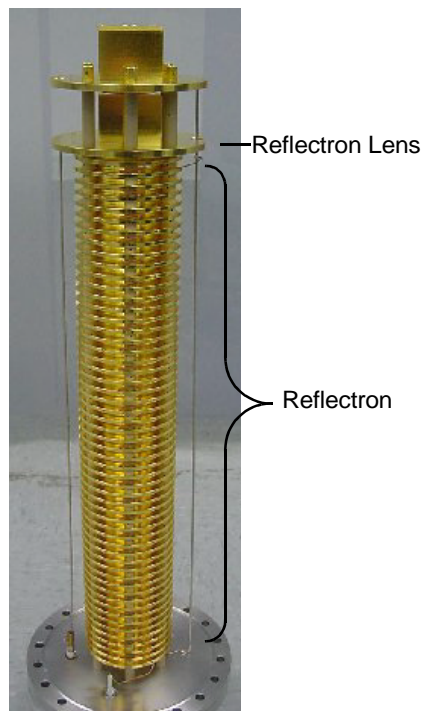


Figure 3-5 Reflectron and Reflectron Lens

The reflected ions travel back through the flight tube. In order to prevent a second deflection in the accelerator, the main steering voltage in the tube is now turned off. The ions penetrate freely through the accelerator and through the Repeller electrode as well, finally striking the detector.

The Reflectron and Reflectron Lens voltage values are set on the Spectrometer tab.

3.3 Detector

The detector consists of dual microchannel plates and an anode. The dual microchannel plates are shown in Figure 3-3 on page 3-3.

The plates are configured as a chevron, that is, the microchannels in the first plate are at an angle to the ion flight path, while the channels in the second plate are at the same angle but in the opposite direction (Figure 3-6). The microchannels are lined with a semiconducting layer.

When an ion enters a microchannel, it collides with the channel wall, causing it to release an electron. The electron then collides with the opposite wall emitting multiple electrons. As the electrons pass through the channel and the opposite-angle channel in the next plate, they create an *avalanche* of electrons.

When the electrons emerge from the microchannel plates, they are collected with a metal anode generating an electrical pulse in the electronic detection system.

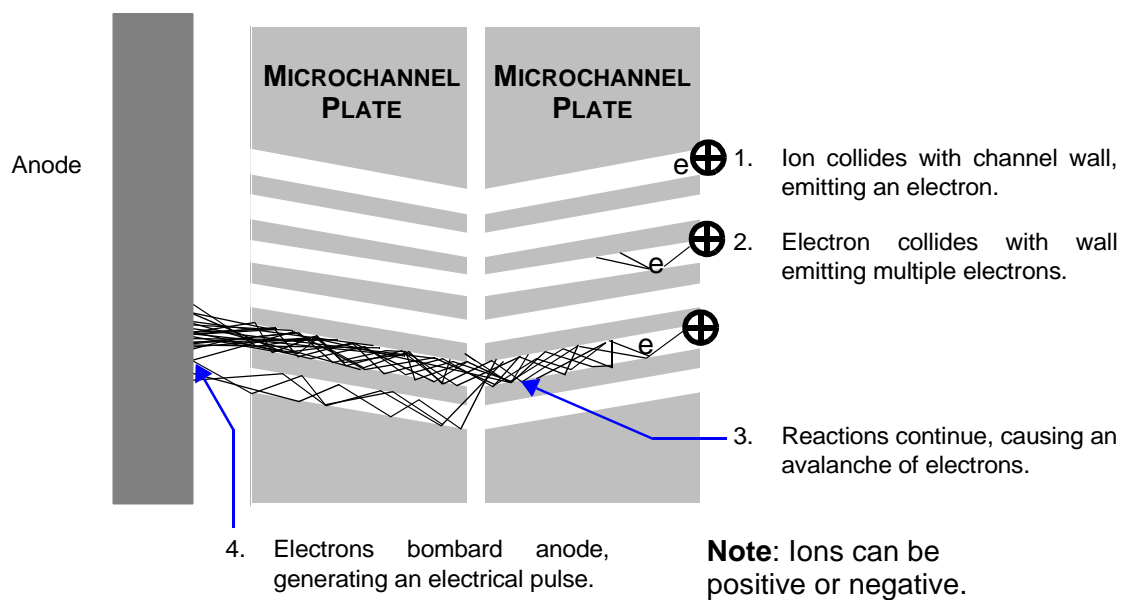


Figure 3-6 Microchannel Amplification

3.4 Digitizer

Recording of the detector signal starts after a preset digitizer delay. The digitizer delay determines the lowest m/z value in the recorded spectrum and shifts the recording window along the mass scale.

The electrical signal from the detector is converted from analog to digital for each **scan**. Multiple scans are aggregated to form a single higher density **record**. The records are summed in the digitizer to form a **spectrum** that is transferred to the workstation.

In *MS Only mode*, the spectra are further accumulated in a single buffer and the buffer contents are displayed in the BioTOF window. At the end of the data acquisition, the contents of the buffer can be saved as a single mass spectrum.

In *Chromatography - MS mode*, the transferred spectra are stored and displayed consecutively, and can be saved as time-stamped mass spectra.

The width of the recording time window is specified by either:

- Selecting a mass range, in which case the BioTOF software automatically determines digitizer delay in keeping with the other timing parameters.
- Specifying the sampling speed and the number of data points per record. The recording window is given by the time per data point multiplied by the number of points to be accumulated in each record. In this case, you must set the appropriate digitizer delay to assure that the mass range includes ion masses of interest.

3.5 Time-of-Flight Calculation

The time of flight calculation is derived as follows:

- All ions are accelerated by the same electrical field in the spectrometer. When entering the drift region, the ions have a kinetic energy equal to the electrical potential applied to the active section of the accelerator (S_0 in Figure 3-7).

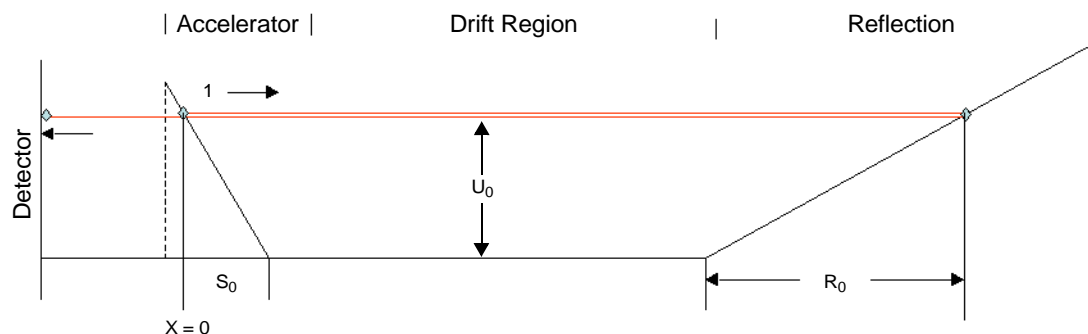


Figure 3-7 Reference Trajectory

- $E_{\text{kin}} = E_{\text{el}} = zeU_0$ where:
 E_{kin} is kinetic energy
 E_{el} is electrical energy
 z is the number of charges (charge state)
 e is the elementary charge
 U_0 is the electrical potential difference
- $E_{\text{kin}} = 1/2m v_0^2$ where:
 m is mass
 v_0 is drift velocity

- Therefore:

$$v_0 = \sqrt{\frac{2eU_0}{\frac{m}{z}}}$$

- Time of Flight:

$$T_0 = \frac{L_{\text{eff}}}{v_0}$$

where: L_{eff} is the effective length of the spectrometer

- TOF is measured in the experiment

$$T_{\text{exp}} = c_0 + c_1 \sqrt{\frac{m}{z}}$$

where: c_0 represents the fixed internal electronic propagation delay of the transistor gates, switches, and cables, and

$c_1 = \frac{L_{\text{eff}}}{\sqrt{2eU_0}}$ is the first order coefficient.

This equation establishes the basic relationship between m/z ratio and the ion's time of flight, and is used for calibration of the spectrometer. The coefficients c_0 and c_1 can be determined experimentally by measuring the time of flight of two ions with different m/z ratios. See "Calibration" on page 9-1.

3.6 Space Focusing

The accelerator and the Reflectron are tuned to compensate for the fact that ions in a given packet do not travel the same distance in the mass spectrometer. When the ions arrive in the orthogonal interface from the ESI source, they are at different distances from the Repeller. Ions closer to the Repeller have a longer round trip in the mass spectrometer than those further away from the Repeller. To ensure that all ions of a given m/z arrive at the detector at the same time, the field in the accelerator is shaped so that ions closer to the Repeller exit the accelerator with higher kinetic energy than those further away (Figure 3-8). The field in the Reflectron mirrors the accelerator field: When the ions enter the Reflectron assembly, those with the greater kinetic energy travel further into the Reflectron before turning around than those with lower kinetic energy.

Space Focusing

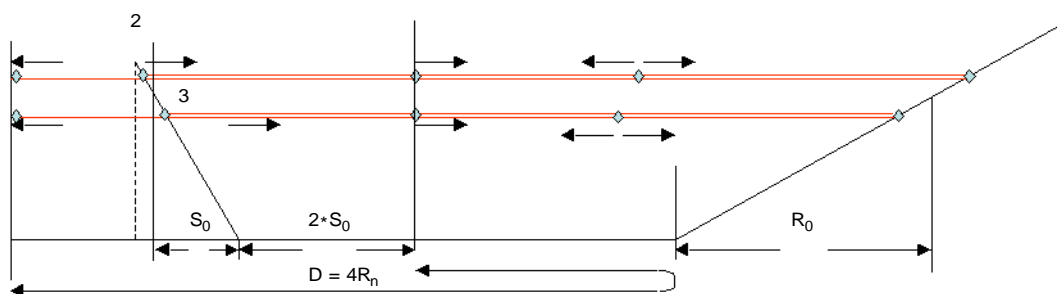


Figure 3-8 Space Focusing in Single Pass Mode

Initially a packet of ions (of identical m/z) in the accelerator is dispersed over a small but finite width in the direction of the flight tube, with some ion being further back (2) and others (3) being further ahead in the accelerator. When the high voltage pulse is applied the ions starting further back gain slightly higher kinetic energies than the ions which started further down. As a result, these ions will have a higher drift velocity in the field free region of the spectrometer and catch up with the slower ions at a point in the drift region. For a single stage accelerator this point is at a distance $2S_0$ away from the accelerator.

A similar process is repeated in the Reflectron: The ions with higher kinetic energy and velocity penetrate deeper into the Reflectron than the ions with lower kinetic energy, and thus spend more time in the Reflectron. Longitudinal space focusing is achieved if the total drift length D measured from the first point of passing at $2S_0$ is equal to four times the mean Reflectron penetration depth ($D=4R_0$).

3.7 Multipass

Multipass is a BioTOF method that enables greater resolution for a specified mass range.

In multipass, the Repeller potential is held high after the ions enter the flight tube. Thus, when the ions return from the Reflectron, they are reflected back into the flight tube for another round trip. After the ions have made the additional round trips in the flight tube, the Repeller voltage is dropped and the ions pass through the accelerator and to the detector (Figure 3-9).

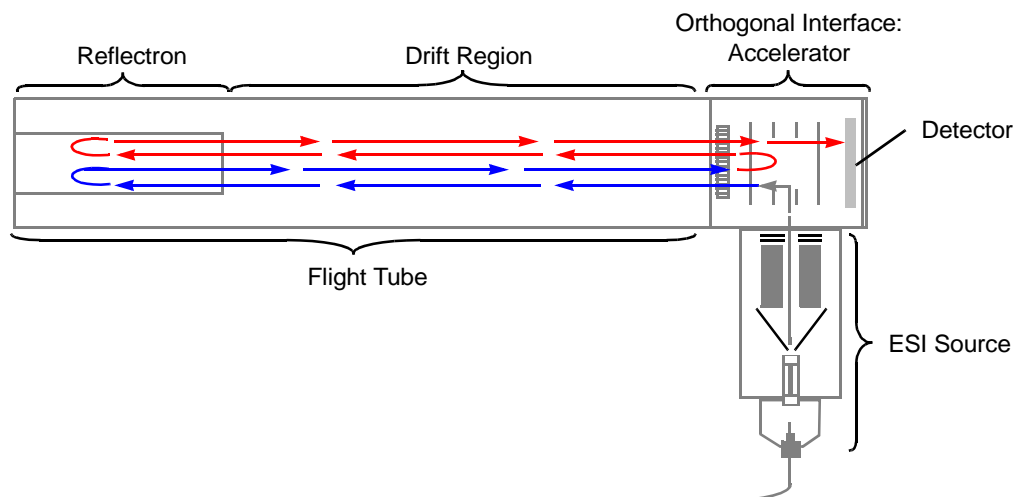


Figure 3-9 Ion Path in Multipass Mode

Multipass effectively creates a much longer instrument. Refer to “Multipass” on page 8-7 for information on setting up multipass.



BioTOF *Series*

Operator Manual Volume II: Operation



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WARNING

Connecting an instrument
to a power source that is
not equipped with a
protective earth contact
creates a shock hazard
for the operator and can
damage the instrument.
Likewise, interrupting the
protective conductor inside
or outside the instrument
or disconnecting the
protective earth terminal
creates a shock hazard for
the operator and can
damage the instrument.

WARNING

The instrument must be
disconnected from its power
source before any cover is
removed or the system is
opened.

WARNING

All connections of the
instrument must be used in
correct way. The instrument
should only be used with
the wires and cables
delivered with the system
or otherwise provided by
the manufacturer.

Noise Declaration

70 dB operator position
normal operation per
ISO 7779: 1988

Instrument Identification

Each instrument is
identified by a unique
serial numbers. These
serial number is located on
a label on the lower left side
near the rear of the
instrument.

When corresponding with
Bruker Daltonics Inc. about
your instrument, be sure to
include the model and the
full serial number.

Write the serial number
of the instrument here for
reference.

Model:

Serial Number:

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BioTOF Series Operator Manual, Version 1.0, Volume II: Operation

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Preface

This document provides detailed information on using the BioTOF system. It consists of two volumes:

- *BioTOF Operator Manual, Volume 1, Theory*
- *BioTOF Operator Manual, Volume 2, Operation*

Three additional manuals in this document set provide information on using the analytical software included with the BioTOF system.

- *DataAnalysis User Manual*
- *QuantAnalysis User Manual*
- *Library Editor User Manual*

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In the U.S.: +1 978 663 3660, Ext. 1445

In Germany: +49 (0) 421 2205 430

In Japan: +81 (0) 45 440 0471

Local Service:

Safety Labels

These labels are placed on the instrument to indicate the respective hazard possibility.



Hazard: Note

Refer to the Operator Manual for details on this hazard.



Warning: High Voltage

A dangerous high voltage or shock hazard exists within this area. Only trained service persons should perform work in this area.



Warning: High Temperature

Hot parts and surfaces are present in this area. Allow the part or surface to cool before performing work in this area. This may require turning off power to selected components or to the entire instrument.



Warning: Biohazard

A biohazard may exist within this instrument or specific area. Handle this biohazard with appropriate caution and protection.

4 System Startup

This chapter describes how to start the BioTOF system.

After installation and calibration, the system is normally placed in standby mode with the pumps running rather than being completely powered down, as it can take several hours to pump down the TOF chamber below the pressure threshold. If this system is already in standby mode, use the instructions in this chapter section to verify that the system is ready for use.

4.1 Start the System

To start the BioTOF system:

1. Open the front cabinet door to access the BioTOF control panels (Figure 4-1).



Figure 4-1 BioTOF Control Panels

The Main Control panel (Figure 4-2) on the upper left contains four pushbutton switches which are illuminated when they are engaged.

MAIN	The main power supply for the ESI Source and TOF Spectrometer.
VACUUM PUMPS	Pump controller
ELECTRONICS	All system electronics.
COMPUTER	Three optional power connections on the bottom left side of the console, one of which can be used to supply power to the computer workstation.



The buttons illuminate when pressed to engage the switches.

Figure 4-2 Main Switch Panel

2. Press **MAIN** pushbutton.

The **MAIN** is illuminated (red) to indicate the main power is On.

3. Press the **VACUUM PUMPS** pushbutton to start the vacuum pumps

When this button is pressed, it lights up and the pumps start sequentially. However, the system will not be ready until the pressure in the vacuum system has dropped below threshold. This may take several hours.

4. Press the **ELECTRONICS** button to initialize the system electronics including the vacuum gauge controller (Figure 4-3).

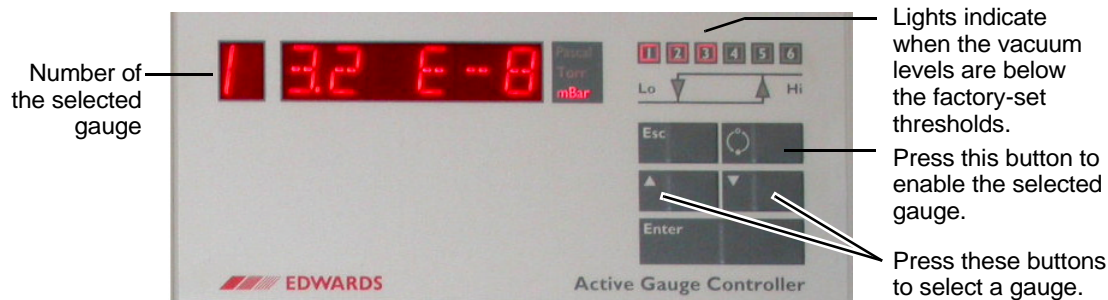


Figure 4-3 Vacuum Gauge Controller

5. Press the ▲ and ▼ buttons on the vacuum gauge controller to cycle through the readouts for the gauges used in the BioTOF system:
 - Gauge 1 reads the flight tube pressure; this gauge is initially off.
 - Gauge 3 reads the pressure in the first pumping stage of the ESI source.

6. Select **Gauge 1** and press the **ON/OFF** button on the gauge controller.

The flight tube pressure must be below 5×10^{-7} mbar to engage the pressure interlock of the high voltage supplies.

7. Select **Gauge 3**.

When the inlet to the vacuum system is open, the pressure should be between 1.5 and 1.9 mbar. When the inlet to the vacuum system is closed, the pressure should be less than 10^{-1} mbar.

The indicators labelled 1 through 6 in the upper right corner of the Vacuum Gauge Controller are lit when the vacuum levels are below the factory-set thresholds. When indicators 1, 2 and 3 are on, you can safely turn the BioTOF system on. Indicators 4, 5 and 6 are not used.

Refer to the separate Vacuum Gauge Controller documentation for information on the controller menu for changing units of measure and making other adjustments to the controller display.

8. Check the **Power Switch** in the lower right corner of the Source Control to make sure that the controller is turned on (Figure 4-4).

The Source Control unit LCD displays the default startup menu.

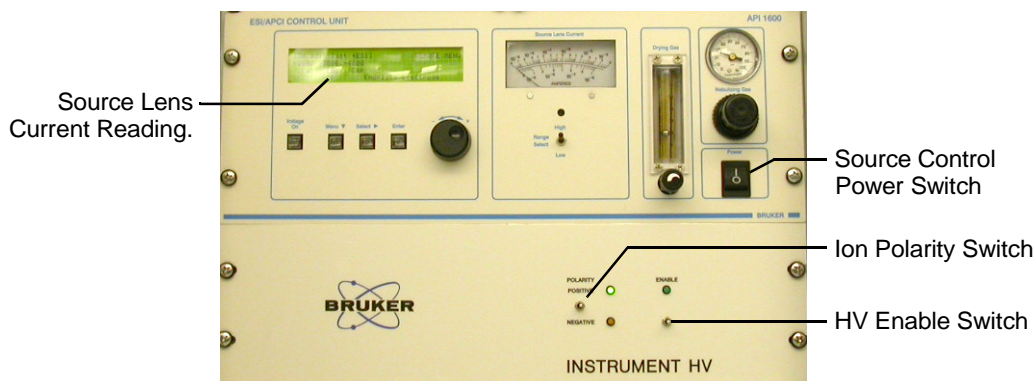


Figure 4-4 Source Control Unit (top) and Instrument HV Panel

9. Use the arrow button below the LCD display to position the display cursor on **End** (Figure 4-5) so that the Source Lens Current gauge on the ESI Source reads the current on the **Endplate Electrode**.

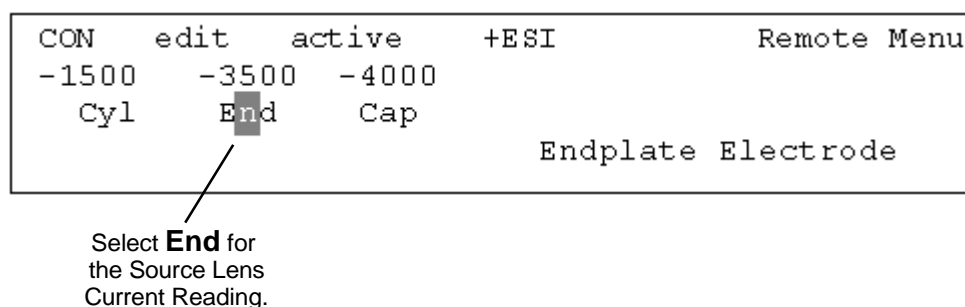


Figure 4-5 Setting the Source Lens Current Reading

10. Set the ion **Polarity** and **Enable** switches on the Instrument HV unit to the middle position.

When the two 3-position switches on the Instrument HV panel (Figure 4-4) are in the middle position, the unit is remotely controlled from the BioTOF window.

11. Check the nitrogen supply.

4.2 Check the Spray Chamber and Sprayer

The next step is to check the Sprayer Assembly on the front of the ESI Source. The assembly is attached to the ESI Source with hinges on the right and locked with a latch on the left. A sample is introduced to the spray chamber via an off-axis spray needle which is held in position by a bayonet connection. Figure 4-6 shows the sprayer assembly unlatched and open, and the off-axis sprayer removed from the assembly.

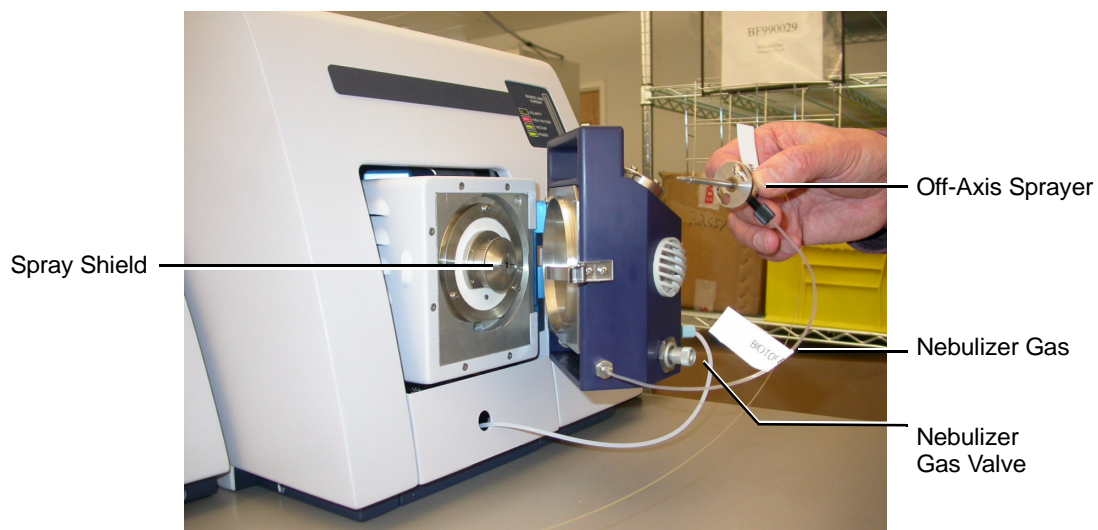



Figure 4-6 Off-Axis Sprayer Assembly

To check the Spray Chamber:

1. Release the latch on the left side of the assembly and swing the assembly out so that the spray shield (Figure 4-6) is visible.
2. Remove the spray shield, remove any plug or rubber seal from the end of the capillary tube, and re-install the spray shield.
3. Remove the off-axis sprayer assembly from the top of the spray chamber, verify that the tip of the inner needle protrudes about 0.5 mm from the body of the sprayer, and reinstall the sprayer.
4. Make sure that the sprayer assembly is connected to the nebulizer gas supply, and turn the gas valve counterclockwise until it is fully open.
5. Close the spray chamber and secure the latch on the left side to engage the source voltage interlock.

4.3 Start the BioTOF Software

To start the BioTOF control software:

1. Turn the workstation on.
2. Do one of the following when the system is initialized:
 - Double-click the BioTOF desktop or tool bar icon ().
 - Choose **Start > All Programs > BIOTOF > BioTOF**.

Refer to Chapter 5, BioTOF Window for a complete description of the user interface.

4.4 Turn the Gases On

The next step is to set the nebulizer and drying gas controls.

To turn the gases on:

1. Click the **Dry Gas** and **Neb Gas** checkboxes in the Gas/Heater Control group (Figure 4-7), if they are not already on.
2. Click the **DG Heat** checkbox in the Gas/Heater Control group to turn the heater on.
3. Click the **ESI Source** tab, and set the Dry Gas Temperature to **200 C**.

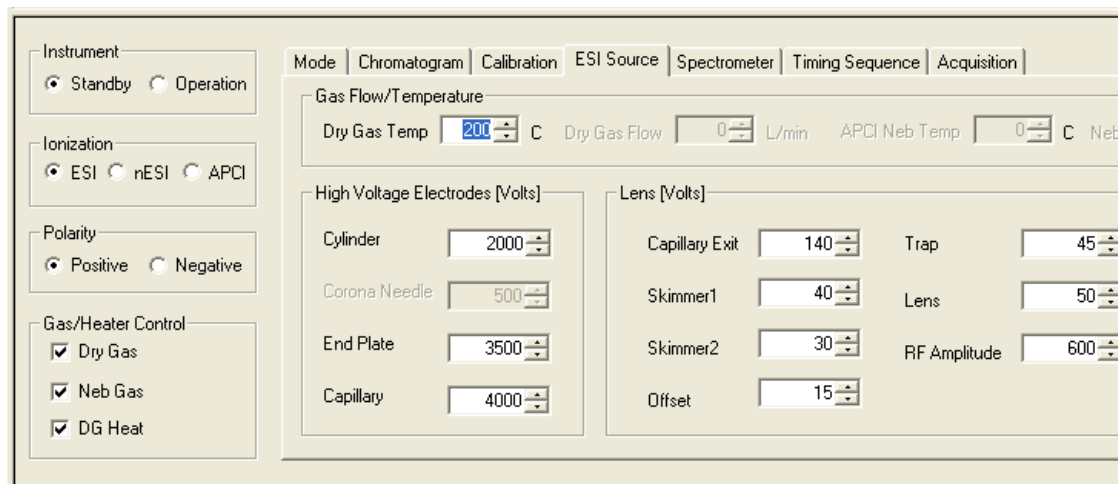


Figure 4-7 Gas/Heater Group and ESI Source Tab

4. At the Source Control panel, set the nebulizer gas pressure to approximately **30 psi**.
5. Set the drying gas flow so that the ball is at about 1/3 scale (see Figure 4-8).



Figure 4-8 Nebulizer and Drying Gas Controls


6. Review Chapter 5, BioTOF Window for a description of the user interface and Chapter 6, Introducing Samples for a information on sample introduction to various ionization methods.

5 BioTOF Window

This chapter gives a detailed description of the BioTOF window including the menus, tool bars and options.

5.1 Window Layout

To view the BioTOF window:

1. Turn the workstation on.
2. Do one of the following when the system is initialized:
 - Double-click ().
 - Choose **Start > All Programs > BIOTOF > BioTOF**

The BioTOF window appears (Figure 5-1). Once you have started the application, you can leave it running, minimizing the window when you are not using the BioTOF system.

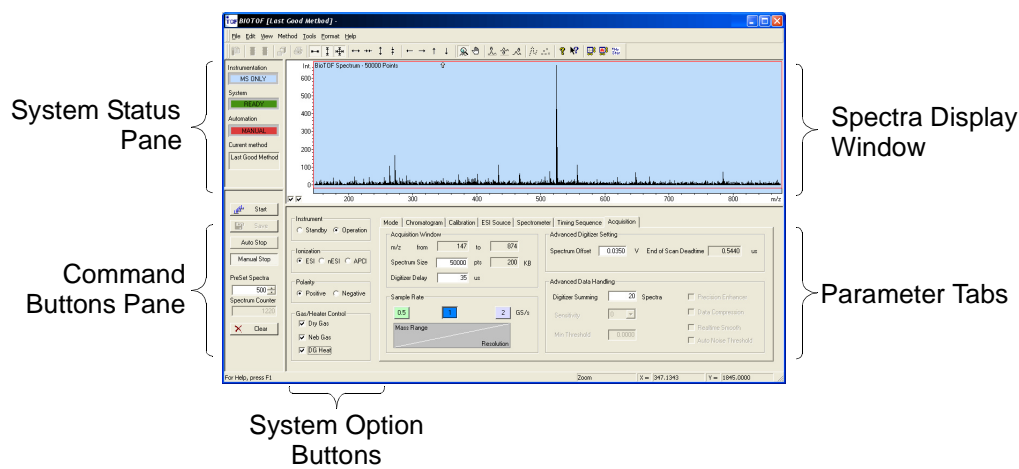


Figure 5-1 Layout of the BioTOF Window

The BioTOF window displays information and controls in five different panes:

- The **Spectra Display** plots the spectra captured by the system. There are a variety of user-selected formats. For example, the graph in Figure 5-2 shows the intensity of ion signals from the detector for each m/z value. The two checkboxes in the bottom left corner of the display control autoscaling for the vertical axis (left) and horizontal axis (right).
- The pane on the upper left side of the window has system status indicators.
- The pane on the lower left side has command buttons for starting and stopping spectra acquisition.
- The four groups immediately to the right of the command buttons includes option button and checkboxes for controlling system operation.
- To the right of these controls are seven tabs for setting system parameters for acquisition and display of the spectra.

Figure 5-2 shows the location of the Autoscaling checkboxes.

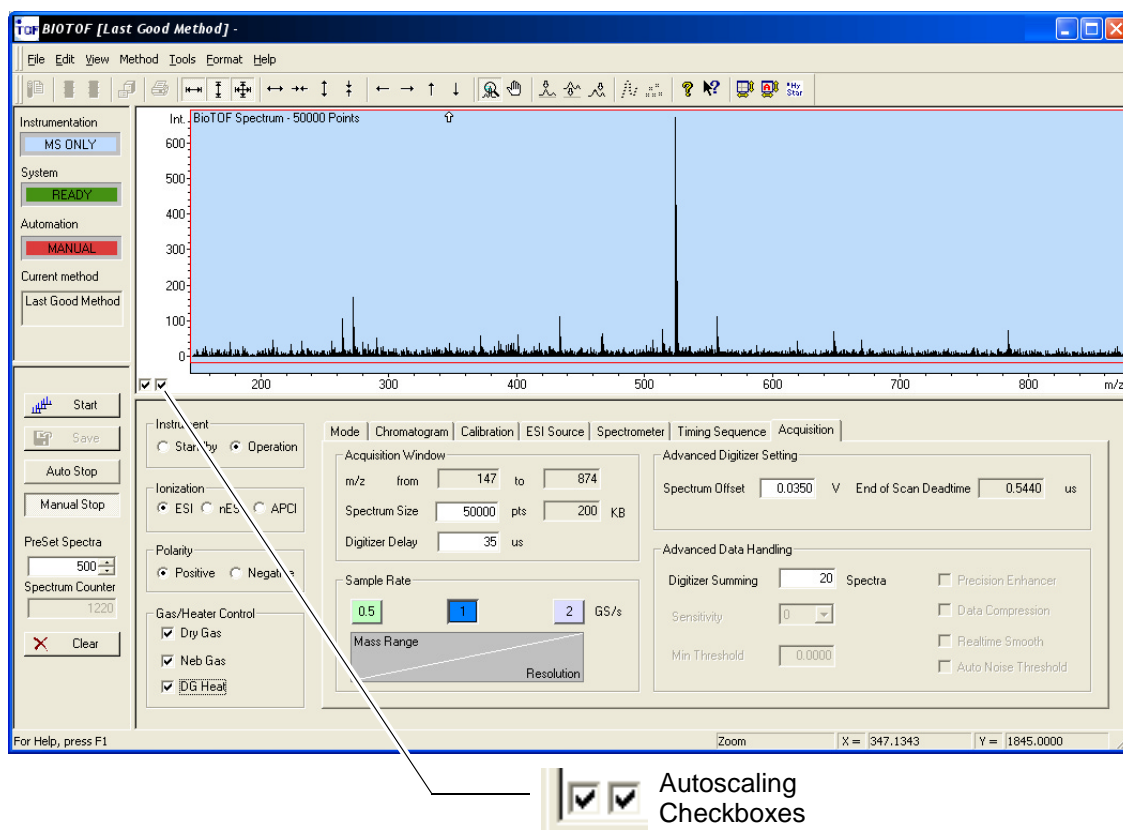


Figure 5-2 BioTOF Window and Autoscaling Checkboxes

5.1.1 Status Indicators Group

The four fields in the Status Indicators group (Figure 5-3) provide essential information about the current status of the BioTOF system:

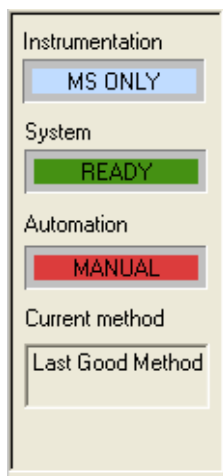


Figure 5-3 Status Indicators

Table 5-1 Status Indicator Descriptions

Function	Description
----------	-------------

Instrumentation	Displays one of three instrumentation modes as set in the Mode tab:
-----------------	---

- **MS ONLY** for mass spectrometry only
- **LC - MS** for chromatography and mass spectrometry when the sample is supplied from an LC system, and you want to display a chromatogram and the mass spectra. The Spectra display window is divided into two panes, the top one showing the spectra and the lower one showing ion chromatograms.
- **Injector - MS** for time controlled mass spectrometry. FIA-MS (Flow injection analysis mass spectrometry) has been selected in the Mode tab to specify a run time. With this mode, you set up the run in the Chromatographic Setting group by specifying the total run time in minutes and a wait time for the solvent to clear.

Table 5-1 Status Indicator Descriptions (Continued)

Function	Description
System	The system modes are indicated by: Yellow - STANDBY Green - READY Red - Not Ready Blue - Running
Automation	Automatic when the instrument is in Injector - MS mode. Otherwise, Manual.
Current method	Identifies the method in use. Initially the field displays "Last Good Method," the settings in use when the BioTOF application was closed. When you load a saved method the Current method displays the method file name. See "Methods" on page 7-1.

5.1.2 Command Buttons Group

Figure 5-4 describes the buttons and fields in the Command Buttons group.

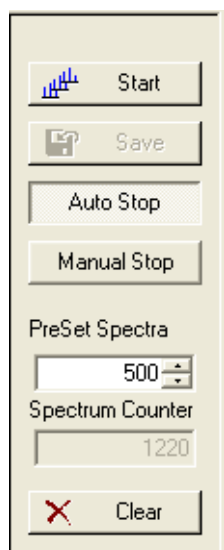


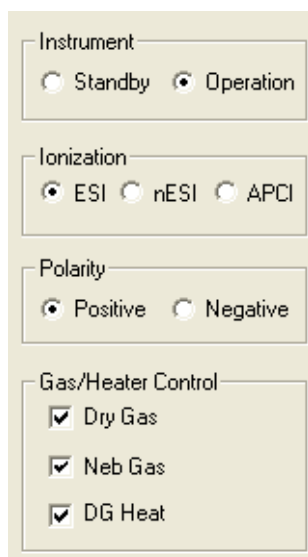
Figure 5-4 Command Buttons

Table 5-1 Command Button Descriptions

Function	Description
Start/Stop	<p>The Start button is enabled when the BioTOF system is in Operation mode. Click Start to begin spectra acquisition. The button changes to Stop when the system is acquiring data, and changes back to Start when acquisition has been stopped either manually or automatically.</p> <p>In MS Only mode, you can stop acquisition and then click Start again to continue acquisition.</p> <p>In Chromatography - MS mode and FIA - MS mode stopping and restarting acquisition clears the buffer and Spectra display.</p>
Save	<p>Save is active when data acquisition has been stopped. Click Save to save spectra from the current session. The spectra are saved in the file formats selected from Format menu as described in "Format Menu" on page 5-26.</p>
Auto Stop Manual Stop	<p>When AutoStop is selected, the spectra acquisition stops automatically once the system has acquired the number of spectra specified in PreSet Spectra.</p> <p>When Manual Stop is selected, data acquisition continues until you click the Stop button. When the system has collected the number of PreSet Spectra, incoming records replace the oldest records in the buffer.</p> <p>Auto Stop and Manual Stop buttons are only active when the Instrumentation mode is MS Only.</p>
PreSet Spectra Spectrum Counter	<p>Use PreSet Spectra to specify the number of records to be accumulated.</p> <p>The Spectrum Counter shows the actual number of records acquired in the current session.</p>
Clear	<p>Clears records from the buffer and display, resets the Spectrum Counter.</p>

5.1.3 System Control Groups

Figure 5-5 describes the option buttons in the four system control groups to the left of the parameter tabs.



The image shows a software interface with four distinct control groups, each enclosed in a light-colored rectangular box. The groups are labeled as follows:

- Instrument:** Contains two radio buttons. The first is labeled "Standby" and is unselected. The second is labeled "Operation" and is selected (indicated by a black dot in the center of the circle).
- Ionization:** Contains three radio buttons. The first is labeled "ESI" and is selected. The second is labeled "nESI" and is unselected. The third is labeled "APCI" and is unselected.
- Polarity:** Contains two radio buttons. The first is labeled "Positive" and is selected. The second is labeled "Negative" and is unselected.
- Gas/Heater Control:** Contains three checkboxes. The first is labeled "Dry Gas" and is checked (indicated by a black checkmark in the box). The second is labeled "Neb Gas" and is checked. The third is labeled "DG Heat" and is checked.

Figure 5-5 System Control Groups

Table 5-2 System Control Group Option Descriptions

Function	Description
Instrument	Sets the instrument mode to Standby HV off, but otherwise ready to acquire data) or Operation (ready to start spectra acquisition).
Ionization	Identifies the source as electrospray ionization (ESI), nanospray (nESI), or atmospheric pressure chemical ionization (APCI).
Polarity	Selects positive or negative ion polarity. The various high voltages in the system are automatically set to the appropriate polarity. The Polarity switch on the Instrument HV panel must be in the neutral position to enable control from the BioTOF window.
Gas/Heater Control	Toggles dry gas and nebulizer gas flow, and dry gas heating. Flow rates are set on the Source Control Unit. Dry gas temperature is set with the ESI Source tab in the BioTOF window.

5.1.4 Spectra Display Window

When Instrumentation is set to MS Only or FIA - MS, the Spectra Display window includes a single pane displaying the accumulated records for each m/z value (Figure 5-6). Units for the horizontal axis can be set to m/z , flight time, or point (see “Chromatogram Tab” on page 7-6).

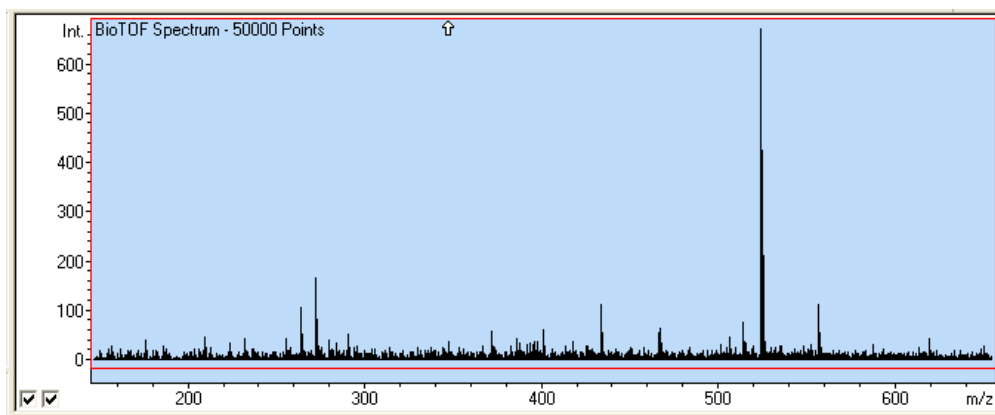


Figure 5-6 Spectra Display Window for MS Only and FIA - MS

When Instrumentation is set to Chromatography - MS, the window also includes the Chromatogram pane that displays ion counts on a time scale (Figure 5-7). In this mode, the display units in the MS pane can only be m/z .

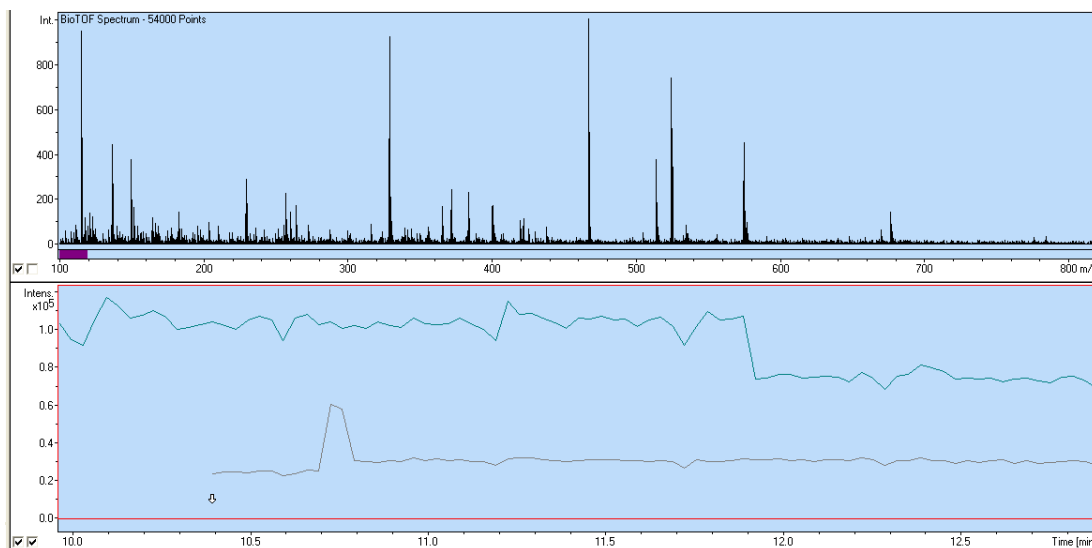


Figure 5-7 Spectra Display Window in Chromatography - MS Mode

5.2 Spectrum Display Pop-up Menu

Several of the Spectrum Display functions in the tool bar are also available from a pop-up menu in the Spectrum Display Window.

To access the menu:

- Right-click anywhere in the Spectrum Display.

The context menu displays the functions listed in Table 5-3.

Table 5-3 Spectrum Display Window Pop-Up Menu Items




Function	Description
Mouse Zoom Rectangle	Enables you to define an area and then zoom in to display that area by dragging the cursor from one corner of the area you want to zoom in on to the opposite corner and then releasing the mouse button.
Mouse Shift	Changes the cursor to a hand icon () in the Spectra display, enabling you to scroll the display by dragging the hand vertically or horizontally.
Mouse Zoom Shift	
Mouse Max Cursor	Changes the cursor to an arrow () and snaps to the next highest peak to the left with each left mouse click.

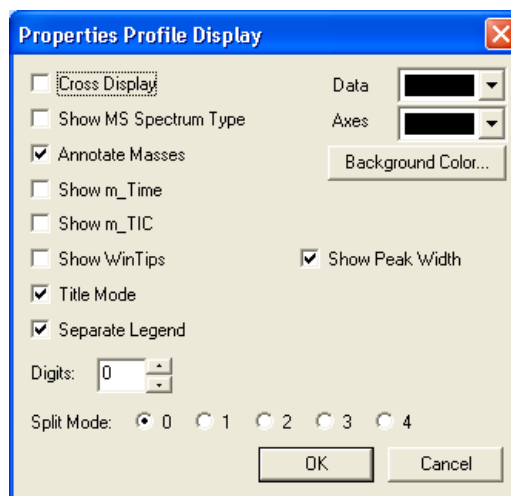
Table 5-3 Spectrum Display Window Pop-Up Menu Items

Function	Description
Mouse Data Cursor	Changes the cursor to an arrow (). The arrow rides along the trace as you move the mouse to the right or left.
Freeze	Stops updates to the display, while data is still collected in the background. Choosing Freeze a second time refreshes the display with current data and resumes real time updates.
Background Color	Opens a standard Windows dialog box for selecting the background for the Spectrum Display (Figure 5-8).

**Figure 5-8 Spectrum Display Background Dialog Box**

Table 5-3 Spectrum Display Window Pop-Up Menu Items

Function	Description
Properties	<p>Opens the Properties Profile dialog box for setting characteristics for the Spectra Display.</p> <p>The properties dialog box (Figure 5-9) for the profile display sets various display characteristics in the Spectra Display window.</p>

**Figure 5-9 Properties Profile Display Dialog Box**

Cross Display	Toggles between the use of crosses and dots to display data points. Use the crosses to make actual data point more obvious.
Show MS Spectrum Type	Replaces the default legend (Profile Spectrum Window).
Annotate Masses	Creates a legend showing the number of points in the mass spectrum.

Table 5-3 Spectrum Display Window Pop-Up Menu Items

Function	Description
Show m_Time	Creates a legend showing the time of the last spectra acquisition.
Show m_TIC	Creates a legend showing the total ion count.
Show WinTips	Enables tooltips, in which the value of data is displayed when you place the cursor over a data point in the display.
Title Mode	Toggles the display of a title bar above the Spectra Display window.
Separate Legend	Creates a separate bar above the Spectra display window for legends that would otherwise appear in the upper left corner of the window.
Digits	Sets the number of digits to the right of the description in the display of data points in the Spectra Display window.
Split Mode	Creates a separate legend above the MS pane rather than displaying the legend data in the top left corner of the MS pane.
Data	Lists the possible colors for the traces and data points.
Axes	Lists the possible colors for the axes.
Background Color	Opens a Windows Color dialog box for selecting the background color for both panes in the Spectra Display window.
Show Peak Width	Displays the width of peaks in Dalton.

Note: Some functions are not available in earlier versions of the BioTOF software.

5.2.1 Parameter Tabs

The seven tabs below the Spectra Display window set the parameters for the collection method:

Tab	Description
Mode	Sets the instrument mode, display units, and mass range.
Chromatogram	Defines a range of ion counts to mark the chromatogram display when Chromatography - MS mode is selected.
Calibration	Calibrates the BioTOF system using reference tables.
ESI Source	Specifies gas temperatures and voltage levels for the ESI Source.
Spectrometer	Sets voltages applied to various components in the mass spectrometer.
Timing Sequence	Sets advanced timing parameters for the mass spectrometer.
Acquisition	Sets sampling rate, spectrum size and other data acquisition parameters.

For more information, see “Methods” on page 7-1.

5.3 Menus and Tool Bar

This section lists the BioTOF functions available from the menu bar and tool bar (Figure 5-10).

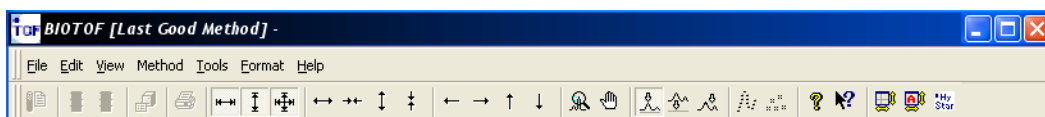



Figure 5-10 BioTOF Menu Bar and Tool Bar

5.4 File and Edit Menus

Exit is the only active item in the File menu; the Edit menu is not used in the BioTOF application.

To close the BioTOF window:

- Choose **File > Exit** from the menu bar, or click  in the upper right corner of the window.

5.5 View Menu

5.5.1 Tool Bar

- Choose **View > Tool Bar** to toggle the display of the BioTOF tool bar (Figure 5-10) on and off.

The tool bar includes functions for controlling the Spectra Display properties and for quick access to functions in the Method and Tools menu. The icons are described in Table 5-4.

Table 5-4 BioTOF Tool Bar Icons





Icon	Function	Description
	Select Method	Displays a Windows Open dialog box for loading a previously saved method. See “Load Method” on page 5-18.
	Start	Starts spectra acquisition.
	Stop	Stops spectra acquisition.
	Get Active Values	Retrieves parameter values from the BioTOF drivers so that tab displays reflect values actually being sent to the system controllers.

Table 5-4 BioTOF Tool Bar Icons (Continued)












Icon	Function	Description
	Print	Opens a Print dialog box for selecting a printer, setting print properties, and printing the current spectra. See “Load Method” on page 5-18.
	Autoscale X Axis	Automatically scales the horizontal axis in the Spectra Display to show the full range of values. Equivalent to checking the left checkbox in the bottom left corner of the Spectra Display.
	Autoscale Y Axis	Automatically scales the vertical axis in the Spectra Display to show the full range of values. Scale increases as additional records are accumulated. Equivalent to checking the right checkbox in the bottom left corner of the Spectra Display.
	Autoscale Both Axes	Automatically scales both axes of the Spectra Display. Equivalent to checking both checkboxes in the bottom left corner of the Spectra Display.
	Increase X Range	Increases the scale of the horizontal axis without changing the vertical axis.
	Decrease X Range	Decreases the scale of the horizontal axis without changing the vertical axis.
	Increase Y Range	Increases the scale of the vertical axis without changing the horizontal axis.
	Decrease Y Range	Decreases the scale of the vertical axis without changing the horizontal axis.
	Scroll Left	Scrolls the Spectra Display to the left.
	Scroll Right	Scrolls the Spectra Display to the right.
	Scroll Up	Scrolls the Spectra Display up.

Table 5-4 BioTOF Tool Bar Icons (Continued)

















Icon	Function	Description
	Scroll Down	Scrolls the Spectra Display down.
	Zoom	<p>Places the Spectra Display in Zoom mode. The display zooms in on the area you mark with the cursor. To mark the area, drag the mouse cursor from the top left corner of the area to the bottom right corner. As you drag the cursor, a dotted line defines a rectangular area of interest. When you release the cursor, the area is enlarged to fill the Spectra Display.</p> <p>Zoom mode can also be invoked by right-clicking anywhere in the Spectra Display and choosing Zoom Rectangle from the pop-up menu.</p>
	Shift	Changes the cursor to a hand icon () in the Spectra display, enabling you to scroll the display by dragging the hand vertically or horizontally.
	Max Cursor	Changes the cursor to an arrow () and snaps to the next highest peak to the left with each left mouse click.
	Perpendicular Cursor	Changes the cursor to an arrow (). The arrow rides along the trace as you move the mouse to the right or left.
	Free Cursor	Changes the cursor to the default cursor used by the computer. Free mode allows you to place the cursor anywhere in the Spectra Display. The cursor may leave the screen when you zoom in.
	Display Dots	Toggles the trace line in the Spectra Display on and off. When the line is off, the dots representing data points are visible. Use Displays Dots to distinguish between a peak (consisting of multiple data points) and a spike (a single point that could be the result of system noise or another anomaly).

Table 5-4 BioTOF Tool Bar Icons (Continued)

Icon	Function	Description
	Display Crosses	Toggles between the use of crosses and dots to display data points. Use the crosses to make actual data points more obvious.
	About TOF Control	Displays version and copyright information for the BioTOF Control software.
	Context Sensitive Help	Launches BioTOF Help
	Default Size - Automatic Mode	Hides the Parameter tabs so that more of the Spectra Display is visible. Drag the bottom edge of the Spectra Display window up to bring the tabs into view.
	Default Size - Advanced Mode	Includes the Parameter tabs in the default BioTOF display. Drag the bottom edge of the Spectra Display window down to hide the tabs.
	Start HyStar	Launches the HyStar Software for Chromatography and Hyphenated Experiments application, placing the BioTOF system under the control of the HyStar application until you exit that application.

5.5.2 Status Bar

The Status Bar displays the following information:

- A short description of the currently selected function or field.
- Any Spectra Display options (such as Max Cursor) that have been selected from the tool bar.
- The X and Y coordinates of the cursor in the Spectra Display window.

To toggle the display of the status bar at the bottom of the window:

- Choose **View > Status Bar**.

5.5.3 Resize Dialog

The two options available from the Resize Dialog determine the initial size of the Spectra Display window:


- Choose **View > Resize Dialog > Automatic Mode** to hide the Parameter tabs so that more of the Spectra Display is visible. Drag the bottom edge of the Spectra Display window up to bring the tabs into view.
- Choose **View > Resize Dialog > Advanced Mode** to include the Parameter tabs in the default BioTOF display. Drag the bottom edge of the Spectra Display window down to hide the tabs.

5.6 Method Menu

When the BioTOF window is opened, the application loads **Last Good Method**, that is, the system settings and parameters that were in use when the system was closed. The Method menu allows you to save the current method to a file and load previously saved methods.

5.6.1 Load Method

To load a previously saved method:

1. Choose **Method > Load Method** from the menu bar, or click () in the tool bar.

An Open dialog box displays the methods that have been saved in **..\Data\Methods** folder (Figure 5-11). The files in the folder are filtered to include only those with the extension ***.tofpar**.

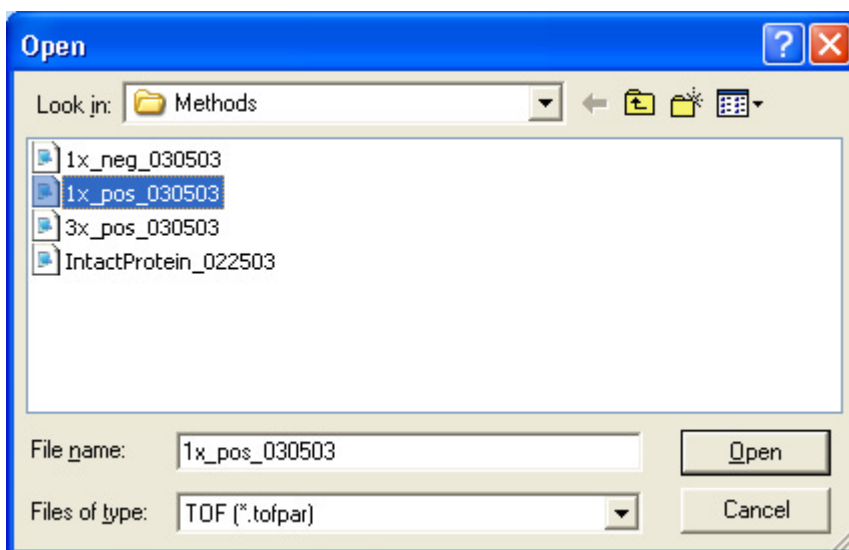


Figure 5-11 Open Dialog Box for Loading a Method

2. If the method is stored in another location, use the dialog box tools to browse to the other folder.

3. Select the method and click **Open**.

The BioTOF system displays a progress gauge as it loads the method (Figure 5-12). It may take several minutes for the data to be loaded. When it is finished, the method file name is shown in the Current Method box on the left side of the BioTOF window.

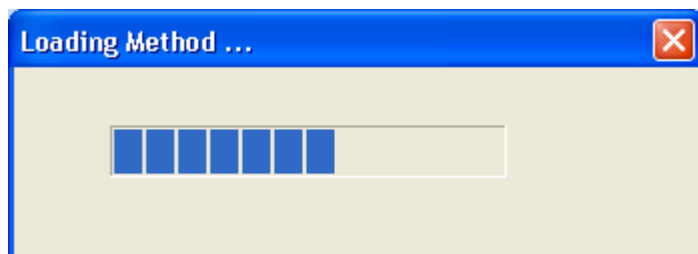


Figure 5-12 Progress Gauge as a Method is Being Loaded

5.6.2 Save Method

Use the Save As function to create a new method or to update a previously saved method. This menu function does not save data. Use the Save button in the Command Buttons pane on the left side of the window to store data for analysis.

To save the current method for later use:

1. Choose **Method > Save As** to open a Save As dialog box (Figure 5-13).

The dialog box displays the methods that have been saved in **..\Data\Methods** folder; the files in the folder are filtered to include only those with the extension ***.tofpar**. You can change the path, but you must use the ***.tofpar** extension.

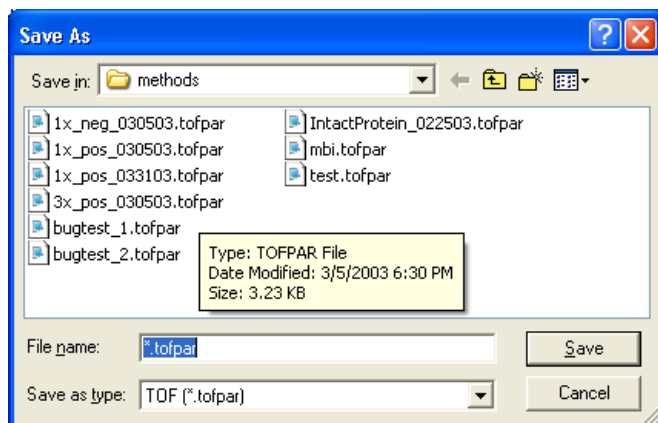


Figure 5-13 Save As Dialog Box for Saving a Method

2. Select an existing method in the dialog box to update the file with the current parameters, or enter an unused name to create a new method.
3. Click **Save**.

If you save to an existing name, a dialog box prompts you to confirm the action.

- Click **Yes** to overwrite the previous file or **No** to cancel the action and select a new name.

5.7 Tools Menu

The Tools menu includes diagnostic functions and a link to the HyStar application, if it is installed on the BioTOF workstation.

5.7.1 Get Actual Values (F5)

Note: This feature is available with Version 3.0 and greater.

The values displayed in the Parameter tabs may differ from the values being sent by the controllers to the BioTOF hardware. For example, the tabs may be displaying ESI Source settings from a just loaded methods file, but the ESI Source controller has not yet apply those settings.

To display the controller values in the BioTOF window:

- Choose **Tools > Get Actual Values** from the menu bar or click  in the tool bar.

5.7.2 Open HyStar NT

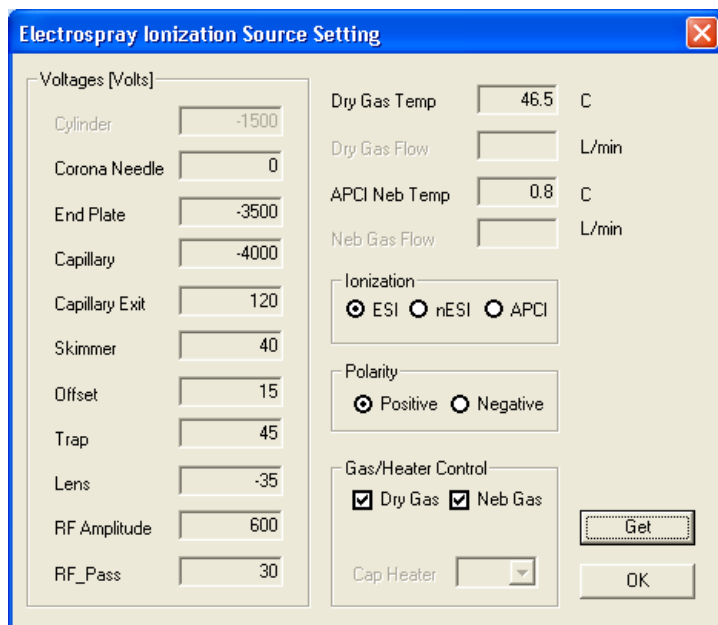
The Tools > Open HyStar NT menu selection is active when the HyStar Software for Chromatography and Hyphenated Experiments application is installed on the BioTOF system host, enabling you to launch the HyStar application and run hyphenated experiments that include BioTOF with the current loaded method.

5.7.3 Diagnostics

Selections in the Tool > Diagnostic menu enable you to compare actual system values with the indicators in the BioTOF window:

To display the parameters set in the ESI Source controller:

1. Choose **Tools > Diagnostics > Ionization Source** from the menu bar to display the Electrospray Ionization Source Setting dialog box (Figure 5-14).



The dialog box, titled "Electrospray Ionization Source Setting", contains the following controls:

- Voltages [Volts]:** A list of input fields for various components:
 - Cylinder: -1500
 - Corona Needle: 0
 - End Plate: -3500
 - Capillary: -4000
 - Capillary Exit: 120
 - Skimmer: 40
 - Offset: 15
 - Trap: 45
 - Lens: -35
 - RF Amplitude: 600
 - RF_Pass: 30
- Dry Gas Temp:** 46.5 C
- Dry Gas Flow:** L/min
- APCI Neb Temp:** 0.8 C
- Neb Gas Flow:** L/min
- Ionization:** Radio buttons for ESI (selected), nESI, and APCI.
- Polarity:** Radio buttons for Positive (selected) and Negative.
- Gas/Heater Control:**
 - Checkboxes for Dry Gas and Neb Gas, both of which are checked.
 - A "Get" button.
 - A "Cap Heater" dropdown menu.
 - An "OK" button.

Figure 5-14 Electrospray Ionization Source Setting Dialog Box

2. Compare the display with the settings in the ESI Source tab.
3. Click **Get** to refresh the display if the dialog box has been opened.
4. Click **OK** to close the dialog box.

5.7.3.1 Spectrometer

The Parameter Settings - Spectrometer dialog box (Figure 5-15) displays the ion polarity, voltage levels and timing parameters in the mass spectrometer as set in the controller, enabling you to compare actual system values with information in the Spectrometer and Timing Sequence tabs in the BiotOF window.

1. Choose **Tools > Diagnostics > Spectrometer** from the menu bar to display the Parameter Settings - Spectrometer dialog box.
2. Click **Get** to refresh the display if the dialog box has been opened.
3. Click **OK** to close the dialog box.

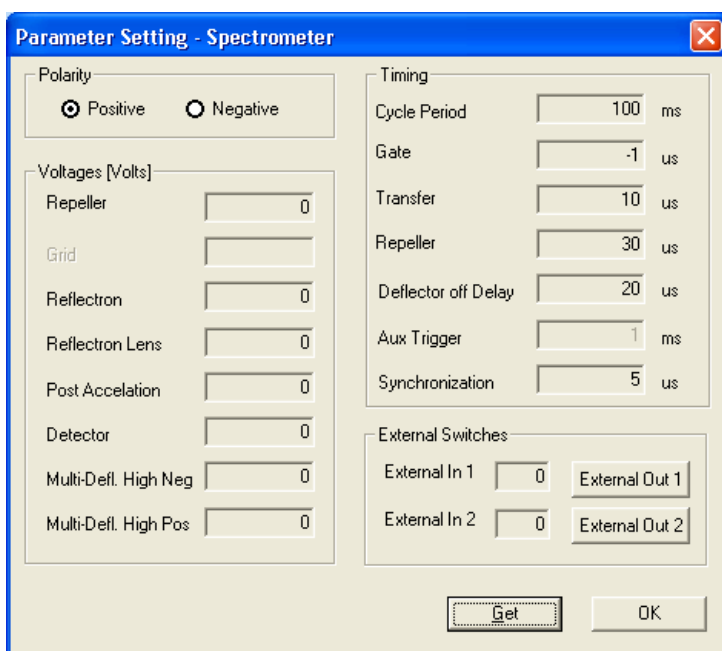


Figure 5-15 Parameter Settings - Spectrometer Dialog Box

External Switches

The BioTOF system supports two external devices. The External Switches group includes two data fields that show the current value of the inputs and two buttons for triggering outputs:

- External In 1 Input from the external device switches the BioTOF control software to transition from Standby to Operation. This is equivalent to clicking the Operation option in the Instrument group.
- External In 2 Input from the external starts spectra acquisition. This is equivalent to clicking the Start button.
- External Out 1 Output to the Ready switch in the external device (however defined in the other system).
- External Out 2 Output to the Ready switch in the external device (however defined in the other system).

The external switches are configured and enabled from the Mode tab. See “Mode Tab” on page 7-3.

5.7.3.2 Digitizer

To compare actual values in the digitizer with settings in the Acquisition tab in the BioTOF window:

1. Choose **Tools > Diagnostics > Digitizer** from the menu bar to display the Parameter Settings - Digitizer dialog box (Figure 5-16).

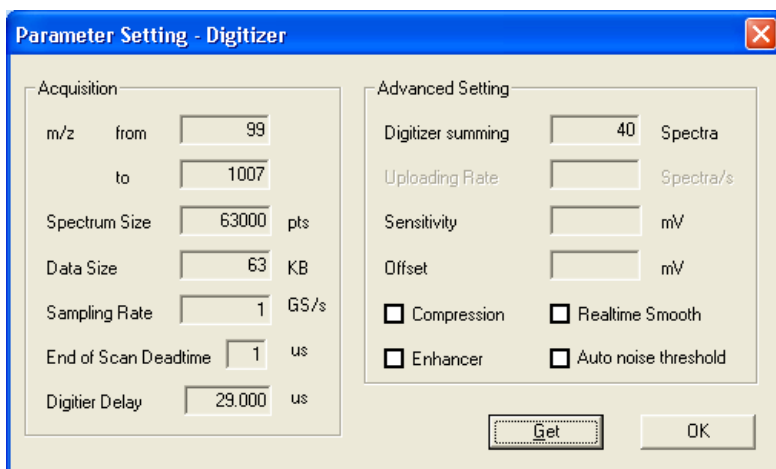



Figure 5-16 Parameter Settings - Digitizer Dialog Box

2. Make changes by typing directly in the value fields and clicking the option checkboxes.
3. Click **OK** or  in the upper right corner to close the dialog box.

5.7.4 Show Servers



The Show Servers selection in the Tools menu provides access to the messages generated by the various servers that make up the BioTOF application:

- Bruker TADS Server - IC1
- Bruker ESI Controller - ESI Con1
- Bruker TREC Server - IC1

To view server message traffic:

1. Choose **Tools > Diagnostics > Show Servers** in the menu bar so that the option is checked.

The Windows task bar displays a button for each server.

2. Click a server button to open a separate window to display the message traffic.
Figure 5-17 is an example of message traffic for the ESI Controller (ESIcon1).
 3. Click  in the upper right corner of the window to minimize the display to the task bar.
- Note:** Do not use  to close the window as this action stops the server program.
4. Choose **Tools > Diagnostics > Show Servers** in the menu bar a second time to uncheck the option and clear the server buttons from the task bar.

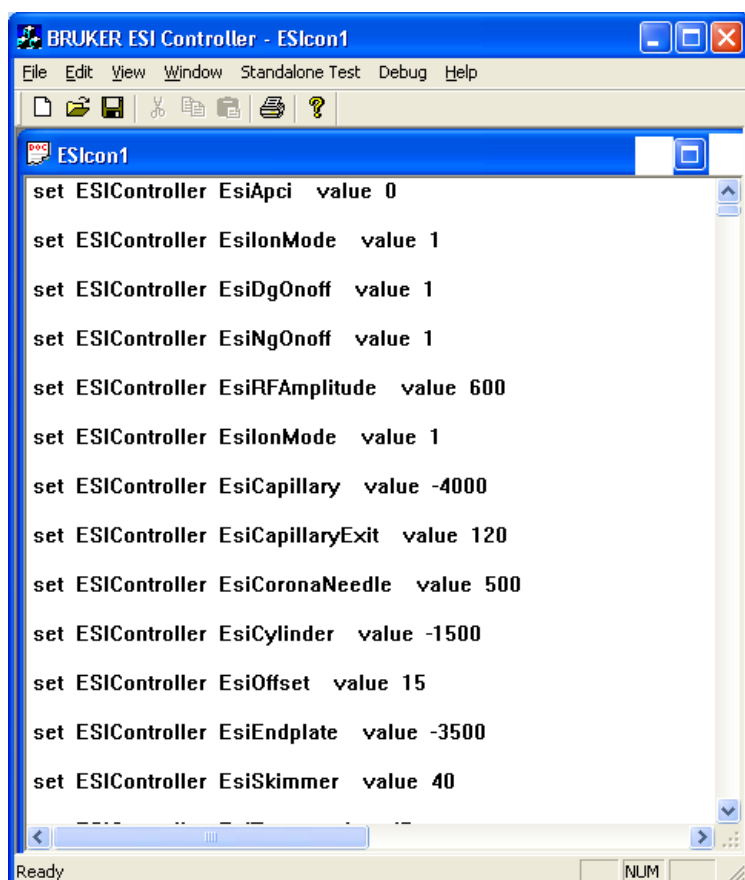


Figure 5-17 ESI Server Window

5.8 Format Menu

By default, the BioTOF application saves spectra to two file formats for use with the data processing applications:

- DataAnalysis
- XMass

To specify formats for saving data:

1. Choose **Format** from the menu bar.
2. Click either or both of the formats listed.

A format is selected when a checkmark appears to the left of the menu option. To deselect an option, click a checkmarked menu option and the checkmark is removed.

5.9 Help Menu

The Help menu has two selections:

- Help Topics provides information on the BioTOF application using a the Windows Help system.
- About TOF Control displays the version and copyright information.

6 Introducing Samples

This chapter describes how to set up sample inlets for Electrospray ionization using the syringe pump and/or a liquid chromatography (LC) system. The chapter then describes the use of the Nanospray and Atmospheric Pressure Chemical Ionization (APCI) options.

6.1 Sample Delivery to Electrospray

Many analytical methods can be accomplished by directly pumping the analyte in solution to the Electrospray using the supplied syringe pump. Often the sample has already undergone some separation or purification process.

Direct infusion can be accomplished at a low flow rate using the syringe pump only and at higher flow rates using the LC system.

- In low-flow mode, the outlet of the syringe needle is coupled through the necessary fittings to the union on the inlet of the Electrospray. Direct low flow rate is capable of higher sensitivity as there is no dilution. Low flow is often associated with small sample volumes.
- In the high-flow configuration the actual delivered flow rate is determined by the LC pump. The flow from the syringe pump is tee'd into the flow from the LC by means of a low dead volume fitting inserted into the flow path directly before the Electrospray union. The addition of solvent from the LC system can reduce certain buffer effects. The increased flow rate also makes it possible to optimally use infusion for APCI work where higher flow rates are optimum. If the target application for a particular analysis is found in LC analysis, then tee'ing into this flow with a representative standard can be useful during method development while optimizing instrument conditions.

One of the most common uses of direct infusion is to provide continuous introduction of sample while optimizing the mass spectrometer for a particular analysis. The most general case is the direct infusion of a standard tuning solution. These solutions can be introduced either in the low flow configuration or in conjunction with the LC pump, depending on whether the target analysis will occur with the LC.

6.1.1 Syringe Pump

The syringe pump (Figure 6-1) can be used to drive glass syringes from 5 μL to 5 mL. The BioTOF system includes a 100 μL syringe suitable for most applications.

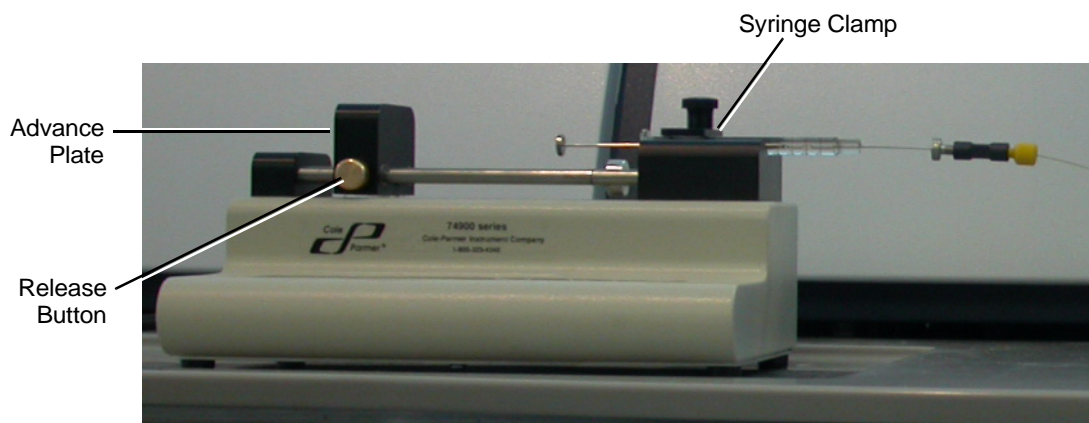


Figure 6-1 Syringe Pump

6.1.2 Setup for Low Flow Direct Infusion

The simplest configuration is a direct connection of the syringe pump to the Electrospray. The flow is started by the keypad on the front of the syringe pump.

To set up the direct infusion to the Electrospray:

1. Fill the syringe with the ESI solvent and sample liquid, avoiding any bubbles.
2. Connect the syringe with the sample inlet of the off-axis sprayer using low volume, clean tubing and fittings (Figure 6-2).
3. Position the syringe in the pump.

Lift the syringe clamp on the pump, place the syringe barrel in the holder as shown in Figure 6-1, and place the clamp on the syringe.

- Press the release button on the front of the drive plate and advance the plate so that it rests against the syringe plunger (Figure 6-2).

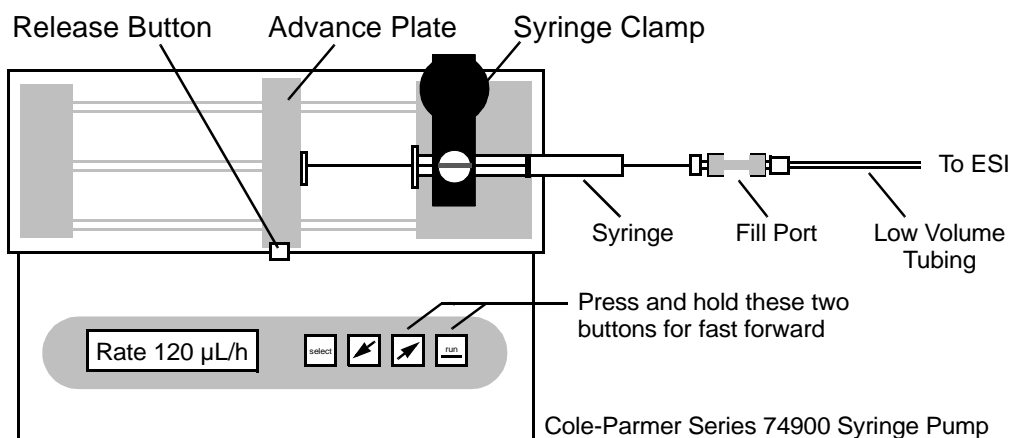


Figure 6-2 Syringe Pump Controls

- Turn the syringe pump on using the rocker switch on back of the pump just above the power cord connection.
- Press **Select** on the pump keypad to access a menu on the small display to the left of the select button.

The displays show three items at a time. To select an item:

- Use the arrow keys to scroll through the menu and highlight the item and then press **Select**.
- Select **Table** to display a list of syringe manufacturers, highlight the syringe manufacturer, and press **Select**.
 - Choose the **Size/Dia** table, select the syringe, and press **Select**.
 - Choose the **Rate** from the menu, and use the arrow keys to set the syringe pump to a flow rate, and press **Select**.

Note that the rate is specified in volume **per hour**, not per minute.

6.1.3 Adjusting the Electrospray Needle

The Electrospray needle consists of two concentric cylinders, the outer one supplying the nebulizing gas and the inner one carrying the sample liquid. The inner needle should protrude from the outer barrel by about 0.5mm. To optimize spray performance for some low flow rate application, it may be necessary to adjust the needle outward.

The Electrospray needle is mounted off-axis in the top of the spray chamber. The Electrospray needle is held in position by a bayonet connection.

To check the Electrospray Needle:

1. Remove the off-axis sprayer assembly from the top of the spray chamber.
2. Verify that the tip of the inner needle protrudes about 0.5 mm from the body of the sprayer.

To adjust the needle position:

1. Loosen the lock nut shown in Figure 6-3.

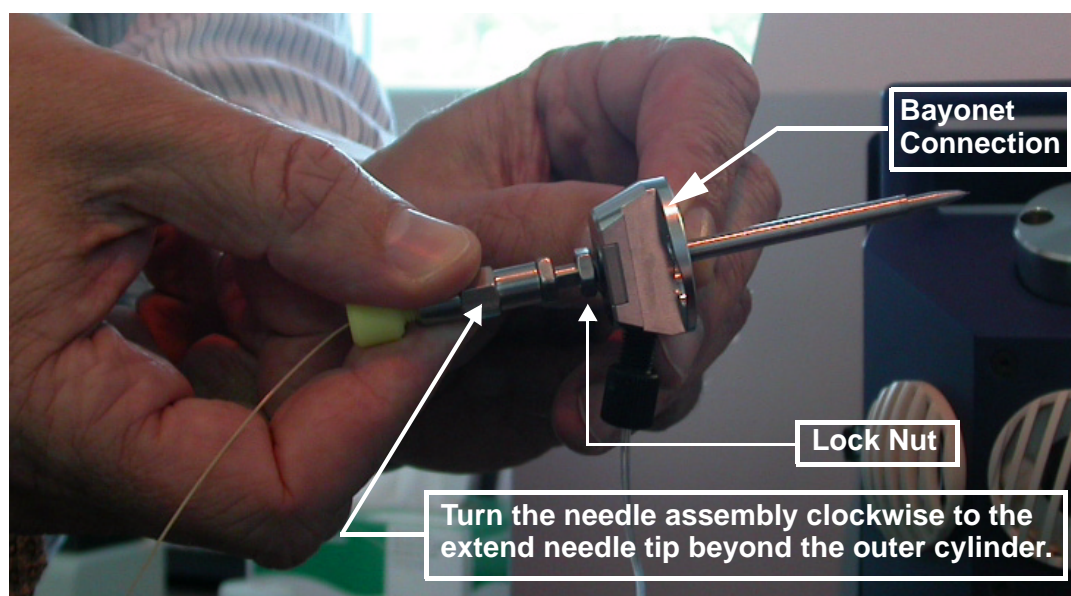


Figure 6-3 Adjusting the Electrospray Needle

2. Hold the bayonet connection with one hand and turn the needle assembly clockwise to extend the inner needle outward, or counterclockwise to draw the needle back into the assembly.
3. Tighten the lock nut and reinstall the assembly in the spray chamber.

6.1.4 Starting the Syringe Pump

To operate the syringe pump:

1. Press **Run** to start the pump.
2. Press the right arrow key and **Run** simultaneously to push the liquid to Electrospray.

This is called *Fast Forward* mode.

3. Release the buttons to stop the pump.
4. Press **Run** to operate at the specified rate.

After the flow has reached the source end of the nebulizer, some additional optimization may be required for low flow rates, such as adjusting the amount of nebulizing gas, drying gas and, in some cases, a slight re-adjustment of the extension of the nebulizer needle.

To adjust the spray needle on-line:

1. Loosen the lock nut.
2. Rotate the body of the needle clockwise or counterclockwise.
3. Make quarter turns and observe the result with the Source Lens Current in the System Status indicators on the ESI Source.
4. When the spray is optimized, tighten the lock nut.

6.1.5 Flushing the Syringe Pump

Unlike FIA or liquid chromatography, direct infusion has no solvent flow between samples to flush the sample path. The entire sample path must be flushed with solvent before and after each sample infusion. If you have enough sample, flush the entire system with the new sample before acquiring data.

To flush the entire system:

1. Fill a syringe with solvent and connect it to the sample tubing.
2. Ensure that the nebulizing and drying gases are on.
3. Inject one full syringe of solvent or sample through the sample path.

Note: Push the liquid through slowly.

4. Check all fittings for leaks.
Tighten fittings if necessary.
5. Verify that the spray is straight with respect to the needle.
If the spray is not straight, the needle may be bent or partially clogged.

6.2 Liquid Chromatography (LC)

The most common form of sample introduction is accomplished by means of an LC system. There are different configurations that are possible with LC which can include use of a pump, automatic sampler, and column. Frequently, the system includes an inline UV detector for dual detection. Certain analyses may not necessarily require the use of an analytical column and are referred to as flow injection analysis (FIA).

The Electrospray system accepts solvent flows up to 1 mL/min. It is compatible with both standard LC columns (2.1 mm to 4.6 mm inner diameter) and microcolumns (320 μ m to 1.0 mm inner diameter). The APCI Source accommodates flow rates up to 2 mL/min.

Reversed-Phase LC

Proteins and peptides exist as amphoteric species which have charged (or chargeable) sites available on relatively hydrophobic molecules. Both charged sites and hydrophobic characteristics are present.

Proteins and peptides are usually separated using mobile phase modifiers such as trifluoroacetic acid (TFA). TFA inhibits positive ion formation in Electrospray. This problem can be overcome through the post-column addition of high concentrations of volatile weak organic acids, such as formic or acetic acids.

Normal-Phase LC

Normal-phase LC can be used with Electrospray. Solvents used are typically non-aqueous and non-polar. The compounds separated are relatively nonpolar. To overcome this, compounds can be protonated $(M+H)^+$ if a proton donor is present in the solution. Alternatively, alkali metal cation adducts can be formed by the addition of sodium acetate, potassium acetate, or other metal acetate salts, as well as some organic compounds.

6.2.1 Operating With Liquid Chromatography

A liquid chromatograph (LC) can serve several functions in an Electrospray interface.

As a Pump

In this mode of operation, only the LC pump is used. The sample is injected manually into a sample loop on the multiport valve. The valve is switched manually to transport the sample into the Electrospray interface. The pump flow can be set manually on the LC keypad or through the system software.

As a Pump and Sample Inlet

This mode is essentially flow injection analysis but the LC performs all of the functions performed by the multiport valve in manual flow injection. The LC serves as a pump and injector. The LC column, if one is present, is switched out of the flow path and no separation takes place. The injections can be manual or automated. This mode of operation can be controlled manually on the LC keypad or through the system software.

As a Liquid Chromatograph

In this mode, the LC operates as a normal liquid chromatograph. A column is installed and sample separation takes place. Setting up an LC for operation with the Electrospray (or APCI) is mostly a matter of determining the correct solution chemistry. The solvents and buffers must provide good chromatography and enhance, or at least not inhibit, ion formation. Buffers can be added before or after separation (pre- or post-column) depending on the chemistry involved. The correct choices are highly sample dependent. The LC is typically controlled through the system software.

As an LC With a Second Detector

If a second detector is used with Electrospray interface, it must be upstream of the mass spectrometer because the sample is otherwise destroyed by the mass spectrometer.

Two configurations are possible:

Serial path

The sample flows first through a flow cell in a non-destructive detector and then to the Electrospray (or APCI). In a parallel path configuration, the sample is split, usually with a standard tee, with part of the flow going to each detector.

A serial configuration provides maximum sensitivity to the alternate detector because it receives the full sample flow. It is also easier to do post-column addition in this configuration. The disadvantages are that the detector must be non-destructive and its flow cell must tolerate high pressures. Also, the flow cell can cause peak broadening that will affect spectrometer data.

Parallel path

A parallel configuration eliminates peak broadening problems and makes sample fraction collection easy. It also allows destructive detectors. Sensitivity is reduced slightly for the alternate detector because it does not receive all of the sample. A parallel configuration also complicates post-column additions and is prone to split-ratio variations.

6.2.2 Using the Syringe Pump at LC Flow Rates

The higher flow (LC compatible) configuration is very similar to the low flow configuration as viewed from the syringe pump. The inclusion of the LC pump is easily accomplished by simply adding a tee directly above the connection made to the Electrospray nebulizer.

Typically the syringe pump is operated at flow rates from 1 $\mu\text{L}/\text{min}$ (60 $\mu\text{L}/\text{h}$) to 10 $\mu\text{L}/\text{min}$ (600 $\mu\text{L}/\text{h}$). The LC pump is typically operated from 100 $\mu\text{L}/\text{min}$ to 1 mL/min . The actual dilution is simply a ratio of the flow rates. Dividing the initial sample concentration by the dilution factor gives the final concentration at the nebulizer.

Concentrations as low as 1 $\text{pg}/\mu\text{L}$ (after dilution) can give good results in infusion experiments. The factors that limit this sensitivity are background contamination that may exist in the LC system, solvents, or sample.

When properly configured, the sample solution is metered into the LC flow stream. Because the flow rates are higher, it is important that all connections are correct and tight. Small leaks may dramatically effect the small infusion amounts coming from the syringe pump.

6.3 Nanospray

For analyzing sample with low concentration and volumes, the ESI source can be configured with the Nanospray assembly in place of the standard Electrospray spray chamber. The Nanospray operates at a flow rate of less than 20 nL/min by delivering the analyte to the source via a small inner diameter needle positioned at the center of the source.

For Nanospray, nebulizing gas is not used, and drying gas is applied at lower temperatures than in Electrospray applications. High voltage is applied to the capillary only.

There are two assemblies in use:

- The **On-Axis Nanospray** assembly (left in Figure 6-4) includes an XYZ stage immediately in front of the spray chamber that accepts a needle assembly for on-line or off-line Nanospray applications. The unit features a camera and LCD screen for monitoring spray operation.
- The **Off-Axis Nanospray** assembly (right in Figure 6-4) delivers the analyte via an off-axis needle assembly. The Off-axis Nanospray assembly includes a microscope for monitoring.

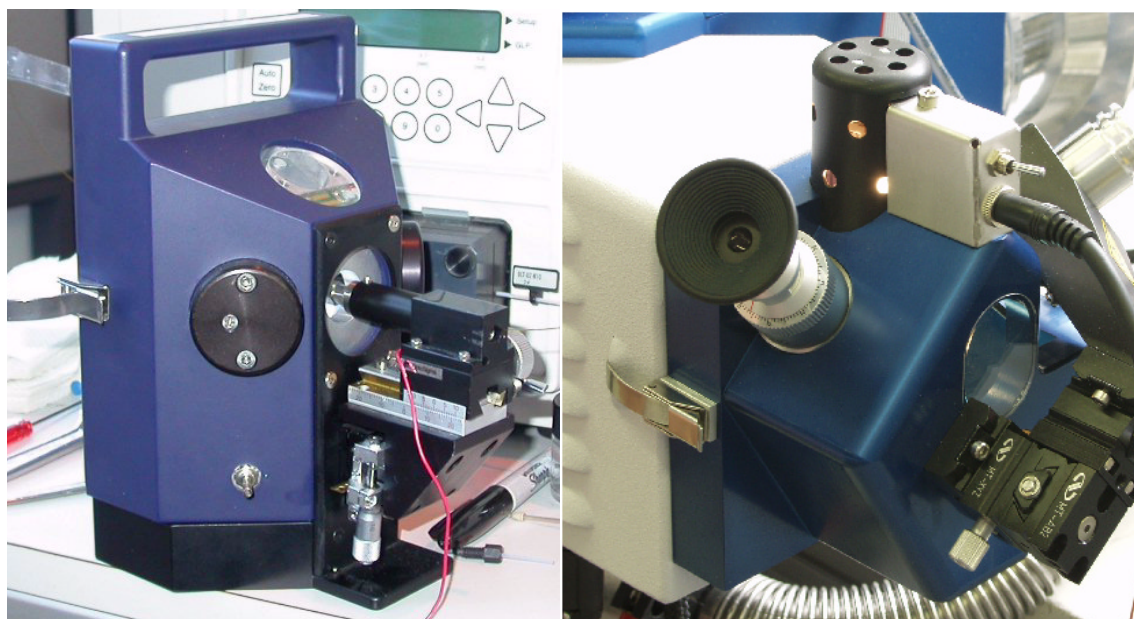


Figure 6-4 On-Axis (left) and Off-Axis (right) Nanospray Assemblies

6.3.1 Installing the Nanospray Assembly

This section describes how to install the On-axis Nanospray Assembly. For detailed instructions, refer to *Apollo On-Line NanoElectrospray Ion Source User Manual*.

To replace the Electrospray with the On-axis Nanospray:

1. At the computer workstation, click **Standby** in the Instrument group to bring the instrument from operation mode to standby mode.

The ESI voltages are turned off.

2. Double-check to ensure that the ESI voltages and the TOF voltages are off.

The light on the high voltage indicator on the ESI front panel must be off and the Instrument HV unit is disabled (Enable LED is off).

3. Click the **ESI Source** tab and set the **Dry Gas Temp** to **30° C** or below.
4. Uncheck **Neb Gas** and **DG Heat** in the Gas/Heater Control Group.
5. Disconnect the nebulizing gas from the Electrospray.
6. Release the latch on the left side of the spray chamber and swing the assembly out so that the spray shield is visible (left in Figure 6-5).
7. After the spray chamber has cooled, remove the spray shield from the end plate and capillary entrance (right in Figure 6-5).

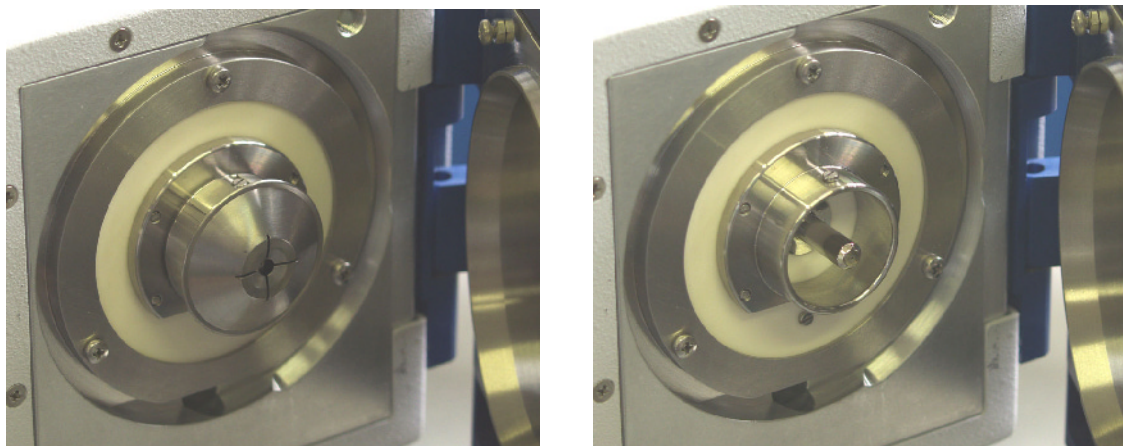


Figure 6-5 Endplate with Spray Shield in Place (left) and with Spray Shield Removed (right)

8. Insert the gas diverter disk into the endplate (left in Figure 6-6), and place the capillary cap over the capillary entrance (right in Figure 6-6).

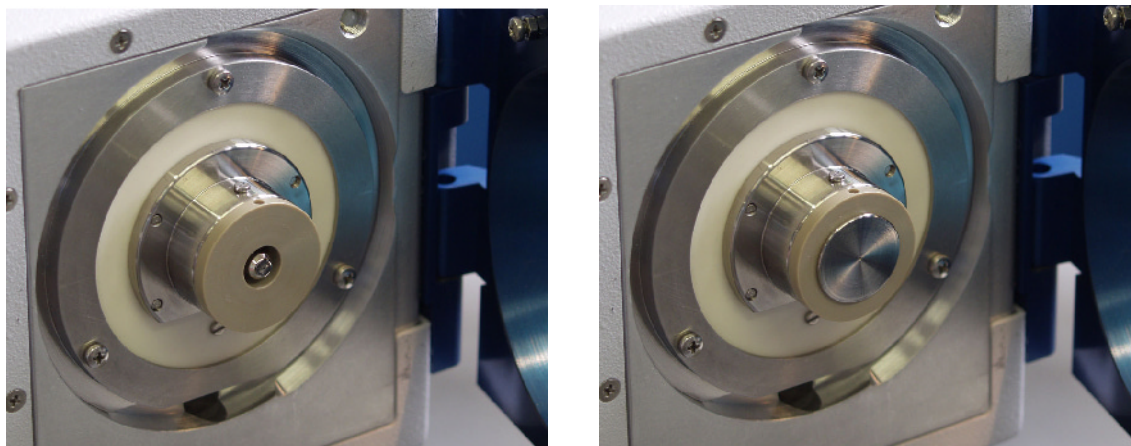


Figure 6-6 Placement of the Gas Diverter (left) and Capillary Cap (right)

9. Lift the spray chamber door from the hinges on the ESI Source assembly.
10. Place the Nanospray assembly on the hinges, and close and latch the spray chamber.

For Nanospray operations, high voltage is applied to the capillary only. After installing the Nanospray assembly, you should use the Source Control Unit to set Source Lens Current display to read the current on the Capillary.

11. Use the arrow buttons below the Source Control Panel LCD display to position the display cursor on Cap (Figure 6-7).

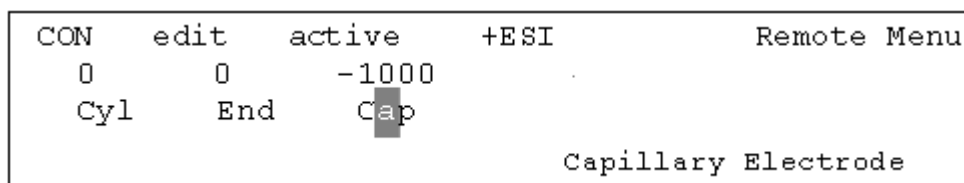


Figure 6-7 Select Cap for the Source Lens Current Reading

6.3.2 On-Line Nanospray

For On-Line Nanospray use Nanospray needles such as 8 micron inner diameter or 15 micron inner diameter New Objective™ Tips.

To install the Nanospray needle for On-Line operation:

1. Use a union to connect the Nanospray needle and the transfer line as shown in Figure 6-8 and either of the two needle configurations in Figure 6-9.

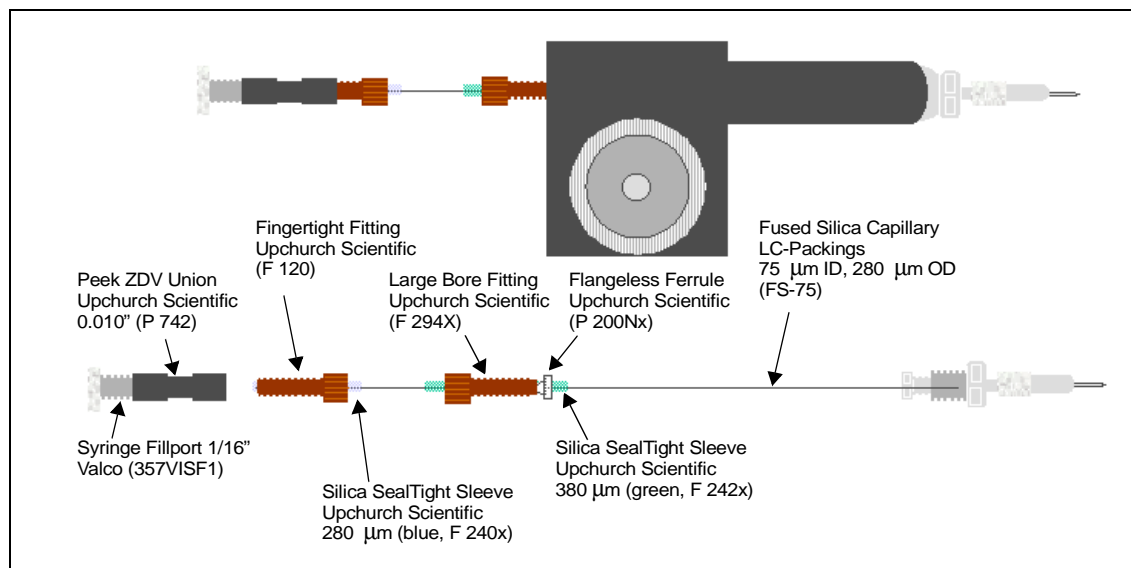


Figure 6-8 Installing the Needle for On-Line Nanospray

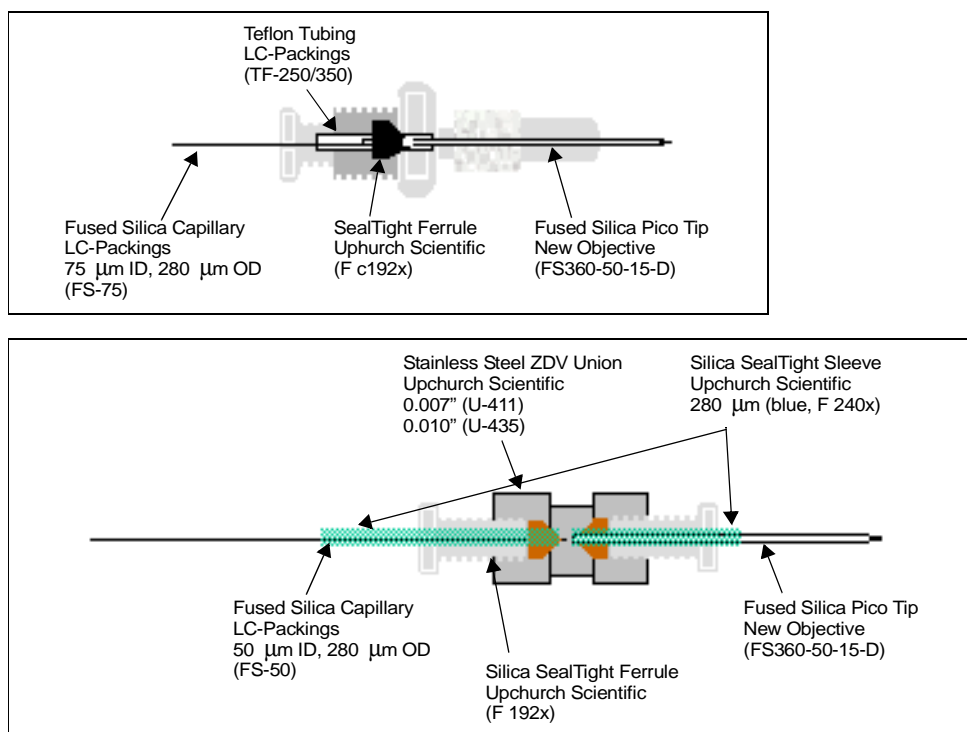


Figure 6-9 Typical Needle Configurations

2. Place the assembly in the XYZ stage.

Note: Be careful not to bump the needle against the capillary cap.

3. Use the stage adjustments and the assembly optics to position the needle on axis with the capillary with the tip about 2.0 mm from the opening in the capillary cap.
4. Connect the transfer line to the nanoLC system or a syringe.
5. Set the Capillary voltage to **600 V** in the ESI Source tab.
6. Start the nanoLC system or syringe pump, and place the system in operation.
7. Increase the Capillary voltage in **50 V** increments until the current is visible in the Source Lens Current indicator.
8. Adjust the XYZ controls on the Nanospray assembly to optimize the spray.
9. In the BioTOF window, check **DG Heat** in the Gas/Heater Control group, and set the Dry Gas Temp in the ESI Source tab to **50 °C**.
10. At the Source Control panel, set the dry gas flow to one-quarter of the scale.
11. Adjust dry gas flow and temperature to optimize for solvent conditions.

6.3.3 Off-Line Nanospray

For Off-Line Nanospray applications, use *Pre-opened* Nanospray needles such as 2 micron inner diameter or 4 micron inner diameter New Objective™ Tips.

For off line Nanospray:

1. Use a micro-pipetter fitted with a gel-loader tip to deliver 1 to 3 μL of analyte solution through the larger diameter end of the tip into the Nanospray needle.
2. Shake down the assembly to allow the liquid to flow forward in the Nanospray tip.
3. Place the filled needle in the fitting and place the fitting in the XYZ stage.
4. Use the stage adjustments and the assembly optics to position the needle on axis with the capillary with the tip about 2.0 mm from the opening in the capillary cap.
5. Set the Capillary voltage in the ESI Source tab to **600 V**.
6. Place the system in Operation.
7. Increase the Capillary voltage in **50 V** increments until the current is visible in the Source Lens Current indicator.

6.4 E-Z Nanospray

The E-Z Nanospray Ion Source allows you to set up an off-line Nanospray application without removing the Electrospray spray chamber. An E-Z Nanospray assembly holding a disposable needle with the analyte solution is inserted on axis into the front of the spray chamber door (Figure 6-10).

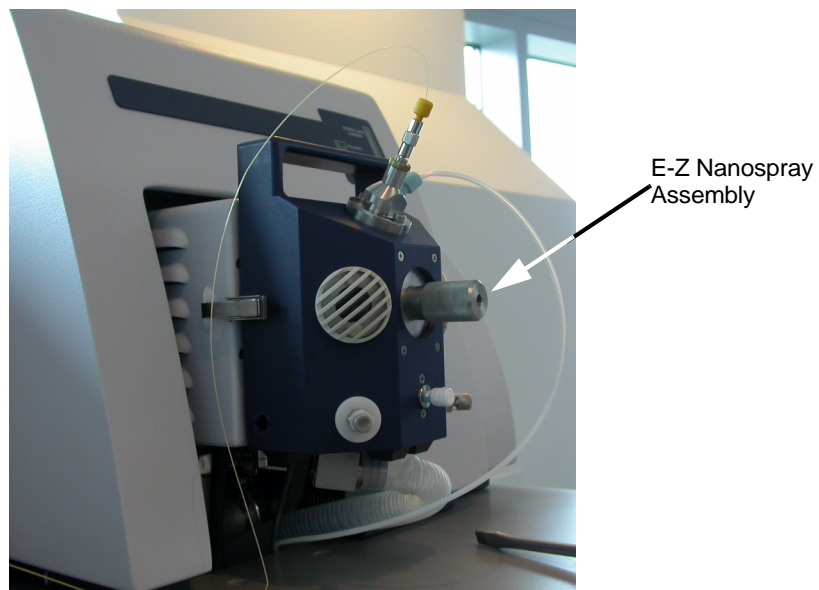


Figure 6-10 E-Z Nanospray Assembly Installed in the Spray Chamber Door

6.4.1 Preparing the Nanospray Needle

Pre-opened Nanospray needles such as 2 micron inner diameter or 4 micron inner diameter New Objective™ Tips, are suggested when using the E-Z Nanospray assembly.

To prepare a Nanospray needle for use:

1. Use a micro-pipetter fitted with a gel-loader tip to deliver 1 to 3 μL of analyte solution through the larger diameter end of the tip into the Nanospray needle (Figure 6-11).

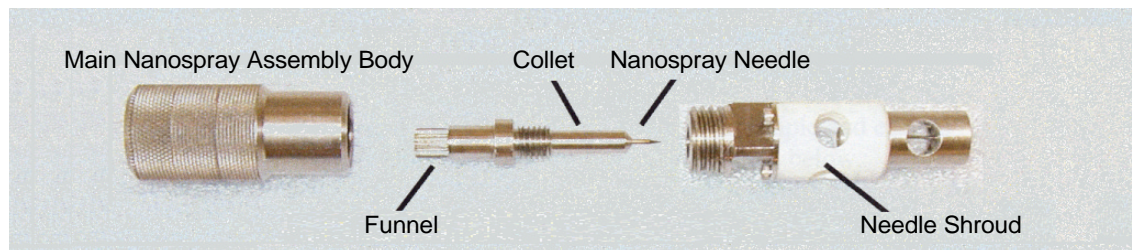


Figure 6-11 Preparing a Nanospray Needle

2. Slide the rear of the needle into the entrance of the collet and mount the collet onto the needle shroud (Figure 6-11).
3. Shake down the assembly to allow the liquid to flow forward in the Nanospray tip (Figure 6-12).

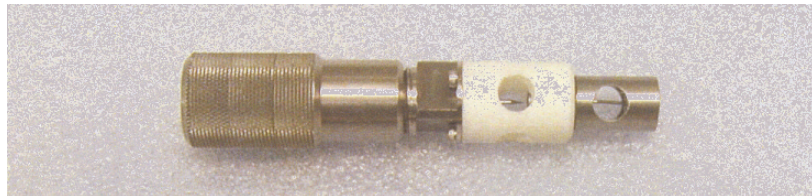


Figure 6-12 Nanospray Assembly

6.4.2 E-Z Nanospray Installation

To insert the E-Z Nanospray Assembly in the ESI Source:

1. Open the spray chamber door, remove the spray shield from the endplate so that the Capillary is visible, and close the spray chamber door (Figure 6-13).

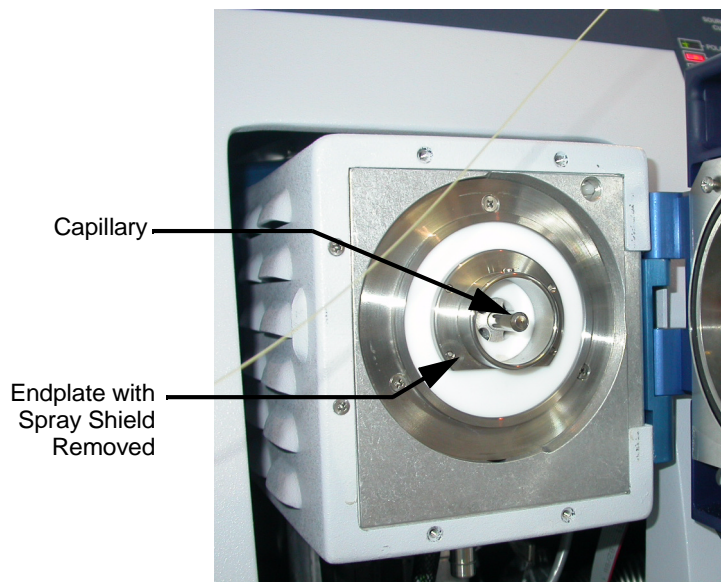


Figure 6-13 ESI Source with Spray Shield Removed

2. Remove the louvered plastic cover and the glass cover from the port on the front of the spray chamber (Figure 6-14).

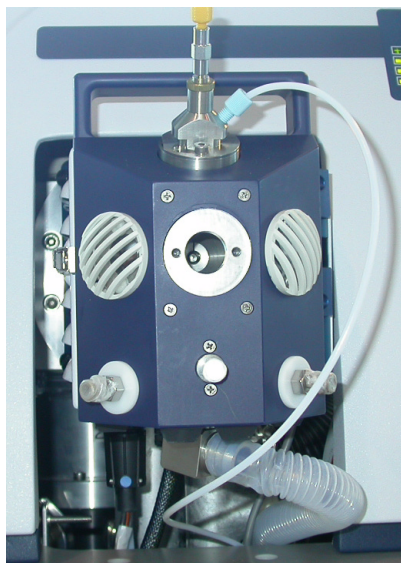


Figure 6-14 Port Covers Removed for E-Z Nanospray

Note: Do not insert the E-Z Nanospray assembly unless the spray chamber door is closed and the latch on the left side is fully engaged.

3. Insert the E-Z Nanospray assembly into the port and push the assembly until it can go no further into the spray chamber.

6.4.3 Nanospray Settings

For Nanospray, only the capillary element is raised to a high voltage. The magnitude of the voltage for Nanospray is lower than that needed for Electrospray applications. For best sensitivity, operate at the lowest possible voltage needed to maintain a stable Electrospray signal.

To set the chamber voltages:

1. Use the arrow buttons below the Source Control Panel LCD display to position the display cursor on **Cap** (Figure 6-15).

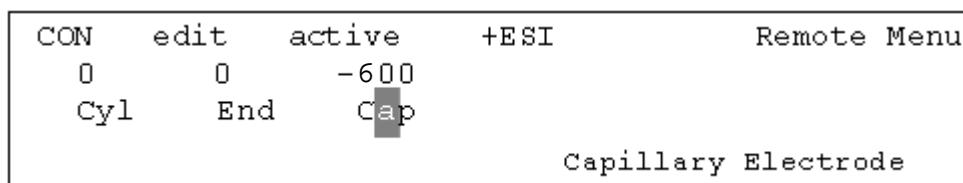


Figure 6-15 Select Cap for the Source Lens Current Reading

2. Set the capillary voltage to **600 V**.
3. With the mass spectrometer in operating mode, slowly increase the capillary voltage in **50 V** increments until the current is registered on the capillary.

At this point, the voltage applied to the capillary affects the flow rate through the needle.

Caution: As the magnitude of the high voltages for negative ESI tends to be lower than those optimal for positive ion mode, caution should be used when adjusting voltages to avoid arcing. Arcing can destroy the coating of the needle tip.

6.5 Configuring the APCI Spray Chamber

This section describes how to convert the ESI Source assembly to use the APCI Source (Figure 6-16).



Figure 6-16 Source Configured with the APCI Source

Note: Installation of the APCI source requires that you open the panels of the instrument.

Before installing APCI:

- Exit the BioTOF acquisition software and then turn off all electronics modules with the power switch labeled **Electronics**.

6.5.1 Installation

To install the APCI chamber:

1. Remove the right front panel section.
2. Disconnect the ESI source high voltage connector (large black round plug) from the cable plug coming from the source controller.

This connection is found inside the frame, by tracing the cable from the source body down to the connector.
3. Disconnect the Nebulizer gas from the ESI spray chamber.
4. Remove the ESI spray chamber from the ESI source body.
5. Place APCI spray chamber on the source hinges and close the latch.
6. Connect the Nebulizer gas to the APCI sprayer.
7. Connect the ESI source high voltage cable to the outlet on the left side of the APCI spray chamber.
8. Connect the high voltage cable from the APCI source to the above mentioned HV outlet cable of the Source controller inside the cabinet.

These plugs are keyed.
9. Make sure the connector are seated and locked properly.
10. Connect the 6 pin APCI heater cable to the mating cable plug in the source controller cable tree.
11. Install the APCI drain and connect it to the outside vent.
12. Close the panel.
13. Open the APCI spray chamber and pull the spray shield forward, approximately 1 mm from the backstop position.
14. If necessary, lock the position with the screw.

6.5.2 APCI Parameters

1. Turn on the power by pressing the **Electronics** switch on the Main Control panel.
2. Restart the BioTOF software.
3. Make sure the instrument is in **Standby** mode and the voltages are off:
 - The Voltage On button on the left side of the Source Control Unit is off.
 - The green Enable LED on the Instrument HV Panel is off.
4. Set to positive ion mode if not already done.
5. Set the source mode to APCI and verify that the source controller mode switches are set to APCI.

The Corona Needle voltage is automatically set to **500 V**.

6. Set the Endplate and Capillary to **-2500** and **-3000 V**, respectively.

The polarity is not displayed in the ESI Source tab, but can be seen on the source controller display.

In APCI mode, the polarity of Capillary and Endplate are opposite of the polarity of Corona Needle.

7. Turn on the nebulizer and the drying gas, and set the nebulizer pressure to **60 psi** on the Source Control unit.
8. Set dry gas flow to **10** units and dry gas temperature to **250 °C**.
9. Connect solvent/sample flow of MeOH/H₂O or CH₃CN/H₂O from the LC system or syringe pump at **20** to **150** µL/min
10. With the liquid flowing, set APCI Heater to **400° C** and allow it to warm up for 10 minutes.

11. The vacuum side voltages should be set for normal operation

The recommended default values are:

Capillary Exit	130 V
Skimmer 1	40 V
Skimmer 2	30 V
Offset	15 V
Trap	45 V
Lens	50 V
RF Amplitude	600 V

12. Set the ampere meter range switch on the source controller to **High**.
13. On the Source Control Panel, select **CNdl** for the Source Lens Current reading.
14. Increase the Corona needle voltage until a stable current appears on the current meter of the source controller.

Using the above values for End and Cap, this should be reached at +1200 V to +1300 V. The current should be in the 10^{-6} A range. Move or turn the corona needle to establish contact if no current is observed at 1400 V.

15. Set mass range to **100 to 700** and start data acquisition.

Observe the spectrum and tune the CNdl voltage to get a stable signal.

Note: *Do not use excessively high differential voltages between CNdl on one side and Endplate/Capillary on the other side. Keep End to Cap differential at or below 500 V, since higher voltages can lead to source arcing.*

16. Switch to *Chromatography* mode and verify the performance with (manual) sample injections (e.g. Reserpine m/z 609, 5 μ L injections, 0.5 μ M, 1 μ M, 2 μ M, 4 μ M series)
17. Lower the dry gas flow carefully to optimize and stabilize the signal, but not lower than 5 units.

7 Methods

In the BioTOF system, the *method* consists of the ESI Source and mass spectrometer operational settings. When the system is started, it uses the Last Good Method, that is, the setting in use when the system was shutdown. You can adjust these settings using the option buttons and the parameter tabs below the Spectra Display window, as described in this chapter.

Additionally, you can save the current settings to a file for use later on similar projects, or restore settings after using variations on a setup.

The Current method is identified in the status group in the upper left of the BioTOF window (Figure 7-1).

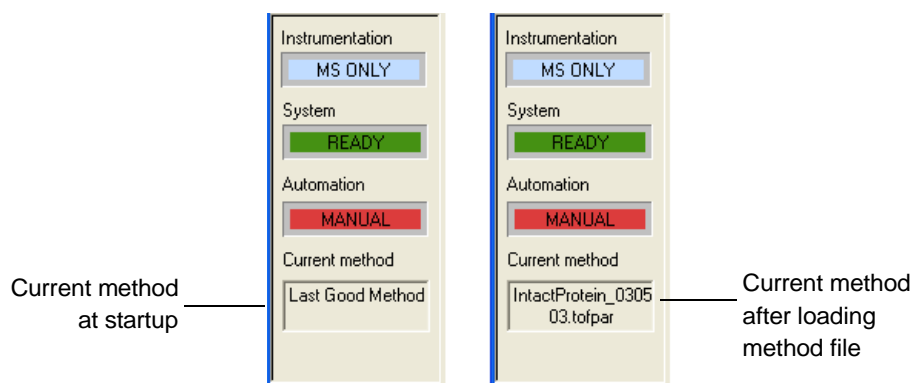


Figure 7-1 Current Method Identified in the System Status Group

This chapter describes how to adjust a method using the option buttons and parameter tabs, and how to save and load methods.

7.1 Control Buttons

The Ionization and ion Polarity option buttons (Figure 7-2) to the left of the parameter tabs set the basic parameters for method.

The image shows a software control panel with four distinct sections, each with a title and a set of controls:

- Instrument:** Contains two radio buttons, **Standby** and **Operation**. The **Operation** button is selected.
- Ionization:** Contains three radio buttons, **ESI**, **nESI**, and **APCI**. The **ESI** button is selected.
- Polarity:** Contains two radio buttons, **Positive** and **Negative**. The **Positive** button is selected.
- Gas/Heater Control:** Contains three checked checkboxes: **Dry Gas**, **Neb Gas**, and **DG Heat**.

Figure 7-2 Ionization and Polarity Groups

To set basic operating parameters:

1. Click the **Ionization** button that is appropriate from the ESI source to be used:
 - **ESI** for the standard Electrospray Ion source
 - **nESI** if you are using the low flow-rate Nano Electrospray source
 - **APCI** if you are using atmospheric pressure chemical ionization
2. Select either **Positive** or **Negative** for Ion **Polarity**.

The appropriate voltages are applied to the ESI Source and Spectrometer. The ion polarity can be set remotely if the three-position Polarity switch on the Instrument HV Voltage Panel is in the middle position.

7.2 Mode Tab

Use the Mode tab (Figure 7-3) to set the instrument mode, display units, and mass range.

Figure 7-3 Mode Tab

Instrument Modes

There are three instrumentation modes:

- **MS Only** (Mass spectrometry only)
- **Chromatography - MS** for when the sample is supplied from an LC system, and you want to display a chromatogram and the mass spectra. The Spectra display window is divided into two panes, the top one showing the spectra and the lower one showing ion counts from the connected LC system.
- **FIA-MS** (Flow injection analysis and mass spectrometry) to specify a run time. With this mode, you set up the run in the Chromatographic Setting group by specifying the total run time in minutes and a wait time for the solvent to clear.

Data Display

The default data display horizontal scale is m/z. When Instrumentation Mode is set to MS Only or FIA - MS, you can also specify Flight Time or Points. When Instrumentation Mode is Chromatography - MS, the scale must be m/z.

Mass Range

The Mass Range group specifies in m/z in Dalton to be recorded, displayed and saved.

Chromatographic Setting

When the Instrument mode is either Chromatography - MS or FIA - MS, you specify the duration of the data acquisition by entering **Total Run Time** and **Solvent Delay** in minutes. The Solvent Delay allows you to flush previous samples from the system before the start of the run. Solvent Delay is not included in Total Run Time.

When the Instrument mode is Chromatography - MS, you set a mass spectra acquisition rate in the Chromatographic Setting group. The rate is between 1 and 70 spectra per minute.

7.2.1 External Start

Use the External Start group in the lower right corner to enable and configure the start of spectra acquisition on a signal from an external device such as an LC system.

To configure the external Start:

1. Click the **Configure** button to open the Configuration dialog box (Figure 7-4).

The dialog box configures two input and two output signals by defining whether a positive or negative signal activates the switch.

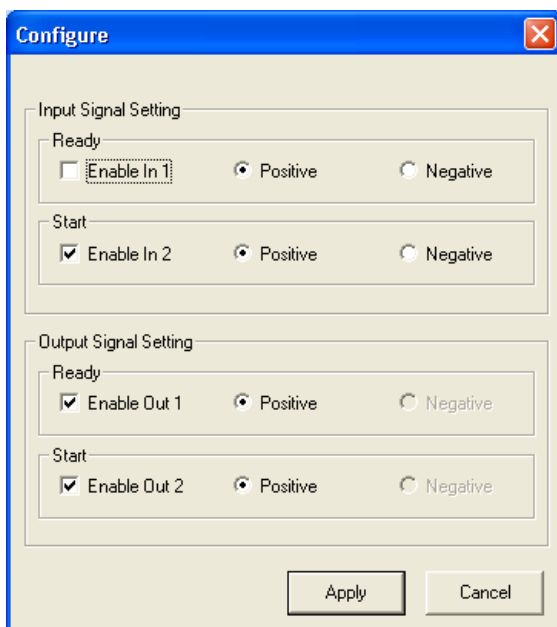


Figure 7-4 External Start Configuration Dialog Box

2. Use **In 1** as a System Ready signal from an external device, and **In 2** for starting spectra acquisition from the connected device.

System Ready is equivalent to clicking the Operation option button in the Instrument group (Figure 7-2 on page 7-2), while receiving a Start input is equivalent to clicking the Start button in the Command Buttons group on the left side of the BioTOF window.

3. Enable **Out 1** to send a ready signal to an external device when the BioTOF system transitions to a ready state, and **Out 2** to switch the connected system on when you click the **Start** button in the BioTOF window.
4. Click **Apply** and close the dialog box to return to the Mode tab.
5. Click **Enable** in the External Start group.

To read the current signals from the external device:

- Choose **Tools > Diagnostics > Spectrometer**.

The current values from the device appear in the lower right section of the Parameter Setting - Spectrometer dialog box. See "Diagnostics" on page 5-21

To manually trigger a signal to a connected device:

1. Choose **Tools > Diagnostics > Spectrometer** to open the Parameter Setting - Spectrometer dialog box.
2. Click **-External Out 1** to send the ready signal or **External Out 2** to send the start signal.

7.3 Chromatogram Tab

Use the Chromatogram tab (Figure 7-5) to define mass ranges from the LC system. These will appear in the Spectra Display window. When the Instrument mode is Chromatography - MS, the Chromatography pane displays a trace for each mass range specified in the tab. Those ranges always include **TIC,MS** (Total Ion Chromatogram, Mass Spectrum).

The control buttons on the right side of the table enable definition of Extracted Ion Chromatograms so you can focus on specific ranges. When you select a range in the tab a color bar at the base of the mass spectrum pane identifies the ions as determined by the mass spectrometer.

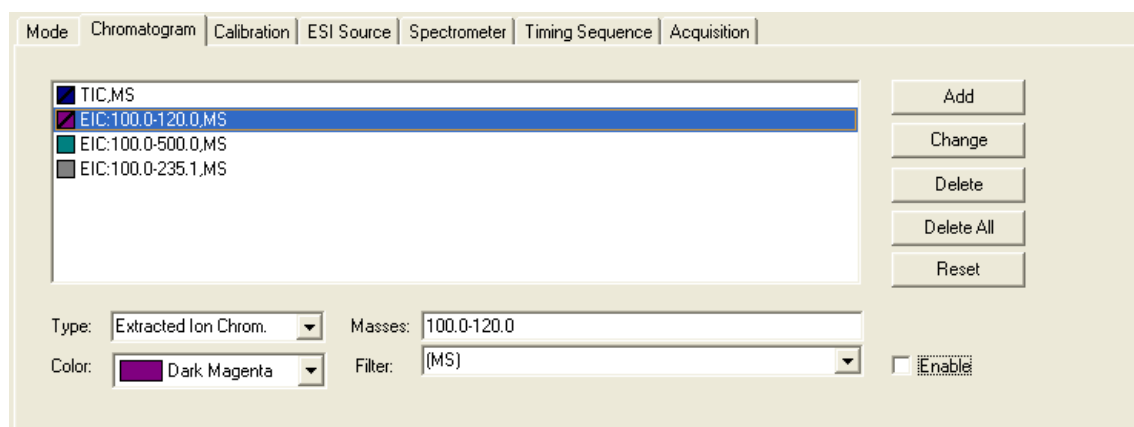


Figure 7-5 Chromatogram Tab

To define an Extracted Ion Chromatogram range:

1. Click the **Mode** tab and select **Chromatography - MS** in the Instrumentation Mode group if the Chromatography pane is not already displayed.
The pane includes a trace from the TIC,MS range.
2. Click the **Chromatogram** tab to display the display the Chromatogram tab.

3. Use the **Type** pull-down list to select **Extracted Ion Chrom.**
4. Click the **Add** button to the right of the list.
5. Change the low and high values in the **Masses** field by doing one of the following:
 - Type over the value on each side of the hyphen.
 - Drag the cursor across a section of the TIC.MS trace in the Chromatography pane. When you release the mouse button, the start and end values are displayed in the Masses field.
6. Use the pull-down list in the **Color** field to select a color for the trace in the Chromatography pane and the marker bar in the Mass Spectrometer pane.

7.4 Calibration Tab

The Calibration tab (Figure 7-6) provides access to reference tables so you can calibrate the spectra displayed in the BioTOF window to the mass of a known constituent in the sample. Use of this tab is described in detail in Chapter 9 “Calibration”.

Reference List: Peptides.ref

Name	Reference Mass	Current Mass	Error [ppm]
• Valine [M+H] ⁺	118.086255		
• Caffeine [M+H] ⁺	195.087653		
• Val-Pro-Leu [M+H] ⁺	328.223632		
→ Arg-Gly-Asp [M+H] ⁺	347.167361		
• tri-Tyrosine [M+H] ⁺	508.207828		
• Leucine-Enkephalin [M+H] ⁺	556.276577	556.2100	119.68
• Methionine-Enkephalin [M+H] ⁺	574.232996		

Fit: Linear Quadratic Preset

Constants:

$t = c_0 + \sqrt{\frac{10^{12}}{c_1}} m + c_2 \cdot m$

Accept Default

Figure 7-6 Calibration Tab

7.5 ESI Source Tab

The ESI Source tab (Figure 7-7) includes groups for setting gas flow, gas temperature, and voltages applied to various ESI Source components.

The active settings on the tab depend on the button selected in the Ionization group (see Figure 7-2 on page 7-2).

The screenshot shows the 'ESI Source' tab selected in a software interface. The tab is part of a larger window with other tabs like 'Mode', 'Chromatogram', 'Calibration', 'Spectrometer', 'Timing Sequence', and 'Acquisition'. The 'ESI Source' tab contains two main sections: 'Gas Flow/Temperature' and 'High Voltage Electrodes [Volts]'. The 'Gas Flow/Temperature' section includes fields for 'Dry Gas Temp' (set to 200 C), 'Dry Gas Flow' (set to 0 L/min), 'APCI Neb Temp' (set to 0 C), and 'Neb Gas Flow' (set to 0 L/min). The 'High Voltage Electrodes [Volts]' section is divided into two columns. The left column lists 'Cylinder' (2000), 'Corona Needle' (500), 'End Plate' (3500), and 'Capillary' (4000). The right column lists 'Capillary Exit' (140), 'Skimmer1' (40), 'Skimmer2' (30), 'Offset' (15), 'Trap' (45), 'Lens' (50), and 'RF Amplitude' (600). A 'Refresh' button is located at the bottom right of the 'High Voltage Electrodes' section.

Gas Flow/Temperature	
Dry Gas Temp	200 C
Dry Gas Flow	0 L/min
APCI Neb Temp	0 C
Neb Gas Flow	0 L/min

High Voltage Electrodes [Volts]	
Cylinder	2000
Corona Needle	500
End Plate	3500
Capillary	4000

Lens [Volts]	
Capillary Exit	140
Skimmer1	40
Skimmer2	30
Offset	15
Trap	45
Lens	50
RF Amplitude	600

Refresh

Figure 7-7 ESI Source Tab

Gas Flow/Temperature

When the Instrumentation is either ESI or nESI, Dry Gas Temp is the only active field in the Gas Flow/Temperature group. For ESI and nESI Dry Gas Temp is typically set between 200° C and 220° C.

When the Instrumentation is APCI, Dry Gas Temp and APCI Neb Temp are active. Typical settings are 250° C and 400° C, respectively.

Dry Gas Flow and Neb Gas Flow are not active in the BioTOF software, as these flow rates are set with control on the Source Control Unit panel.

High Voltage Electrodes (Volts)

When Instrumentation is ESI or nESI, high voltage is applied to three electrodes:

- Cylinder
- End Plate
- Capillary

When Instrumentation is APCI, high voltage is applied to:

- Corona Needle
- End Plate
- Capillary

When Ion Polarity is Positive, the actual ESI spray chamber voltages in the High Voltage Electrodes group are negative.

Note: The software limits the difference between any two of the spray chamber voltages to a maximum of 2000 V. You may need to increase the voltages sequentially and in steps.

Lens (Volts)

The voltages in the Lens group in Figure 7-7 are typical settings.

- The Offset must be **15 V**.

Refresh button

- Click the **Refresh** button to send the displayed gas and voltage values to the ESI controller.

7.6 Spectrometer Tab

The High Voltage settings on the **Spectrometer** tab (Figure 7-8) are set during installation and configuration of the system and should not be modified.

Note: Changing the parameters in the Spectrometer tab changes the mass calibration. These advanced parameters were set during installation and are specific to each instrument. Modifying these parameters can adversely affect system performance.

The BioTOF system must be re-calibrated if any of the Spectrometer tab values are changed.

The screenshot displays the 'Spectrometer' tab within a software interface. The tab is part of a sequence including 'Mode', 'Chromatogram', 'Calibration', 'ESI Source', 'Spectrometer', 'Timing Sequence', and 'Acquisition'. The 'Spectrometer' tab is active, showing a 'High Voltage [Volts]' section. This section contains eight parameters, each with a numerical input field and a small up/down arrow icon. The parameters and their values are: Repeller (8500), Grid (0), Reflectron (9300), Reflectron Lens (100), Post Acceleration (2000), Detector (1600), Multi Deflector Low (5.0), and Multi Deflector High (71.0).

High Voltage [Volts]	
Repeller	8500
Grid	0
Reflectron	9300
Reflectron Lens	100
Post Acceleration	2000
Detector	1600
Multi Deflector Low	5.0
Multi Deflector High	71.0

Figure 7-8 Spectrometer Tab

7.7 Timing Sequence Tab

The Timing Sequence tab is used to set the cycle delay time and duration used in the mass spectrometer. The values shown in Figure 7-9 are the default values, which are appropriate for most cases.

The screenshot shows the 'Timing Sequence' tab selected in a software interface. The tab bar at the top includes 'Mode', 'Chromatogram', 'Calibration', 'ESI Source', 'Spectrometer', 'Timing Sequence', and 'Acquisition'. The main area is titled 'Advanced Spectrometer Timing' and contains several input fields and a button. The 'Cycle Period' is set to 25 ms, 'Repeller' is set to 10 us, 'Gate' is set to 100 us, 'Transfer' is set to 100 us, and 'Deflector off Delay' is set to 25 us. A 'Pulse ON/OFF' button is located in the bottom right corner of the timing group.

Parameter	Value	Unit
Cycle Period	25	ms
Repeller	10	us
Gate	100	us
Transfer	100	us
Deflector off Delay	25	us

Pulse ON/OFF

Figure 7-9 Timing Sequence Tab

Pulse ON/OFF

The Pulse ON/OFF button in the lower right corner of the Advanced Spectrometer Timing group toggles operation of the HV pulser that supplies a high-voltage pulse to the Repeller in the accelerator. This pulse changes the trajectory of the ion and sends them into the TOF flight tube. The pulser must be on for the BioTOF to collect mass spectra. However, when troubleshooting unexpected results, it may be useful to turn off the pulser so that there are no ions travelling through the tube and reflected back to the detector. The digitizer continues to send data to the BioTOF window allowing you to identify data points that are not a result of detecting reflected ions.

7.8 Acquisition Tab

The settings on the Acquisition tab (Figure 7-10) determine display and data recording resolution.

The screenshot shows the 'Acquisition' tab selected in a software interface. The tab bar at the top includes: Mode, Chromatogram, Calibration, ESI Source, Spectrometer, Timing Sequence, and Acquisition. The main area is divided into several sections:

- Acquisition Window:** Contains input fields for 'm/z from' (147), 'to' (874), 'Spectrum Size' (50000 pts), and '200 KB'. Below these is a 'Digitizer Delay' field set to 35 us.
- Advanced Digitizer Setting:** Contains 'Spectrum Offset' (0.0350 V) and 'End of Scan Deadtime' (0.5440 us).
- Sample Rate:** Features three buttons for 0.5, 1, and 2 GS/s. The '1' button is currently selected.
- Mass Range:** A graphical representation showing a linear increase in 'Resolution' as the mass range expands.
- Advanced Data Handling:** Contains a 'Digitizer Summing' field set to 20 Spectra.

Figure 7-10 Acquisition Tab

Acquisition Window

The m/z range is set on the Mode tab (Figure 7-3 on page 7-3). The m/z range and the Spectrum Size set on this tab determine total size of a the spectra file.

The Spectrum Size is set in the number of data points per record. The maximum number of data points per record is 252,000. The number of points determines the disk space required for a single spectrum, as shown in kilobytes in the field to the right.

The Digitizer Delay is specified in μs from the start of each data acquisition cycle until the digitizer begins recording data. This delay establishes the low end of the m/z range. When you change the delay, the mass range is shifted accordingly.

Sample Rate

There are three sampling rates: 0.5, 1.0 and 2.0 gigasamples per second. The higher the rate, the greater the digitizer resolution, that is, the number of data points across the ion peak. However, as you increase the sampling rate, the upper end of the m/z range is decreased. If you increase resolution, you may need to adjust either Spectrum Size or Digitizer Delay to capture the correct m/z range.

Advanced Digitizer Setting

- Spectrum Offset adjusts the zero point of the vertical scale in the Spectra Display window. Increasing the value excludes data points that may be noise. The offset should be in the range -0.4 to 0.4.
- End of Scan Deadtime is a delay at the end of a cycle before the next cycle begins. This value cannot be changed from the BioTOF window.

Advanced Data Handling


- Digitizer Summing sets the number of records the digitizer accumulates before it sends the summed records to the BioTOF software as a single Spectrum. A setting of 20 is usually appropriate.

7.9 Method Files

When the BioTOF window is opened, the application loads the Last Good Method, that is, the system settings and parameters that were in use when the system was closed. The Method menu allows you to save the current method to a file and load previously saved methods.

7.9.1 Save Method

Use the Save Method function to create a new method or to update a previously saved method.

This menu function does not save mass spectrum data. Click  **Save** in the Command Buttons pane on the left side of the window to store data for analysis.

To save the current method for later use:

1. Choose **Method > Save As** to open a Save As dialog box (Figure 7-11).

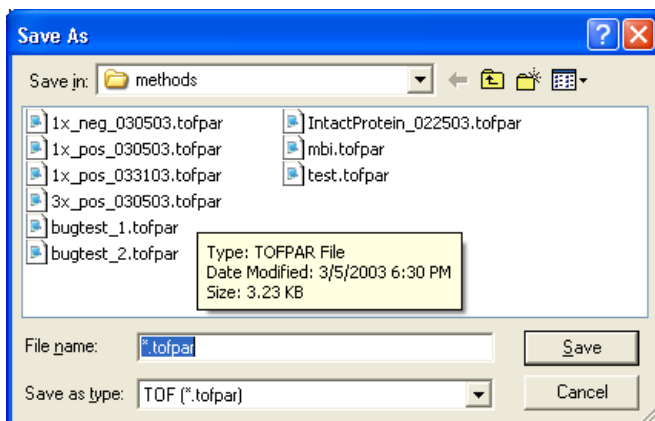


Figure 7-11 Save As Dialog Box for Saving a Method

The dialog box displays the methods that have been saved in **..\Data\Methods** folder; the files in the folder are filtered to include only those with the extension ***.tofpar**. You can change the path, but you must use the ***.tofpar** extension.

2. Select an existing method in the dialog box to update file with the current parameters, or enter an unused name to create a new method.
3. Click **Save**.

If you save to an existing name, a dialog box prompts you to confirm the action.


- Click **Yes** to overwrite the previous file or **No** to cancel the action and select a new name.

The Current method field changes to the name of the saved file.

When you exit the BioTOF window, the system saves the parameters and presents them as the Last Good Method next time the window is opened.

7.9.2 Load Method

To load a previously saved method:

1. Choose **Method > Load Method** from the menu bar, or click () in the tool bar.

An Open dialog box displays the methods that have been saved in **..\DataMethods** folder (Figure 7-12). The files in the folder are filtered to include only those with the extension ***.tofpar**.

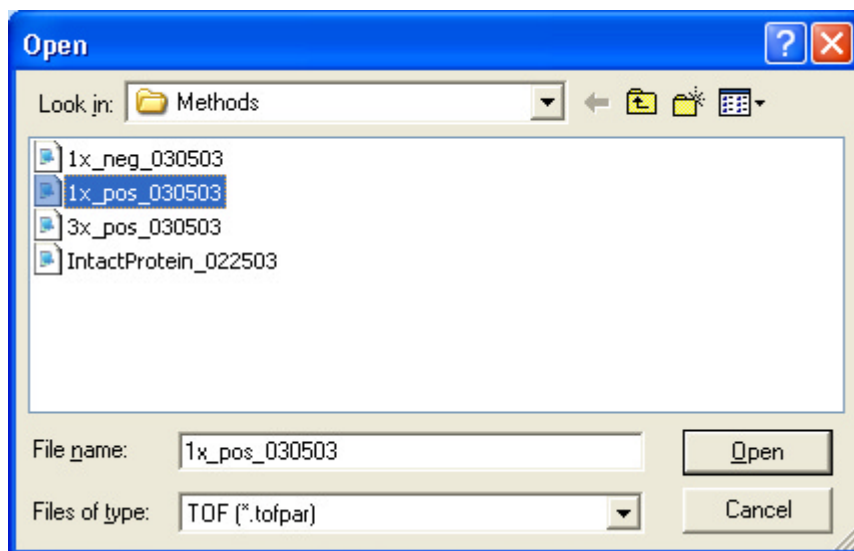


Figure 7-12 Open Dialog Box for Loading a Method

2. If the method is stored in another location, use the dialog box tools to browse to the other folder.

3. Select the method and click **Open**.

The BioTOF system displays a progress gauge as it loads the method (Figure 7-13). It can take several minutes for the data to be loaded. When finished, the method file name is shown in the Current Method box on the left side of the BioTOF window.

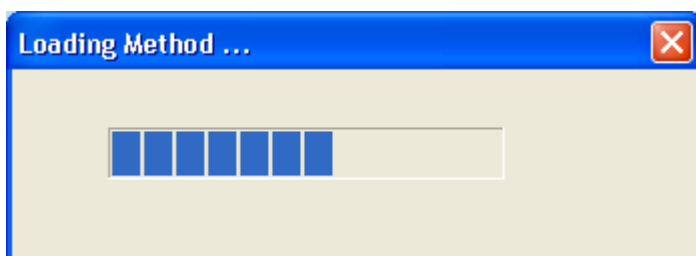


Figure 7-13 Progress Gauge as a Method is Being Loaded

8 Acquiring Data

After introducing the sample to the ESI Source and selecting the acquisition method, the next step is to acquire and save spectra. Topics covered by this chapter include:

- Starting system operation
- Acquiring and saving spectra
- Acquiring spectra in MS - Chromatogram mode
- Multipass

8.1 Starting System Operation

To start BioTOF operation:

1. In the BioTOF window, click the **Operation** option button in the Instrument group (Figure 8-1).

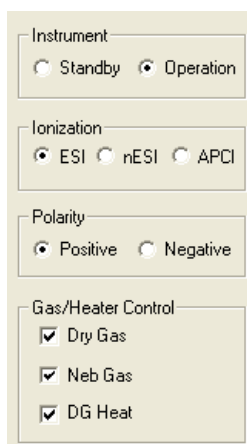


Figure 8-1 Instrument Control Group

The instrument goes from Standby to Operation.

The Enable LED on the Instrument HV unit lights up (Figure 8-2) and the high voltages ramp up. All ESI source voltages turn on.

You may also be prompted to press the **Voltage On** button on the Source Control unit (Figure 8-2). This switch lights up to indicate that the source voltages have been turned on.

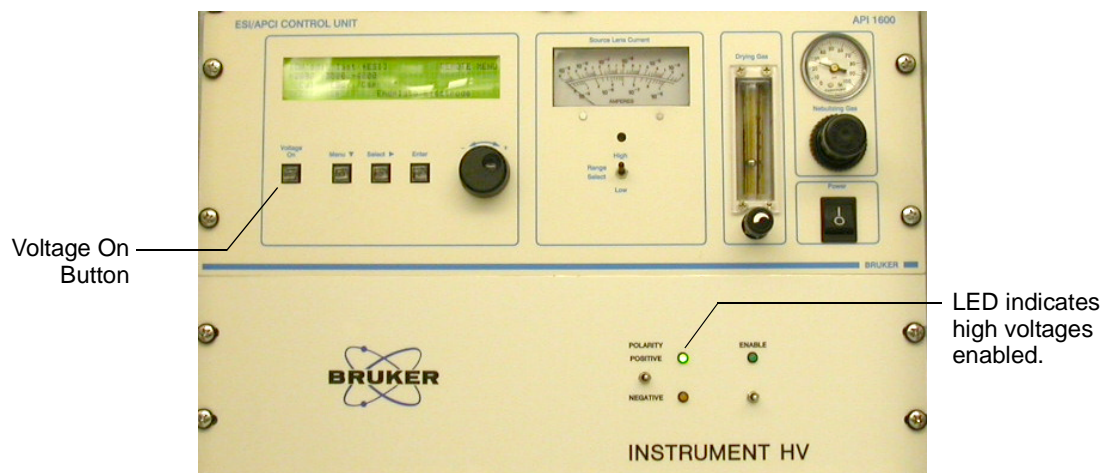


Figure 8-2 Source Control Unit and Instrument HV Panel

2. Start the sample flow from the syringe pump or LC system.

3. If using the syringe pump, press and hold the two right buttons on the syringe pump to fast-forward the solution to the ESI.

When sample is flowing through the tip of the spray needle assembly, and nebulizer gas and spray chamber voltages are turned on, the current meter (Figure 8-3) of the ESI source controller indicates the spray current.

If there is no reading:

- Check to make sure that the meter is set to read the endplate electrode, as described in “Start the System” on page 4-1.
 - Adjust the needle “Adjusting the Electrospray Needle” on page 6-4
 - Verify that the spray shield is installed.
4. Release the two syringe pump buttons to stop fast forward, and press **Run** the pump at the specified rate.

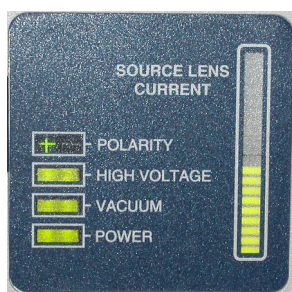


Figure 8-3 **Source Lens Current Reading**

8.2 Acquiring and Saving Spectra

Controls and indicators for starting and stopping spectrum acquisition are located in the two panes on the left side of the BioTOF window (Figure 8-4). You can start acquisition when the status bar is green and displays **Ready**.

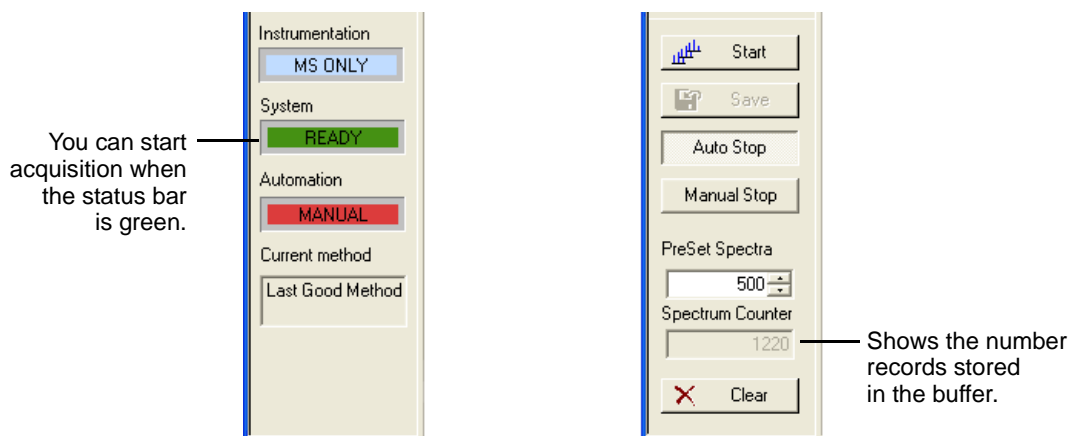


Figure 8-4 Indicators and Controls on the Left Side of the BioTOF Window

To run spectra acquisition:

1. Click the **Start** button. Data acquisition and transfer begin, and the Spectra Display plots the results. The Spectrum Counter indicates the total number of records accumulated in the buffer. The Start button changes to a Stop button, enabling you to manually stop acquisition when a sufficient number of records have been stored in the buffer.

2. Click the two checkboxes in the lower left corner of the Spectra Display to enable autoscaling for both axes of the display (Figure 8-5). The BioTOF continues to accumulate records until the buffer contains the number of records specified in PreSet Spectra.
 - If **Manual Stop** is selected, the buffer is cleared and a new acquisition is started.
 - If **Auto Stop** is selected, acquisition stops and the data in the buffer is maintained.

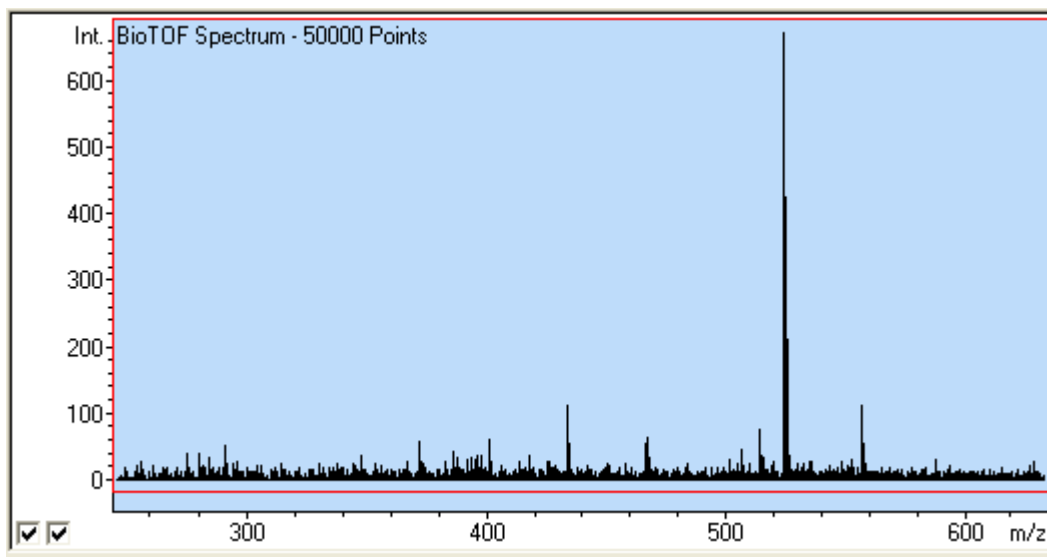
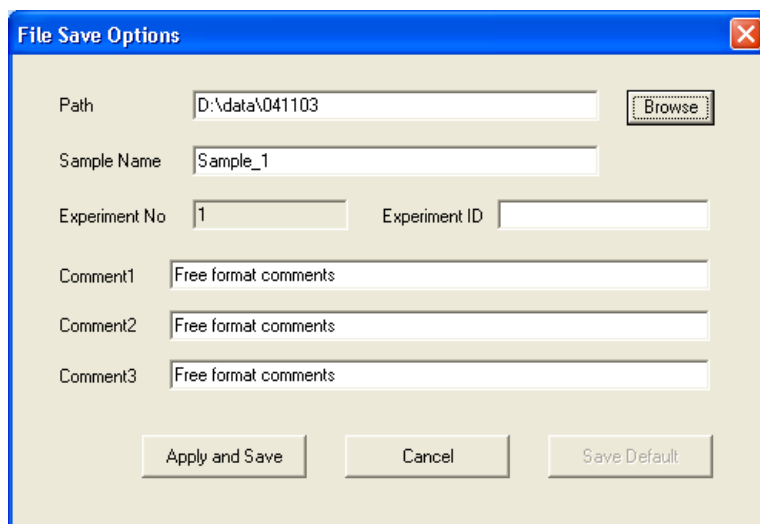


Figure 8-5 Spectra Display

3. Click the **Stop** button to manually stop the acquisition when there sufficient records. The Save button becomes active.

- Click **Save** in the control panel.

The BioTOF software opens a dialog box for setting the file options (Figure 8-6).



The dialog box titled "File Save Options" contains the following fields and controls:

- Path:** A text box containing "D:\data\041103" and a "Browse" button.
- Sample Name:** A text box containing "Sample_1".
- Experiment No:** A text box containing "1".
- Experiment ID:** An empty text box.
- Comment1:** A text box containing "Free format comments".
- Comment2:** A text box containing "Free format comments".
- Comment3:** A text box containing "Free format comments".
- Buttons:** "Apply and Save", "Cancel", and "Save Default" at the bottom.

Figure 8-6 File Save Options Dialog Box

The BioTOF application creates two files, one formatted for the DataAnalysis application, the other compatible with the XMass.

To set the application to use either or both formats:

- Choosing **Format > DA format** and/or **Format > Xmass format** from the menu bar.

8.3 Multipass

Multipass is a patented technology available in Bruker's BioTOF Series Time-of-Flight mass spectrometers. Multipass effectively multiplies the length of the flight tube by sending the ions through the tube multiple times creating a much longer instrument with enhanced mass resolution.

Multipass is accomplished by keeping the Repeller voltage up when the ions return from the Reflectron, thereby sending them back into the flight tube for another turn. After the desired number of turns, the Repeller voltage is dropped and ions pass through the Accelerator and on to the Detector. As ions move at different velocities through the flight tube, the timing of the Repeller voltage depends on the mass range of interest. High and low m/z ions may separate so far that they are no longer in the same cycle and the free spectral range of ions with the same number of turns is limited as shown in Table 8-1.

Table 8-1 Spectral Range for Multiple Passes

Passes	Ratio of High m/z to Low m/z
2	4.4
3	2.3
4	1.9
5	1.6

8.3.1 Using a Predefined Multipass Method

Multipass methods are saved on the BioTOF workstation during final test in the factory and re-confirmed during installation. These methods are defined typically for a standard mass range. To change the mass range in multipass mode, the digitizer acquisition delay and the Repeller timing must be reconfigured as described below.

To set up multipass using a predefined method:

1. Click **Standby** in the Instrument group, load the predefined multipass method, and then click **Operation** in the Instrument group.
2. Select the **Mode** tab and enter the low end of the desired mass range in the Mass Range [m/z] group.
3. Calculate the high end of the new range using the ratios in Table 8-1 on page 8-7 and enter the value in the Mass Range [m/z] group.
4. Click the **Acquisition** tab and find the new **Digitizer Delay** in the Acquisition Window group.
5. Click the **Timing Sequence** tab and set the set **Repeller** to **5 μ s** lower than the new Digitizer Delay value.
6. Test the new settings with a standard sample.
7. Fine-tune Multideflector High, Multideflector Low and Reflectron voltages, as necessary.
8. Recalibrate the instrument.
9. Save the parameters as a new method.

8.3.2 Creating a Multipass Method

The flight times multiply approximately with the number of turns in Multipass mode, that is, for two passes flight times approximately double, for three passes they triple, and so on. However, the focusing conditions change and it is necessary to retune the Reflectron voltage in order to achieve best performance. In general, the Reflectron voltage increases with the number of turns. Set up Multipass methods sequentially for two, three or more passes.

To set up multipass without a predefined method:

1. Start in normal mode (single pass, positive ions) using a strong sample with only one or two well known peaks chosen in accordance with the Table 8-1 on page 8-7.
2. Click the **Mode** tab and select **Flight Times** in the Data Display group.
3. Note the flight times for the peaks of interest.
4. Click the **Acquisition** tab and set the Digitizer Delay **5 to 10 μ s** below the doubled flight time of the (low m/z) peak.
5. Change to the **Timing Sequence** tab and set the Repeller duration to **5 μ s** lower than the new Digitizer Delay.
6. Record a spectrum.
Out-of-focus peaks will appear at approximately doubled flight times.
7. Retune the Reflectron, Reflectron Lens and multideflector voltages for optimized performance, as described in "BioTOF Reflectron Tuning" on page 10-6.
8. Click the **Mode** tab and select **m/z** in the Data Display group.
9. Record a spectrum and recalibrate the instrument (two passes).
10. Save spectrum and save the method with new parameters and new calibration.
11. Proceed to setting up a three-pass method accordingly.

8.3.3 Tips on Using Multipass

Tuning

Experience shows that the sensitivity of the Reflectron voltage tuning increases with the number of passes as well as the importance of fine-steering of the ion beam provided by Multideflector Low voltage.

Eliminating un-wanted ions from the mass spectrum:

In a complex sample, ions of m/z higher or lower than the accepted mass range may appear in the spectrum after the wrong number of passes. Those ions are typically not in focus, but may be of significant intensity particularly if their number of passes is lower (that is they are higher m/z ions) than specified by the multipass method.

To eliminate higher m/z ions:

- Switch to the **Timing Sequence** tab and set **Deflector off Delay** to a low value such as **2 or 3 μ s**.

This setting turns off the steering voltage before high m/z ions have passed through the multideflector into the flight tube. The trajectories of those ions are not be corrected for the orthogonal drift, and the ions are effectively eliminated from the spectrum.

Try this approach in single pass mode first and observe the affect on the mass range transmission.

To eliminate lower m/z ions:

- Switch to the **Timing Sequence** tab and increase the **Transfer** delay.

9 Calibration

As part of the BioTOF installation, the system was calibrated using a table of reference masses included in the software. The Calibration tab enables you to check the calibration after acquiring spectra from a known sample.

See “Time-of-Flight Calculation” on page 3-9 for any explanation of the calibration formula.

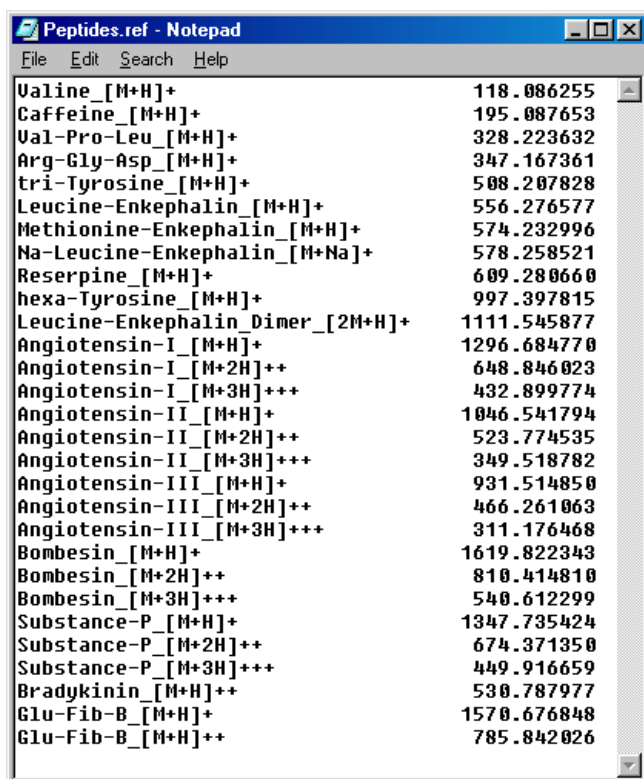
9.1 Reference Tables for Calibration

The following tables are supplied with the BioTOF software:

Table 9-1 Reference Tables for Calibration

Reference File	Description
Peptides.ref	Positive ions, Peptides
Negative_Ions.ref	Negative Ions Peptides, Assorted
NA-TFA_neg.ref	Sodium-TFA (trifluoroacetic acid), negative ions
NA-TFA_pos.ref	Sodium-TFA, positive ions
PEG_Na.ref	Sodiated Polyethylene Glycol, positive ions
HPESIPOS.ref	Agilent ESI Tune mixture, positive ions

The reference files are ASCII text file consisting of a two-column list of elements and their masses. Figure 9-1 shows the file format.



The screenshot shows a Notepad window with the title 'Peptides.ref - Notepad'. The window contains a two-column list of peptides and their corresponding mass values. The peptides are listed on the left, and the mass values are on the right. The list includes various peptides such as Valine, Caffeine, Val-Pro-Leu, Arg-Gly-Asp, tri-Tyrosine, Leucine-Enkephalin, Methionine-Enkephalin, Na-Leucine-Enkephalin, Reserpine, hexa-Tyrosine, Leucine-Enkephalin Dimer, Angiotensin-I, Angiotensin-II, Angiotensin-III, Bombesin, Substance-P, and Bradykinin, along with their Glu-Fib-B derivatives. The mass values are in scientific notation, ranging from 118.086255 to 785.842026.

Valine_[M+H] ⁺	118.086255
Caffeine_[M+H] ⁺	195.087653
Val-Pro-Leu_[M+H] ⁺	328.223632
Arg-Gly-Asp_[M+H] ⁺	347.167361
tri-Tyrosine_[M+H] ⁺	508.207828
Leucine-Enkephalin_[M+H] ⁺	556.276577
Methionine-Enkephalin_[M+H] ⁺	574.232996
Na-Leucine-Enkephalin_[M+Na] ⁺	578.258521
Reserpine_[M+H] ⁺	609.280660
hexa-Tyrosine_[M+H] ⁺	997.397815
Leucine-Enkephalin Dimer_[2M+H] ⁺	1111.545877
Angiotensin-I_[M+H] ⁺	1296.684770
Angiotensin-I_[M+2H] ⁺⁺	648.846023
Angiotensin-I_[M+3H] ⁺⁺⁺	432.899774
Angiotensin-II_[M+H] ⁺	1046.541794
Angiotensin-II_[M+2H] ⁺⁺	523.774535
Angiotensin-II_[M+3H] ⁺⁺⁺	349.518782
Angiotensin-III_[M+H] ⁺	931.514850
Angiotensin-III_[M+2H] ⁺⁺	466.261063
Angiotensin-III_[M+3H] ⁺⁺⁺	311.176468
Bombesin_[M+H] ⁺	1619.822343
Bombesin_[M+2H] ⁺⁺	810.414810
Bombesin_[M+3H] ⁺⁺⁺	540.612299
Substance-P_[M+H] ⁺	1347.735424
Substance-P_[M+2H] ⁺⁺	674.371350
Substance-P_[M+3H] ⁺⁺⁺	449.916659
Bradykinin_[M+H] ⁺	530.787977
Glu-Fib-B_[M+H] ⁺	1570.676848
Glu-Fib-B_[M+H] ⁺⁺	785.842026

Figure 9-1 Sample Reference File

The files are located in the `..\biotofsystems\list` directory.

9.2 Calibrating the System

To calibrate the BioTOF system:

1. Acquire spectra from a sample with known constituents.
2. Click the **Calibration** tab to display the table of reference masses.
3. Use the pull-down list at the top of the tab to select the reference file for display in the Reference in the tab.
4. Select the first known sample used in acquiring the spectra (Angiotensin II in Figure 9-2).

A red vertical line appears in the Spectra Display at the calculated position of the selected compound on the m/z scale, and the cursor in the Spectrum Display changes to the Max cursor (↑).

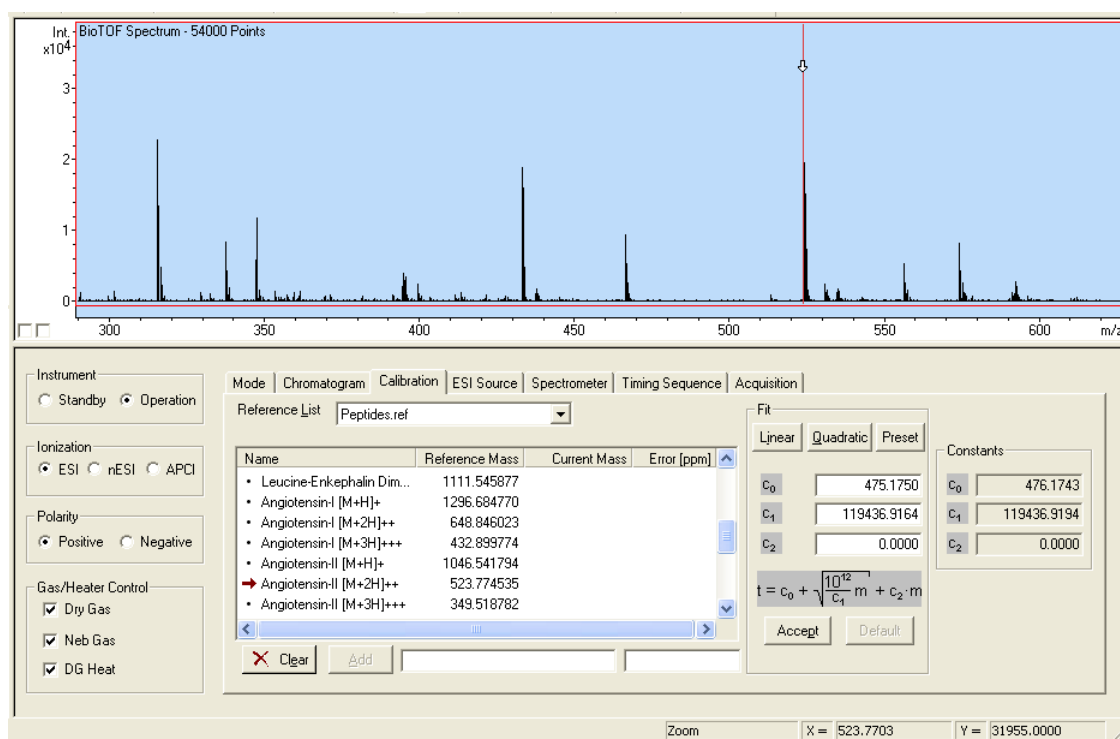


Figure 9-2 Calibrating the BioTOF System

5. Drag the cursor immediately to the right of the mono-isotopic peak of the reference compound and click the left mouse button.

The cursor snaps to the peak and the mass appears under the Current Mass in the reference table in the Calibration tab.

6. Use the same technique to snap the line to the next peak, but keeping second cursor below the top of the first peak.

A minimum of two peaks is required for linear calibration. If you add more than two peaks, the software computes a least-squares fit.

7. Click the **Linear** button in the Fit group to display the new calibration constants.
8. Check for obvious errors, such as negative constants.

These errors can occur if the peaks selected are not the proper compounds or the max cursor snapped to the wrong mass.

To restart a calibration:

1. Click **Clear** in the bottom left corner of the tab to erase values in the Current Mass column and clear the three constants in the Fit group.
2. Repeat steps 4 through 8.
3. Click the **Accept** button in the Fit group.

The new values appear in the Constants group to the right and the mass scale in the Spectra Display changes accordingly.

To calibrate using a curved line:

- Snap at least three peaks and click **Quadratic**.

To temporarily add a reference mass to a table:

1. Enter a name in the field immediately to the right of the Add button.
2. Enter a mass in the next field.
3. Click **Add**.

The new reference mass is listed in the table. However, the mass is deleted when you click Clear, select a new table, or exit the BioTOF software.

Note: The **Preset** button is a service tool that allows a rough calibration based mainly on instrument voltages. Use **Preset** if you do not have any calibration and need a starting point.

9.3 Creating a Custom Reference File

You can set up a custom reference file by copying one of the reference files and adding reference masses for items of interest.

To create a new file:

1. Use Notepad or a similar text editor to open one of the supplied reference files.
2. Choose **File > Save As** from the menu bar and save the file in the same directory with a different name and the extension **.ref**.

Note: Do not include any spaces in the file name.

3. Delete any line that you do not want in the reference table, but keep at least one line to use as a template.
4. For each new constituent, add a line consisting of the name and the reference mass, aligning the reference masses on the decimal.
5. Follow these rules:
 - Use the space bar, not Tab, to insert spaces between the name and the reference mass value.
 - Use the underscore character instead of spaces in the compound names.
 - Do not press **Enter** immediately after the last reference mass.

10 Maintenance

This chapter covers routine maintenance, and basic and advanced troubleshooting.

10.1 Daily Maintenance

Daily cleaning of the ESI Source is necessary to maintain optimum performance and sensitivity for the BioTOF system.

To clean the ESI Source:

1. Click **Standby** in Instrument group to turn off the high voltage.
2. Turn off Dry Gas Heat and allow the system to cool.
3. Flush the transfer line from the syringe pump or LC system.

Generally, you can flush the line with the solvent last used in operating the source. It is recommended to flush with a volume of at least 1 to 2 mL of solvent.

4. Open the spray chamber and wipe it with a methanol-soaked tissue.
5. Remove the spray shield and wipe it clean with a methanol-soaked tissue.
6. Wipe the capillary entrance with a methanol-soaked tissue and reinstall the spray shield.
7. If the project has included high flow rate applications, empty the high flow rate bucket behind the system and follow approved procedures for disposing the LC solvents.

10.2 Monthly Maintenance

This section describes maintenance that should be performed on a monthly basis. You may adjust the frequency of this routine maintenance depending on usage and sample concentrations. Monthly maintenance of the BioTOF system includes:

- The cleaning specified in daily maintenance
- Checking the oil level in the vacuum pump
- Cleaning the Capillary and Skimmer 1 in the ESI Source

The last task required that you shut down the vacuum pumps.

10.2.1 Stopping the Vacuum System

The BioToF system is normally left running in Standby mode when it is not in use so that the ESI Source and TOF Spectrometer pressure can be maintained below thresholds.

To shut down the vacuum system:

1. At the control station, stop data acquisition and bring the BioTOF system into Standby mode if you have not already done so.
Standby Mode turns off the ESI source and spectrometer voltages.
2. Check the **Enable** light on the High Voltage unit and the light in the ESI **High Voltage** switch to ensure that the voltages are in fact off.
Use the manual switches on the Instrument HV panel and the Source Control Unit.
3. When both lights are off, allow the source to cool down and seal the inlet of the capillary.
4. Select **gauge 1** on the gauge controller and press **ON/OFF**.

Note: It is recommended (but not required) that you turn off all electronics by pressing the **ELECTRONICS** switch on the main switch panel. This may also require shutdown of the BioTOF software and a restart the next time the system is turned on.

5. Release **VACUUM PUMPS** switch on the main switch panel.
As soon as the Turbo Pumps have slowed sufficiently, the vacuum system automatically vents with air filtered through an air dryer cartridge.
6. Allow approximately 10 to 15 minutes for the vacuum pump to spin down and for the system to vent to atmosphere completely.

10.2.2 Cleaning the Capillary and Skimmer 1

To clean the Capillary:

1. Raise the hood on the ESI source and use a screwdriver to loosen the two holding screws on the left side of the source housing and swing the housing open (See Figure 5).

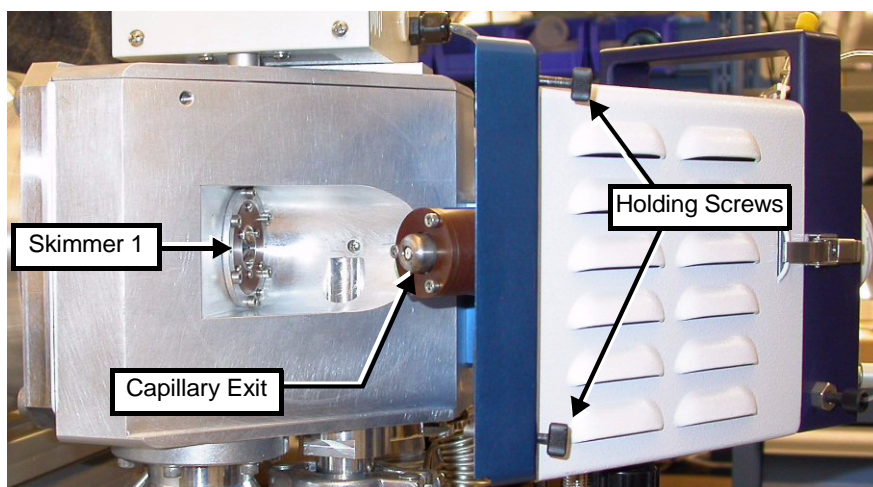


Figure 10-1 Skimmer 1 and Capillary Exit in the ESI Source

2. Open the spray chamber door and remove the end-cap that covers the face of the glass capillary.

The glass capillary is removed from the source by unscrewing the knurled nut that is behind the *capillary exit* and pulling the capillary out from the front (spray chamber side).

Note: Pull the capillary straight out until it is completely removed from the assembly.

3. Once removed, flush the capillary with an appropriate electrospray solvent.
Wiping with a lint-free cloth, Kim-Wipe™ or similar, moistened with solvent will sufficiently clean the metal-coated ends of the glass capillary.
4. Reinstall the Capillary, reverse the procedure above.
Cleaning Skimmer 1 does not require removing it from the source.

To clean Skimmer 1:

1. Moisten a lint-free cloth with solvent and gently polish the exposed face of the Skimmer.

Depending on the amount of material deposited on the skimmer, it may be necessary to perform this step a few times. The round metal shroud in which the capillary exit resides should also be cleaned in a similar fashion.

2. Inspect the skimmer and remove any fibers left from the cleaning.
3. Close the hinged door and tighten the two housing screws. The source is now ready to be pumped down and turned on for operation.

10.2.3 Checking the Oil Supply

At least monthly, check the oil supply for the vacuum pump. The pump is located in the right rear of the BioTOF system. The gauge is on the front of the pump.

To check the oil:

1. Open the door in the lower right corner of the right side of the cabinet to access the pump motor (Figure 10-2).

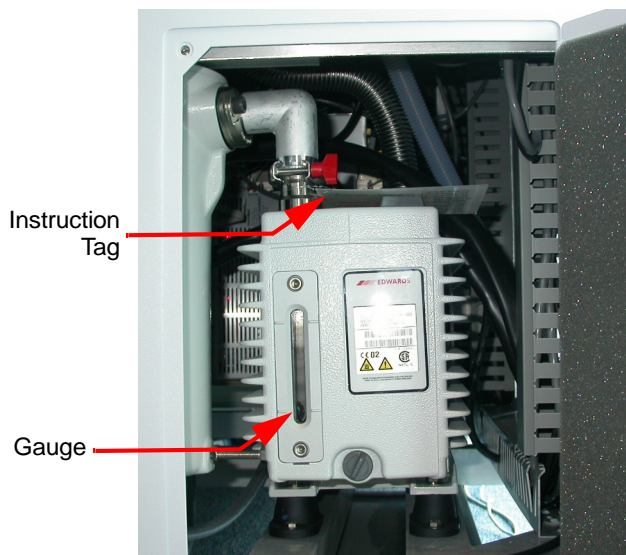


Figure 10-2 Vacuum Pump Motor and Oil Gauge

2. Follow the instructions on the tag attached to the pump.
3. If a refill is indicated, contact Bruker Daltonics customer service for assistance and to ensure that you have the correct oil.

Note: Use only the specified oil.

10.3 BioTOF Reflectron Tuning

This procedure describes tuning of the BioTOF time-of-flight analyzer for resolution and peak sensitivity. This procedure is completed during final test and reproduced during installation and acceptance, and method files with the default parameters are stored on the BioTOF workstation. Furthermore, the components of the BioTOF time-of-flight analyzer are configured and mechanically aligned in the factory, or at installation by a trained field service engineer. Once set, the tuning of the BioTOF system is stable and does not require user interaction. The procedure is described here for reference and for the purpose of retuning the instrument after extensive periods of use or shutdown of the instrument.

Note: Before re-tuning the instrument, save the current parameters and calibration in a method file. Changing any of the TOF voltages will affect the instrument calibration and re-calibration is required after the instrument tuning.

10.3.1 Setup for Tuning

To set up the BioTOF system to tune Reflectron voltages:

1. Save the current parameters and calibration to a method file as described in "Method Files" on page 7-13.
2. Click **Operation** in Instrument Group and **Positive** in the ion Polarity group, and infuse a standard sample from the syringe pump.
3. Select the **ESI Source** tab to verify that the **Offset** in the Lens group is set to **15 V**, and that standard values are set for all other source parameters.
4. Select the **Spectrometer** tab and set the **Detector** to **1600 V**.
Post Acceleration may be set to 2000 V but is not required at this point.
5. Use the **Acquisition** tab to verify that the Digitizer offset is set properly to give only a 1-2 count electronic noise band per scan and set the **Sample Rate** to **1 Gsa**.

6. Set the voltages in the **Spectrometer** tab as follows:

Repeller: 8500 V
Multideflector High: 67 V (BioTOF II) or the default value
Multideflector Low: 5 V (that is, no secondary deflection)
Reflectron Lens: 300 V
Reflectron: 9100 V

7. Start data acquisition.

The instrument should show good ion signal of at least 1000 counts for 200 spectra at the expected flight times (Leucine Enkephaline or Reserpine around 70 us).

With good signal proceed as described below; otherwise, enter the troubleshooting procedure.

- If the ion signal is not strong enough, set Reflectron Lens to **500 V**, and then to **700 V**.
- If signal is better with the higher Reflectron Lens voltage, proceed.

Note: Make sure that the spray and the spray current are stable. Check spray regularly during the tuning procedure

8. Pre-tune Multideflector High voltage in steps of 2 V up and down from the default value for maximum signal intensity.

10.3.2 Reflectron Tuning Procedure

The goal of the Reflectron tuning procedure is to optimize the Reflectron voltage for mass resolution and simultaneously balance Multideflector High and Reflectron Lens voltages for peak intensity.

Note: Do not change Repeller voltage during the tuning; leave it set at 8500 V.

1. Tune the Reflectron voltage as follows:
 - Acquire a decent spectrum. Typically 200 to 500 scans should be sufficient with standard sample.
 - Set data acquisition to AutoStop with acquisition counter preset to an appropriate number.
 - Acquire a spectrum and DO NOT CLEAR.
 - Set Reflectron voltage in steps of 100 V higher and lower from the starting value covering the range from 8600 to 9600 V. This procedure generates an envelope of peaks, with each peak (or group of isotope peaks) representing a Reflectron tuning voltage.
 - Settle for the voltage that gives the highest intensity in that envelope, which coincides with the narrowest peak shape, squeezing all the ions into a narrower package on the detector.
 - Click the Clear button.
2. Optimize peak intensity by tuning the Multideflector High deflector voltage for highest intensity initially in steps of 2 V, note the peak intensity, and then click the Clear button.

For the BioTOF II, the Maximum intensity should be achieved somewhere between 63 and 72 V.
3. Change the Multideflector High to optimal voltage and acquire a new spectrum.

4. Optimize peak intensity by tuning Reflectron Lens voltage in steps of 100V, noting the peak intensity, then clear; change the voltage and restart acquisition.

Check peak shapes for splits, bumps or other irregularities. For good signal to noise ratio always check intensities, resolution and peak shapes on the most intense peak of the isotope distribution.

5. Go back to step 1 and repeat procedure, starting from the Reflectron voltage determined in the first round.
6. Zoom in on the most intense peak, turn on the data point markers, and check resolution by estimating the (fractional) number of data points across the width of the peak at half height.

At the 1 Gsa sampling rate, the time between data points is 1ns. Mass resolution is equal to the flight time divided by 2 times the peak width in the same time units.

For example, at a flight time of 70 us or 70,000 ns, a peak width of 3.5 ns or 3.5 data points corresponds to a mass resolution of 10,000.

At this point in the tuning resolution should be at least 8,500 to 9,000 or approximately 4 data points for the Reserpine or Leucine Enkephalin peaks.

7. Save a spectrum and check resolution with the data processing software.
8. Start again with the voltage from the maximum peak, again not clearing the spectrum between acquisitions.
9. Tune in steps of 50 V up and down, over a range of -150 V to +150 V from previous optimum.
10. Follow the envelope of the tuning peaks and pick a voltage which correspond to the visual maximum of the envelope. This may be in between the recorded steps.
11. Clear and fine tune **Multideflector High** in 1 V steps as described above, optimizing peak shapes and resolution without giving up intensity by more than 10%
12. Fine-tune **Reflectron Lens** in steps of 50 V, optimizing peak shapes and resolution without giving up intensity by more than 10%, then repeat from step 8.

13. Clear and fine tune **Reflectron** voltage in steps of 25 V from previous best value.

Figure 10-3 shows a Spectra Display with 1000 spectra each for seven different Reflectron voltages. The maximum at 9350 V corresponds to the best tuning conditions.

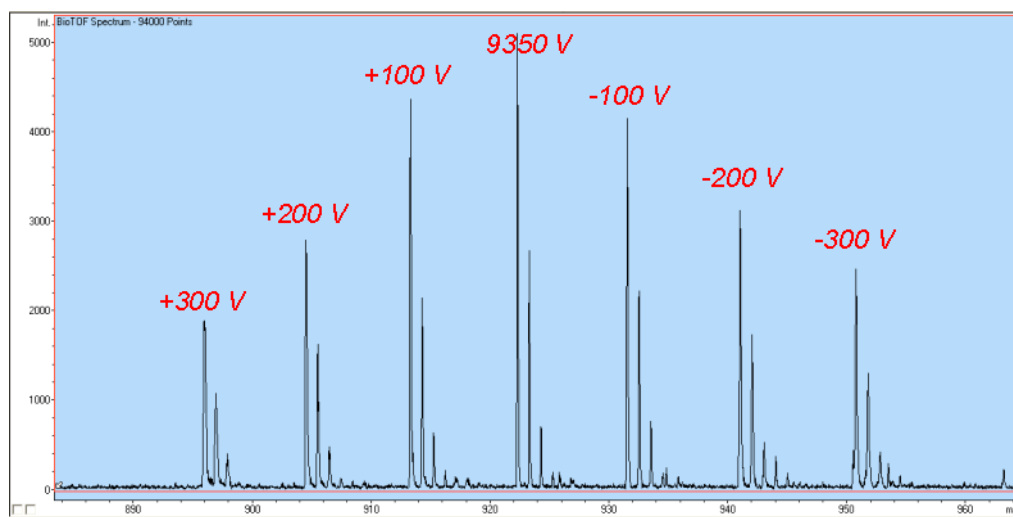


Figure 10-3 Determining Reflectron Voltage Providing Maximum Resolution

14. Re-calibrate the instrument as described in Chapter 9 “Calibration”.
15. Save spectrum and save parameters and calibration in a new method file.

10.4 Basic Troubleshooting

Figure 10.4 describes an algorithm for basic troubleshooting of the ESI Source. The flowchart is meant for new users of the source and provides a simple, step-wise guide based on observations of key parameters.

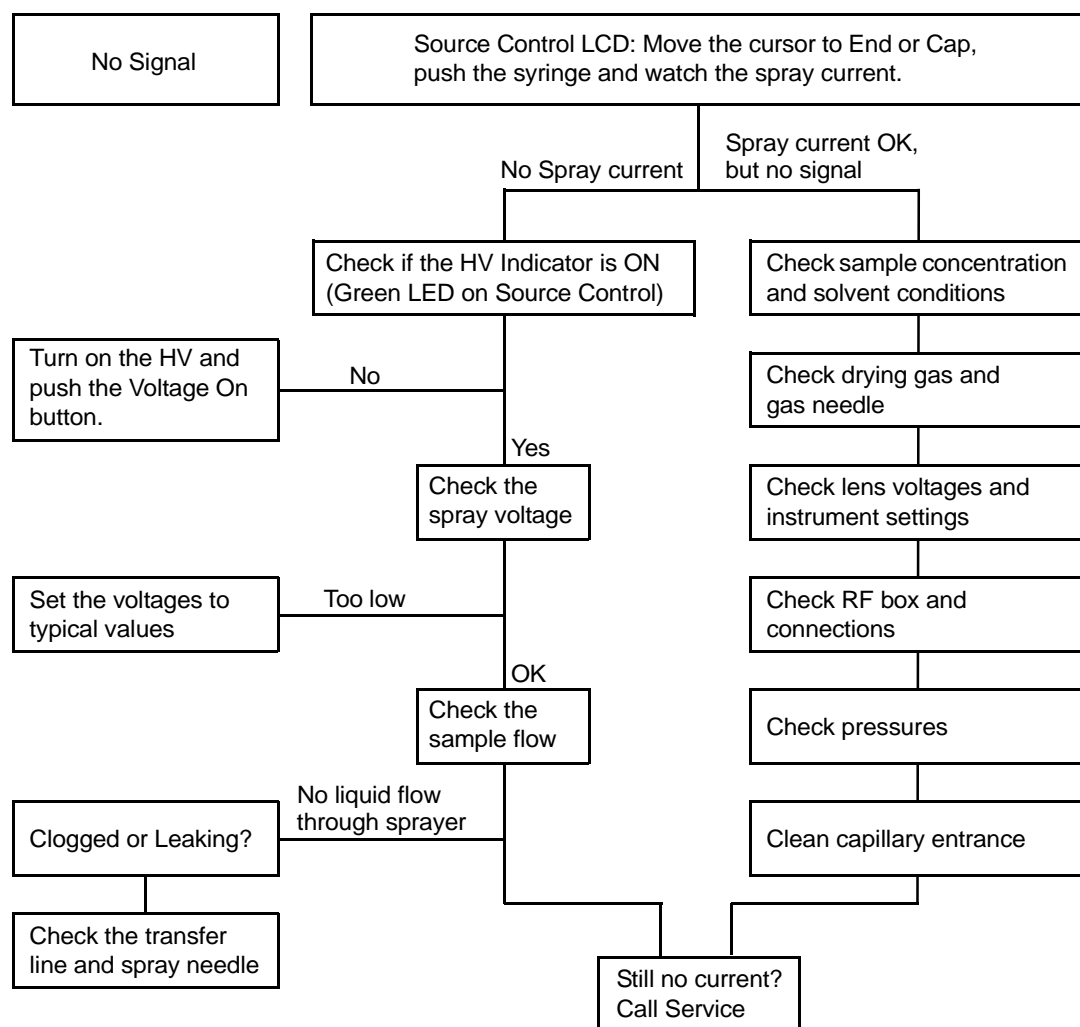


Figure 10-4 ESI Source Troubleshooting Flowchart

10.5 Advanced Troubleshooting

A more extensive guide to troubleshooting is presented in Table 10-1. Diagnostics and solutions to many of the problems depicted in these tables may require electrical tests or cleaning of the source.

Table 10-1 Advanced Troubleshooting Table

Symptom	Diagnostic	Possible Cause	Solution
Unstable signal and current	<ul style="list-style-type: none">• Unstable signal• Unstable current	Air bubbles	Purge the solvent.
	<ul style="list-style-type: none">• Liquid droplet on the Endcap	Drying gas flow, temperature and nebulizing gas not optimized	Adjust drying gas, nebulizing gas, and temperature.
Poor sensitivity or weak signal	<ul style="list-style-type: none">• Weak signal for standard	Source out of tune	Tune the Source.
	<ul style="list-style-type: none">• Unusual settings needed for Cap Exit or Skimmers	Skimmer may be dirty	Clean the Skimmer.
	<ul style="list-style-type: none">• Low signal intensity through the entire range of voltage settings of Cap Exit	Bad electrical contact or bad cable	Check the continuity of the Capillary Exit spring contact inside the 45° door and cable
	<ul style="list-style-type: none">• Low signal intensity through the entire range of voltage setting on Skimmer 1	Bad electrical contact or bad cable Dirty Capillary or Endcap	Check the continuity between pin 6 of the 6-pin feedthrough and the first skimmer. Clean the Capillary and Endcap.

Table 10-1 Advanced Troubleshooting Table (Continued)

Symptom	Diagnostic	Possible Cause	Solution
Error 151	• Check the Spray Chamber	Spray Chamber is not closed properly	Close the Spray Chamber.
	• Check the HV cable	HV cable is not plugged in or has a defect	Plug in or change the cable.
Error 120	• Check RF box and cable	RF box and cable not connected properly	Fix the connection.
	• Check the RF feedthrough, it is a short circuit to ground or to each other	Damaged hexapole	Call for service.
	• RF amplitude is above a certain value	Defective capacitor	Call for service.
	• None of the above	Defective RF driver	Replace the RF driver.
Error 122 - No heat from drying gas	• Check heater and TC cable	Loose cable	Tighten the cable.
	• Check the resistance of the heater. Should be 6 E0.5		
	• Check the heater fuse	Blown fuse	Replace the fuse.
	• If the Temperature setting is over 300 °C	Heater has insufficient power	Lower the setting temperature or apply an extra 24 V/100 W power supply on the second heater.

Table 10-2 provides the test points and values for all high voltage and low voltage elements to the source. When performing an electrical test, use the software to set the voltage of the element to a typical value listed in Table 10-2. The voltage interlock to the source must be **[Active]**. The **[Active]** state indicates that the high voltage button to the API 100/1600 is **[On]** and the spray chamber door is close.

Table 10-2 Electrode Test Points and Typical Values

Electrode	Pin and Connector	Pin to Element Resistance	Typical Voltage
Capillary	Pin 3 of HV Connector	< 5 Ohms	DC 3 kV ~ 5 kV *
Endcap	Pin 2 of HV Connector	< 5 Ohms	DC 3 kV ~ 5 kV *
Capillary Exit	Pin 1 of lens Connector	< 5 Ohms	DC 50 V ~ 200 V *
Skimmer 1	Pin 6 of lens Connector	< 5 Ohms	DC 10 V ~ 20 V
Skimmer 2	Pin 2 of lens Connector	< 5 Ohms	DC 5 V ~ 10 V
Trap/(Extract)	BNC (SMA)	< 5 Ohms	Pulsed 0 ~ 50 V
Lens (BioTOF) only	Pin 4 of lens Connector	< 5 Ohms	10 V ~ 40 V

*Extreme caution should be used when measuring these voltages. Interlock must be defeated for measuring high voltages. Set all high voltages to <700 V before measuring.

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