AP/MALDI *PDF+* Source for Thermo Finnigan LCQ XP - Ion Trap Mass Spectrometers

Installation, Operation and Maintenance Manual

February 2007

Warning

Optical parts of the AP/MALDI *PDF+* 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 to 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 *PDF+* 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 8) 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 *PDF+* source manufacturer, MassTech, or your sales agent. ONLY replace the fiber with an exact replacement from the manufacturer, MassTech (Replacement Part number 6140004)

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: support@apmaldi.com

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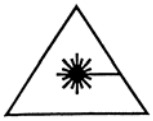
PREFACE

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 8, 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 *PDF+* A NEW SOURCE OF ATMOSPHERIC PRESSURE IONS

Atmospheric Pressure Matrix-assisted Laser Desorption/Ionization – AP/MALDI:

The AP/MALDI source is designed to produce molecular ions of analytes under normal atmospheric pressure conditions from a mixture of matrix/analyte microcrystals by irradiating these crystals with nitrogen laser pulses. These ions are analyzed by Thermo Finnigan's LCQ ion trap 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 Thermo Finnigan mass spectrometry instruments like ESI, APCI, nanospray, etc.

- The replacement of target (sample) plates is a simple and quick process, because the AP/MALDI source operates under atmospheric pressure, without the need to pump down or break vacuum.

- The AP/MALDI source is designed as an additional external source for Thermo Finnigan's LCQ trap instrument . The process of mass spectra measurement is completely decoupled with the 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 2 kDa in Normal mode or 4 kDa in High Mass mode (requires Xcalibur 1.4 and enabled High Mass mode from Thermo Finnigan). 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 4,000 Da. [NOTE: There are other versions of AP/MALDI sources adopted for different MS instruments (see website: www.apmaldi.com/ap_maldi.htm). In each of these cases, the analytical capabilities of the AP/MALDI-MS combination are based on the particular mass analyzer.]

- 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 [1,2,3].

Pulsed Dynamic Focusing Technology – PDF:

AP/MALDI has conventionally used continuous electric fields to extract ions into a mass spectrometer. Pulsed Dynamic Focusing (PDF) Technology applies a new electric field scheme whereby the extraction field is applied for only a brief timed interval after the laser pulse. By removing the electric field while ions are in transit from the target plate surface to the entrance of the mass spectrometer, ions avoid being lost to the entrance tip and walls and are instead entrained by the gas flow into the MS. The technique is termed Pulsed Dynamic Focusing because the electric field (between the target plate and MS inlet) is *pulsed* to zero at an optimal time, so that ions are *dynamically focused* into the MS. PDF Technology significantly improves the signal level and reliability of AP/MALDI [4].

Advantages of PDF are:

- Increased transmission efficiency of ions into MS.
- Higher Ionization Efficiency at greater Voltage setting.
- Greater sample throughput when larger laser spot size is applied.
- Insensitivity to laser misalignments.

PDF is integrated directly into the AP/MALDI PDF+ ion source and is controlled via the Target Control software.

1.1 QUICKSTART OPERATION

This section covers basic operation of the AP/MALDI PDF+ source after the AP/MALDI PDF+ source, Target software, and the 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 5 of this manual, the operation steps are as follows. NOTE: 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 4 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 with these initial settings:
   Plate voltage: 3.0kV; Accu Time=220ms,
   PDF Pulse Delay Time 20μs.

3. Prepare a MALDI Sample according to Section 6 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 6.1 of this manual. Ensure that you close and "click" in the Source securely.

5. Use the Target software to fire the laser and test your samples. To operate in Manual mode (spot by spot spectra measurement), make sure that the AutoSequence toolbar icon is unchecked, and choose a desirable spot using the Target software. Adjust the position of the laser using the target image on the Computer screen, if necessary. Start LCQ data Acquisition. Start the Laser firing and (optionally) spiral/raster motion (in the Target program).

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). Now you can repeat the procedure for other spots (a detailed explanation of automatic

operation is included as Section 7.5 of this manual).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 *PDF*+ source is not strictly necessary for successful practical use of the source. However, a brief summary of the apparatus and operating mechanisms are provided here for completeness. The description of the AP/MALDI *PDF*+ source is better understood by explaining the AP/MALDI operation separately from the PDF technology. Thus this section will first focus on the description of the AP/MALDI process, while Section 3 will describe the PDF operating principles.

Ion Source:

A simplified scheme of the AP/MALDI *PDF*+ source is presented in Fig. 2-1 below.

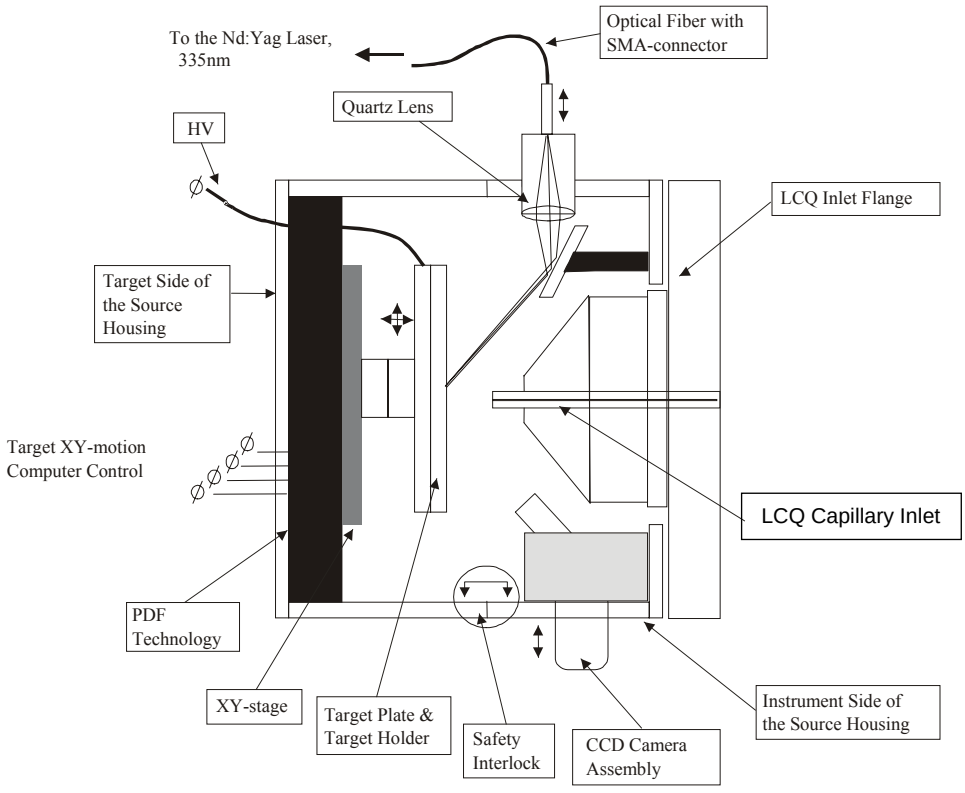


Fig. 2-1. Simplified schematic diagram of the AP/MALDI *PDF*+ source installed on Thermo Finnigan's LCQ trap instrument.

The following explanation of AP/MALDI basics will become clearer as you set up your unit. The AP/MALDI *PDF*+ ion source is mounted inside a Housing. The source **Housing** is attached to the **LCQ Inlet Flange.** Ions produced inside the source **Housing** travel toward the inlet orifice of the **LCQ** with a stream of gas. The source Housing consists of two connected halves, 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 96 sample spots can be deposited on the surface of each **Target Plate**. High Voltage (typically, 3 kV) is applied to a **Capillary Extension** 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 laser light pulses. High repetition rate all-solid-state Nd:YAG laser (wavelength 355nm) is mounted inside a Control Unit (not shown in Fig. 2-1) and is connected to the AP/MALDI *PDF*+ source by **Optical Fiber**. UV laser 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 imaging optics enable the user to monitor the target plate motion and the sample evaporation processes from a COMPUTER's video capture screen (not shown in Fig. 2-1). Inside the source Housing there is also a source of visible light (not shown in Fig. 2-1) to illuminate the target plate surface. The AP/MALDI *PDF*+ 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**.

Control Unit:

The second important part of the AP/MALDI *PDF*+ 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 USB port that controls the target plate motion and laser firing. Either a separate (PC) computer or the LCQ control computer can be used to operate the AP/MALDI *PDF*+ source. The USB connection is used to communicate between the Control Unit and a computer. Inside the Control Unit is all-solid-state Nd:YAG laser (wavelength 355nm). (Appendix A is a list of specifications for this OEM laser).

3 PDF PRINCIPLE OF OPERATION

Pulsed Dynamic Focusing (PDF) is an added feature to AP/MALDI which changes the electric field scheme in the ion source so that ions are focused into the MS inlet. PDF technology allows more reliable operation of AP/MALDI and improves performance.

The voltage scheme for PDF is described in Figure 3-1. Each laser pulse is used to trigger a high voltage switch, after a user-defined timed interval (pulse delay). The switch immediately removes the electric field between the target plate and capillary for a hold time of >1ms, and then afterwards allows the electric field to return back to its original level. The electric field is removed by pulsing the target plate to the same voltage as the capillary for the set hold time.

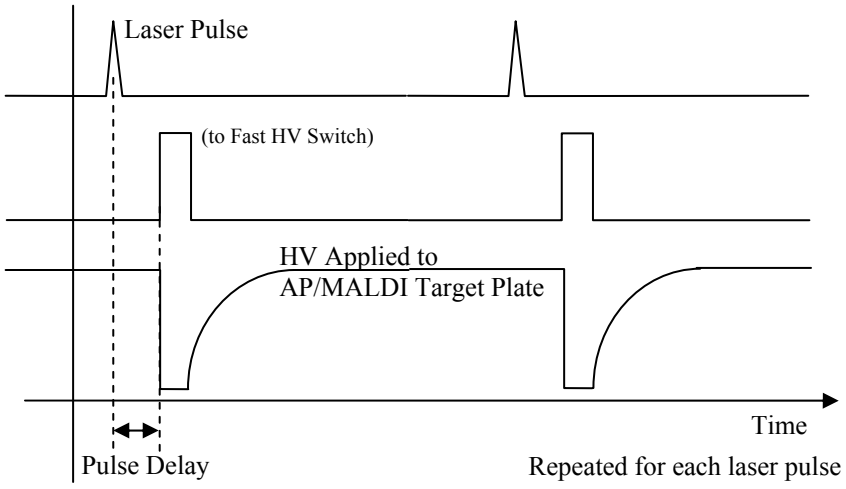


Figure 3-1. Voltage scheme for AP/MALDI PDF+

PDF improves S/N ratio at higher voltages. PDF also allows larger laser spot sizes to be effectively utilized. The combination of working at higher voltages and utilizing larger laser spot areas result in a sensitivity improvement in comparison to classical AP/MALDI without PDF. In addition, PDF technology allows misalignments in the laser position, of up to 1.2 mm (radial off-axis from the capillary axis) to not greatly affect sensitivity.

The user controls the "Pulse Delay" time through Target software so that ion signal is optimized. This is described in Section 7.7.

4 SAFETY PROCEDURES WHILE USING AP/MALDI PDF+

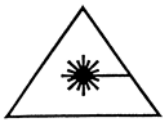
If operated properly, the AP/MALDI PDF+ source is safe. No special knowledge of laser safety or electrical safety is necessary to operate the source. There are two potentially hazardous factors connected with AP/MALDI *PDF*+ source installation, operation and maintenance/ troubleshooting:

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 components from UV radiation and High Voltage, provided that the AP/MALDI *PDF*+ source Power is TURNED OFF during installation/ uninstallation.

4.1 Safety Precautions

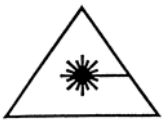
This section describes important precautions that must be observed during AP/MALDI *PDF*+ source ***installation, operation, and maintenance***. Appropriate precautions can be divided into the following stages:



- **Installing/Uninstalling:** Before the source is installed onto the LCQ instrument, uninstalled, or replaced, the LCQ instrument must be in either "Standby" or "Shutdown" mode. 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 *PDF*+ Control Unit before the source is ***completely installed***, and optical fiber properly connected at **both ends**.

When uninstalling, again: make sure that the LCQ is in Standby or Shutdown mode, switch OFF the power at the rear panel of the AP/MALDI *PDF*+ Control Unit; then start any disassembling operations or source detachment. The AP/MALDI *PDF*+ 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 *PDF*+ source to load or unload the target plate. It is recommended that you first switch the LCQ instrument to either "Standby" or "Shutdown" mode, stop laser firing (Click on the "Stop " button in the AP/MALDI PDF+ 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 6.1 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 *PDF*+ source safety interlocks automatically switch the High Voltage and the Laser OFF.

  In addition, depending on the capillary temperature and gas flows used in the source, the sample plate may become hot.

Caution: Target plate may be hot**!**

- **Mass Spectra recording:** Normally, the recording of AP/MALDI *PDF*+ 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 *PDF*+ source safety interlocks automatically switch the High Voltage and the Laser OFF.

- **Maintenance and troubleshooting:** The AP/MALDI *PDF*+ 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 8) of the present manual.

DO NOT ATTEMPT services or repairs that are not covered in the Troubleshooting section, Section 8 of this Manual. For services and repairs beyond those specifically provided in Section 8, 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 *PDF*+ source is not properly attached to the LCQ instrument or the optical fiber is not properly installed.**

4.2 Operator Controls and Indicators

The two figures below illustrate the front and back plate of the AP/MALDI *PDF+* Control Unit.

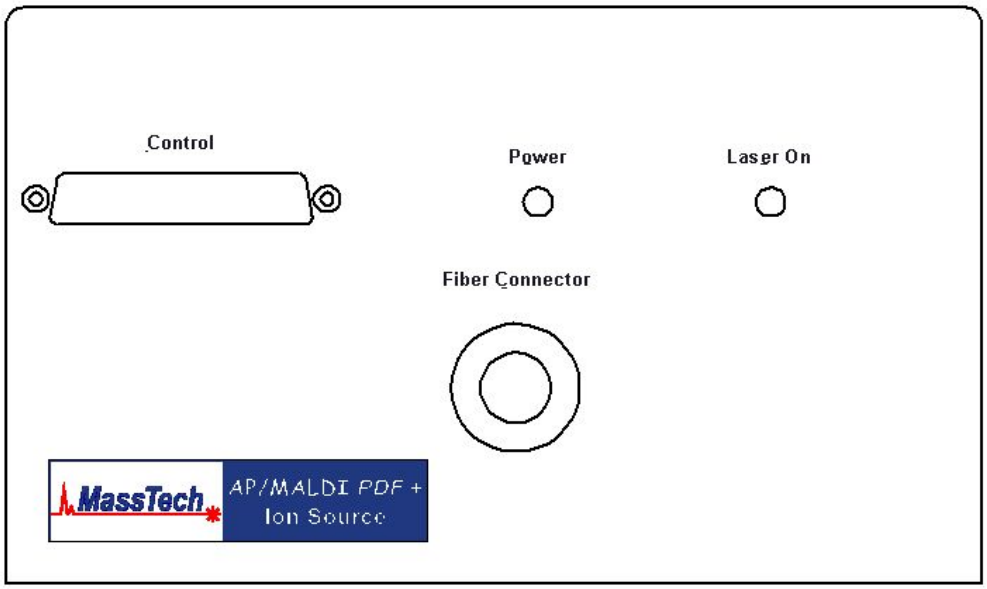


Fig. 4-1 The Control Unit Front Plate

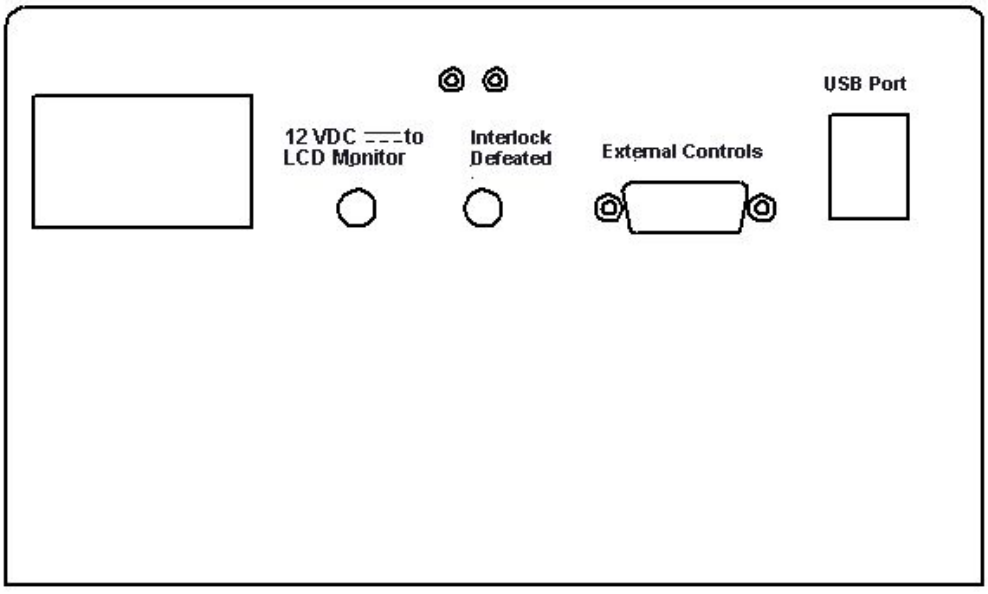


Fig. 4-2 The Control Unit Back Plate

5 SOURCE INSTALLATION

5.1 Checking that all components have been received.

Before you start installing your source, ensure that all necessary Parts and Accessories have been delivered. Figures 5-1 through 5-7 below show these components and introduce some definitions and part names used in the installation explanations.

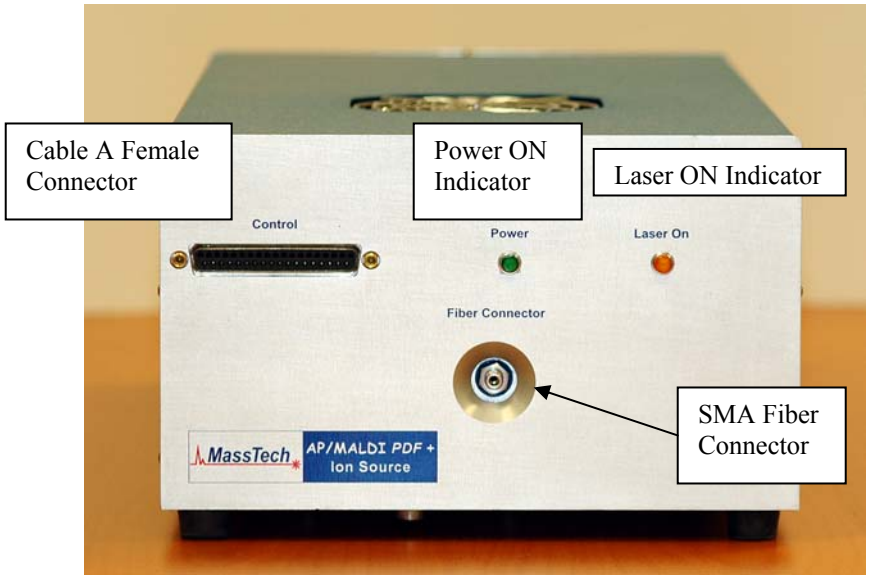


Fig. 5-1 Control Unit Front View

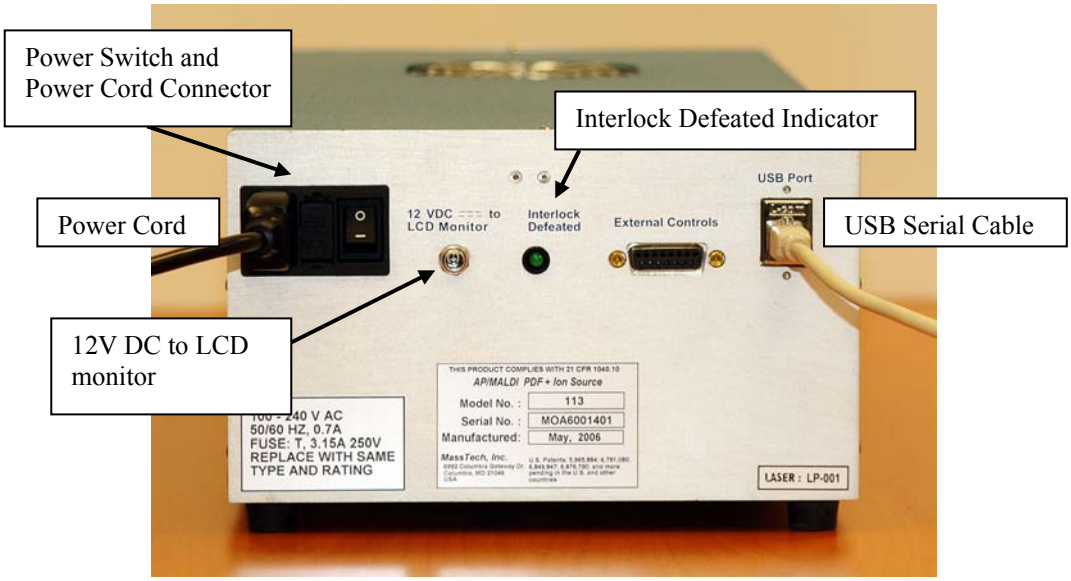


Fig. 5-2 Control Unit Rear View

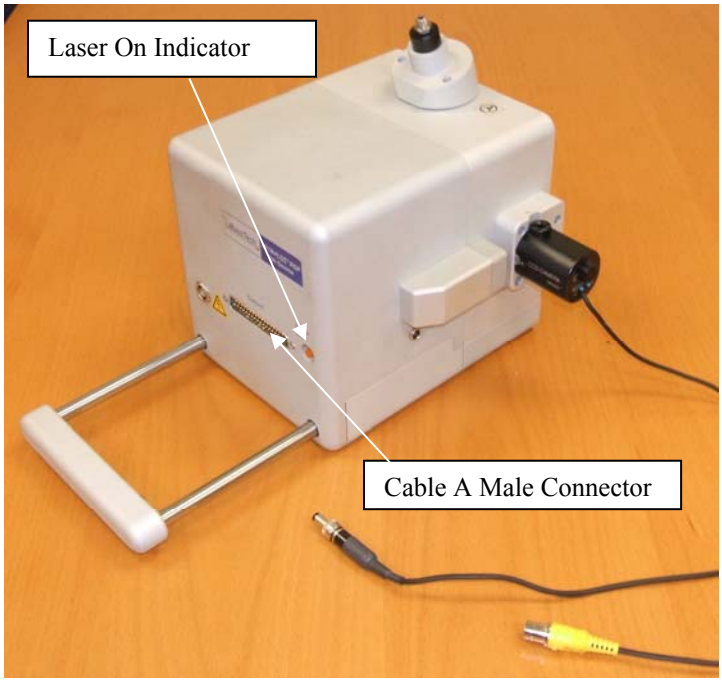
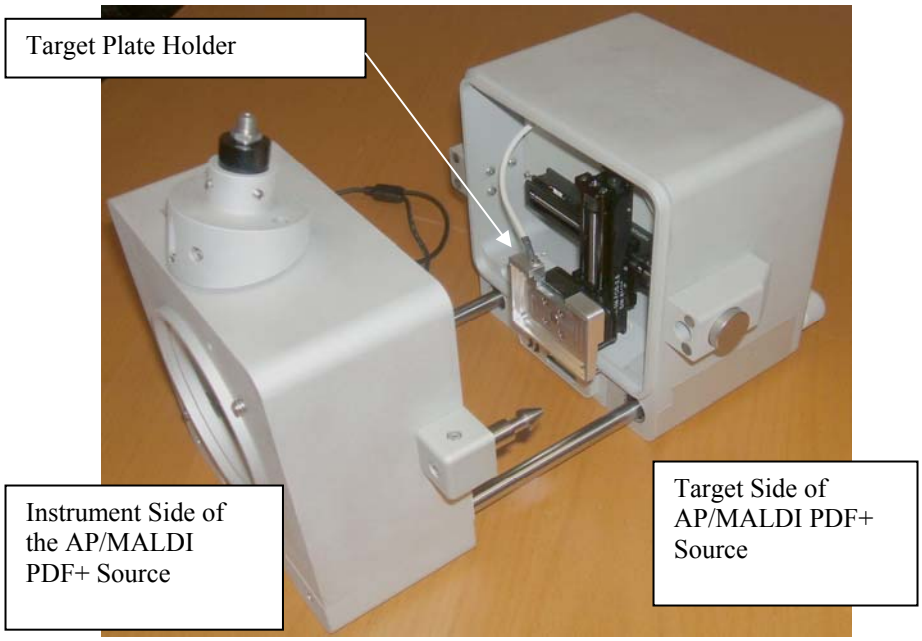


Fig. 5-3 Ion Source for Thermo Finnigan's LCQ trap



F**ig. 5-4** The AP/MALDI ion source opened illustrating the 96-spot target plate holder.

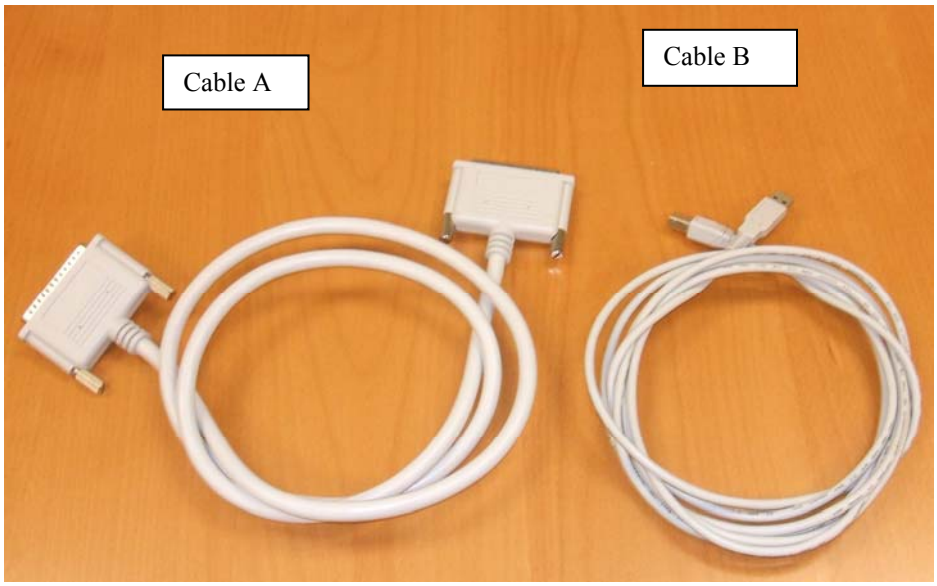


Fig. 5-5 Cable A (Control Unit – to – Source) and Cable B (Control Unit – to – PC, standard USB Cable).

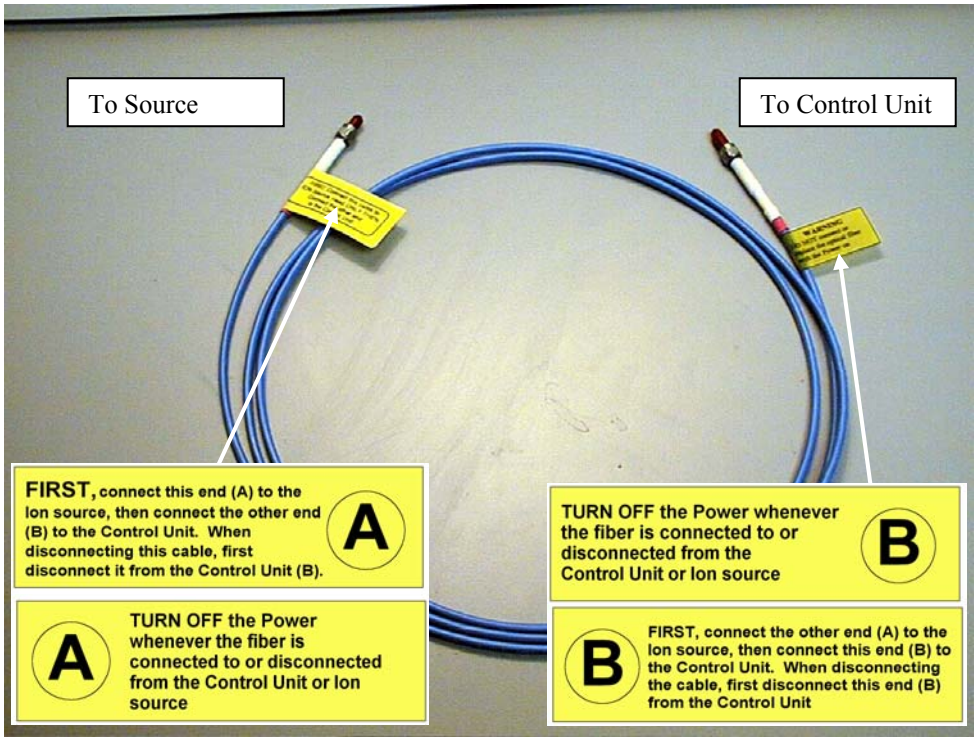
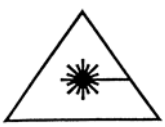


Fig. 5-6 Optical UV-grade Fiber with SMA-connectors labeled on both sides. SMA-connectors on both sides are covered with protective plastic caps. (The shipment includes one spare Optical cable, not shown in the figure).



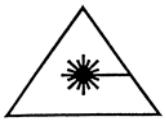
You must turn OFF the Control Unit (so the laser cannot be accidentally fired) whenever you have the optical fiber

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 6140004).

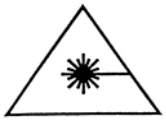
5.2 Installation of the Source

Installing/Uninstalling: Before the source is installed onto the LCQ instrument, uninstalled, or replaced, the LCQ instrument must be in either "Standby" or "Shutdown" mode. 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 PDF+ Control Unit before the source is ***completely installed***, and optical fiber properly connected at **both ends**, to the AP/MALDI PDF+ source.

When uninstalling, again, make sure that LCQ is in Standby or Shutdown mode; switch OFF the power at the rear panel of the AP/MALDI *PDF*+ Control Unit; then start any disassembly operations or source detachment. The AP/MALDI *PDF*+ 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.**

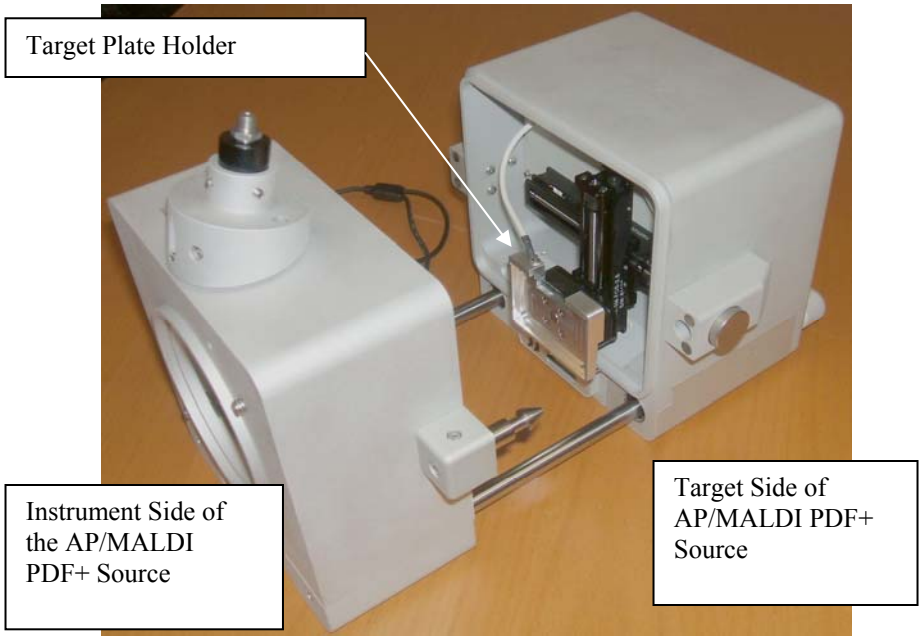


Fig. 5-7 The two sides of the AP/MALDI *PDF*+ Ion Source.

Remove your current source from the LCQ leaving an empty inlet flange (Fig 5-8). Unscrew the two inserts and remove the source rails and support.Your LCQ inlet should look like Fig. 5-9 when it is ready for AP/MALDI-*PDF* installation.

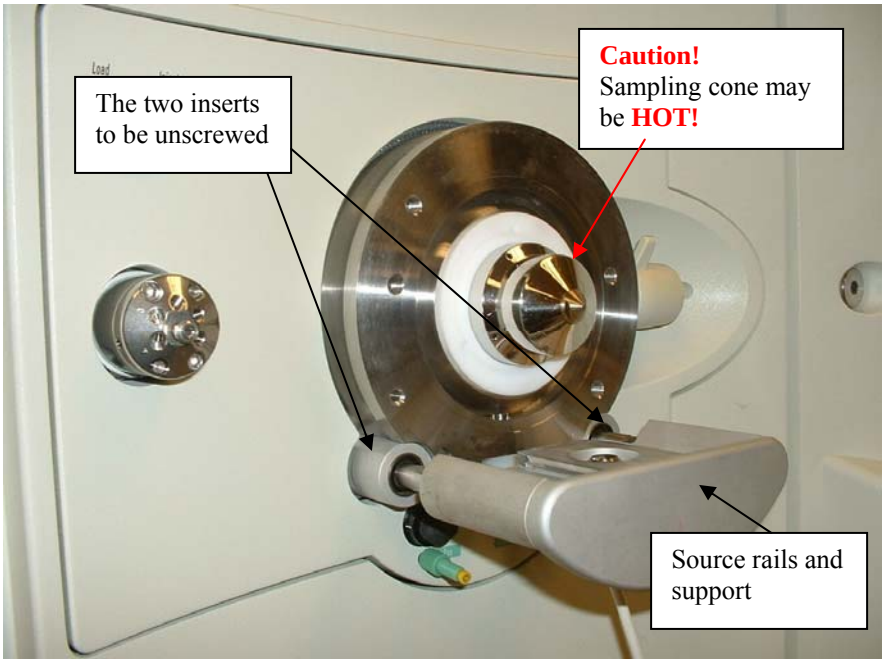


Fig 5-8 LCQ without any ion source attached.

Fig 5-9 LCQ instrument ready for AP/MALDI source installation.

Next, mount the AP/MALDI-PDF ion source (see Fig. 5-7) onto the LCQ, lining up the AP/MALDI-PDF source with the LCQ's inlet flange, and then screw the two screws using a screw driver (see Fig. 5-10).

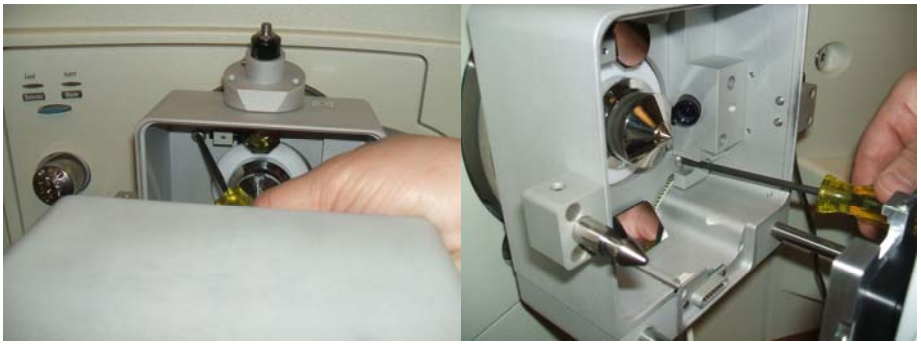


Fig 5-10 Screwing AP/MALDI source onto the LCQ inlet flange.

5.3 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.

Connect the black power cord and Cable B to the corresponding connectors at the rear plate of the Control Unit (Fig. 5-11)**. No adjustment is necessary for ~110/~127/~220/~240V AC!**

Fig. 5-11 The black power cord and USB Cable B connected to the back of the Control Unit. **No adjustment is necessary for ~110/~127/ ~220/ ~240V AC!**

Connect the other end of Cable B to a free USB port on your PC. Either the LTQ instrument computer or a separate PC can be used (Fig. 5-12).

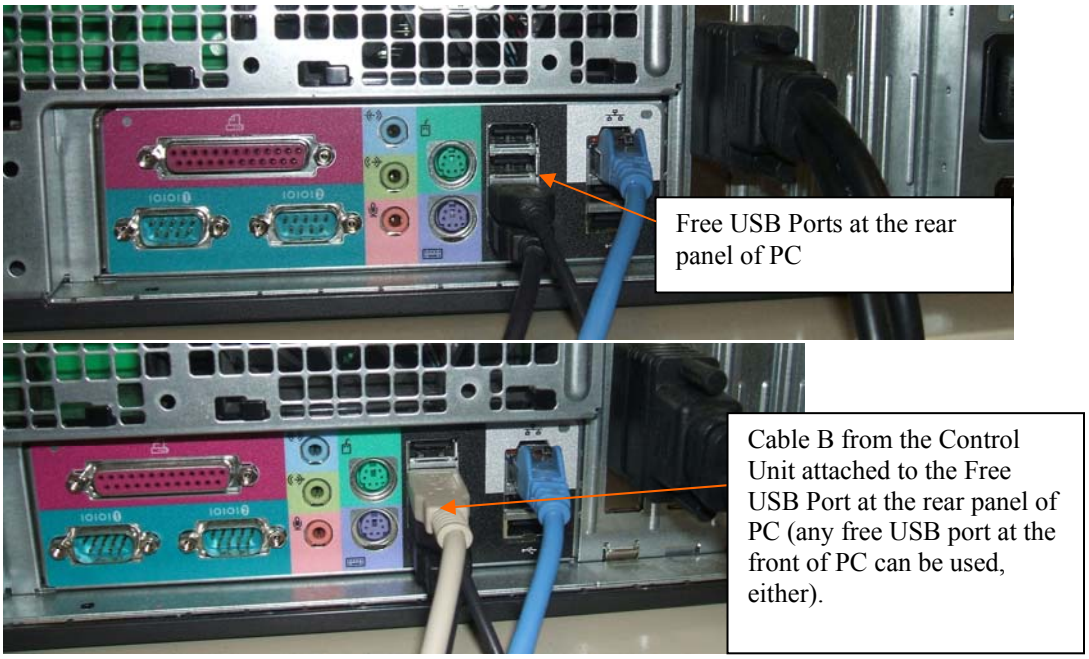


Fig. 5-12 The other end of Cable B connected to a free USB Port on a PC. Either the LTQ-instrument computer or a separate PC can be used.

The Video Out cable (Fig. 5-13) should be attached to either a video card on your computer or via a USB video adapter cable to your computer. The Camera Power Cable (Fig. 5-13) should be connected to its receptacle below the CCD camera as shown in Fig. 5-14.

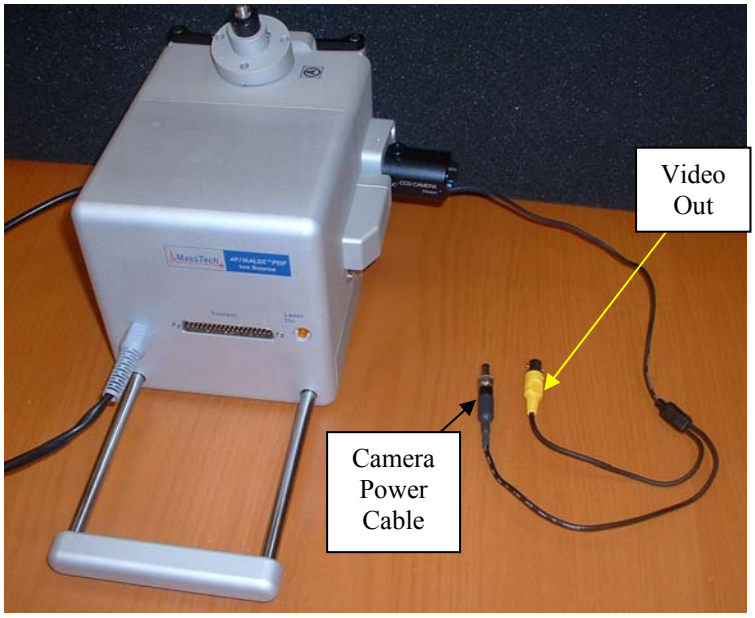


Fig. 5-13 CCD camera cables.

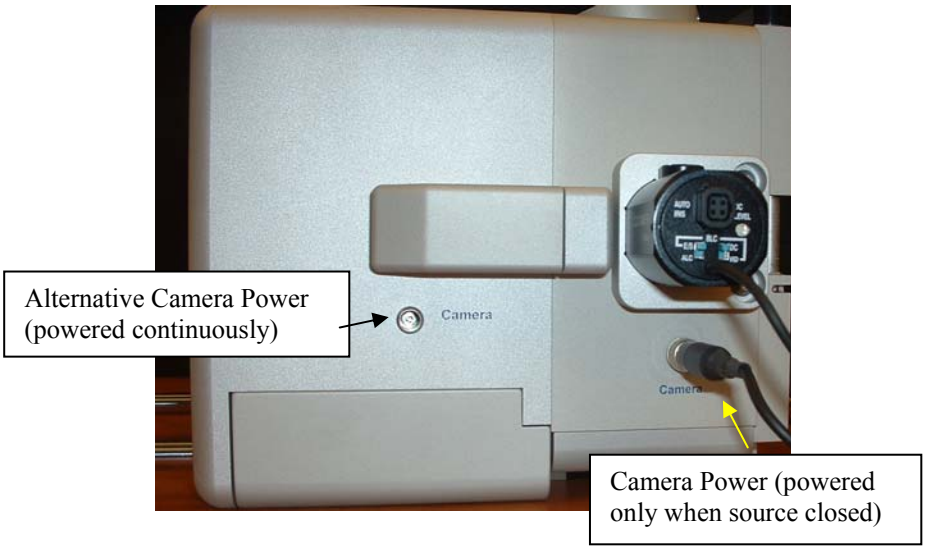


Fig. 5-14 Normal connection for Camera power cable. Alternative power port is to maintain imaging while source is open, and is used for testing/tuning purposes only.

Now, attach the other wires and cables to the source according to Figure 5-15 below. Refer to Fig. 5-16, 5-17 to help with the optical Fiber connections. Refer to Fig. 5-18 to help with the HV connection.

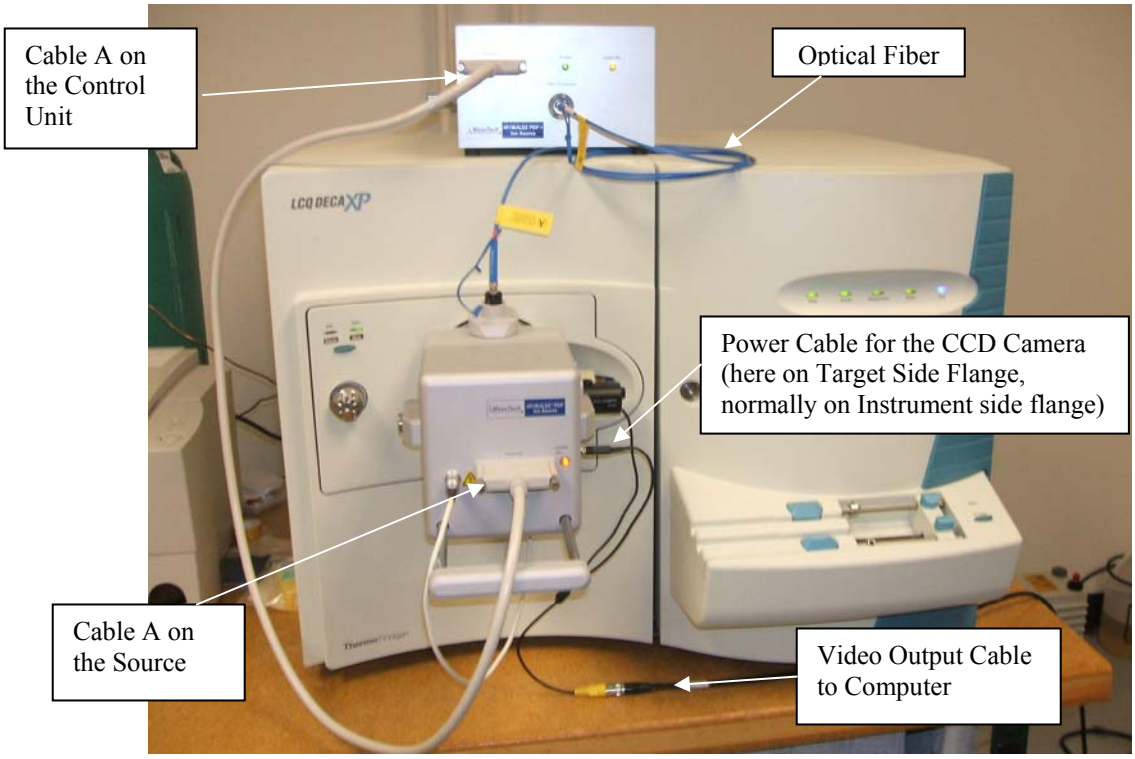
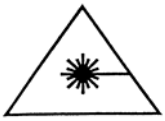


Fig 5-15 The Source with wiring connections completed.



When you install and uninstall the source on the LCQ, you must connect or disconnect the optical cable from both the Source and the Control Unit as shown in the figures below.

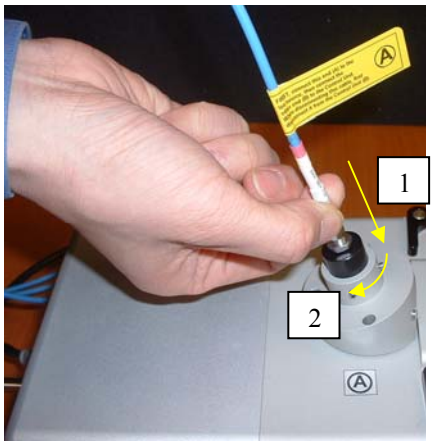


Fig. 5-15 Connecting fiber to Source (A to A)

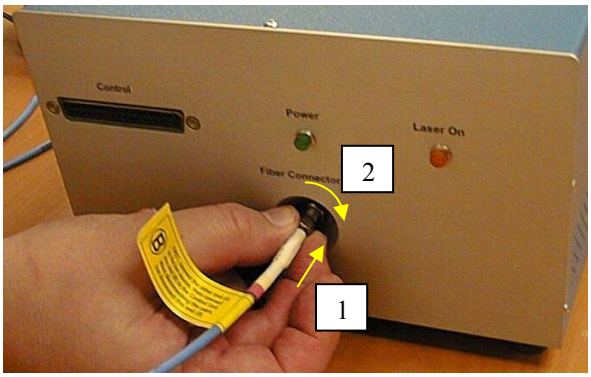


Fig. 5-16 Connecting the fiber to the Control Unit (B to B)

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

- Remove the plastic protection cap.
- Attach the optical fiber securely to the SMA-connector at the Source by pushing the fiber into the connector (1), and then tightening (2) the nut tightly according to the picture.

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.
- Insert the fiber, pushing it in, then tighten the nut.

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. However, safety interlocks are present which are designed to turn off the laser when an optical fiber is disconnected.

The HV Cable is connected from the Target Side to the Instrument Side of the ion source (Fig. 5-18).

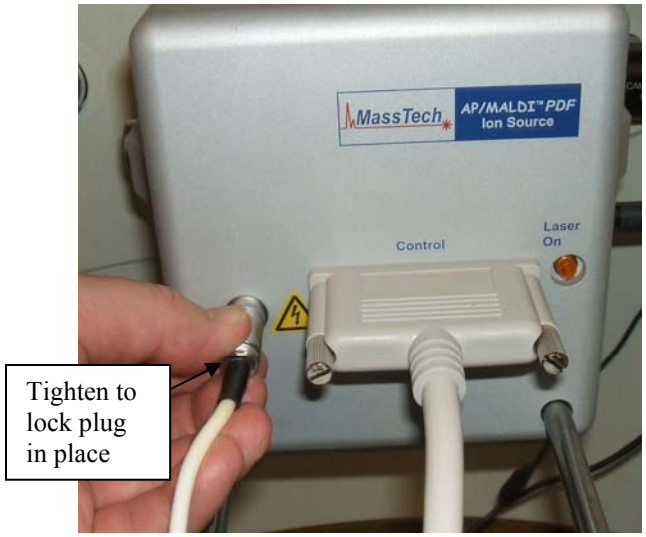


Fig. 5-18. HV Cable Connection. Tightening the knurled back end of the HV plug locks the cable in place.

When complete, your LCQ with the AP/MALDI PDF+ source will look like it is shown in Fig. 5-15 above.

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.

5.4 Fine Adjustments to Source

If necessary, fine adjustments to the imaging (e.g. Sharpness of focus) can be made as shown in Figs 5-19 and 5-20. But the unit comes pre-tuned.

Fig. 5-19 CCD camera alternative power cable connection for adjustments

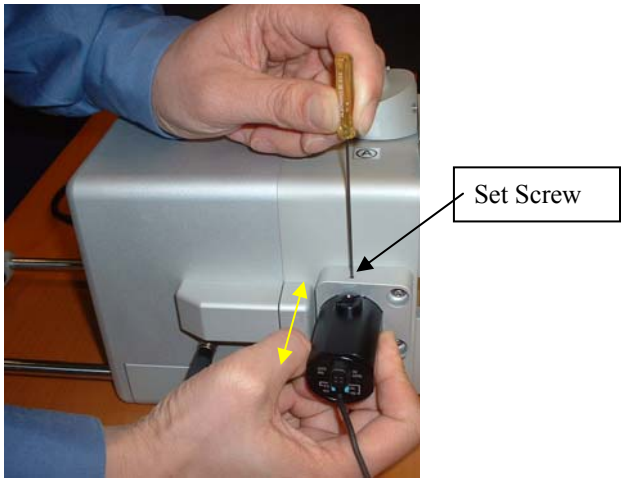


Fig. 5-20 CCD camera focusing.

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

NOTE: The Video Capture software also has brightness and contrast controls that can be adjusted to improve image quality. These controls are under the "Video Capture" menu located on the Video Capture box.

If necessary, fine adjustments to the laser positioning and focusing can be made as shown in Fig. 5-21 and Fig. 5-22, respectively but the unit comes pretuned. More details are provided in Sections 8.2 and 8.3.

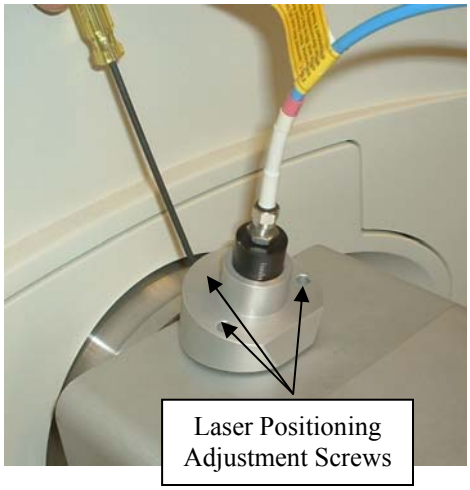


Fig. 5-21. Laser Positioned by adjusting three top screws.

Fig. 5-22.Laser Focused by loosening side set screw, adjusting focusing optic up/down, and then retightening set screw.

5.5 Source Removal 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, uninstall the source by reversing the installation procedure just described in Section 5.2.

6 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. 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.

Prepare several standard samples for testing the AP/MALDI PDF+. The following is a typical sample preparation procedure :

- 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, Gramicidin S and/or similar) with a concentration of around 500-1000 fmole/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).

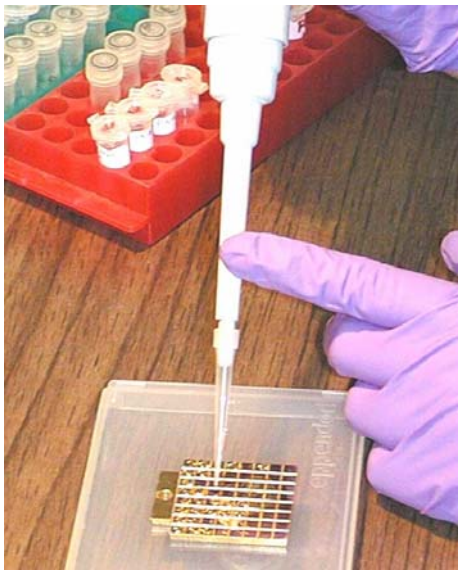


Fig. 6-1 Spotting of several standard samples on a target (sample) plate for testing by AP/MALDI. The sample preparation procedure is basically the same as for original MALDI experiments.

6.1 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 "Shutdown" mode, 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 PDF+ source safety interlocks automatically switch the High Voltage and the Laser OFF.

To open the source press the round silver button, located on the right hand side of the source, and pull the Target Side of the AP/MALDI *PDF* source away from the instrument.

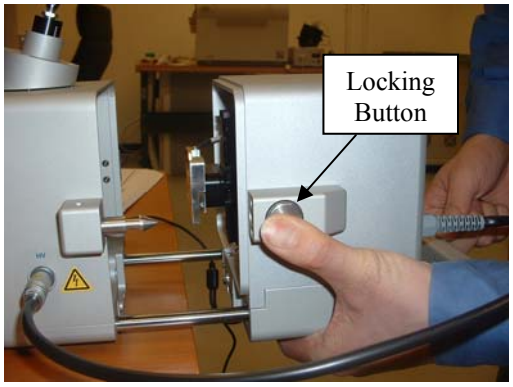


Fig. 6-2 Opening the source by pulling the Target Side of the source away from the instrument.

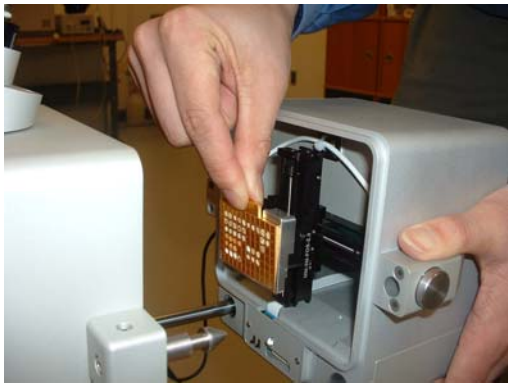


Fig. 6-3 Handling the Target Plate with the prepared sample spots into the Target Plate Holder. The Plate is held in place by a magnet.
Caution – the Target Plate may be hot when unloading.

When the source is closed, its two sides are held together with magnetic latches. There should be no visible gap between the two source flanges (see Fig. 6-4).

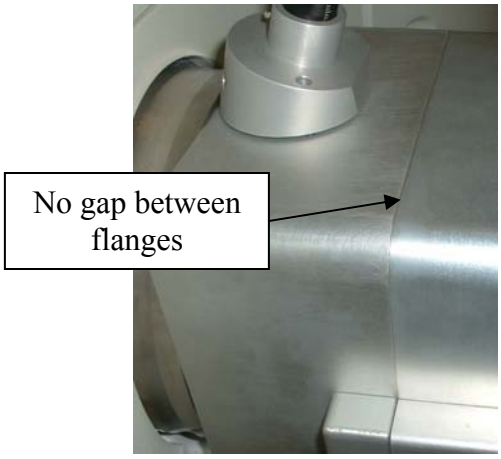


Fig. 6-4 The Source into the close position after target (sample) plate is loaded. Notice that there is no gap between the two halves of the ion source.

If your Target software is already running, you should see the following on the video capture image if the target plate is loaded properly.

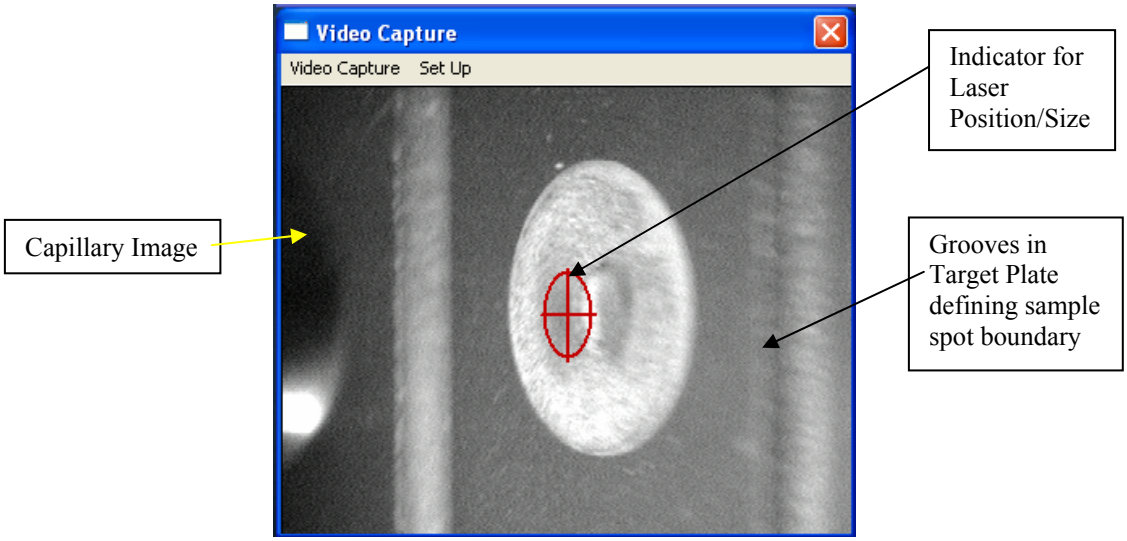


Fig. 6-5 MALDI sample ready for AP/MALDI PDF+ analysis.

7 AP/MALDI *PDF*+ OPERATION

7.1 Installing the TARGET *software and* USB Drivers

Before starting the installation of Target AP/MALDI source control software, install hardware/software for video capture to the computer. Refer the video capture Manual for the installation.

Target Version 6.0 software is used to control the AP/MALDI target motion, laser firing and PDF operation.

To install the software for AP/MALDI *PDF*, follow these steps:
(under Windows XP 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. Follow the next few dialog boxes to completion.

After the Target software installation process is completed, create a shortcut if you wish on your desktop to Target.exe.

Because USB port is used to communicate between the Control Unit and computer, you will also need to install the USB Drivers for this communication method.

To install the software to enable USB communication on the Control Unit, follow these steps: (under Windows NT or 2000 you will need Administrator access)

1. DO NOT connect the control unit to the PC computer through the USB cable (cable B). If the cable is plugged, unplug it at any side.
2. Browse the provided Installation CD "Target v. 6.0" to the directory ../USB To RS232 Serial Cable/. Run the installation program "PL-2303 Driver Installer.exe" from the directory. Follow the next dialog boxes to completion.
3. Connect the Control Unit and computer with USB cable (cable B).
4. Turn on the power to the Control Unit.
5. The computer should detect "New Hardware Found"
6. When asked for drivers insert the CD, go to the directory …\drivers to select the driver
7. Follow the next few dialog boxes to completion.

7.2 Starting the TARGET *software*

Start the Target program by either double-clicking on the desktop shortcut, or the target.exe file. The Target window (Fig. 7-1) appears.

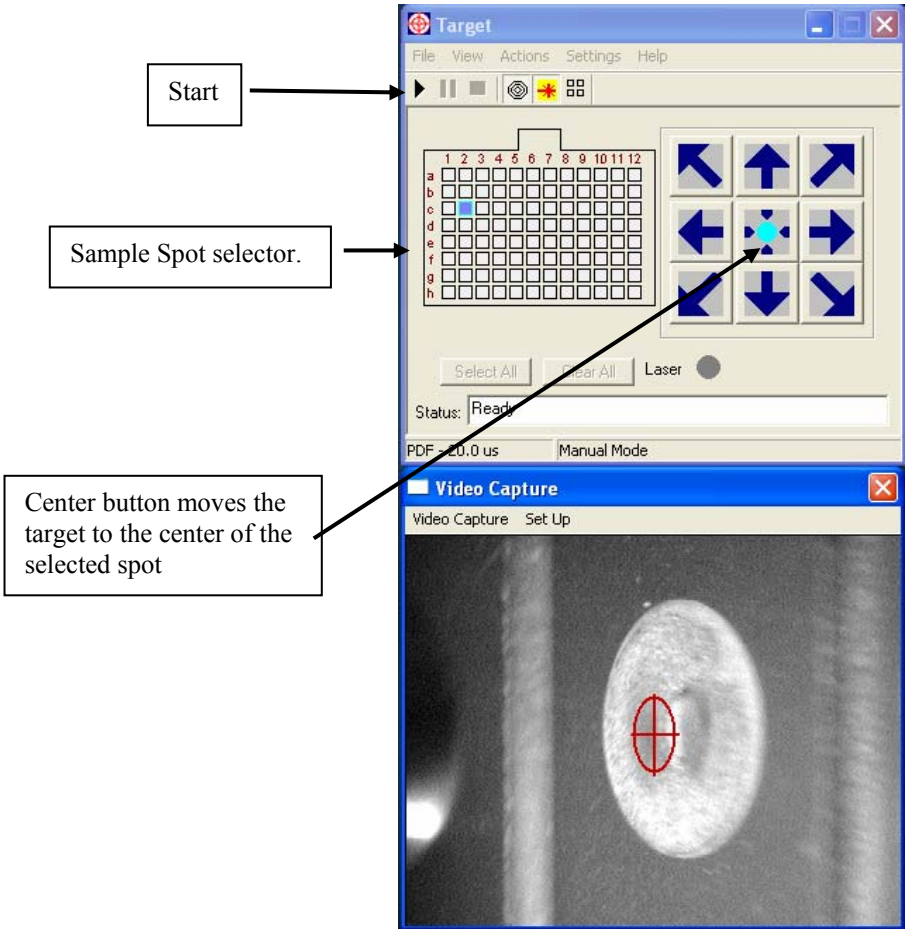


Fig. 7-1. Target software started, properly initialized and ready

At this moment the initialization of the XY stages will start automatically. If everything has been connected properly, you will see target motion on the Video Capture image. During initialization the target moves to its limit positions. After initialization, the target plate's first sample position is A1 (the upper left hand corner of the target (sample) plate).

If the Power On indicator on the Control Unit is OFF, or if the Control Unit is not properly wired to the computer, you will get the message shown in Fig. 7-2. Correct the problem, then reinitialize the software by going to Settings>Set Parameters>General>Init Motors. The Target software can also be reinitialized by exiting and restarting.

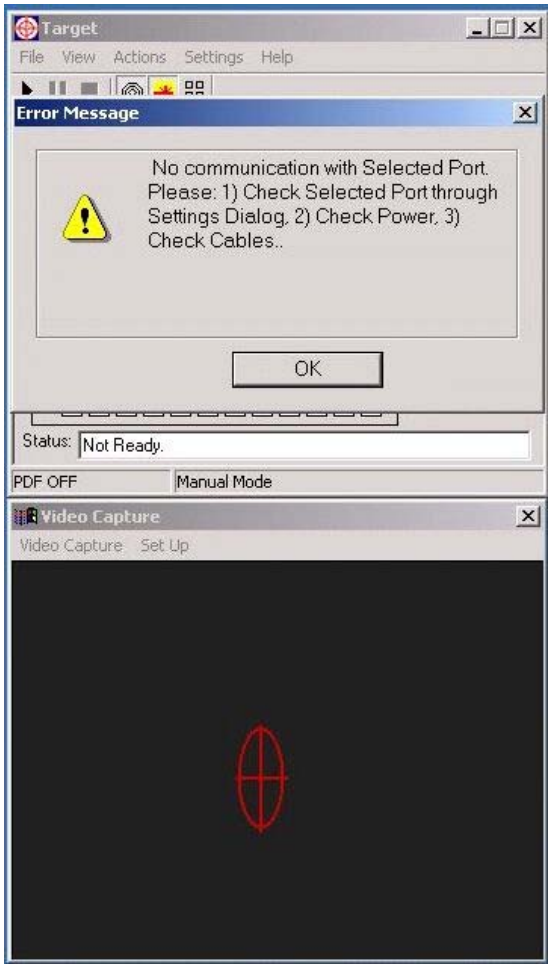


Fig. 7-2 Error Message if control unit is off, connections/ communications problem.

After the AP/MALDI *PDF+* source initializes, there can still be instances when the system is in a "Not Ready" state (see Fig. 7-3). The "Not Ready" status can be simply due to the source housing being open – in which case simply close the source for operation. Another possibility for the "Not Ready" status is that an interlock is open. Check to make sure the fiber optic is tightly secured to the connector if this message appears when the source housing is closed.

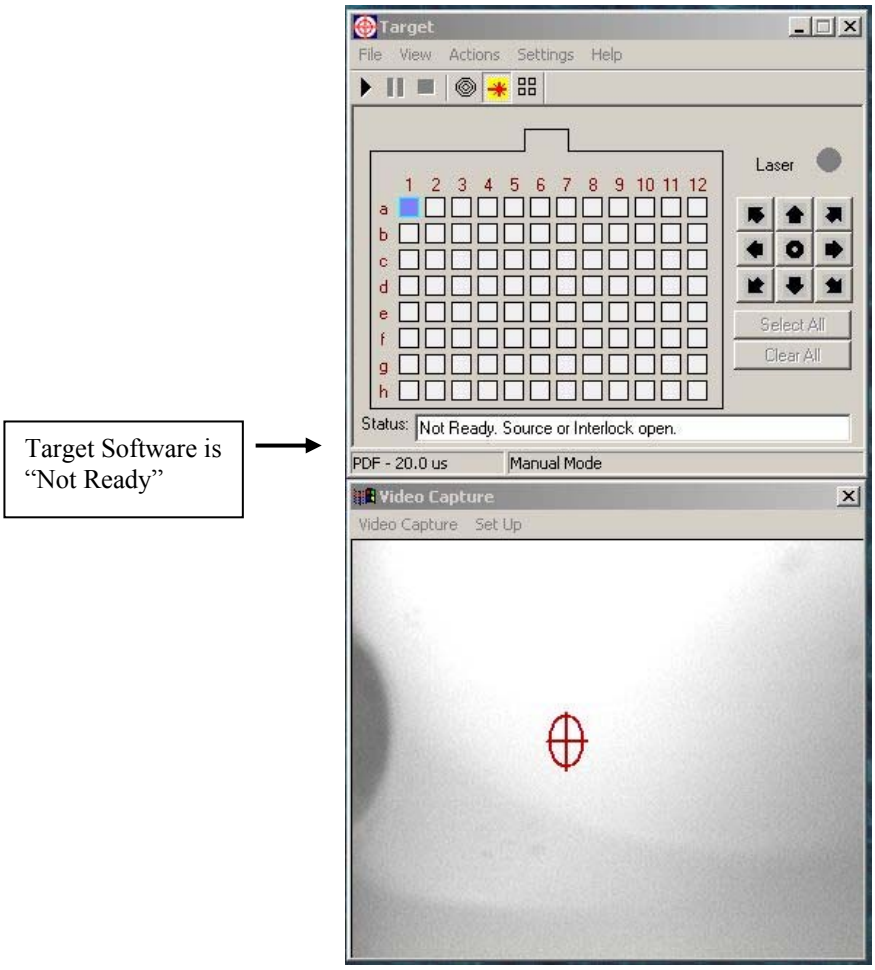


Fig. 7-3 Target software showing "Not Ready" state.

7.3 Positioning/Sizing of the Red (Laser) Cross-Hairs

The Red-Cross Hairs on the Video Capture image are used as an indicator for the laser position and size. The position and size of the Red Cross-Hairs in the Video Capture image should correspond to where the laser is firing and the approximate size of the laser beam area. NOTE: These Red Cross-Hairs are simply an indicator and do not physically adjust the position of the laser.

Adjust the position of the Red Cross-Hairs to coincide with the burn mark of the laser. It is easy to do this with Spiral/Raster Motion deactivated, and using a dense matrix. Hold down the *Ctrl* key and drag and drop the Red Cross-Hairs to the *position* where the laser is firing (Fig. 7-4).

For the size of the Red Target, Hold down the *Shift* key and drag to change the *size* of the laser beam (Fig. 7-5).

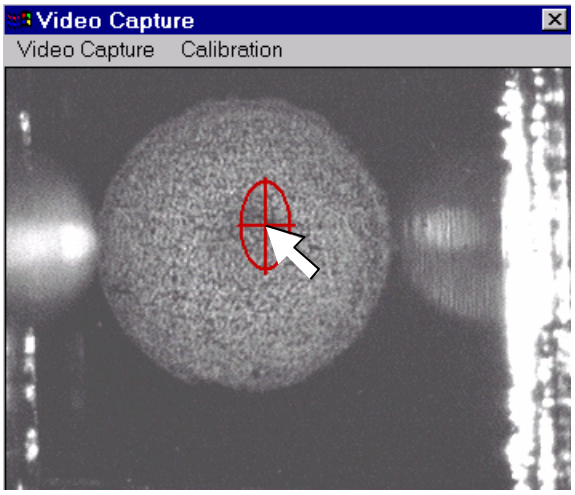


Fig. 7-4 *Positioning* of the Red (Laser) Cross Hairs by placing the mouse at the center of the Red Cross Hairs, *pressing Ctrl* and dragging the Red Cross Hairs to the position of the laser reflection/ablation.

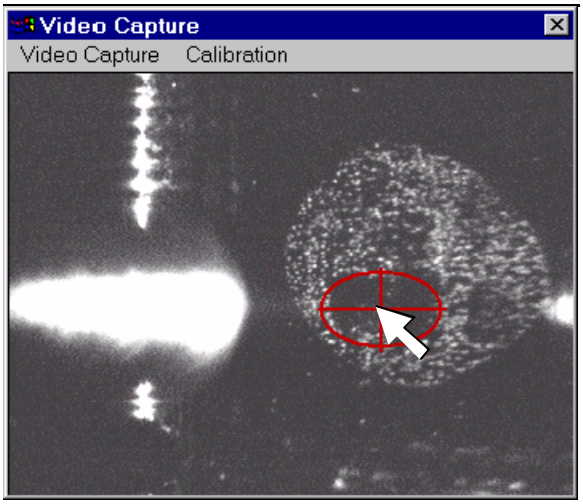


Fig. 7-5 *Resizing* of the Red (Laser) Target by placing the mouse at the center of the Red Cross Hairs, *pressing Shift* and dragging the mouse to resize the area.

7.4 Calibration of the Video Capture Sample Positioning System

To move the position of the sample that the user desires to be laser irradiated, a "point-and-click" system has been developed through the Video Capture imaging system. This "point-and-click" system uses the **mouse pointer** to **choose** a desired location on the sample image, and **double-clicking the left mouse button** to **move** the sample to the desired location. Before this system can be accurately utilized, it must be calibrated. To calibrate the positioning of the "point-and-click" positioning system, under the Video Capture menu bar item: "Calibration", choose "Start" (Fig. 7-6).

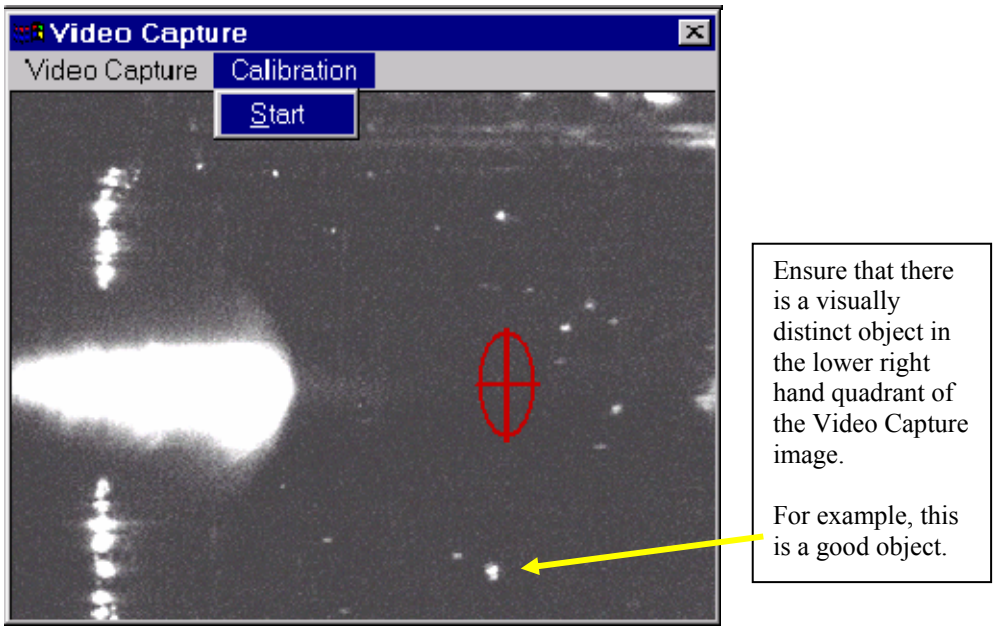


Fig. 7-6 Start of Video Capture sample positioning Calibration Procedure.

After beginning Calibration, a five step procedure will be described in dialog boxes. The first step is to ensure that there is a distinct visual object in the lower right hand quadrant of the screen (see Fig. 7-6). A sample plate with ablated matrix or dilute matrix can be used. If there is no distinct visual object, select another spot, or prepare a new sample (Section 6). Advance through Step 1 by clicking "Next" (Fig. 7-7).

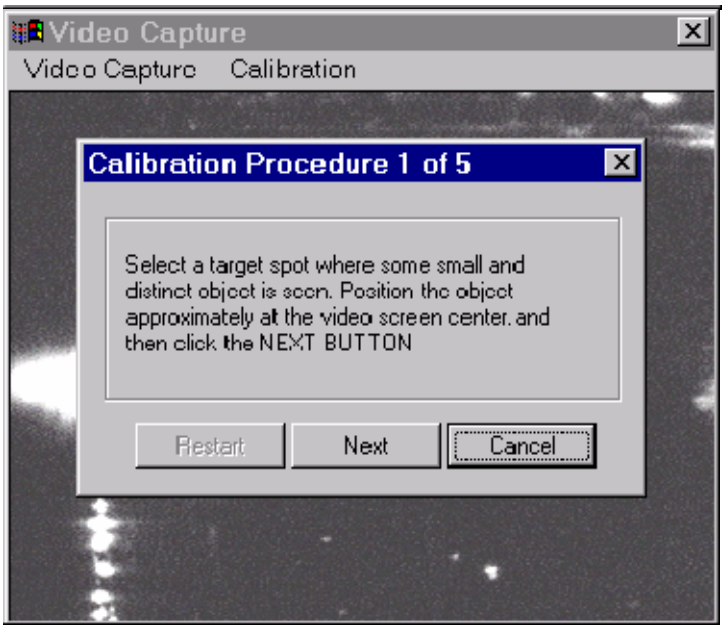


Fig. 7-7 Step 1 of the Video Capture Sample Positioning Calibration

In Step 2 (Fig. 7-8), drag and drop the green target icon to the visually distinct object in the lower right hand quadrant. Then click "Next".

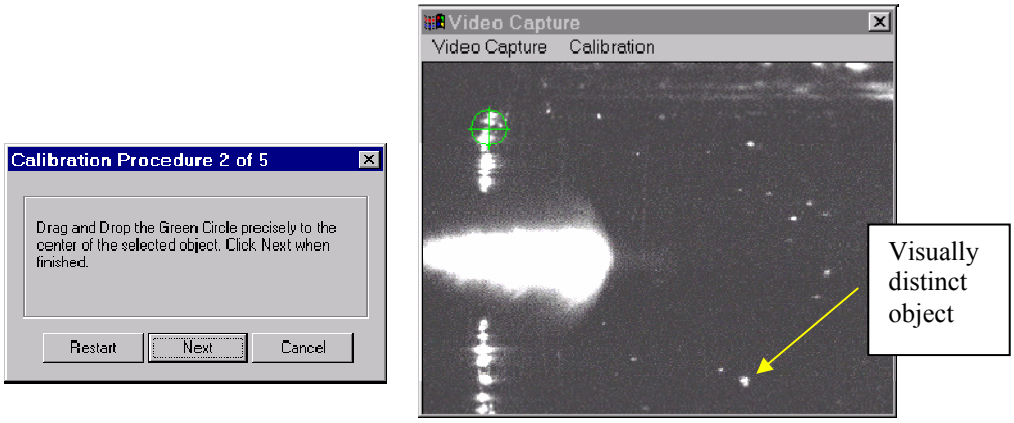


Fig. 7-8a Step 2 of the Video Capture Sample Positioning Calibration showing the position of the original green target.

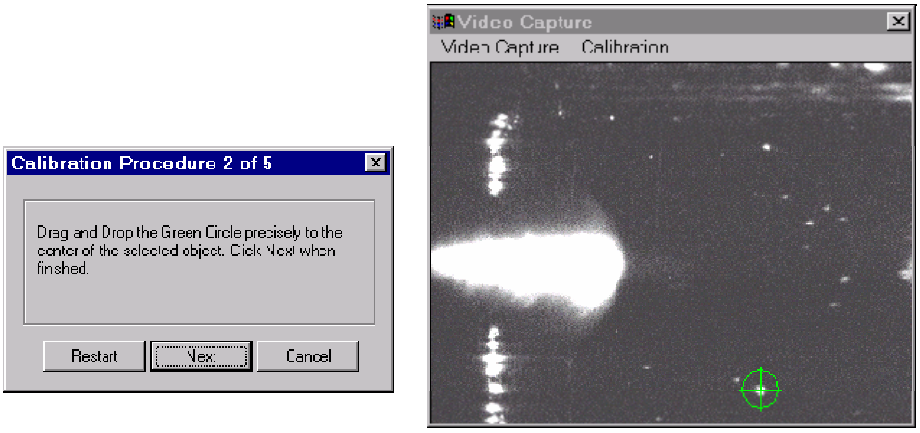


Fig. 7-8b Step 2 of the Video Capture Sample Positioning Calibration showing the green target dragged to a visually distinct object in the lower right hand quadrant.

In Step 3 (Fig. 7-9), the target plate moves horizontally. Watch where the visually distinct object moves to. Drag and drop the green target icon to the same visually distinct object. Then click "Next".

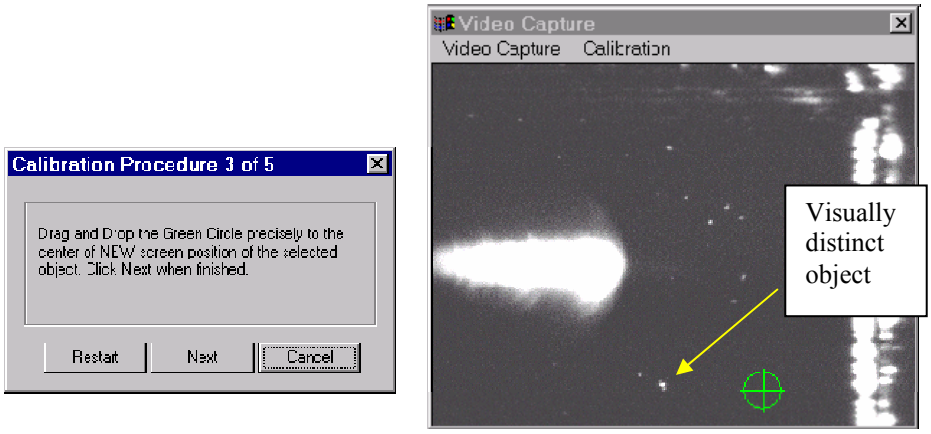


Fig. 7-9a Step 3 of the Video Capture Sample Positioning Calibration showing the visually distinct object moved *horizontally* away from the green target.

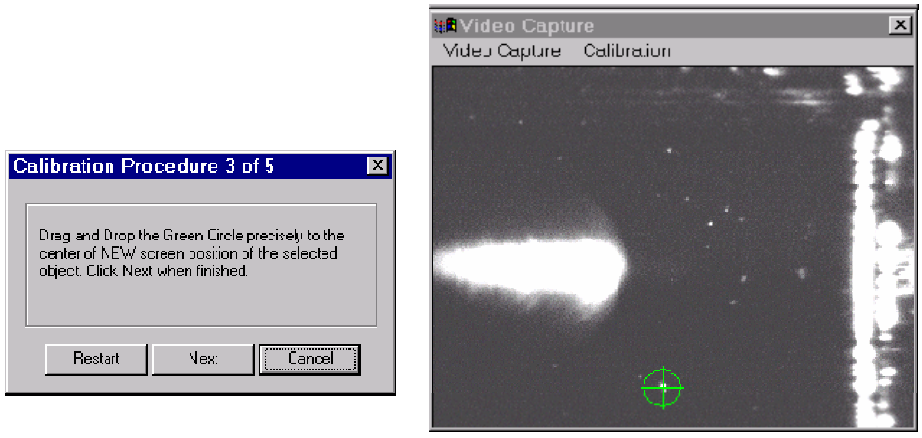


Fig. 7-9b Step 3 of the Video Capture Sample Positioning Calibration showing the green target dragged and dropped back on top of the visually distinct object.

In Step 4 (Fig. 7-10), the target plate the target plate moves again. This time it moves vertically. Watch where the visually distinct object moves to. Drag and drop the green target icon to the same visually distinct object. Then click "Next".

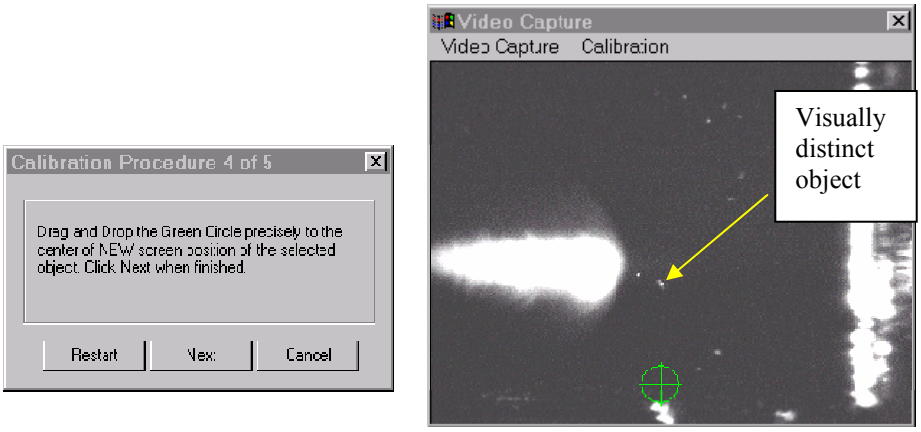


Fig. 7-10a Step 4 of the Video Capture Sample Positioning Calibration showing the visually distinct object moved *vertically* away from the green target.

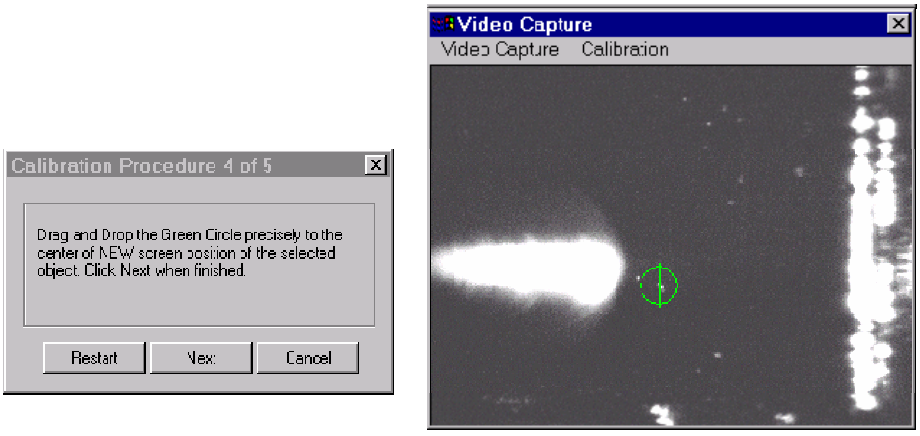


Fig. 7-10b Step 4 of the Video Capture Sample Positioning Calibration showing the green target dragged and dropped back on top of the visually distinct object.

In Step 5 (Fig. 7-11), click "Finish" to accept the new calibration. "Cancel" keeps the original calibration. "Restart" allows the user to recalibrate the system again.

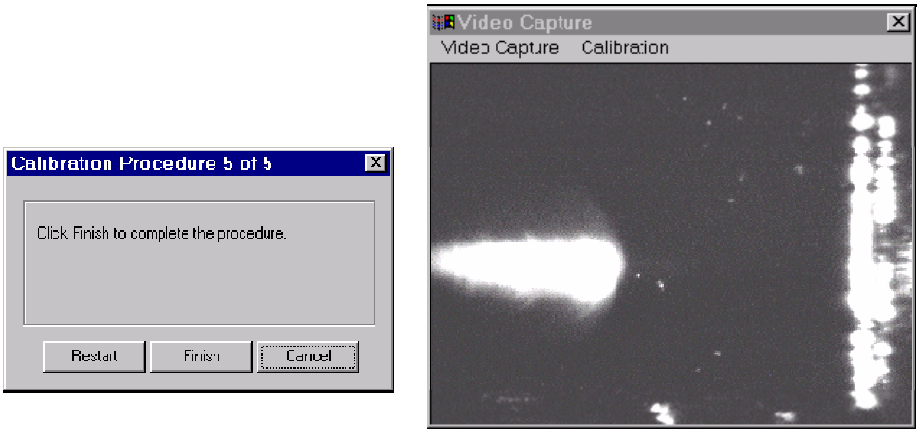


Fig. 7-11 Step 5 of the Video Capture Sample Positioning Calibration.

Now use the mouse pointer and double-click the left mouse button to verify that the sample moves to the desired location.

7.5 Running AP/MALDI on the LCQ instrument.

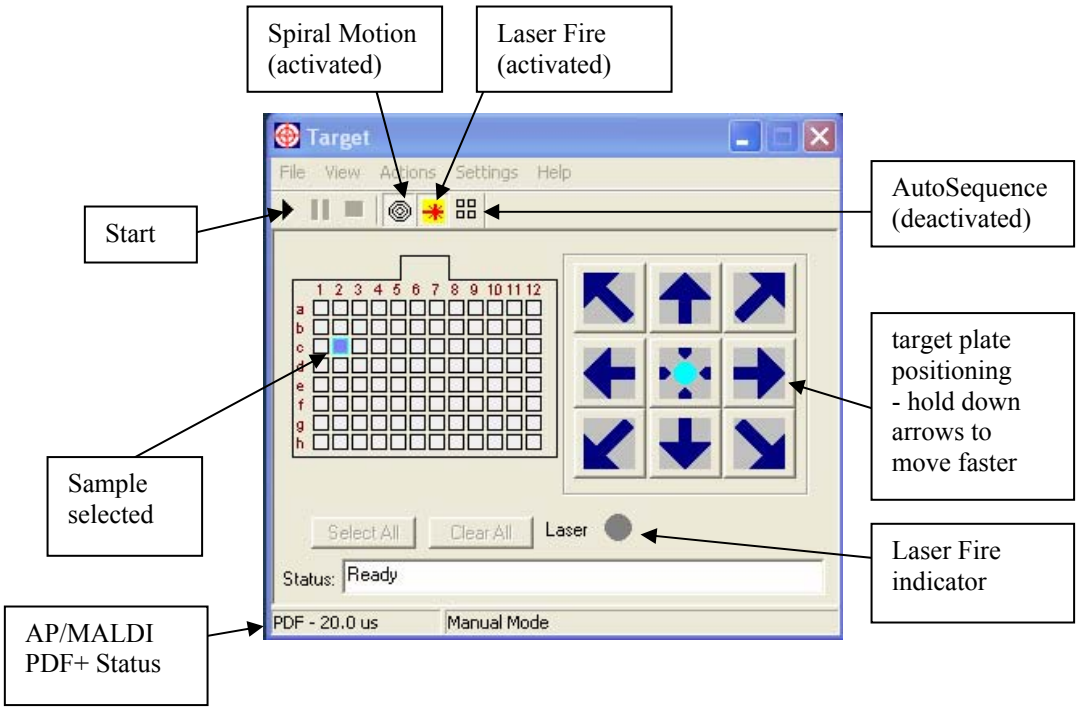


Fig. 7-12. Target software controls.

There are two modes of operation for the Target software: Manual (AutoSequence toolbar icon is deactivated) and AutoSequence. Switch between the modes by pressing the AutoSequence toolbar icon  (Fig. 7-12). In manual mode, only one sample on the "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. 7-12) or holding down on the arrow buttons to advance faster. 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 a continuous series of spots, click the first spot, then pressing SHIFT, click the last spot; to choose selected spots, press CTRL and click the spots you want to select.

To start actions, press the “PLAY”  icon. The PLAY button also activates other features depending on what other toolbar icons (AutoSequence/Laser Fire/Spiral/Raster Motion) are activated. To stop ALL activated actions, press the “STOP”  icon.

Note, that even AFTER the actions are started (i.e., PLAY has been pressed), you can manually shift the spot by clicking on the Video Capture image. You can additionally switch the Laser ON/OFF and start/stop spiral motion by activating/deactivating the appropriate button.

In AutoSequence Mode, after the PLAY 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 the default spiral and laser fire buttons are used (i.e. activated)). 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 the default spiral and laser fire buttons are used (i.e. activated)). The process is repeated 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, spiral/raster motion properties, laser frequency, AutoSequence Mode timing and so on, click the "Settings” button and edit the parameter(s) as it is shown in Figs. 7-13 to 7-18, below.

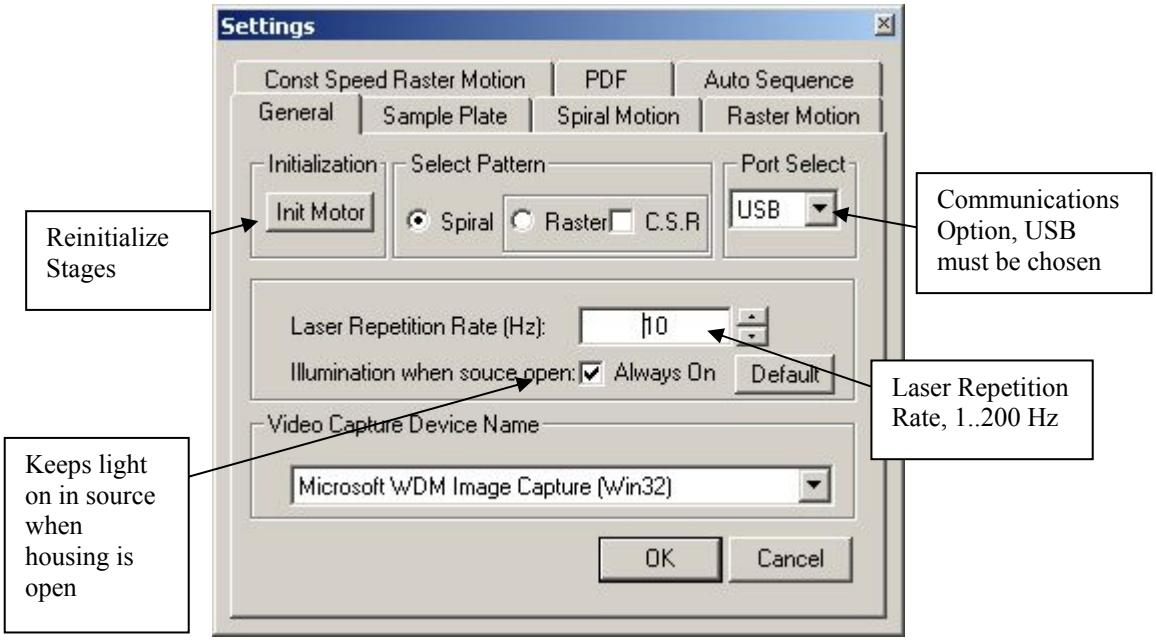


Fig. 7-13 General settings in the Target software.

There are three different sample plate patterns that can be selected during MS data acquisition to desorb fresh regions of sample surface by laser radiation: Spiral motion (selected in Fig. 7-13) and two different Raster patterns – Raster Motion and Constant Speed Raster Motion (C.S.R. control in Fig. 7-13 checked/unchecked). The parameters of the Patterned Motion can be adjusted using the three separate pages of the Setting dialog: Spiral Motion; Raster Motion and Constant Speed Raster Motion, correspondingly. Spiral or Raster motion can be used if the acquisition of long enough MS signal is desirable (for signal accumulation, relatively weak MS/MS data recording etc.). Raster/C.S.R (Constant Speed Raster) motion is designed for special applications like recording of AP MALDI images. For MALDI imaging, smooth target plate motion with constant speed is important. Another important feature of Constant Speed Raster Motion is the possibility of precise calculation of xy-coordinates of exposed position (WHERE the spectrum was recorded) based on the time elapsed from the Motion start ("retention time", WHEN a spectrum was recorded). This feature enables the conversion of recorded mass-chromatogram into a two-dimensional plot of a particular peak intensity distribution (MS image). In Fig. 7-14, Constant Speed Raster Motion page of Settings dialog that controls the mode is shown.

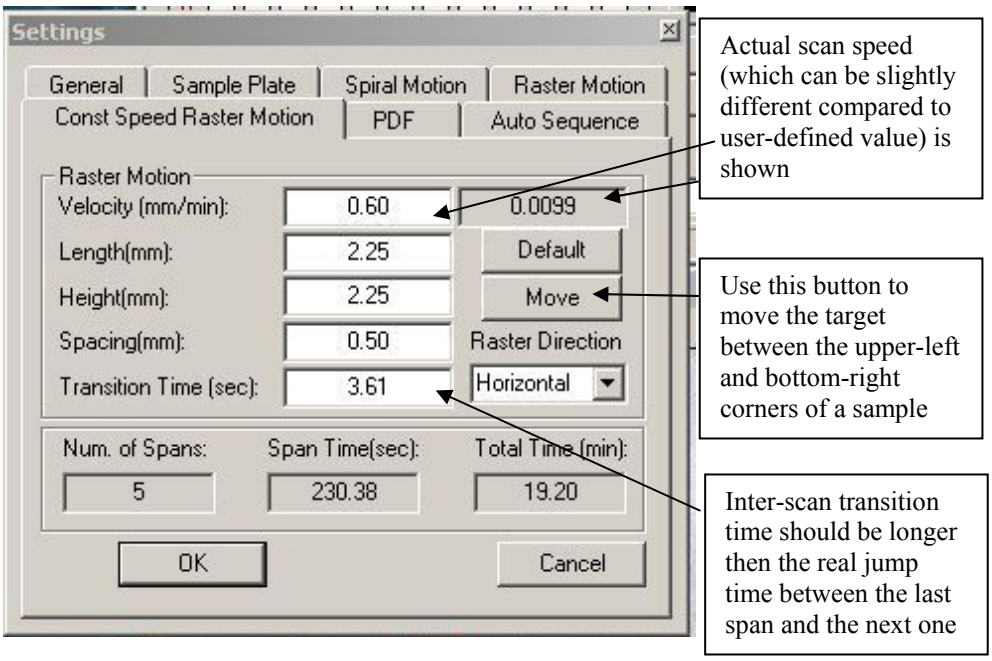


Fig. 7-14. Constant Speed Raster Motion control page.

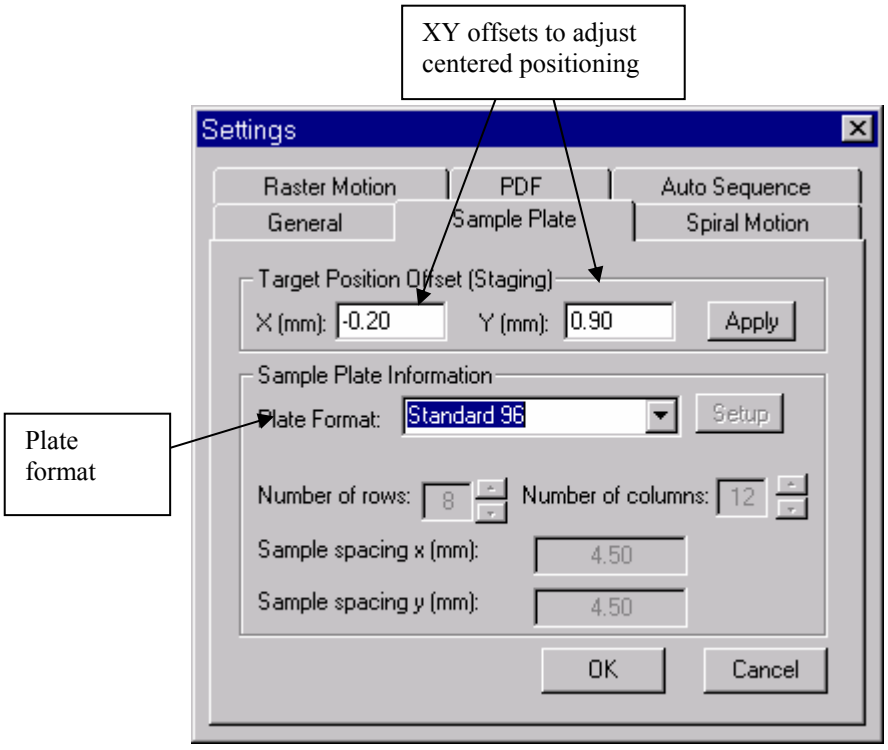


Fig. 7-15 Sample Plate settings in the Target software.

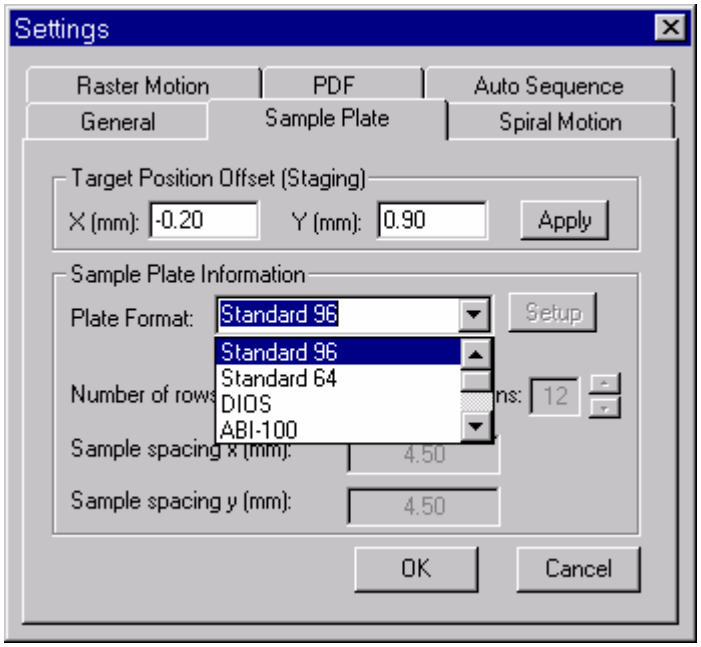


Fig. 7-16. Plate formats supported by Target version 6.0 and the AP/MALDI PDF ion source

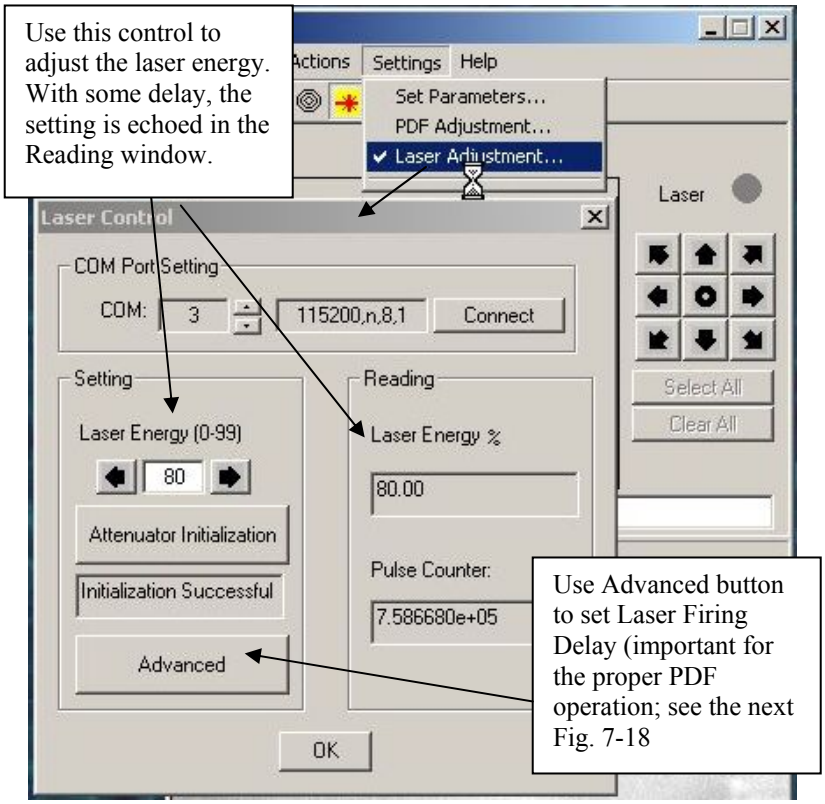


Fig. 7-17. Laser Adjustment dialog enables to choose an appropriate laser attenuation.

To adjust laser attenuation, use the dialog box shown in Fig. 7-17 (accessed through Settings -> Laser Adjustment menu item). There are more controls included in Laser Control dialog (Fig. 7-17) like COM Port Settings etc. They are included for technological purposes; the user has no needs to use them.

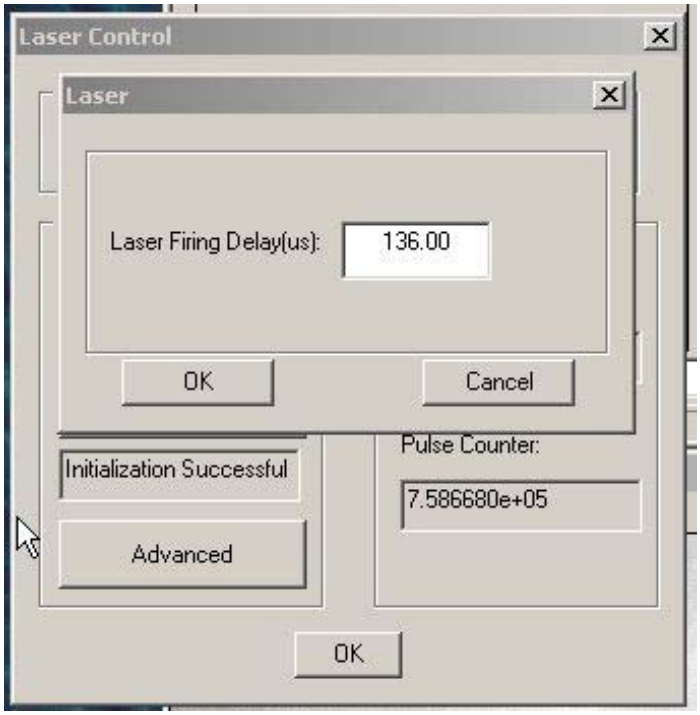


Fig. 7-18. Enter the proper Laser Firing Delay (specified at the bottom of the Control Unit) for the proper functioning of PDF. The operation should be performed only once during software installation.

Important. During the first run of Target software, the proper Laser Firing Delay value should be set through Advanced button (see Figs 7-17 and 7-18). The delay is important for proper PDF operation. Its value is unique for every Control Unit and is specified on a special sticker placed at the factory at the bottom of Control Unit housing.
After you have finished hardware/software installation, sample preparation, and successfully run the Target software, everything is ready to operate the LTQ instrument in AP/MALDI mode.

7.6 Setting the LCQ Parameters

To run AP/MALDI on the LCQ instrument optimally, the following tuning procedure of the LCQ Trap Control 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 LCQ Operator's Manual.
- The selection of nanospray (NSI) source enable to switch off Sheath and Aux gas flow off (Fig. 7-19). Generally, AP/MALDI source does not consume any gases. Use Setup -> Change API source Type menu to configure LCQ for NSI.

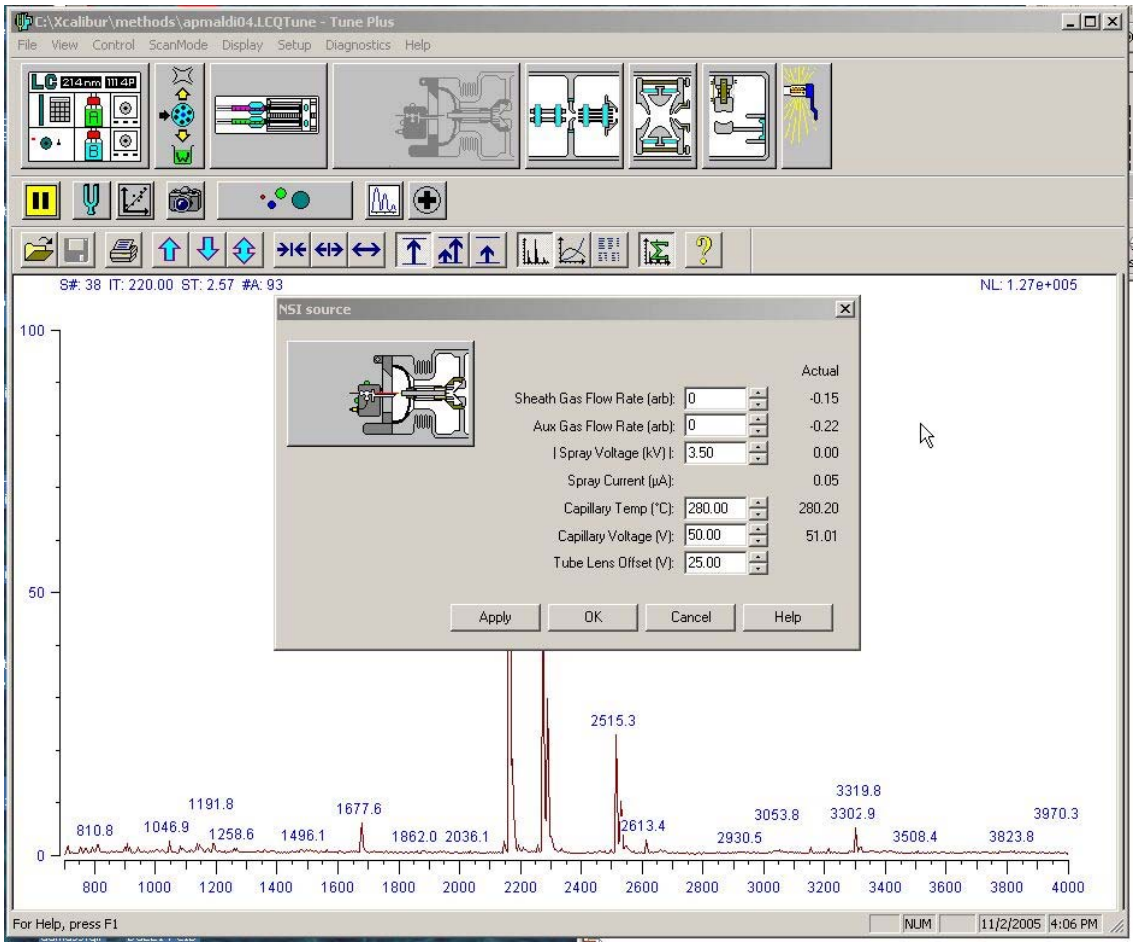


Fig. 7-19 Good parameters for the capillary HV (3-3.5 kV) and Capillary Temperature (200-280^0 C) in the ion trap are shown above.

- **Capillary Temperature settings**: The heated capillary is used to evaporate the clusters formed during the MALDI process. The capillary temperature is typically set to 200°C to 280°C (Fig. 7-19).

- The AP/MALDI PDF+ source typically generates a much weaker ion current compared with Electrospray. Therefore, for the highest sensitivity, switch Automatic Gain Control (AGC) off and choose Injection time manually around 220ms. Sometimes you may have to increase the Injection Time up to 500-1000 ms. If the sample concentration is too high

(NL parameter in the spectrum reaches 1e7 value or above), it is your responsibility to decrease Injection Time to avoid the trap saturation (Fig. 7-20).

- 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

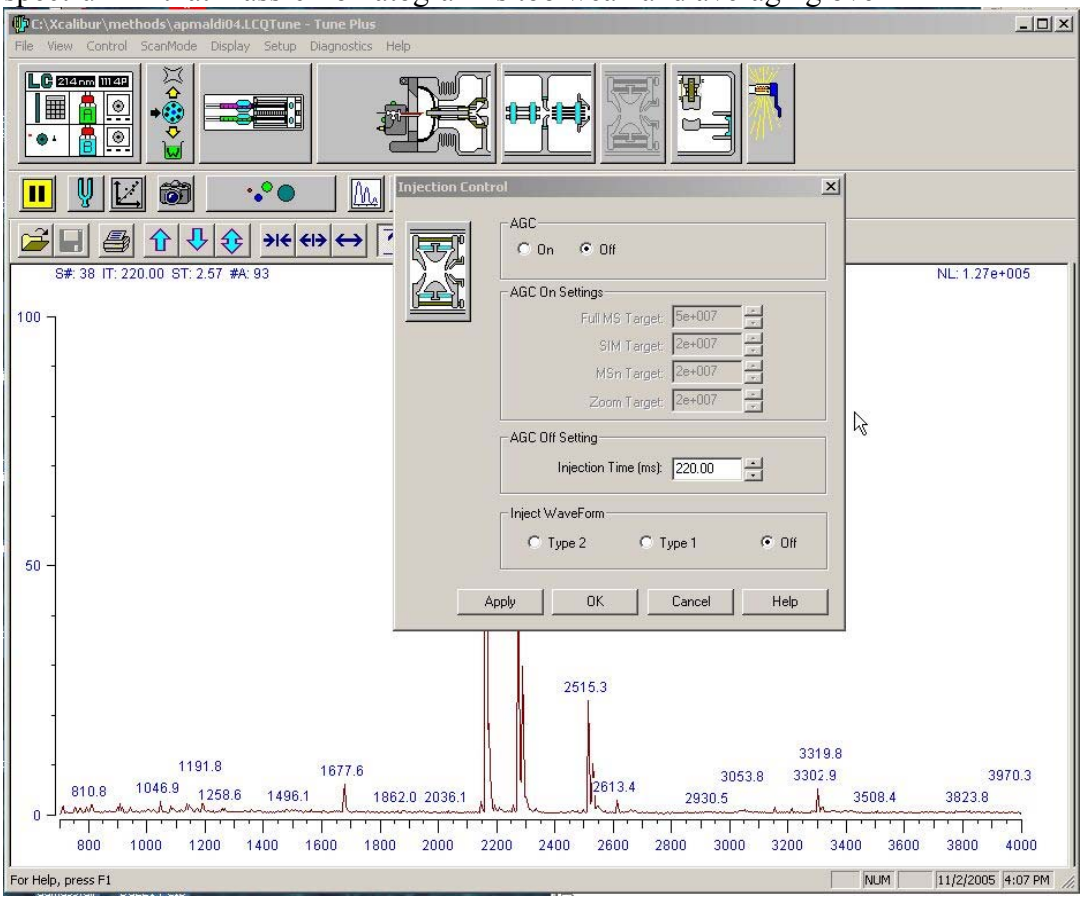


Fig. 7-20 Switch off AGC and set Injection Time to 220ms or even more

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. Therefore 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. 7-21.

- Laser pulse energy may be easily tuned through Target software (see Fig. 7-17).

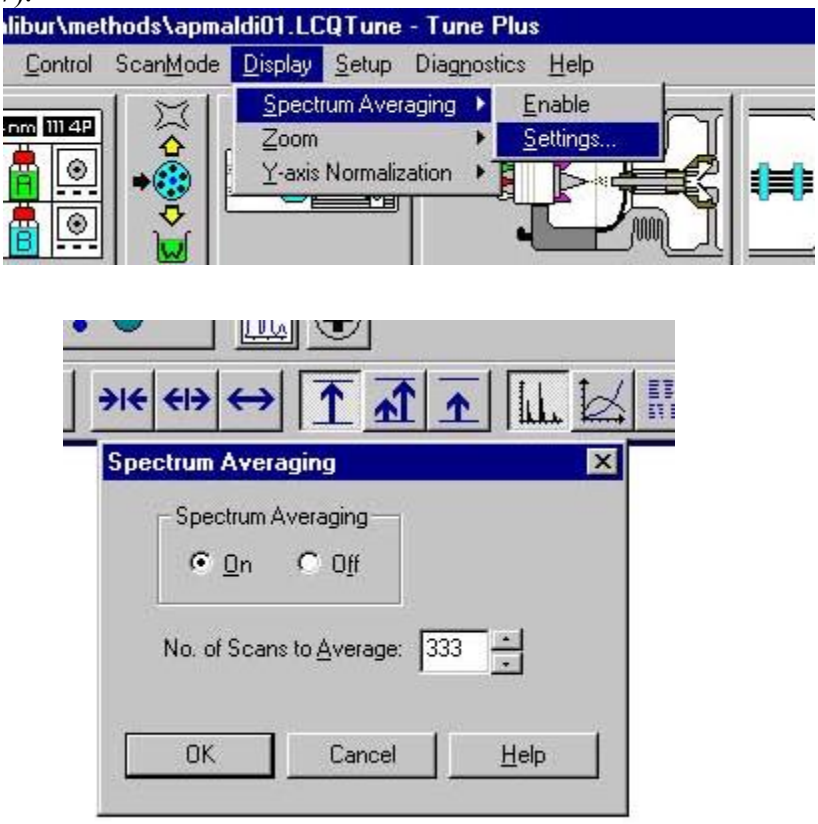


Fig. 7-21 Increasing the maximum number of spectra to Average value to 333..

Typically you should tune the attenuation for the maximum signal only once for every matrix type (α-CHC, DHB and so on).

- The final recommendation is how to choose between manual and spiral/raster 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 ion signal that is unstable over the acquisition time. If you need a long and stable signal, start the laser firing and then start either of the predefined target motion patterns of spiral or raster. This mode will enable you to continuously expose fresh parts of the sample to laser irradiation. Spiral motion will give you a stable

AP/MALDI signal for 10-20 minutes. It is sufficient for MS, MS/MS, and MS^n experiments.

7.7 PDF Operation

The PDF technology, integrated into the ion source holding, allows the user to adjust the delay time interval before the electric field is removed from the AP/MALDI ion source. The delay time interval is controlled by typing in a value in the "Pulse Delay" box in the "Settings" menu (this is the *delay* time before the electric field between the Target Plate and capillary is rapidly *pulsed* to zero) as shown in Fig. 7-22. This timed interval is also later displayed in microseconds on the lower left-hand corner of the Target operating software.

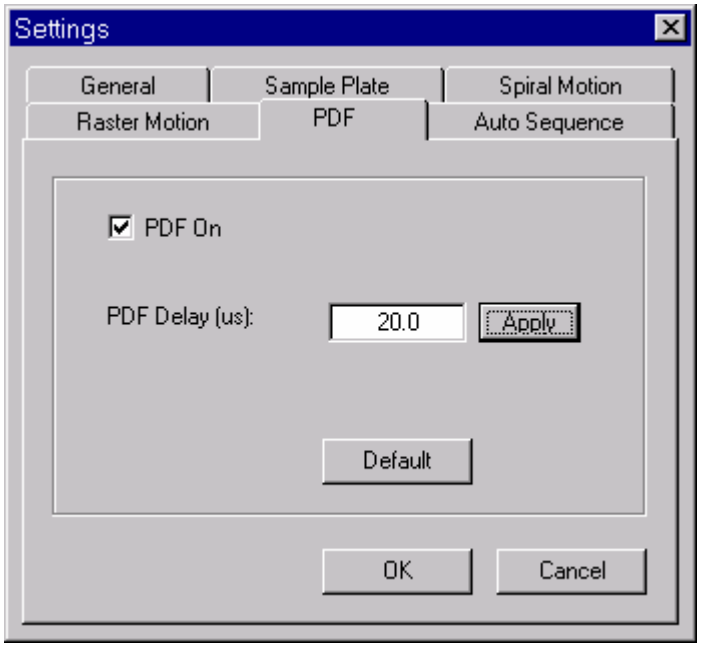


Figure 7-22. Pulse Delay adjustment

To achieve the best performance from applying the PDF technique, the user should first operate the PDF in the "OFF" mode by unchecking the PDF box (Fig. 7-22 shows PDF box checked ON). A standard peptide at 100 fmol level can be used to tune the AP/MALDI *PDF+* setup (see sample preparation procedures (Section 6)).

Record the spectrum from classical AP/MALDI operation, making note of the signal level. Change the HV on the Tune page of the LCQ software, so that the "Capillary Voltage" is increased from classically 3.0kV to 3.5kV. Adjust the Pulse Delay to around 20 microseconds, and adjust it +/-15 microseconds to determine the time for the highest signal level. You can confirm that the PDF is operational by switching the Pulse Delay time to 1 μs

which should show a dramatic drop in ion signal. This is correct because there is virtually no electric field to transport ions to the MS inlet with such a short Pulse Delay time. When the Pulse Delay is too long (for example >200 μs) ions will have already entered into the MS inlet. Thus there is optimal Pulse Delay time. Once determined, this should not need to be changed.

To further enhance the throughput of the AP/MALDI *PDF*+ setup, de-focus the UV laser by loosening the set screw and pushing in the focusing tube on the ion source about 1-2mm (Fig. 7-23). There is a scale on the side of the focusing tube to facilitate this adjustment.

Fig. 7-23. Adjustment of the focusing tube on the AP/MALDI ion source to defocus the laser beam.

Then increase the laser energy so that roughly the same laser fluence (energy/area) is maintained. The purpose behind this exercise is to generate more analyte ions per laser shot. With the PDF Technology, ions generated far away from the MS entrance can even be entrained into the MS.
Similar to the operation for optimizing the Pulse Delay, tune the focusing tube position and the laser energy, while optimizing the signal intensity for a standard chemical. AP/MALDI is sensitive to the laser fluence. If the fluence is too high, there can be increased chemical noise, and poor analyte S/N. If the fluence is too low, analyte peaks may not be present due to insufficient ionization energy. Carefully adjust the laser energy and laser focusing until the signal is optimal. An improvement in signal intensity by a factor of an additional 2 to 3 is reasonable in these laser-related adjustments.

7.8 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 *LCQ Xcalibur TunePlus* program and *TARGET* features. The data acquisition in *LCQ Xcalibur* is started independently from the target position and laser control in the *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 capillary, octopole and ion optics voltages, etc. accessible via *LCQ Xcalibur*). Saving the spectral data is your responsibility and is done using appropriate *LCQ Xcalibur* functions.

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

1. Deactivate the "AutoSequence" button in the *TARGET* software window (See Fig. 7-12 for location of AutoSequence button).

2. Start data acquisition using the *LCQ Xcalibur* software (see the previous *Setting Parameters* section (Section 7.5) in this manual or *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), activate the "Laser Fire" button and "Spiral Motion" button (if desired) and PDF (if setup/desired).

4. Click on the desired sample using the sample spot selector (map) provided in the *TARGET* software window (see Fig. 7-1). The target plate will move to this sample position and stop near its center (this is observable on the Video Capture imaging system).

5. Press the PLAY button in the *TARGET* software window to start AP/MALDI operation (and PDF if activated).

6. Adjust the desired laser energy and frequency, position the laser spot on the sample (using the "Point-and-Click" sample positioning system or the "Manual Motion Control" arrow buttons in the *TARGET* software window while observing the sample on the Video Capture screen).

7. Save data acquired, when necessary, using LCQ *LCQ Xcalibur* 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.

7.9 Automated Mode of Operation

In Manual mode of operation described in the previous section, the user acquires the spectra of different target spots (samples) one by one. Automated mode of operation enables unattended recording of multiple spectra for multiple samples as a batch. "Target" AP/MALDI source control software provides two modes of automated data acquisition.
The first mode, that is called "Internal timing" mode, there is no synchronization of AP/MALDI source operation with LCQ data acquisition. On LCQ side, the user starts the continuous data acquisition/recording. On AP/MALDI side, the user can select multiple spots, set the acquisition time per spot and run all the selected spots automatically one by one. The timing of the experiment is controlled by Target software; after the acquisition of last spot is finished, the user should stop data acquisition of LCQ manually. Major disadvantage of this regime is that the spectra for different samples are recorded in a single spectrum under the same file name. The user can (during data processing) extract the spectra for individual samples based on the retention time of the corresponding spectra.

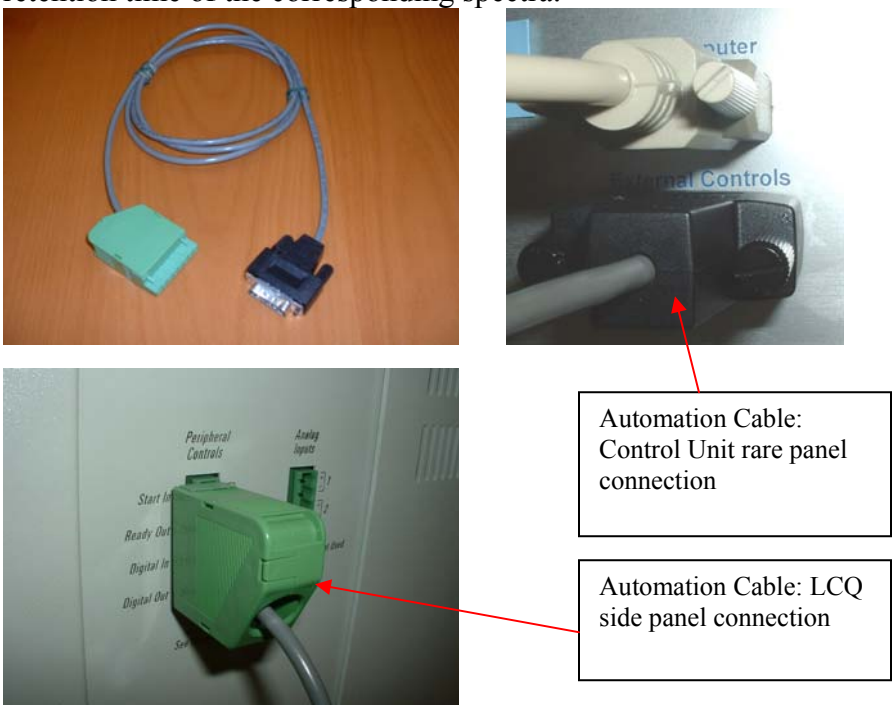


Fig. 7-24 External Control cable and its connection to the rare panel of AP/MALDI control unit and to the side panel of LCQ.

The second automated mode (that is called “External timing”) enables to record the spectra of a number of individual samples in separate files under separate user-defined names. Now LCQ communicates with AP/MALDI source through exchange of bi-directional “Start” and “Ready” signals. 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 Controls” connector. The cable and its connection are shown in the Fig. 7-24.
The selection between the two Automation modes is implemented in the “Auto Sequence” tab of “Settings” dialog box of Target software (the dialog is activated through “Settings” menu item). It is shown in Fig. 7-25.

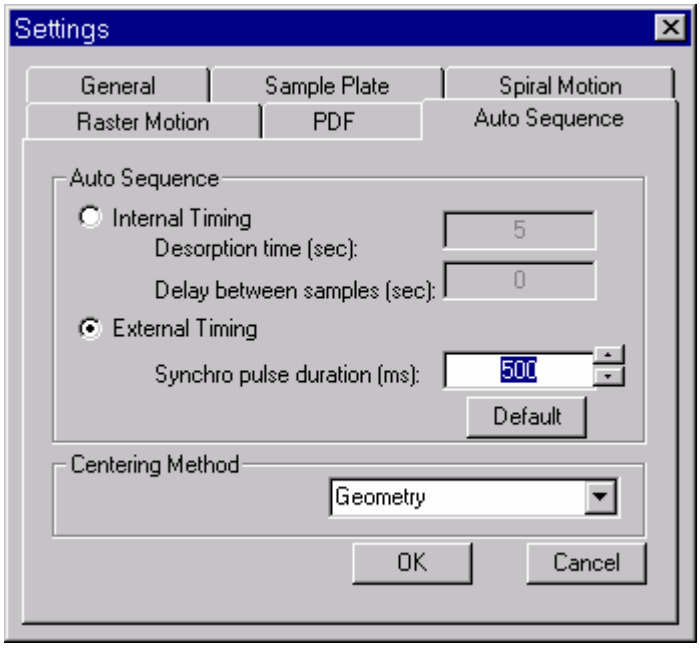


Fig. 7-25 Auto Sequence settings

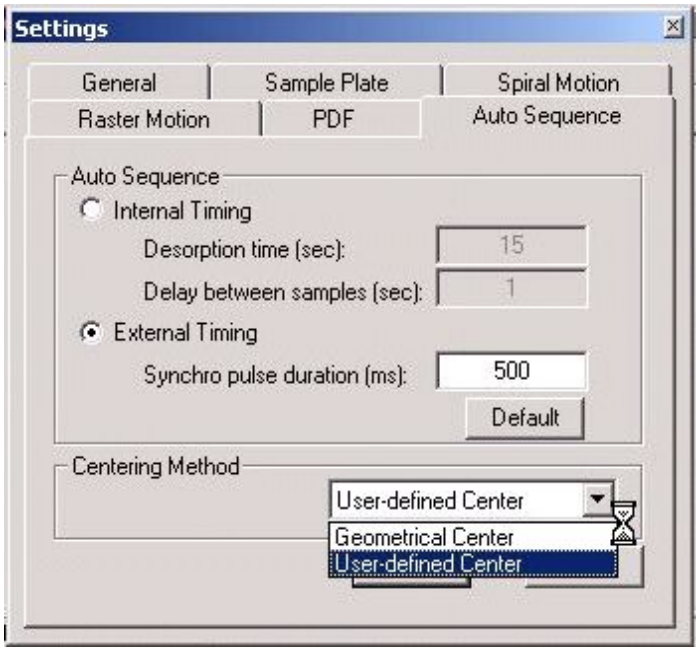


Fig. 7-26 Selection of sample Centering.

Different modes of AutoSequence are available (Fig. 7-24) and include: Geometrical Centering (Geometry) and User-defined Centering (Manual). The present modes are used to determine the center position of samples on a target plate.

Geometrical Centering mode is used when the center of all sample spotted on a plate are precisely in the same spot in each sample cell. This is useful for automated, precisely controlled spotters.
When manually hand spotting of samples is conducted the User-defined Centering mode may be useful. The User-defined Centering mode is used to allow the user to tell the Target software where the sample center is for each spot to be analyzed.

Following is the procedure for operating in the AutoSequence mode:

1. For External Timing mode, **connect** the "External Control" connector on the Control Unit's rear panel to the LCQ "Peripheral Controls" port (Fig. 7-24).

2. **Check** the "AutoSequence" button is activated in the main *TARGET* software window (see Fig. 7-14) and **check** the "External Timing" radio button in the *TARGET*'s "Settings Dialog" window (see Fig. 7-26).

3. **Check** the "AutoSequence" button is activated in the main *TARGET* software window (see Fig. 7-14). For Internal Timing, **check** the "Internal

Timing" radio button in the *TARGET*'s "Settings Dialog" window (see Fig. 7-26). You don't need any External Control cable in this case. For External Control, **check** the "External Timing" radio button. 500ms in the figure is the appropriate duration of Start pulse.

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. **For the "Internal Timing" mode**, start "Continues" data acquisition/recording in Tune-plus program of Xcalibur package. Then click on the PLAY button in the TARGET software window to start AP/MALDI (PDF) operation. The data acquisition will start and continue during the time specified for the spot ("Desorption time" in Fig. 7-26). When the data acquisition from the first sample is done, the laser firing is stopped and the target moves to the next sample spot. The ***current*** sample is indicated by a blinking color. The process will be repeated until the last sample spot has been analyzed. Finish the data recording in Tune-plus program.

6. **For the "External Timing" mode**, you need first properly configure Xcalibur software (both Instrument Setup and Sequence setup). Tune-plus program cannot be used for automated data acquisition in External Timing mode. **The procedure of Xcalibur software tuning for automated analysis is described in the chapter 7.10 below in details.** After Xcalibur is properly configured, you need first to start the sequence run; wait for status message: "Waiting for contact closure" and then click on the PLAY button in the TARGET software window to start AP/MALDI (PDF) operation. The data acquisition will start and continue during the time specified for the segment in Xcalibur software. When the data acquisition from the first sample is done, the laser firing is stopped and the target moves to the next sample spot. The process will be repeated until the last sample spot has been analyzed. The sample positions on the map where the data have been collected are shown by a solid color.

7.10 Automated Mode of Operation: configuring Xcalibur for External Timing mode.

The first step is Instrument Setup. If you are currently running Tune-plus, close the program. Run the *Xcalibur/Instrument Setup* program. Create/edit an Instrument Method or open an Instrument Method file appropriate for your experiment (see Thermo Finnigan's *Xcalibur* software manual for details). Then click on the Contact Closure page tab (see Fig. 7-27 below).

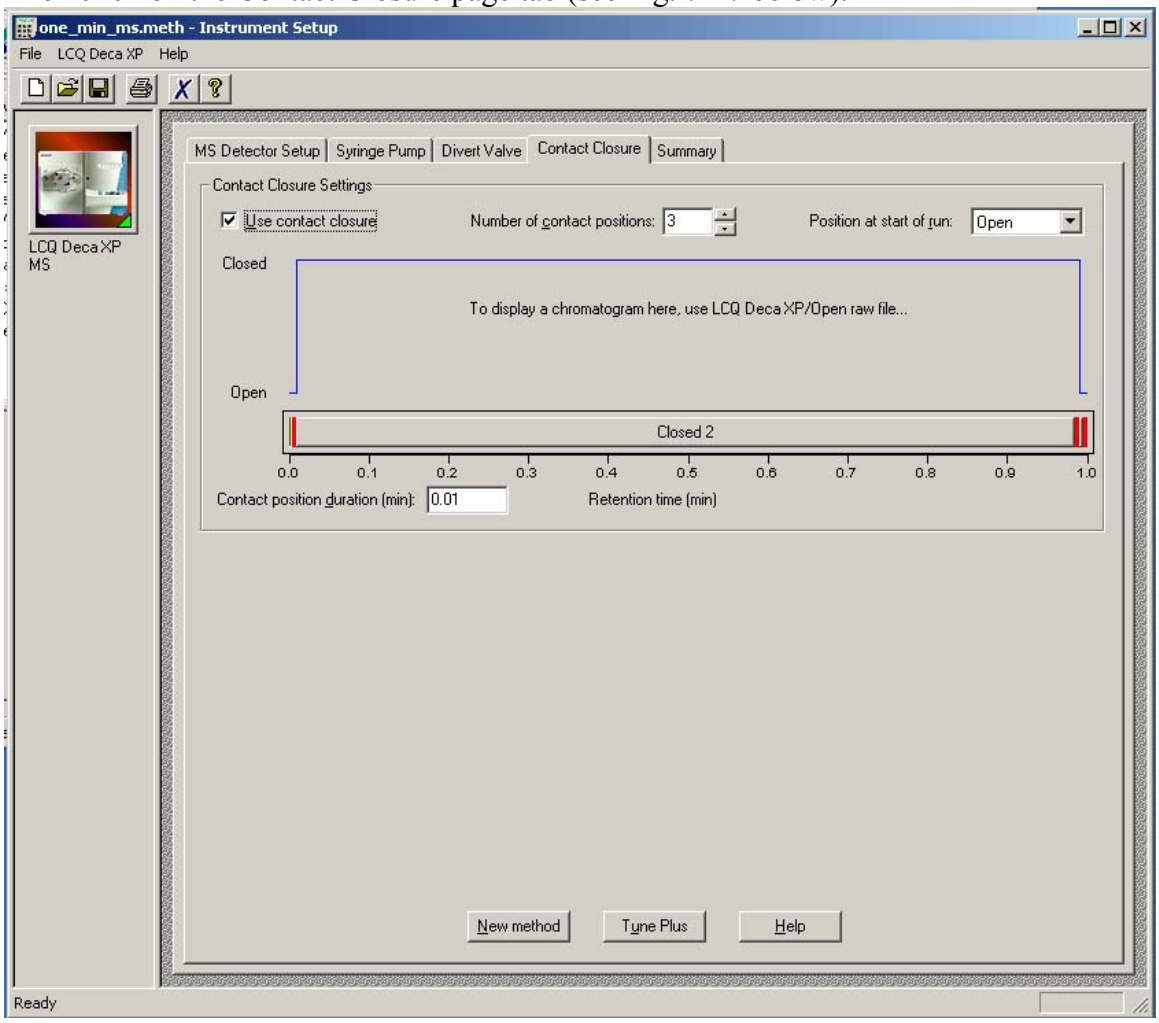


Fig. 7-27 *Xcalibur/Instrument Setup/Contact Closure* configured for External Timing automated AP/MALDI data recording.

Make changes to this page so it looks exactly like that shown in Fig. 7-25 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. You can prepare several different Instrument Methods for different types of experiments and experiment durations (Fig. 7-27, for example, define the experiment with 1-minute data acquisition); make sure that the Contact Closure profile for every Method looks like it is shown in Fig. 7-27.

The next step is Sequence setup. 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. 7-28) should correspond to the total number of samples selected for analysis.

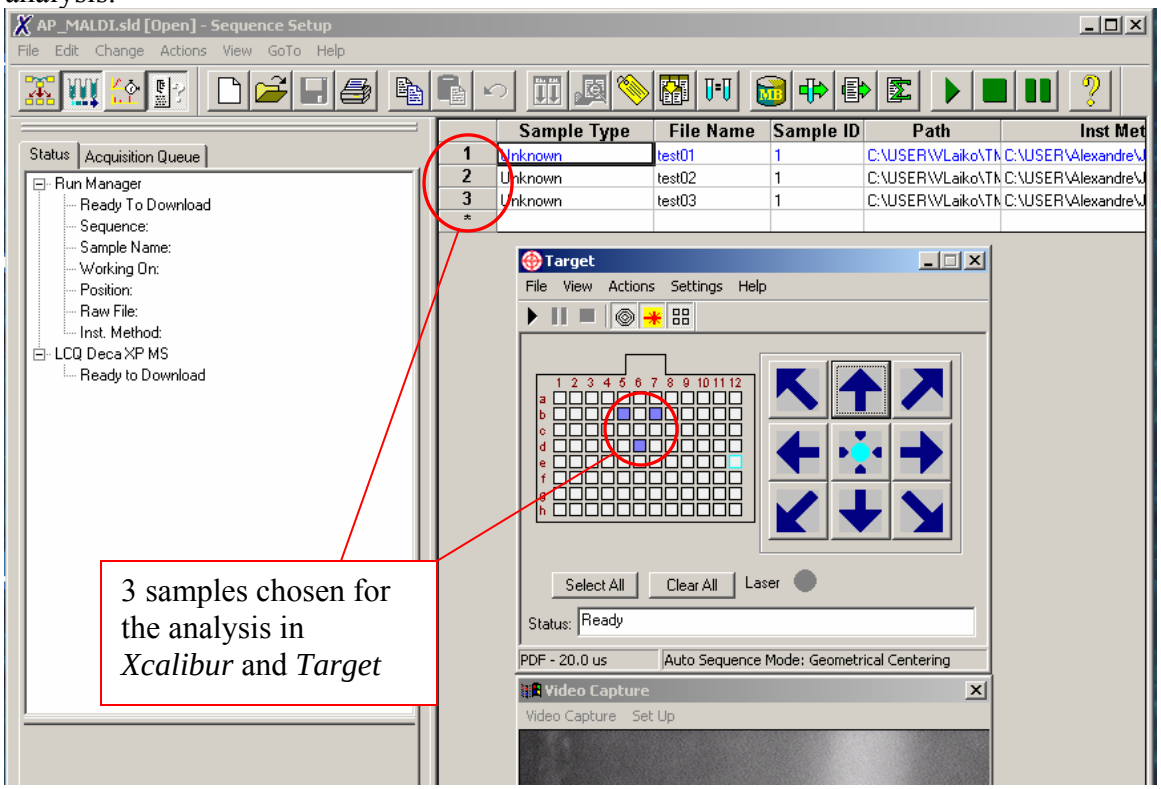


Fig. 7-28 *Xcalibur/Instrument Setup/Sequence Setup* and *Target* configured for External Timing automated AP/MALDI 3-sample data recording.

The lines in the Sequence table are run sequentially and every line in the table corresponds to the sample selected in *Target* window as they are run one-by-one (Fig. 7-28). 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 above (Fig. 7-27). The files where the acquired data will be saved are shown in the File Name column
The final step is to start the Sequence data acquisition. Go to the *Actions/Run Sequence* menu to open the Run Sequence dialog window (see Fig. 7-29 below).

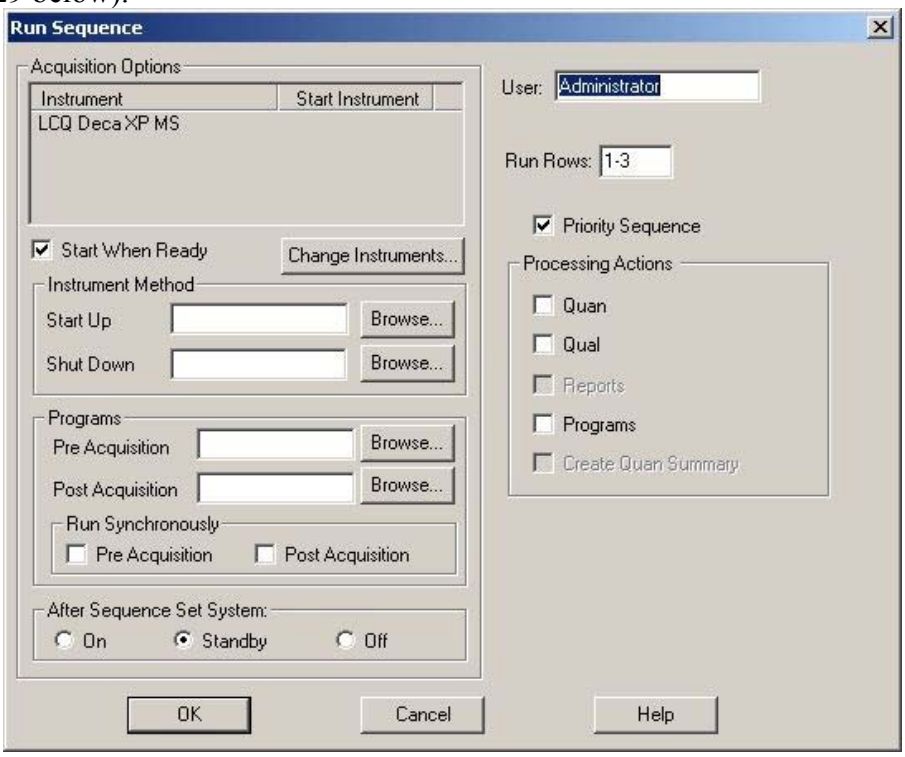


Fig. 7-29 *Xcalibur/Instrument Setup/Sequence Setup,* Actions->Run Sequence Dialog. Starting the Sequence Run.

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. Now click "Change Instruments…" button (see Fig. 7-29). The dialog "Change Instruments In Use" starts. The first column should contain only one instrument: LCQ Deca XP or Duo MS, and possibly some other instrument in the strings below. The column "In Use" for MS instrument (the first string in Fig. 36a) must contain "Yes"; the column "Start Instrument" **must be empty**. If it is not, highlight and delete (by pressing "Del" keyboard key)"Yes" mark in "Start Instrument" column. Click OK button.
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.
After the “Waiting for contact closure” message is displayed in the “Sequence setup” window (See Fig. 35), press the PLAY 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.

8 MAINTENANCE —TROUBLESHOOTING THE SOURCE.

Maintenance and troubleshooting: The AP/MALDI *PDF*+ source does not require regular maintenance, except for the cleaning of the optical fiber cable ends every six weeks. (Section 8.5 of this manual describes a cleaning procedure). Please refer to Section 5.3 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.

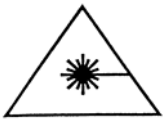
DO NOT ATTEMPT services or repairs that are not covered in this Troubleshooting section. For services and repairs beyond those specifically provided in the Troubleshooting section, contact the manufacturer, MassTech, Inc. 6992 Columbia Gateway Dr, Columbia, MD, 21046 (443)539-1758

The AP/MALDI *PDF*+ 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 8.5. It is recommended that you clean the fiber ends every six weeks to avoid deposit accumulation (preferably using the method described in Section 8.5).

8.1 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 computer's Video Capture screen.
4. If you can see a blinking spot on the computer's Video Capture 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 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 8.2

iii. Try to improve the position of the laser beam relative to the inlet Section 8.3

If these actions do not help, call MassTech for assistance.

NOTE: On the Video Capture menu there is an "Save Image" feature that saves bitmap images to the directory where the Target software program was saved. When contacting MassTech about laser ablation issues, use the Save Image feature to show the extent of matrix ablation.

8.2 PROBLEM: The laser beam is not well-focused

Goal: To increase the laser fluence, i.e. energy per unit area (J/cm^2) of the irradiation spot by adjusting the laser spot size.

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

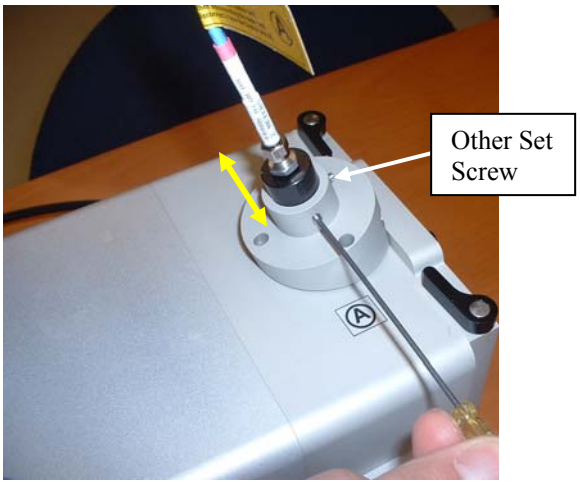


Fig. 8-1. Adjusting the laser focusing by moving the fiber optic connector up or down.

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

3. Push the fiber optic connector down about 1 or 2 mm (Fig. 8-1).
4. Disable the spiral/raster 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 1 or 2 mm 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.

8.3 PROBLEM: The laser beam focal point at the target plate is not aligned with the capillary extension.

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 Capillary extension.

Safety: The procedure is performed from outside the source housing with the source closed. The position of the laser beam is monitored on the Video Capture screen. As a result, the **procedure is safe** and can be performed with both the LCQ instrument and AP/MALDI PDF+ source switched ON.

Step 1. First, you need to determine if the laser spot 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) on a shiny gold target plate. 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 Video Capture screen should look as follows (Fig. 8-2):

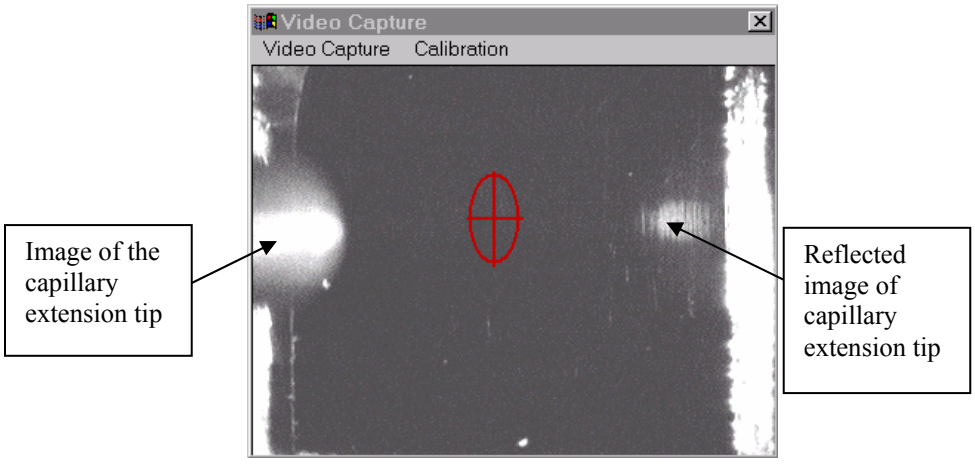


Fig. 8-2 Blank sample spot on the Video Capture screen

Both the capillary extension 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 capillary extension tip and its reflection are still, while the image of the target plate moves.

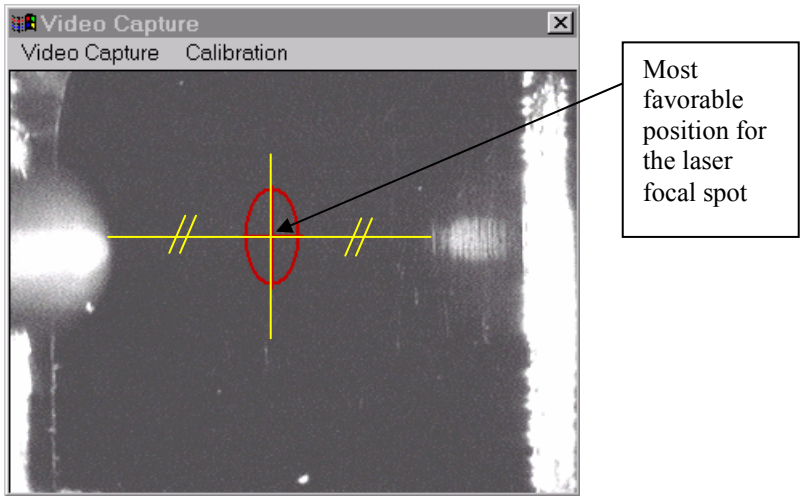


Fig. 8-3 Ideal laser positioning, on-axis with the capillary extension.

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 capillary extension tip and the image of its reflection (see Fig. 8-3). 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:

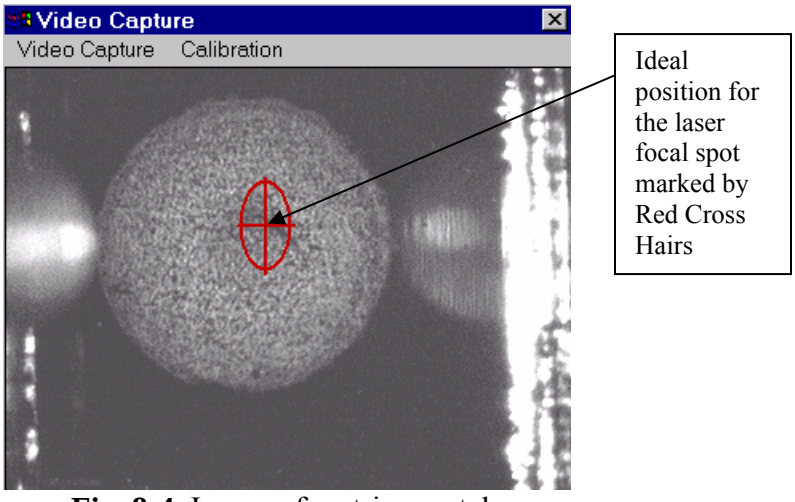


Fig. 8-4 Image of matrix crystals

Step 2. Switch ON the laser, spiral/raster motion OFF. Set the maximum laser power. Now you should see the matrix crystal's evaporation at the place where the laser beam is focused (Fig. 8-4)

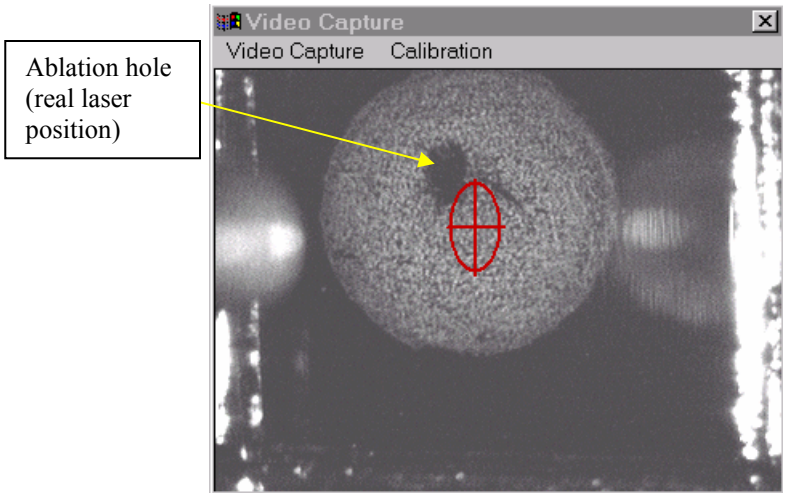


Fig. 8-5 Ablation of the matrix sample away from the ideal laser position.

By comparing the location of the ablation hole with the ideal position shown by the Red Cross-Hairs, we can see that the laser focus is close to its ideal position, but slightly above and to the left. The deviation of the focal point shown in Figures 8-4 and 8-5 is acceptable, especially with PDF activated, but the source sensitivity can possibly be improved by fine tuning.

Step 3. Continue with the same spot. Switch ON the laser at maximum power (minimum attenuation). Using a hexagonal screwdriver, turn the three screws (see Fig. 8-6).

Look at the Video Capture 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 capillary extension tip and the image of its reflection (see Fig. 8-3). The position in Fig. 8-7 below is a good alignment.

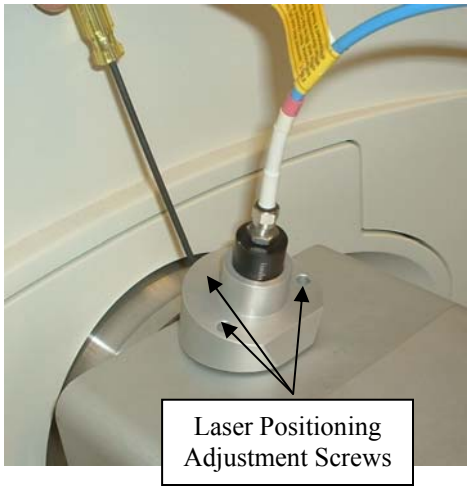


Fig. 8-6. Adjustment of the Laser position by tightening/loosening screws on the fiber connector tilt mount.

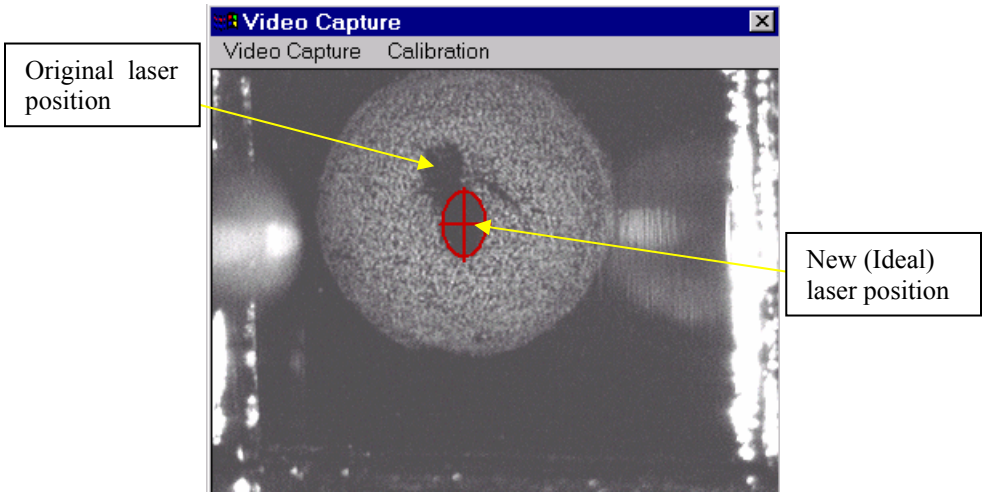


Fig. 8-7 Good alignment of the laser position.

Now you can set the best laser energy, appropriate for your matrix, 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. 8-6) based on the quality of the MS signal by a trial-and-error method.

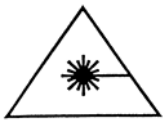
8.4 PROBLEM: the Ion transport into the LCQ instrument is clogged/blocked.

Goal: Determine if ion transport path to MS is blocked

1. To test for clogged ion transport into the LCQ 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 COMPUTER screen.
4. If you can see a blinking spot on the COMPUTER 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 LCQ interlock is operating properly.
6. Ensure that the LCQ Control program (Tune-plus) is configured as described in this Manual.
7. Ensure that your probe preparation & matrix material are being used properly.
8. If all the above are checked, the system should show at least spectral noise. If there is no chemical noise, the capillary may be clogged and require cleaning with a thin wire as described in LCQ manual.
9. Finally, ensure that your LCQ instrument operates properly with the electrospray instrument attaches. The problem may be with the LCQ instrument rather than the source.

8.5 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, 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) has been included with your shipment. To use this product:

i. Press the optical fiber end onto the Fiberclean tape and rub in figure 8 motions.

ii. After about three figure 8 motions, inspect the optical fiber end with a microscope.

iii. Repeat as necessary.

iv. Advance the tape after cleaning each optical fiber end.

8.6 PROBLEM: Spectral Response with high background and mass-shifted ion peaks

Because AP/MALDI PDF+ allows many more ions into the ion trap than does classical AP/MALDI, it is important to be aware of space-charge effects that can occur in the trap. It may be necessary to reduce the injection time of the ion trap to 100ms so as to not saturate the trap. An alternative way to solve this problem would be to adjust the Laser Energy and frequency so that fewer ions are generated.

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

9 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.

3. Susanne C. Moyer, Robert J. Cotter, "Atmospheric Pressure MALDI", Analytical Chemistry, Sept 2002, pp. 469A-476A.

4. Phillip V. Tan, Victor V. Laiko, Vladimir M. Doroshenko, "Atmospheric Pressure MALDI with Pulsed Dynamic Focusing for High Efficiency Transmission of Ions into a Mass Spectrometer", Analytical Chemistry, v. 76, No. 9, 2004, pp. 2462-2469.

Additional References

Miller CA; Yi DH; Perkins PD. "An Atmospheric Pressure Matrix-assisted laser Desorption/Ionization Ion Trap with enhanced sensitivity" Rapid Commun. Mass Spectrom. 2003, 17 (8): 860-868.

Moyer SC; Marzilli LA; Woods AS; Laiko VV; Doroshenko VM; Cotter RJ. "Atmospheric Pressure Matrix-assisted laser desorption/ionization (AP MALDI) on a Quadrupole Ion Trap Mass Spectrometer" Int. J. Mass Spectom. 2003, 226(1); 133-150.

Doroshenko VM; Laiko VV; Taranenko NI; Berkout VD; Lee HS. "Recent developments in atmospheric pressure MALDI mass spectrometry" Int. J. Mass Spectrom. 2002, 221(1):39-58.

10 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 PDF+ 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.

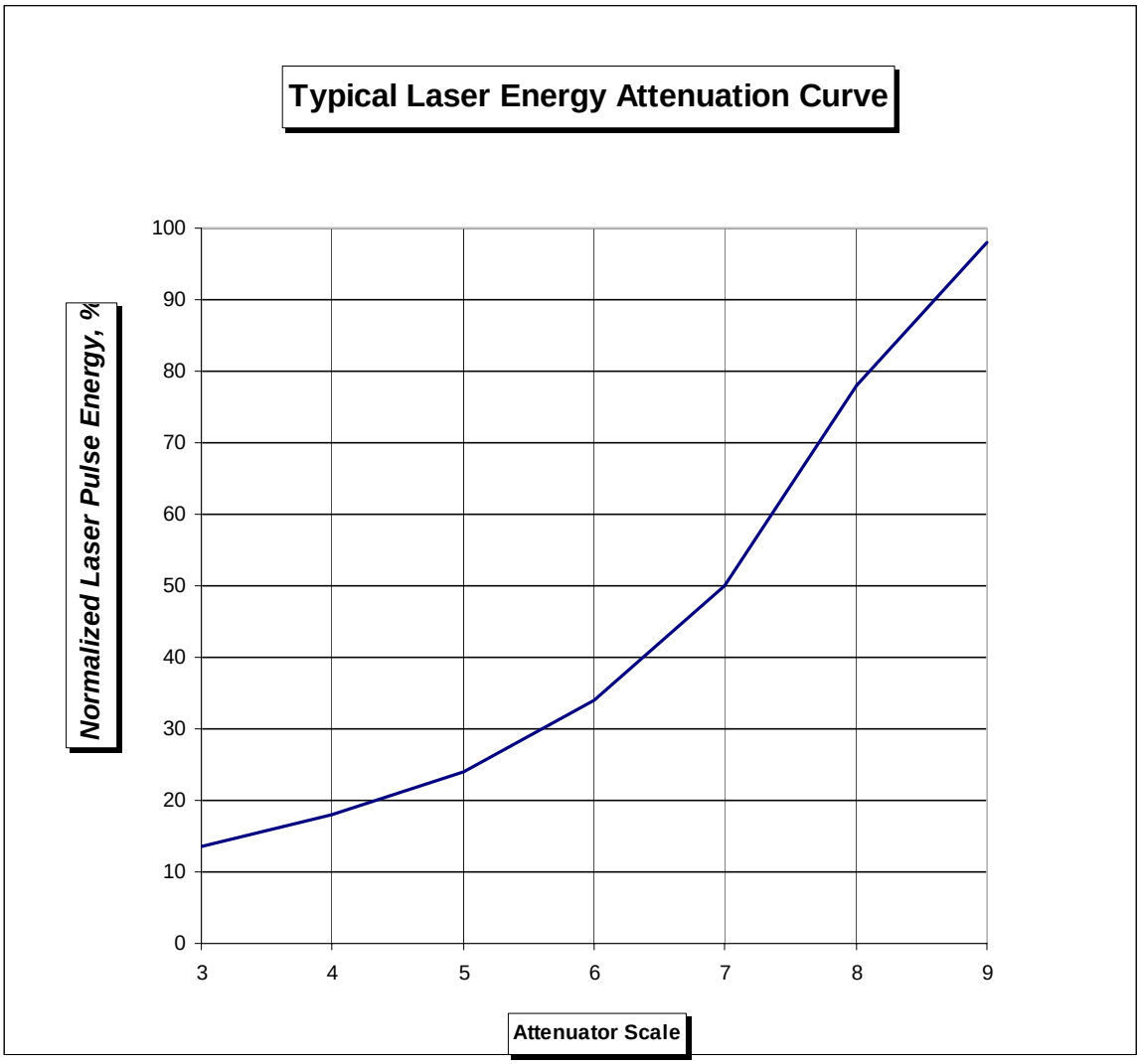
This limited warranty contains the entire obligation of MassTech, Inc. and no other warranties expressed, implied, or statutory are given. No representative or employee of MassTech, Inc. is authorized to assume any further liability or grant any further warranties except as set herein.

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 HIGH REPETITION RATE ALL-SOLID-STATE ND:YAG LASER SPECIFICATION

Part Number	**NAPLES 355**
Wavelength	355 nm
Repetition Rate	Up to 200 Hz, software controlled
Pulse Width, FWHM	3-5 nsec
Pulse Energy	200 μJ
Build-in attenuator	0-100% in 2-3% increments (software controlled)
Lifetime	> 1 billion pulses

APPENDIX B ILLUSTRATION OF THE LASER ENERGY ATTENUATION CURVE



APPENDIX C WARNING AND IDENTIFICATION LABELS

Labels Concerning the Optical Fiber

These are the two Warining labels 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 #110-AC0004 Optical Fiber cable
ONLY replace with an exact replacement part:
(Part # 110-AC0004 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: AOA000055

Warning label 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

High Voltage Warning labels placed on the outside of the Source

Control Unit Labels

Warning label for Control Unit Shutter

Turn Off the Power Before Connecting or Disconnecting the Optical Fiber

This is for placing inside the Control Unit on the Optics box

DO NOT OPEN
No Serviceable Parts Inside

This is for placing inside the Control Unit on top of the LSI laser

LSI Laser 337-Si Inside
Serial No.: S070247
MFG: July, 2002

Identification and Certification label on Control Unit

This product complies with 21 CFR 1040.10

AP/MALDI PDF+ Ion Source

Model No.:	132
Serial No.:	MOA5000401
Manufactured:	March, 2005

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

U.S. Patents: 6,791,080 and more pending

Electrical Information on the Control Unit

CE

110-240 V ~
50/60 HZ, 1.9A
FUSE: F, 3.0A 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