AP/MALDI Source for LCQ Deca XP ion trap

Installation, Operation and Maintenance Manual

April, 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: (301) 879-6994
Fax: (301) 879-4887
Email: msms@apmaldi.com

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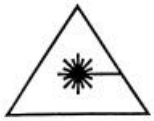
PREFACE

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
(301) 879-6994.

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 an LCQ 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 LCQ 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 LCQ Deca XP ion traps manufactured by Thermo Finnigan. 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 LCQ ion traps: **high sensitivity, the stability of calibration, MS^N capability, powerful data processing, and spectra interpretation software**. However, it also inherits all the limitations of LCQ ion traps: the M/Z range of LCQ is limited to 2kDa in normal mode or 4kDa in a High Mass mode (if Finnigan's Excalibur software is installed). The AP/MALDI source, like the conventional MALDI source, produces mostly singly-charged ions. As a result, the present capability of the Finnigan ion trap limits the mass range of the AP/MALDI-LCQ combination to 4,000Da.

- 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 LCQ 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 unistallation 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 LCQ software is normally optimized for the Electrospray source, you must adjust the LCQ software's parameters so it is optimized for AP/MALDI:

   Set the LCQ software for "nanospray" with these changes:
   Source voltage: 2-3kV; IT=300 to 1000ms; AGC off.

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 and bolt the Source, 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 LCQ data acquisition (using LCQ 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 LCQ data acquisition (by using the LCQ 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.

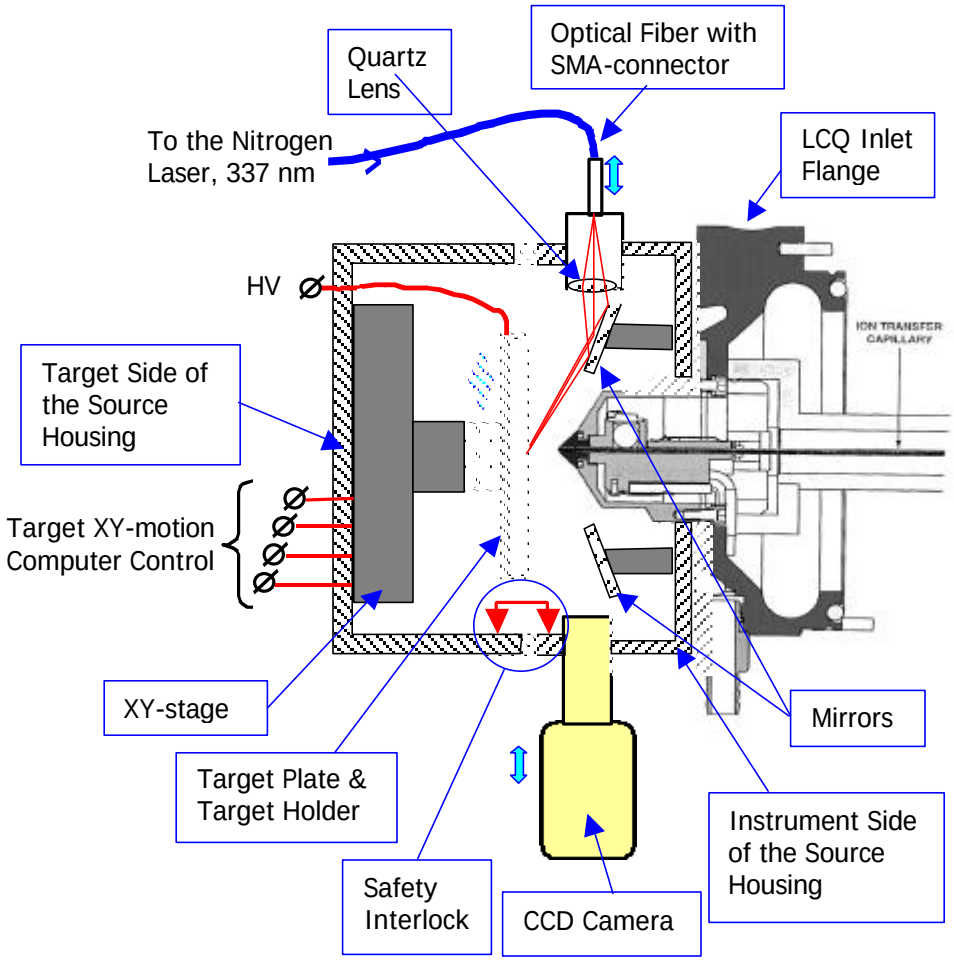


Fig. 1. Simplified schematic diagram of AP/MALDI source installed at LCQ Deca XP.

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 **LCQ Inlet Flange.** Ions produced inside the source **Housing** are dragged toward the inlet orifice of the **LCQ** with a stream of 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, 2-2.5kV) 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 an LCQ control computer can be used to operate the AP/MALDI source. Inside the Control Unit is a nitrogen laser made by Thermo Laser Science. (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

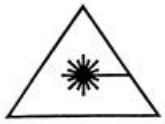
If operated properly, the AP/MALDI source is safe. No special knowledge of laser safety or electrical safety is necessary to operate the source. There are two potentially hazardous factors connected with AP/MALDI source installation, operation and maintenance/troubleshooting:

1. **Invisible coherent UV irradiation** 337nm, up to 300ìJ 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

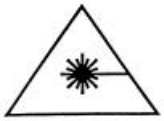
This section describes important precautions that must be observed during AP/MALDI source ***installation, operation, and maintenance***. Appropriate precautions can be divided into the following stages:



- **Installing/Uninstalling:** Before the source is installed onto the LCQ instrument, uninstalled, or replaced, the LCQ instrument must be in either "Standby" or "Off" mode (the "Scan" light on the LCQ front panel ***must*** be turned OFF). The same rules, described in the LCQ 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 LCQ 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. It is recommended that you first switch the LCQ instrument to either "Standby" or "Off" mode (the "Scan" light at the LCQ front panel OFF), stop laser firing (Click on the "Stop " button in the AP/MALDI source "Target" software) ***so that the "Laser ON" indicator on the front panel of Control Unit is OFF***. 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 LCQ 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.

- **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 LCQ instrument, which excludes any possibility of High Voltage shock or laser radiation exposure. Once again, if by accident you open the source while the LCQ 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 (301) 879-6994.

<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 LCQ instrument or the optical fiber is not properly installed.**

3.2 Operator Controls and Indicators

The two figures below illustrate the front and back plate of the AP/MALDI Control Unit. Additional warning and Identification labels are illustrated in Appendix B 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 2-9 below show these components and introduce some definitions and part names used in the installation explanations.

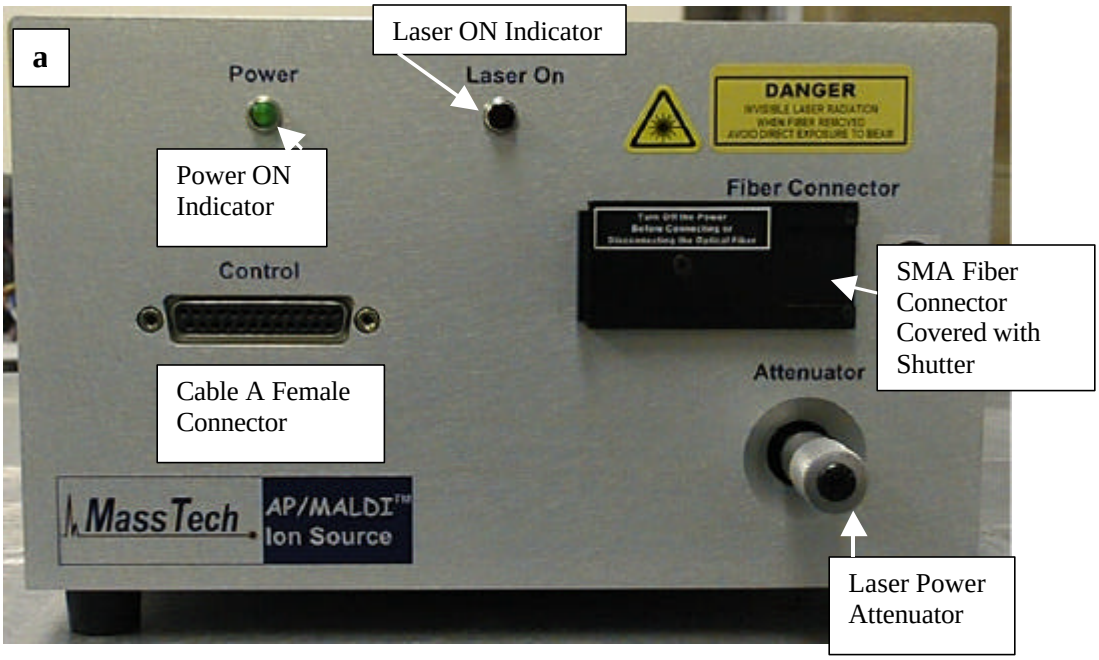


Fig. 2a. Control Unit Front View

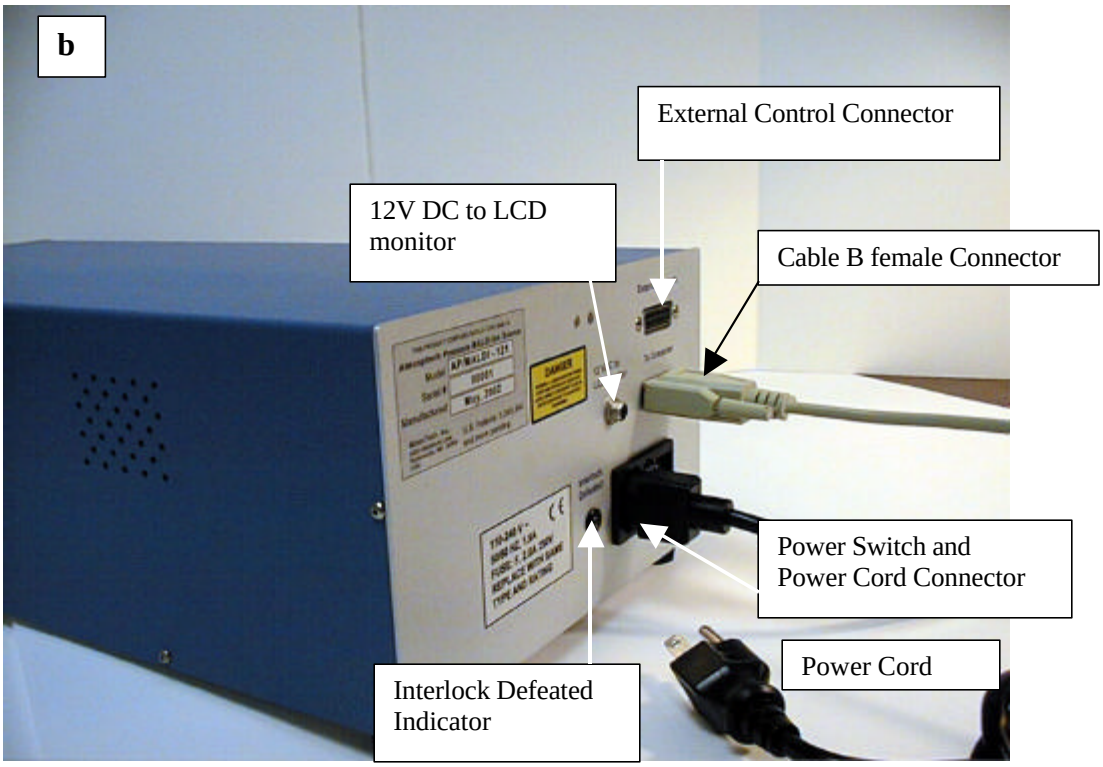


Fig. 2b. Control Unit Rear View

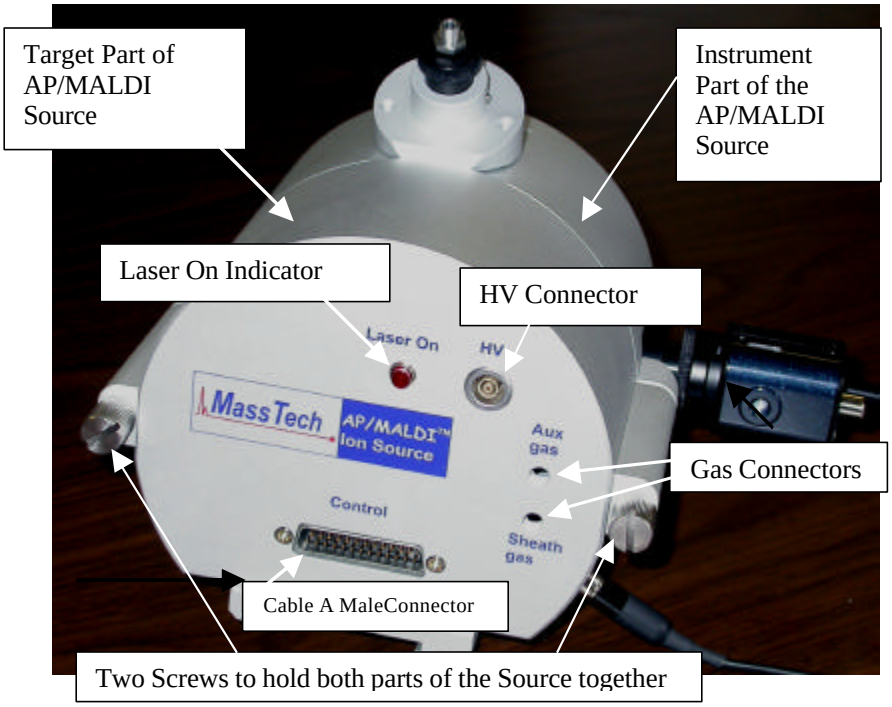


Fig. 3. Ion Source for Deca XP

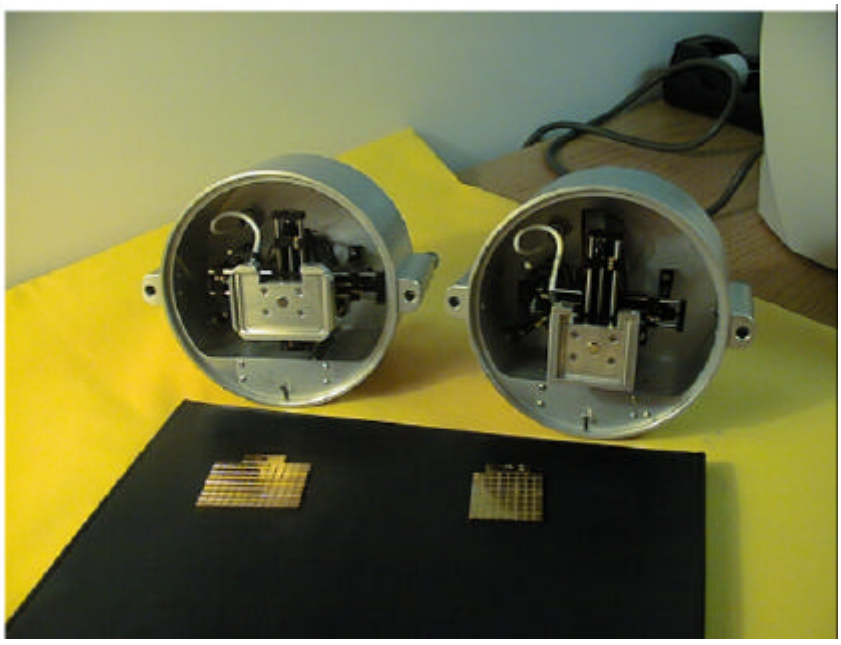


Fig. 4. The 64-spot- target plate and target flange (on the right) is the standard configuration with the 96-spot plate/flange as an optional purchase.

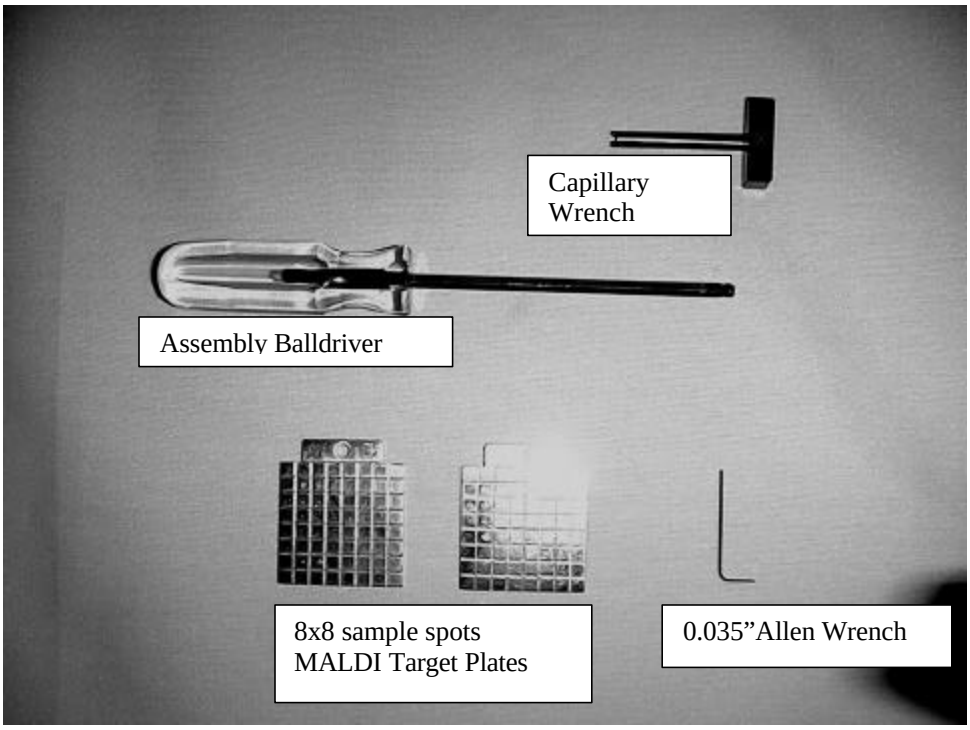


Fig.5. Necessary Tools (included in shipment) and Target Plates.

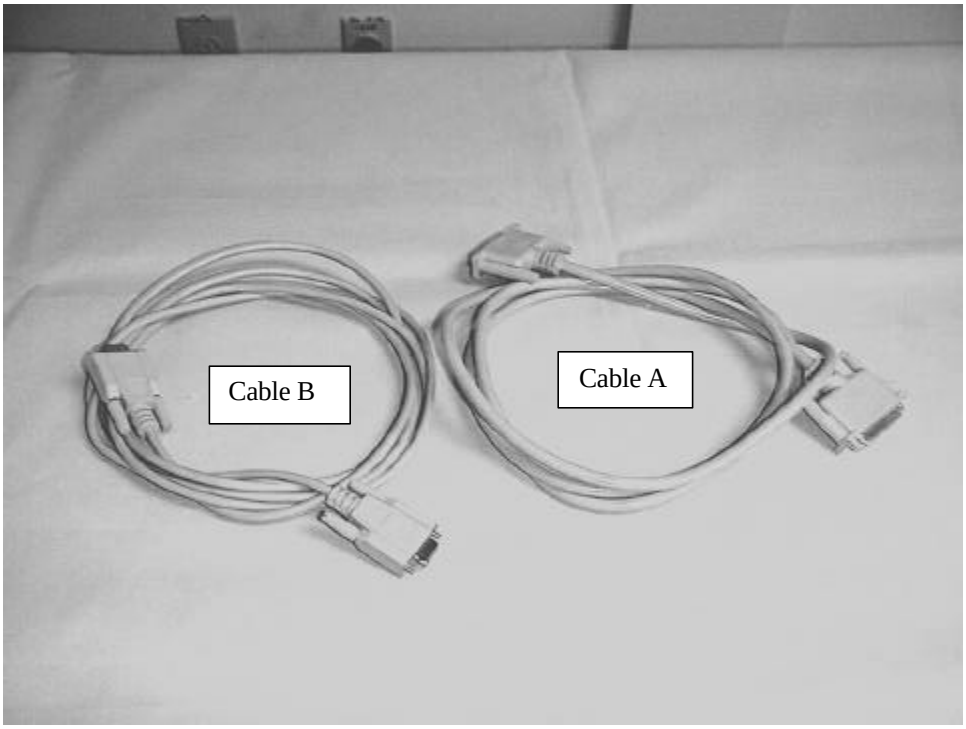


Fig. 6. Cable A (Control Unit – to - Source Cable) and Cable B (Control Unit – to - Serial Port of PC computer Cable).

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

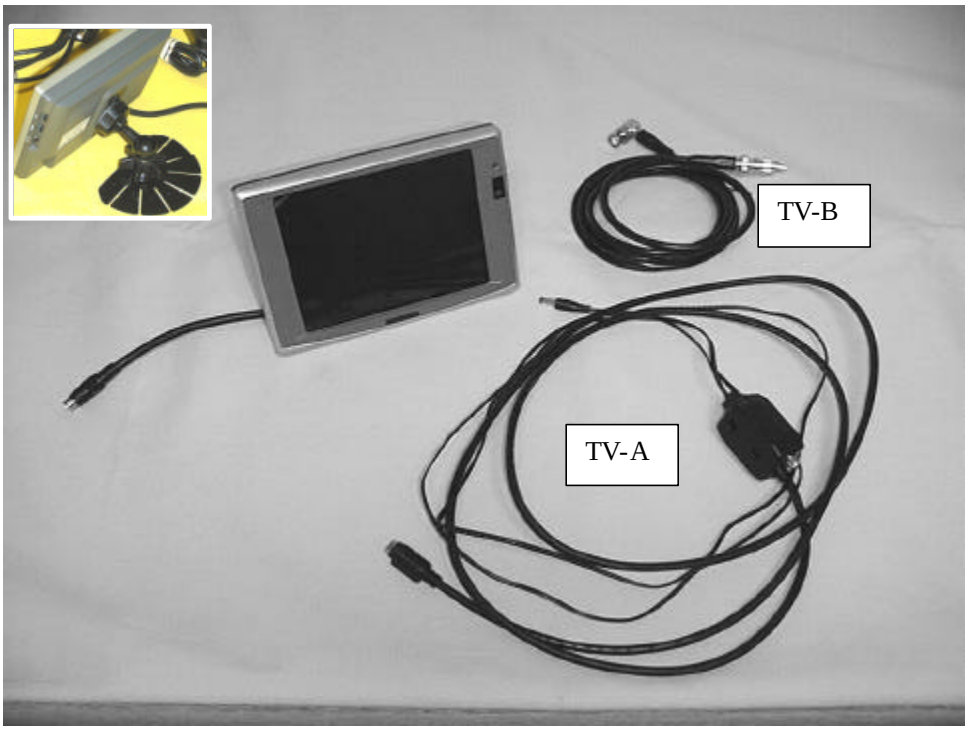


Fig. 8. Flat TV-screen with Power cables TV-A and TV-B.
Insert: Rear view.

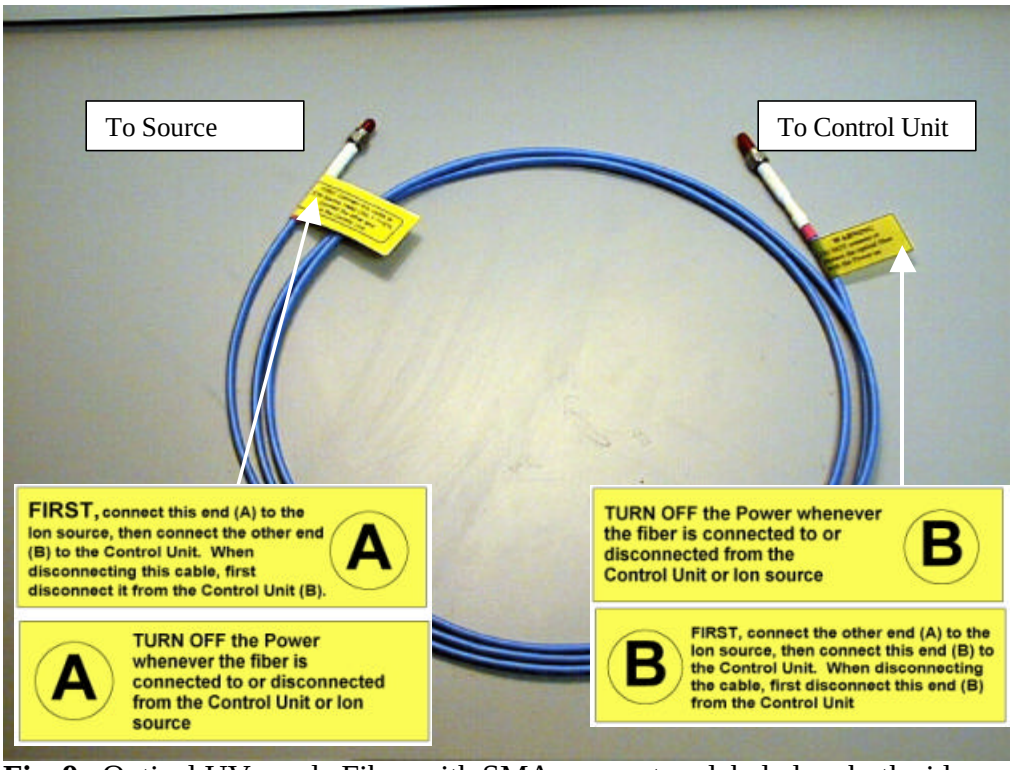
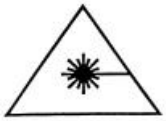
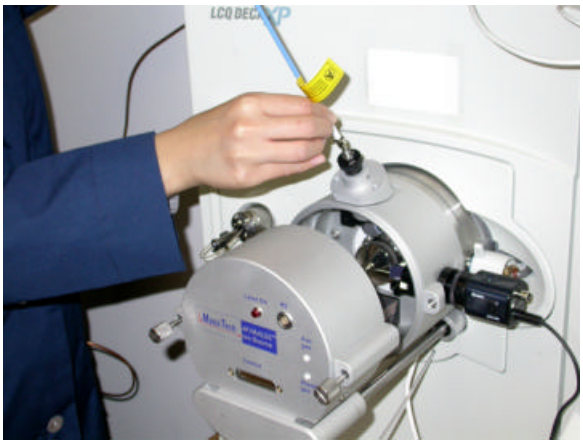


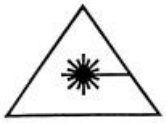
Fig. 9. Optical UV-grade Fiber with SMA-connectors labeled on both sides. SMA-connectors on both sides are covered with protective plastic caps. (The shipment includes one spare Optical cable, not shown in the figure).



When you install/uninstall the source on the LCQ, 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.



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.

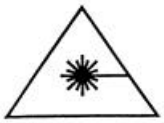


You must turn OFF the Control Unit (so the laser cannot be accidentally fired) whenever you have the optical fiber disconnected from either end or plan to disconnect or connect it.

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)

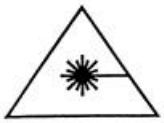
4.2 Installation of the Source

Installing/Uninstalling: Before the source is installed onto the LCQ instrument, uninstalled, or replaced, the LCQ instrument must be in either "Standby" or "Off" mode (the "Scan" light on the LCQ front panel ***must*** be turned OFF). The same rules, described in the LCQ 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 LCQ 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 10 below.

Fig 10. The Ion Source right out of the box.

Separate the Ion Source into three components as shown below.

Fig 11. The two components of the Ion Source with Shipping base.

Remove your current ESI instrument from the LCQ Deca leaving an empty inlet flange as shown on the picture below.

Fig 12. LCQ Deca ready to receive AP/MALDI Ion Source

Tighten the two screws with the Assembly Balldriver tool (Fig. 5). Some gentle rotation of the Source around the LCQ flange might be necessary

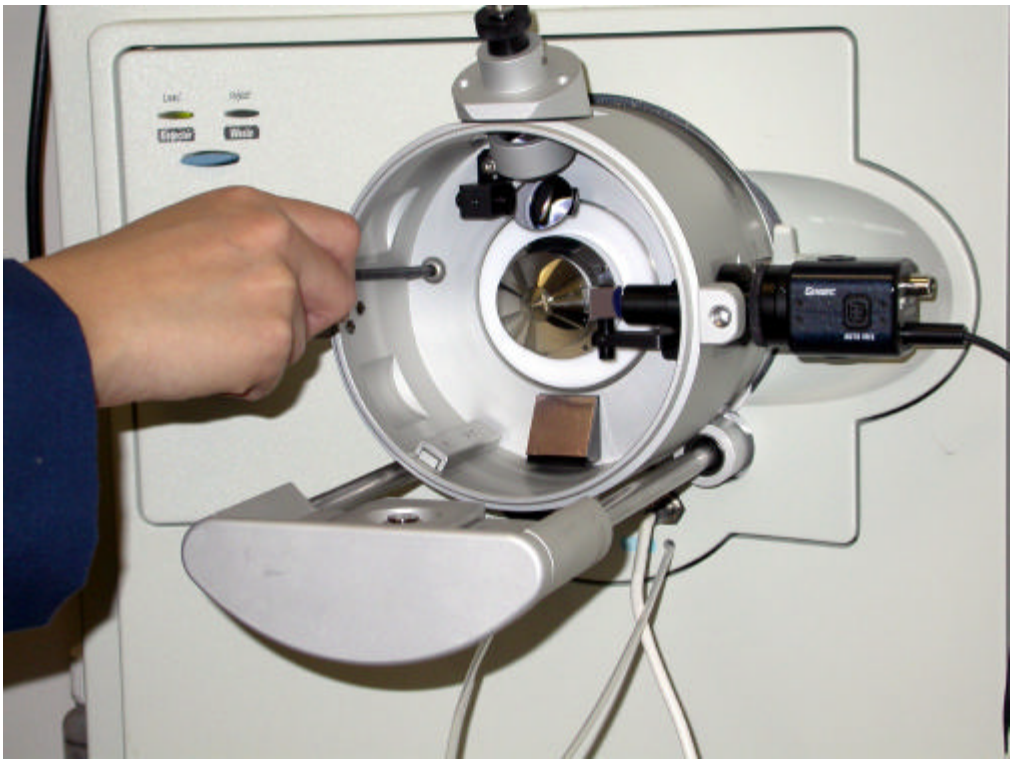


Fig. 13. Screwing on the Source to the LCQ Deca Instrument.

Finally, install the Target part of the Source to the rail like you would install a standard ESI source. Connect Cable A, the HV connector, and the gas pipes to the Source. It doesn't matter in which order the two gas pipes are connected

Fig 14. Installing the Source Target Part to the LCQ Deca XP rails.

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.

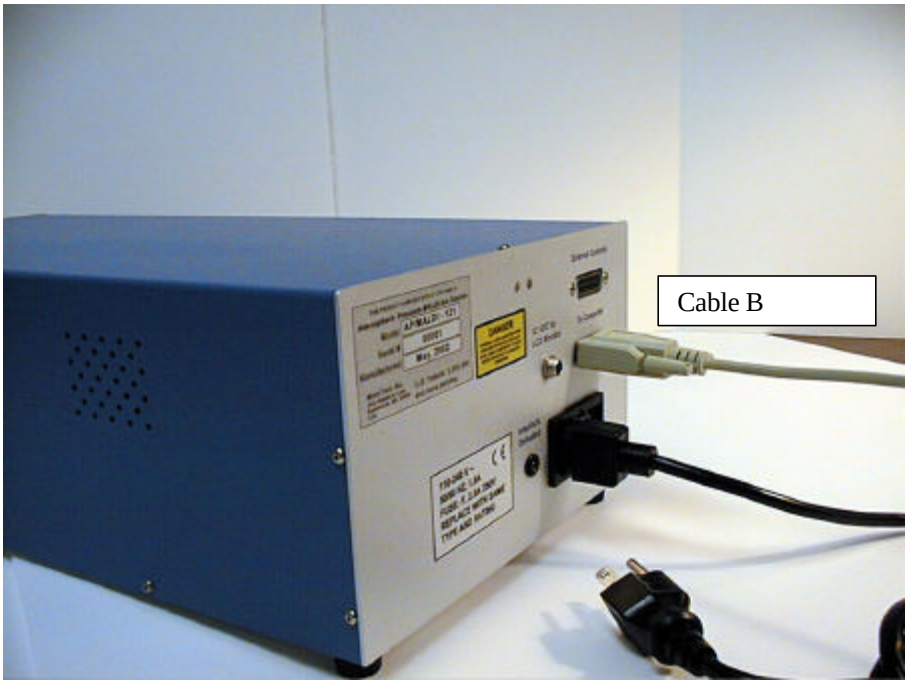
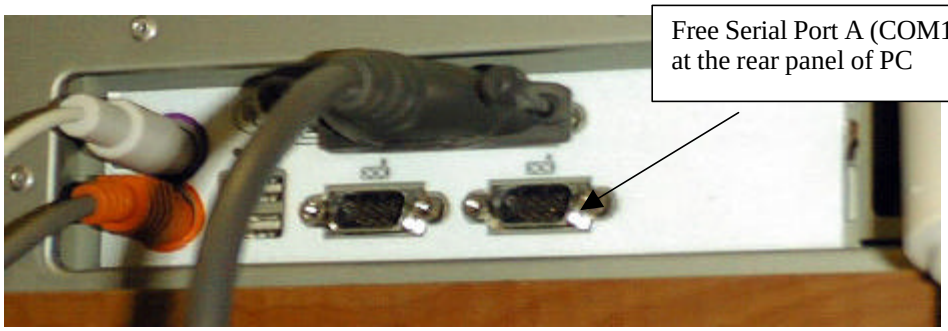
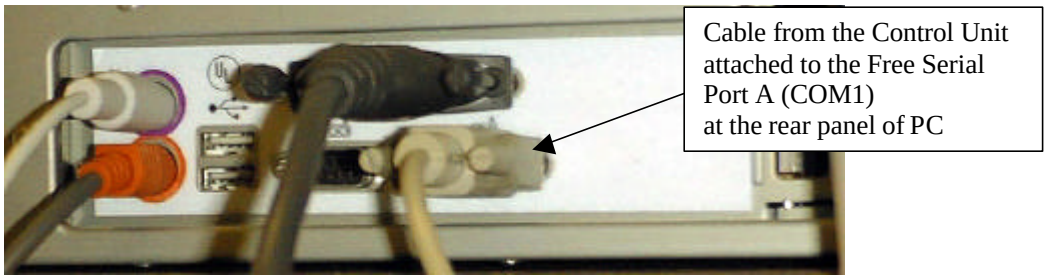


Fig. 15. Connect the black power cord and Cable B to the corresponding connectors at the rear plate of the Control Unit**. No adjustment is necessary for ~110/~127/~220/~240V AC!**

Fig. 16. Connect the other end of Cable B to a free Serial Port on your PC. Either an LCQ-instrument computer or another separate PC can be used.





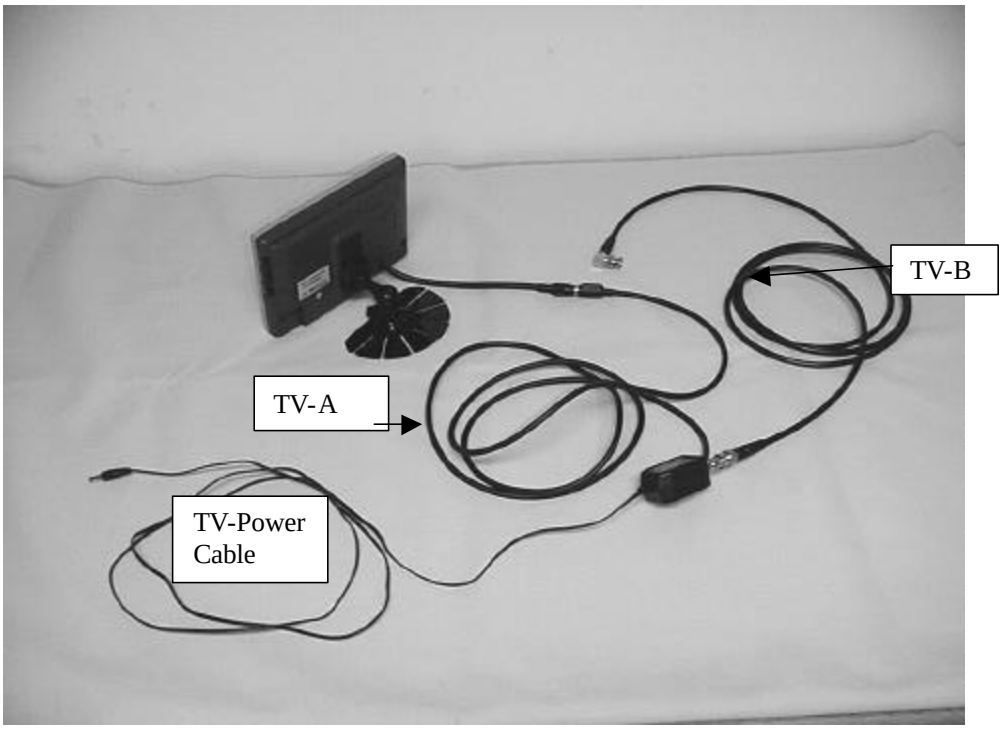
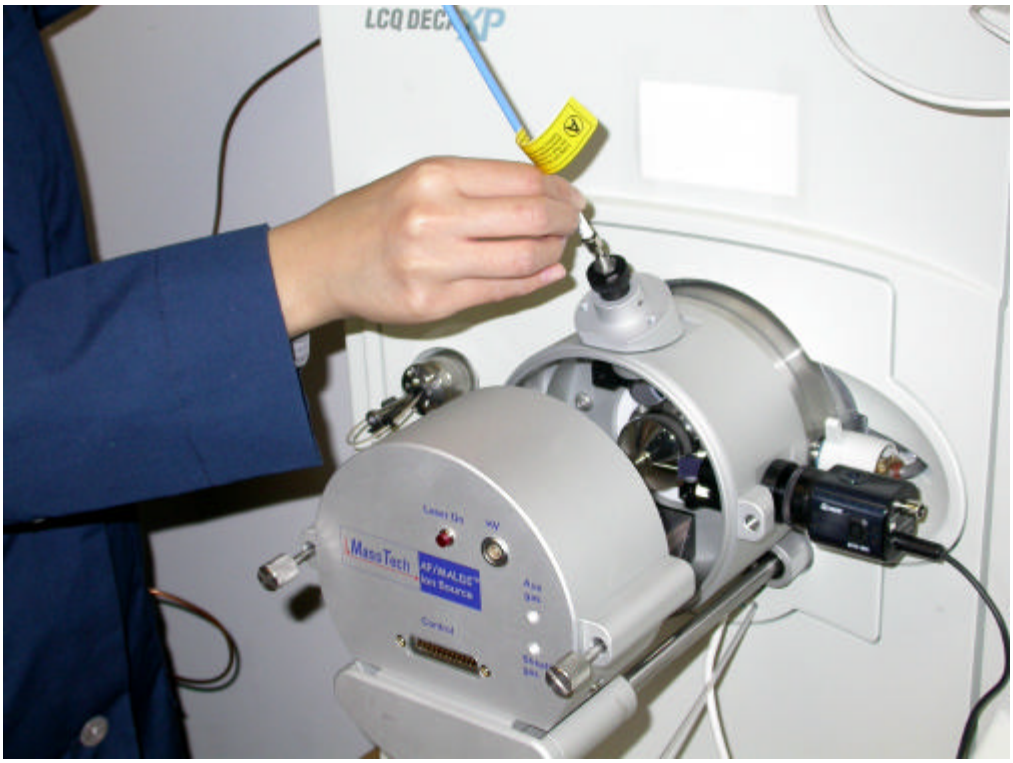


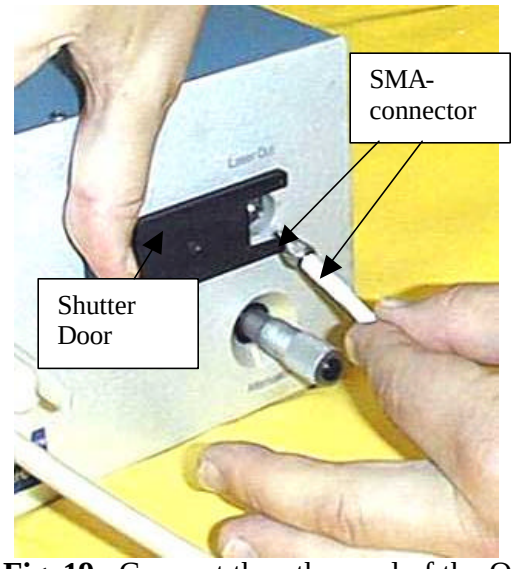
Fig. 17. Connect the TV monitor cables as shown in the picture.



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

Fig. 18. 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.



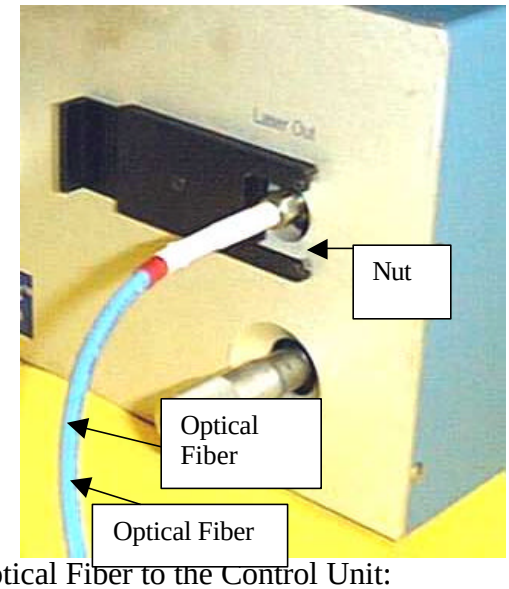


Fig. 19. Connect the other end of the Optical Fiber to the Control Unit:

- 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.

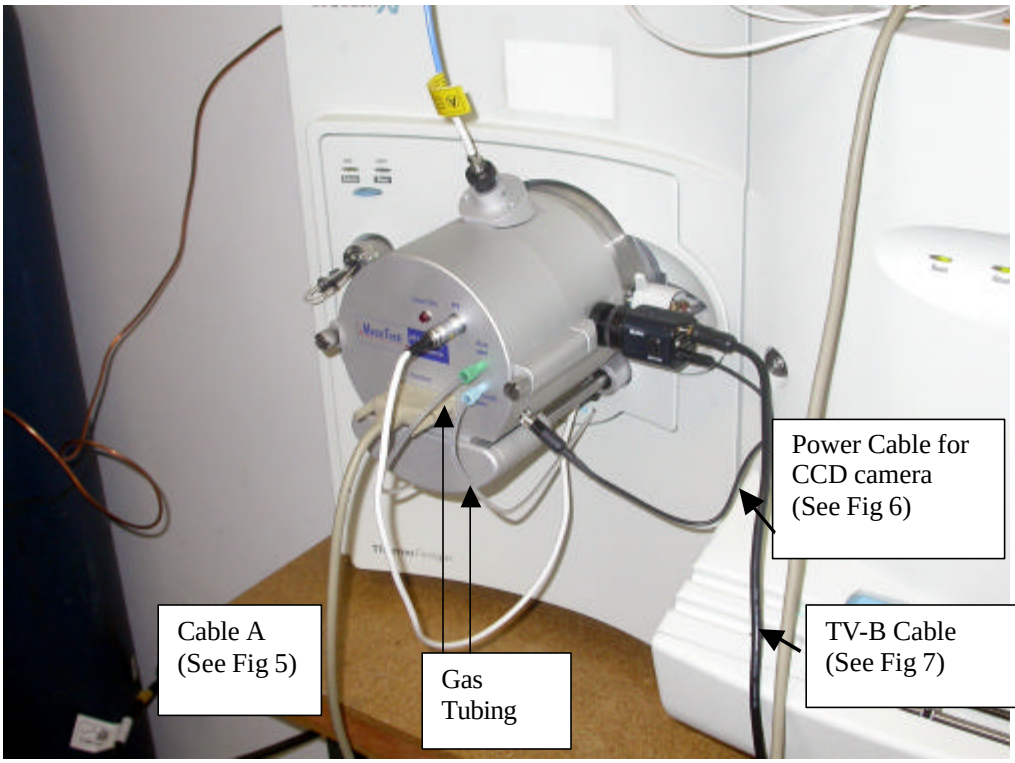


Fig 20. Wire the Source accordingly

The position of the Fiber Connector with respect to the Source housing can be adjusted by easing the Set Screw. This position determines the laser beam focusing at the target surface. There is a scale engraved at the cylindrical surface; approximately 4-5 lines should be visible. This position was already adjusted at the factory so tuning is necessary only if the position was changed during transportation or installation. Fine-tuning of the Fiber position can be performed later based on the beam focus image and spectra quality.

Now, attach the wires and cables to the source according to Figure 20 above. The order in which you attach the gas tubing does not matter with this Source.

When complete, your LCQ Deca XP with AP/MALDI source will look like this:

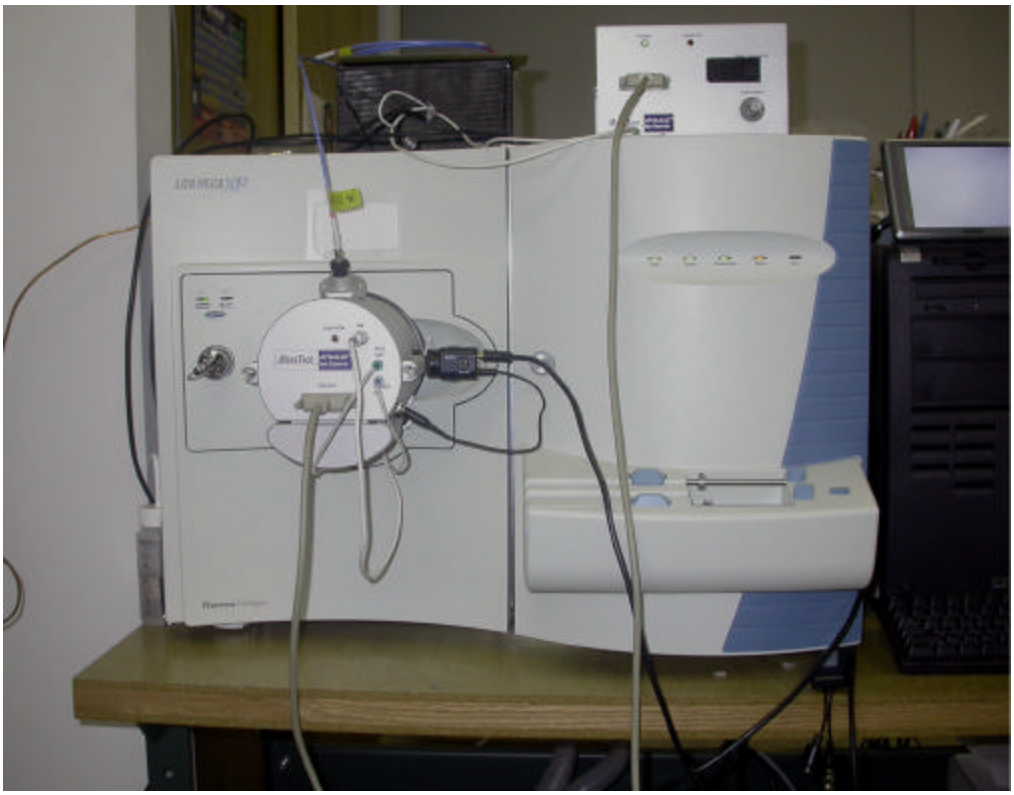


Fig 20a. Completed source installation

Before switching on the Power on the Control Unit:

1. Ensure that the HV connector is 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 LCQ, **First**,

1. Set the LCQ 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. 21. 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, á-Cyano-4-hydroxycinnamic acid (á-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 Fmole/ìL.
- Deposit a droplet of 0.5-2 ìL 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. It is recommended that you first switch the LCQ instrument to either "Standby" or "Off" mode (the "Scan" light at the LCQ front panel OFF), stop laser firing (Click on the "Stop " button in the AP/MALDI source "Target" software) ***so that the "Laser ON" indicator on the front panel of Control Unit is OFF***. After that, proceed with loading or unloading of the target plate. If by accident you open the source while the LCQ 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.

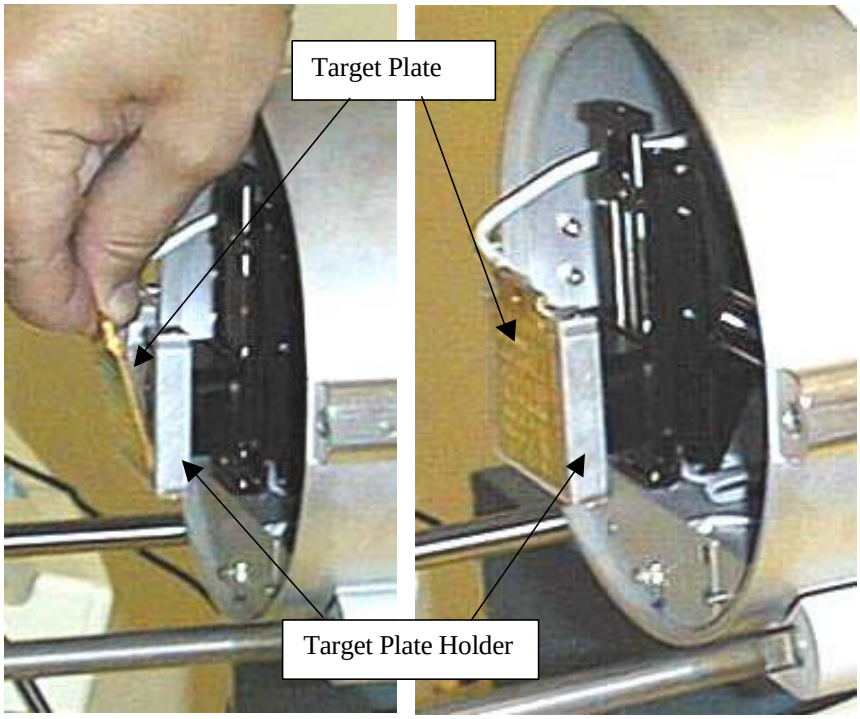


Fig. 22. Insert the Target Plate with the prepared sample spots into the Target Plate Holder. The Plate is held in place by a magnet.

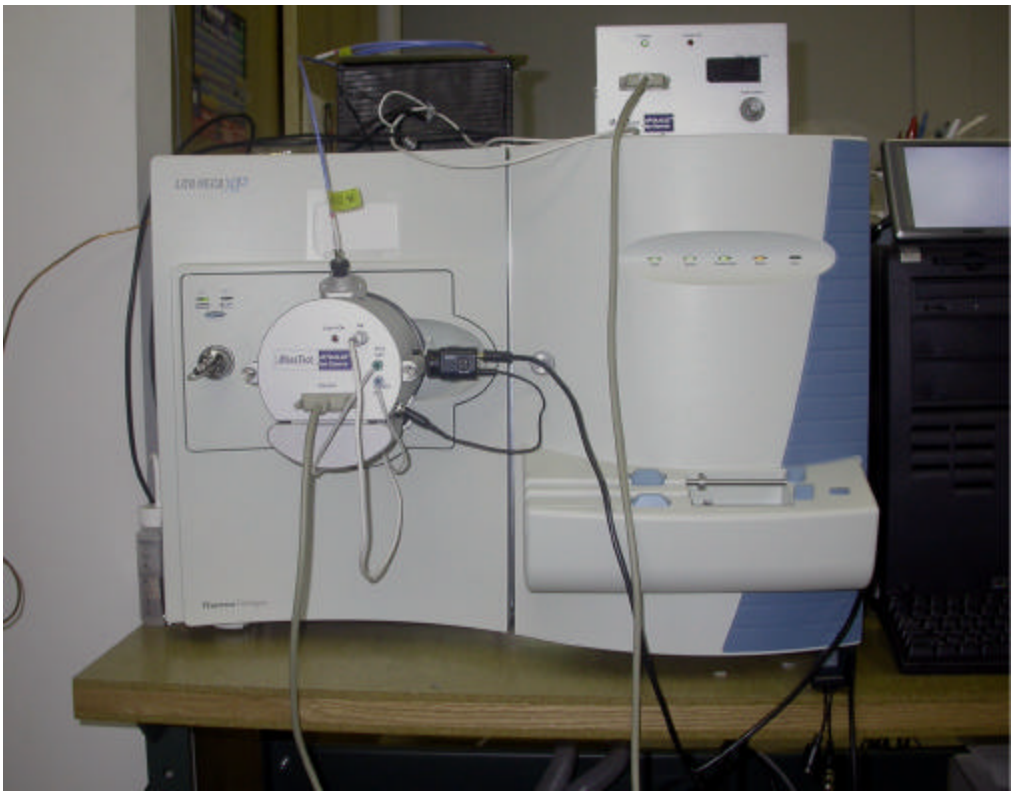


Fig. 23. Screw the Source together. Plug in the Control Unit and switch it on (rear panel switch) and turn on the TV monitor (at the side).

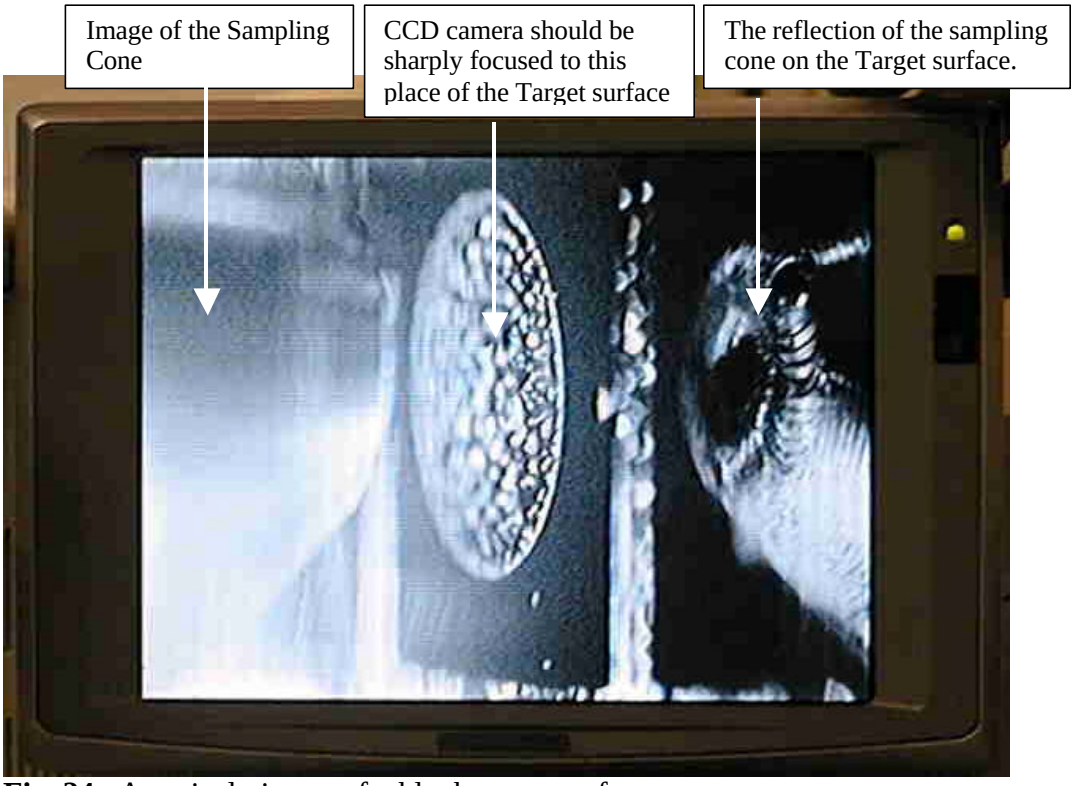


Fig. 24. A typical picture of a blank target surface.

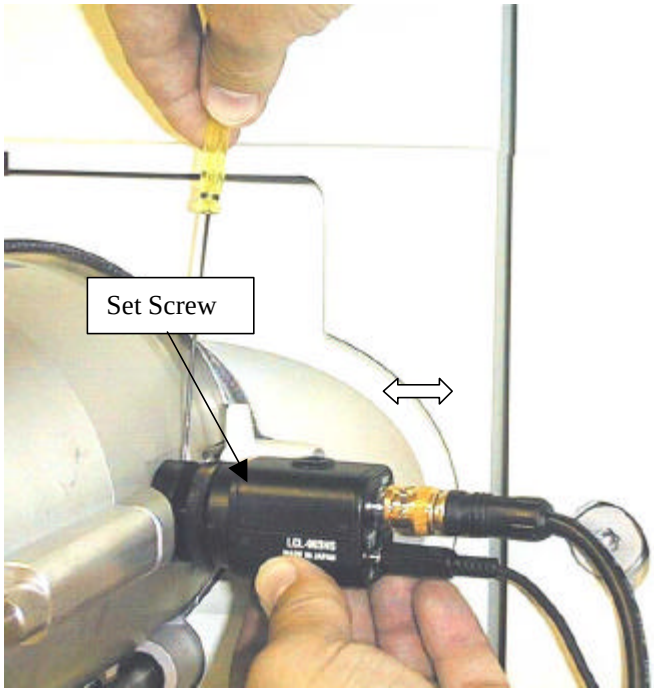


Fig. 25. CCD camera focusing.
This procedure can be safely performed even if the source is ON and the laser is firing.
Ease the Set Screw, move the camera, refasten the Set Screw.

6 AP/MALDI OPERATION

6.1 Using the TARGET *software*

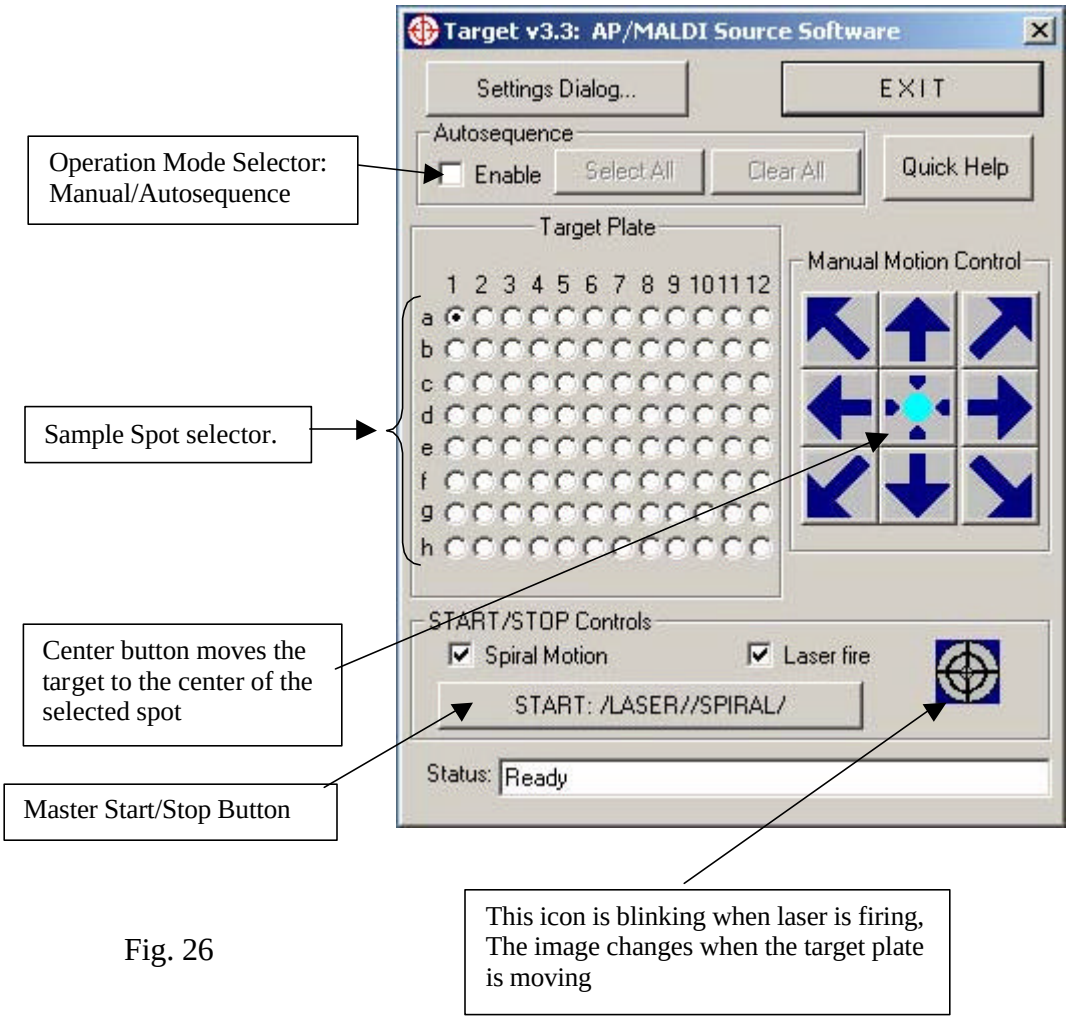


Fig. 26

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.26 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

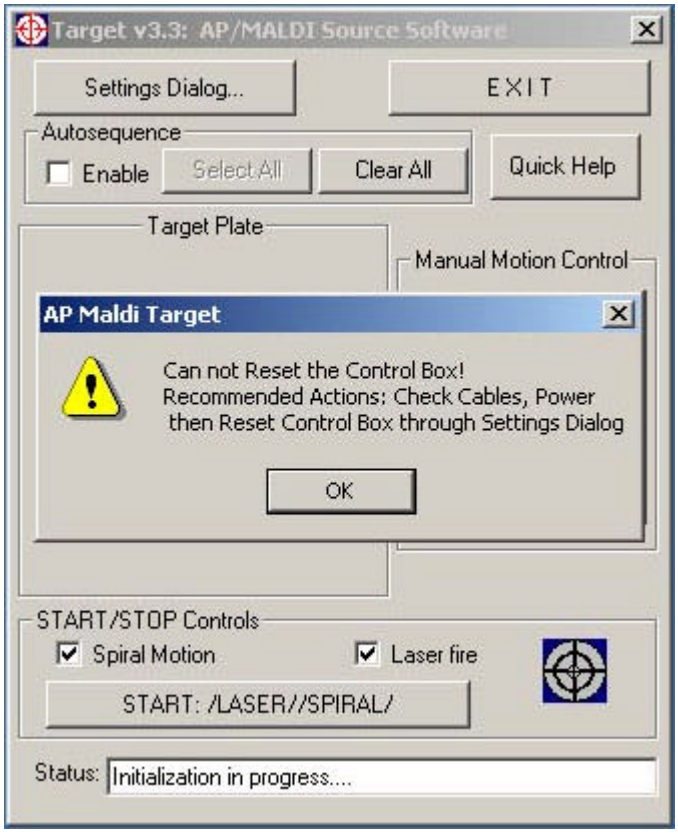


Fig. 27

OFF, or if the Control Unit is not properly wired to the computer, you will get the messages shown in Fig. 27. 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. 26). 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. 28, below.

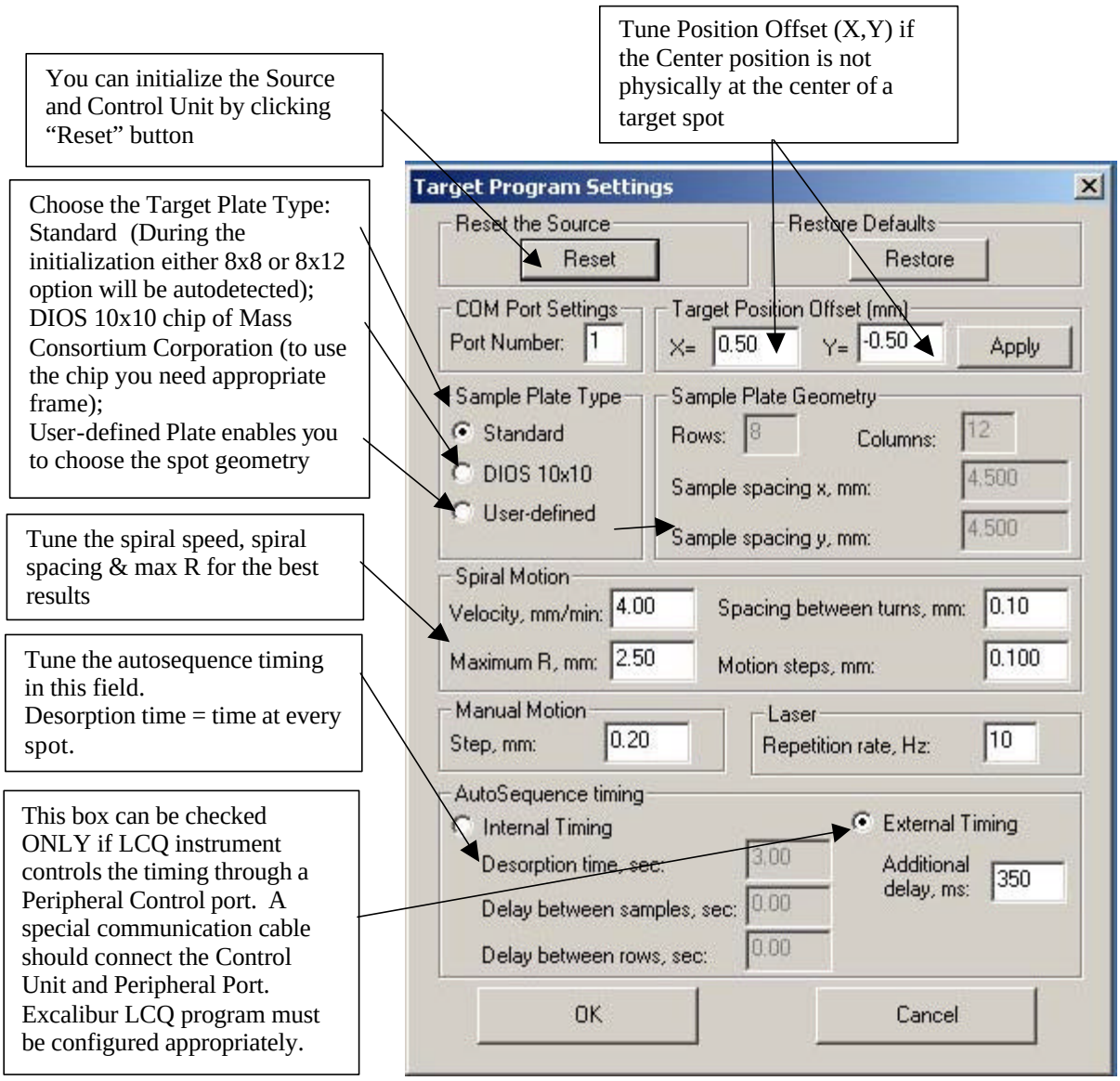

You can initialize the Source and Control Unit by clicking "Reset" button
Tune Position Offset (X,Y) if the Center position is not physically at the center of a target spot
Choose the Target Plate Type:
Standard (During the initialization either 8x8 or 8x12 option will be autodetected);
DIOS 10x10 chip 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 LCQ instrument controls the timing through a Peripheral Control port. A special communication cable should connect the Control Unit and Peripheral Port. Excalibur LCQ program must be configured appropriately.
Target Program Settings
Reset the Source
Reset
Restore Defaults
Restore
COM Port Settings
Port Number: 1
Target Position Offset (mm)
X= 0.50
Y= -0.50
Apply
Sample Plate Type
Standard
DIOS 10x10
User-defined
Sample Plate Geometry
Rows: 8
Columns: 12
Sample spacing x, mm: 4.500
Sample spacing y, mm: 4.500
Spiral Motion
Velocity, mm/min: 4.00
Spacing between turns, mm: 0.10
Maximum R, mm: 2.50
Motion steps, mm: 0.100
Manual Motion
Step, mm: 0.20
Laser
Repetition rate, Hz: 10
AutoSequence timing
Internal Timing
External Timing
Desorption time, sec: 3.00
Additional delay, ms: 350
Delay between samples, sec: 0.00
Delay between rows, sec: 0.00
OK
Cancel

Fig. 28

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

6.2 Running AP/MALDI on the LCQ instrument.

6.2.1 Setting the LCQ Parameters

To run AP/MALDI on the LCQ instrument optimally, the following tuning procedure of the Tune Plus program is recommended:

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

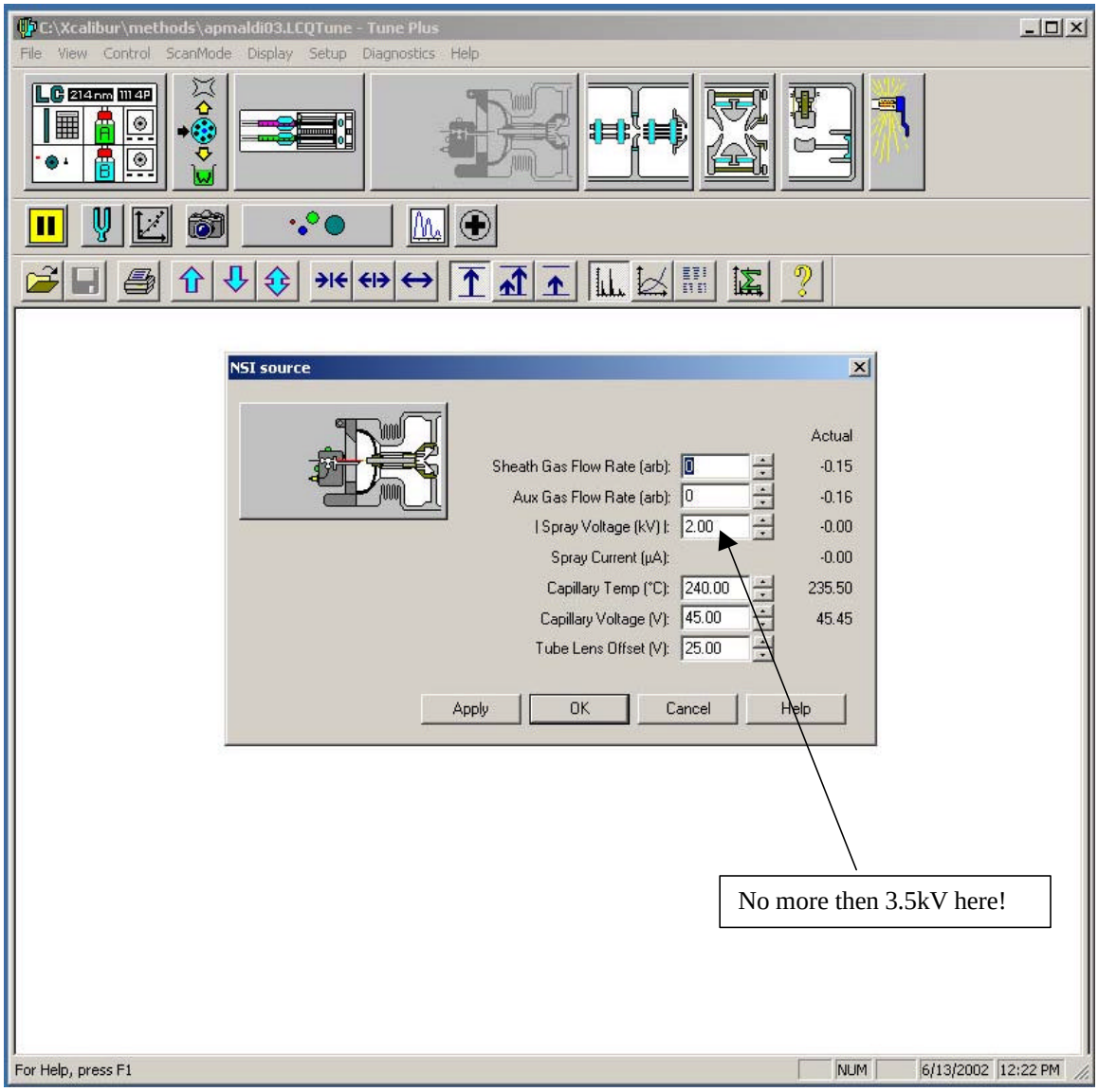


Fig. 29.

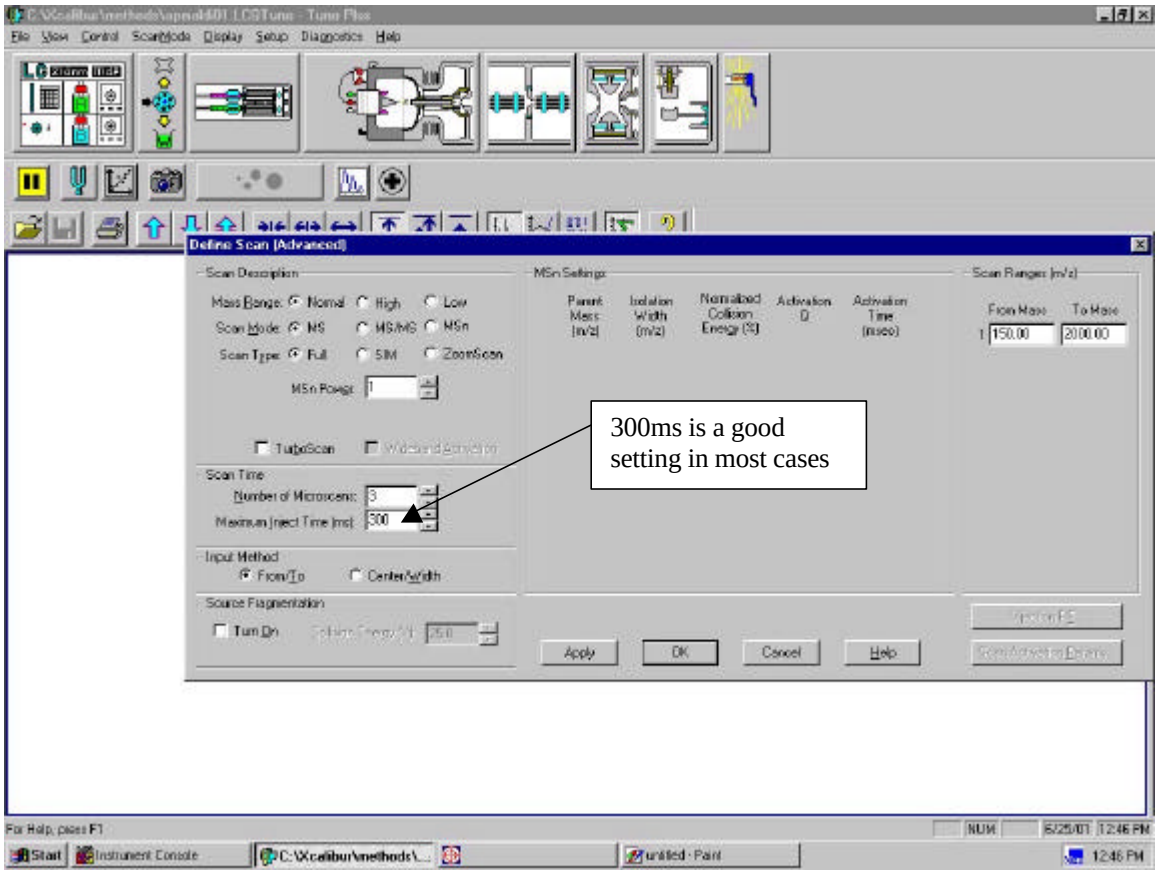


Fig. 30.

- The AP/MALDI source typically generates a much weaker ion current compared with Electrospray. So it is recommended that you increase the Maximum Inject Time to 200-500ms or even more (see Fig. 30 above). More experienced users can try to switch the Automatic Gain Control (AGP) to achieve the highest sensitivity for very diluted samples. In this case, Inject Time should be around 500ms. MS/MS and Zoom Scan does not work if AGP is set to OFF with some releases of the Tune Plus program. If AGP is OFF, you are responsible for decreasing the Injection Time manually for samples with higher analyte concentration (typically, 500fMole/spot or more) to avoid Trap saturation.

- **Sheath Gas and Aux Gas Flow settings**: Both gas lines (Sheath & Aux) attached to the AP/MALDI source can be used for only one purpose: that is to fill the Source housing with dry nitrogen. This dry nitrogen atmosphere may improve the AP/MALDI spectra quality sometimes (typically, this is the case if the air humidity in the lab is too high). Our recommendation is to try to reduce the nitrogen gas flow from 10 units to zero while recording some standards. Typically no differences in spectra quality can be detected. There is some small inconvenience in switching the gas flows to OFF: the Tune Plus program was designed for electrospray source and there is a protection that prevents system operation with Sheath Gas flow less than 20 units. To bypass this

- protection, we recommend to configure LCQ for nanospray source (NSI). Follow the example in Fig. 31 below:

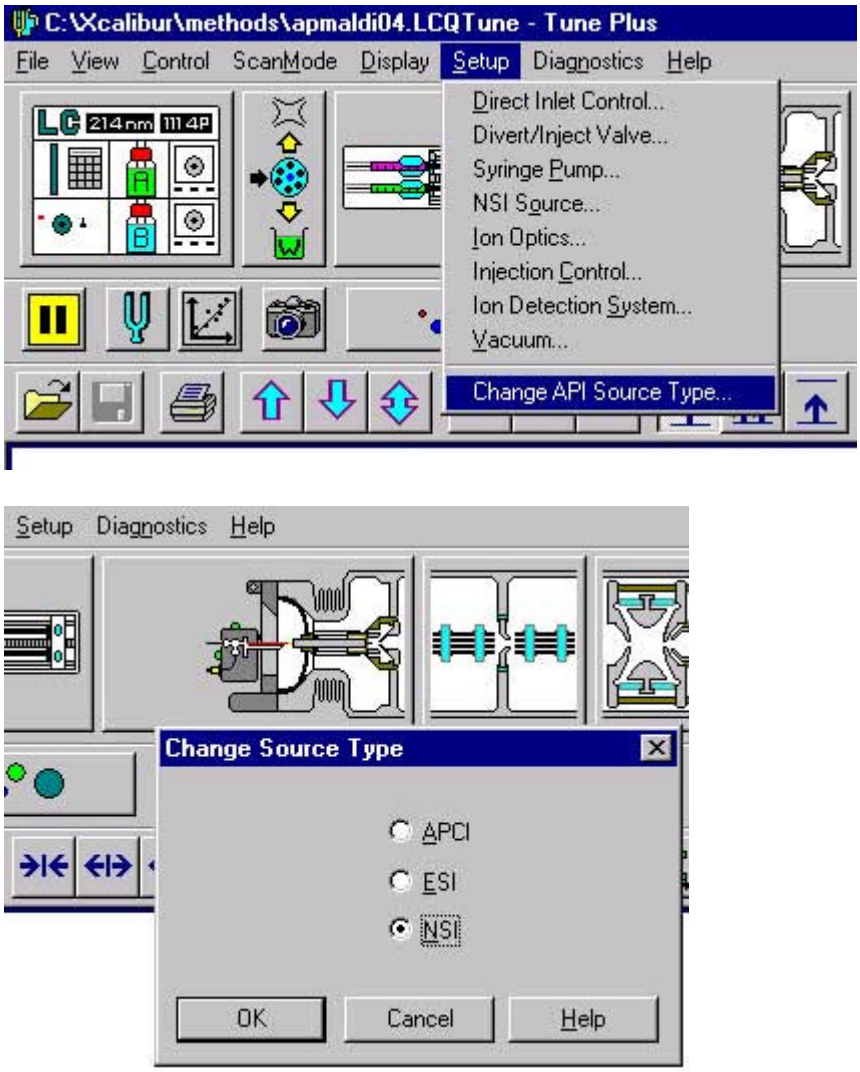


Fig. 31.

- There are two ways of recording and saving AP/MALDI spectra. To begin with, you may treat (record and save) an AP/MALDI signal like a signal from an HPLC/electrospray combination. Typically a single spectrum in that mass-chromatogram is too weak and averaging over several spectra is desirable (this operation can be performed in the Qual data browser). The disadvantages of this approach are: too much hard drive space consumption; you cannot estimate in advance if the collected data quality is good enough so you can go ahead and terminate the recording. So we suggest another approach, that is, recording in the Tune Plus program with the "Spectrum Averaging" button on the toolbar ON. After you are satisfied with the data quality, record the final (averaged) spectra for several seconds. By default, the Tune Plus program is averaging the last 15 spectra. If the analyte concentration is too low, this setting may not be sufficient to record spectrum with good signal/noise. It is recommended that you change the above setting to 333

(maximum allowed value). If you are satisfied sooner than when 333 spectra are recorded – just save your spectrum and stop the data acquisition and laser firing. The way to increase Max. Averaging is shown in Fig. 32.

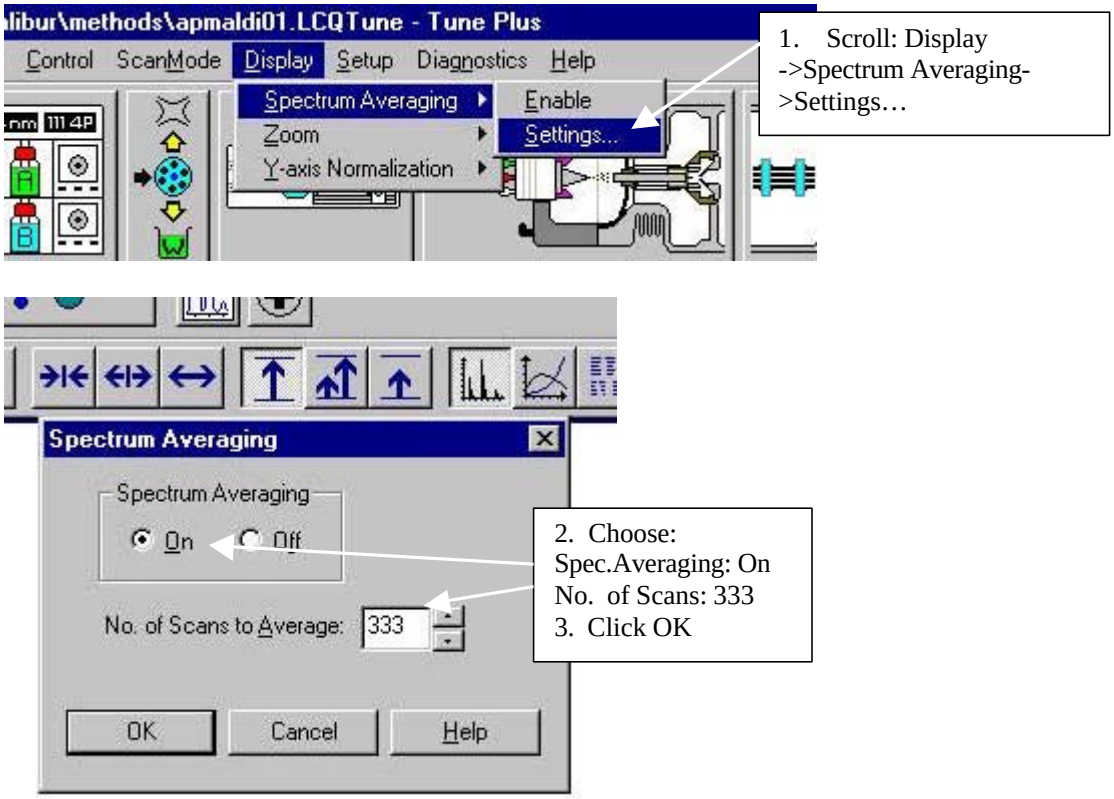


Fig. 32.

- 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 (á-CHC, DHB and so on).
- If Thermo Finnigan's *Excalibur* software is not installed on your LCQ instrument, M/Z range of the instrument is limited to 2,000Da. With *Excalibur* an extended M/Z range can be used to record spectra up to 4,000Da. Call Finnigan for an upgrade of your software.
- 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 in-time 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 in time. Spiral motion will give you a stable AP/MALDI signal for 10-20 minutes. It is enough for MS, MS/MS, and MS^N experiments. Even the Autotune feature of the Tune Plus program can be used for the instrument's tuning in AP/MALDI mode.

- Fig. 33 represents a screen copy made during an AP/MALDI spectrum measurement. (An older version of *TARGET* is shown in the Figure below). You can easily switch between the *Tune* Plus and *TARGET* programs to operate both LCQ and AP/MALDI source from the same computer. Or alternatively, separate computers can be used to run *TARGET* software and operate AP/MALDI.

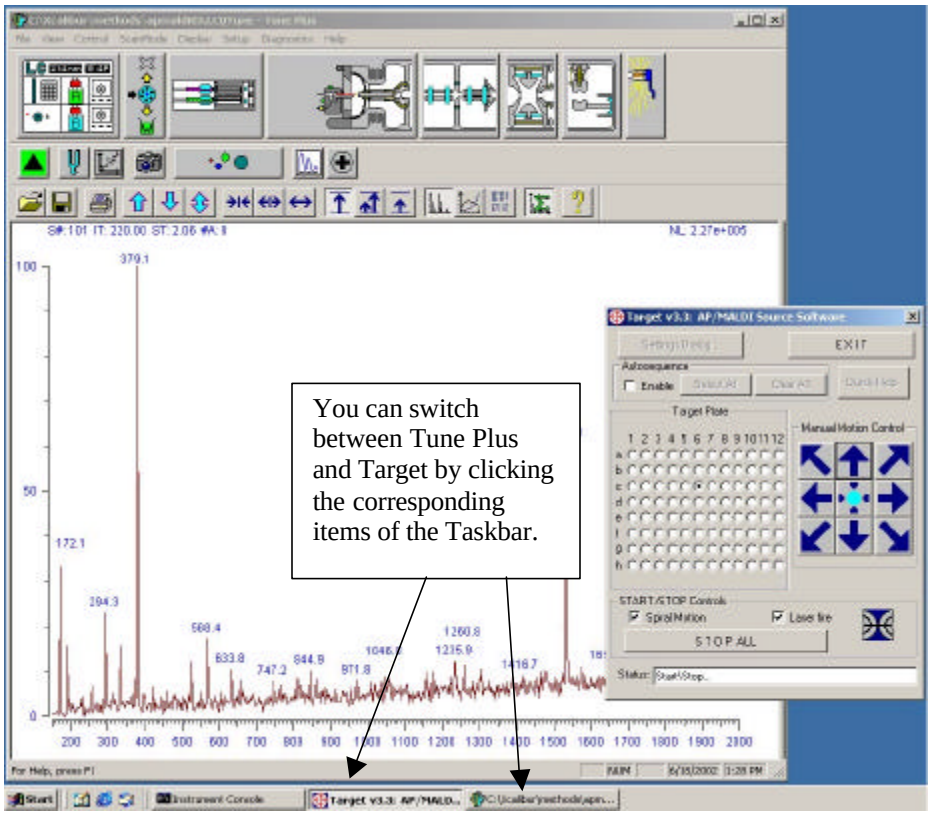


Fig. 33.

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 *Xcalibur* and *TARGET* features. The data acquisition in *Xcalibur* (or *LCQ Tune*) is started independently from the target position and laser control in *TARGET* software. The spectra acquired will depend on what sample is currently located near the inlet capillary 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, octopole and ion optics voltages, etc. accessible via *Xcalibur*). Saving the spectral data is your responsibility and is done using appropriate *Xcalibur* 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. 32).

2. Start data acquisition using *Xcalibur* or the *LCQ Tune* software (see the previous *Setting LCQ Parameters* section in this manual or Thermo Finnigan's *Xcalibur* 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. 26). 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. Save data acquired, when necessary, using LCQ software.

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

9. Repeat steps 3-8 to acquire one more spectrum from the same or another sample.

10. Stop data acquisition on the LCQ.

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 LCQ 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 LCQ's *Xcalibur* or *LCQ Tune* software is started independently from the *TARGET* software. Synchronization of the start of the data acquisition and the start of the *TARGET* operation is a your responsibility. This synchronization is important for subsequent assignment of the acquired data (in the chroma togram) 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. 26) and **uncheck** the "Contact Closure Timing" check box in the *TARGET*'s "Settings Dialog" window (see Fig. 28).

2. Set the "AutoSequence Timing" parameters in the *TARGET*'s "Settings Dialog" window (see Fig. 28): "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 than 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 *Xcalibur* or *LCQ Tune* software (see previous *Setting LCQ Parameters* section in this manual and Thermo Finnigan's *Xcalibur* 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 LCQ when the 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 LCQ's "Peripheral Control" connector. Synchronization of the LCQ and AP/MALDI source operations is achieved via bi-directional signal communication between the LCQ 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 LCQ's data acquisition process and turns on the laser firing; then, the LCQ 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 LCQ 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. Using one supplied cable, **connect** the "External Control" connector on the Control Unit's rear panel with the LCQ's "Peripheral Control" connector.

2. **Check** the "Autosequence-Enable" check box in the main *TARGET* software window (see Fig. 26) and **check** the "Contact Closure Timing" check box in the *TARGET*'s "Settings Dialog" window (see Fig. 28).

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 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 operation system. If the Ctrl button is depressed than 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. Run the *Xcalibur/Instrument Setup* program. Create an Instrument Method or open an Instrument Method file (see Thermo Finnigan's *Xcalibur* software manual for details). Click on the Contact Closure page tab (see Fig. 34 below).

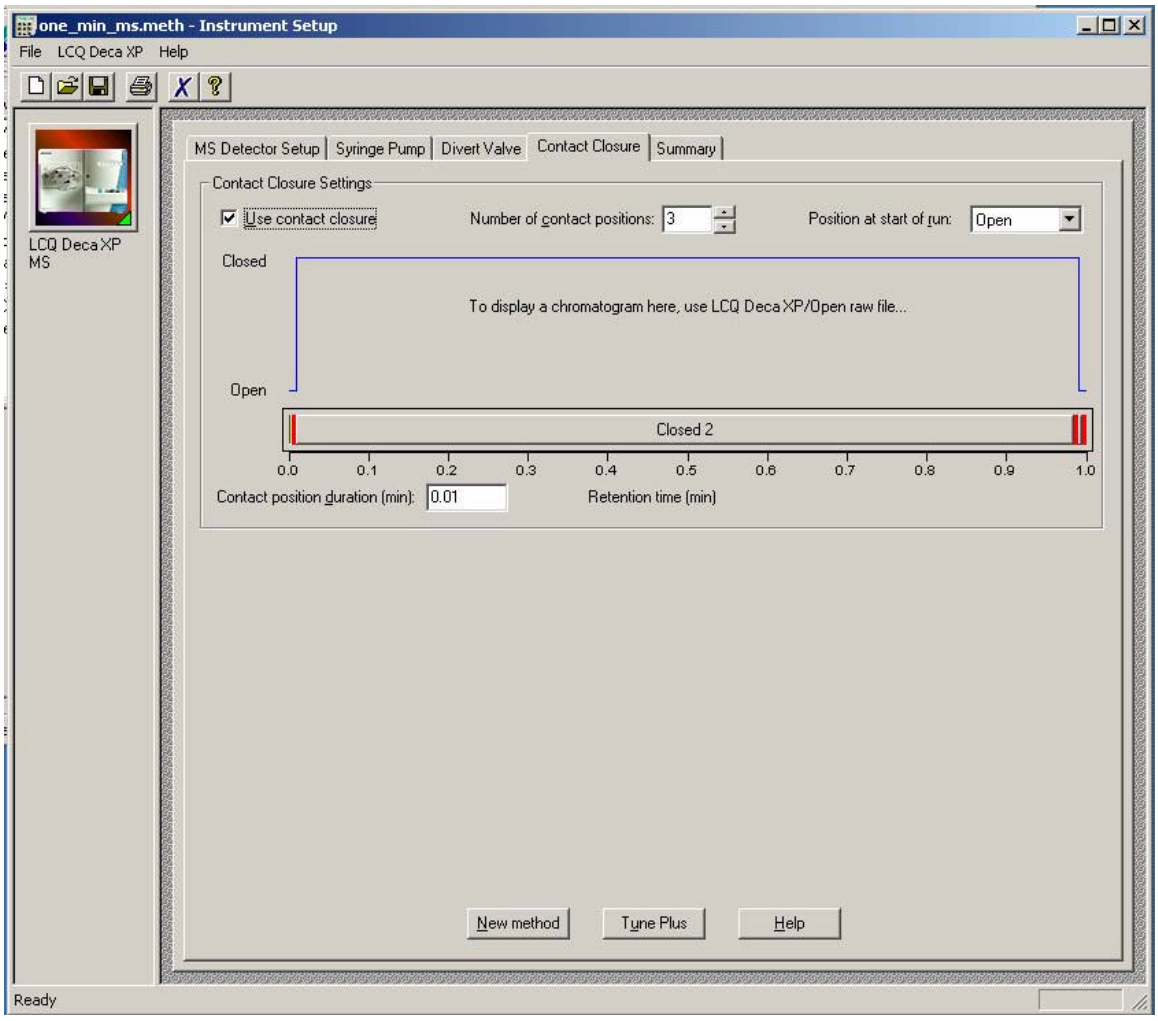


Fig. 34

Make changes to this page so it looks exactly like that shown in Fig. 34 above including:

- Checking the "Use contact closure" check box
- "Number of contact positions:" 3
- "Position at start of run:" Open
- "Contact position duration (min):" 0.01 when Position 1 or 3 is selected.

Save the settings to the Instrument Method file and close the *Instrument Setup* program. These settings ensure the generation of the LCQ proper control signals on contacts 1-2 of the "Peripheral Control" connector for controlling laser firing by the *TARGET* software. They will be used later during data acquisition.

6. Run the *Xcalibur/Sequence Setup* program. Create an Xcalibur Sequence or open an Xcalibur Sequence file (see Thermo Finnigan's *Xcalibur* software manual for details). The number of samples in the Sequence Setup window table (see Fig. 35) should correspond to the total number of samples selected for analysis in step 4.

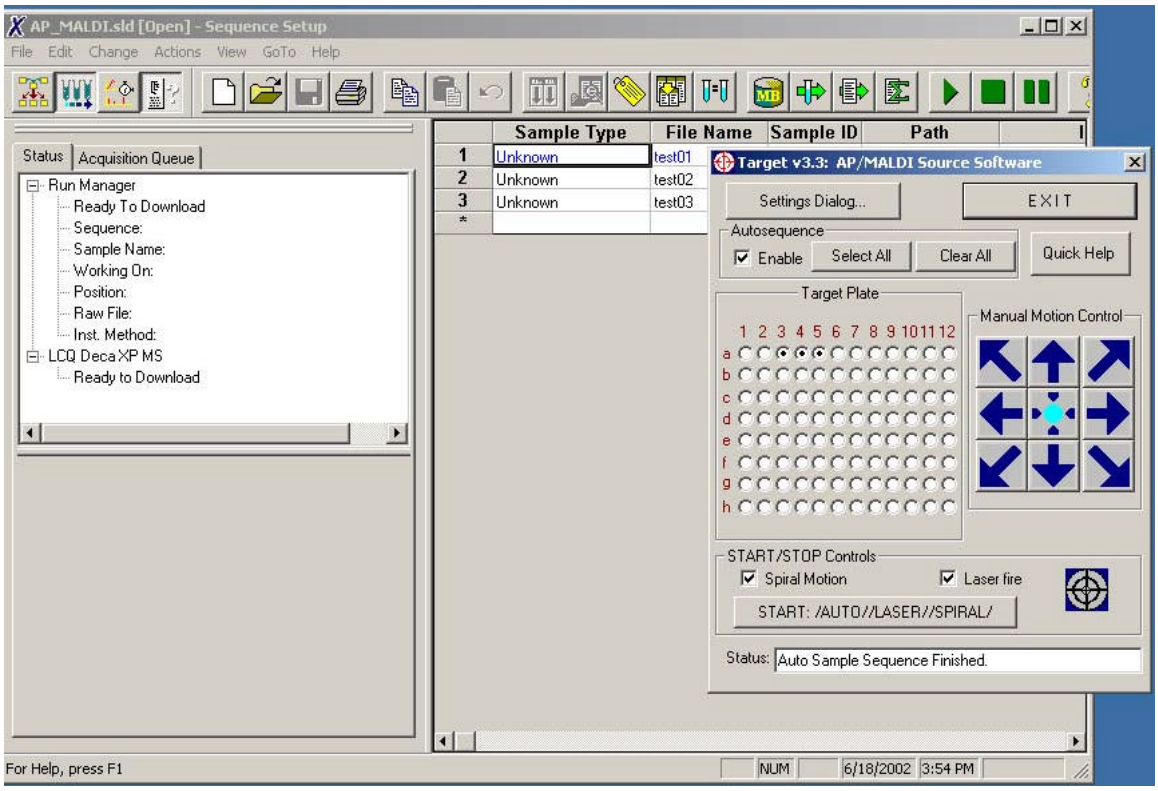


Fig. 35

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 Help/Sequence Setup menu in the *Xcalibur/Sequence Setup* program for more details on the creation and editing of the Sequence Setup table. Make sure that all files in the Instrumental Method column of the Sequence table were saved with Contact Closure settings described in previous step 5. The files where the acquired data will be saved are described in the File Name column.

7. Go to the *Actions/Run Sequence* menu to open the Run Sequence window (see Fig. 36 below).

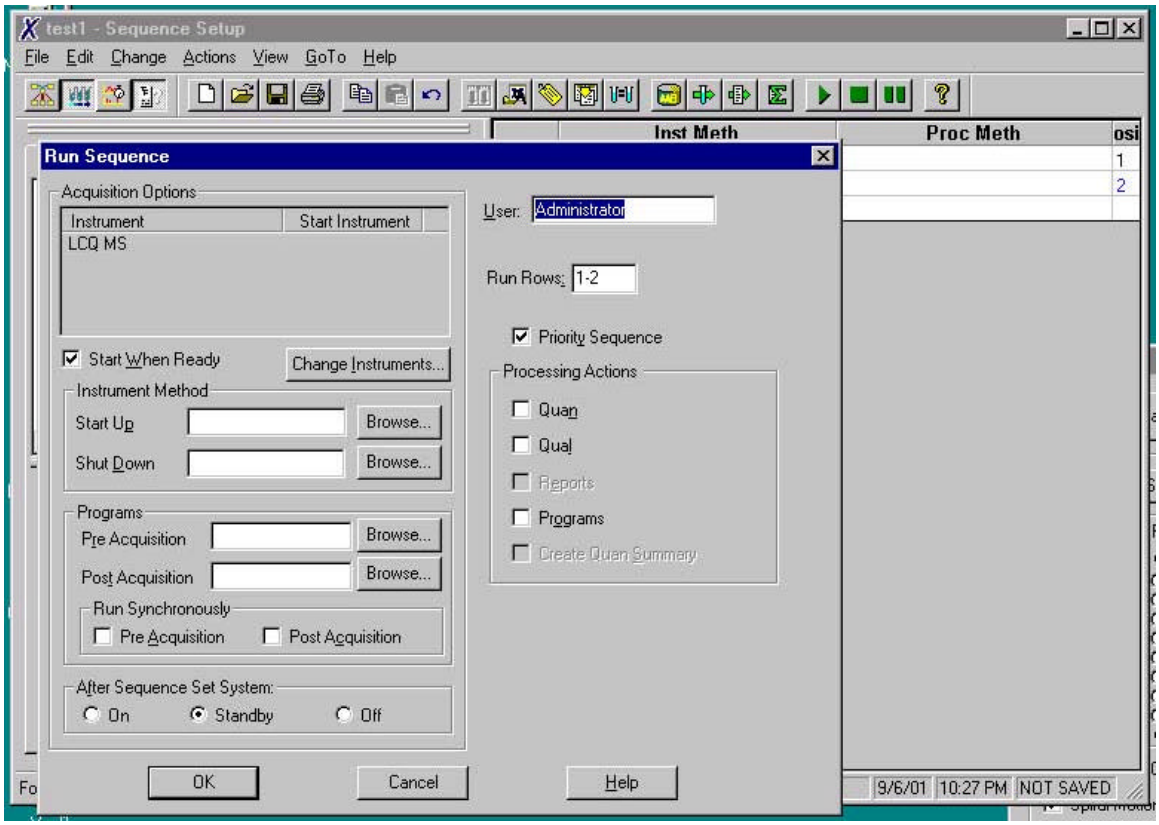


Fig. 36

Make sure that LCQ is **not** chosen as the Start Instrument (no "Yes" in the Start Instrument column against the LCQ line) and that the "Start When Ready" check box is checked (like in the example above). Leave empty the "Start Up" and "Shut Down" fields in the "Instrument method" box. Describe all rows, e.g., 1-20, in the "Run Rows:" field. Check the "Priority Sequence" check box to run the sequence immediately. Please use the *Run Sequence* Help if you have any problems changing the settings. The settings in the Run Sequence window ensure that the LCQ is properly triggered by the AP/MALDI hardware. Click the OK button to run the sequence. In the Status page of the *Xcalibur/Sequence Setup* program window you will see a "Downloading" message at the LCQ status line first and then a "Waiting for contact closure" message.

8. After the "Waiting for contact closure" message is displayed in the "Sequence setup" window (See Fig. 35), press the START button in the *TARGET* software window to start AP/MALDI operation. The "Running" message will be displayed in the LCQ status line which later will be replaced by the "Waiting for contact closure" 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 LCQ and AP/MALDI after data acquisition is completed. 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 STOP ANALYSIS button in the *Xcalibur/Sequence Setup* 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.

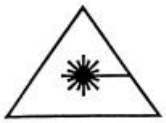
DO NOT ATTEMPT services or repairs that are not covered in this Troubleshooting section. For services and repairs beyond those specifically provided in the Troubleshooting section, contact the manufacturer, MassTech, Inc. 6992 Columbia Gateway Dr; Columbia, MD 21046; (301) 879-6994.

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 á -CHCA matrix. (á-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 á -CHCA matrix without the beam attenuation the crystals should disappears 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).



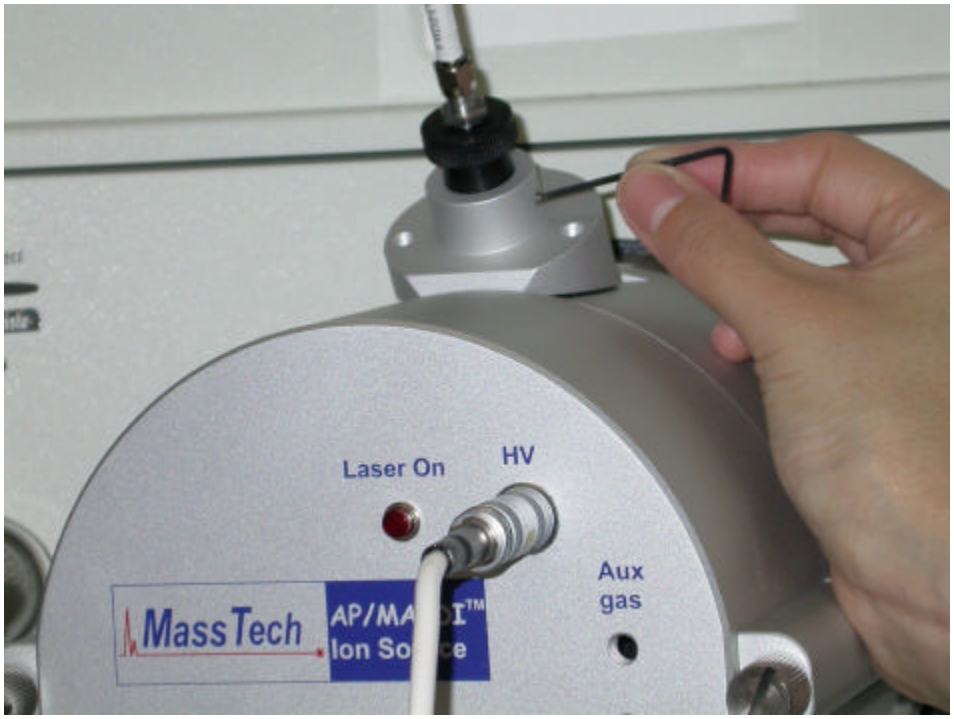
IMPORTANT: If you choose to replace the optical fiber, turn the power OFF on the Control Unit.

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.



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 LCQ 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 follows:

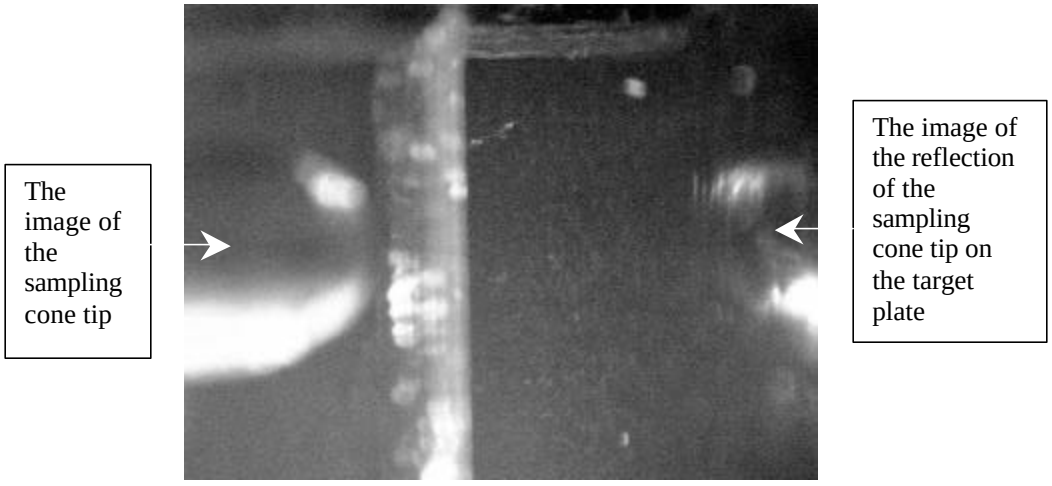


Fig. 37

Both the sampling cone tip 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:

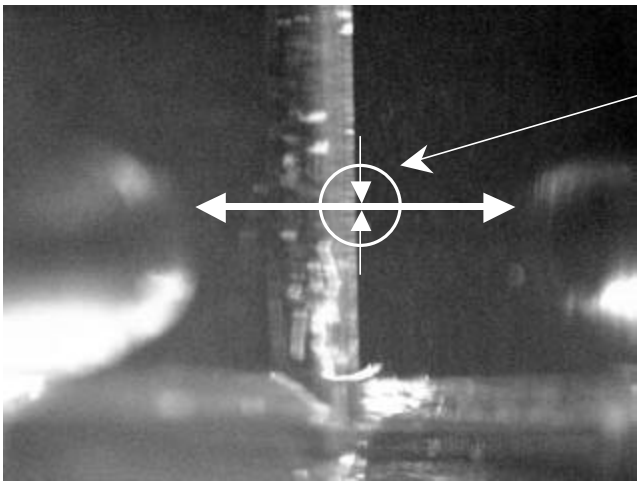


Fig. 38

The **ideal** position for the laser focal spot on the target surface plane is at the middle of the imaginary line that connects the image of the the sampling cone tip and the image of its reflection (see Fig.38). Now we need to determine the **real** position of that spot.

Move the target plate to a position where a matrix was deposited. Now the picture at the screen should look like the following:

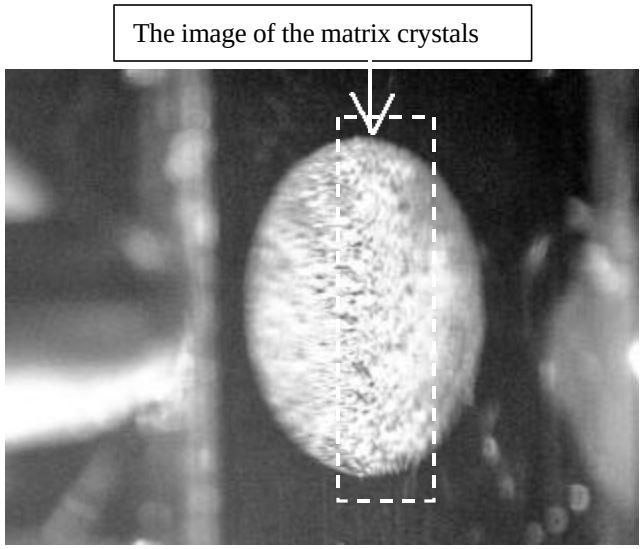


Fig. 39

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

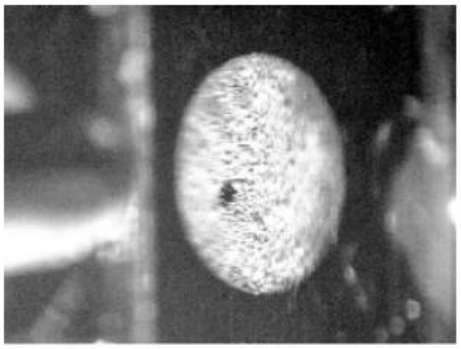


Fig. 40

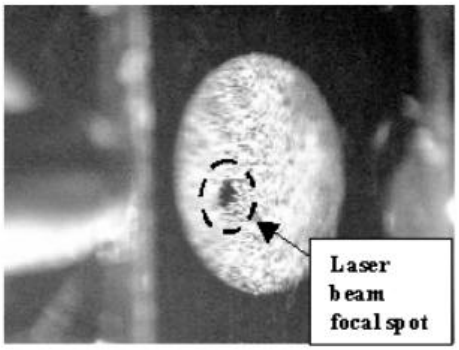


Fig. 41

By comparing Figures 40, 41 and 38 we can see that the laser focus is close to its ideal position, but slightly below and to the left. The deviation of the focal point shown in Figures 40 and 41 is acceptable, but the source sensitivity can possibly be improved by fine tuning.

Step 3. Move the target plate to a fresh spot, like in Step 1 (Fig. 39). If you follow the procedure, you can continue with the same spot that was used for the laser spot position determination (Figs. 40, 41). Switch ON the laser at maximum power (minimum attenuation). Using a hexagonal screwdriver 3/32" turn the allen screw (see Fig. 42).

Fig. 42

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 at the middle of the imaginary line that connects the image of the sampling cone tip and the image of its reflection (see Fig. 38). For example, the position in Fig. 43 below is good enough.

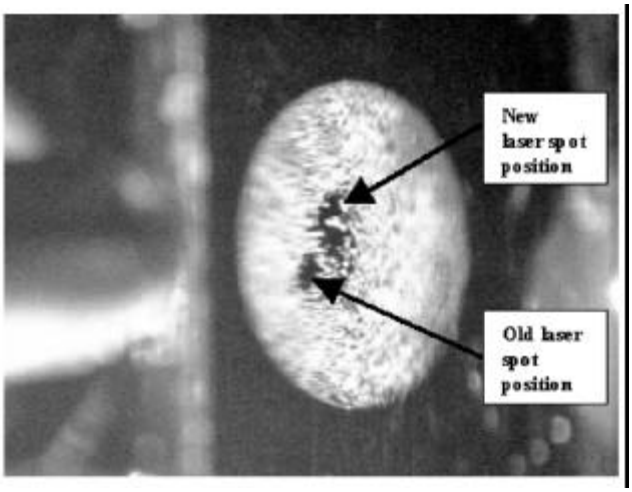


Fig. 43

Now you can set the best attenuation, appropriate for the matrix you use, shift the target to a new fresh spot and prove that the sensitivity is better.
Alternatively, the position of the laser focal spot can be adjusted by a rotation

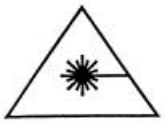
of tuning screws (Fig. 43) based on the quality of MS signal by a trail-and-error method.

7.4 PROBLEM: the Ion transport into the LCQ instrument is interrupted.

1. To test for interrupted ion transport into the LCQ instrument, prepare several target spots with a dense á -CHCA matrix. (á-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 á -CHCA matrix without the beam attenuation the crystals should disappears in 5-15 seconds). If they disappear, then:
5. Ensure that the LCQ interlock is operating properly. To do this Open the source. The "Vacuum" green light on the LCQ instrument should be off/green. If this is not the case, call MassTech for assistance.
6. Ensure that the high voltage cable of the LCQ instrument is attached tightly to the HV connector of the source.
7. Ensure that the Tune Plus (or Xcalibur) program is configured as described in this Manual.
8. Ensure that your probe preparation & matrix material are being used properly.
9. Finally, ensure that your LCQ instrument operates properly with the electrospray source attached. The problem may be with the LCQ 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 (301) 879-6994 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 (301) 879-6994 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.

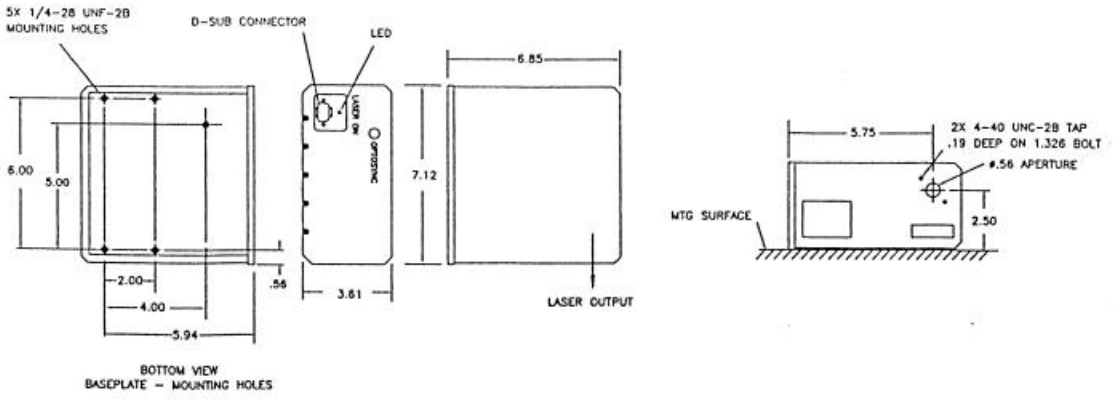
This limited warranty is void if the instrument has been damaged by accident, misuse, negligence, act of God, or serviced by any other person not authorized by MassTech, Inc. The warranty also does not apply to units that have had the serial lot number altered, defaced or removed.

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.

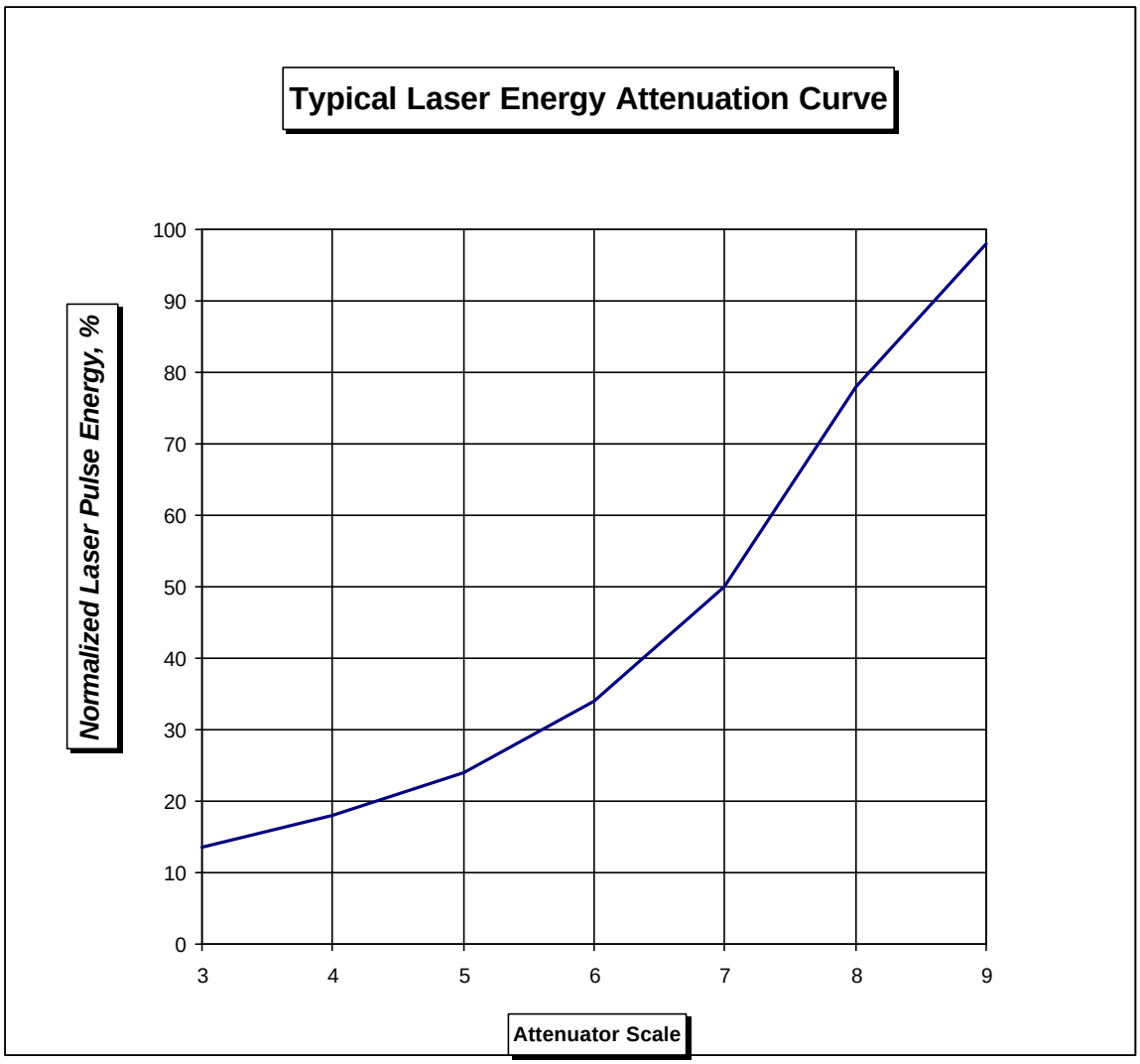
MassTech, Inc. disclaims liability for indirect, incidental or consequential damages. Exclusion or limitation of incidental or consequential damages are not permitted by some states and this limitation or exclusion may not apply to you. Warranty rights vary from state to state; and, therefore, you may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

APPENDIX A THERMO LASER SCIENCE 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 ì J
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 ì sec, <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
Optical Fiber cable

Part #6140004

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
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