AccuTOF™-DART™ Training Guide

JEOL USA, Inc.
11 Dearborn Road
Peabody, MA 01960-6043
(978) 535-5900 / ms@jeol.com
www.jeolusa.com

Version 20070618

JEOL AccuTOF™-DART™ Training Guide
Revision 20070618

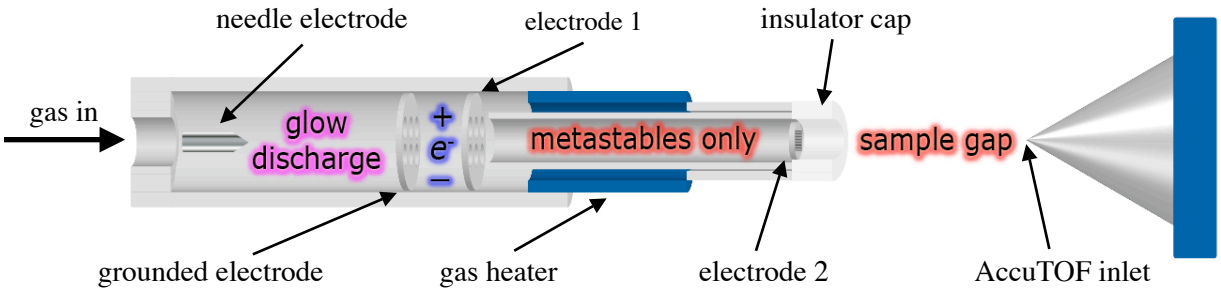
Analytical Instruments Division
11 Dearborn Road
Peabody, MA 01960
(978) 535-5900

Direct Analysis in Real Time (DART™) U.S. Patent Numbers 6,949,741 and 7,112,785

Table of Contents

Basic Hardware Overview

The DART™ Ion Source



Demonstrative Schematic of the DART™ Ion Source

A simplified schematic of the Direct Analysis in Real Time (DART™) ion source is shown above. A gas is introduced (usually helium) and passed by a needle electrode with a potential. A glow discharge is produced, which creates both charged particles and excited-state species (metastable gaseous atoms or molecules). This gas stream then passes through an additional electrode (electrode 1) to filter out the charged particles, leaving only the metastables. As the metastables continue through the source, they flow through a tube that can be heated. The ability to heat the gas allows for control of both thermal desorption and pyrolysis of samples in the sample gap. Next, the metastables pass through a final grid electrode (electrode 2) that is used to prevent any positive and negative ions from recombining as they exit the source. Finally, the gas exits through the insulator cap. The insulator cap ensures that the operator is well protected from any of the high voltages isolated within the source. DART™ ionization of samples occurs in the sample gap. The ions formed are directed to the AccuTOF mass spectrometer inlet by both the gas flow and a slight vacuum on the spectrometer inlet.

DART™ Ionization Mechanisms

Reference: *Anal. Chem.* **2005**, *77*, 2297-2302

As mentioned previously, DART™ ionization occurs in the sample gap. Because of this, both the DART™ gas stream and the analyte are exposed to open air. Different ionization mechanisms occur depending on the type of sample being analyzed (and its concentration), the nature of the carrier gas used and the polarity of the ions formed. A brief summary of mechanisms for both positive and negative ion formation, when helium is used as the carrier gas, is given below. For a complete discussion of the DART™ technique and its ionization mechanisms, please consult the Analytical Chemistry paper cited above.

Positive Ion

The metastable helium atoms formed in the source react with atmospheric water to produce ionized water clusters:

$$He(2^3S) + H_2O \rightarrow H_2O^{+\bullet} + He(1^1S) + e^-$$
$$H_2O^{+\bullet} + H_2O \rightarrow H_3O^+ + OH^\bullet$$
$$H_3O^+ + nH_2O \rightarrow [(H_2O)_{n+1}H]^+$$
$$[(H_2O)_nH]^+ + M \rightarrow MH^+ + nH_2O$$

The $He(2^3S)$ electronic excited state has an energy of 19.8 eV and a reaction cross-section of 100Å for water ionization. The protonated water formed after reacting with the excited-state helium mestable can then react with the analyte to form a protonated molecule.

Negative Ion

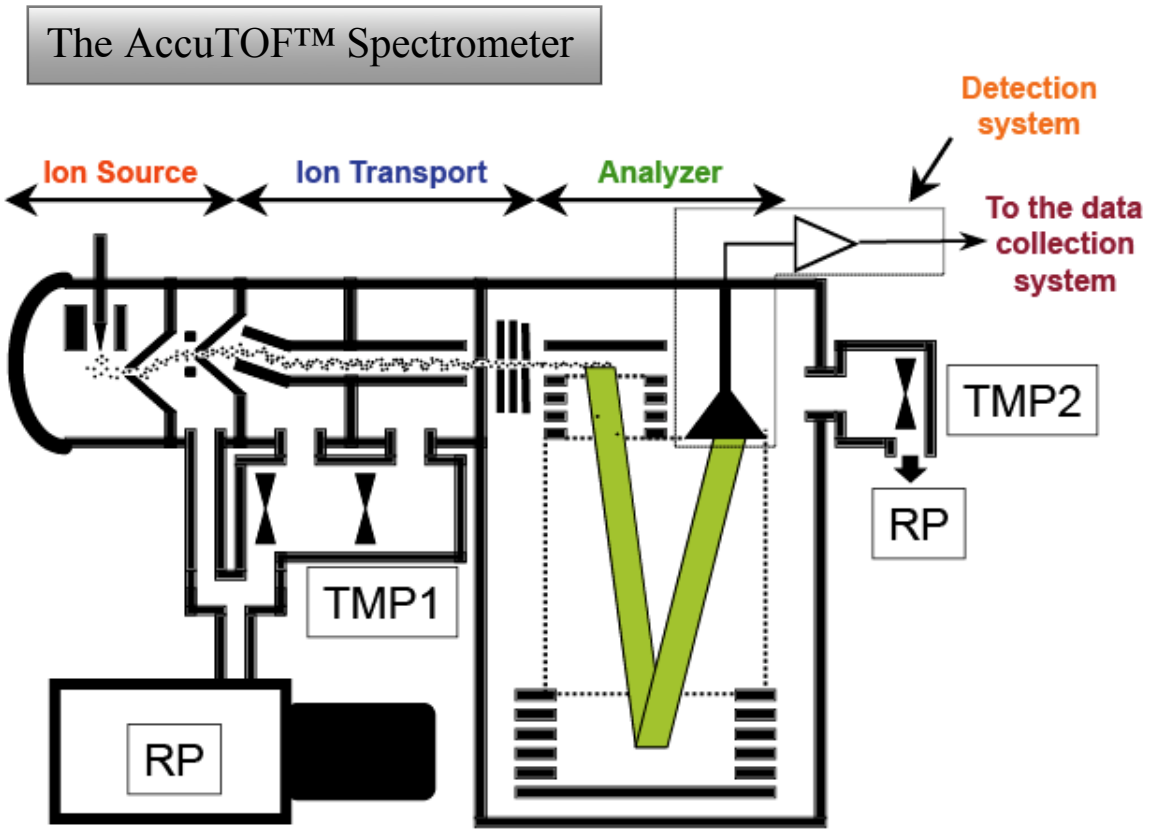
Metstable helium atoms can react with a neutral (N), such as the grid electrode, or another neutral species to form electrons through Penning ionization:

$$M^* + N \rightarrow N^{+\bullet} + M + e^-$$

The electrons formed are rapidly thermalized by collisions with atmospheric gases (G) and react with gaseous oxygen to produce ionized oxygen anions. These oxygen anions can then react with sample molecules (S) to produce analyte negative ions:

$$e^{-*} + G \rightarrow e^{-} + G^{*}$$
$$e^{-} + O_2 \rightarrow O_2^{-\bullet}$$
$$O_2^{-\bullet} + S \rightarrow [S\text{-}H]^{-} + OOH^{\bullet}$$
$$O_2^{-\bullet} + S \rightarrow S^{-\bullet} + O_2$$
$$O_2^{-\bullet} + S \rightarrow [S+O_2]^{-\bullet *} + G \rightarrow [S+O_2]^{-\bullet} + G^{*}$$

where: S is assumed to be a sample that contains hydrogen.



AccuTOF™ Spectrometer Schematic

A simple schematic for the AccuTOF™ spectrometer is given above. A brief description of the various spectrometer parts shown in the schematic is given in the

following paragraphs. A comprehensive description of the AccuTOF™ hardware and capabilities entitled “New Generation LC-TOF/MS “AccuTOF™” can be found on our website.

Ion Source

Both a DART™ and orthogonal spray ESI ion source come standard with the AccuTOF™-DART™ system. If you have questions about additional ion source choices available on the AccuTOF™ platform, please contact JEOL applications.

Ion Transport

Ion transport in the AccuTOF™ is accomplished using a quadrupole ion guide with three focusing lenses. Two off-axis skimmers and a bent RF ion guide are employed to efficiently transport ions from atmospheric pressure to high vacuum, while simultaneously keeping neutral contamination out of the vacuum system. A ring lens is also used to help to collimate multiple-charge ions and reduce space charge (ion-ion repulsion).

Analyzer

The analyzer is an orthogonal, two-stage acceleration, time-of-flight mass spectrometer incorporating a single-stage reflectron.

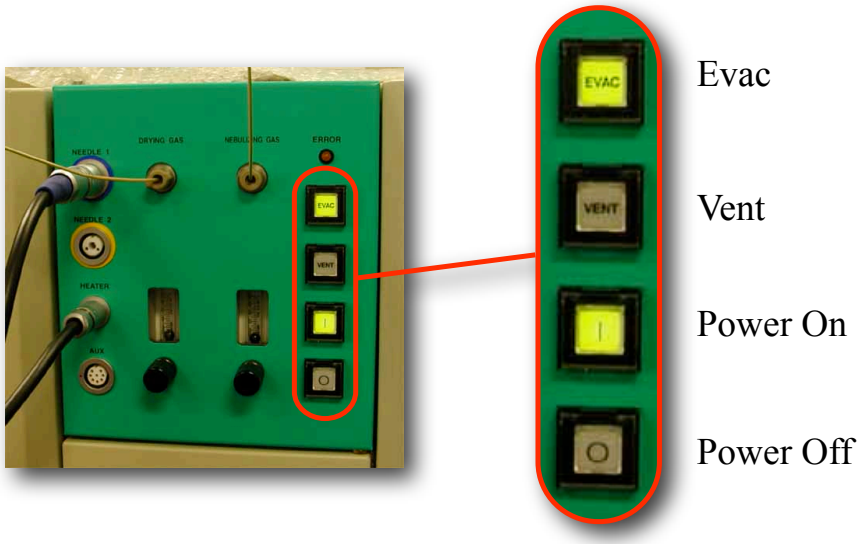
Detection System

The detector comprises two micro channel plates (MCP) with a continuous digital averager (ADC) as the data acquisition system. This provides high sensitivity, fast detection and high dynamic range.

System Start-up/Shut-down

Starting up after a Complete Shutdown

Use this procedure to power up the instrument after a power outage or after the instrument has been completely shut down.



The Front Panel on AccuTOF™

Powering up the system

a. If it is off, turn on the main breaker switch on the back of the console. Hit the **Power ON** switch on the front panel (**VENT** will be illuminated).

b. Hit **EVAC** to start pumping down the spectrometer. The **VENT** button will blink until the analyzer reaches a pressure of $\leq 2.0 \times 10^{-5}$ Pa. Note that you must wait until the analyzer reaches this pressure before proceeding with ***Conditioning the MCP***.

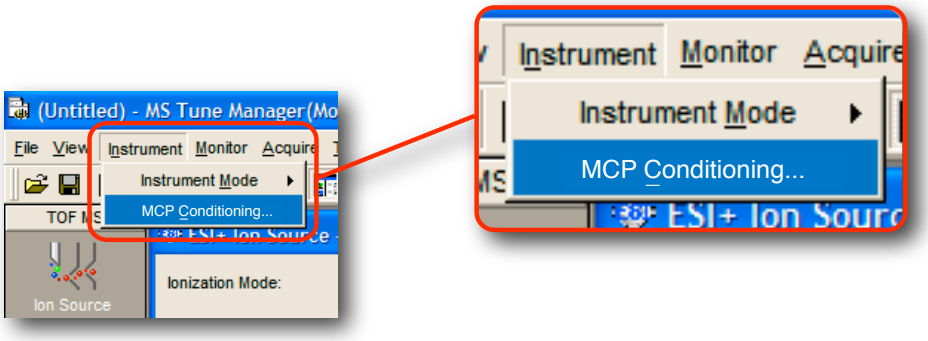
c. Turn on the P.C. and printer.

d. Toggle on the switch on the DART™ power supply console.

Conditioning the MCP

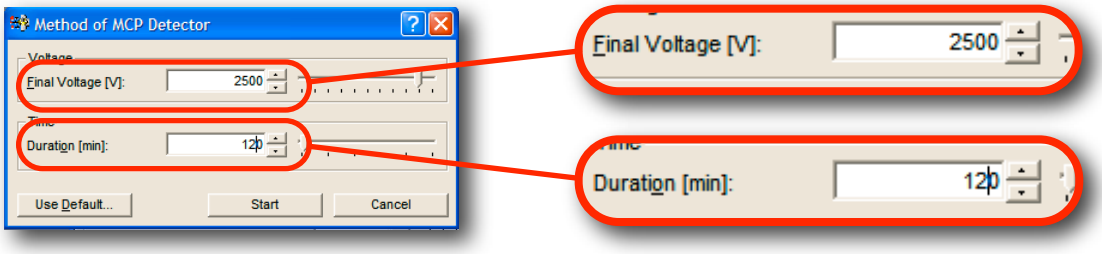
Note: MCP conditioning should always be done when starting up the system or after it has been vented.

You must wait until the analyzer reaches a pressure of ≤ 2.0 x 10^{-5} Pa before conditioning the MCP. Failure to do so can damage the MCP.



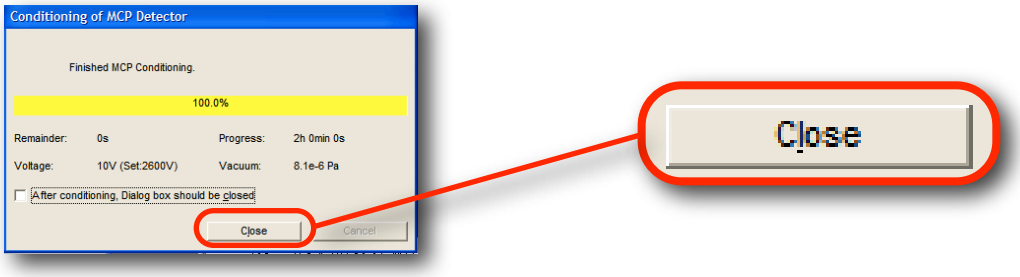
Selecting the MCP Conditioning Option from MS Tune Manager

e. From ***MS TUNE MANAGER***, choose ***INSTRUMENT-> MCP CONDITIONING***. If this option is grayed out, then the spectrometer has not yet reached an operational vacuum pressure or the MCP conditioning has already been done.



Setting the MCP Conditions

f. Set the MCP conditioning parameters as shown above or click ***Use Default*** and then Click ***START***.

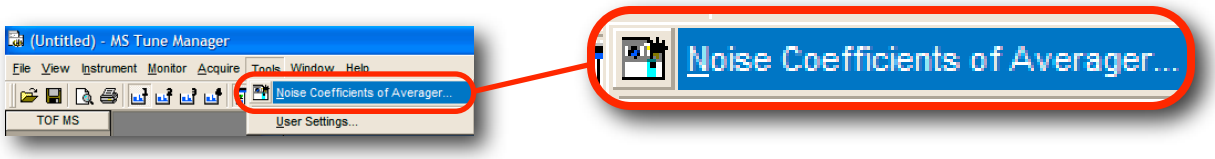


MCP Conditioning Progress Window

g. When MCP conditioning is complete, the ***CLOSE*** button will be available in the MCP Conditioning progress window. Click ***CLOSE*** to exit.

Reducing Baseline Noise on FastFlight (Optional)

Note : This procedure is performed by the JEOL Service Engineer at installation and is normally not needed when restarting the system. However, it can be done at any time if there is a question about the magnitude of baseline noise.



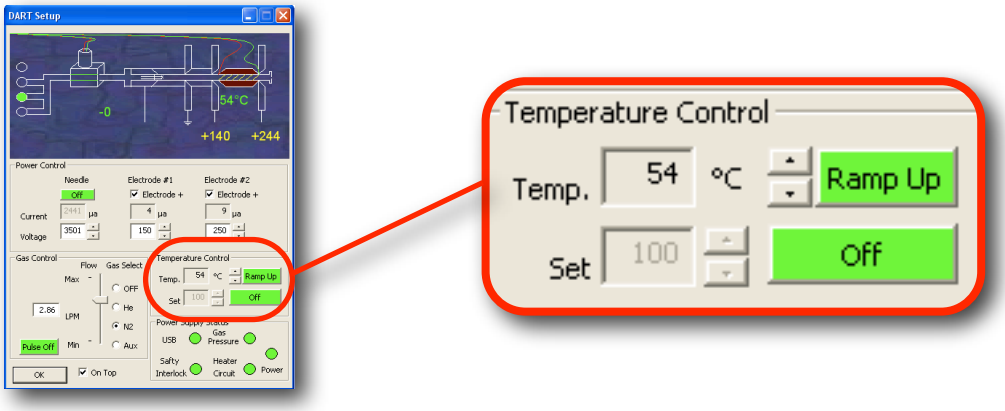
Reducing Baseline Noise on FastFlight

h. From ***MS TUNE MANAGER***, choose ***Tools-> Noise Coefficient of Averager***. If this option is grayed out, then you have not yet reached an operational vacuum pressure. Click ***Start***. This process takes 1-2 minutes to complete.

Performing a Complete Shutdown

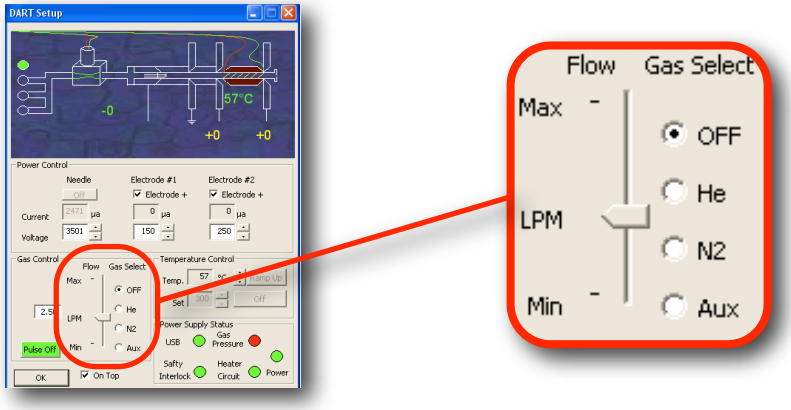
Use this procedure to completely power down the instrument for long-term time periods or if you expect a planned power outage.

DART™ Source Shutdown



Turning off the Temperature in Dart Setup

a. In ***DART Setup***, click the On icon in ***Temperature Control*** so that it switches to Off .



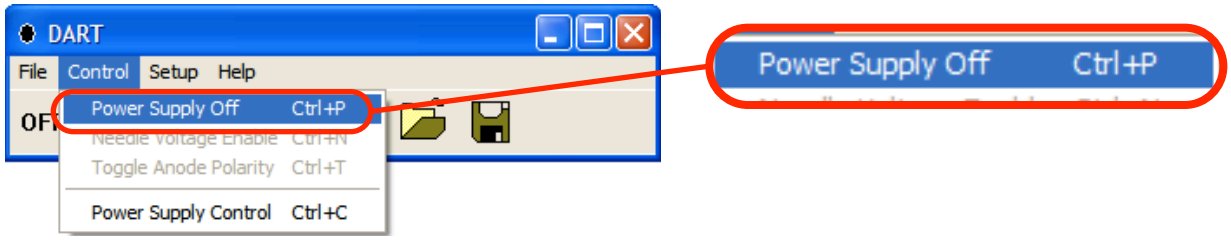
Turning Off the Gas Flow in Dart Setup

b. In ***DART Setup*****,** wait until the DART temperature is ≤ 200°C. Click the

Off radio button in the ***Gas Select*** section to turn off the gas flow.

Turning off the gas flow before the temperature falls below 200°C *will damage the source!*

c. Turn off main valve on the helium gas cylinder.

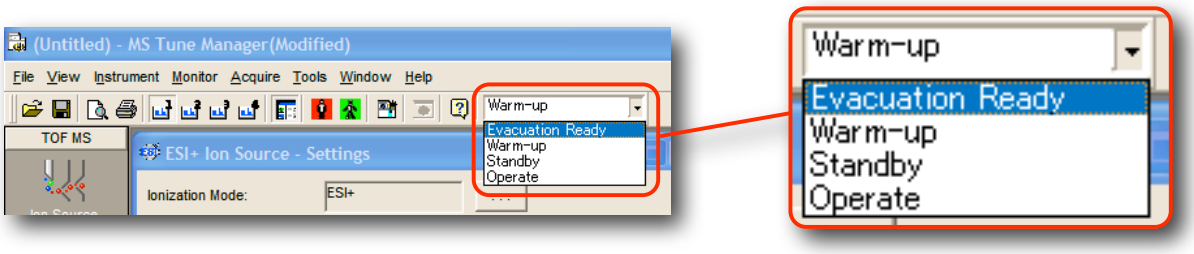


Turning Off the DART™ Power Supply

d. In the ***DART Bar***, choose ***Control*** -> ***Power Supply Off***. Close the DART™ software by right-clicking on the miniaturized icon in the Windows tool tray and choosing ***Exit***. Note that closing with the tool tray icon is the proper way to completely close the DART™ software.

e. Turn off the switch on the DART™ power supply console.

AccuTOF™ Shutdown



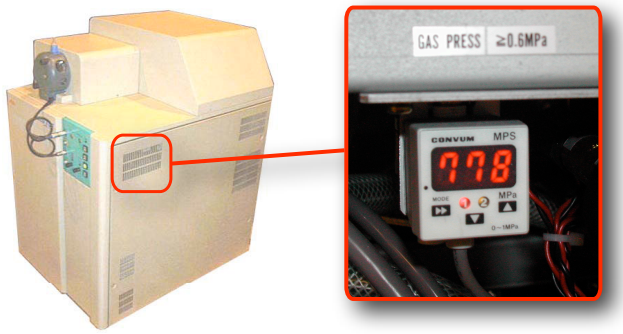
Setting the Instrument to Evacuation Ready

a. In ***MS Tune Manager***, set the instrument mode to ***Evacuation Ready***.

b. Close ***MS Tune Manager*** and quit ***Mass Center***.

Note: If using the ESI source at time of shutdown, make sure that both the

Nebulizing Gas and ***Desolvating Gas*** valves are closed by unchecking them in ***ESI+ Ion Source Settings*** before shutting down MassCenter.



Checking Vent Gas Pressure

f. Make sure that the N_2 venting gas pressure is ≥ 600kPa. Look through the grating on the right-hand side of the console to see the red LED gauge. It is not necessary to remove the side panel.

g. Push the ***Vent*** switch on the front panel. The ***Vent*** light will blink for about 10 minutes indicating that dry nitrogen gas is filling the chamber. Wait until the light stops blinking before proceeding to the next step.

f. Turn off the P.C. and printer.

g. Push the **Power OFF** switch on the front panel.

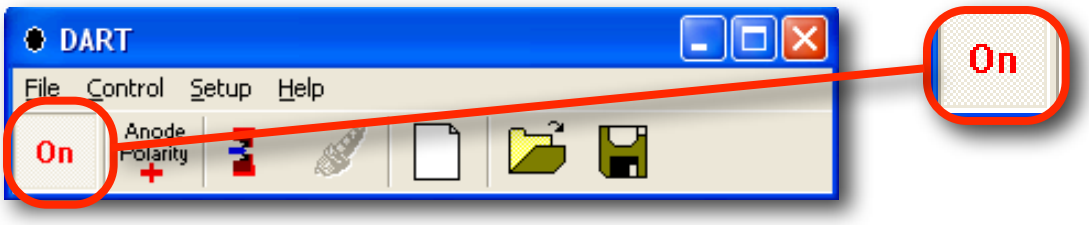
h. Turn off nitrogen gas supply.

Optional

i. Turn off the main breaker switch on the back of the AccuTOF console.

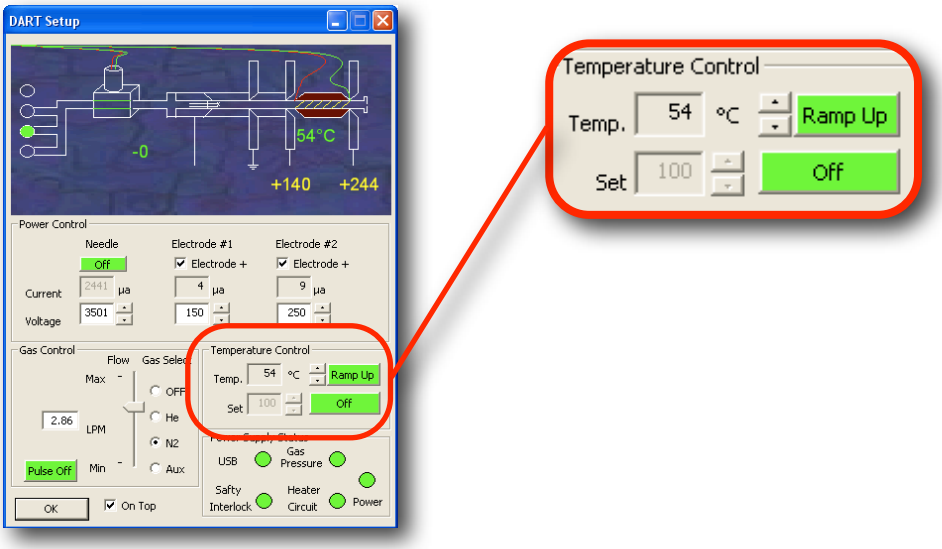
Leaving the Instrument Idle

Use this procedure to leave the instrument idle when you have completed your analyses and will be leaving the AccuTOF powered on.



Switching to Standby Mode in Dart Setup

a. In the ***Dart Bar***, hit the On icon so that it switches to , which is the standby icon. In this mode, the needle voltage will be turned off and the gas source will switch to N_2.

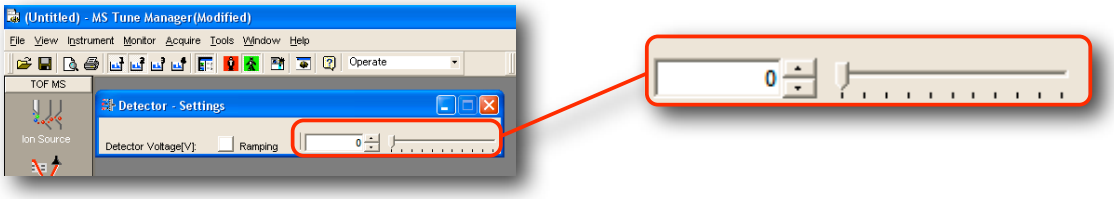


Turning off the Temperature in Dart Setup

b. In ***DART Setup***, click the On icon in ***Temperature Control*** so that it switches to Off . Before turning off the gas flow you must make sure that

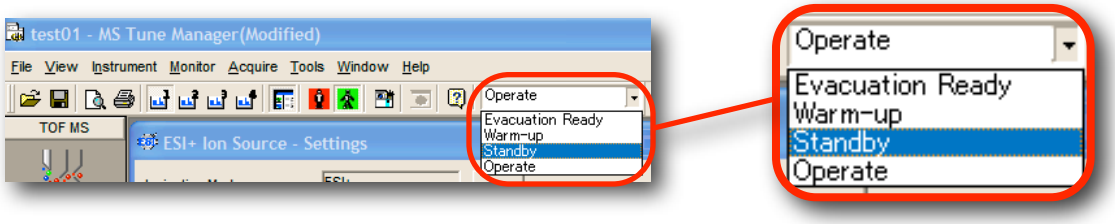
the temperature indicated in ***Temperature Control*** falls below 200°C.

Turning off the gas flow before the temperature falls below 200°C *will damage the source!*



Setting the Detector Voltage to 0

c. In ***MS Tune Manager***, set the detector voltage to 0V.



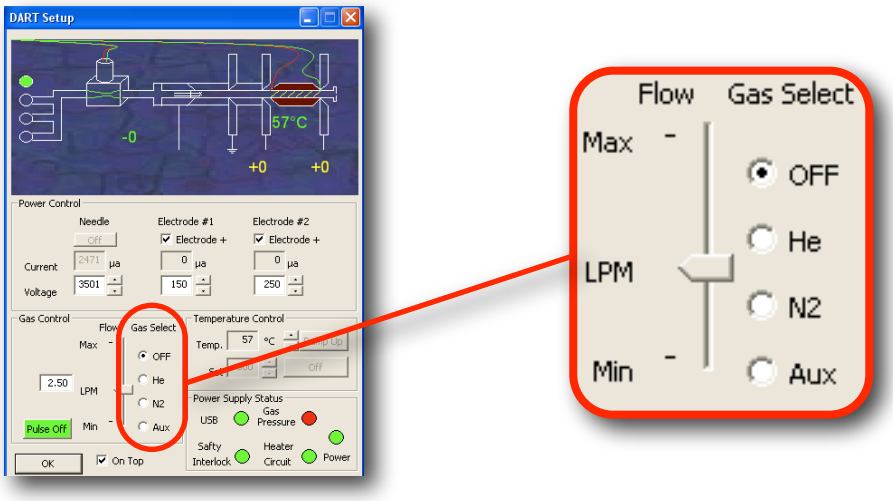
Setting the Instrument Mode to Standby

d. In ***MS Tune Manager***, set the instrument mode to ***Standby***.

Note: For extended periods of idle time, you can also set the instrument mode to ***Warm-up***. The settings for the various instrument modes are summarized in the table below.

Instrument Mode	High Voltage			Temperature (Orifice 1)
	MCP	Acceleration	Ion Source	
Evacuation Ready	OFF	OFF	OFF	OFF
Warm-Up	ON	OFF	OFF	OFF
Standby	ON	ON	OFF	ON
Operate	ON	ON	ON	ON

Instrument Modes and Corresponding Settings



Turning Off the Gas Flow in Dart Setup

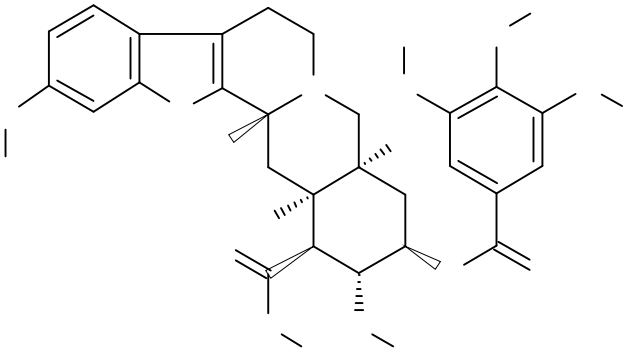
e. In ***Dart Setup,*** when the DART temperature is ≤ 200°C, click the ***Off*** radio button in the ***Gas Select*** section to turn off the gas flow.

Optional

f. Turn off the main valve on the helium gas cylinder.

Tuning the AccuTOF™

Spectrometer Tuning Using Reserpine -ESI

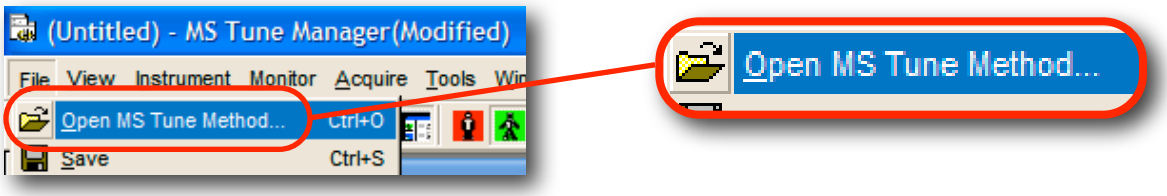


Reserpine FW 608.69

Sample: reserpine, $C_{33}H_{40}N_2O_9$, $[M+H]^+$ at *m/z* 609.2812
Concentration: 50 or 100 ppb in acetonitrile, you will need ~3mL
Syringe pump flow rate: 200µL /min
Source: Electrospray (ESI)

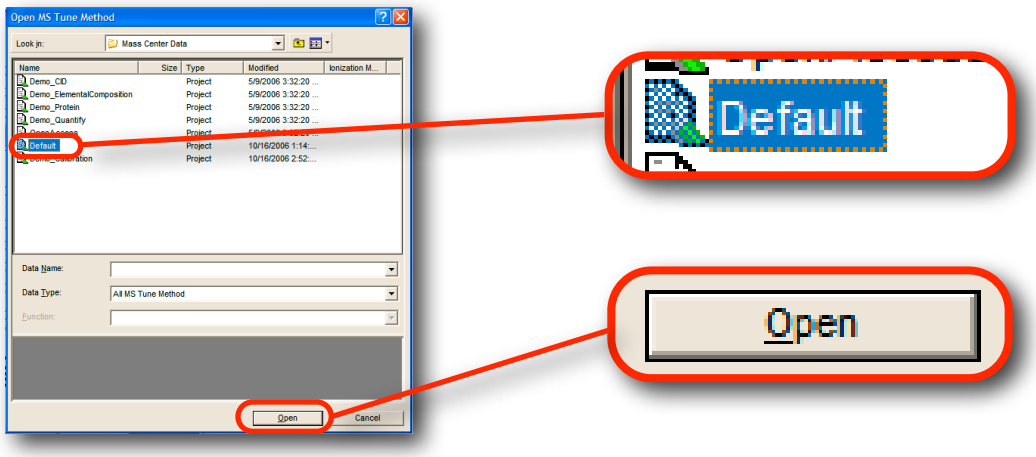
AccuTOF™ Tuning Setup

a. Install the ESI source and connect the syringe pump line.



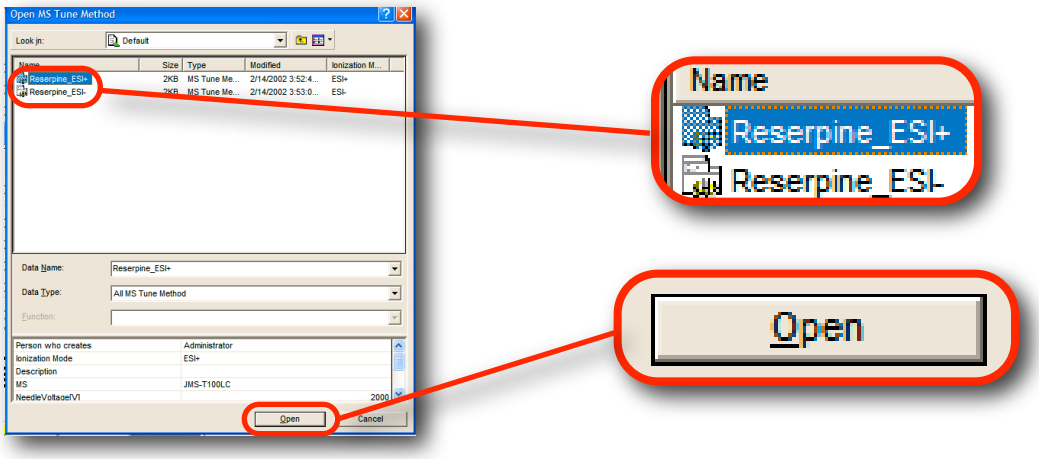
Opening a Default MS Tune Method

b. Open a default tuning file by choosing ***MS Tune Manager*** -> ***File*** -> ***Open Tune Settings...***



Select Default and then Open

c. Select project ***Default*** and then click ***Open***.



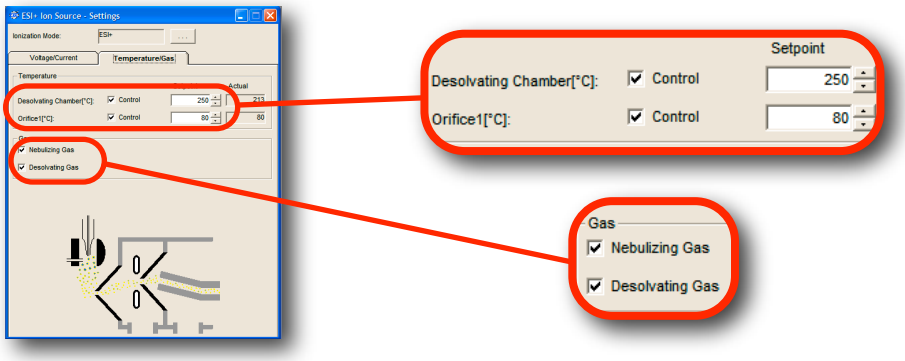
Choosing the Default Reserpine Tuning File

d. Choose ***Reserpine_ESI+*** and then click ***Open***.

e. In ***MS Tune Manager***, click 

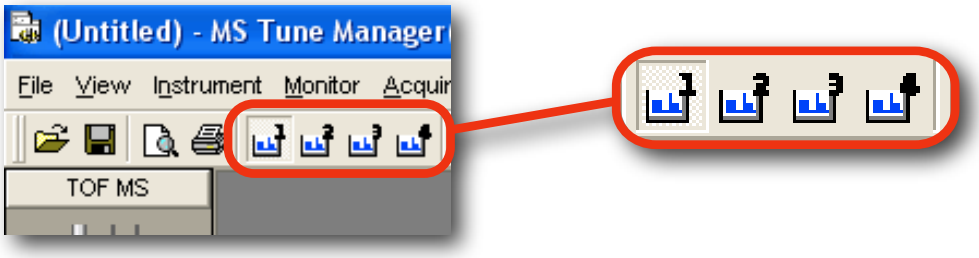
and 

to open the ***Analyzer*** and ***ESI+ Ion Source Settings*** windows, respectively.



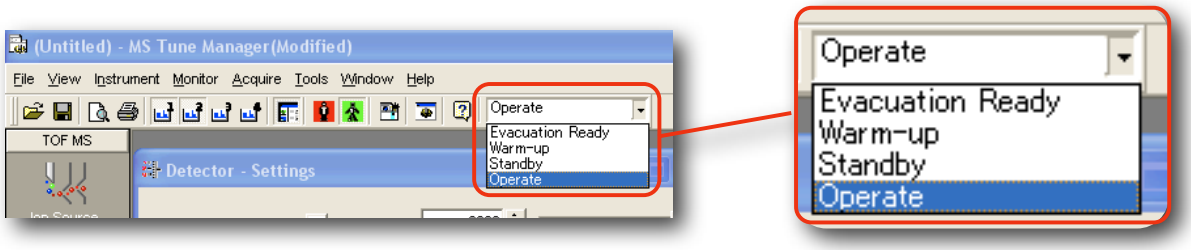
Setting Parameters for the Ion Source

f. On the ***ESI+ Ion Source Settings -> Temperature/Gas*** tab, click the box where it says ***Control*** to turn the temperature on for both ***Desolvating Chamber[°C]*** and ***Orifice1 [°C]***: set the temperatures to 200-250°C and 80°C, respectively. Open the ***Nebulizing Gas*** and ***Desolvating Gas*** valves by clicking the check boxes.



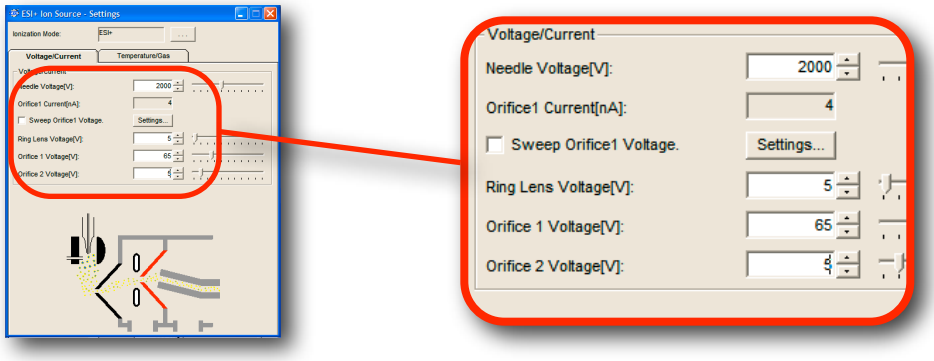
Opening a Spectrum Monitor from MS Tune Manager

g. In ***MS Tune Manager***, click one of the  icons to open a ***Spectrum Monitor***. A real-time spectrum monitor window will appear.



Setting the Instrument Mode to Operate

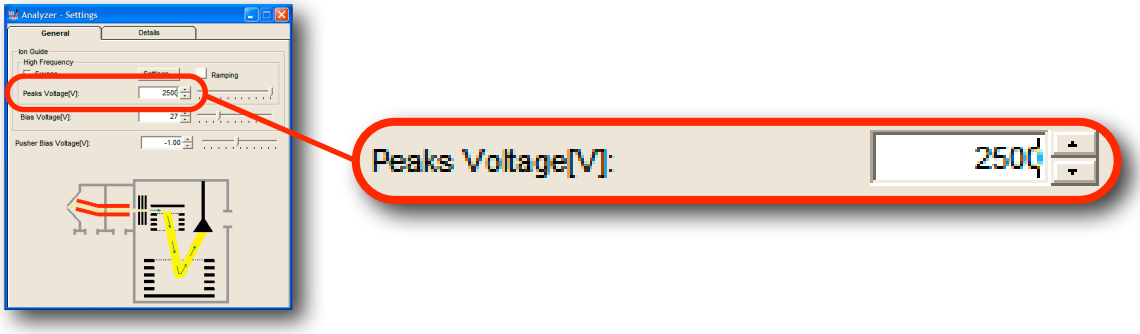
h. In ***MS Tune Manager***, set the instrument mode to ***Operate*** and wait until the temperature is ready.



Setting Parameters for the Ion Source

i. On the ***ESI+ Ion Source -Settings -> Voltage/Current*** tab, check that the voltages are set to these values:

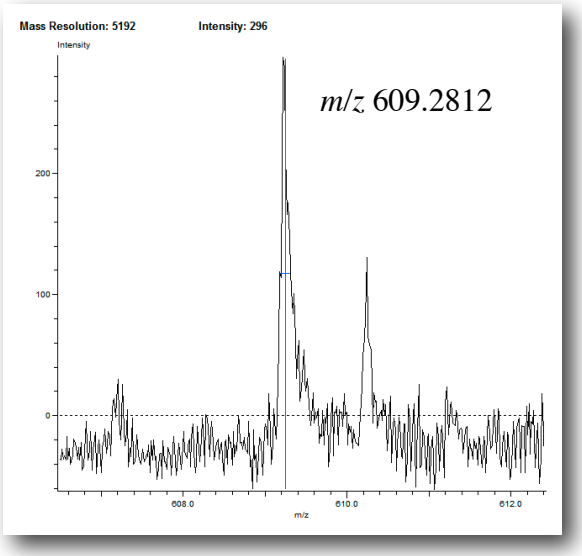
Needle Voltage[V]: 2000V
Ring Lens Voltage[V]: 10V
Orifice 1 Voltage[V]: 65V
Orifice 2 Voltage[V]: 5V



Setting Peaks Voltage[V] in Analyzer - Settings

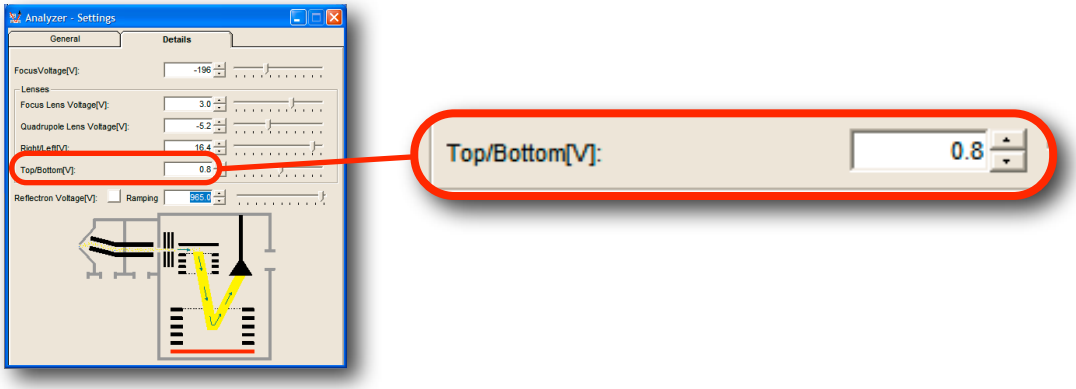
j. In ***Analyzer***, set ***Peaks Voltage[V]*** to 2500V.

AccuTOF Tuning Procedure



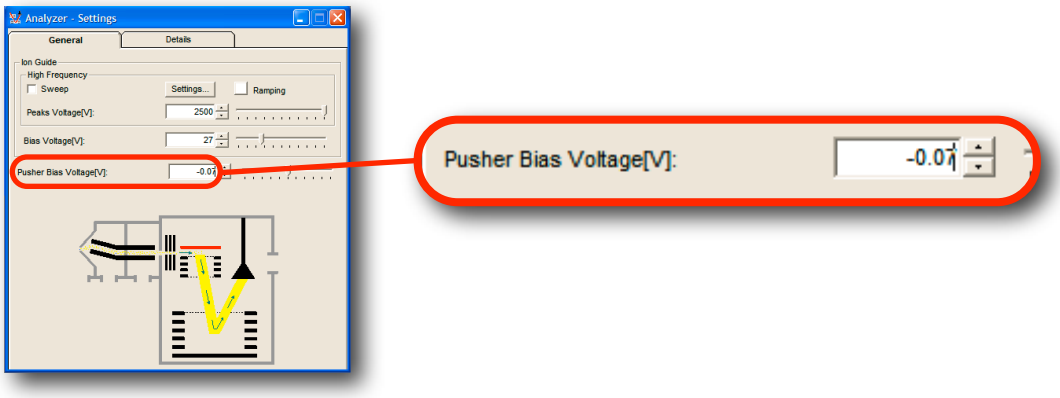
Monitoring the Reserpine $[M+H]^+$ at m/z 609.2812 for AccuTOF Tuning

a. Turn the syringe pump on. In this tuning procedure, you will monitor the reserpine $[M+H]^+$ at *m/z* 609.2812 in the ***Spectrum Monitor*** window. A poorly tuned, real-time spectrum is shown in the figure above.



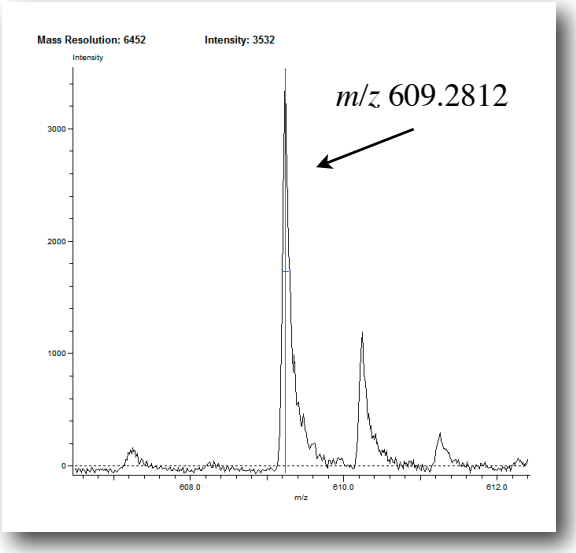
Adjusting Top/Bottom[V]: in Analyzer

b. On the ***Analyzer*** -> ***Details*** tab, adjust ***Top/Bottom[V]:*** so as to maximize the intensity of the peak. You can adjust in roughly 0.5V steps by clicking on the up/down buttons. The maximum change should be ± 5V.



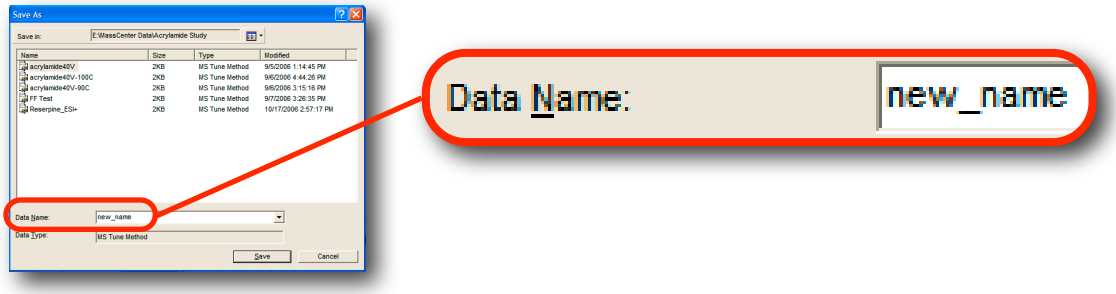
Adjusting Pusher Bias Voltage[V]: in Analyzer

c. On the ***Analyzer*** -> ***General*** tab, adjust ***Pusher Bias Voltage[V]*** so as to maximize both the intensity and the resolution. You can first adjust in roughly 0.2V steps, and then do a fine adjust using 0.05V steps.



Adjusting Pusher Bias Voltage[V]: in Analyzer

d. The tuning at this point should yield sufficient resolution and sensitivity. The figure above shows the result of an adequate tuning.



Saving Your Optimized Tune Settings

e. If you are satisfied with your tuning you should save the settings. In ***MS Tune Manager***, choose ***File*** -> ***Save As...*** and type a file name where it says ***Data Name*** and then click ***Save***.

Extended Tuning Procedure (Optional)

f. If you are not satisfied with the tuning, you can perform further adjustments on the following voltages:

FocusVoltage[V] and ***Quadrupole Lens Voltage[V]***: These two potentials are interrelated. Adjust the focus potential in 10V steps; set the quadrupole lens potential to 0 initially and then adjust it in 1V steps.

Right/Left[V]: Adjust the voltages carefully.

Reflectron Voltage[V]: Adjust the reflectron in 20V steps as necessary to maximize the resolution. Note that if you choose to adjust this voltage you will need to repeat steps a-e.

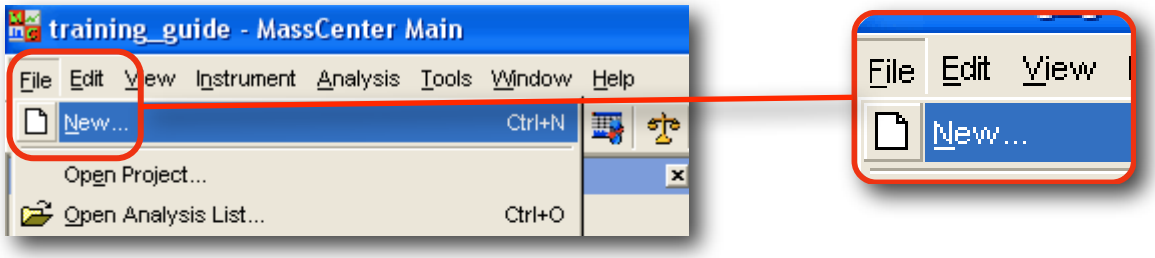
Data Acquisition and Processing

Use these procedures to collect and process mass spectra using the Direct Analysis in Real Time (DART™) ionization source.

Creating a New MassCenter Project

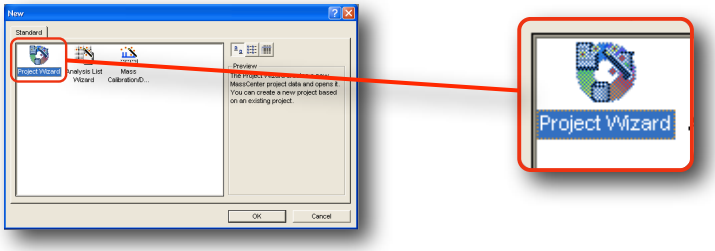
a. Double-click the 

icon on the desktop to open ***MassCenter***.



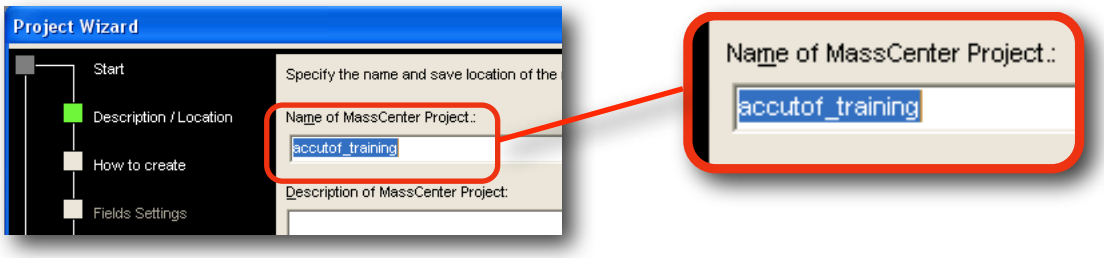
Opening the New Project Wizard

b. If you are starting a new set of analyses, you can create a new ***MassCenter*** project to help keep your data organized. In ***MassCenter Main***, choose ***File->New*** to open a new ***Project Wizard***.



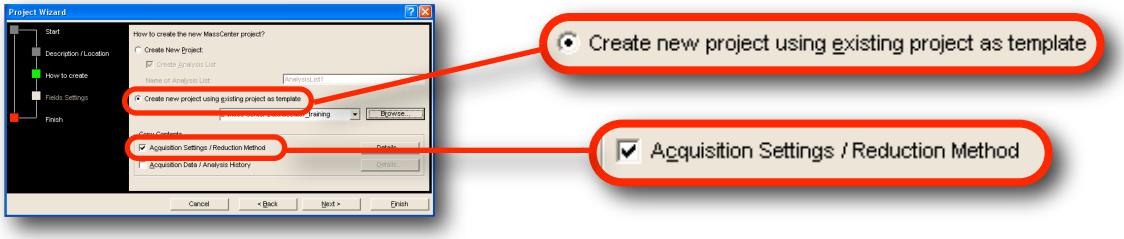
c. In the window that appears, click the 

icon and then click ***OK***.



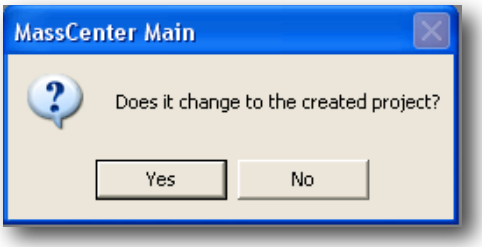
Naming a New MassCenter Project

c. Click ***Next*** and then type a project name. You can optionally enter a project description and specify a new location.



Specifying New Project Settings

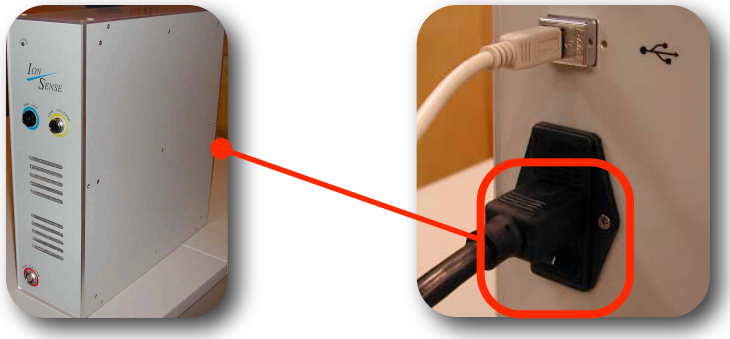
d. Click ***Next***. Make sure that ***Create new project using existing project as template*** and ***Acquisition Settings / Reduction Method*** are selected. Continue to click ***Next*** and then ***Finish*** to close the ***Project Wizard***.



Confirming the New Project

e. Click ***Yes*** to confirm changing the current project to the new one.

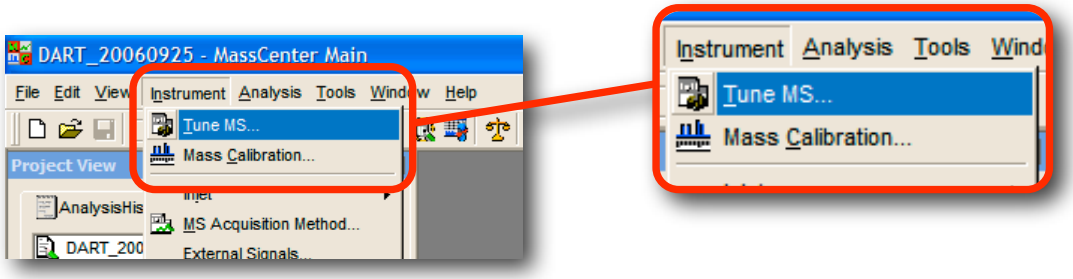
Starting up the DART™ Source - Positive Ion Analysis



Front Back

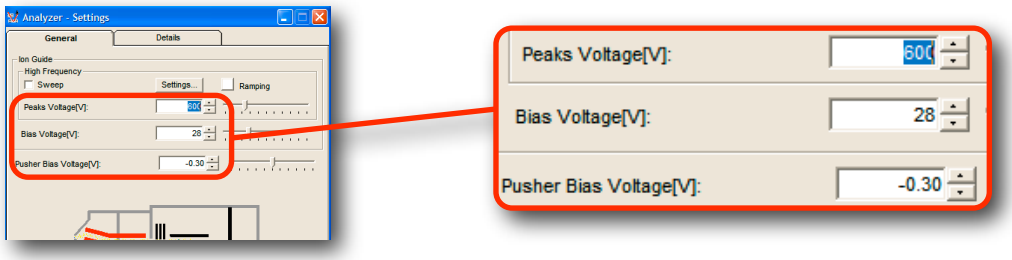
DART™ Power Supply Toggle Switch (photos courtesy IonSense, Inc.)

a. If off, toggle on the switch on the back of the DART™ power supply.

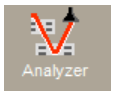


Selecting Tune MS to open MS Tune Manager

b. Choose ***MassCenter Main*** -> ***Instrument*** -> **Tune MS** to open ***MS Tune Manager***.

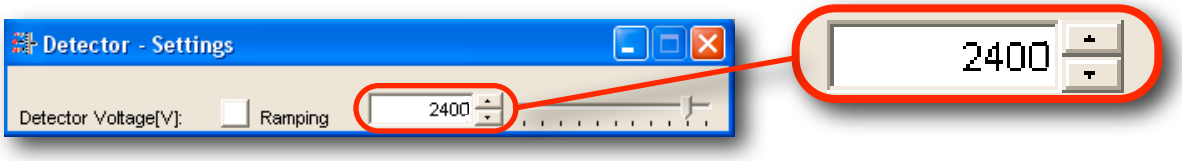


Setting Voltages in Analyzer

c. In ***MS Tune Manager***, click the 

icon to open the ***Analyzer*** window. Set **Peaks Voltage(V)** so that is roughly 10x that of the smallest

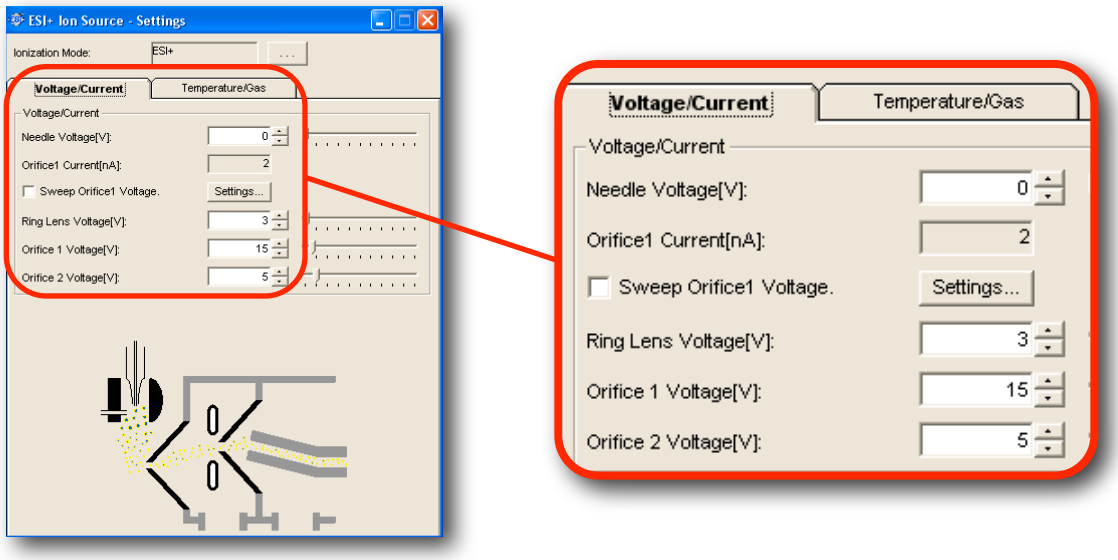
mass you wish to observe. The values for both ***Bias Voltage(V)*** and ***Pusher Bias Voltage(V)*** should not be changed from their defaults.



Setting the Detector Voltage

d. In ***MS Tune Manager***, click the 

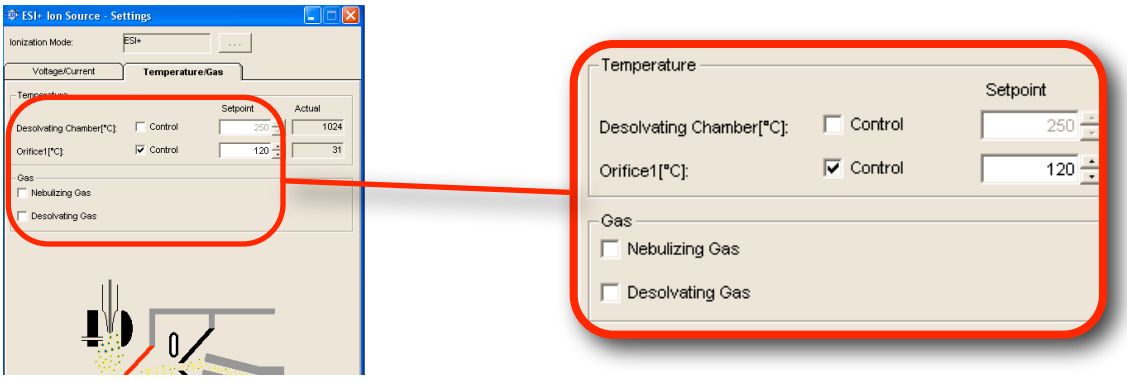
icon to open the ***Detector*** window. Set the voltage to 2400V. This value can vary from instrument to instrument.



Setting the Ion Source Voltages

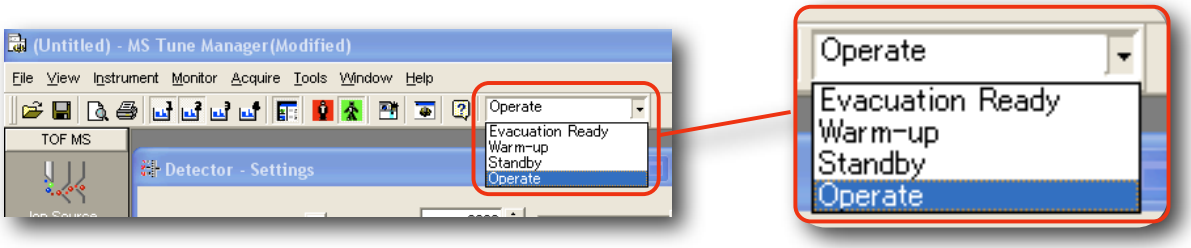
e. In ***MS Tune Manager***, click the 

icon to open the ***ESI Ion Source*** window. For DART™ analyses, set ***Needle Voltage(V)*** to 0. The ***Orifice1 Voltage(V)*** can be set to values from 15-25V - setting higher values of this voltage can result in greater ion fragmentation. Both ***Ring Lens Voltage(V)*** and ***Orifice 2 Voltage(V)*** can usually be left at the default settings.



Verifying the Temperature/Gas Settings

f. On the ***Temperature/Gas*** tab, set ***Orifice1[°C]*** to 120°C. Make sure that ***Desolvating Chamber [°C]***, ***Desolvating Gas*** and ***Nebulizing Gas*** are not checked.

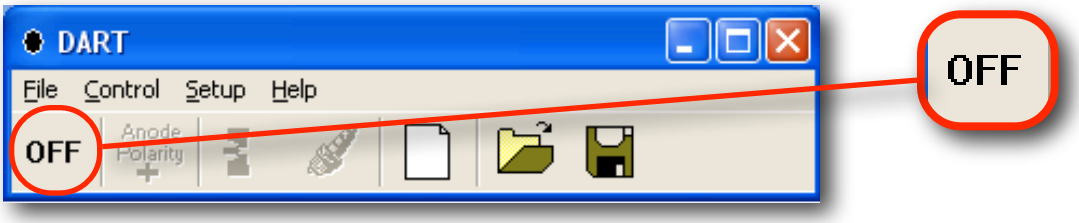


Setting the Instrument Mode to Operate

g. In ***MS Tune Manager***, set the instrument mode to ***Operate***.

h. Double-click the 

icon on the desktop to start the ***JEOL DART*** control program



Turning on the DART™

i. In the ***DART Bar*** window, click the 

icon to toggle it to 

.

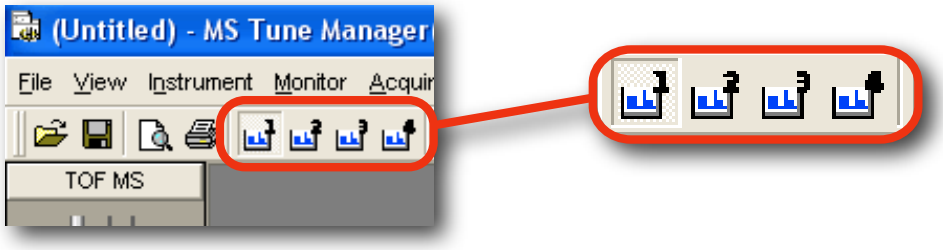
Setting the DART™ Polarity in Dart Bar

j. Make sure that the anode polarity is set correctly in the ***Dart Bar***. You can toggle the anode polarity button to make sure of the status. For a positive ion measurement, it should look like the figure above. If it does not click the ***Anode Polarity*** icon so that it changes to  .

k. Turn on the helium gas supply.

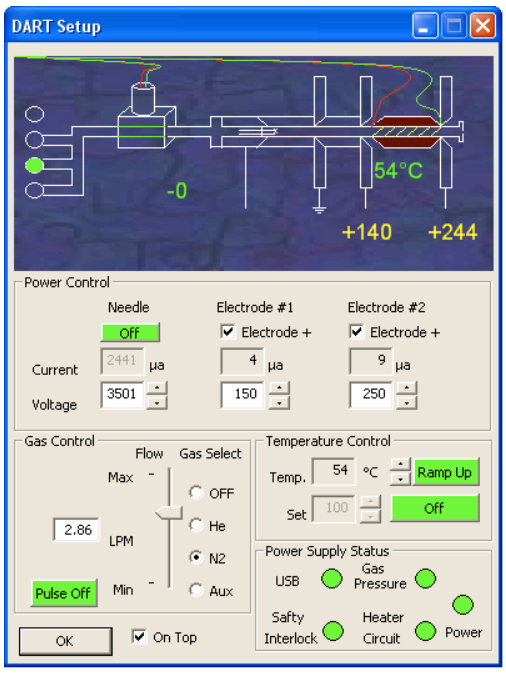
With the exception of setting up experimental conditions, you are now ready to start DART™ analyses. The following sections describe further DART™ setup.

Testing the DART™ Acquisition Conditions



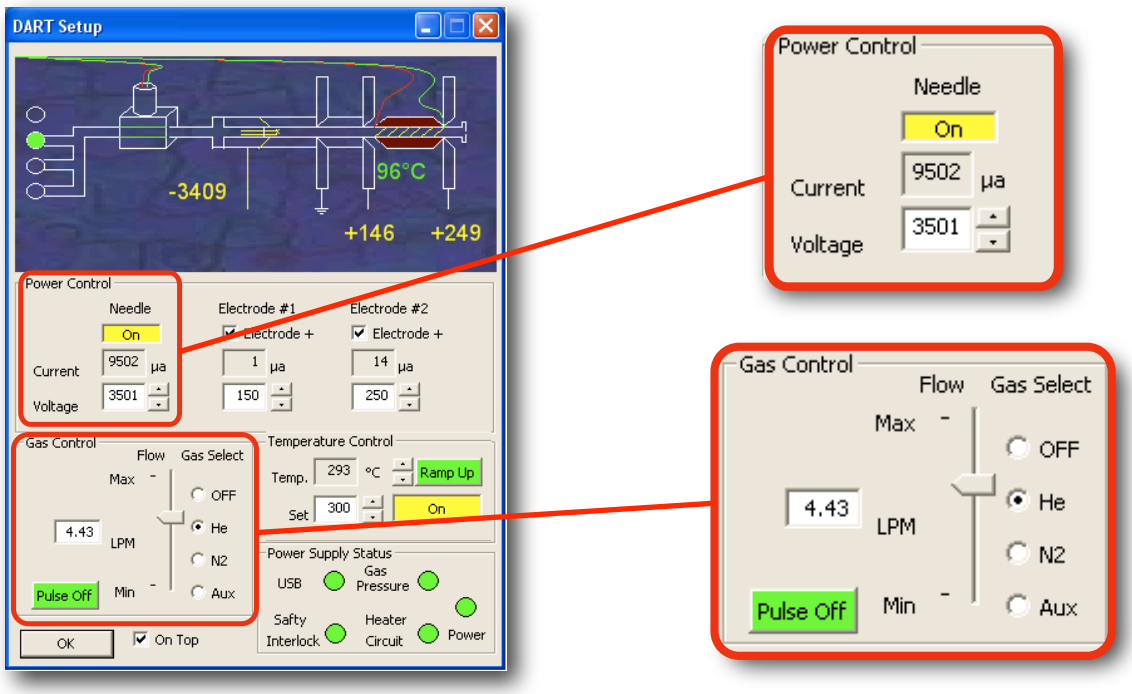
Opening a Spectrum Monitor from MS Tune Manager

a. Using AccuTOF-DART™, it is possible to perform a real-time test of the ionization conditions without actually writing data to disk. In ***MS Tune Manager***, click one of the  icons to open a ***Spectrum Monitor***. A real-time spectrum monitor window will appear.



Turning on the Temperature in DART Setup

b. In ***DART Setup***, click the Off icon in the ***Temperature Control*** section so that it toggles to On. Enter the desired temperature where it says ***Set***. In this example, we will use a temperature of 300°C. Allow the temperature to reach the target value. This should take just a few moments.

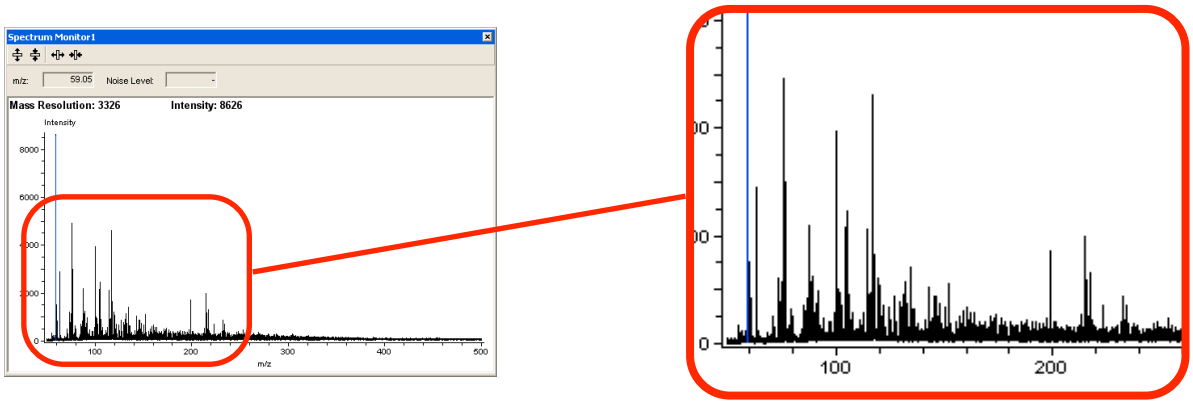


Turning on the He gas flow and Needle in DART Setup

c. In ***DART Setup***, click the radio button for ***He*** and ensure that gas is flowing. You should be able to hear the gas flow. Then hit the ***Needle*** 

icon so that it toggles to 

.

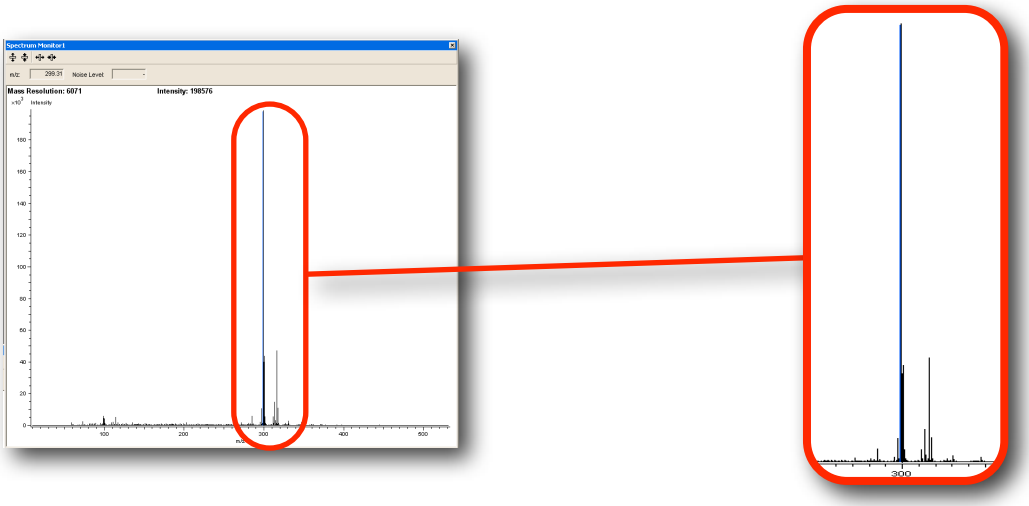


Typical DART™ Background Ions

d. You should now be able to see ions in the ***Spectrum Monitor***. The figure above shows an example of typical background ions formed.

Dangling a Capillary Tube in the Sample Gap

d. Dip the blunt end of a capillary into the methyl stearate so that some crystals adhere to the blunt end of the tube. Dangle your sample in the gap as shown above. Take care not to completely block the He gas flow. You will be able to hear a change in the gas flow when the sample just reaches the stream.



Methyl Stearate Peaks

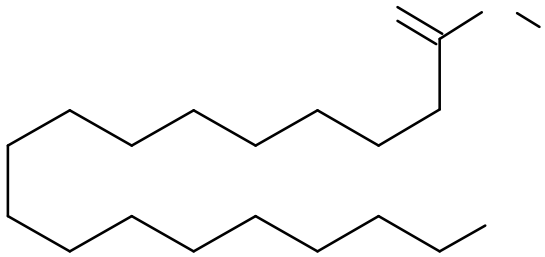
e. Peaks like those shown above should appear in the ***Spectrum Monitor***. The nominal mass of methylstearate is 298, so in the AccuTOF-DART™ spectrum you should see a peak for the $[M+H]^+ = 299$ protonated molecule, and other background or adduct peaks.

Note: If desired, it is possible to continue using this procedure to further

tweak the DART™ ionization source conditions. For more information regarding the effect of the different ionization source conditions, see ***Appendix A Optimizing DART™ Ionization Conditions*** .

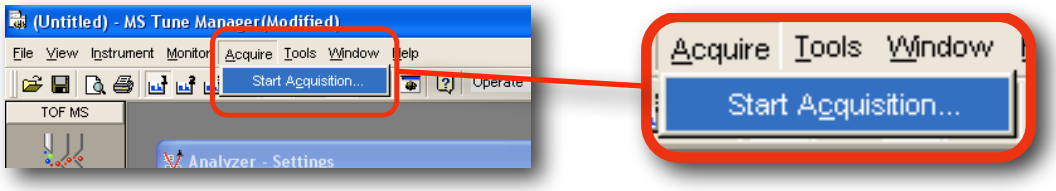
f. To save on the consumption of helium gas, click the On icon to switch to  , which is the DART standby mode. In standby mode, the heater will continue to operate and a low flow of N_2 will be enabled to purge the DART source. All voltages are off in this mode. Note that when toggling between these two modes,the software will remember the last manually set gas state when returning to the On state.

Collecting a Positive-Ion Mass Spectrum



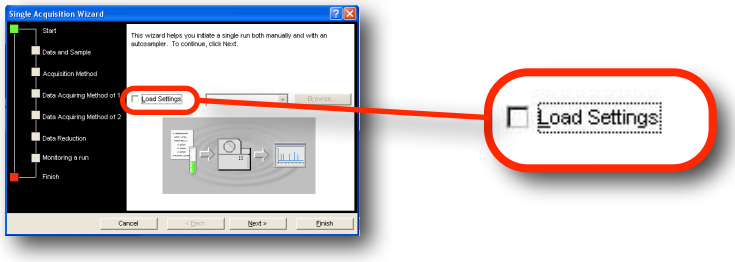
Methylstearate FW 298.50

Sample: methyl stearate, $C_{19}H_{38}O_2$, $[M+H]^+$ at *m/z* 299.29498
Internal Standard: solution of PEG 600 in methanol/methylene chloride (50:50 v/v)
Sampling Method: glass capillary tube



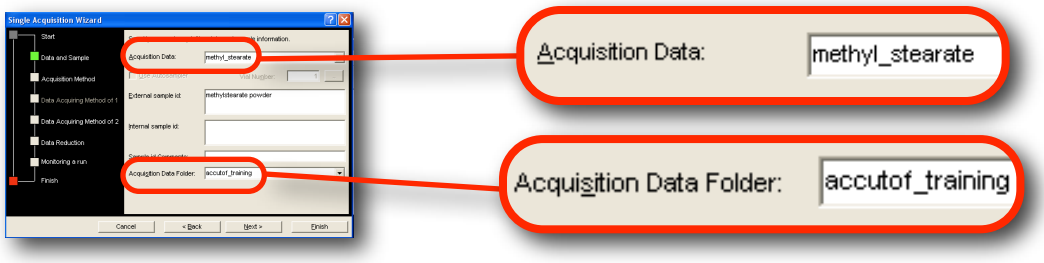
Opening the Single Acquisition Wizard

a. Choose ***MS Tune Manager*** -> ***Acquire*** -> ***Start Acquisition*** to open the ***Single Acquisition Wizard***.



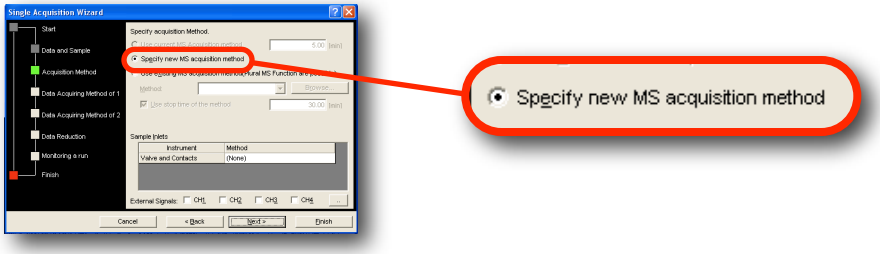
Acquisition Wizard Start Screen

b. If you have some special conditions saved, click ***Load Settings***. Otherwise, just click ***Next***.



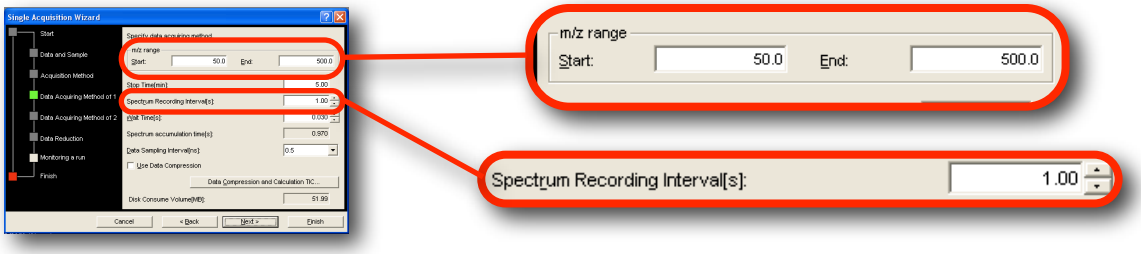
Entering a Filename and Data Folder

c. Click ***Next*** and enter a filename in ***Acquisition Data:***. If desired, you can can also fill in the comment fields and specify a new data folder in ***Acquisition Data Folder:***.



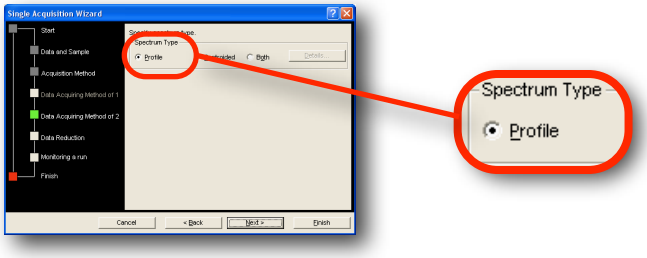
Specifying Acquisition Mass Range

d. Click ***Next*** and make sure that ***Specify new MS Acquisition method*** is checked.



Specifying Acquisition Mass Range

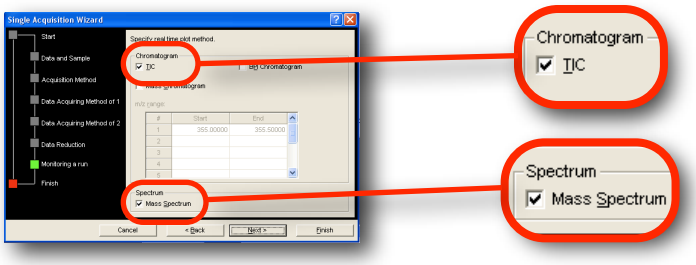
e. Click ***Next*** and then enter a mass range into ***m/z range*** in which to look for ions. You can optionally set ***Stop Time[min]:***, which sets the time when data will automatically stop acquiring. You can also set ***Spectrum Recording Interval***. A typical value is one second per spectrum.



Specifying Profile Data

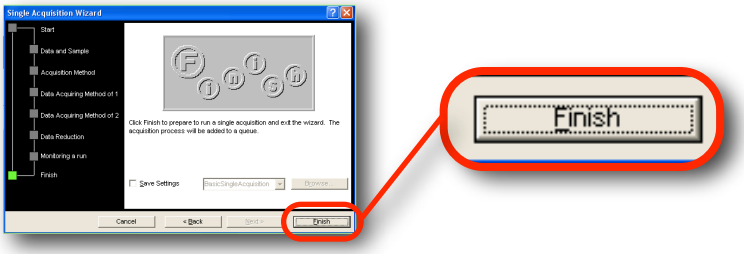
f. Click ***Next*** and then click the radio button where it says ***Profile***.

g. Click ***Next*** until you reach the real-time options window (i.e skip over the the Data Reduction screen). Click so that both ***TIC*** and ***Mass Spectrum*** are checked.



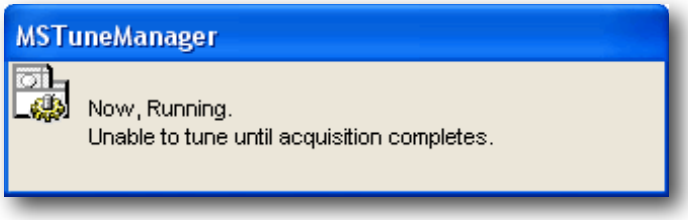
Specifying Real-Time View Options

g. Click ***Next*** until you reach the real-time options window (i.e skip over the the Data Reduction screen). Click so that both ***TIC*** and ***Mass Spectrum*** are checked.



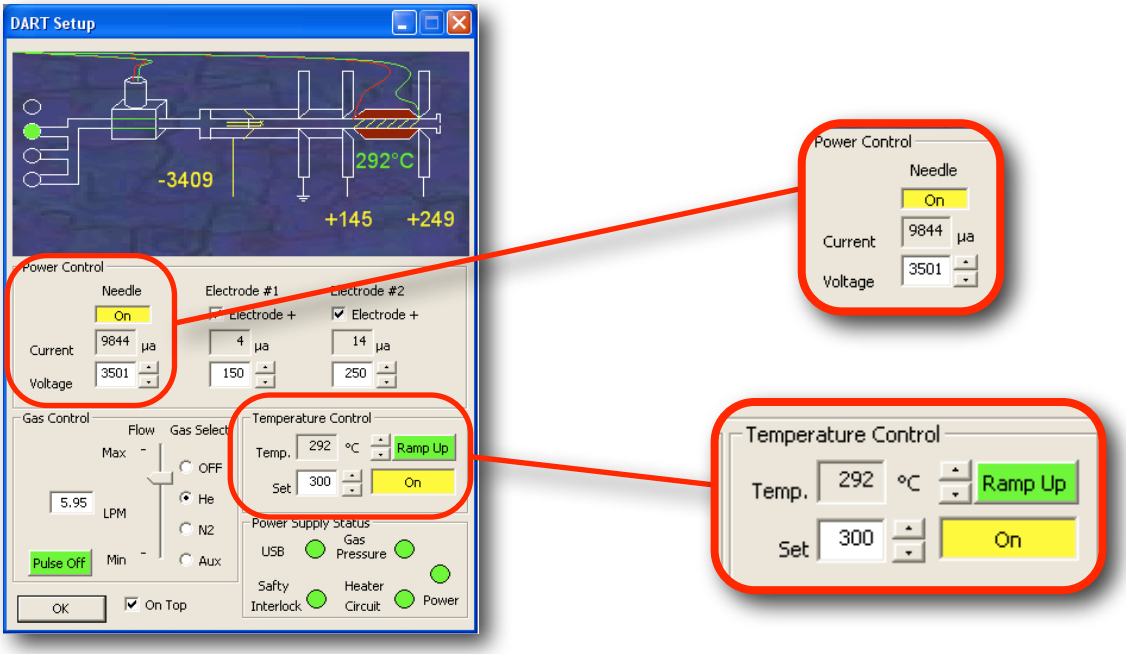
Closing the Acquisition Wizard

h. Click ***Next*** and then click ***Finish*** to close the wizard.



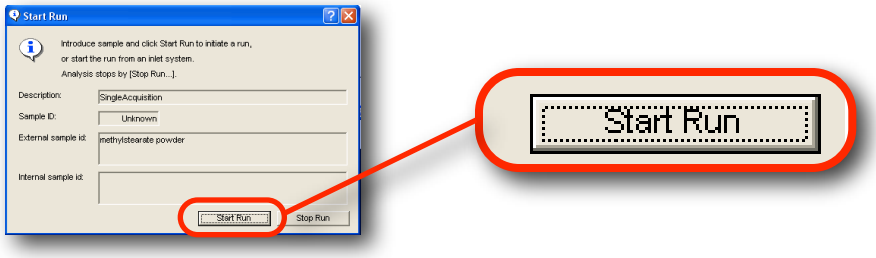
Dialog Box for Preparing for acquisition

h. A dialog box will appear informing you that ***MassCenter*** is preparing the instrument for acquisition.



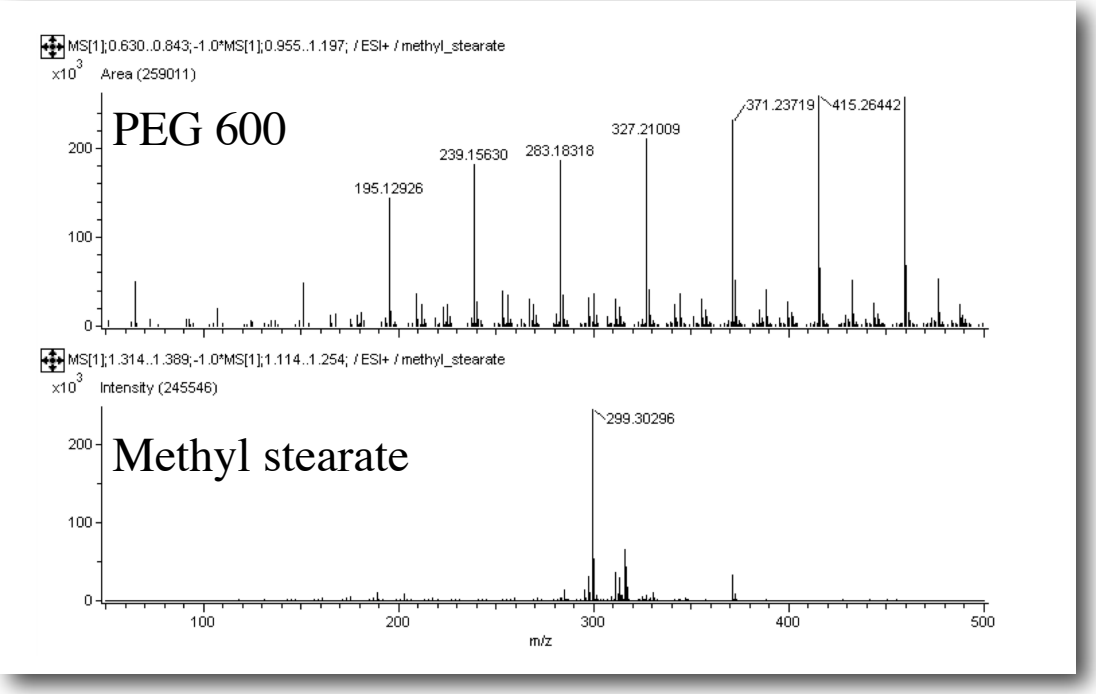
Turning on the Needle, Gas Flow and Temperature in DART Setup

i. If ***DART Setup*** is not already visible, click the  icon on the ***DART Bar*** to toggle it open. In ***DART Setup***, click the radio button for ***He*** and ensure that He gas is flowing. You should be able to hear the change in gas flow. Then hit the ***Needle*** Off icon so that it toggles to On. Make sure that ***Temp.*** reads around 300°C.



Hit Start Run to Begin the Analysis

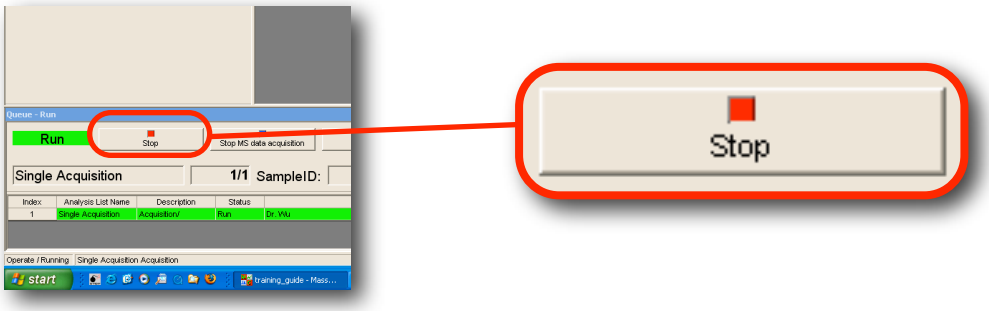
j. When you are ready to perform a measurement, go to ***MS Tune Manager*** and click ***Start Run*** in the ***Start Run*** dialog window.



Typical Spectra for PEG Standard and Methyl Stearate

k. Dip a glass capillary in the PEG solution and then dangle the capillary in the gap. You should see a series of PEG peaks appear in the ***Spectrum Viewer*** and a change in the intensity of the TIC (total ion chromatogram) in ***Chromatogram Viewer***. It is useful to note at roughly what time the peak intensity changes in ***Chromatogram Viewer***. This will make it easier to find the PEG peak when processing. You can repeat the sampling process a couple of times to make sure that you get a strong PEG signal. The figure above shows a typical PEG spectrum.

l. Dip a fresh capillary into the methyl stearate bottle so that a few crystals adhere to the sealed end of the tube. Again dangle the capillary into the gap. You should see methyl stearate peaks appear in the ***Spectrum Viewer*** and a change in the intensity of the TIC in ***Chromatogram Viewer***. Visually note at roughly what time the peak intensity changes in ***Chromatogram Viewer***. You can sample a couple of times to make sure that you get a strong methyl stearate signal. The figure on the previous page shows a typical spectrum.

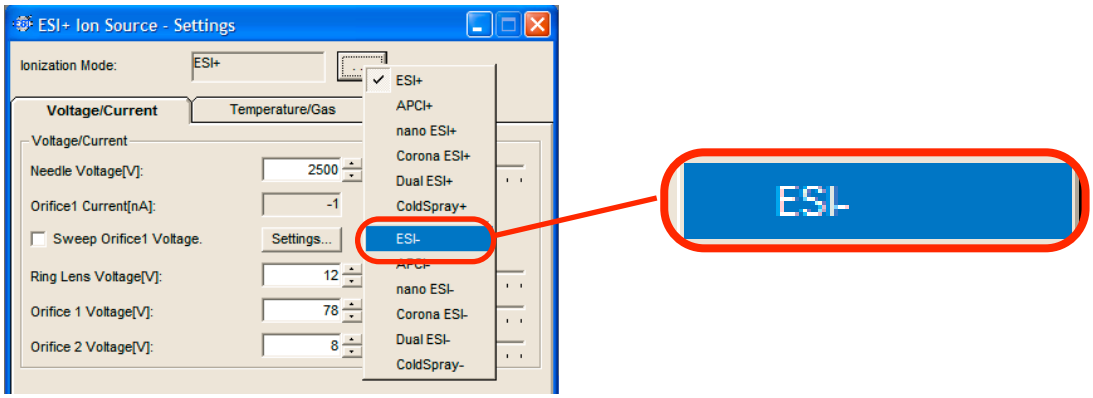


Stopping the Acquisition in MassCenter Main

m. To stop the acquisition, hit the 

icon in ***MassCenter Main***.

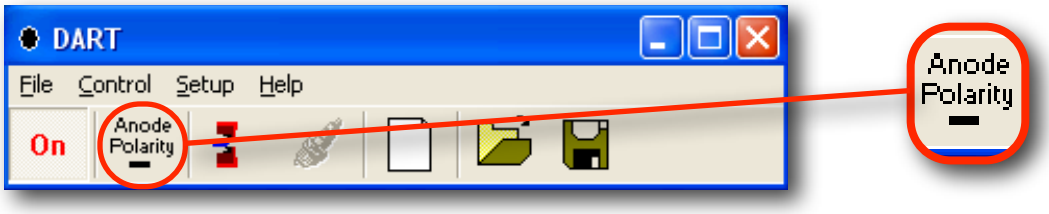
Configuring DART™ for Negative-Ion Analysis



Changing the AccuTOF Ionization Mode to ESI-

a. If you have a negative-ion tuning file, load it now. Otherwise, in ***MS Tune Manager***, change the ***Instrument Mode*** by clicking the 

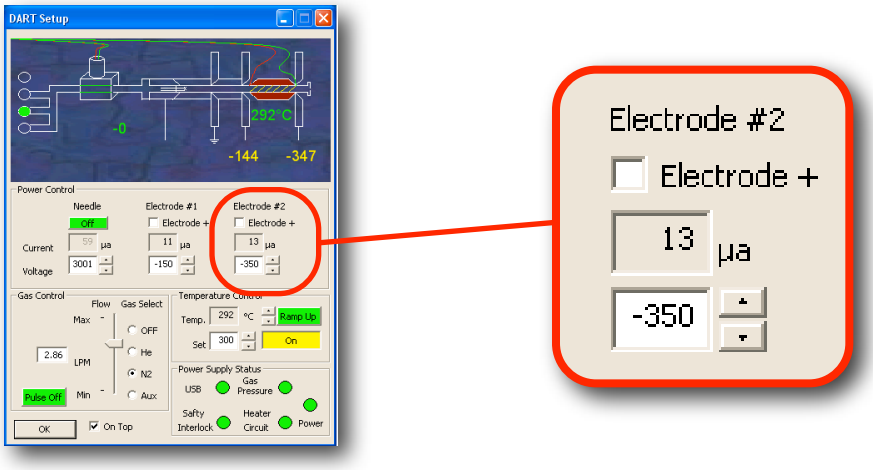
icon. Choose ***ESI-*** from the menu that appears.



Setting the DART™ Polarity in Dart Bar

b. Make sure that the anode polarity is set correctly in the ***Dart Bar***. It should look like the figure above. If it does not, click the ***Anode Polarity*** icon so that it changes to 

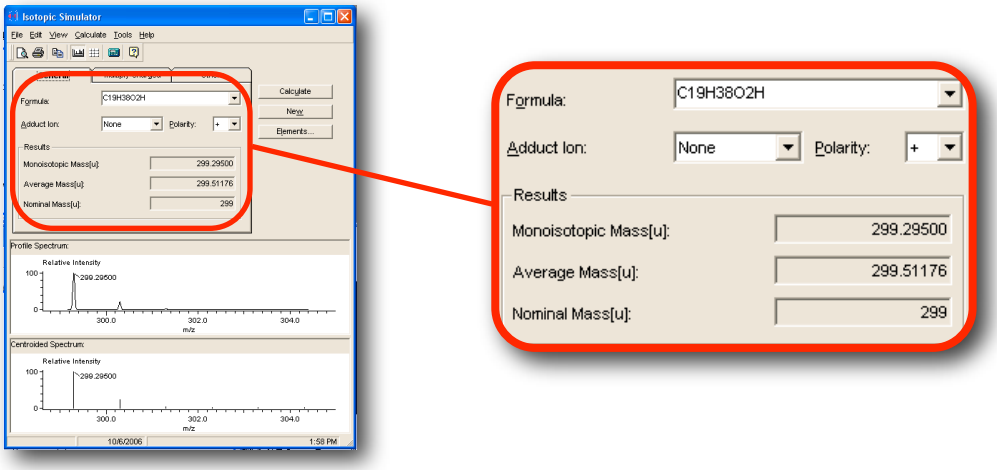
.



Setting Electrode #2 Voltage in DART Setup

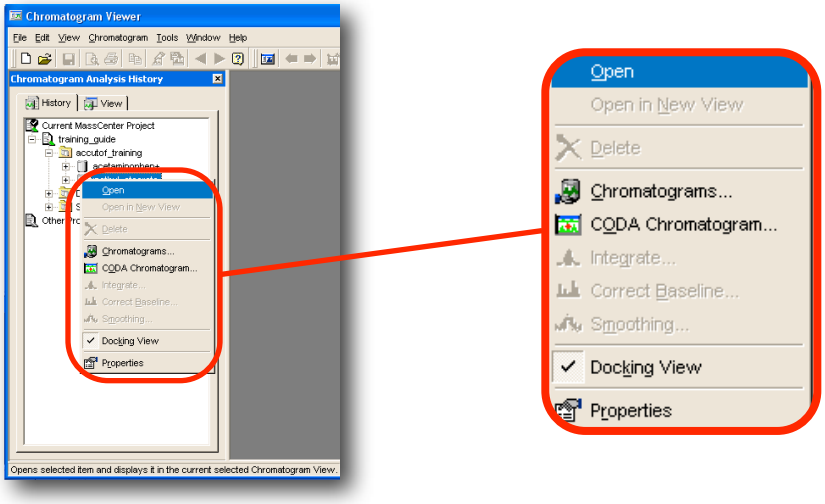
c. When you switch the polarity, the voltages will automatically switch to negative values. However, for negative ion mode you should also change ***Electrode #2*** to be -350V in ***DART Setup***. Changing this voltage will improve negative-ion sensitivity.

Determining an Accurate Mass



Using Isotopic Simulator to Calculate the Monoisotopic Mass

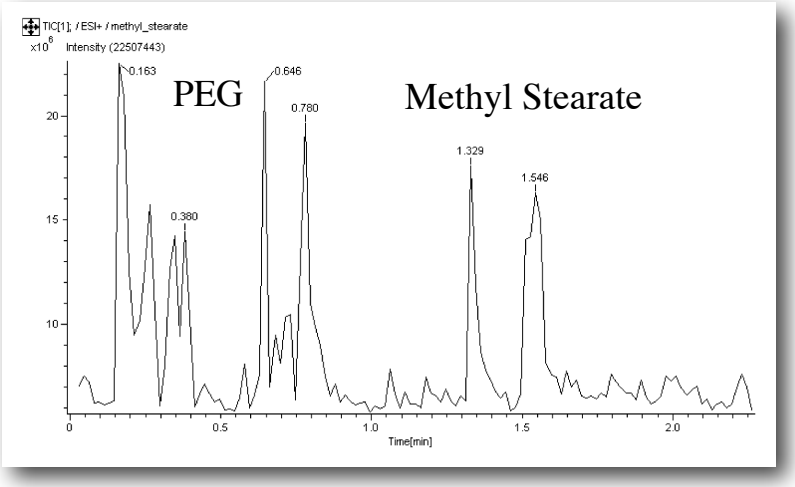
a. Calculate the monoisotopic mass for methyl stearate. To calculate the monoisotopic mass, use ***MassCenter*** -> ***Tools*** -> ***Isotopic Simulator***. Enter the numbers of atoms contained in the compound, $CH_3(CH_2)_{16}CO_2CH_3$, into the ***Formula*** field, plus one more hydrogen. Remember that we are expecting to see the $[M+H]^+$ methyl stearate peak so an extra hydrogen is required in the calculation. Hit the ***Calculate*** button to update the masses.



Opening a Chromatogram in Chromatogram Viewer

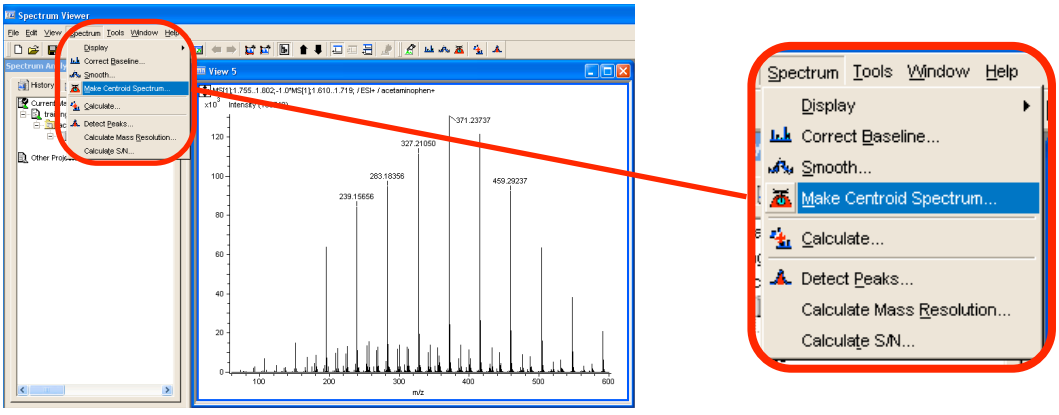
b. If ***Chromatogram Viewer*** is not already open, open it by choosing

MassCenter Main -> ***Tools*** -> ***Chromatogram Viewer***. You can also open ***Spectrum Viewer*** from the same menu. In ***Chromatogram Viewer***, navigate to the data you wish to open and then ***right-click*** the name and choose ***Open***. The Total Ion Chromatogram (TIC) will appear in the window.



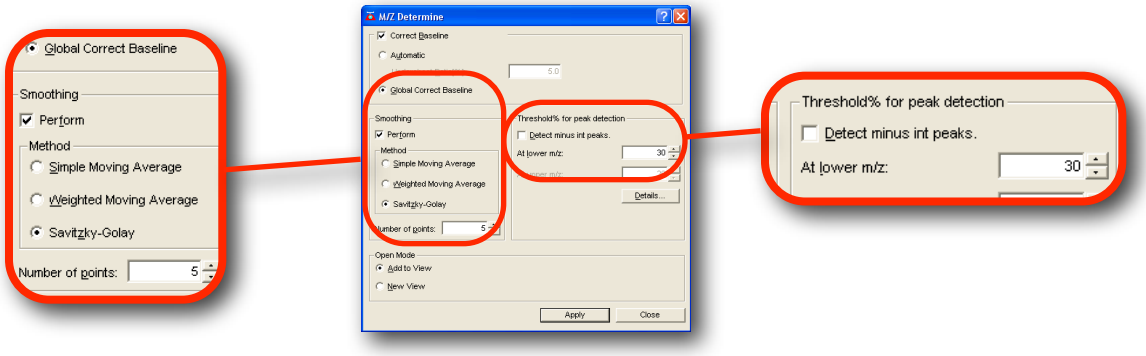
TIC for PEG and Methyl Stearate

c. To create a mass spectrum, ***right-click-drag*** over a desired region. The TIC shown above shows two samplings of both PEG and methyl stearate. To create a background-subtracted mass spectrum, hold down **SHIFT** and then ***right-click-drag*** over a peak region. Then to subtract the background, hold down **CTRL** and ***right-click-drag*** over a noise region. The background-subtracted spectrum will appear in ***Spectrum Viewer***.



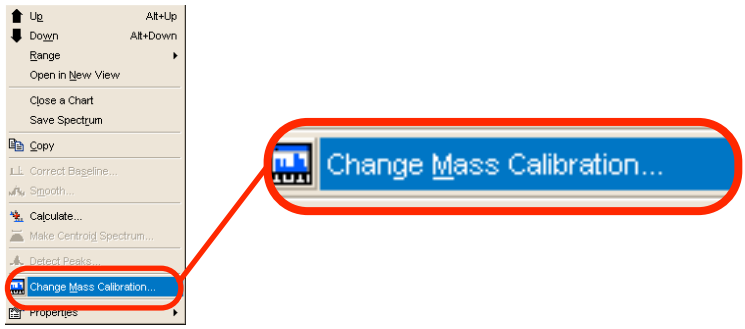
Choosing Make Centroid Spectrum from Spectrum Viewer

d. In ***Spectrum Viewer***, choose ***Spectrum*** -> ***Make Centroid Spectrum***.



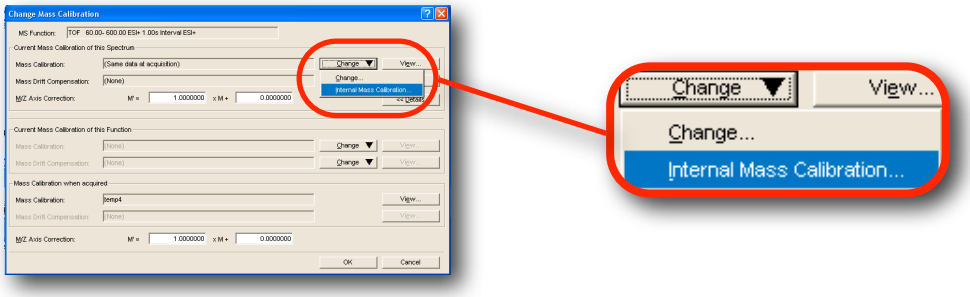
Centroiding Properties

e. A menu will appear allowing you to change centroiding properties. For example, you may wish to modify the ***Threshold % for peak detection***. If you are happy with the parameters, just click ***Apply***.



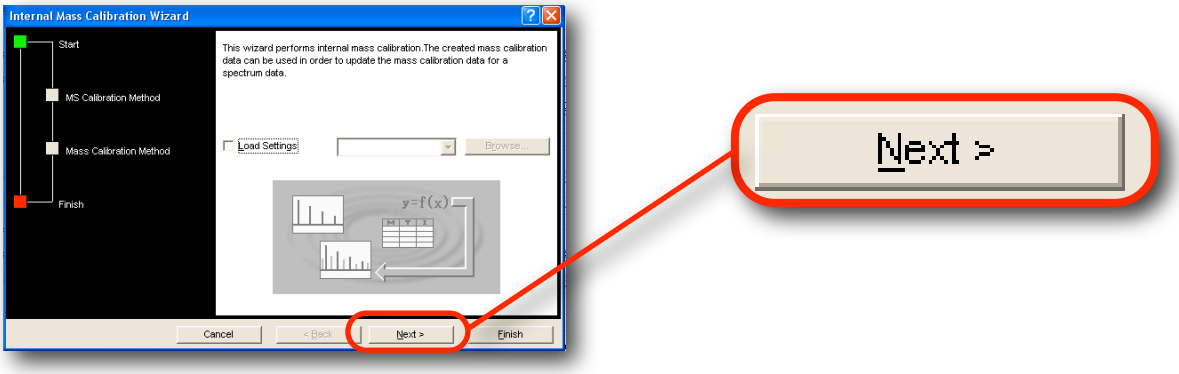
Choosing Change Mass Calibration from the Right-Mouse Menu

f. ***Right-click*** on the centroided spectrum and choose ***Change Mass Calibration*** from the menu that appears.



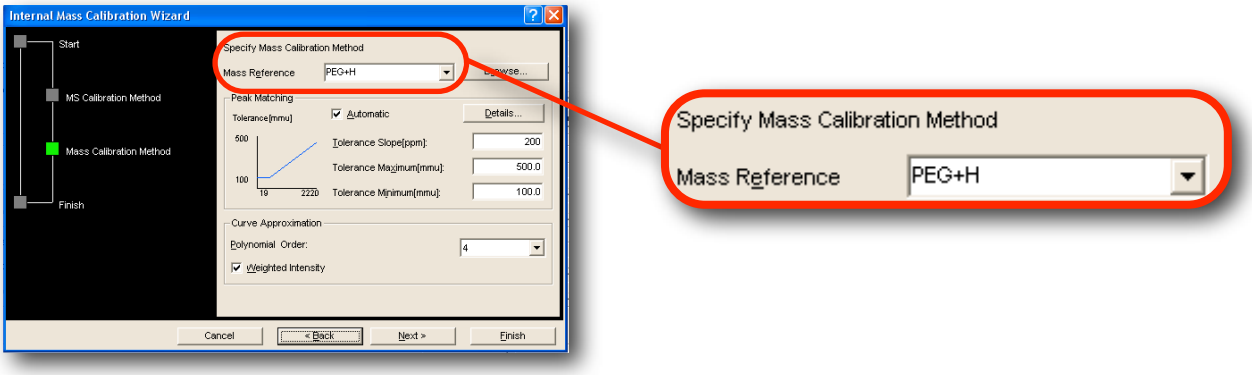
Choosing Internal Mass Calibration

g. Choose ***Mass Calibration -> Change -> Internal Mass Calibration***.



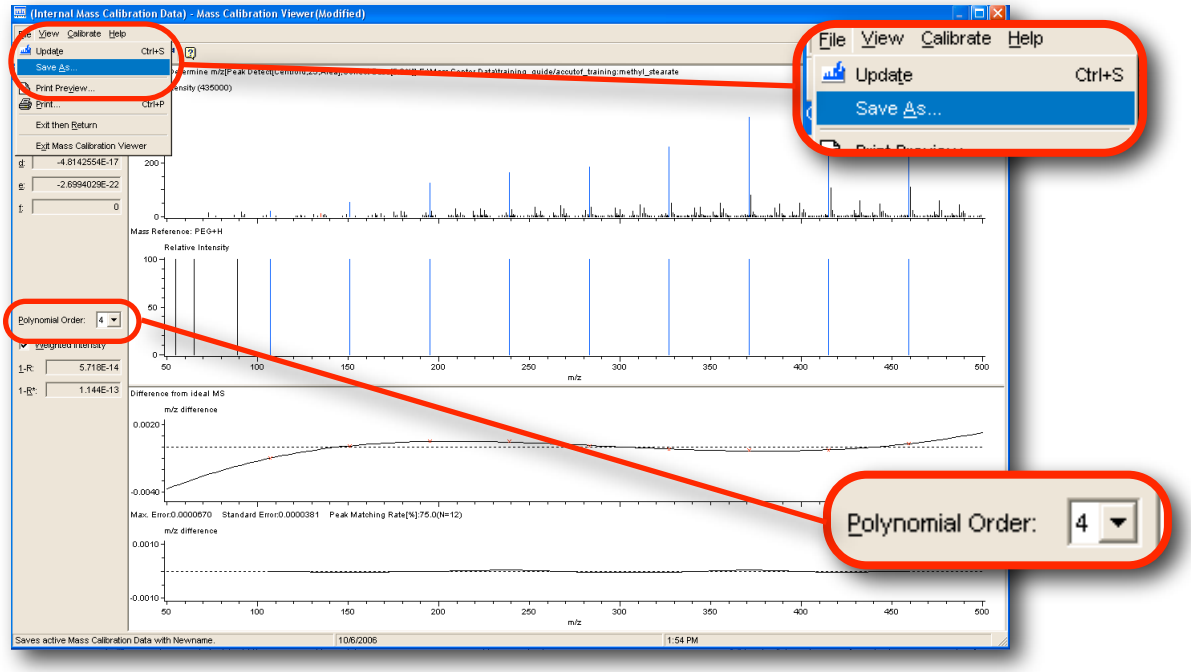
Internal Mass Calibration Wizard

h. When the ***Calibration Wizard*** appears, click ***Next***.



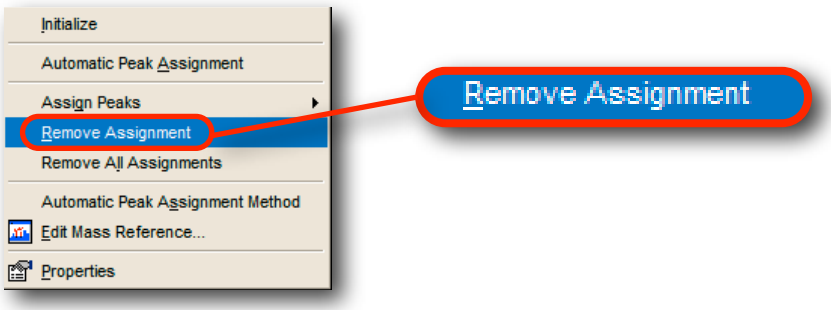
Choosing the Mass Reference

i. Make sure that ***PEG+H*** is chosen for ***Mass Reference***. Click ***Finish***.



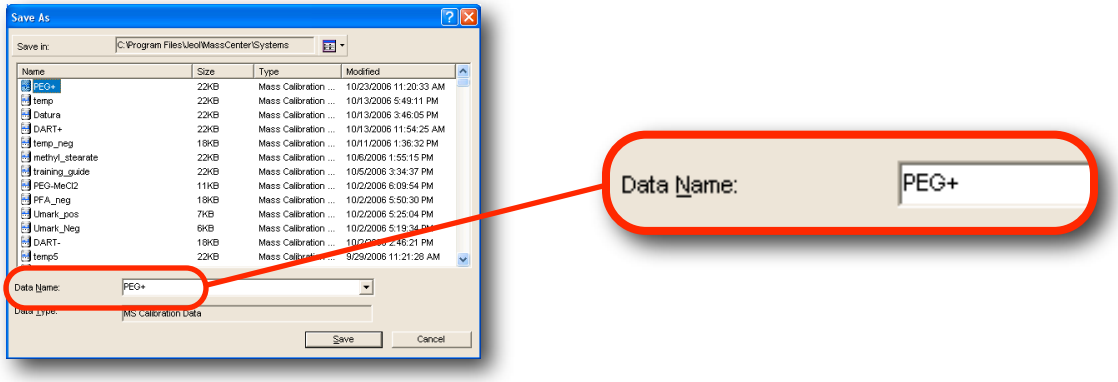
Checking the Calibration in Mass Calibration Viewer

j. The ***Mass Calibration Viewer*** should now be visible. This window displays (from top to bottom) the PEG mass spectrum, the PEG theoretical spectrum, the calibration curve and a residual curve. If you are happy with the calibration choose ***File*** -> ***Save As...*** to save the calibration. However, if you would like to tweak the calibration you can change ***Polynomial Order***, which changes the number of coefficients used in the



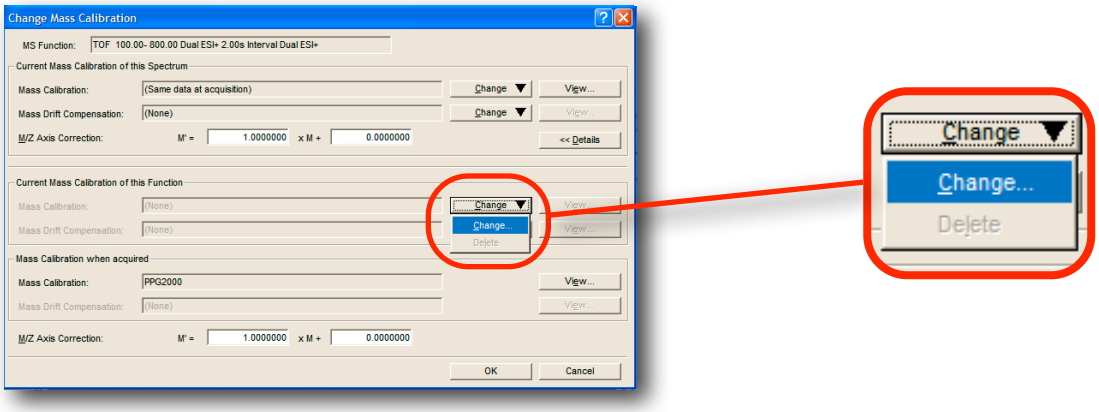
Right-mouse Menu in Calibration Viewer

curve fitting routine (normally 3 or 4 terms are used). If there are points that you wish to discard (i.e.outliers), select the corresponding peak in the top spectrum using ***left-click***. The peak will turn red. Then using ***right-mouse-hold***, choose ***Remove Assignment***. If you want to get the assignment back, choose ***Automatic Peak Assignment*** re-pick all of the original peaks.



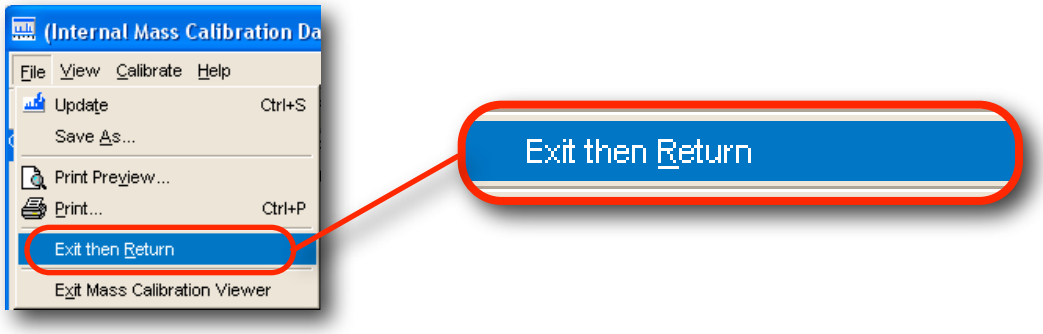
Naming the Mass Calibration File

k. After choosing ***Save As*** you will see a window prompting you to enter a filename. You can enter either a unique or generic filename. For this example, call the calibration file "PEG+". Now close ***Calibration Viewer*** by choosing ***File -> Exit then Return***.

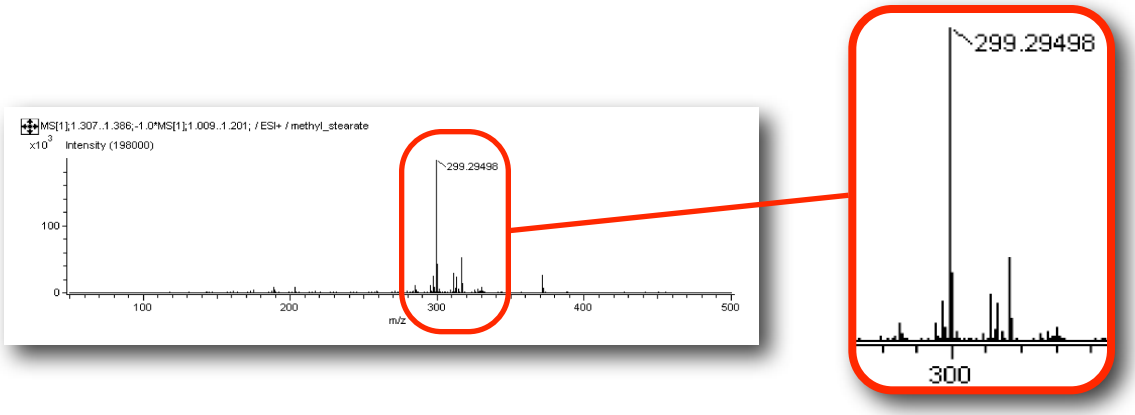


Changing the Mass Calibration File

l. ***Right-click*** on the centroided PEG 600 spectrum and choose ***Current Mass Calibration of this Function*** -> ***Change*** -> ***Change***. Choose ***Peg+*** from the window that appears. Click ***Open***. The default calibration file will now be set to the calibration made for "Peg+".



m. Close ***Calibration Viewer*** by choosing ***File*** -> ***Exit then Return***



Methyl Stearate Spectrum with Calibration Applied

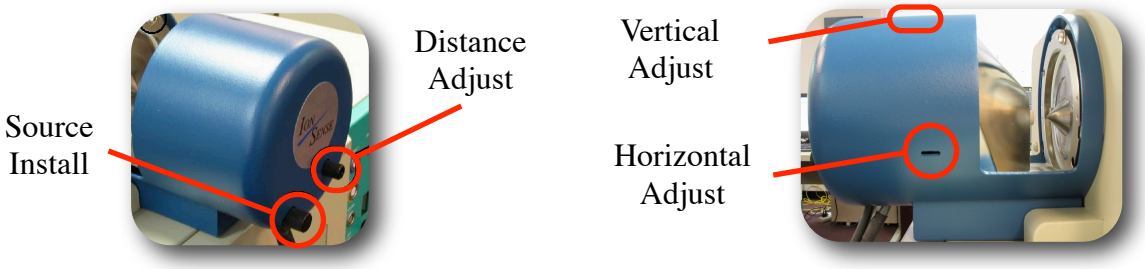
n. Create a centroided mass spectrum for methyl stearate (refer to steps c-e if you do not remember the procedure). The masses should be correct. Note that in the spectrum above, the $[M+H]^+$ at *m/z* 299.29498 is within 0.002 *m/z* units of the monoisotopic mass calculated previously (*m/z* 299.29500).

Appendix A

Optimizing DART™ Conditions

Peaks Voltage: a rough rule-of-thumb is to set this voltage to ~10x the lowest mass you wish to observe. Note that the software allows this voltage to be swept.

Gas temperature: normal range is 200-350°C, with a maximum of 500°C. Can use higher temperatures to desorb or pyrolyze analytes (i.e. polymers). One practical example of note is that it is more difficult to see higher mass PEG 600 peaks at 'low' temperatures when obtaining a calibration spectrum.



Photos Courtesy IonSense™

Dart tip positioning: the gas stream position emanating from the DART™ tip can be tweaked. One can monitor the water dimer at *m/z* 37 to adjust the x,y,z positioning. The figure above shows where to make these adjustments:

> **Distance Adjust**: distance from the source tip to the inlet of the spectrometer, adjust by turning the knurled knob.
>
> **Vertical/Horizontal Adjust**: Adjust by inserting a 7/64" (or 5/64") Allen wrench and turning the screw.

Orifice 1: use 15-25V normally. At higher voltages of, say 60-90V, you can start to produce fragmentation. This voltage can be varied using function switching.

Detector voltage: the voltage for the MCP varies per instrument and may need to be increased as the MCP ages. A value of 2400-2600V is common.

Needle voltage: 3500V for positive or negative mode

Electrode 2: -350V for negative-ion mode and +250V for positive-ion mode

Gas flow rate: a higher flow rate will introduce more ions into the spectrometer but also cools the gas stream.

Appendix B

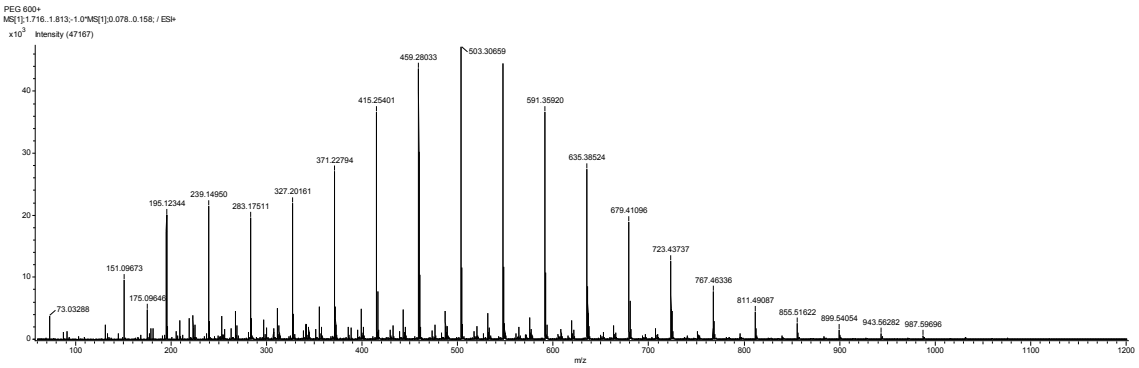
DART™ Reference Ions

PEG 600
(DART™, positive-ion)

Sample: 50 µL of PEG 600 (neat) dissolved in 10 mL of a 1:1 (v/v) methanol/ methylene chloride solution.

Exact Mass	Composition
65.06025	$[(CH_3OH)_2+H]^+$
107.07081	$[(C_2H_4O)_2+H_2O+H]^+$
151.09703	$[(C_2H_4O)_3+H_2O+H]^+$
195.12324	$[(C_2H_4O)_4+H_2O+H]^+$
239.14946	$[(C_2H_4O)5+H_2O+H]^+$
283.17567	$[(C_2H_4O)_6+H_2O+H]^+$
327.20189	$[(C_2H_4O)_7+H_2O+H]^+$
371.2281	$[(C_2H_4O)_8+H_2O+H]^+$
415.25432	$[(C_2H_4O)_9+H_2O+H]^+$
459.28053	$[(C_2H_4O)_{10}+H_2O+H]^+$
503.30675	$[(C_2H_4O)_{11}+H_2O+H]^+$
547.33296	$[(C_2H_4O)_{12}+H_2O+H]^+$
591.35918	$[(C_2H_4O)_{13}+H_2O+H]^+$
635.38539	$[(C_2H_4O)_{14}+H_2O+H]^+$
679.41161	$[(C_2H_4O)_{15}+H_2O+H]^+$
723.43782	$[(C_2H_4O)_{16}+H_2O+H]^+$

Exact Mass	Composition
767.46404	$[(C_{22}H_4O)_{17}+H_2O+H]^+$
811.49025	$[(C_2H_4O)_{18}+H_2O+H]^+$
855.51647	$[(C_2H_4O)_{19}+H_2O+H]^+$
899.54268	$[(C_2H_4O)_{20}+H_2O+H]^+$
943.5689	$[(C_2H_4O)_{21}+H_2O+H]^+$
987.59511	$[(C_2H_4O)_{22}+H_2O+H]^+$
1031.62132	$[(C_2H_4O)_{23}+H_2O+H]^+$
1075.64754	$[(C_2H_4O)_{24}+H_2O+H]^+$
1119.67375	$[(C_2H_4O)_{25}+H_2O+H]^+$

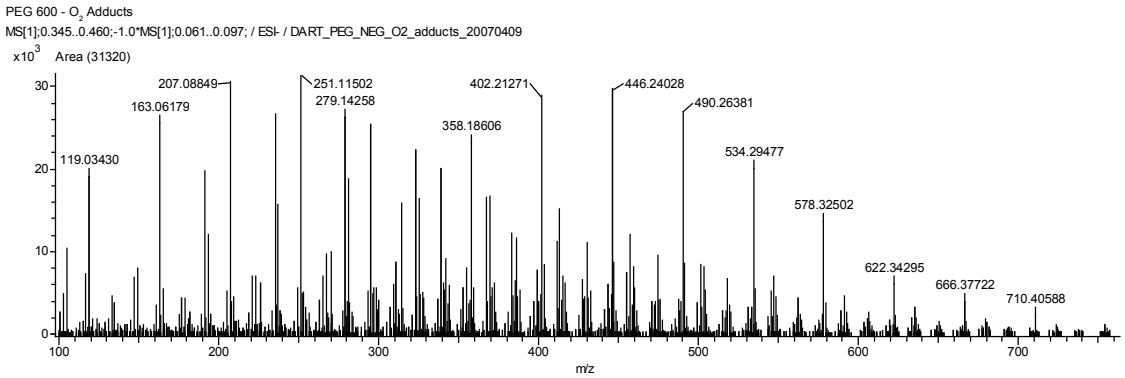


PEG 600 Positive Ion Mass Spectrum Collected on AccuTOF™-DART™

PEG 600
(DART™, negative-ion, O_2 adducts)

Sample: 50 µL of PEG 600 (neat) dissolved in 10 mL methanol

Exact Mass	Composition
119.03443	$[(C_2H_4O)_2+O_2-H]^-$
163.06064	$[(C_2H_4O)_3+O_2-H]^-$
207.08686	$[(C_2H_4O)_4+O_2-H]^-$
251.11307	$[(C_2H_4O)_5+O_2-H]^-$
295.13929	$[(C_2H_4O)_6+O_2-H]^-$
490.26254	$[(C_2H_4O)_{10}+H_2O+O_2]^-$
534.28875	$[(C_2H_4O)_{11}+H_2O+O_2]^-$
578.31497	$[(C_2H_4O)_{12}+H_2O+O_2]^-$
622.34118	$[(C_2H_4O)_{13}+H_2O+O_2]^-$
666.36739	$[(C_2H_4O)_{14}+H_2O+O_2]^-$
710.39361	$[(C_2H_4O)_{15}+H_2O+O_2]^-$
754.41982	$[(C_2H_4O)_{16}+H_2O+O_2]^-$
798.44604	$[(C_2H_4O)_{17}+H_2O+O_2]^-$
842.47225	$[(C_2H_4O)_{18}+H_2O+O_2]^-$
886.49847	$[(C_2H_4O)_{19}+H_2O+O_2]^-$



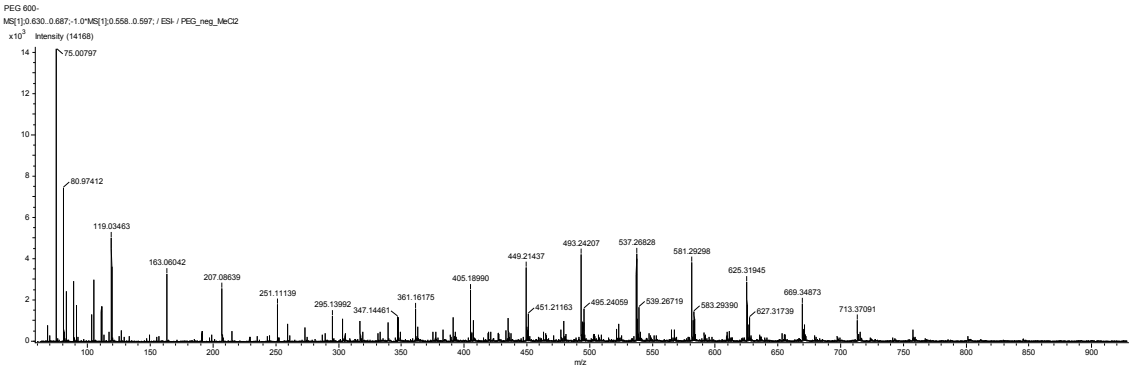
PEG 600 Negative Ion, O_2 Adducts Mass Spectrum Collected on AccuTOF™-DART™

PEG 600
(DART™, negative-ion, Cl adducts)

Sample: 50 µL of PEG 600 (neat) dissolved in 10 mL of a 1:1 (v/v) methanol/ methylene chloride solution.

Exact Mass	Composition
75.00821	$[C_2H_4O+O_2-H]^-$
119.03443	$[(C_2H_4O)_2+O_2-H]^-$
163.06064	$[(C_2H_4O)_3+O_2-H]^-$
207.08686	$[(C_2H_4O)_4+O_2-H]^-$
251.11307	$[(C_2H_4O)_5+O_2-H]^-$
273.11049	$[(C_2H_4O)_5+H_2O+Cl]^-$
295.13929	$[(C_2H_4O)_6+O_2-H]^-$
317.1367	$[(C_2H_4O)_6+H_2O+Cl]^-$
339.1655	$[(C_2H_4O)_7+O_2-H]^-$
361.16292	$[(C_2H_4O)_7+H_2O+Cl]^-$
383.19172	$[(C_2H_4O)_8+O_2-H]^-$
405.18913	$[(C_2H_4O)_8+H_2O+Cl]^-$
449.21535	$[(C_2H_4O)_9+H_2O+Cl]^-$
493.24156	$[(C_2H_4O)_{10}+H_2O+Cl]^-$
537.26778	$[(C_2H_4O)_{11}+H_2O+Cl]^-$
581.29399	$[(C_2H_4O)_{12}+H_2O+Cl]^-$
625.32021	$[(C_2H_4O)_{13}+H_2O+Cl]^-$
669.34642	$[(C_2H_4O)_{14}+H_2O+Cl]^-$

Exact Mass	Composition
713.37264	$[(C_2H_4O)_{15}+H_2O+Cl]^-$
757.39885	$[(C_2H_4O)_{16}+H_2O+Cl]^-$
801.42507	$[(C_2H_4O)_{17}+H_2O+Cl]^-$
845.45128	$[(C_2H_4O)_{18}+H_2O+Cl]^-$
889.4775	$[(C_2H_4O)_{19}+H_2O+Cl]^-$
933.50371	$[(C_2H_4O)_{20}+H_2O+Cl]^-$
977.52993	$[(C_2H_4O)_{21}+H_2O+Cl]^-$



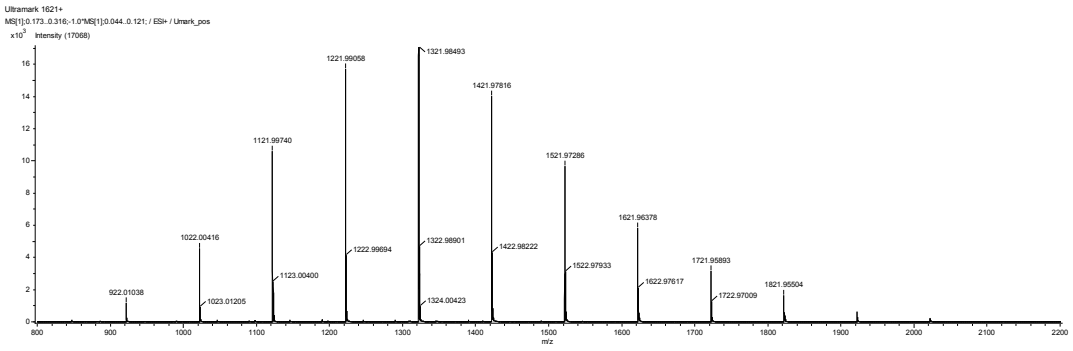
PEG 600 Negative Ion Mass Spectrum Collected on AccuTOF-DART™

Note: $[M+Cl]^-$ usually observed at higher masses, while $[(C_2H_2O)+O_2-H]^-$ are usually observed at lower masses.

Ultramark 1621® (Lancaster Synthesis) (DART™, positive-ion)

Sample: undiluted sample of Ultramark 1621® (perfluoroalkylphosphazine)

Exact Mass	Composition
922.01035	$C_{18}H_{19}O_6N_3P_3F_{24}$
1022.00397	$C_{20}H_{19}O_6N_3P_3F_{28}$
1121.99758	$C_{22}H_{19}O_6N_3P_3F_{32}$
1221.99119	$C_{24}H_{19}O_6N_3P_3F_{36}$
1269.97235	$C_{25}H_{17}O_6N_3P_3F_{38}$
1321.98481	$C_{26}H_{19}O_6N_3P_3F_{40}$
1421.97842	$C_{28}H_{19}O_6N_3P_3F_{44}$
1521.97203	$C_{30}H_{19}O_6N_3P_3F_{48}$
1621.96564	$C_{32}H_{19}O_6N_3P_{17}F_{52}$
1721.95926	$C_{34}H_{19}O_6N_3P_3F_{56}$
1821.95287	$C_{36}H_{19}O_6N_3P_3F_{60}$
1921.94648	$C_{38}H_{19}O_6N_3P_3F_{64}$
2021.94013	$C_{40}H_{19}O_6N_3P_3F_{68}$

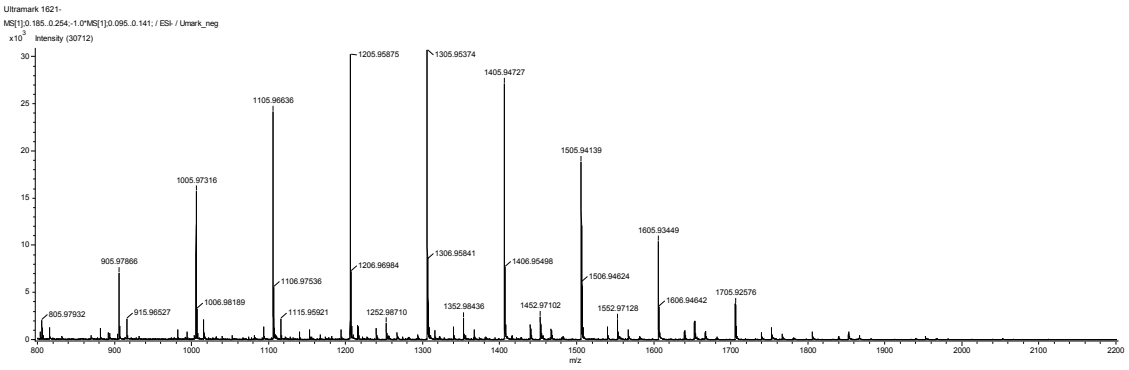


Ultramark 1621 Positive Ion Mass Spectrum Collected on AccuTOF-DART™

Ultramark 1621® (Lancaster Synthesis) (DART™, negative-ion)

Sample: undiluted sample of Ultramark 1621® (perfluoroalkylphosphazine)

Exact Mass	Composition
805.98544	C15H15O6N3P3F20
905.97905	C17H15O6N3P3F24
1005.97267	C19H15O6N3P3F28
1105.96628	C21H15O6N3P3F32
1205.95989	C23H15O6N3P3F36
1305.95351	C25H15O6N3P3F40
1405.94712	C27H15O6N3P3F44
1505.94073	C29H15O6N3P3F48
1605.93434	C31H15O6N3P3F52
1705.92796	C33H15O6N3P3F56
1805.92157	C35H15O6N3P3F60



Ultramark 1621 Negative Ion Mass Spectrum Collected on AccuTOF-DART™

Possible DART™ Background Compounds

Note: This table lists a sampling of compounds that exist in ubiquity. These compounds may or may not be present in your DART™ spectra.

Name	Formula	Observed m/z	Function	Where Found	Polarity
acetone	$C_3H_6O_3$	59.04969	solvent	background	positive
bis(ethylhexyl phthalate)	$C_{24}H_{38}O_4$	391.284835	plasticizer	everywhere	positive
cholestadiene	$C_{27}H_{44}$	369.352127	(cholesterol)	fingerprints, sweat	positive
decamethylcyclo pentasiloxane	$C_{10}H_{30}O_5Si_5$	371.101791	silicones	background	positive
dioctyladipate	$C_{22}H_{42}O_4$	371.316135	plasticizer	melting point tube package	positive
erucamide	$C_{22}H_{43}NO$	338.34229	slip agent	plastic bags	positive
lactic acid	$C_3H_6O_3$	89.023869		fingerprints, sweat	negative
myristic acid	$C_{14}H_{28}O_2$	227.201105	fatty acid	fingerprints, solvents	negative
oleic acid	$C_{18}H_{34}O_2$	281.248056	fatty acid	fingerprints, solvents	negative
oxygen	O_2	31.989829		air	negative
palmitic acid	$C_{16}H_{32}O_2$	255.232405	fatty acid	fingerprints	negative
squalene	$C_{30}H_{50}$	411.399077	lipid	fingerprints	positive
stearic acid	$C_{18}H_{36}O_2$	283.263706	fatty acid	fingerprints, solvents	negative
water dimer	H_5O_2	37.028954	$(H_2O)_2+H$	air	positive

Appendix C

AccuTOF™-DART™ Standard Samples

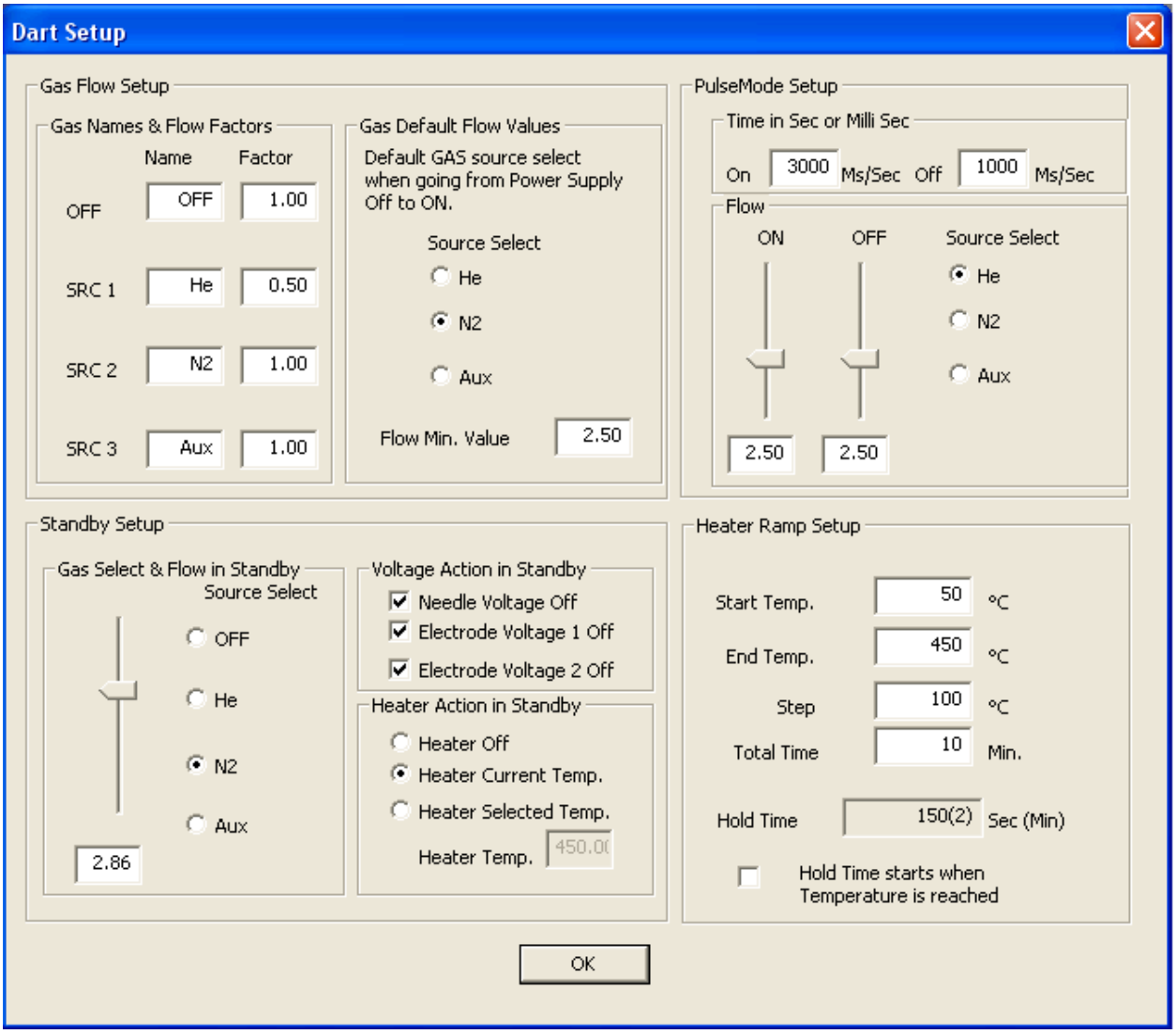
Use the following if you wish to reorder any of the standard samples.

Part Number	Sample
JU200729	reserpine 10ng/mL in methanol
JU2007530	reserpine 100ng/mL in methanol
JU2007531	PEG 600 100ng/mL in methanol
JU2007532	PEG 600 neat
JU2007533	methylstearate 100ng/mL in hexane

Appendix D

DART™ Source Standard Settings

To access this window, from ***DART Bar*** choose ***Setup*** -> ***DART Setup...***. These are the default settings set by the engineer at installation.



Setup Page for the DART™ Source

Appendix E

Consumables Part Numbers

The following table lists part numbers for some commonly used items. If you have any questions, please contact JEOL Applications or Service for advice.

Part Description	Vendor	Part #
Inland 45 pump oil	SIS JEOL	INV451 JU2002045
EMF-20 oil mist filter element	SIS	A223-04-199
EMF-20 odor element	SIS	A223-04-077
ceramic end cap	IonSense	MA05130
exit grid	JEOL	JU2005669
ESI needle 0.2mm OD x 0.1mm ID x 120mm	JEOL Small Parts	812162498 *HTX-33R (120mm)
PEEK ferrule	JEOL Alltech	4030227365 30661
needle hold tubing	Upchurch	1529
Hamilton gas-tight syringe 1005TLL 5.0mL	Restek Agilent	20178 5183-4551
PEEK tubing, redstripe 1/16 in. OD x 0.0025 in. ID	Restek	25065
PEEK union 1/16 in. connector	Restek	25323

* Denotes a “special order” part number. Specify when ordering.

Appendix F

Troubleshooting

Problem: No signal.

Software Checks

Make sure instrument is in ***Operate*** mode.

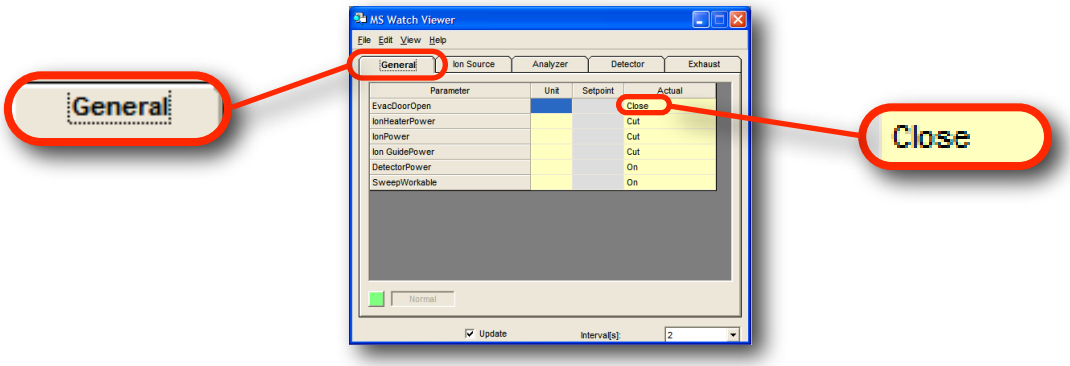
Make sure detector voltage is set to operating voltage (e.g. 2400V).

Make sure that you have loaded a valid set of tune parameters.

Check the DART™ electrode potentials. ***E1*** and ***E2*** should both be positive for positive-ion mode, negative for negative-ion mode.

Make sure that the hight voltage is on for the discharge needle.

Check the MS interlock. In ***MassCenter Main***, choose ***MS Status Panel*** -> ***Status***. In ***MS Watch Viewer*** on the ***Gernal*** tab, ***Evac Door Open*** should say ***Close***.



Setup Page for the DART™ Source

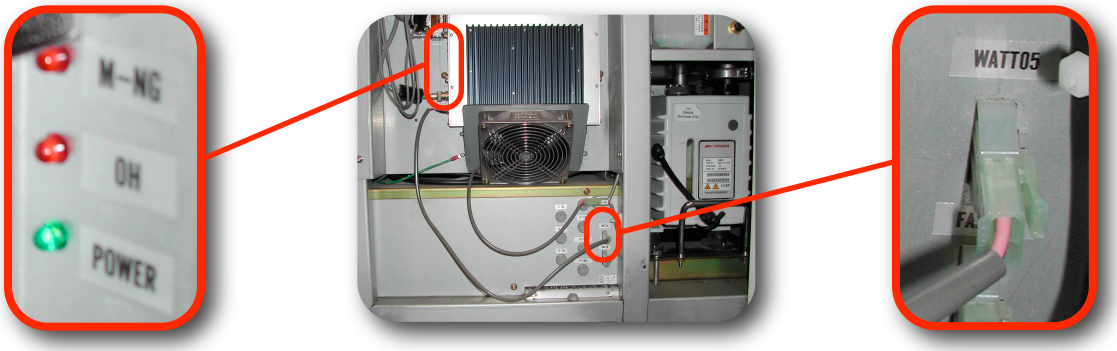
In ***MS TUNE Manager***, switch between ***Operate*** and ***Standby***, then switch back. If there is an interlock problem, you will see an error message in ***MS Watch Viewer***.

Note: ***MS Watch Viewer*** is an excellent, general troubleshooting tool. Only a couple of error state examples are covered here. But the remaining tabs contain a wealth of information about the AccuTOF's current status. Noting other error states will be very useful when contacting JEOL for advice.

Hardware Checks

Check to see that the DART™ power supply is turned on. The power switch is on the rear of the controller. Make sure that the AccuTOF orifice is not blocked. You can unclog the orifice with an old electrospray needle or similar O.D. wire without shutting down the instrument.

Check the RF ion guide status. Choose ***Mass Center Main*** -> ***MS Status Panel*** -> ***Status***. On the ***MS Watch Viewer Analyzer*** tab, ***Ion GuideConformNG*** should say ***Normal***. If instead the message says ***Incorrect Matching***, you will need to reset the ion guide power supply. Remove the front panel of the AccuTOF by removing the two (2) screws located inside the door. Check the status of the LED on the ion guide power supply. The green LED labeled ***Power*** indicates a normal condition. If a red LED labeled ***M-NG*** is illuminated, shut down ***MassCenter***. Disconnect the white Molex connector labeled ***Watt05***. After five seconds, reconnect the Molex connector. Restart ***MassCenter***.



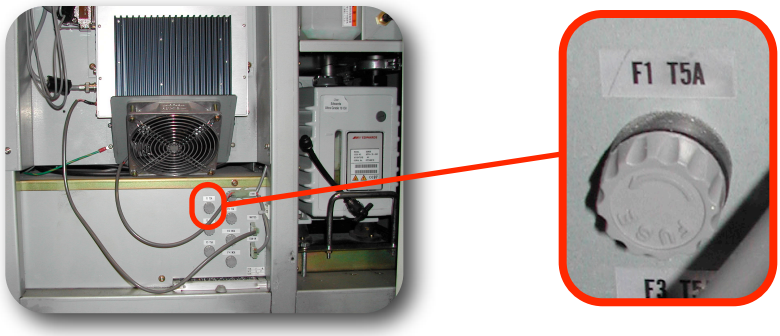
AccuTOF front with panel removed: LED status (left) and Watt-5 connector (right)

Problem: ***MassCenter*** shows the error message "MassCenter is unstable. Please restart and try again."

Shut down ***MassCenter***. If necessary, click on the 

icon to ensure that all processes are stopped. Reboot the workstation.
If restarting ***MassCenter*** does not resolve the issue, you may need to reset

the acquisition processor (APU). Shut down ***MassCenter***. Remove the front panel. Remove and then replace the fuse labeled ***F1***. Restart ***MassCenter***.



Locating fuse ***F1***

Problem: You can not get a good calibration. That is, the correlation coefficient displayed in ***Mass Calibration Viewer*** is not 10^{-11} or less.

In ***Mass Calibration Viewer***, check to see if any reference peaks are mis-assigned. Try deleting reference peaks that are weak or in the background noise.

Check the mass spectral peak shapes and resolution. If the resolving power has decreased significantly, it may be time to re-tune the mass spectrometer. If re-tuning does not work, it may be time to clean the atmospheric pressure interface. Refer to the ***AccuTOF Introduction*** manual or contact JEOL for information on this procedure.