AP/MALDI Source for the Micromass Q-Tof

Installation, Operation and Maintenance Manual

December, 2003

Warning

Optical parts of the AP/MALDI source should be handled with **extreme** care. Touching them with bare fingers, storing them in or exposing them to dirty or dusty environments can result in permanent damage of some optical components. Be aware that the warranty does not extend to the fiber optical cable, which requires special care during storage, installation, and operation of the AP/MALDI source. Any finger tapping, dirt deposition, or exposing to a dirty environment will result in burning the fiber ends. An optical fiber is shipped with special protective caps on its ends. After removing the fiber optic protective caps, please keep them in clean conditions and put the protective caps back on the fiber ends immediately after the cable is detached from a connector or the cable is not used. If cleaning of the fiber end is required please refer to the Maintenance/Troubleshooting section (Section 7) of this manual for a cleaning procedure. It is a good idea to proceed with fiber end cleaning every time an exposure to dirt or a contamination of a fiber end surface is suspected. In normal operation with proper care an optical fiber will have a long lifetime. We've included a spare optical fiber cable in case your first optical fiber cable is accidentally damaged. Additional fiber cables **MUST** be ordered from the AP/MALDI source manufacturer, MassTech, or your sales agent. ONLY replace the fiber with an exact replacement from the manufacturer, MassTech (Replacement Part number 6100004)

This product is to be used only in laboratory conditions by trained and skilled technicians and in accordance with these operating and maintenance instructions. Primary safety is ensured by correct assembly of the instrument and by following the instructions and warnings in this manual. To ensure continued safety, please ensure that any voltage source connected to this product has a suitable safety ground and complies with local and national electrical regulations

Attention: Users of the 8x12 target plate AP/MALDI Source Option:

1. **You can start running the Target program only with the Source *closed*. Otherwise you will get a warning message prompting you to close the source.**
2. **After you start running the Target software, the XY stages are being initialized. Do not try to open the source before this process is finished and you have a "Ready" message in the Status window! *Forceful Opening of the 8x12 Source during a stage initialization may result in the Source mechanism having mechanical damage!***

For maintenance or repair please contact your sales agent or the manufacturer directly:

MassTech, Inc.
6992 Columbia Gateway Dr.
Columbia, MD 21046
USA
Phone: (443) 539-1758
Fax: (443) 539-1759
Email: msms@apmaldi.com

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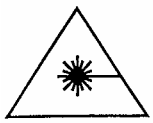
PREFACE

The following symbols are used in this manual to indicate material that should especially be noted because it relates to safety issues.

This symbol in the manual margin is used to emphasize the presence of very important operating instructions related to safety especially during installation, uninstallation, maintenance and troubleshooting.



This symbol in the manual margin is used to alert the operator to potential dangerous exposure to hazardous invisible laser radiation.

Operators are strongly encouraged to read this manual before installation, uninstallation, operation, maintenance, or troubleshooting. Operators should pay special attention to paragraphs marked by  and  .

DO NOT ATTEMPT services or repairs that are not covered in the Troubleshooting section, section 7, of this Manual. For services and repairs beyond those specifically provided in the Troubleshooting section, contact the manufacturer:
MassTech, Inc. 6992 Columbia Gateway Dr; Columbia, MD 21046
(443) 539-1758.

1 INTRODUCTION: AP/MALDI – A NEW SOURCE OF ATMOSPHERIC PRESSURE IONS

The AP/MALDI source is designed to produce molecular ions of analytes under normal atmospheric pressure conditions from a mixed matrix/analyte microcrystals by irradiating these crystals with nitrogen laser pulses. These ions are analyzed by a Q-Tof instrument by recording corresponding mass spectra. The mechanism of **AP/MALDI** ion production is similar to that of **conventional MALDI**. The main difference is that AP/MALDI produces ions under atmospheric pressure conditions **outside** of the instrument vacuum housing. The main consequences are:

- The AP/MALDI source is an external ionization source. It is designed to be easily interchangeable with other sources of Q-Tof instruments like ESI, APCI, nanospray, etc.

- Because the AP/MALDI source operates under atmospheric pressure, the replacement of target (sample) plates is a simple and quick process.

- The AP/MALDI source is designed as an additional external source for Q-Tof mass spectrometers manufactured by Micromass. There are other versions of AP/MALDI sources adopted for some instruments. The process of mass spectra measurement is completely decoupled with sample ionization process. Thus AP/MALDI inherits all the power of the Q-Tof mass spectrometers: **high sensitivity, high m/z range, the stability of calibration, MS/MS capability, powerful data processing, and spectra interpretation software**.

- AP/MALDI is a softer ionization technique compared with conventional vacuum MALDI. This is an important advantage when unstable molecular mass of analyte in a gas phase is to be measured. A detailed discussion of this phenomenon and some examples may be found in publications [1,2].

The AP/MALDI source operates under normal ambient pressure conditions similar to ESI sources. AP/MALDI and ESI sources are interchangeable and typically provide complimentary analytical information. Appropriate use of both ESI and AP/MALDI sources provides the opportunity to cover the broad range of problems of modern analytical chemistry [1,2].

1.1 QUICKSTART OPERATION

This section covers basic operation of the AP/MALDI source after the AP/MALDI source, Target software, and Q-Tof mass spectrometer have been properly installed and set-up.

Once the Ion source and control unit are installed and connected to each other and the mass spectrometer according to Section 4 of this manual, the operation steps are as follows. All installation and uninstallation procedures **must** be done with the Power TURNED OFF. Before proceeding you are strongly urged to read the Safety procedures in Section 3 of this manual.

1. Close the Ion source, turn on the Control unit, and run the Target software on the PC connected to the Mass Spectrometer. Wait until the initialization is completed and "Ready" is displayed in the status field of the Target software.

2. Since the Q-Tof software is normally optimized for the Electrospray source, you must adjust the Q-Tof software's parameters so it is optimized for AP/MALDI:

With settings optimized for the ESI, then make the following changes: ConeV=155V; EntranceV=85V; Aperture2=16V; MCP: ~100V higher than that typically applied for ESI

3. Prepare a MALDI Sample according to Section 5 of this manual. (a typical sample preparation procedure is the same as is done for conventional vacuum MALDI).

4. Load the Target plate containing the samples into the Ion source target plate holder according to Section 5.1 of this manual. Ensure that you close the Source flush, otherwise the laser will not fire.

5. Use the Target software to start firing the laser and test your samples. To operate in Manual mode (spot by spot spectra measurement), make sure that the AutoSequence check box is unchecked, and choose a desirable spot using the Target software. Adjust the position of the laser using the target image on the TV screen, if necessary. Start Q-Tof data acquisition (using MassLynx software). Start the Laser firing and (optionally) spiral motion (in the Target program). After satisfactory data collection, write the spectrum to a hard drive. Now you can repeat the procedure for other spots. (A detailed explanation of automatic operation is included as Section 6 of this manual).

6. When you finish the data acquisition, stop Q-Tof data acquisition (by using the Q-Tof's MassLynx software), and stop laser firing and target motion (by using the Target software). Open the source and remove the used target plate.

7. Replace the target plate, close the Ion source, and repeat step 5 to get spectra from a new target plate.

2 AP/MALDI BASIC PRINCIPLES

Understanding the basic principles of the AP/MALDI source is desirable, but not strictly necessary for successful practical use of the source. A simplified scheme of the AP/MALDI source is presented in Fig. 1 below.

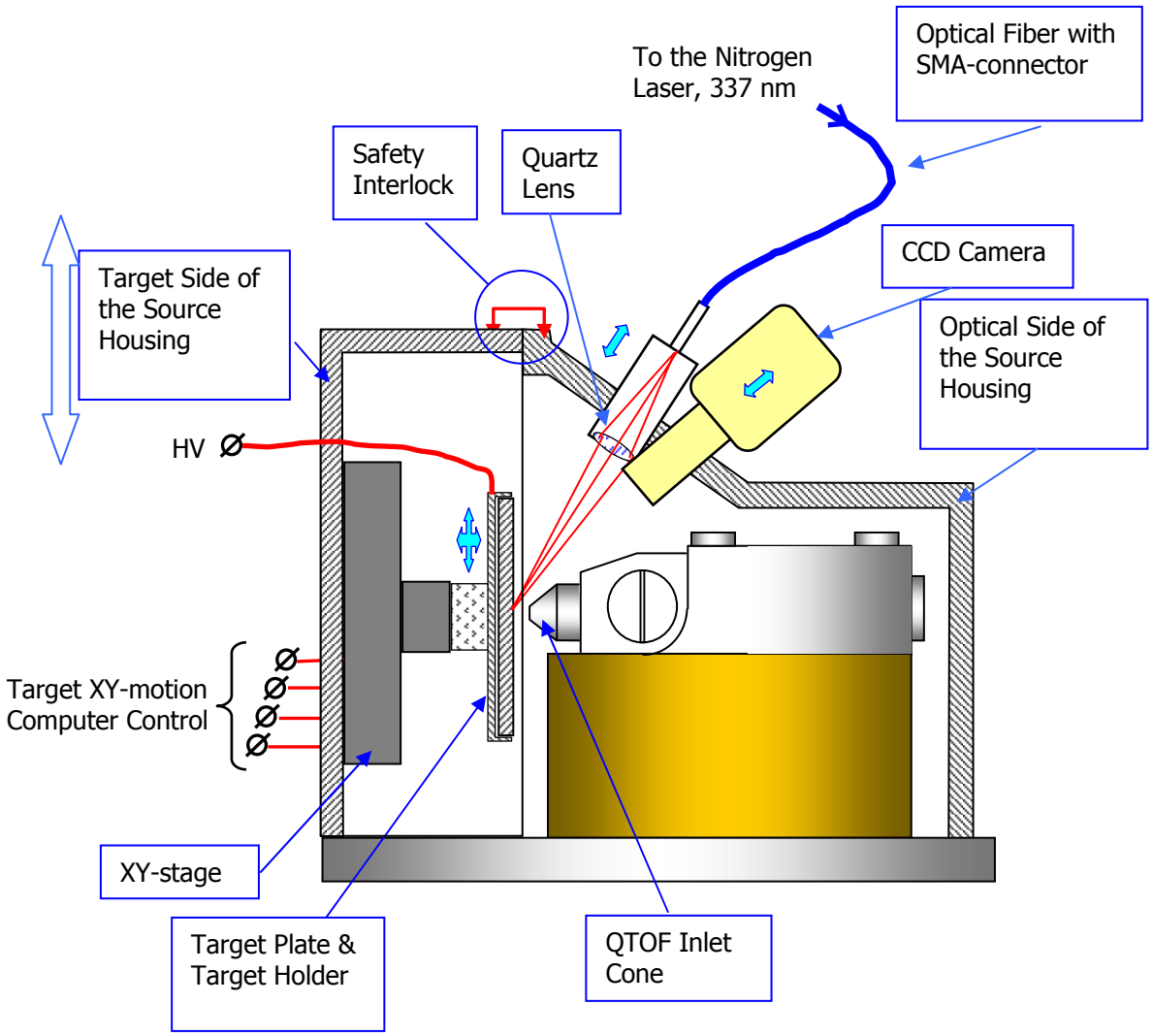


Fig. 1. Simplified schematic diagram of the AP/MALDI source installed on a Q-Tof instrument.

The following explanation of AP/MALDI basics will become clearer as you set up your unit. The AP/MALDI source is mounted inside a Housing. The source **Housing** is attached to the **Q-Tof Inlet Flange.** Ions produced inside the source **Housing** are directed toward the inlet orifice of the **Q-Tof** with an electric field and the intake gas. The source Housing consists of two separate parts, the **Target Side of the Source Housing** and the **Instrument Side of the Source Housing.** MALDI samples are deposited onto the surface of a replaceable **Target Plate** that is slipped into a **Target Plate Holder**. Up to 64 sample spots (or 96 spots if Option 1 was purchased) can be deposited on the surface of each **Target Plate**. High Voltage (typically, 2-3kV) is applied to a **Target Plate** to assist the transportation of produced ions toward the inlet orifice. Sample material deposited on the surface of a **Target Plate** is irradiated with UV light pulses. A Nitrogen Laser (wavelength 337nm) is mounted inside a Control Unit (not shown at Fig. 1) and is connected to the AP/MALDI source by **Optical Fiber**. UV light pulses transmitted through the **Optical Fiber** are focused by a **Quartz Lens** and directed onto the target surface with a **Mirror**. A **CCD Camera** and one more additional **Mirror** enable the user to monitor the target plate motion and the sample evaporation processes from a TV screen (not shown in Fig.1). Inside the source Housing there is also a source of visible light and one more additional Mirror (not shown in the Fig.1) to illuminate the target plate surface. The AP/MALDI source can be easily opened to replace **Target Plates**. A **Safety Interlock** prevents the laser from being switched **ON** or **HV** to be applied to a **Target Plate** if the source is **OPENED**.

The second important part of the AP/MALDI unit is a **Control Unit** (not shown in the figure). UV laser and XY-stage controllers are mounted inside it. The Control Unit is connected to the source by an Optical Fiber and electrical cable. One more cable connects the Control Unit with a PC computer's serial (COM) port that controls the target plate motion and laser firing. Either a separate (PC) computer or a Q-Tof control computer can be used to operate the AP/MALDI source. Inside the Control Unit is a nitrogen laser made by Spectra-Physics. (Appendix A is a list of specifications for this OEM laser).

3 SAFETY PROCEDURES WHILE USING AP/MALDI

This product is to be used only in laboratory conditions by trained and skilled technicians and in accordance with these operating and maintenance instructions. Primary safety is ensured by correct assembly of the instrument and by following the instructions and warnings in this manual. To ensure continued safety, please ensure that any voltage source connected to this product has a suitable safety ground and complies with local and national electrical regulations

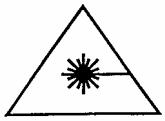
If operated properly, the AP/MALDI source is safe. No special knowledge of laser safety or electrical safety is necessary to operate the source. There are two potentially hazardous factors connected with AP/MALDI source installation, operation and maintenance/troubleshooting:

1. **Invisible coherent UV irradiation** 337nm, up to 300µJ per pulse
2. **High Voltage** up to 5kV DC

To provide the necessary safety, the manufacturer of this product has provided careful protection to users by shielding (housing) and reliable interlocking of the source component from UV radiation and High Voltage, provided that the AP/MALDI source Power if TURNED OFF during installation/uninstallation.

3.1 Safety Precautions

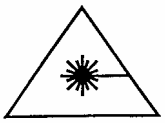
This section describes important precautions that must be observed during AP/MALDI source ***installation, operation, and maintenance***. Appropriate precautions can be divided into the following stages:



- **Installing/Uninstalling:** Before the source is installed onto the Q-Tof instrument, uninstalled, or replaced, the Q-Tof instrument must be in either "Standby" or "Off" mode (the "Scan" light on the Q-Tof front panel ***must*** be turned OFF). The same rules, described in the Q-Tof operator's Manual for the replacement of the standard sources (Electrospray/Nanospray/APCI), are applicable for AP/MALDI, too.

Never switch the power ON at the rear panel of the AP/MALDI Control Unit before the source is ***completely installed***, optical fiber properly connected at **both ends**, and the HV connector properly connected to the AP/MALDI source.

When uninstalling, again: make sure that the Q-Tof instrument is in Standby or Off mode ("Scan" is not blinking); switch OFF the power at the rear panel of the AP/MALDI Control Unit; then start any disassembling operations or source detachment. The AP/MALDI source safety interlocks safeguard the user from accidental application of High Voltage or Laser Radiation when the source and Control Unit are covered in their housing.



IMPORTANT: Whenever the optical fiber is being detached from or connected to the Control Unit or the Source housing, **MAKE SURE the power switch on the Control Unit is OFF.**

- **Target plate loading/unloading:** You need to open the AP/MALDI source to load or unload the target plate. This should be done with laser OFF, the mass spectrometer acquisition stopped, and the Capillary voltage set to zero (0) volts. After that, proceed with loading/unloading of the sample as described in Section 5 of this Manual. If by accident you open the source while the Q-Tof instrument is in "Operate" mode (HV is ON) and/or the Laser is firing, the AP/MALDI source safety interlocks automatically switch the High Voltage and the Laser OFF.

- **Mass Spectra recording:** Normally, the recording of AP/MALDI spectra is the computer's job. The source at that time is closed and attached to the Q-Tof instrument, which excludes any possibility of High Voltage shock or laser radiation exposure. Once again, if by accident you open the source while the Q-Tof instrument is recording the spectrum (HV is ON) and/or the Laser is firing, the AP/MALDI source safety interlocks automatically switch the High Voltage and the Laser OFF.

- **Maintenance and troubleshooting:** The AP/MALDI source does not require any maintenance, except cleaning of the optical fiber ends. It is strongly recommended that you follow the maintenance and troubleshooting procedures that are described in the "Troubleshooting" section (Section 7) of the present manual.

DO NOT ATTEMPT services or repairs that are not covered in the Troubleshooting section, section 7 of this Manual. For services and repairs beyond those specifically provided in section 7, contact the manufacturer, MassTech, Inc. 6992 Columbia Gateway Dr; Columbia, MD 21046 (443) 539-1758.

<u>Remember:</u> Only personnel specifically qualified for laser/high voltage jobs can ignore the following safety rules:

- **Never defeat or bypass interlocks**
- **Never open the cover of the Control Unit**
- **During the Optical Fiber replacement or removal, the Power at the Control Unit must be OFF**
- **Never switch the Power ON at the Control Unit if the AP/MALDI source is not properly attached to the Q-Tof instrument or the optical fiber is not properly installed.**

3.2 Operator Controls and Indicators

The two photos below illustrate the front and back plate of the AP/MALDI Control Unit. Additional warning and Identification labels are illustrated in Appendix C of this manual.

The Control Unit Front Plate

The Control Unit Back Plate

4 SOURCE INSTALLATION

4.1 Checking that all components have been received.

Before you start installing your source, ensure that all necessary Parts and Accessories have been delivered. Figures 2a-9 below show these components and introduce some definitions and part names used in the installation explanations.

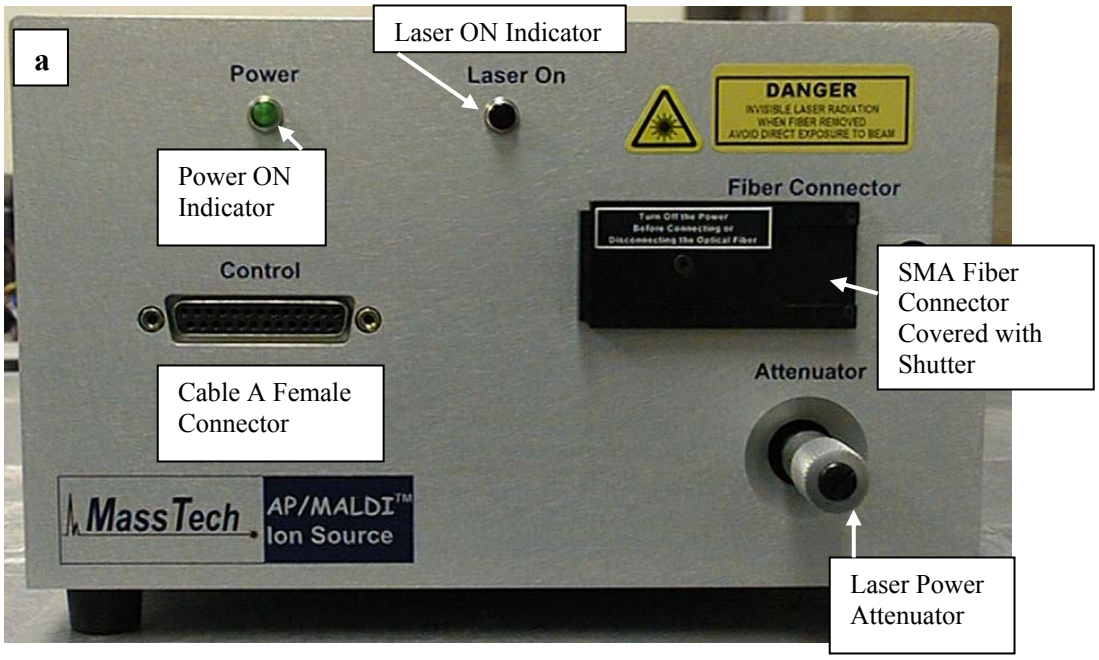


Fig. 2a. Control Unit Front View

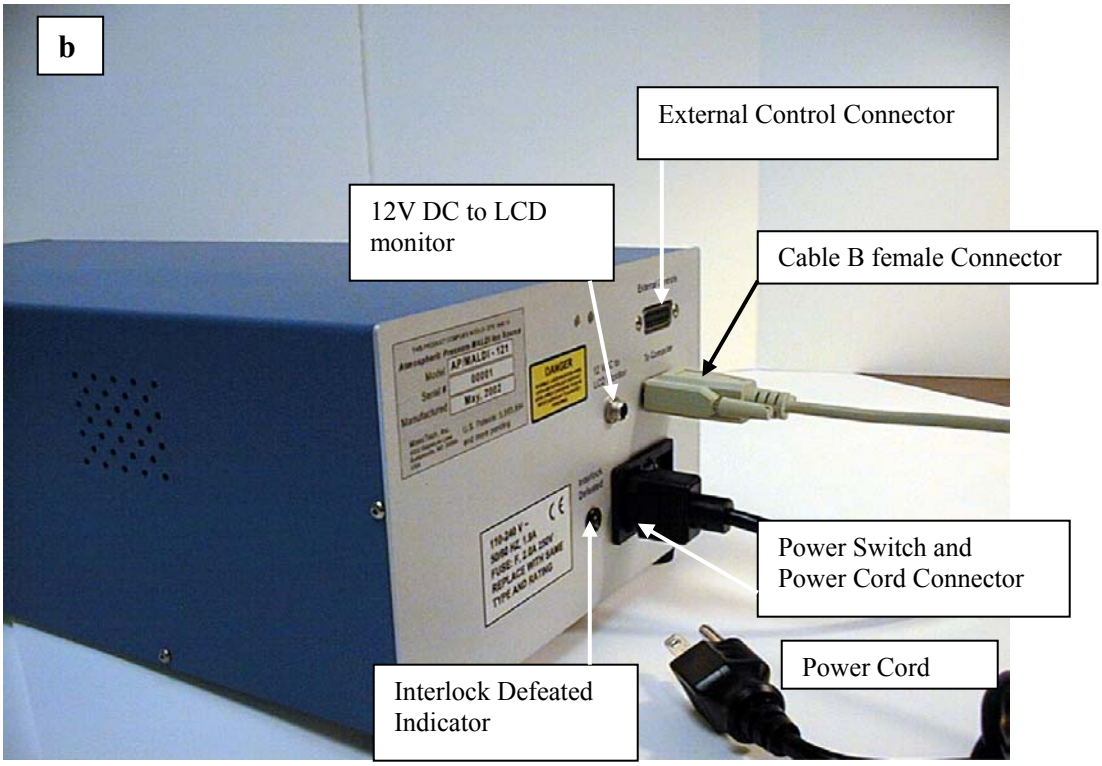


Fig. 2b. Control Unit Rear View

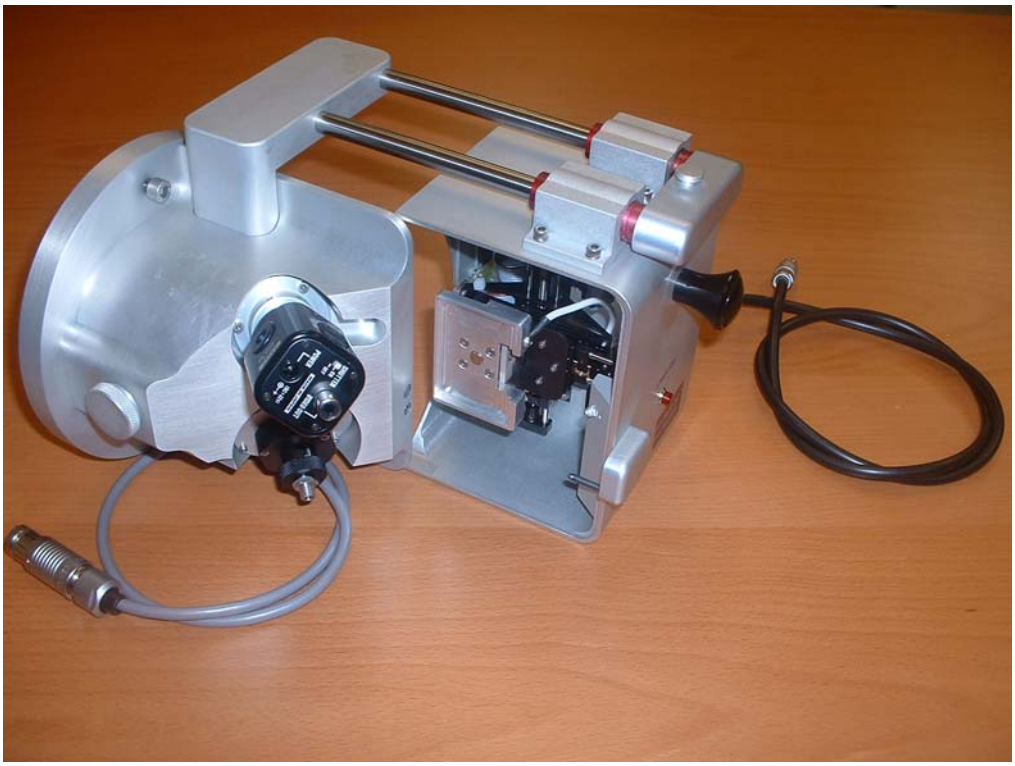


Fig. 3. Ion Source for the Micromass Q-Tof instrument

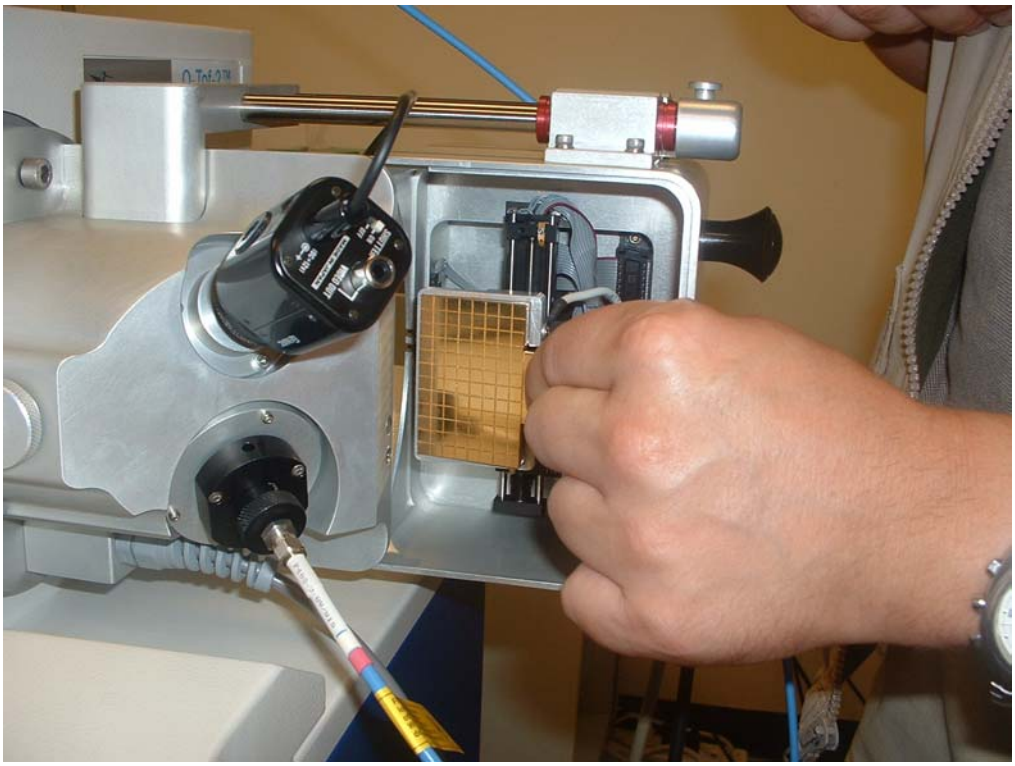


Fig. 4. The 96-spot- target plate and target flange

Fig. 5. Screw that ships for attaching the source to the Micromass Q-Tof instrument

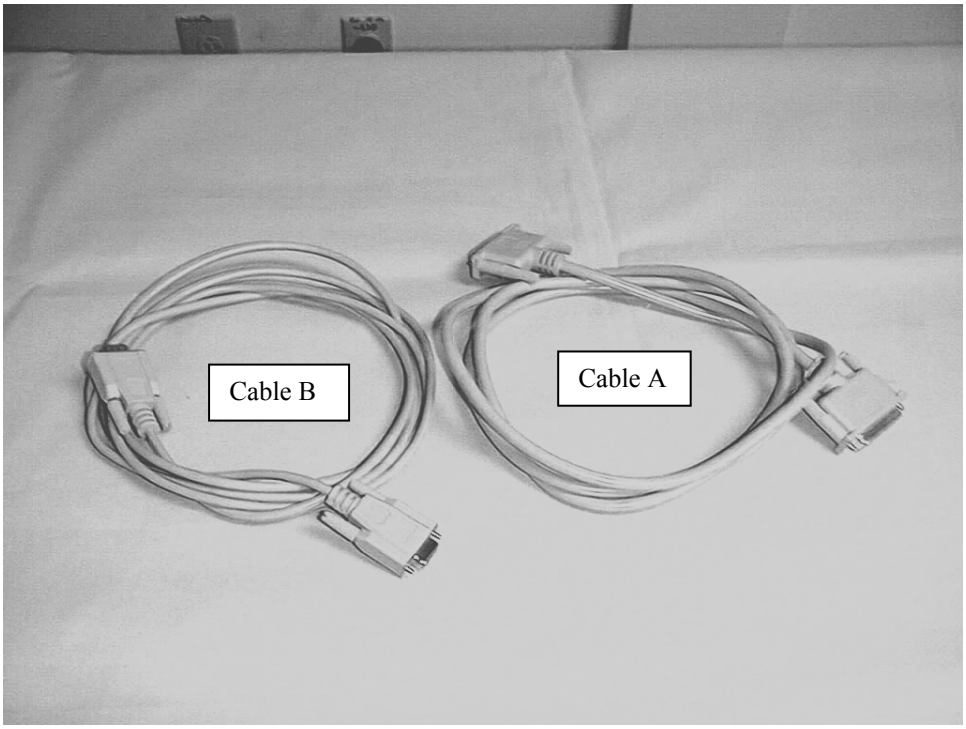


Fig. 6. Cable A (Control Unit – to - Source Cable) and Cable B (Control Unit – to - Serial Port of PC computer Cable).

Fig. 7. CCD Camera and Power Cable for CCD Camera.

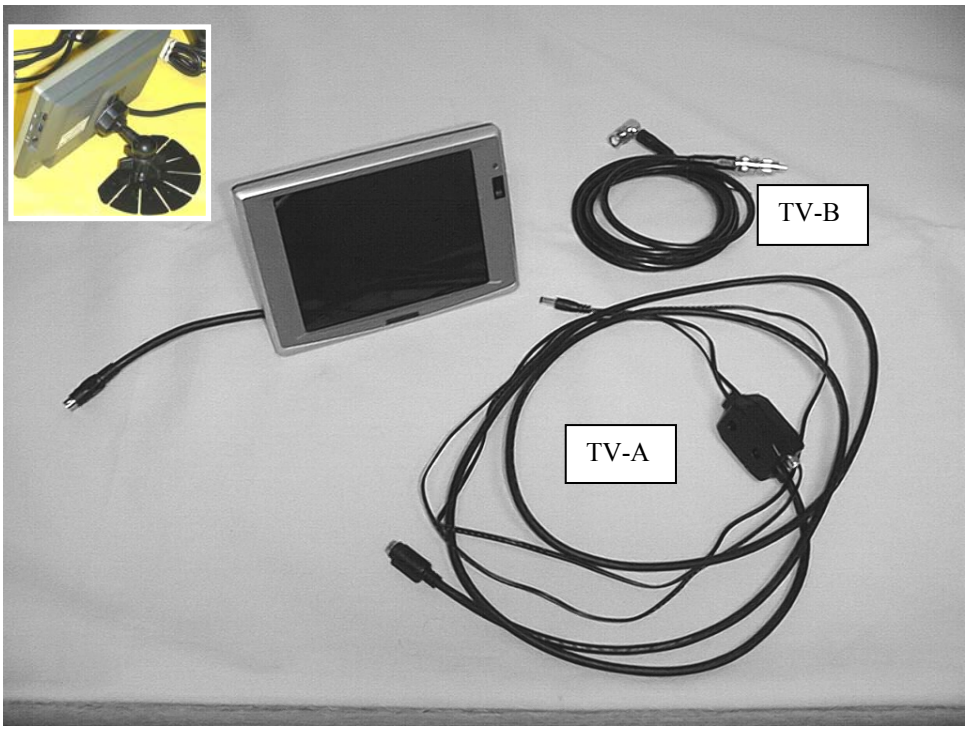


Fig. 8. Flat TV-screen with Power cables TV-A and TV-B.
Insert: Rear view.

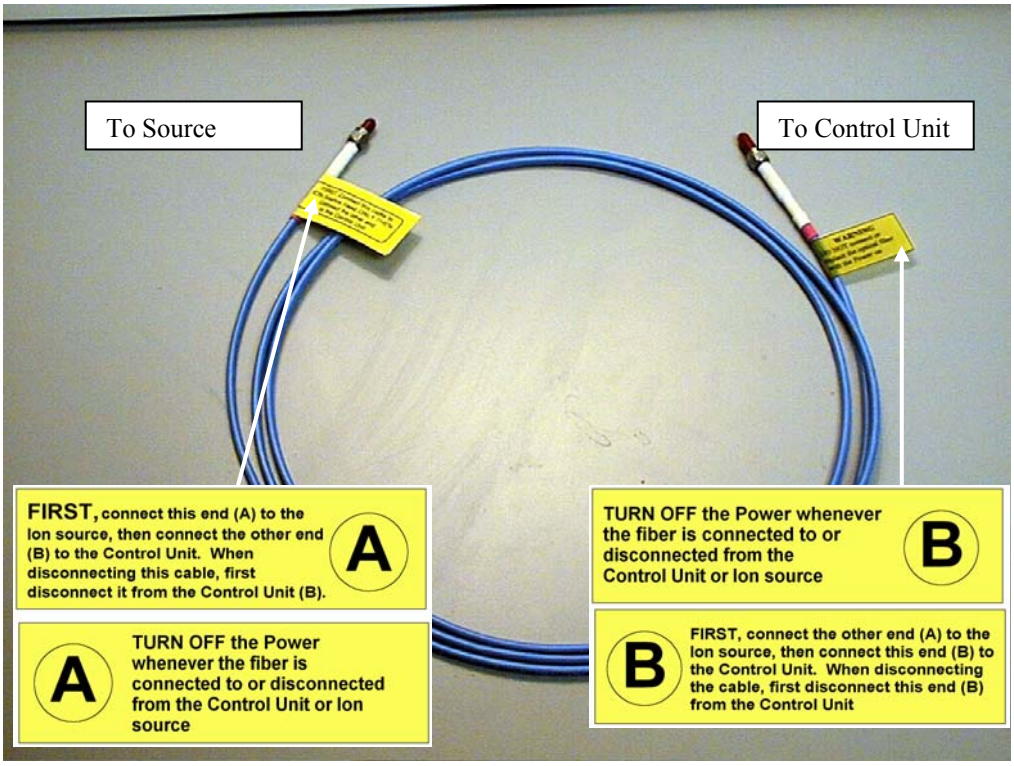
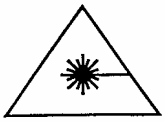


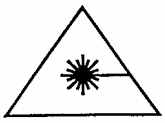
Fig. 9. Optical UV-grade Fiber with SMA-connectors labeled on both sides. SMA-connectors on both sides are covered with protective plastic caps. (The shipment includes one spare Optical cable, not shown in the figure).



When you install/uninstall the source on the Q-Tof instrument, the Power switch **must** be in the Off position. The figures below illustrate how to connect/disconnect the optical fiber from the Source and the Control Unit.

Fig. 10. Proper connection/disconnection of the optical fiber from the Source and the Control Unit.

When the optical cable is disconnected, any laser fire can emit invisible laser radiation from the ends of the optical cable. Therefore, throughout this manual we warn you of this danger.

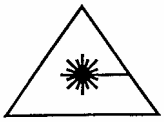


You must turn OFF the Control Unit (so the laser cannot be accidentally fired) whenever you have the optical fiber disconnected from either end or plan to disconnect or connect it.

In the event that you need to purchase another optical fiber cable, ONLY replace the fiber with an exact replacement from the manufacturer, MassTech (Replacement Part number 6100004)

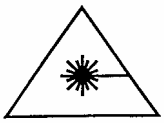
4.2 Installation of the Source

Installing/Uninstalling: Before the source is installed onto the Q-Tof instrument, uninstalled, or replaced, the Q-Tof instrument must be in either "Standby" or "Off" mode (the "Scan" light on the Q-Tof front panel ***must*** be turned OFF). The same rules, described in the Q-Tof operator's Manual for the replacement of the standard sources (Electrospray/Nanospray/APCI), are applicable for AP/MALDI, too.



> ***Never*** switch the power ON at the rear panel of the AP/MALDI Control Unit before the source is ***completely installed***, optical fiber properly connected at **both ends**, and the HV connector properly connected to the AP/MALDI source.

When uninstalling, again: make sure that the Q-Tof instrument is in Standby or Off mode ("Scan" is not blinking); switch OFF the power at the rear panel of the AP/MALDI Control Unit; then start any disassembling operations or source detachment. The AP/MALDI source safety interlocks safeguard the user from accidental application of High Voltage or Laser Radiation when the source and Control Unit are covered in their housing.



> **IMPORTANT:** Whenever the optical fiber is being detached from or connected to the Control Unit or the Source housing, **MAKE SURE the power switch on the Control Unit is OFF.**

When you take the source out of the box, it will look like Figure 11 below.

Fig 11. The AP/MALDI Ion Source right out of the box.

Fig 12. The Micromass Q-Tof with the ESI mounted

Remove your current ESI source according to your Micromass Q-Tof instructions, leaving an empty inlet flange as shown on the picture below. Removing the three large black screws will allow you to detach the ESI source.

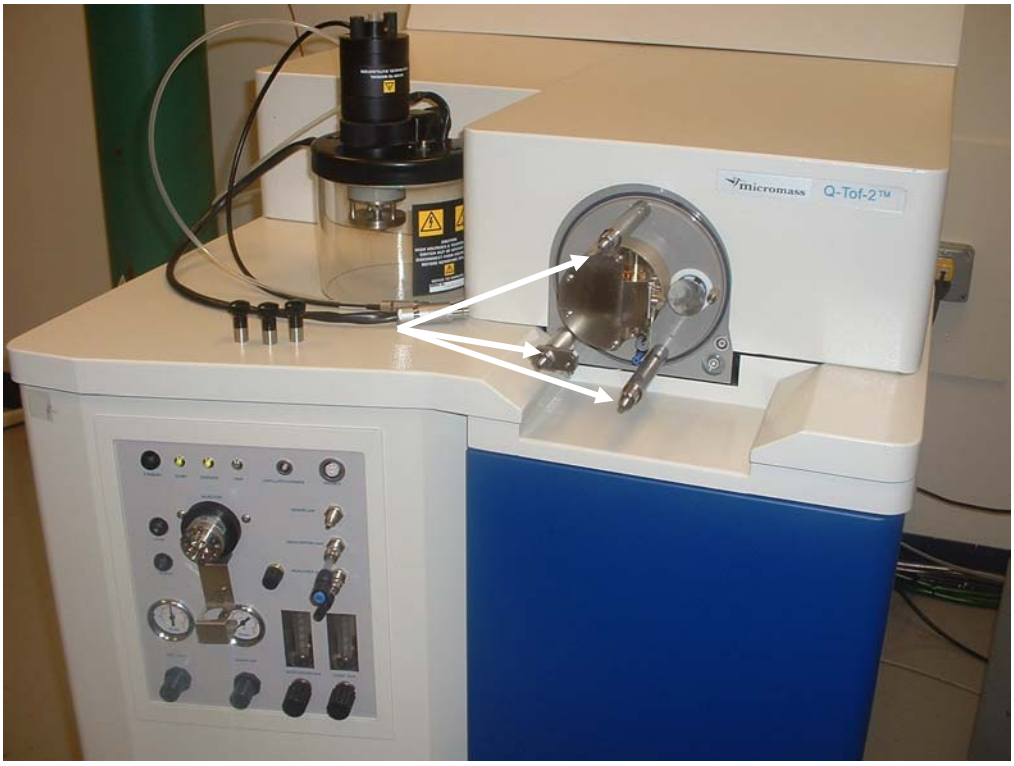


Fig 13. Q-Tof with the ESI source housing removed

Also, remove the three steel rods (see arrows above) by simply unscrewing them.

Fig. 14. The Micromass Q-Tof with ESI and steel rods removed

Remove the two pieces indicated below by pulling them straight out. The lower blue plastic cap requires you to push in the outer black connector while pulling the cap simultaneously.

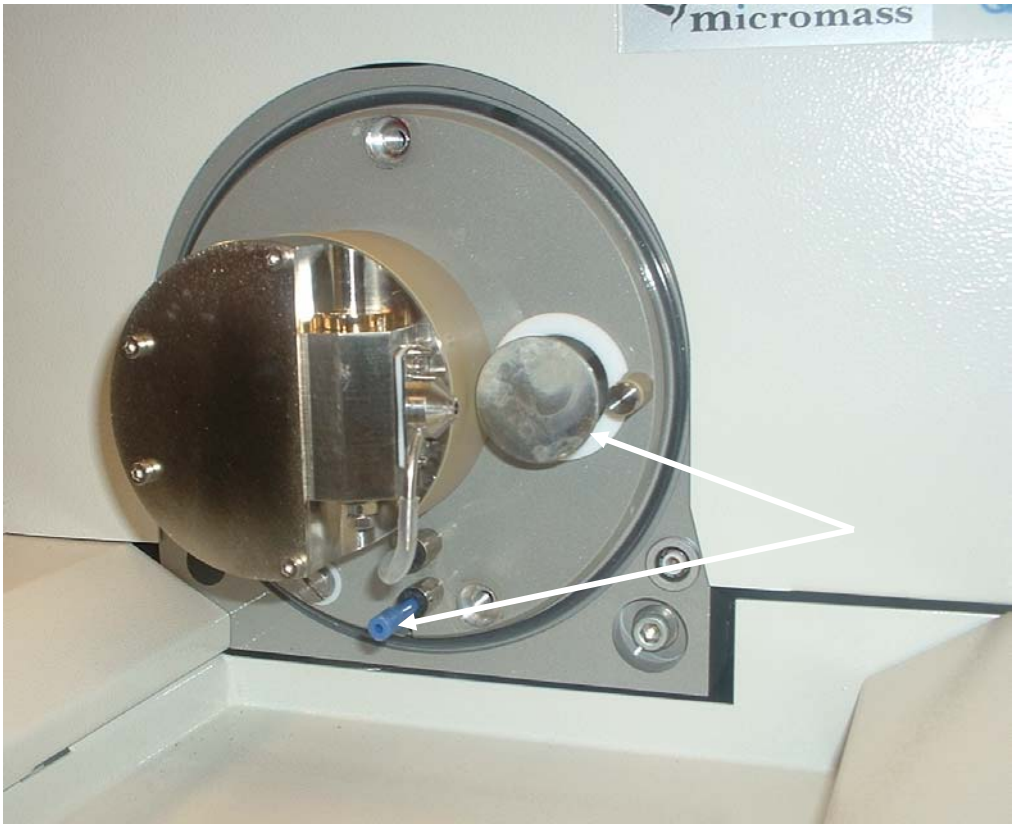


Fig 15. Removal of ESI components

Insert the Screw shown below which was shipped separately with your AP/MALDI Source. Screw it in so it is finger-tight.

Fig 16. Insertion of screw during installation.

Line the source up with the hole being pointed to below.

Make sure this alignment is done with the Target flange in the Open and Locked position (see arrow below).

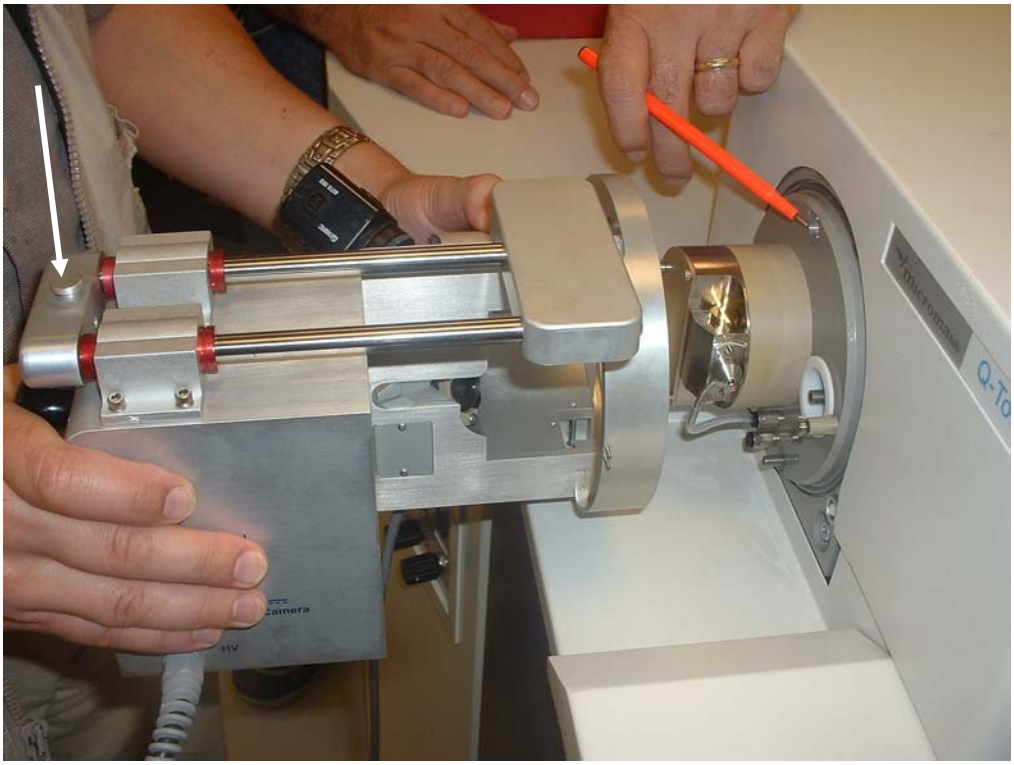


Fig 17. Lining up the Source

Fig 18. Birds-eye view of lining up the Source

Align source so it is flush with the Micromass Q-Tof.

Fig 19. Engaging the Source to the Micromass Q-Tof

Now, screw in the two screws illustrated in Figures 20 and 21.

Fig 20. Screwing in the top screw

Fig 21. Screwing in the left-side screw

Ensure that the source is flush to the Micromass Q-Tof, as shown in the Figure below.

Fig 22. The Source shown flush to the Q-Tof

Attach the CAPILLARY/CORONA cable and PROBES cable to the Micromass Q-Tof as illustrated below.

Fig 23. Attaching the Target Plate HV and Interlock cables from the Source to the Micromass Q-Tof

4.3 Wiring of the Control Unit and the Source:

Ensure that the Power on the Control Unit is OFF until the source is completely wired to it.

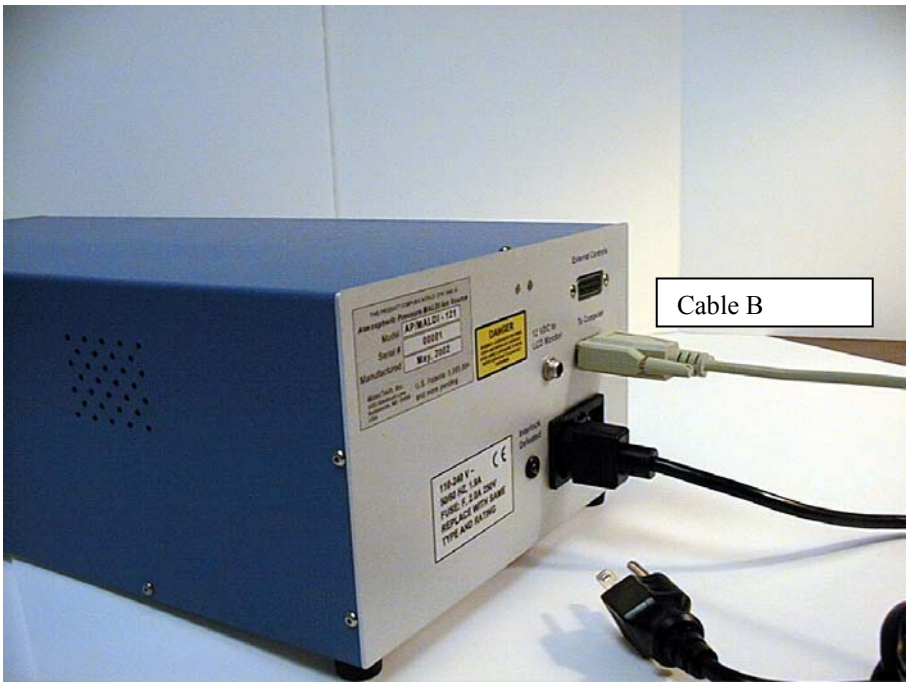
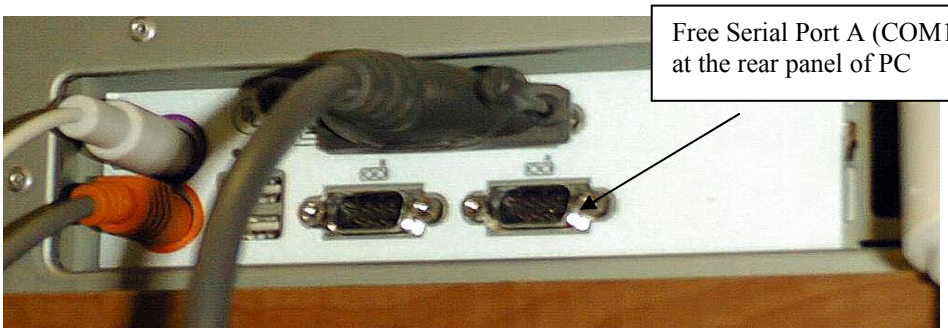
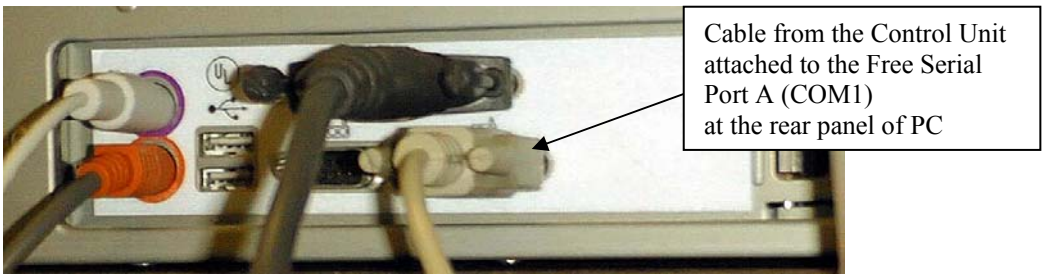


Fig. 24. Connect the black power cord and Cable B to the corresponding connectors at the rear plate of the Control Unit**. No adjustment is necessary for ~110/~127/~220/~240V AC!**

Fig. 25. Connect the other end of Cable B to a free Serial Port on your PC. Either a Q-Tof-instrument computer or another separate PC can be used.





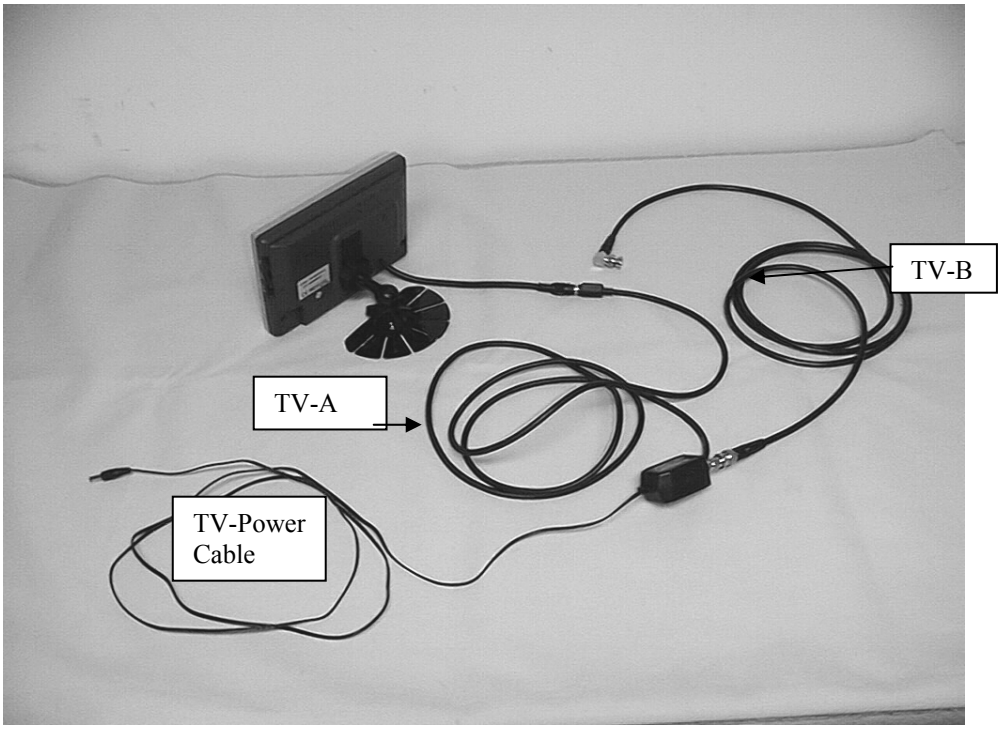
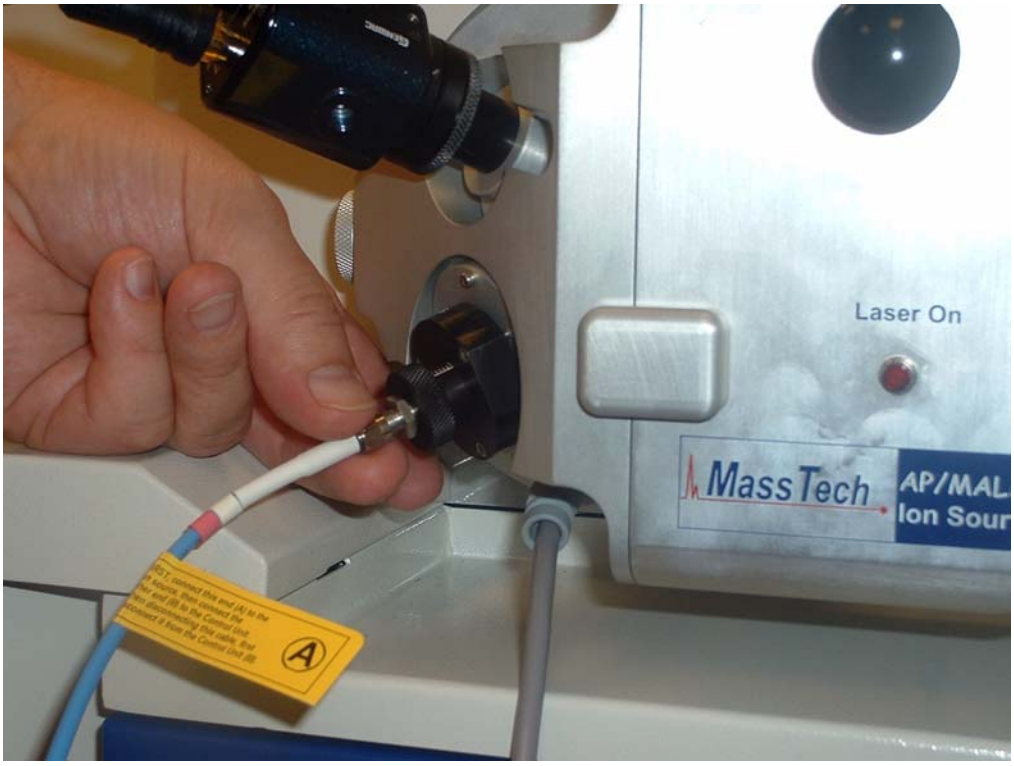


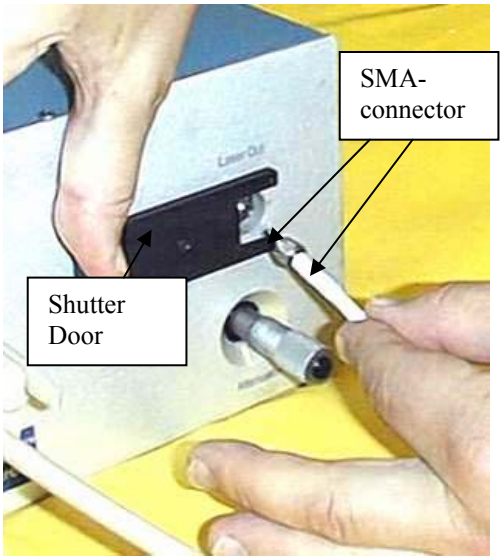
Fig. 26. Connect the TV monitor cables as shown in the picture.



Make sure the Power is shut off while connecting the Optical Fiber

Fig. 27. Connecting the Optical Fiber to the SMA-connector at the Source.

- Remove the red plastic protection cap.
- Attach the optical fiber securely to the SMA-connector at the Source.



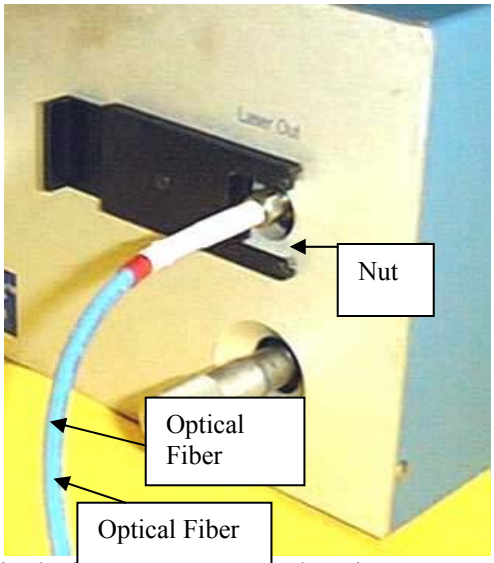


Fig. 28. Connect the other end of the Optical Fiber to the Control Unit:

- Carefully remove the plastic protection tip from the SMA connector. **Do not touch the optical surface of the Fiber with your fingers**. If you did

by mistake, clean the surface with ethanol or methanol, as described in Section 7 of this manual.

- Open the shutter door with one hand; insert the SMA and fix it tightly with the nut according to the picture.

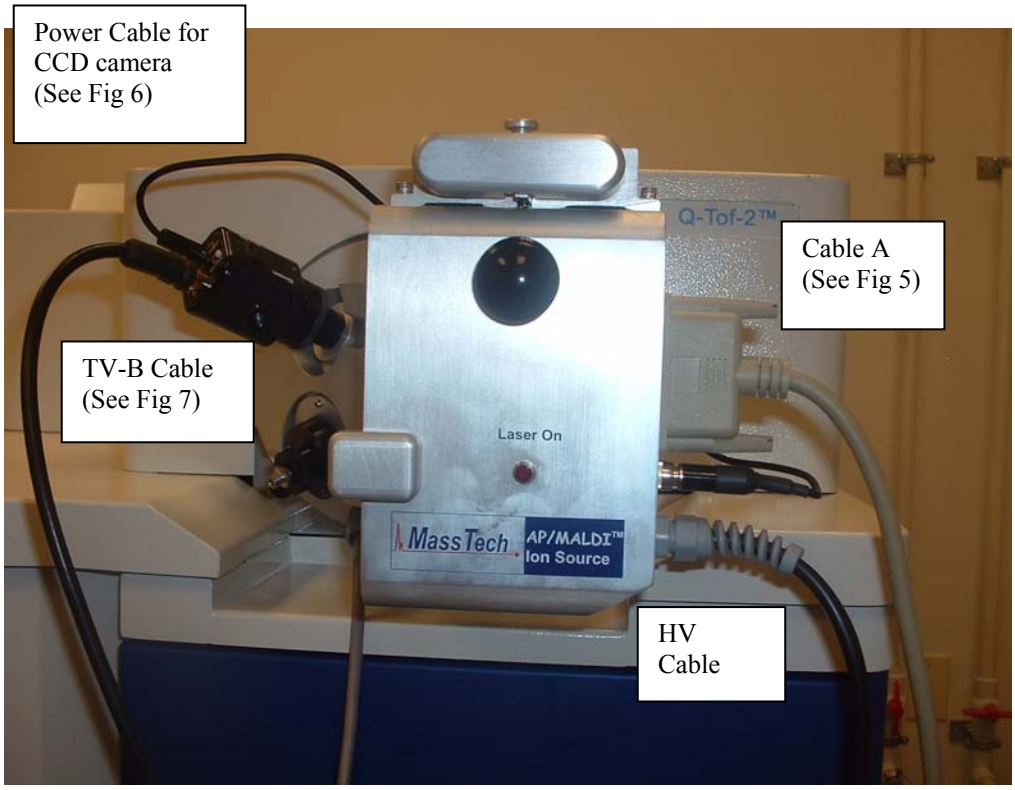


Fig 29. Wire the Source accordingly

Now, attach the wires and cables to the source according to Figure 31 below. The order in which you attach the cables does not matter with this Source.

When complete, your Q-Tof instrument with the AP/MALDI source installed will look like this:

Fig 31. Completed source installation

Before switching on the Power on the Control Unit:

1. Ensure that the CAPILLARY/CORONA cable and PROBES cable are firmly connected
2. Ensure that both ends of the optical fiber are firmly connected

NOW it is safe to turn on the Control Unit.

4.4 Source Disassembly and Uninstallation

When you need to remove the AP/MALDI ion source in order to put another device on the Q-Tof, **First**,

1. Set the Q-Tof instrument to Standby or OFF mode
2. Turn off the Power on the Control Unit

Then, unassemble the source by reversing the installation procedure just described in Section 4.3.

5 SAMPLE PREPARATION

The same sample preparation techniques and the same matrix used for conventional vacuum MALDI can be used successfully for AP/MALDI sample preparation. This procedure was briefly described in the previous section. The main difference is that the crystal size has no direct influence on the spectrum quality. A typical molar ratio of a sample-to-matrix is between 1:100 and 1:10,000.

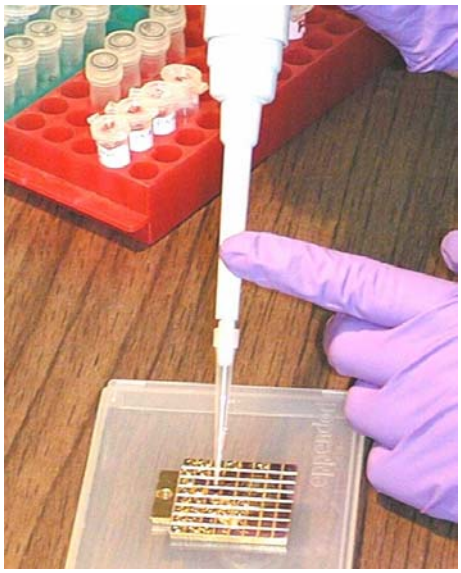


Fig. 32. Prepare several standard samples for testing in the AP/MALDI target plate. The sample preparation procedure is basically the same as for original MALDI experiments.

- Carefully clean the Target Plate surface
- For the standards test, α-Cyano-4-hydroxycinnamic acid (α-CHCA) matrix is recommended
- Mix 1:1 matrix solution and analyte solution of some standard peptides (Angiotensin, Bradykinin, Grammicidin S and/or similar) with a concentration of around 500-1000 fmol/μL.
- Deposit a droplet of 0.5-2 μL of the mixture on the target surface and allow it to dry. (Alternatively, matrix and analyte solutions can be deposited on the target separately and then allowed to dry).

5.1 Loading/Unloading the Target Plate

You need to open the AP/MALDI source to load or unload the target plate. Ensure that the Laser is OFF, the mass spectrometer acquisition stopped, and the Capillary voltage set to zero (0) volts. After that, proceed with loading or unloading of the target plate. Insert the Target Plate with the prepared sample spots into the Target Plate Holder. The Plate is held in place by a magnet.
If by accident you open the source while the Q-Tof instrument is in "Operate" mode (HV is ON) and/or the Laser is firing, the AP/MALDI source safety interlocks automatically switch the High Voltage and the Laser OFF.

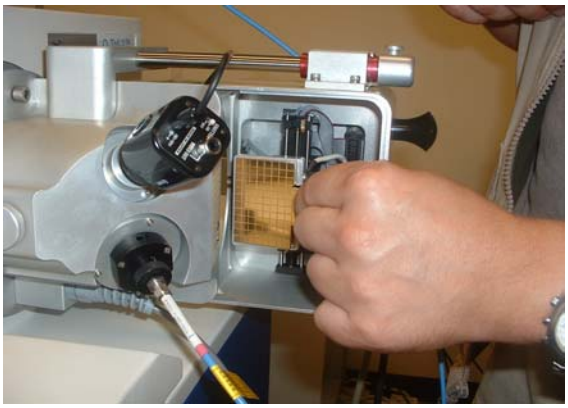


Fig. 33a. Inserting the Target Plate

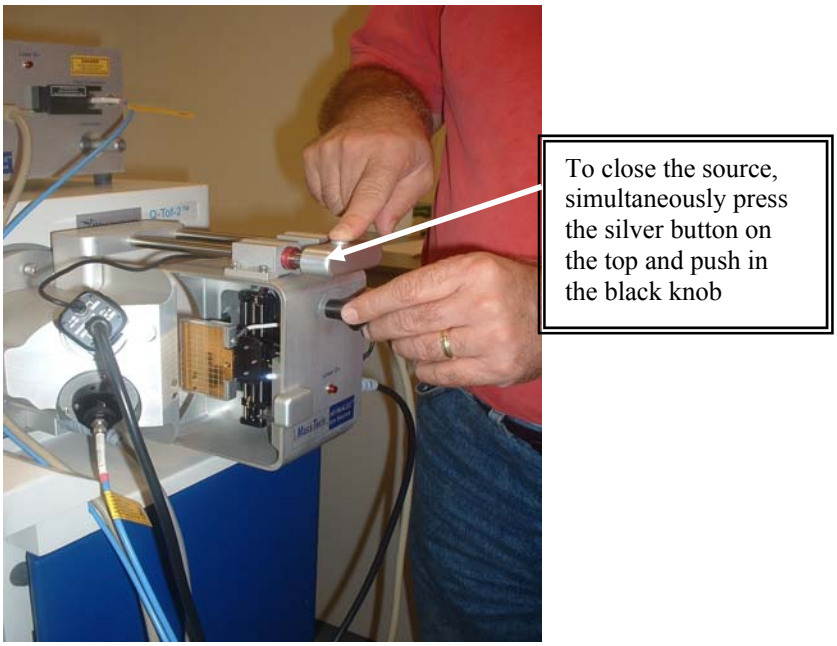


Fig. 33b. Closing the AP/MALDI source

Fig. 34. Push the Source together (see Figure 33b). Plug in the Control Unit and switch it on (rear panel switch) and turn on the TV monitor (at the side).

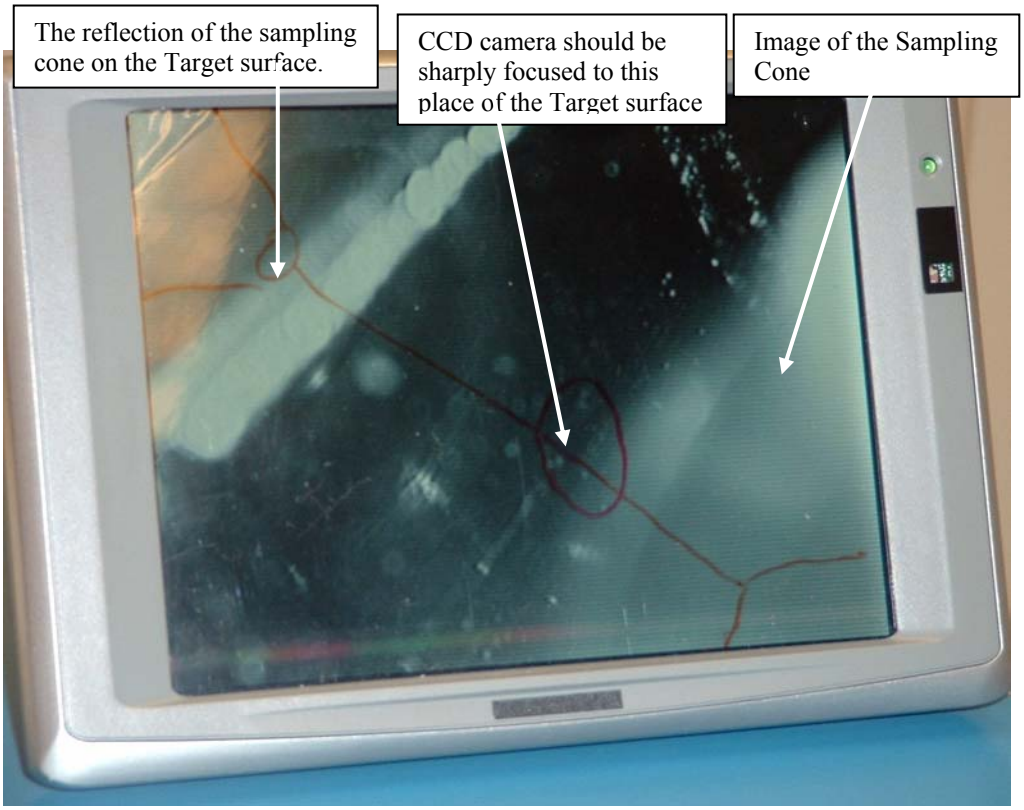


Fig. 35. A typical picture of a blank target surface.

Fig. 36. CCD camera focusing.

This procedure can be safely performed even if the source is ON and the laser is firing.

Ease the Set Screw, move the camera, refasten the Set Screw.

6 AP/MALDI OPERATION

6.1 Using the TARGET *software*

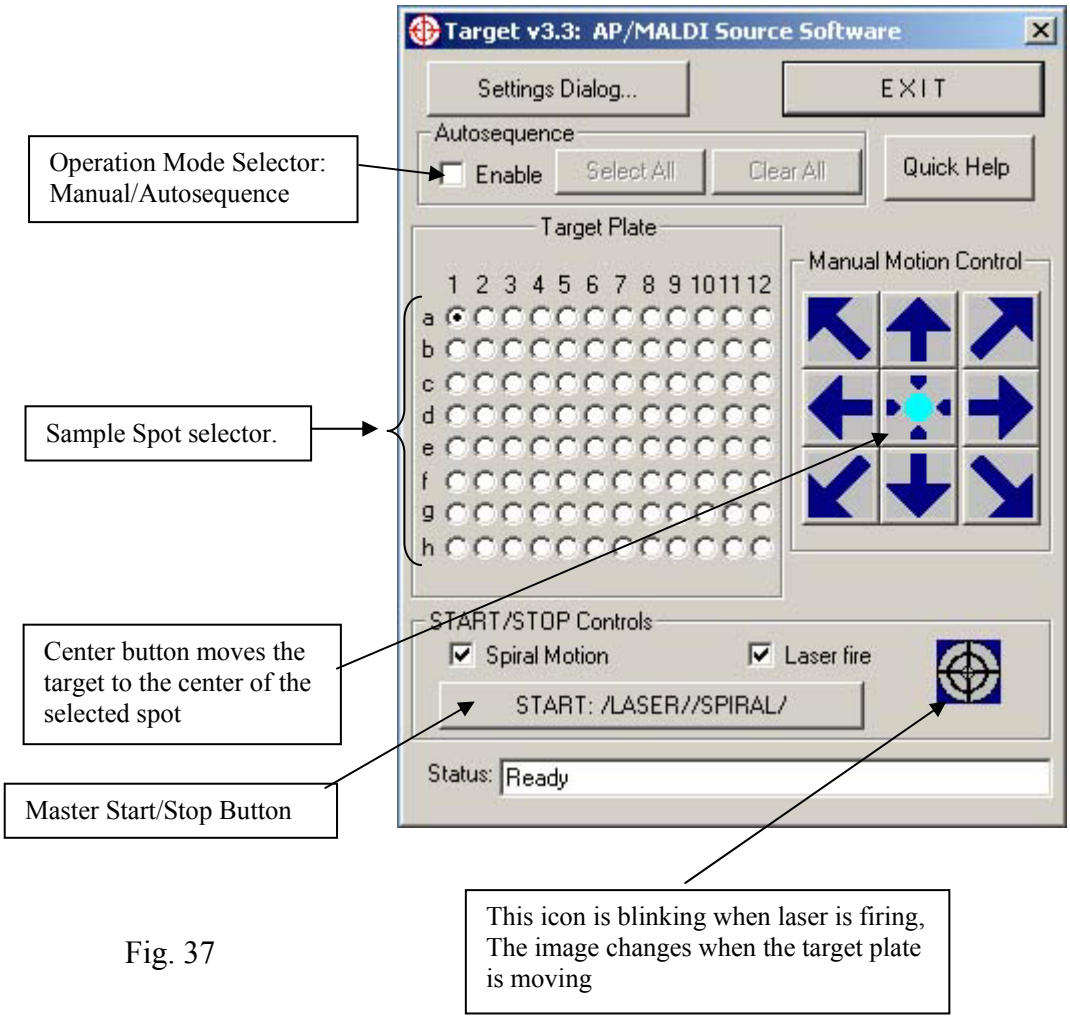


Fig. 37

This software is used to control the AP/MALDI target motion and laser firing.

To install the software for AP/MALDI, follow these steps:
(under Windows NT or 2000 you will need Administrator access)

1. Insert the installation CD and run the Setup.exe program from your CD drive.
2. Chose the desirable location and folder name for the Target software. By default, the folder is: C:\Program Files\MassTech\
3. Answer OK at the next few dialog boxes.

After the installation process is completed, start the Target program in your conventional way. The Target window (Fig.37 above) appears.

At this moment the initialization of the XY stages will start automatically. If everything has been connected properly, you will see the target motion at the TV monitor: for the initialization the target first moves to its most down, then – to most left position, then – to the spot specified by the spot selector. If the Power On indicator on the Control Unit is

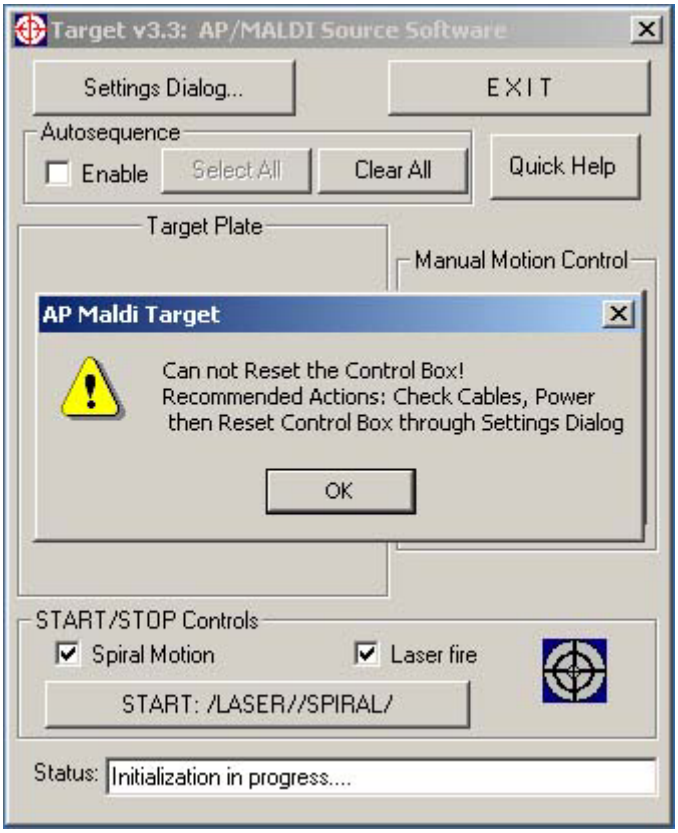


Fig. 38

OFF, or if the Control Unit is not properly wired to the computer, you will get the messages shown in Fig. 38. Click OK if you get these error messages; the stages can be initialized manually later through the "Settings Dialog…", described later.

There are two modes of operation for the Target software: Manual (Enable/Autosequence Check Box is clear) and Autosequence. Switch between the modes by checking/unchecking the "Enable" Box. In manual mode, only one Radio Button of the group "Target plate" can be selected. Click any spot in the Target Plate field and the target plate will move to the selected position. You can shift the position of the spot by clicking the arrow buttons placed around the Center button (See Fig. 37). Click the Center button to restore the central spot position.

In Autosequence mode, multiple samples can be pre-selected. Use the ClearAll/SelectAll Buttons to select/clear all spots. To select all spots, click the first spot, then pressing SHIFT, click the last spot; to select selected spots, press CTRL and click the spots you want to select.

To start actions, press the START button. Depending on what check boxes (Autosequence/Laser Fire/Spiral Motion) are checked, the capture on the START button shows which actions will be activated. To stop ALL activated actions, press the same button (It will be labeled "STOP ALL" at that time).

Note, that even AFTER the actions are started (i.e., START has been pressed), you can manually shift the spot clicking arrow buttons. In Manual Mode ONLY, you can additionally switch the Laser ON/OFF and start/stop spiral motion by checking/uncheching the appropriate Box.

In Autosequence Mode, after the START button is pressed, the target plate moves to the upper left of the selected spots. Then the laser starts firing and the target plate spirals slowly around the initial position (if corresponding check boxes in START/STOP Controls group are marked). After a pre-selected time, all actions stop and the target moves to the next pre-selected spot. Again, the laser starts firing and the target plate spirals slowly around the initial position (if corresponding check boxes in START/STOP Controls group are marked). The process repeats until the last spot is finished (or the STOP button is pressed). The order of sample testing is from left to right in every row, from top to bottom rows. Additional time delays can be introduced between the samples and between the rows.

To change various program parameters like manual step, spiral motion, laser frequency, Automatic Mode timing and so on, click the "Settings Dialog..." button and edit the parameter(s) as it is shown in Fig. 39, below.

You can initialize the Source and Control Unit by clicking "Reset" button

Tune Position Offset (X,Y) if the Center position is not physically at the center of a target spot

Choose the Target Plate Type:
Standard (During the initialization either 8x8 or 8x12 option will be autodetected);
DIOS 10x10 chip of Mass Consortium Corporation (to use the chip you need appropriate frame);
User-defined Plate enables you to choose the spot geometry

Tune the spiral speed, spiral spacing & max R for the best results

Tune the autosequence timing in this field.
Desorption time = time at every spot.

This box can be checked ONLY if Q-Tof instrument controls the timing through a Peripheral Control port. A special communication cable should connect the Control Unit and Peripheral Port. MassLynx Q-Tof program must be configured

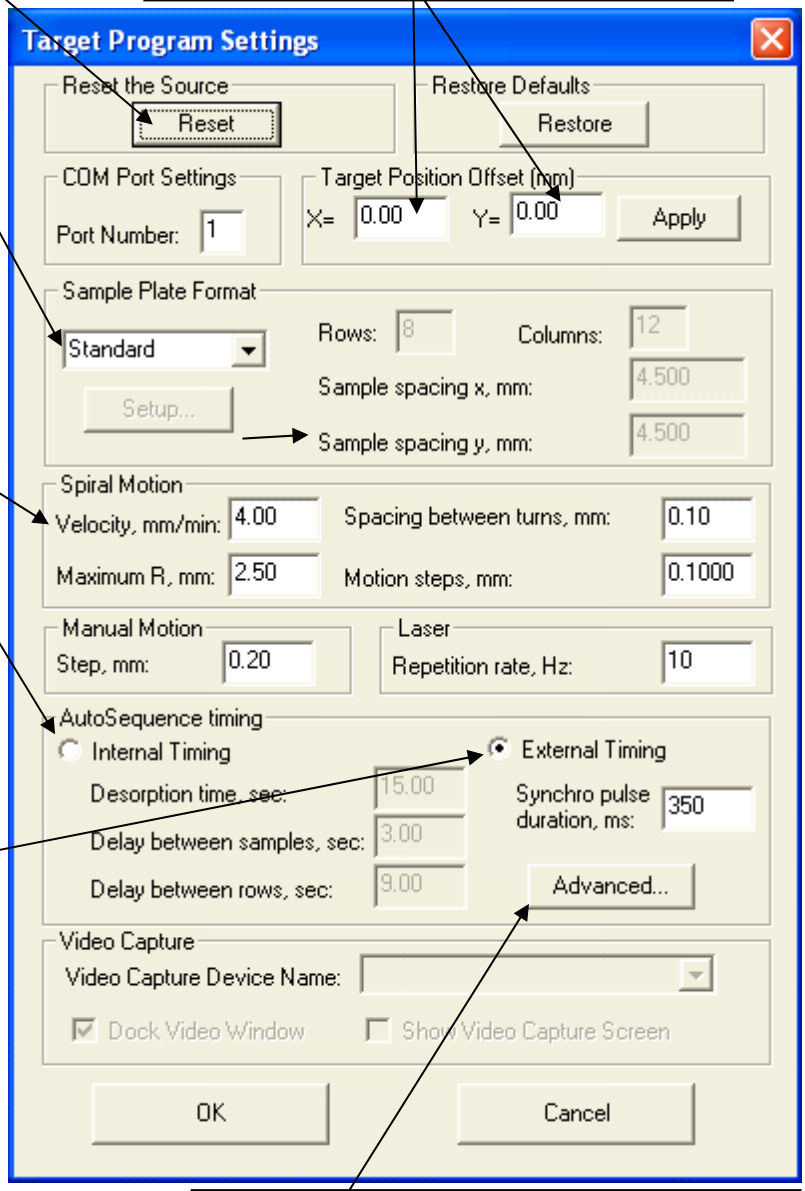
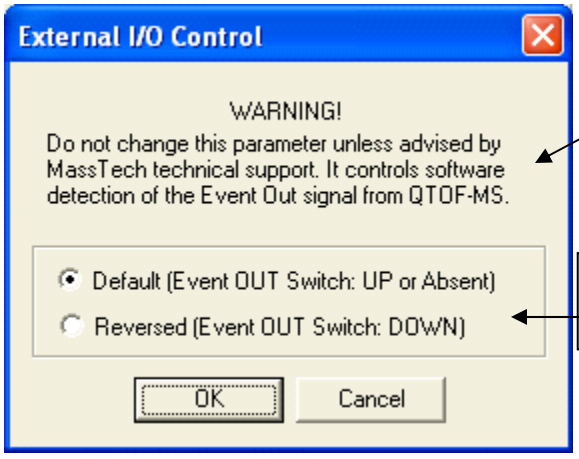


Fig. 39



This button allows manipulation of the external I/O controls in the software, and should only be used under the advisement of MassTech.

The setting should correspond to the hardware setup described in Section 6.5 (Figs 44A and 44B).

After you have finished hardware/software installation and sample preparation, everything is ready to run the Q-Tof instrument in AP/MALDI mode.

6.2 Running AP/MALDI on the Q-Tof instrument.

6.2.1 Setting the Q-Tof Parameters in MassLynx

To run AP/MALDI on the Q-Tof instrument optimally, the following tuning procedure of the MassLynx program is recommended:

- Autotuning the instrument in ESI mode before switching the source to AP/MALDI and saving the corresponding file is a good idea. See the MassLynx Operator's Manual.

- The following Q-Tof settings work well with AP/MALDI:

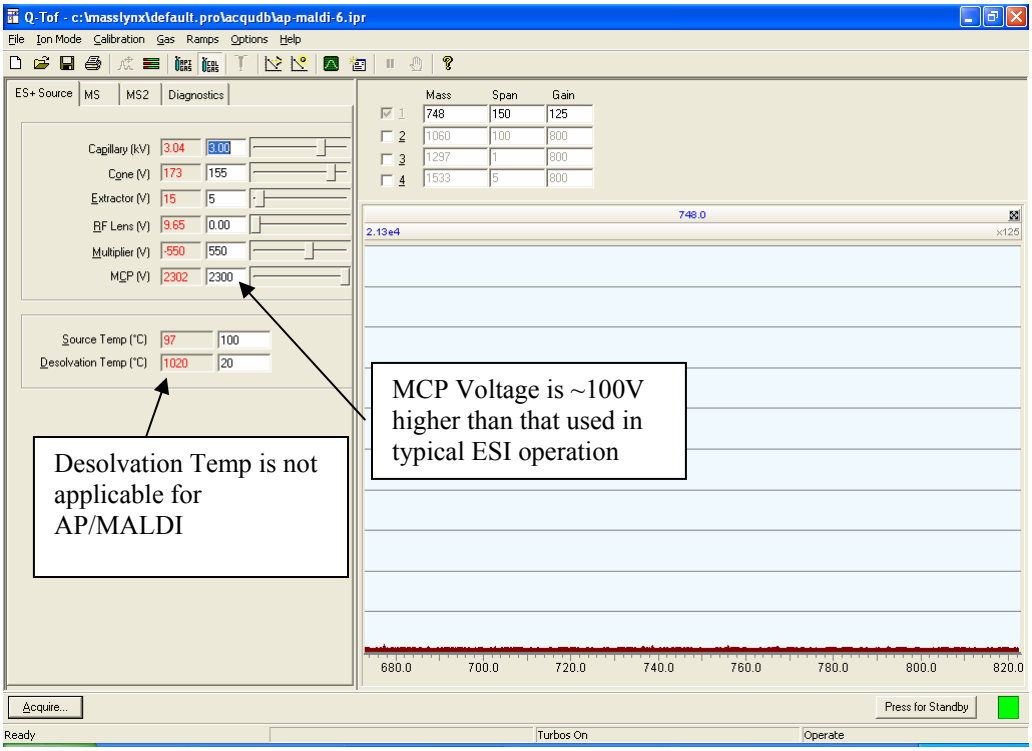


Fig. 40. MS Tune page in MassLynx with ES+Source tab showing

- The desolvation temperature in FIG. 40 is not meaningful in AP/MALDI operation, and thus correctly shows a value of >1000°C to indicate it is not functional. Notice that in FIG. 40, the capillary voltage is set to 3kV, and the MCP voltage is set to 2300V (higher MCP voltage is beneficial for AP/MALDI over ESI).

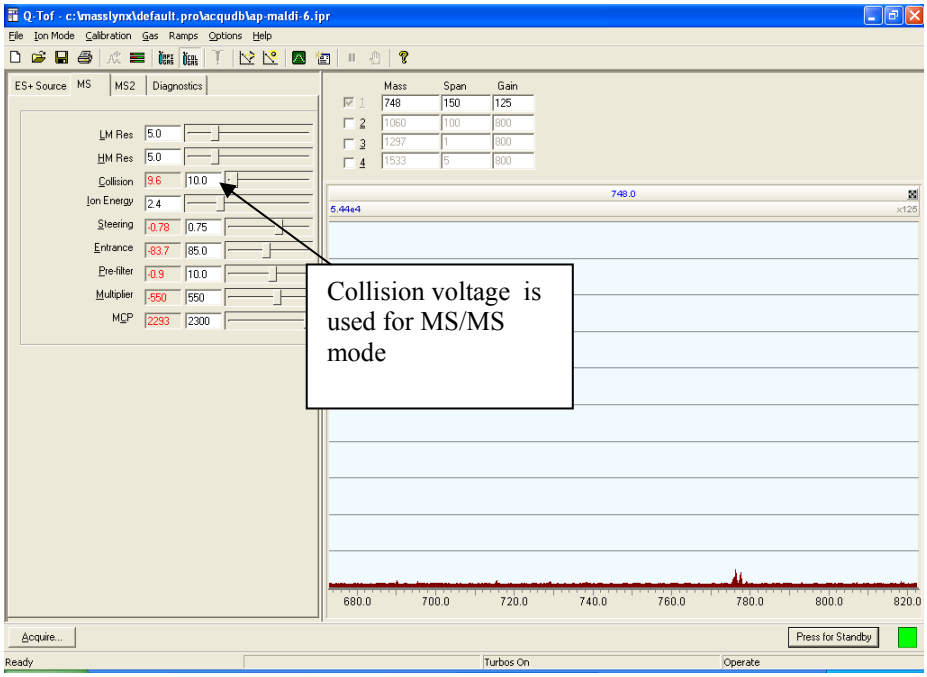


Fig. 41. **MS Tune page in MassLynx with MS tab showing**

- The Collision voltage in FIG. 41 is used in tandem MS/MS. Voltages higher than that typically used in ESI are required to break down the singly-charged MALDI ions. The Entrance voltage in FIG. 41 is set to 85V (higher Entrance voltage is beneficial for AP/MALDI over ESI).

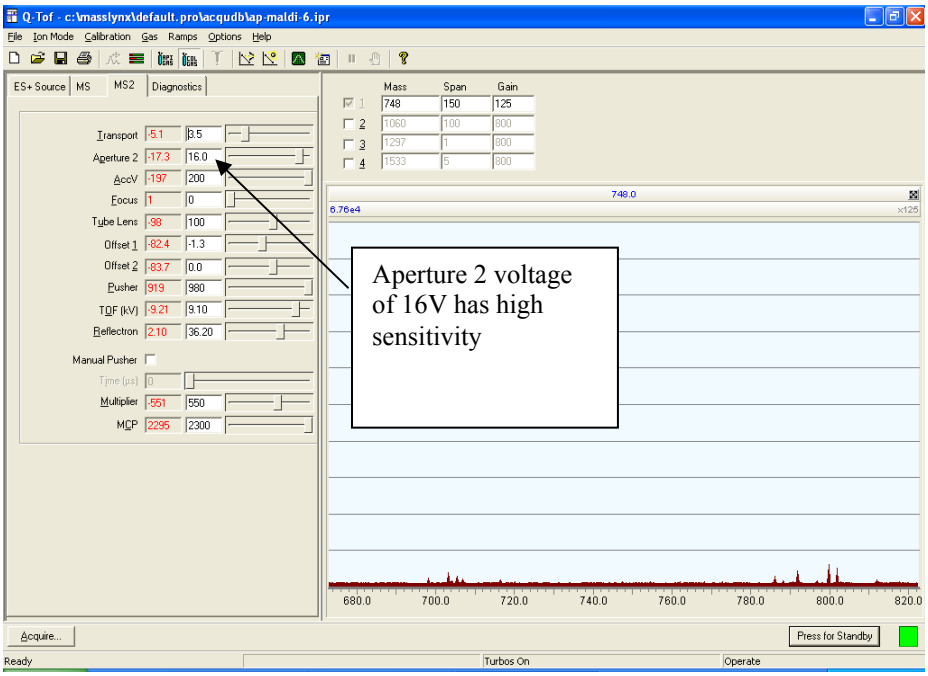


FIG. 42. MS Tune page in MassLynx with MS2 tab showing

- The Aperture 2 voltage has a significant influence on sensitivity of the AP/MALDI with the Q-Tof instrument. At a recommended setting of 16 V, higher sensitivities are realized (higher Aperture 2 voltage is beneficial for AP/MALDI over ESI).

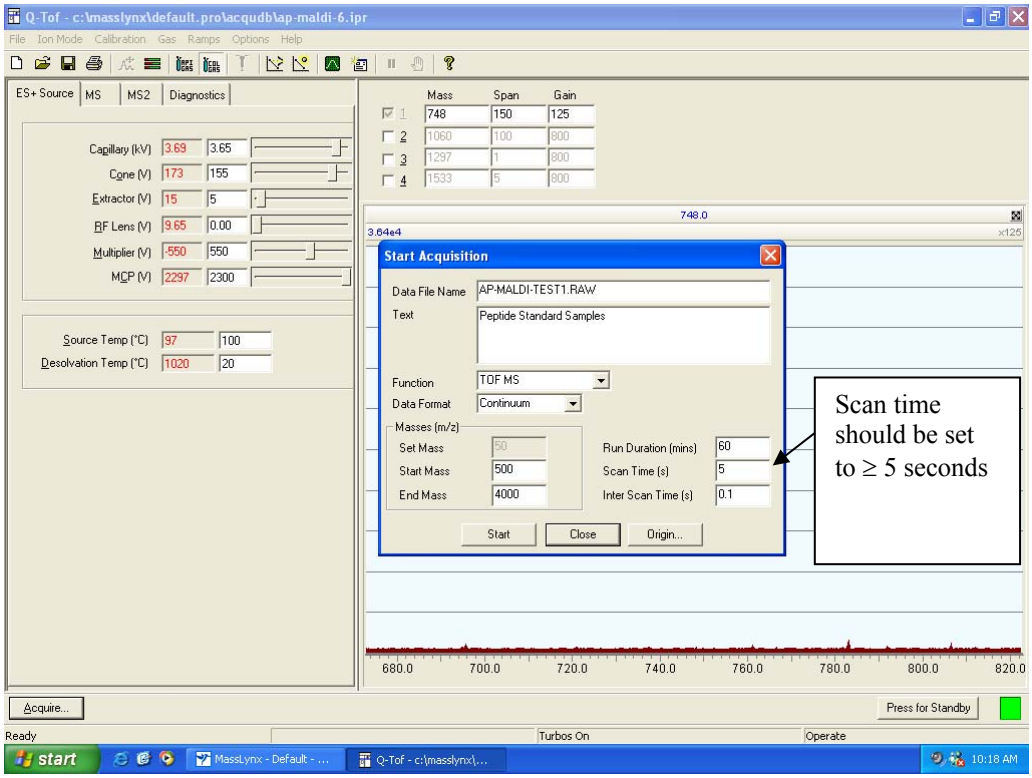


FIG. 43 MS Tune page in MassLynx with Start Acquisition dialog box showing. Scan Time should be set to 5 seconds to see discernable mass peaks during real-time updates.

- The AP/MALDI source typically generates a much weaker ion current compared with Electrospray. So it is recommended that you increase the Scan Time to 5s or more (see Fig. 43 above) to see a reasonable spectrum during real-time spectral updating.

- Laser pulse energy may be easily tuned at the front panel of the Control Unit by using the Attenuator handle (see Fig. 2a). This handle has a scale; its position can vary from 1-3 to approximately 12 (mm). The rotation of the handle changes the position of the lens that focuses the laser beam to a fiber surface. 12(mm) corresponds to complete focusing conditions (that is, maximum pulse energy). Lens motion is limited to approx. 12(mm) to avoid fiber surface damage. Typically you should tune the attenuation for the maximum signal only once for every matrix type (α-CHCA, DHB and so on). For α-CHCA, typical attenuator settings are 5-8mm.

- The final recommendation is how to choose between manual and spiral target motion control in the ***TARGET*** program. Typically, the signal from one spot deteriorates in 5-20 seconds (depending on the matrix, sample preparation, and laser attenuation). The target can be shifted manually to another spot within the same sample; but manual target motion will produce an unstable signal. If you need a long and stable

signal, start the laser firing and then start the spiral target motion. This mode will enable you to expose the fresh sample spot parts to the laser irradiation continuously. Spiral motion will give you a stable AP/MALDI signal for 10-20 minutes. It is enough for MS and MS/MS experiments.

- Fig. 44 represents the operation of both MassLynx and AP/MALDI simultaneously on the same computer. You can easily switch between the *MassLynx* and *TARGET* programs. Or alternatively, separate computers can be used to run *TARGET* software and operate AP/MALDI. *TARGET* software is an independent, stand-alone user interface.

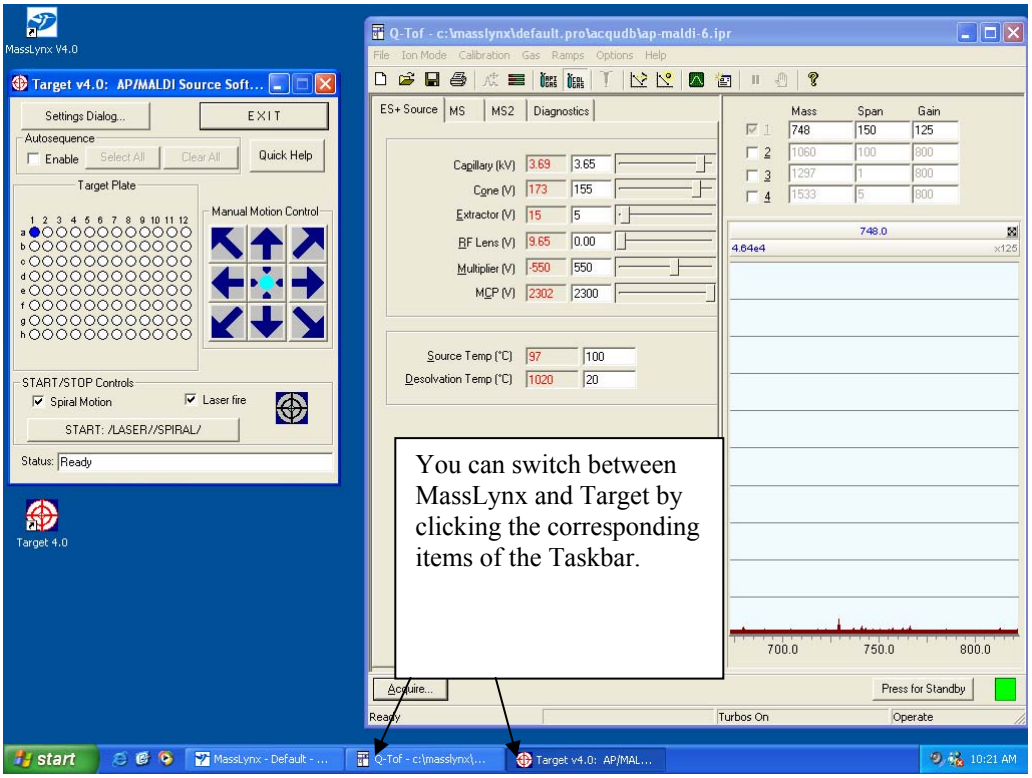


Fig. 44. Operation of both AP/MALDI Target software and MassLynx

6.3 Manual Mode of Operation

Manual control means that you control the data acquisition in an interactive real-time manner. Most of the acquisition parameters can be accessed and changed during the data acquisition using the *MassLynx* and *TARGET* features. The data acquisition of the *MassLynx* program is started independently from the target position and laser control for the *TARGET* software. The spectra acquired will depend on what sample is currently selected and what parameters (like laser frequency and energy, speed of motion of the target plate accessible via *TARGET* software, *or* voltage on the target plate, hexapole and ion optics voltages, etc. accessible via *MassLynx*). Saving the spectral data is your responsibility and is done using appropriate *MassLynx* functions.

The procedure for operating in manual mode consists of several basic steps:

1. *Uncheck* the "Autosequence-Enable" check box in the *TARGET* software window (see Fig. 43).

2. Start data acquisition using the *MassLynx* software (see the previous *Setting Q-Tof Parameters* section in this manual or Micromass's *MassLynx* software manual for details).

3. Set desired *TARGET* settings (using the Settings Dialog window). Set the desired laser energy (using the micrometer knob on the Control Unit front panel), check the "Laser Fire" check box and "Spiral Motion" Control Unit (if desired).

4. Click on the desired sample using the sample spot selector (map) provided in the *TARGET* software window (see Fig. 37). The target plate will move to this sample position and stop near its center (this is observable on the LCD monitor screen).

5. Press the START button in the *TARGET* software window to start AP/MALDI operation.

6. Adjust the desired laser energy (using the micrometer knob on the Control Unit front panel), or position the laser spot on the sample (using the "Manual Motion Control" arrow buttons in the *TARGET* software window while observing the sample on the LCD monitor screen).

7. Press the STOP button in the *TARGET* software window to stop AP/MALDI operation.

8. Stop data acquisition on the Q-Tof.

6.4 Automated Mode of Operation - writing the data from all samples into a single file

In this mode of operation the data are acquired in automated (*unattended*) mode by you selecting a sample pattern on the sample map in the *TARGET* software window which then moves the target plate sequentially from one sample to another sample according to the sample map you've selected. All data are recorded into a single file which Q-Tof's MassLynx software saves automatically.

The laser is temporarily turned off by *TARGET* software while the target plate moves from one sample position to another one. This allows you to distinguish the data acquired from different samples since turning off of the laser cuts off all ions and creates notches on the data acquisition chromatogram separating the data from different samples. As in the manual mode of operation the data acquisition in this mode using Q-Tof's *MassLynx* software is started independently from the *TARGET* software. Synchronization of the start of the data acquisition and the start of the *TARGET* operation is your responsibility. This synchronization is important for subsequent assignment of the acquired data (in the chromatogram) to different samples on the target plate.

This is the procedure for operating in the automated mode, writing to a single file:

1. **Check** the "Autosequence-Enable" check box in the main *TARGET* software window (see Fig. 37) and **check** the "Internal Timing" radio button in the *TARGET*'s "Settings Dialog" window (see Fig. 39).

2. Set the "AutoSequence Timing" parameters in the *TARGET*'s "Settings Dialog" window (see Fig. 39): "Desorption time" – time for laser firing per sample; "Delay between samples" – time for moving to another sample position; "Delay between rows" – time for moving between samples in different rows (it is recommended that you set extra time for moving from one row to another row – this simplifies sample data assignment in the chromatogram after completion of the data acquisition). All times are specified in seconds.

3. Set other *TARGET* settings (using the Settings Dialog window). Set the desired laser energy (using the micrometer knob on the Control Unit front panel); check the "Laser Fire" check box and "Spiral Motion" Control Unit (if desired).

4. Select the desired position(s) on the sample spot selector (map) in the main *TARGET* software window by first using the "Clear All" or "Select All" buttons in the *TARGET* software window and then depressing the Shift or Ctrl keyboard buttons to select spots by clicking on the sample map. (Selecting sample spots is similar to using the mouse for file selection in standard dialogs of the Windows operation system. If the Ctrl button is depressed, then clicking of the mouse button changes the selection to the opposite (to Selected if Not selected and vise versa). If the Shift button is depressed, then clicking of the mouse button will selects a contiguous group of samples). The "Clear All" and "Select All" buttons in the *TARGET* software

window are there for convenience. The selected samples will be executed in the left-to-right order starting from the highest row on the map and then moving to the next lower row.

5. Start data acquisition using the *MassLynx* software (see previous *Setting Q-Tof Parameters* section in this manual and Micromass's *MassLynx* software manual for details) including saving of the acquired data into a file.

6. Press the START button in the *TARGET* software window to start the AP/MALDI operation. The sample positions on the map where the data have been collected are shown by a solid color. The current sample is shown by a blinking color.

7. Stop data acquisition on the Q-Tof when all data acquisition is finished. The *TARGET* software operation is stopped automatically upon completion of sample analysis or can be stopped manually by clicking on the STOP button in the *TARGET* software window (if the interruption of the data acquisition is desired).

6.5 Automated Mode of Operation - writing the data from different samples to separate files

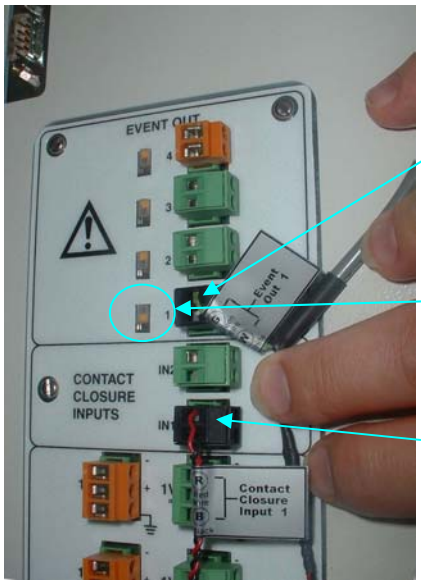
This mode of operation requires a special "External control cable" for connecting the AP/MALDI's "External Control" connector on the Control Unit rear panel with the Q-Tof's "Peripheral Control" connector. Synchronization of the Q-Tof and AP/MALDI source operations is achieved via bi-directional signal communication between the Q-Tof and AP/MALDI control electronics. Like the automated mode described in Section 6.3, in this mode of operation the data are acquired in automated (unattended) mode by you selecting a sample pattern on the sample map in the *TARGET* software window which then moves the target plate sequentially from one sample to another sample according to the sample map you've selected.

However, in contrast to the previous case, all data from different samples are recorded into ***separate*** files which simplifies data processing after acquiring the data. In this mode the *TARGET* software initiates the Q-Tof's data acquisition process and turns on the laser firing; then, the Q-Tof tells the *TARGET* software when it finishes the acquisition of the data from the current sample. The *TARGET* software turns off the laser, moves the plate to the next sample position, and this process starts over again until the last sample is finished. For proper operation in this mode it is important to do things in this order: first start the Q-Tof data acquisition process and then start *TARGET* software operation.

Following is the procedure for operating in the automated mode by saving the data from different samples into separate files:

1. The Automation cable for Q-Tof has one connector on its first side and two connectors on the other side marked with the labels "Event Out 1" and "Contact Closure Inputs, In1". Using one supplied cable, **connect** the "External Controls" connector on the Control Unit's rear panel with the first side of the Automation

Cable. Locate "Event Out" and "Contact Closure Inputs" panels on the rear side of the Q-Tof instrument. Remove any plugs from "Event Out 1" and "Contact Closure Inputs, In 1" connectors if present. Keep the Switch located beside "Event Out 1" in the **UP** position (default) if such a microswitch is present. However, if your AP/MALDI unit was received prior to December, 2003, move the Switch located beside the "Event Out 1" connector to the DOWN position. Plug in the two connectors of Automation Cable according to the labels (see Fig. 44 A and B).



A: The connection of the Automation Cable to the Q-Tof's Rear Panel

Out 1 Connector

Leave this Microswitch (if present) in the default **UP** position, unless you received your AP/MALDI prior to December, 2003, in which case put this Microswitch in **DOWN** position. Software settings in Fig. 39 should correspond

Input 1 Connector

B: The connection of the Automation Cable to the Control Unit Rear Panel

Fig. 44 A and B

2. **Check** the "Autosequence-Enable" check box in the main *TARGET* software window (see Fig. 37) and **check** the "External Timing" check box in the *TARGET*'s "Settings Dialog" window (see Fig. 39).

3. Set other *TARGET* settings (using the Settings Dialog window). Set the desired laser energy (using the micrometer knob on the Control Unit front panel), check the "Laser Fire" check box and "Spiral Motion" check box (if desired).

4. Select desired position(s) on the sample spot selector (map) in the main *TARGET* software window by first using the "Clear All" or "Select All" buttons in the *TARGET* software window and then depressing Shift or Ctrl keyboard buttons and clicking on the sample map. (Selecting sample spots is similar to using the mouse for file selection in standard dialogs of the Windows operating system. If the Ctrl button is depressed, then clicking of the mouse button changes the selection to the opposite (to Selected if Not selected and vise versa). If the Shift button is depressed, then clicking of the mouse button will selects a contiguous group of samples). The "Clear All" or "Select All" buttons in the *TARGET* software window are there for convenience. The selected samples will be executed in the left-to-right order starting from the highest row on the map and then moving to the next lower row.

5. Open the *MassLynx* program. Open its "Instrument Configurations…" Dialog Box through Status->Inlet Method->Tools->Instrument configurations (see Fig. 45 below). Click on the "Events & Triggering" button to open Events and Triggering Wizard as shown in Fig. 46. Click on the "Next" button there. In a "Choose Events" window (see Fig. 47 at the left), check Event In number 1 and Event Out number 1 as shown in Fig. 47 (left). Click on the "Next" and then on "Finish" (see Fig. 47, right). Click on the "Finish" to close the "Inlet Configuration", then close the "Inlet Method" window, too (see Figs. 45-46). When properly configured, the contact closure indicator or the left tool bar should be green, and say "configured" (as shown on figures 45 & 46).

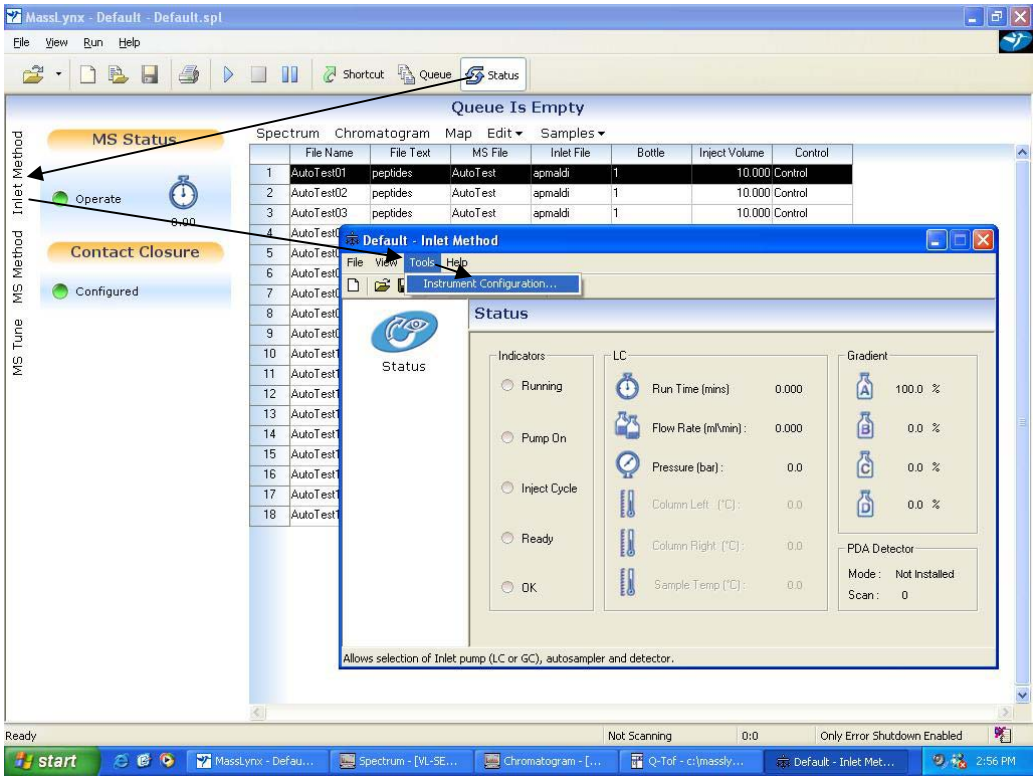


Fig. 45

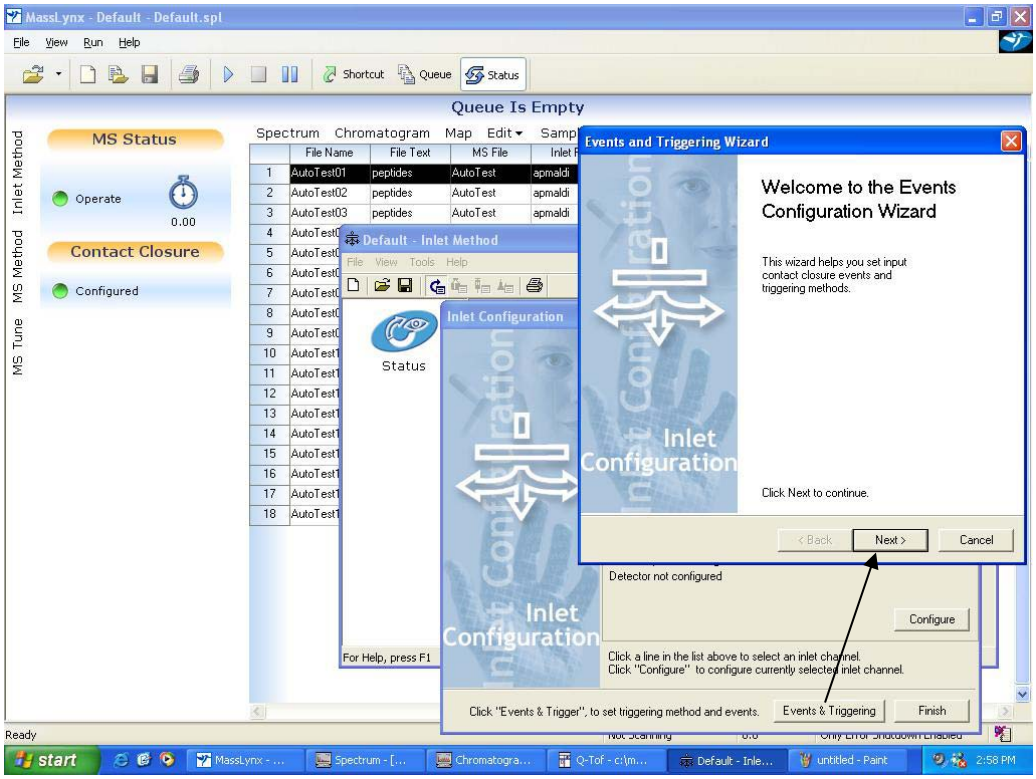


Fig. 46

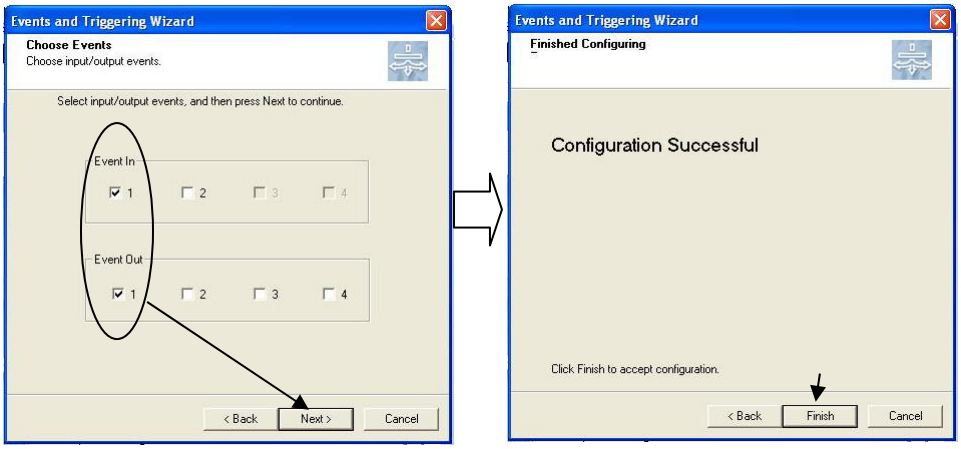


Fig. 47

6. Configure the *MassLynx* to run a desirable number of samples using necessary MS methods and record them using a desirable file names. Check the "Status" button in the *MassLynx* window. Use "MS Method", "Edit" and "Samples" menu items to edit MS method(s) and the peak list (see Fig. 48). The number of samples in the Peak List table (for example, this number is 18 in Fig. 48) should correspond to the total number of samples selected for analysis in the Target program (step 4).

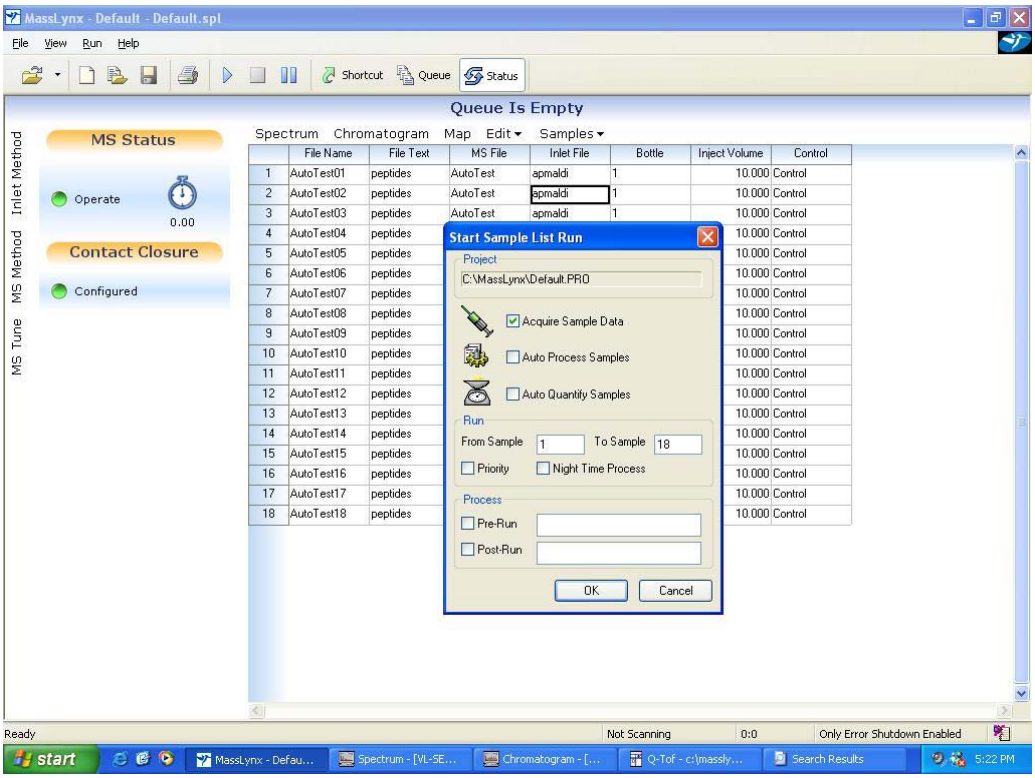


Fig. 48.

The lines in the Sequence table are run sequentially and every line in the table corresponds to some sample selected in step 4 as they are run one-by-one. Please use the *MassLynx* Software Manual for more details on the creation and editing of the Sample List table. The files where the acquired data will be saved are described in the File Name column. The MS Method used for each analysis is defined in the MS File column and can be accessed through he "MS Method" tab.

7. Go to the *Run->Start* to open the Start Sample List Run window, or click "►" button at the toolbar (see Fig. 48).

   Make sure that the appropriate number of samples are assigned for the run (check the fields "From Sample" and "To Sample". The check box "Acquire Sample Data" is to be checked (see Fig. 48). Click on the "OK" button and wait for the Status Bar message "Wait for the inlet start 1" to appear (see Fig. 49)

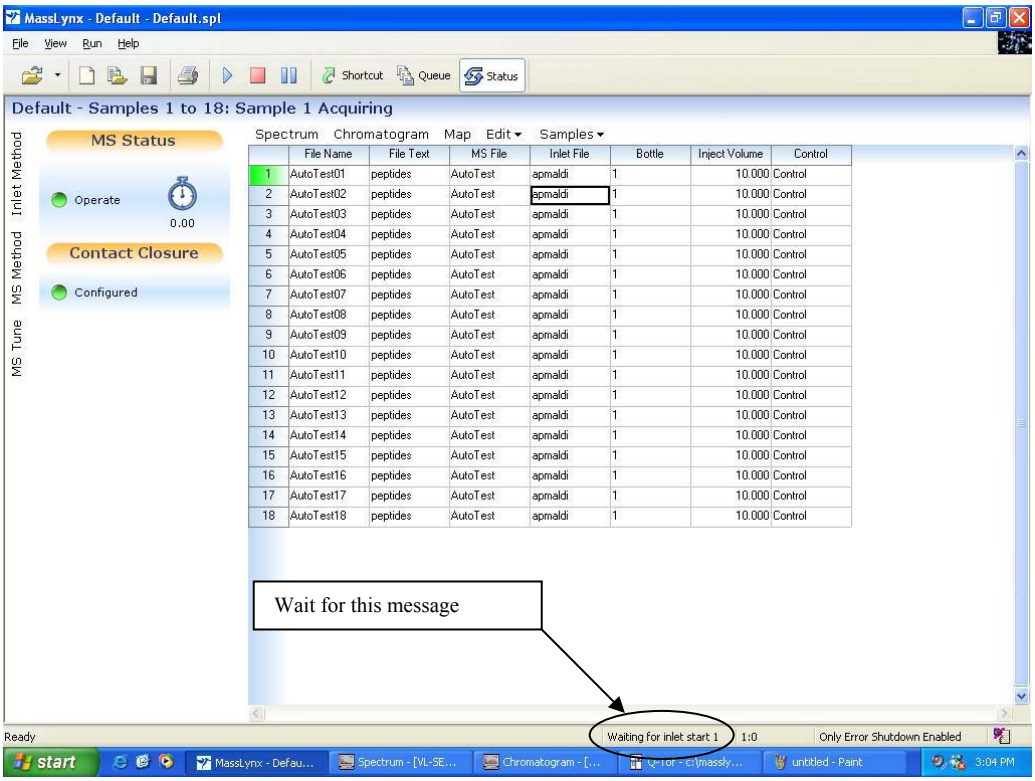


Fig. 49.

8. After the "Wait for the inlet start 1" message is displayed in the Status bar of the *MassLynx* window (See Fig. 49), press the START button in the *TARGET* software window to start AP/MALDI operation. The "Acquiring…" message will be displayed in the *MassLynx* status line which later again will be replaced by the "Wait for the inlet start 1" message when the data acquisition from the first sample is completed. This process will be repeated until the last sample is analyzed. The sample positions on the map where the data have been collected are shown by a solid color. The current sample is shown by a blinking color.

9. The data acquisition process will stop automatically on the Q-Tof and AP/MALDI after data acquisition is complete. The *TARGET* software operation can also be stopped manually by clicking on the STOP button in the *TARGET* software window (if the interruption of the data acquisition is desired) and the Run->STOP menu item or "■" toolbar button in the *MassLynx* program window.

7 MAINTENANCE —TROUBLESHOOTING THE SOURCE.

Maintenance and troubleshooting: The AP/MALDI source does not require regular maintenance, except for the cleaning of the optical fiber cable ends every six weeks. (Section 7.5, Method 4 of this manual describes a cleaning procedure). Please refer to Section 4.3 of this manual for instructions about connecting and disconnecting the optical fiber. It is strongly recommended that you follow the troubleshooting procedures that are described below.

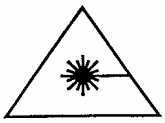
DO NOT ATTEMPT services or repairs that are not covered in this Troubleshooting section. For services and repairs beyond those specifically provided in the Troubleshooting section, contact the manufacturer, MassTech, Inc. 6992 Columbia Gateway Dr; Columbia, MD 21046; (443) 539-1758.

The AP/MALDI source is supplied completely tuned and ready for operation. Still there are several reasons why the MS signal might decrease significantly or even disappear at times. The following sections describe possible symptoms with their remedies

Remember: any contamination of the optical fiber's opened ends results in irreversible fiber damage during the source operation. Get in the habit of putting the protective plastic caps back on the optical fiber ends immediately after you disconnect the optical fiber from the source and Control Unit. If by accident you touch (or contaminate) the opened ends of the fiber, clean it according to the procedure in Section 7.5.

7.1 PROBLEM: Insufficient ion production - lack of laser power being delivered to the target spot.

1. To test for a lack of laser power hitting the target spot, prepare several target spots with a dense α -CHCA matrix. (α-CHCA provides the brightest fluorescence and the lowest pulse energy necessary.)
2. Set the attenuation to full laser power. (e.g., 10-11mm).
3. Fire the laser and watch the TV screen.
4. If you can see a blinking spot on the TV screen, see if the matrix crystals at that spot are disappearing. (For α -CHCA matrix without the beam attenuation the crystals should disappear in 5-15 seconds). If they disappear, then laser power is sufficient.
5. If they don't disappear in 5-15 seconds at the blinking spot, then laser power is NOT sufficient.
6. If the laser power is NOT sufficient, you have three options
   i. Try another optical fiber (one spare was shipped with your unit).



IMPORTANT: If you choose to replace the optical fiber, turn the power OFF on the Control Unit.

ii. Try to improve the focus of the laser beam on the target. To do this, attempt to adjust the position of the source fiber connector with respect to the source housing as described in Section 7.2

iii. If this does not help, call MassTech for assistance.

7.2 PROBLEM: The laser beam is not well-focused

1. Locate the allen screw on the fiber optic mounting connector, as shown in the photo below.

Fig 50. Adjusting the laser focus

2. Loosen the screw; notice that the fiber optic cable can now be moved up and down.

3. Push the fiber down into the connector.

4. Disable the spiral so that the laser light strikes the same spot each time.

5. Start the laser firing at maximum power.

6. Using the camera, you will be able to see how fast the matrix desorbs.

7. If the spot does not desorb quickly, pull the fiber optic cable up a millimeter and repeat the experiment.

8. Once you see that the matrix is desorbed in less than a minute, screw in the allen screw to relock the position of the fiber optic cable.

7.3 PROBLEM: The laser beam focal point at the target plate is not aligned with the sampling cone.

The goal of this procedure is to improve the source's sensitivity by aligning the laser beam focal point at the target plate surface with the Sampling Cone.

Safety: The procedure is performed from outside the source housing with the source closed. The position of the laser beam is monitored on a CCD monitor. As a result, the **procedure is safe** and can be performed with both the Q-Tof instrument and AP/MALDI source switched ON.

Step 1. First, you need to determine if the source is misaligned or not. Prepare several target spots with 1-2 µL of undiluted matrix (it could be either pure matrix solution or matrix/any analyte mixture). After drying, insert the target plate into the source, close it, switch it ON (if it was not switched before) and run the Target software (if this was not done already). Choose any empty (blank) target position. The picture on the CCD screen should look as shown in FIG. 51, with the corresponding cones shown in FIG. 52 for clarity:

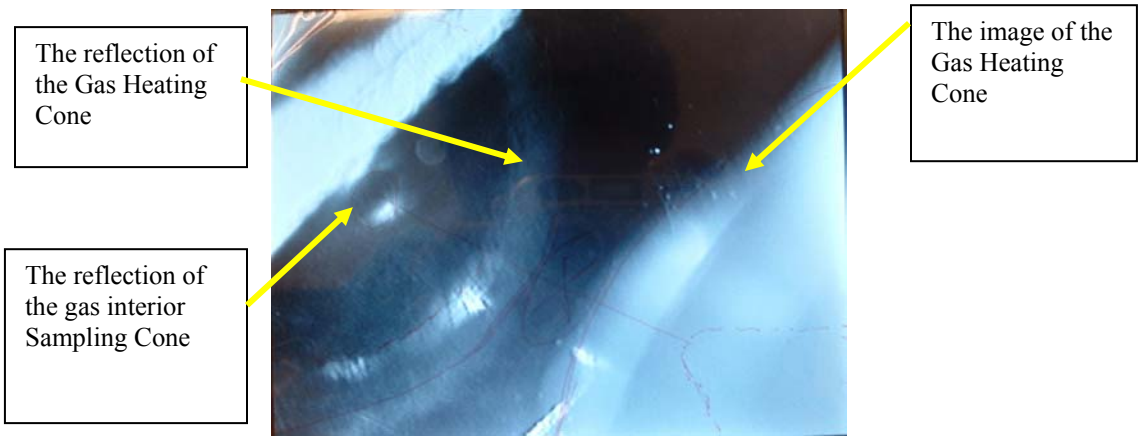


FIG. 51 Image of Gas Heating Cone and its reflection as seen with a blank Target spot on the TV screen

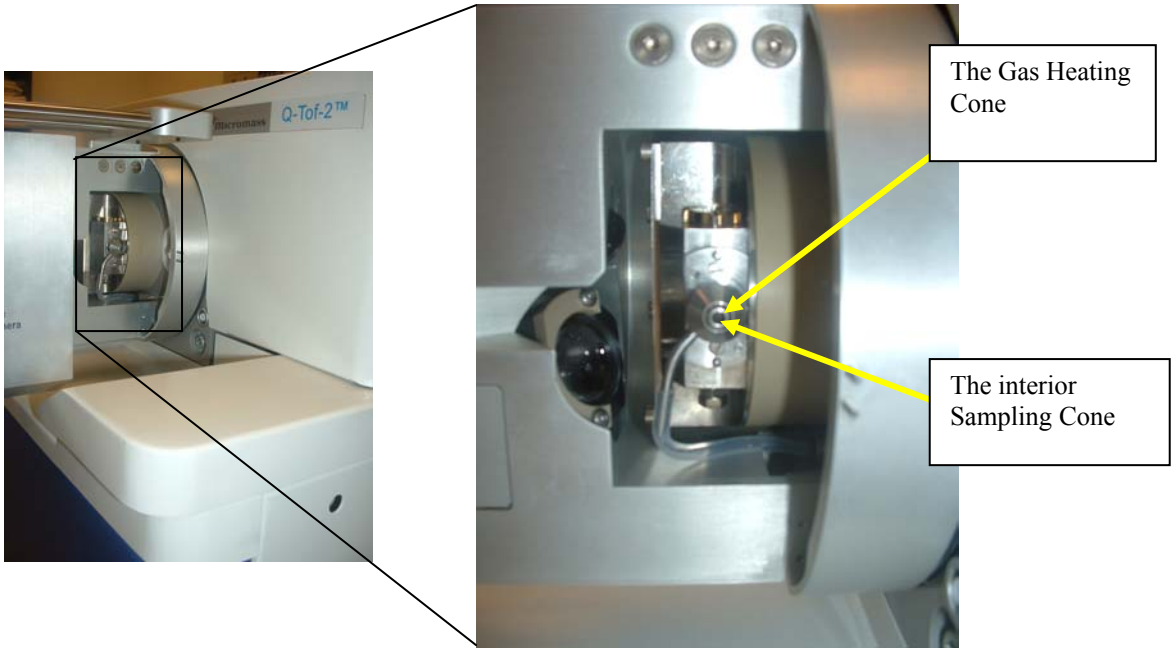


FIG. 52. The Gas Heating Cone and Sampling Cone that are seen on the TV screen in FIG. 51

Both the cone image and its reflection are not well-focused; to ensure that you identify the images correctly, just move the target in any direction with the arrow keys of the Target program. The images of the sampling cone tip and its reflection are still, while the image of the target plate moves:

The **ideal** position for the laser focal spot on the target surface plane is directly in front of the image of the heating gas Cone and in line with the reflection of the interior Sampling Cone. The ideal position is the narrowest position between the Heating Gas Cone and its Reflection (FIG. 54).

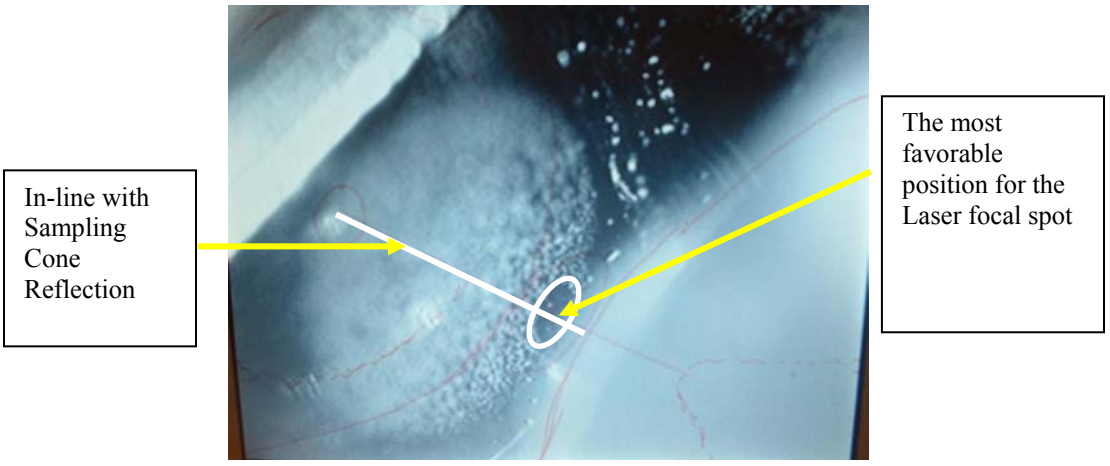


FIG. 53 Image showing the most favorable position for the laser focal spot. The position of the target plate was chosen to show the reflection of the

interior Sampling cone, however, the matrix sample spot is not centered in this position.

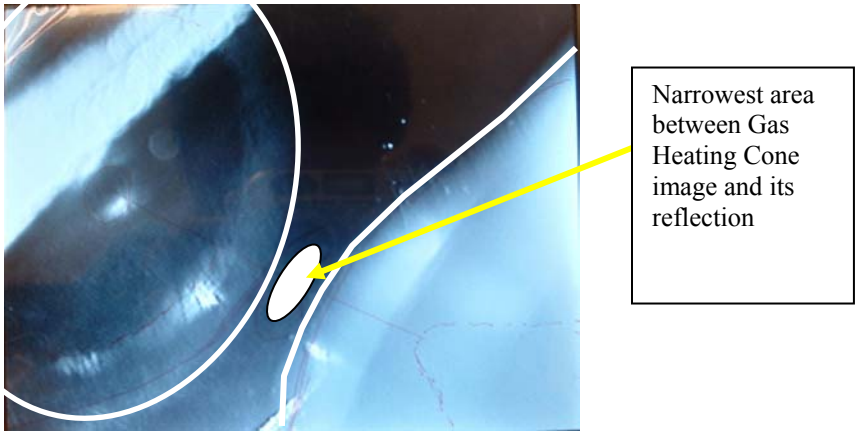


FIG. 54 An outline of the narrowest area between the Gas Heating Cone image and its reflection. This is the ideal position for the laser focal point.

Now we need to determine the **real** position of the laser spot. Move the target plate so that the most favorable laser position is "centered" on a spot with matrix. The picture at the screen should look like the following:

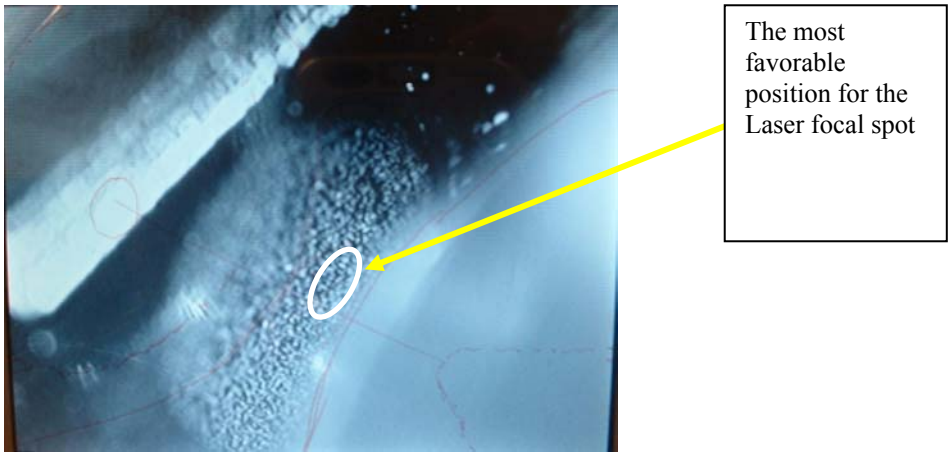


FIG. 55 Picture on the TV screen with the sample spot centered. The obstruction of a portion of the sample by the image of the Heating Gas Cone is correct.

Step 2. Switch ON the laser with spiral motion OFF. Set the maximum laser power with attenuator screw. Now you should see the matrix crystals evaporate at the place where the laser beam is focused:

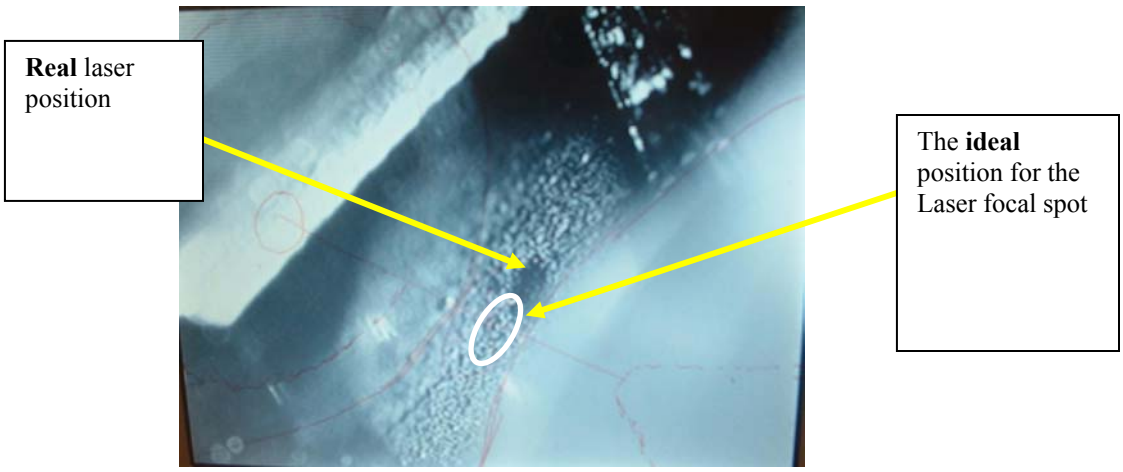


FIG. 56 Image of burn mark on an α-CHCA matrix, with the laser focused away the optimal location.

By comparing Figures 55, and 56 we can see that the laser focus is close to its ideal position, but slightly to the right and up. The deviation of the focal point will likely produce ion signal, however, fine tuning can be attempted to improve sensitivity.

Step 3. Move the target plate to a fresh spot, like in Step 1 (Fig. 55). Switch ON the laser at maximum power (minimum attenuation). Using a hexagonal screwdriver 3/32" turn the allen screws to try to improve the position (see Fig. 57).

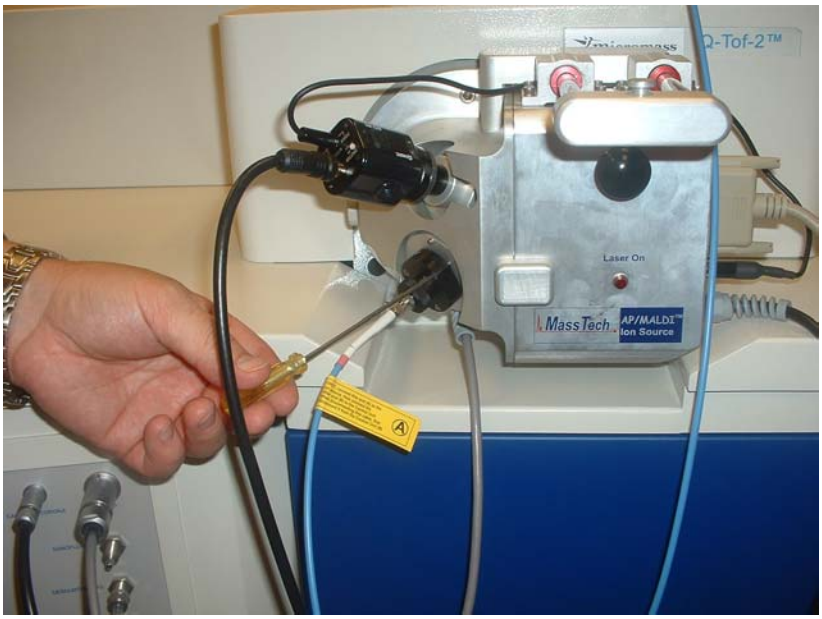


FIG. 57 Adjustment of the laser focal position using external screws. Look at the CCD screen for the corresponding motion of the laser focal spot. Your objective is to move that spot as close as possible to its ideal position (see Fig. 55). For example, the position in Fig. 58 below is a good position.

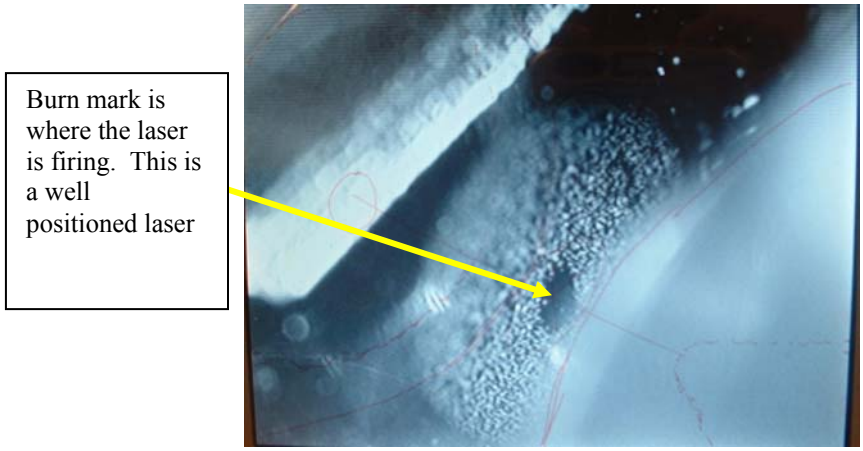


FIG. 58. Well positioned laser focus.

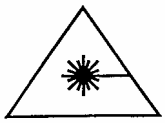
Now you can set the best attenuation, appropriate for the matrix you use, shift the target to a sample standard spot and prove that the sensitivity is better. Alternatively, the position of the laser focal spot can be adjusted by a rotation of tuning screws (Fig. 57) based on the quality of MS signal by a trail-and-error method.

7.4 PROBLEM: The Ion transport into the Q-Tof instrument is interrupted.

1. To test for interrupted ion transport into the Q-Tof instrument, prepare several target spots with a dense α -CHCA matrix. (α-CHCA provides the brightest fluorescence and the lowest pulse energy necessary.)
2. Set the attenuation to full laser power. (e.g., 10-11mm).
3. Fire the laser and watch the TV screen.
4. If you can see a blinking spot on the TV screen, see if the matrix crystals at that spot are disappearing. (For α -CHCA matrix without the beam attenuation the crystals should disappears in 5-15 seconds). If they disappear, then:
5. Ensure that the Q-Tof interlock is operating properly. To do this check to ensure that the Capillary voltage readback on the MS tune page of MassLynx is correct at 2-3 kV when the source is closed. When the source is open, the Capillary voltage readback should read close to 0V. If this is not the case, call MassTech for assistance.
6. Ensure that the high voltage cable of the Q-Tof instrument is attached tightly to the HV connector of the source.
7. Ensure that the MassLynx program is configured as described in this Manual , and also ensure that you can read "Instrument Present" in the status toolbar.
8. Ensure that your Target plate preparation and both sample & matrix materials are being used properly.
9. Finally, ensure that your Q-Tof instrument operates properly with the electrospray source attached. The problem may be with the Q-Tof instrument rather than the AP/MALDI source.

7.5 PROBLEM: The optical fiber ends need to be cleaned

It is vital that the cleanliness and surface quality of the fibers be maintained during the life of the product in order to ensure optimal performance. ***The optical fiber end protective caps should be used for cable protection anytime the optical fiber is removed from the operational position.*** One spare optical fiber cable has been shipped with your source.



IMPORTANT: Whenever the optical fiber is being detached from or connected to the Control Unit or the Source housing, **MAKE SURE the power switch on the Control Unit is OFF.**

Materials required for Cleaning the Optical fiber ends:

1. Lint-free lens tissue (e.g., from Edmund Industrial Optics, Barrington, NJ, Stock No L60-375)
2. Spectroscopic grade alcohol-based lens cleaner (e.g., Edmund's Stock No. L53-881)
3. Powder-free gloves for handling optical components (e.g., Edmund's Stock No L54-808)
4. An optional Inspection microscope, 50x to 100x is typical strength.

While the exposed fiber ends are handled, fiber gloves must be worn at all times.

1. Prior to cleaning the fibers it is advisable to inspect the fiber ends for damage or burn areas using a microscope.
2. Inspection of the fiber should reveal a uniform, blush, smooth and shiny surface (maybe, with minor scratches, inclusions or dust particles).
3. After inspection, the fiber ends should be cleaned by one (or all) of the four methods described below, as needed to achieve the desired results.

   (1) The first method should be used to remove contaminants *not tightly bound to the surface* of the optical fiber. Put a single drop of the cleaning solvent near the center of a small piece of lens tissue and rub the fiber end slowly and steadily, moving either the tissue or the fiber until no more liquid remains at the point of contact between the fiber and tissue.

(2) The second method is similar to the first one except that the one end of the lens tissue strip (2-3 cm wide) is fixed to the desk edge by adhesive tape and the other end pulled away by hand from the desk edge to create tension along the tissue strip. This tension allows more force to be applied to the cleaned surface.

(3) The third method is to fold lens tissue to form a small wiper approximately 3-4 mm wide, which may be trimmed as necessary; put 2-3 drops of cleaning solvent on the end of this "wiper" and gently draw across the fiber end surface. This method can be used to remove more tightly bound contaminants, but care must be taken with this method since it also applies more stress to the fiber ends. It is often advisable to inspect the progress of fiber cleaning process using the microscope.

(4) A cleaning product called Fiberclean (made by HellermannTyton) was included with your shipment. To use this product:

1. Press the optical fiber end onto the Fiberclean tape and rub in figure 8 motions.
2. After about three figure 8 motions, inspect the optical fiber end with a microscope.
3. Repeat as necessary.
4. Advance the tape after cleaning each optical fiber end.

We are ready to provide you any technical assistance! Call us at (443) 539-1758 or e-mail the problem to: msms@apmaldi.com

8 LITERATURE

1. Victor V. Laiko, Michael A. Baldwin, Alma L. Burlingame, "Atmospheric Pressure Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry", Analytical Chemistry, Vol. 72, No.4, 2000, pp. 652-657.

2. Victor V. Laiko, Susanne C. Moyer, Robert J. Cotter, "Atmospheric Pressure MALDI/Ion Trap Mass Spectrometry", Analytical Chemistry, v.72, No.21, 2000, pp. 5239-5243.

9 WARRANTY INFORMATION – SIX MONTH LIMITED WARRANTY

MassTech, Inc. provides to the original purchaser the following limited warranty from date of invoice.

MassTech, Inc. warrants each AP/MALDI instrument and its components to be free from defects in material and workmanship. Liability under this warranty covers servicing of the instrument when returned from the customer's facility within the United States pre-paid to our factory. MassTech, Inc. will repair any component(s) or part(s), except the optical cables, that it finds to be defective during the period of this limited warranty, which is six months from the date of invoice. Should a defect become apparent, the original purchaser must first notify MassTech, Inc. at (443) 539-1758 of the suspected defect and request a Return Merchandise Authorization number (RMA#). The instrument (or suspect components) should be carefully packaged in the original container (if the original shipping container has been lost, trashed, or damaged, another one must be purchased from MassTech, Inc. prior to shipping). Then, mark the original container with the RMA#, and ship prepaid to:

MassTech, Inc.
6992 Columbia Gateway Dr;
Columbia, MD 21046
Attn: Service Dept.

The instrument will be repaired in the shortest possible time and returned prepaid by the same shipping method as received by the factory. During the warranty period, no charge will be made to you for parts, service, or labor.

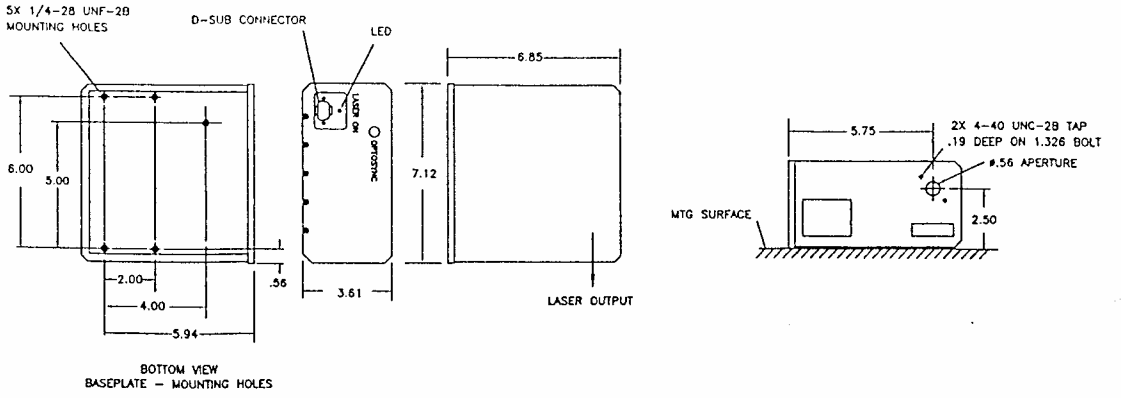
This limited warranty is void if the instrument has been damaged by accident, misuse, negligence, act of God, or serviced by any other person not authorized by MassTech, Inc. The warranty also does not apply to units that have had the serial lot number altered, defaced or removed.

This limited warranty contains the entire obligation of MassTech, Inc. and no other warranties expressed, implied, or statutory are given. No representative or employee of MassTech, Inc. is authorized to assume any further liability or grant any further warranties except as set herein.

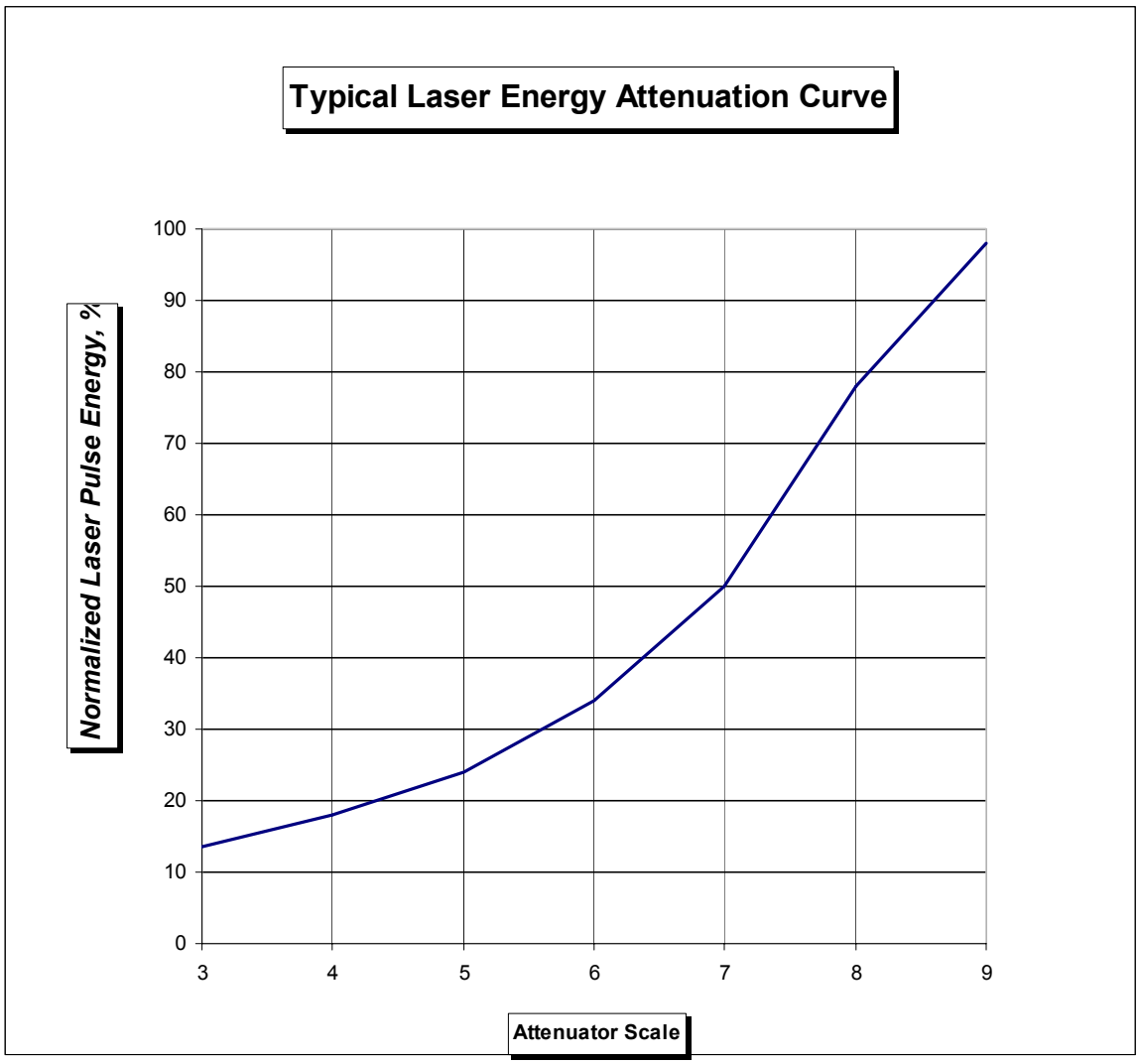
MassTech, Inc. disclaims liability for indirect, incidental or consequential damages. Exclusion or limitation of incidental or consequential damages are not permitted by some states and this limitation or exclusion may not apply to you. Warranty rights vary from state to state; and, therefore, you may have other rights in addition to those provided by this warranty.

APPENDIX A SPECTRA-PHYSICS OEM 337-SI NITROGEN LASER SPECIFICATIONS

Part Number	337203
Wavelength	337.1 nm
Spectral Bandwidth	0.1 nm
Repetition Rate	Up to 10 Hz, user-supplied trigger
Pulse Width, FWHM	4 nsec
Pulse Energy	300 µJ
Pulse to Pulse Energy Stability	3% std. dev. at 10 Hz
Peak Power	75 kW
Average Power	3mW at 10 Hz
Beam Area	35 mm^2
Beam Divergence, Full Angle	0.3 mrad
External Trigger Input	TTL, opto-isolated
Trigger In to Optical Pulse Out	<1 µsec, <40 nsec std. dev. Jitter
Power Requirements	+24 volts DC, 600 mA average at 10 Hz, <1 A peak
Power Consumption	15W at 10Hz
Dimensions, L x W x H	7.1 x 6.8 x 3.6 in; 18.1 x 17.4 x 9.2 cm
Weight	9 lbs; 4.1 kg



APPENDIX B ILLUSTRATION OF THE LASER ENERGY ATTENUATION CURVE



APPENDIX C WARNING AND IDENTIFICATION LABELS

Labels Concerning the Optical Fiber

Two Warning labels (one for each end of the optical fiber)

A	**TURN OFF the Power whenever the fiber is connected to or disconnected from the Control Unit or Ion source**	**FIRST,** connect this end (A) to the Ion source, then connect the other end (B) to the Control Unit. When disconnecting this cable, first disconnect it from the Control Unit (B).	A
B	**FIRST, connect the other end (A) to the Ion source, then connect this end (B) to the Control Unit. When disconnecting the cable, first disconnect this end (B) from the Control Unit**	**TURN OFF the Power whenever the fiber is connected to or disconnected from the Control Unit or Ion source**	B

This is the Identification label to place on the Optical fiber ZIPLOC bag

Mass Tech AP/MALDI Ion Source
Part #6140004 Optical Fiber cable
ONLY replace with an exact replacement part:
(Part # 6140004 from MassTech, Inc.)
Tel. 301-879-6994

The A and B below go on the Ion Source and Control Unit, respectively

A

B

Ion Source Labels

Serial Number Identification label on Ion Source

S/N: TOT000086

Warning labels placed on the outside of the Source

Turn Off the Laser Before Opening the Ion Source

Turn Off the Power Before Connecting or Disconnecting the Fiber

Control Unit Labels

Warning label for Control Unit Shutter

Turn Off the Power Before Connecting or Disconnecting the Optical Fiber

Placed inside the Control Unit on the Optics box

DO NOT OPEN
No Serviceable Parts Inside

Placed inside the Control Unit on top of the LSI laser

LSI Laser 337-Si Inside
Serial No.: T031247
MFG: March, 2003

Identification and Certification label on Control Unit

THIS PRODUCT COMPLIES WITH 21 CFR 1040.10
Atmospheric Pressure MALDI Ion Source

Model	**TF - 121**
Serial #	**TOT000086**
Manufactured	**April, 2003**

MassTech, Inc.
6992 Columbia Gateway Dr.
Columbia, MD 21046
USA

U.S. Patents: 5,965,884 and more pending

Electrical Information

230 V ~
50/60 HZ, 1.9A CE
FUSE: S, 2.0A 250V
REPLACE WITH SAME TYPE AND RATING

Danger Labels placed on the Control Unit

DANGER
INVISIBLE LASER RADIATION WHEN OPEN AND INTERLOCK DEFEATED. AVOID DIRECT EXPOSURE TO BEAM. REFER SERVICING TO QUALIFIED PERSONNEL.

DANGER
INVISIBLE LASER RADIATION
WHEN FIBER REMOVED
AVOID DIRECT EXPOSURE TO BEAM