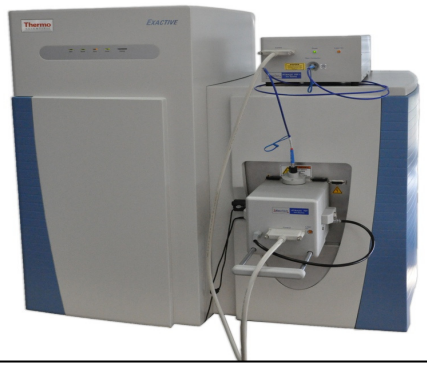
# AP/MALDI *PDF+* and AP/MALDI *HR* Source for Thermo Mass Spectrometers

## (LTQ Ion Trap, LTQ Orbitrap, Orbitrap Elite, Velos, Velos Pro, Exactive, and Q Exactive, Orbitrap Fusion, TSQ Quantiva, TSQ Endura)

Installation, Operation, and Maintenance Manual



***Warning***

The optical parts of the AP/MALDI *PDF+* or AP/MALDI *HR* sources should be handled with **extreme** care. Touching the optical parts with bare hand/fingers, storing, or exposing them to dirty /dusty environments can result in permanent damage to the optical components. These actions can result in the fiber ends getting burned. 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 ion source. Do not bend the fibers beyond its natural flexing. Bending the fiber ends will break the fiber inside the connector damaging them irreversibly. An optical fiber with special protective caps on its ends is included in the shipment; please keep them in dust free conditions. After removing the fiber optic protective caps, please put the protective caps back on the fiber ends immediately after the cable is detached from a connector or if the cable is not use. If cleaning of the fiber end is required, refer to the Maintenance/Troubleshooting section (Section 8) of this manual for the proper cleaning procedure. Every time the fiber is exposed to dirt or contamination occurs/is suspected, cleaning the fiber needs to be completed. *Note: High energy fibers that come with AP/MALDI* HR *cannot be cleaned due to the style of fiber termination, therefore, do not attempt to clean them. If damage or dirt is suspected, a new one need to be used. Using high energy fiber(s) with excess laser energy as specified on the product labels will damage them.* In a normal operation with proper care, an optical fiber will have a long lifetime. MassTech Inc. has included a spare optical fiber cable in case the optical fiber cable (that is supplied) is accidentally damaged. Additional fiber cables **MUST** be ordered from the AP/MALDI source manufacturer, MassTech, or your sales agent.

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


**MASSTECH, INC.**

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Last revision: January 28, 2015

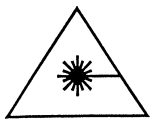
MassTech

# Table of Contents

**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, un-installation, 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, un-installation, 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.**
**ATTN: Service Department**
**6992 Columbia Gateway Drive**
**Suite 160**
**Columbia, MD 21046 USA**

**Phone #: (443) 539-1758**
**E-Mail: msms@apmaldi.com**

# 1 INTRODUCTION: AP/MALDI PDF+ AND AP/MALDI HR : AN ION SOURCE FOR MASS SPECTROMETERS WITH ATMOSPHERIC PRESSURE INTERFACE

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

The AP/MALDI source is designed to produce ions of different analytes under atmospheric pressure conditions from a mixture of matrices/analyte microcrystal by irradiating these crystals with UV laser pulses. These ions are analyzed by the mass spectrometer through recording their corresponding mass spectra. The mechanism of the **AP/MALDI** ion production is similar to that of a **conventional (vacuum) MALDI**. The main difference is that the AP/MALDI produces ions under the atmospheric pressure conditions **outside** of the instrument's vacuum housing.The main consequences are as follows:

- The AP/MALDI source is an external ionization source. It is designed to be easily interchangeable with other sources of the mass spectrometers such as the ESI, APCI, nanospray, etc.

- The replacement of the target (sample) plates is a simple and quick process because the AP/MALDI source operates under atmospheric pressure; the source does not need to establish vacuum.

- The AP/MALDI source is designed as an additional external source for the mass spectrometer. The process of the mass spectra measurement is completely decoupled with the sample ionization process. Thus, the AP/MALDI inherits all the power of the Thermo instruments: the high sensitivity, stability of calibration, $MS^n$ capability, powerful data processing, and spectra interpretation software. However, it also inherits all the limitations such as the mass range. The AP/MALDI source, like the conventional MALDI source, produces mostly single-charged ions.

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

- The AP/MALDI source operates under the normal ambient pressure conditions similar to the ESI sources. The AP/MALDI and ESI sources are interchangeable and typically provide complimentary analytical

information. The appropriate use of both the ESI and AP/MALDI sources provide the opportunity to cover the broad range of problems in modern analytical chemistry [1-3].

*Pulsed Dynamic Focusing Technology – PDF:*

The AP/MALDI has conventionally used continuous electric fields to extract ions into a mass spectrometer. The 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 is initiated. 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. Instead, the ions are 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 those ions are *dynamically focused* into the MS. Overall, PDF technology significantly improves the signal level and reliability of the AP/MALDI [4].

**Advantages of PDF Include:**

- Increased the transmission efficiency of ions into MS
- Higher ionization efficiency at a greater voltage setting
- Greater sample throughput when the larger laser spot size is applied
- Insensitivity to the laser misalignments

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

AP/MALDI *HR:*

The AP/MALDI *HR* source is designed to significantly reduce the spot size for the laser beam on the sample. This enable “High Resolution” more specifically higher lateral resolution. By employing the Zoom Mode, one can create and monitor ion signal and relate it to its location. More information about the zoom mode is included in Chapter 7.12.

## *1.1 QUICKSTART OPERATION*

This section covers the basic operation of the AP/MALDI PDF source after the AP/MALDI PDF source, Target Software, and the Thermo Mass Spectrometer have been properly installed and set-up.

Once the ion source and control unit are installed/connected to each other according to Section 5 of this manual, the operation steps are as follows in the section below.

**NOTE:** All installation and uninstallation procedures **must** be done with the power turned "Off." Before proceeding, the user is strongly urged to read the safety procedures in Section 4 of this manual.

1. Close the ion source, turn the control unit "On", and run the Target Software on the PC connected to the mass spectrometer. Wait until the initialization is completed and the "Ready" notification is displayed in the status field of the Target Software.

2. Since the Thermo Software is normally optimized for the electrospray source, the user must adjust the software's parameters so it is optimized for the AP/MALDI:

| Set the software to the following initial settings: |
|---|
| Plate Voltage: 2 kV |
| InjectionTime: 200ms |
| PDF Pulse Delay Time: 20µs (if equipped) |

3. Prepare a MALDI sample according to Section 6 of this manual; a typical sample preparation procedure has the same procedure for a conventional (vacuum) MALDI.

4. Load the target plate containing the samples into the target plate holder according to Section 6.1 of this manual. Ensure that the user closes and locks the source securely by pressing the "Close" button. When closed, the user should hear a low click sounds when the source is locked into place.

5. Use the Target Software to fire the laser and test the samples. To operate in the manual mode (spot by spot spectra measurement), make sure that the auto sequence toolbar icon is unchecked and chose a desirable spot using the Target Software. Adjust the position of the laser using the target image on the computer screen, if necessary. Start the laser firing and (optionally)

spiral/raster motion (in the Target Program). After satisfactory data collection, switch to data acquisition. Now the user can repeat the procedure for other spots (a detailed explanation of automatic operation is included as Section 7.5 of this manual).

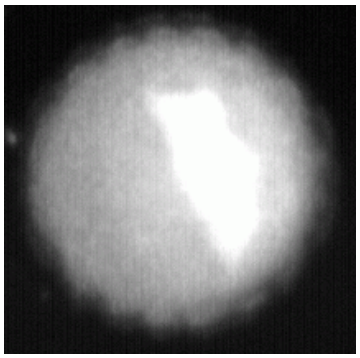


*Figure 1 An Ideal Starting Sample Spot*

6. When the user finishes the data acquisition, he/she must complete the following: stop the data acquisition (by using the Thermo Software), put the mass spectrometric instrument to the "Standby" mode, and stop the laser firing and target motion (by using the Target software). Then, open the source and remove the used target plate.

7. Replace the old target plate with one that has new sample spots, close the ion source, and repeat step 5 to get the spectra from a new target plate.

## 2 AP/MALDI BASIC PRINCIPLES

The description of the AP/MALDI ion 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 the following section will describe the PDF operating principles.

*Ion Source:*
A simplified scheme of the AP/MALDI *PDF+* source is represented in Fig. 2-1.

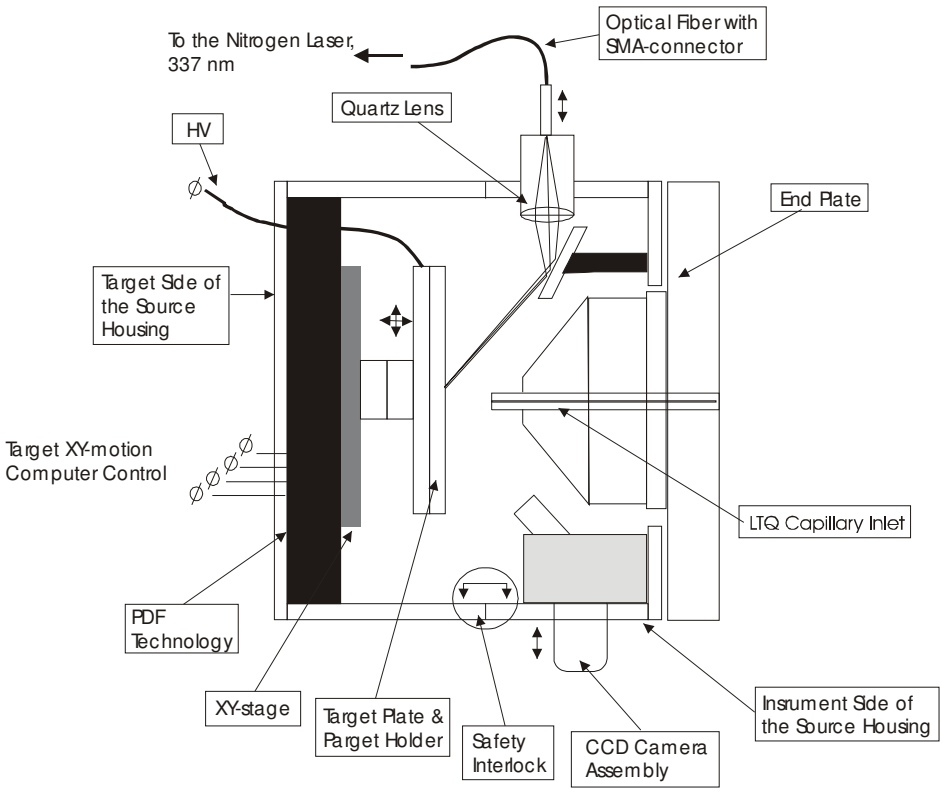



*Figure 2.1 Simplified Schematic Diagram of the* AP/MALDI *PDF+ Source that is Installed on Thermo Instrument*

The AP/MALDI Ion Source is mounted inside a housing source; the housing source is attached to the inlet flange. All ions produced inside the housing source travel toward the inlet orifice of the mass spectrometer with a stream of steady gas flow created by the suction provided by the mass spectrometer inlet. The housing source consists of two connected halves: the target side of the housing source and the instrument side of the housing source. The MALDI samples are deposited onto the surface of a replaceable target plate that is slipped into a target plate holder; the samples can be deposited on the surface of each target plate. The high voltage (typically 2 kV) is applied between the sample plate and capillary inlet of the mass spectrometer to assist the transportation of the ions toward the inlet orifice.

The sample material deposited on the surface of a target plate is irradiated with the laser light. The high repetition rate (all-solid-state Nd:YAG laser) has a wavelength 355 nm and is mounted inside a control unit. (50-200 Hz for AP/MALDI *PDF+*; 500-1000 Hz for AP/MALDI *HR*) The sample material is also connected to the AP/MALDI ion source by an optical fiber. The laser light pulses transmitted through the optical fibers, which 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 on the computer monitor (not shown in Fig. 2.1). Inside the housing source there is also a source of visible light (also not shown in Fig. 2.1) to illuminate the target plate surface. The AP/MALDI ion source can be easily opened to replace the 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:*

Another important part of the AP/MALDI ion source is the control unit; the UV laser and XY-stage controllers are mounted inside it. The control unit is connected to the source through an optical fiber and electrical cable. One more cable connects the control unit to the computer’s USB port, which controls the target plate motion and laser firing. Inside the control unit is a high repetition rate all-solid-state Nd:YAG laser (Appendix A contains a list of specifications for the laser).

## 3 PULSED DYNAMIC FOCUSING (PDF)

The 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. The PDF technology allows for a more reliable operation of the AP/MALDI and improves its overall performance.

The voltage scheme for the 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) has occurred. The switch immediately removes the electric field between the target plate and capillary for a hold time of >1ms. Immediately following, the electric field returns 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.

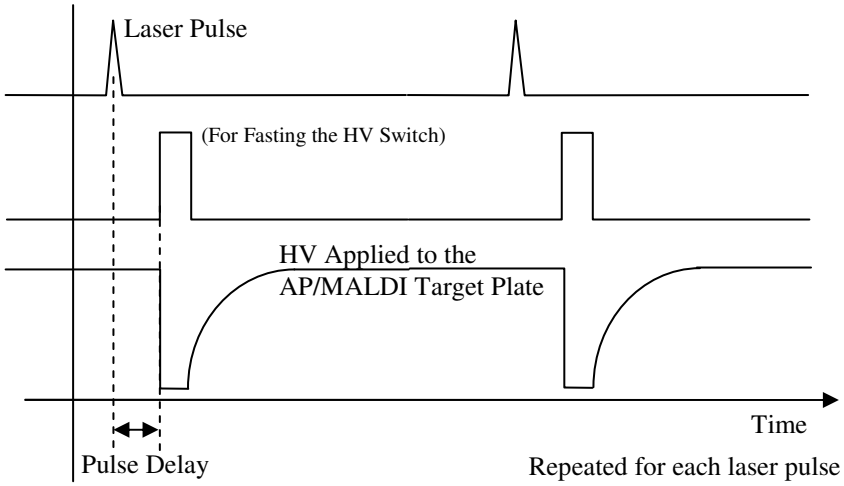



*Figure 3.1 Voltage Scheme for the AP/MALDI PDF*

The PDF improves S/N ratio at higher voltages and also allows larger laser spot sizes to be effectively utilized. The combination of working at higher voltages and utilizing larger laser spot areas, results in a sensitivity improvement in comparison to a classical AP/MALDI without the PDF. In addition, the PDF technology allows for misalignments in the laser position, of up to 1.2 mm (radial off-axis from the capillary axis) without significant compromise in 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 THE AP/MALDI ION SOURCE

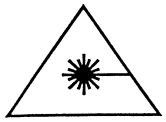
If operated properly, the AP/MALDI ion source is safe. No special knowledge of laser or electrical safety is necessary to operate the source. However, there are two potentially hazardous factors connected with the AP/MALDI source installation, operation, and maintenance/ troubleshooting:

1. Invisible coherent UV irradiation 355 nm, up to 300 µJ per pulse
2. High voltage up to 5 kV DC

In order to insure the necessary safety measures, the manufacturer of this product has provided careful protection for users by housing (shielding) and reliable interlocking of the source components from the UV radiation and high voltage. Therefore, the AP/MALDI source power needs to be turned "OFF" during installation/ uninstallation.

## *4.1 Safety Precautions*

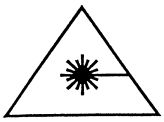
This section describes important precautions that must be observed during the AP/MALDI PDF source ***installation/un-installation, operation, and maintenance***. Appropriate precautions can be divided into the following stages:



- **Installing/Un-installing:** Before the source is installed/un-installed onto the mass spectrometer, the mass spectrometer must be in either in the "Standby" or "Shutdown" mode. The same rules, described in the operator's manual for the replacement of the standard sources (electrospray/nanospray/APCI), are applicable for AP/MALDI as well.

> Never switch the power "ON" at the rear panel of the AP/MALDI control unit before the source is completely installed and the optical fiber properly connected at both ends.

As stated earlier, when uninstalling the device, make sure that the MS is in the "Standby" or "Shutdown" mode, switch "OFF" the power at the rear panel of the AP/MALDI control unit; then start any disassembling operations or source detachment. The AP/MALDI source safety interlocks the safeguard mode, which prevents the user from accidental application of the high voltage or laser radiation when the source and control unit are covered in their housing units.



**IMPORTANT:** Whenever the optical fiber is being connected/detached to or from the control unit, **the user must MAKE SURE the power switch on the control unit is "OFF." The same rules are applicable when using the housing source.**

- **Target Plate Loading/Unloading:** The user needs to open the AP/MALDI source to load/unload the target plate. For safety purposes, it is mandated that the user first switch the instrument to either the "Standby" or "Shutdown" mode and stop the laser firing (click on the "Stop" button on the AP/MALDI ion source in the 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 the source is opened while the instrument is recording the spectrum (HV is ON) and/or the laser is firing, the AP/MALDI *PDF*+ source safety interlocks automatically switches the high voltage and the laser to the "OFF" mode.

**Caution:** The target plate may be hot**!**

- **Mass Spectra Recording:** Normally, the recording of the AP/MALDI spectra is done by the computer. During the data acquisition the source is closed and attached to the MS instrument, which excludes any possibility of high voltage shock or laser radiation exposure. If the source is opened by accident while the MS instrument is recording the spectrum (HV is "ON") and/or the laser is firing, the AP/MALDI source automatically initiates the safety interlocks switch the high voltage and the laser to the "Off" mode.
- **Maintenance and Troubleshooting:** The AP/MALDI source does not require any maintenance, except cleaning of the optical fiber ends. It is strongly recommended that the user follows the maintenance and troubleshooting procedures that are described in the "Troubleshooting" Section (Section 8) of this 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.**
**Suite 160**
**Columbia, MD 21046, USA**
**+1 (443) 539-1758 – Please ask to be directed to the MassTech Sales Department.**

**<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 must be turned "OFF"**
- **Never switch the power "ON" at the control unit if the AP/MALDI PDF source is not properly attached to the mass spectrometer or if 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.

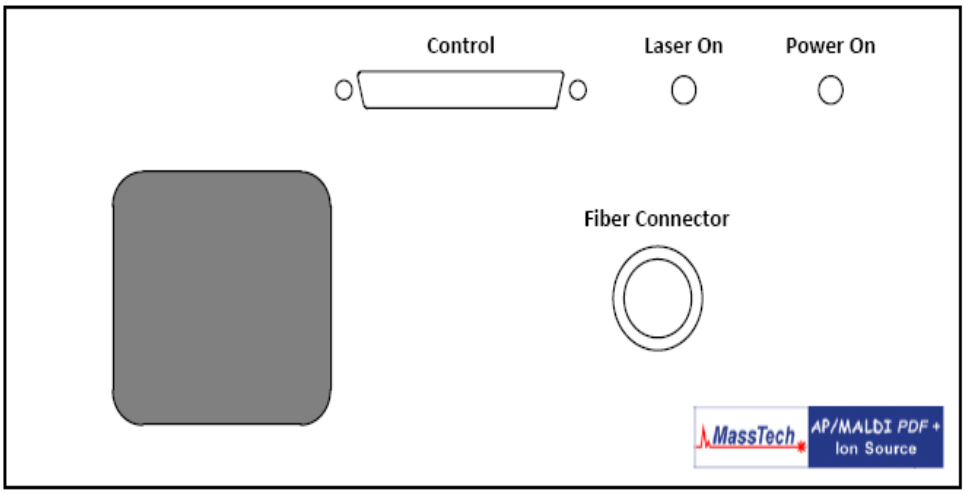



*Figure 4.1 The Control Unit's Front Plate*

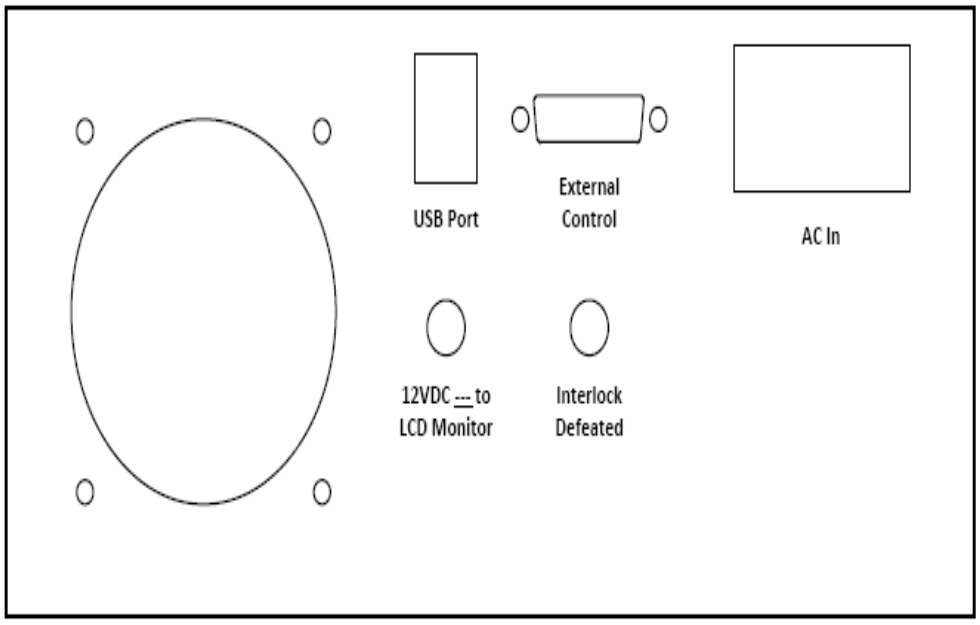



*Figure 4.2 The Control Unit's Back Plate*

# 5 SOURCE INSTALLATION

## *5.1 Checking that all Components have been Received*

Before the user start installing the source, he/she must ensure that all necessary parts and accessories have been delivered. Figures 5.1 through 5.7 (below) show these components and introduce some definitions as well as the part names used in the installation explanations.

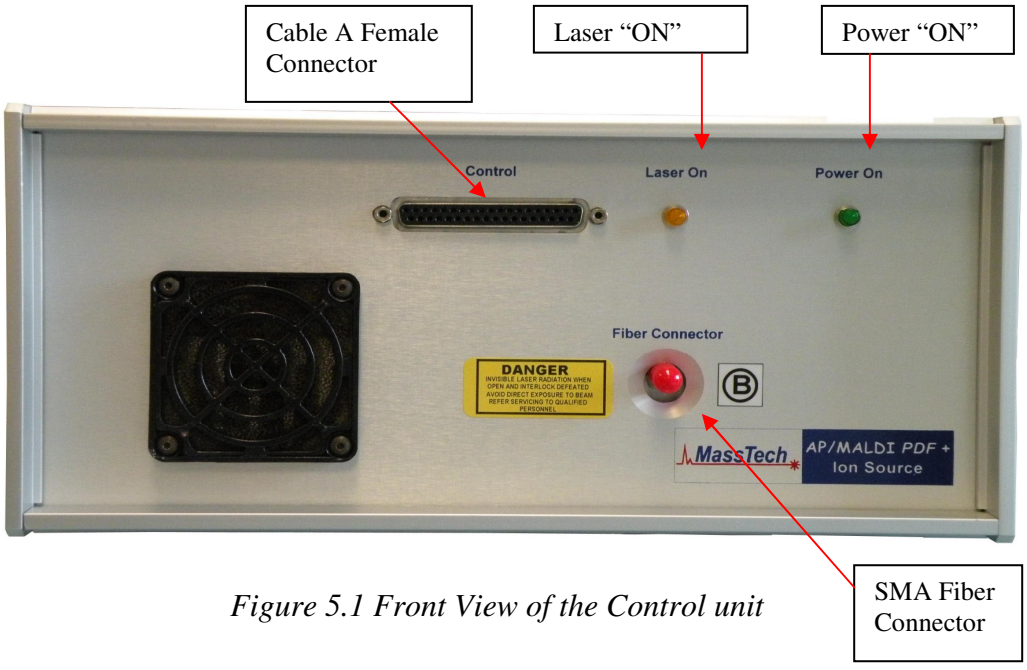



*Figure 5.1 Front View of the Control unit*

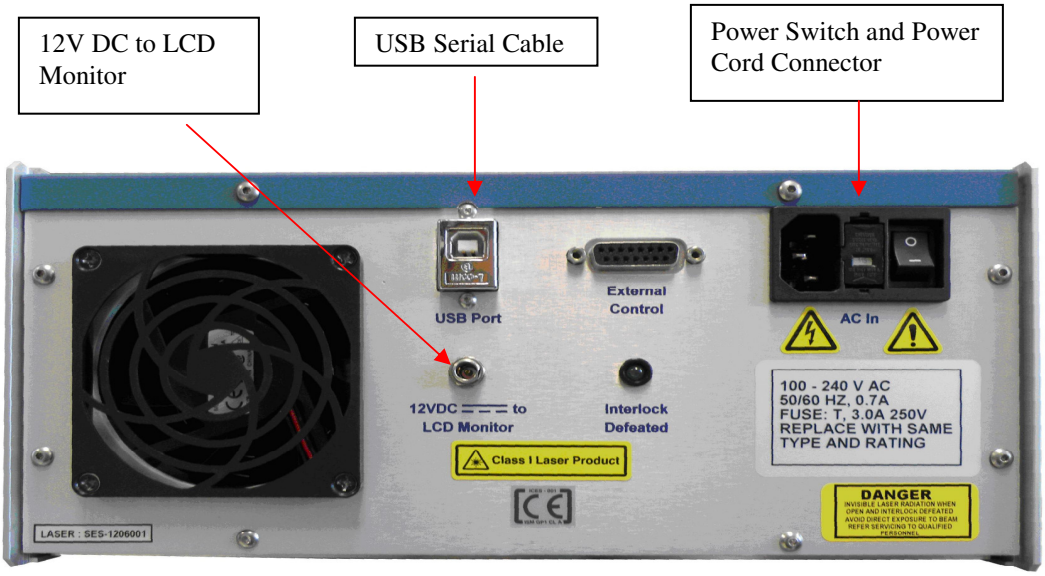



*Figure 5.2 Control Unit Rear View*

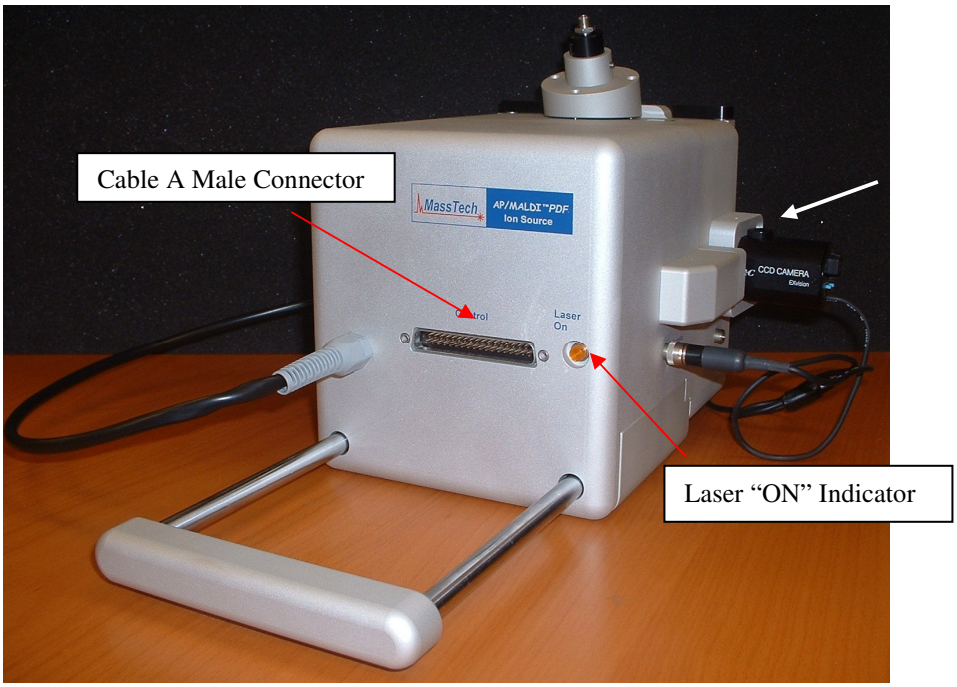



*Figure 5.3 Ion Source for the Thermo Mass Spectrometer*

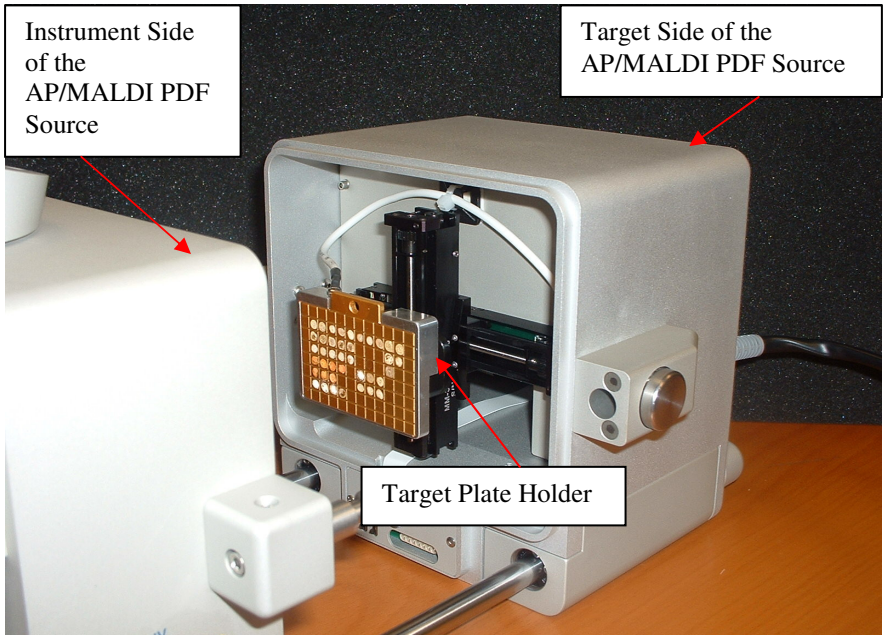



*Figure 5.4 AP/MALDI Ion Source Opened*

Figure 5.4 illustrates the AP/MALDI ion source opened, which contains a 96-spot target plate holder with gold-coated target plate loaded.

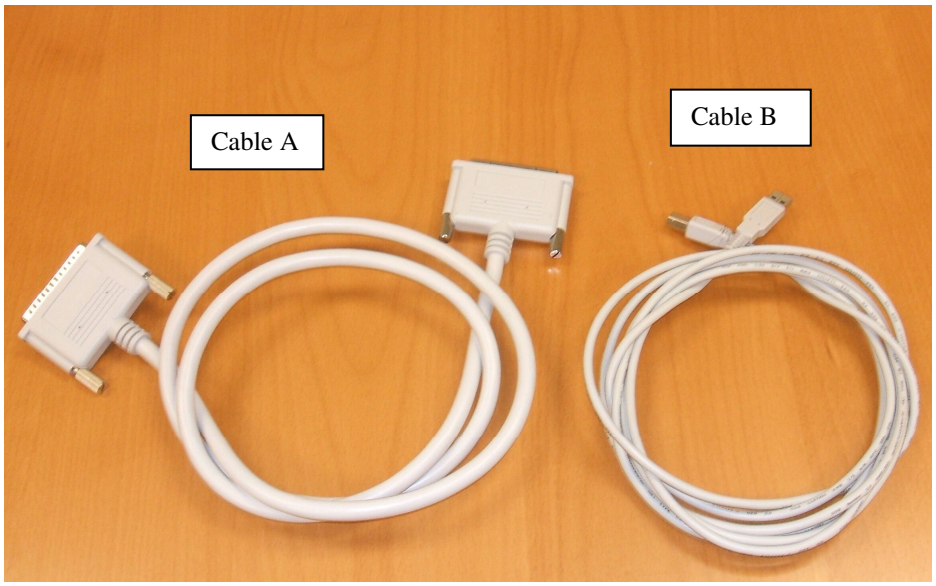



*Figure 5.5 Cable A (Control Unit – to – Source) and Cable B (Control Unit – to – PC, standard USB Cable)*

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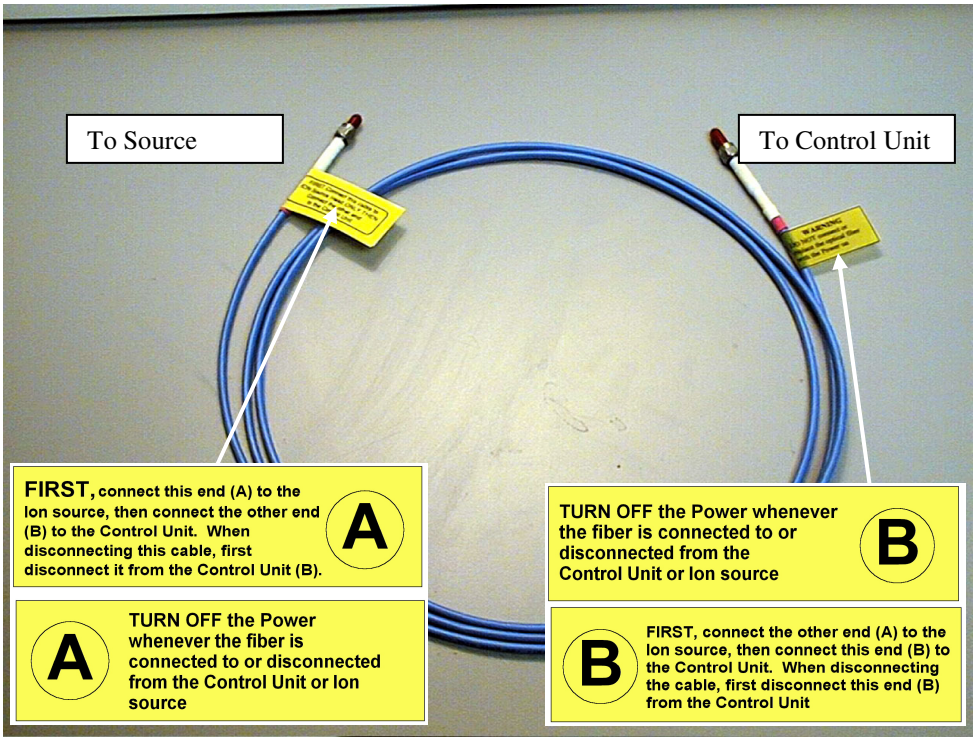
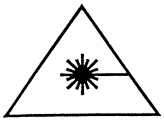



*Figure 5.6 Optical UV-Grade Fiber with SMA-Connectors*

The Optical UV-grad fibers have SMA-connectors on both sides that are covered with protective plastic caps. (The shipment includes one spare Optical cable, not shown in the figure).



***The user must turn "Off" the control unit (so the laser cannot be accidentally fired) whenever the optical fiber is disconnected from either end, or if the user plans to disconnect or connect it***.

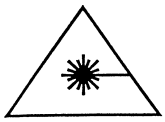
In the event that the user needs to purchase another optical fiber cable, ONLY replace the fiber with an exact replacement from the manufacturer, MassTech Inc.:

**Replacement part numbers:**

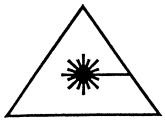
- **OF-300 for AP/MALDI *PDF+;***
- **OF-H100 for AP/MALDI *HR***

### *5.2 Installation of the Source*

**Installing/Uninstalling:** Before the source is installed, uninstalled, or replaced onto the mass spectrometer the mass spectrometer must be in either the "Standby" or "Shutdown" mode. The same rules, as described in the mass spectrometer operator's manual for the replacement of the standard sources (electrospray/nanospray/APCI), are applicable for the AP/MALDI as well.



***Never*** switch the power "ON" at the rear panel of the AP/MALDI PDF control unit before the source is ***completely installed***. The optical fiber needs to properly connected at **both ends** to the AP/MALDI PDF source.



**IMPORTANT:** Whenever the optical fiber is being detached from or connected to the control unit/ source housing, **MAKE SURE the power switch on the control unit is "Off."**

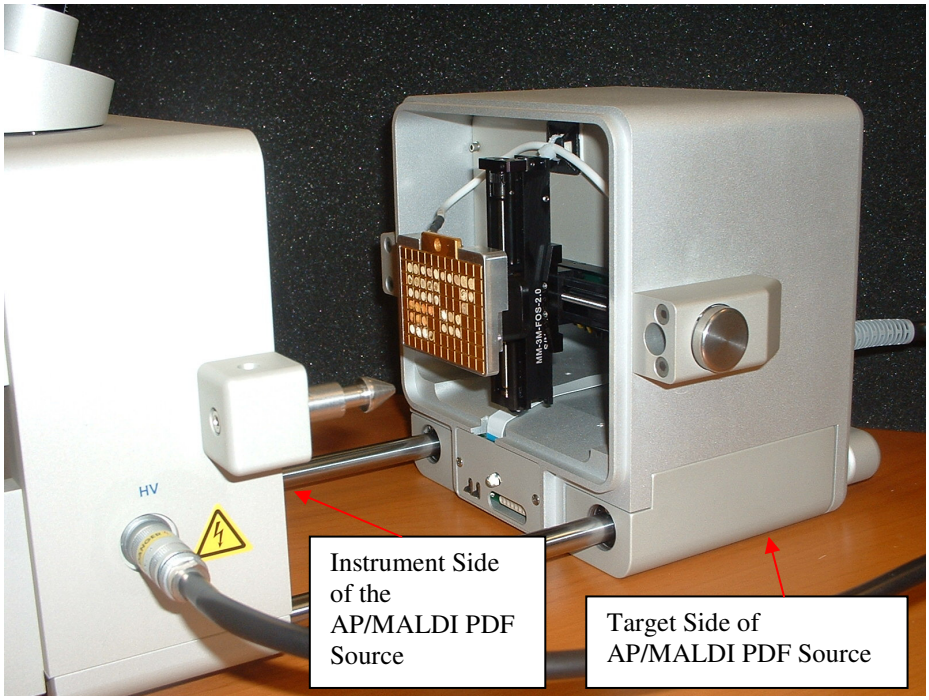



*Figure 5.7 The Two Sides of the AP/MALDI PDF Ion Source*

Remove the current source from the mass spectrometer; this action will result in an empty inlet flange (Fig. 5.8). **The ion sweep cone must also be removed (CAUTION – the ion sweep cone may be hot)**. The mass spectrometer inlet should look like Fig. 5.9 when it is ready for the AP/MALDI-PDF installation.


*Figure 5.8 The Mass Spectrometer without the Ion Source and Ion Sweep Cone Attachment*

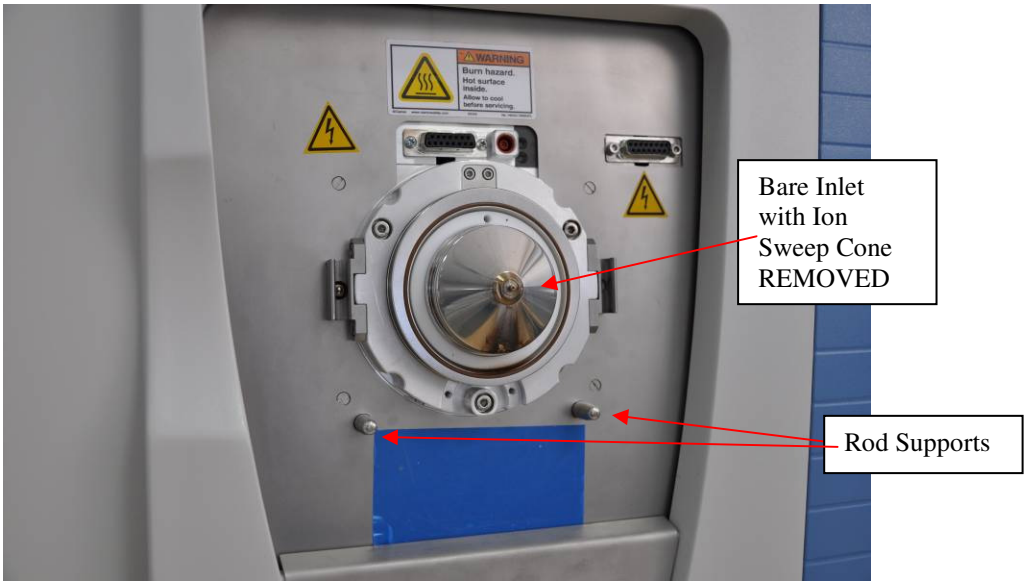



*Fig 5.9a Bare Inlet of the Mass Spectrometer*

Figure 5.9a shows the bare inlet of the mass spectrometer before the AP/MALDI ion source is attached. NOTE: That the ion sweep cone(shown below) has been removed in Figure 5.9b.

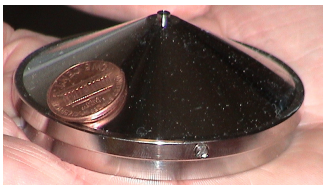


*Fig 5-9b The Ion Sweep Cone Removed Prior to Source Installation*

**CAUTION: The ion sweep cone may be hot!**

Next, mount the AP/MALDI ion source onto the mass spectrometer as one would do for a standard ESI source, lining up the AP/MALDI source with the rod supports (see Fig. 5.9). To lock the source into place, the user turns the black levers inwards toward the machine (Fig. 5.10).

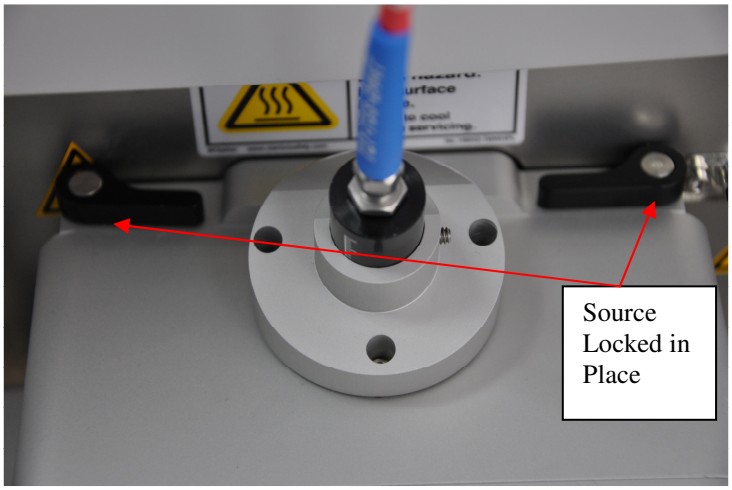



*Fig 5.9 Locking the AP/MALDI-PDF Source onto the Mass Spectrometer*

### *5.3 Wiring of the Source and Control Unit:*

Ensure that the power on the control unit is "OFF" until the source is wired to the control unit.

Connect the black power cord and USB Cable B to the corresponding connectors at the rear plate of the control unit.

**No adjustment is necessary for ~110/~127/~220/~240V AC!**


*Figure 5.10 Back of the Control Unit*

Connect the other end of Cable B to a free USB port on the PC (Fig. 5.11).

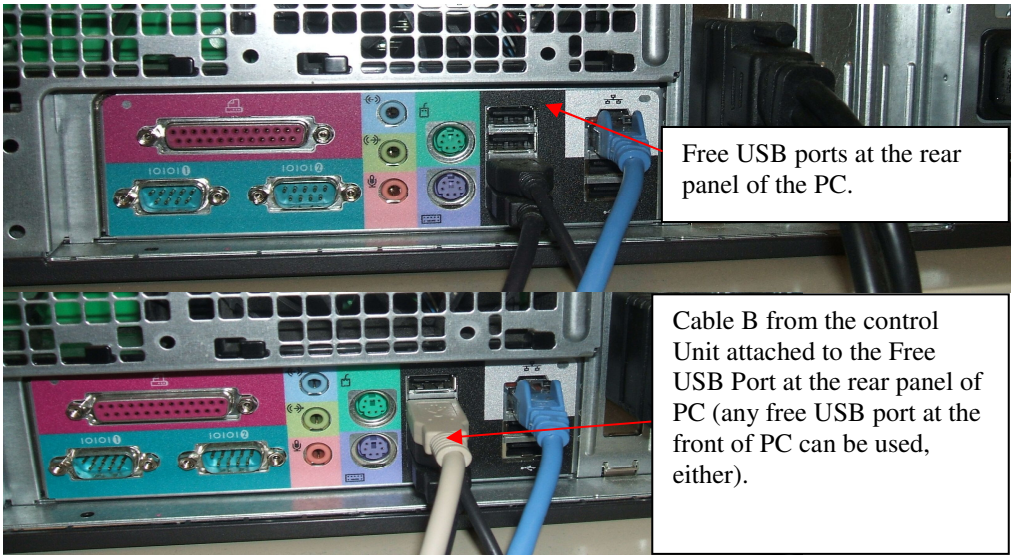



*Fig. 5-11 The Other End of Cable B Connected to a Free USB Port on a PC*

The video out cable (Fig. 5.12) should be attached to either a video card or USB video adapter cable to the user's computer. The camera power cable (Fig. 5.12) should be connected to its receptacle below; the CCD camera as shown in Fig. 5.13.

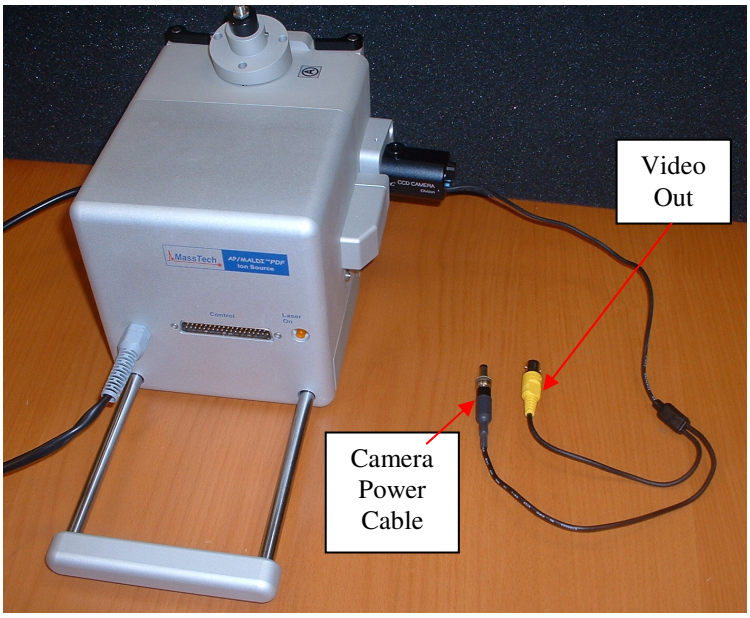



*Figure 5.12 CCD Camera Cables*


*Figure 5.13 Normal Connection for the Camera Power Cable*

An alternative power port maintains the imaging while the source is open.

Now, attach the other wires and cables to the source, according to Figure 5.14 below. Refer to Figs. 5.15 and 5.16 to help with the optical fiber connections and refer to Fig. 5.17 to help with the HV connection.

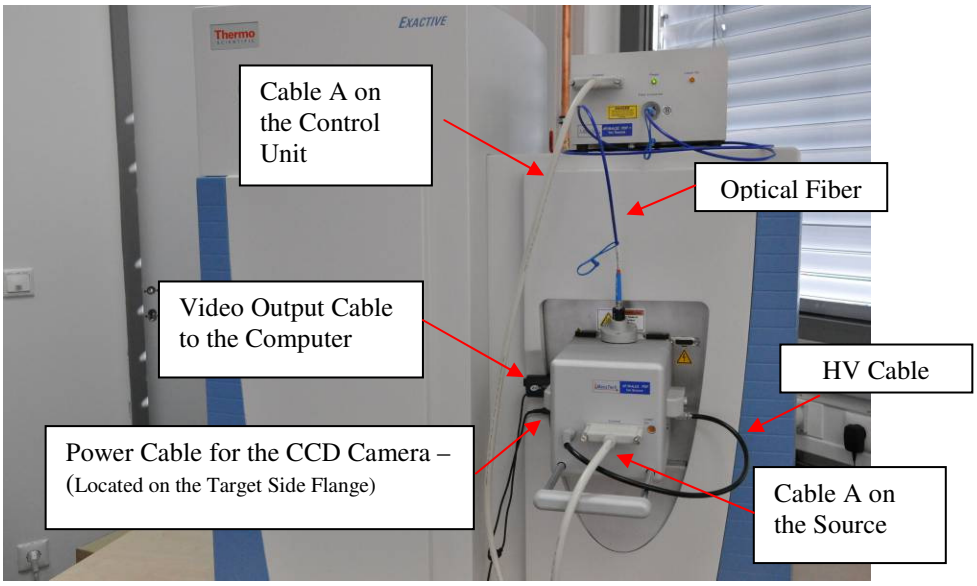
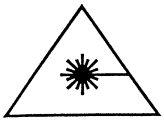



*Figure 5.14 The Source with the Wiring Connections Completed*



When the user installs and uninstalls the source on the mass spectrometer, he/she must connect or disconnect the optical cable from both the source and control unit as shown in the figures below.

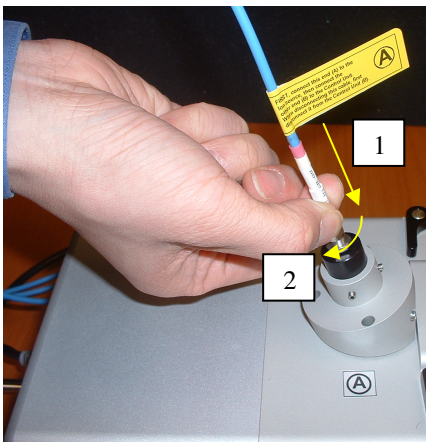



*Fig. 5.15 Connecting the Fiber to the Source (A to A)*

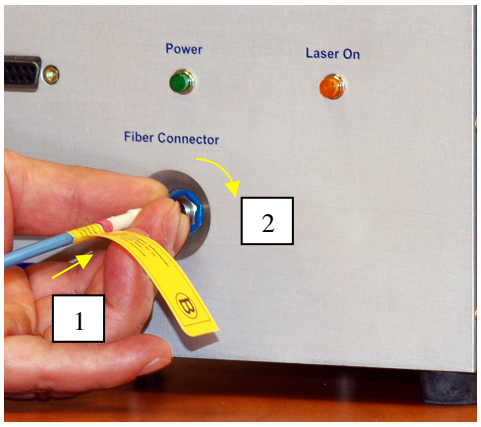


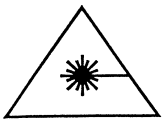

*Fig. 5.16 Connecting the Fiber to the Control Unit (B to B)*

**<u>Connecting the Optical Fiber to the SMA-Connector at the Source:</u>**

- 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 tighten (2) the nut <u>firmly</u> (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. **The user needs to make sure he/she does not touch the optical surface of the fiber with his/her fingers.** If the user touches the fiber with his/her hands did by mistake, the surface needs to be cleaned with ethanol or methanol, as described in Section 7 of this manual.
- Insert the fiber (pushing it in) and then tighten the nut.



When the optical cable is disconnected, any laser firing can emit invisible laser radiation from the ends of the optical cable. Therefore, throughout this manual, MassTech warns the user of this potential 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. 5.17).

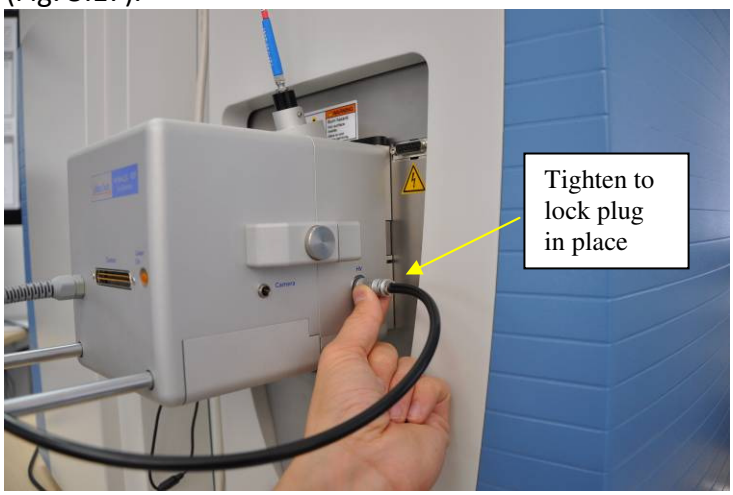



*Figure 5.17.HV Cable Connection*

Figure 5.17 shows the HV cable connection by tightening the knurled back end of the HV plug, which locks the cable in place.

When complete, the mass spectrometer with the AP/MALDI source should look like Figure 5.18:


*Figure 5.18 Completed Source Installation*
(Note: In this picture, camera power cable is plugged into Target Side Flange, although it is normally plugged into Instrument Side Flange)

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.
3. Now it is safe to turn on the control unit.

### *5.4 Fine Adjustments to the Source*

The unit comes pre-tuned, but if necessary, the fine adjustments to the imaging (e.g. sharpness of an image) can be made as shown in Figs. 5.19 and 5.20.

*Figure 5.19 The Alternative Power Cable Connection for the CCD Camera*

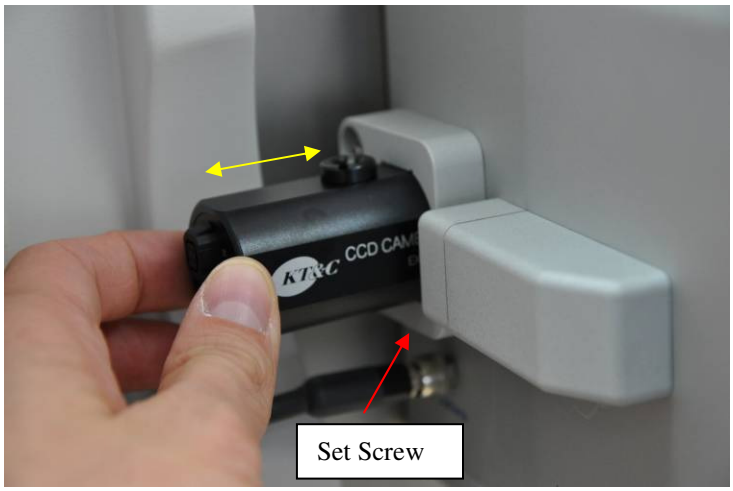



*Figure 5.20 CCD Camera Focusing*

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 the image quality. These controls are under the video capture menu located on the video capture box.

If necessary, the fine adjustments to the laser positioning and focusing can be made as shown in Fig. 5.21 and Fig. 5.22. More details are in Sections 8.2 and 8.3.

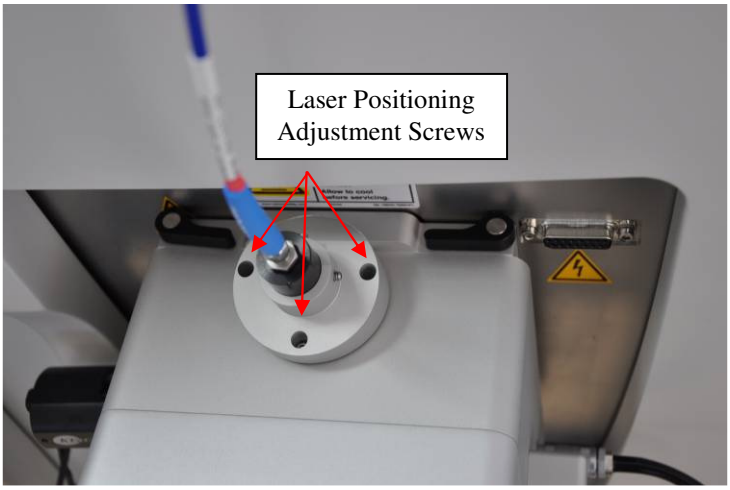



*Figure 5.2 Laser Positioned by Adjusting the Three Top Screws*

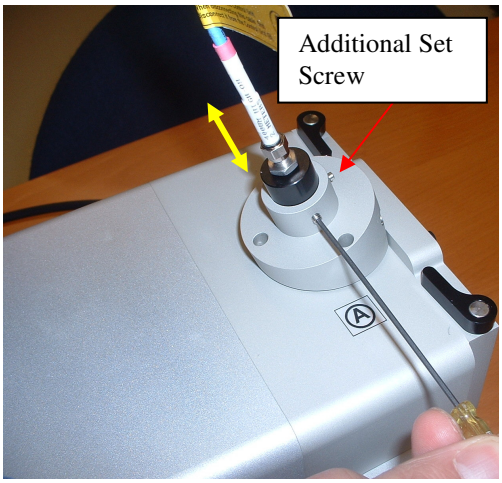



*Figure 5.22 Laser Focused*

The laser is focused by loosening the side set screw, adjusting focusing optic up/down, and then retightening set screw.

### *5.5 Source Removal and Uninstallation*

When the user needs to remove the AP/MALDI ion source in order to put another device on the MS, he/she must follow the directions below:

1. Set the MS Instrument to either the “standby” or “OFF” mode.
2. Turn the power on the control unit to the “OFF” mode.
3. Then, uninstall the source by reversing the installation procedure as described in Section 5.2.

## 6 SAMPLE PREPARATION

The same sample preparation techniques and the same matrix used for the conventional MALDI vacuum can be used for the AP/MALDI sample preparation. The main difference, with the vacuum AP/MALDI, 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 steps below are deemed as a typical sample preparation procedure:

- Carefully clean the target plate surface
- For the standards test, a α-Cyano-4-hydroxycinnamic acid (α-CHCA) matrix is recommended
- Mix a 1:1 matrix and analyte solution that is composed of the standard peptides (Angiotensin, Bradykinin, Gramicidin S and/or similar peptides) with a concentration of approximately 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, the matrix and analyte solutions can be deposited on the target separately and then allowed to dry).

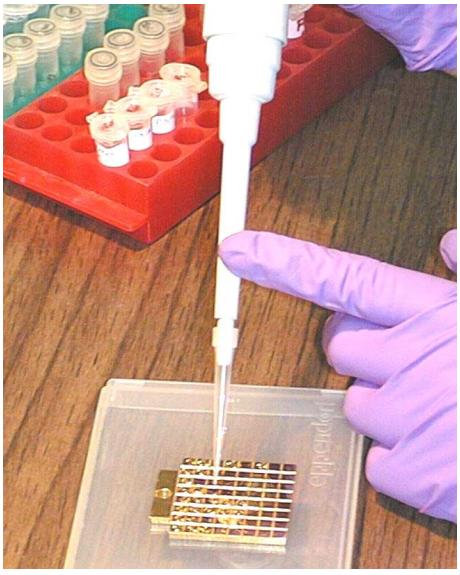


*Figure 6.1 Spotting of Several Standard Samples*

Figure 6.1 shows the spotting of several standard samples on a target (sample) plate for testing by the AP/MALDI. The sample preparation procedure is similar to the original MALDI experiments.

### *6.1 Loading/Unloading the Target Plate*

The user needs to first open the AP/MALDI source to load or unload the target plate. The user must then put the mass spectrometer in either the "Standby" or "Shutdown" mode. To stop the laser firing, click on the "Stop" button in the 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.

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

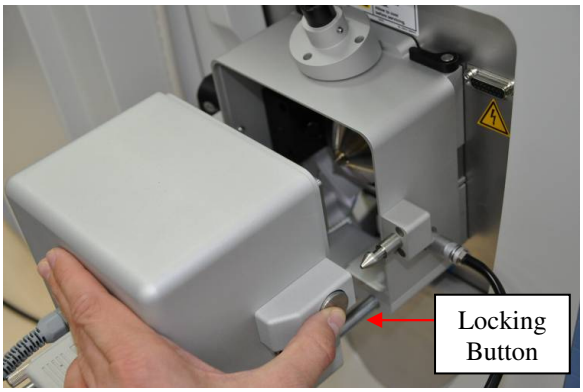



*Figure 6.2 Opening the Source*

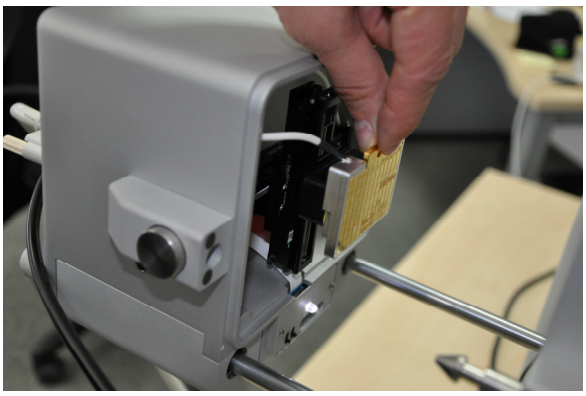


*Figure 6.3 Handling the Target Plate with the Prepared Sample Spots*

Figure 6.3 shows the user inserting 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, there should be a "click" sound, which corresponds to the two halves of the source locking together. After closing the source, pull back on the target side flange gently, to ensure that the two halves of the source housing are locked.

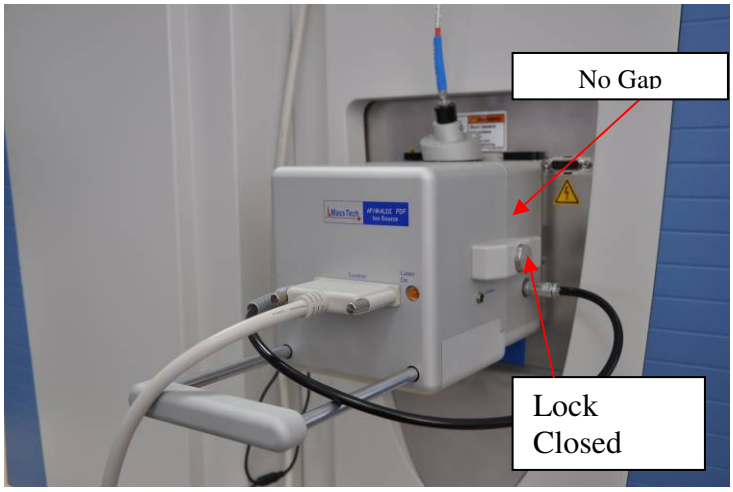



*Figure 6.4 Source "Clicked" into the Close Position*

Figure 6.4 shows the source "clicked" into the close position after the target (sample) plate is loaded. Notice that there is no gap between the two halves of the ion source.

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

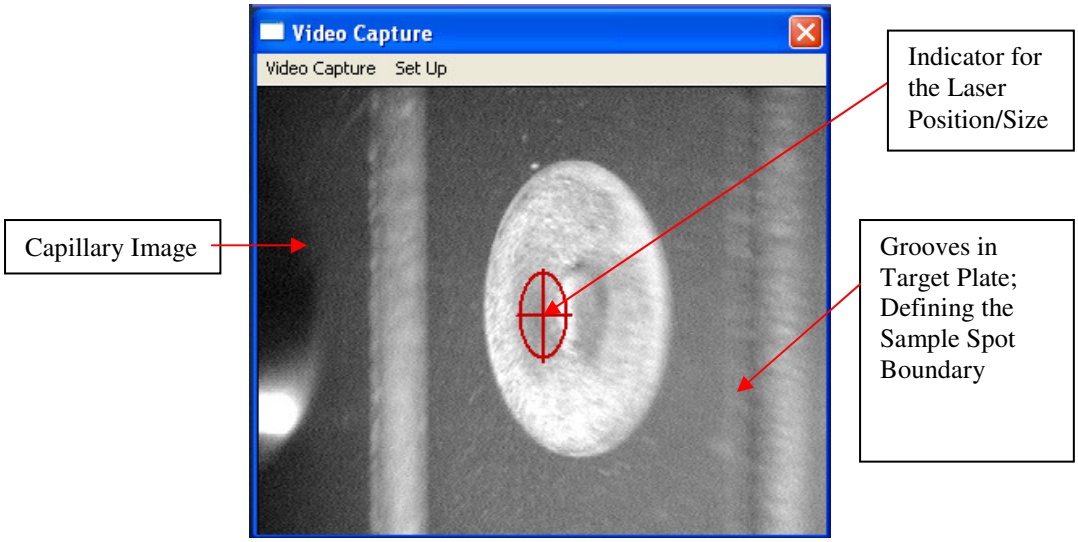



*Figure 6.5 MALDI Sample ready for the AP/MALDI Analysis*

For a properly loaded target plate that has the software already running, the user should see the following on the video capture image seen in Figure 6.5.

# 7 AP/MALDI OPERATION

## *7.1* Installing the TARGET Software and USB Drivers:

**Before starting the installation of AP/MALDI Software, install hardware/software of video capture device on the computer designated to control AP/MALDI source; this is according the appropriate Operational Manual of the video capture device shipped within AP/MALDI system.**

The Target Version 7.0 Software is used to control the AP/MALDI target motion, laser firing, and PDF operation.

To install the Target Software, follow these steps (under the Windows operating system, the user will need the administrator's access):

1. Download the Installer Package (can be found on the Downloads page) from apmaldi.com; the Installer Package will be under the Downloads for AP/MALDI HR and AP/MALDI *PDF+* Section.
2. Insert the installation CD and run the Setup.exe Program from the user's CD drive.
3. Choose the desirable location and folder name for the Target Software. By default, the folder name is: **C:\Program Files\MassTech**
4. Follow the next few dialog boxes to completion.

After the Target Software installation process is completed, it is recommended for the user to create a shortcut from the desktop to the Target.exe.

To install the software and enable the USB communication on the control unit, follow these steps (under Windows, the user will need the administrator's access):

1. **DO NOT** connect the control unit to the PC through cable B. If the cable is plugged in, unplug it at from any and all sides.
2. Download the Target Software self installation package from the www.apmaldi.com
3. Connect both the control unit and computer with cable B.
4. Turn "ON" the power to the control unit.
5. The computer should detect the "New Hardware Found."
6. Follow the next remaining 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 window (Fig. 7.1) will soon appear.

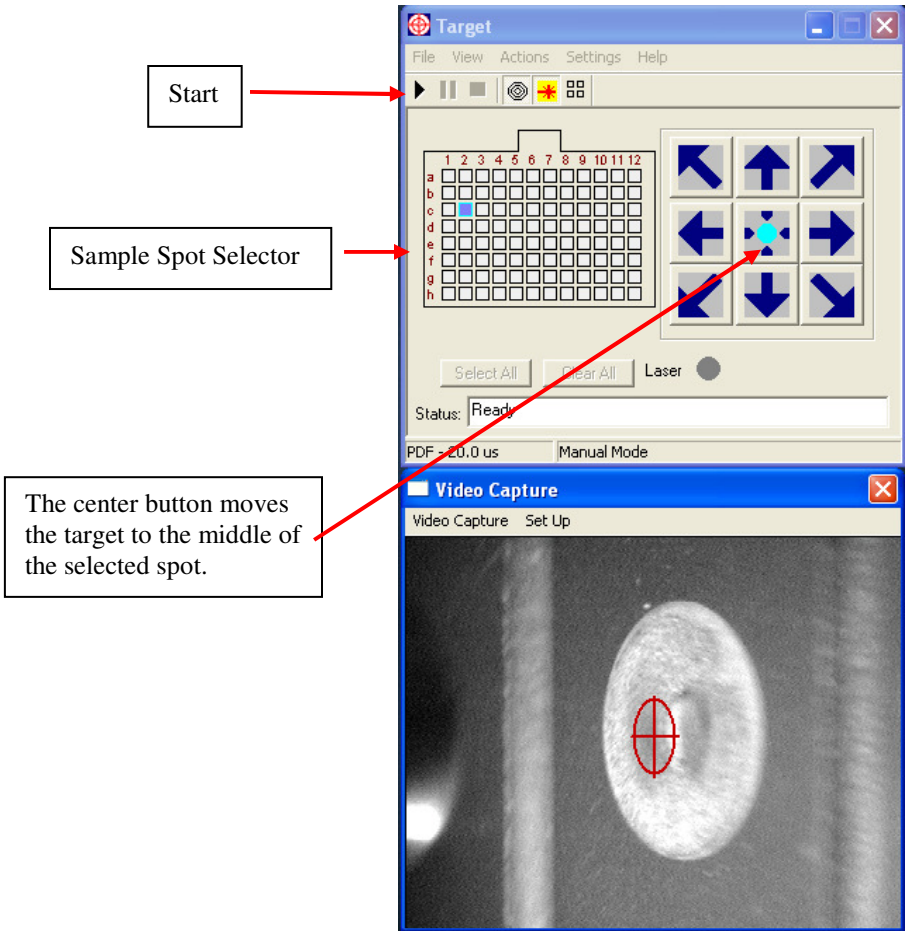



*Figure 7.1 The Target Software Started, Properly Initialized, and Ready*

At this moment, the initialization of the XY stages will start automatically. If everything has been connected properly, the user will see target motion on the video capture screen. During initialization, the target moves to its different 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 in the "Off" mode, or if the control unit is not properly wired to the computer, the user will get the message shown in Fig. 7.2. Once the problem has been corrected, then reinitialize the software by going through the following steps: **Settings>Set Parameters>General>Init Motors**.

The Target Software can also be reinitialized by exiting and restarting the program.

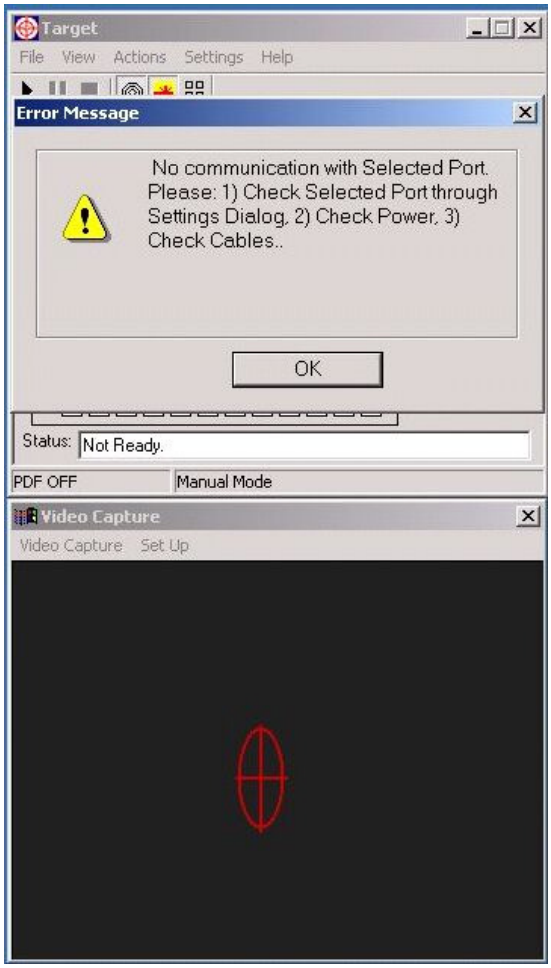



*Figure 7.2 Error Message*

After the AP/MALDI 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 a result of the housing source being open – in which case simply close the source. Another possibility for the "Not Ready" status is that an interlock is open. Check the housing source, when close, to make sure the fiber optic is tightly secured to the connector.

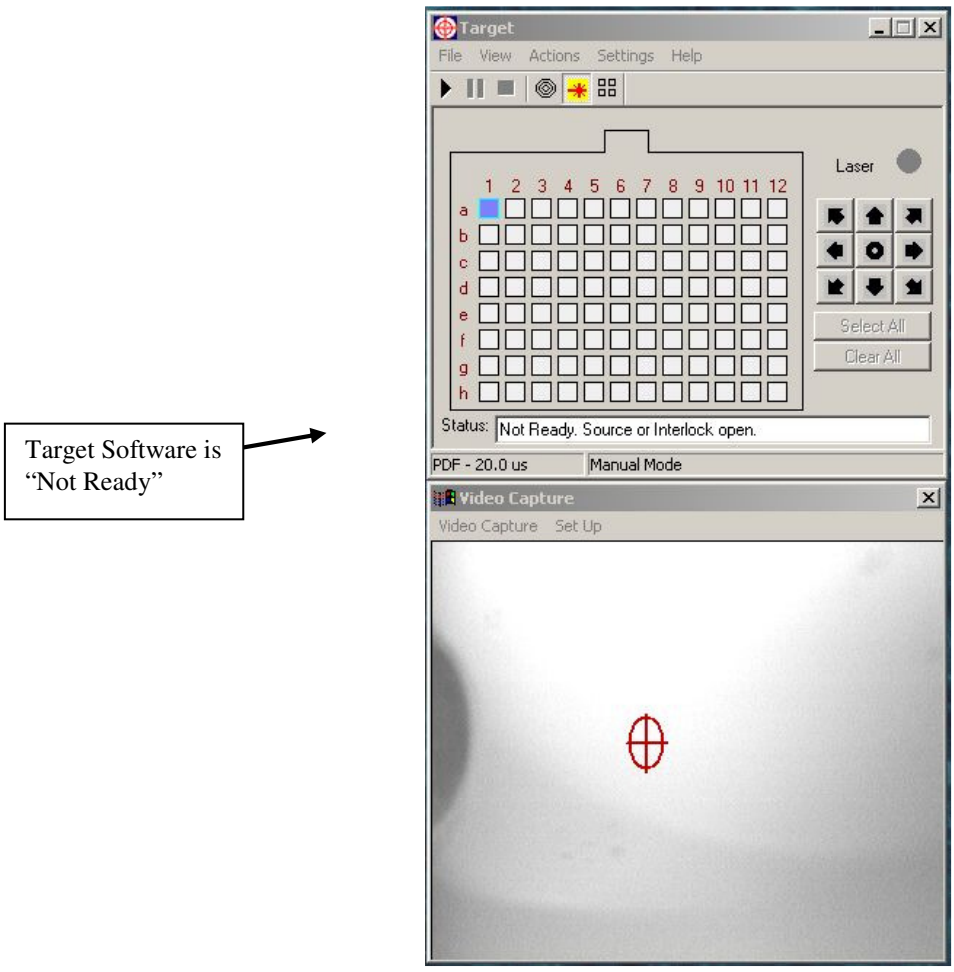



*Figure 7.3 Target Software Showing the "Not Ready" State*

### *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**: The 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 crosshairs to the position where the laser is firing (Fig. 7.4).

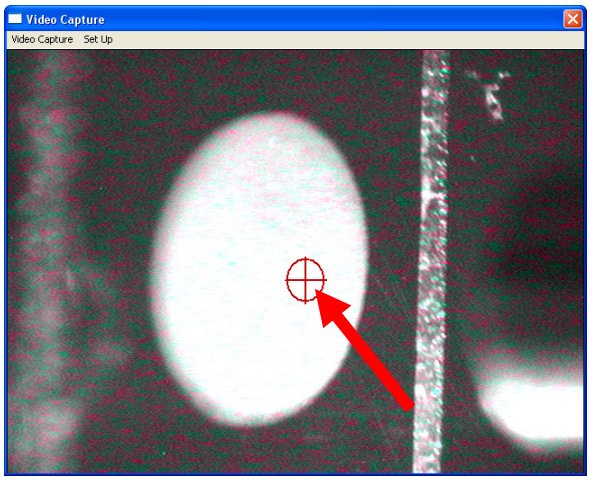



*Figure 7.4 Positioning the Red (Laser) Cross Hairs*

Positioning the red laser cross hairs is done by placing the mouse at its center, *pressing Ctrl,* and dragging the red cross hairs to the desirable position.

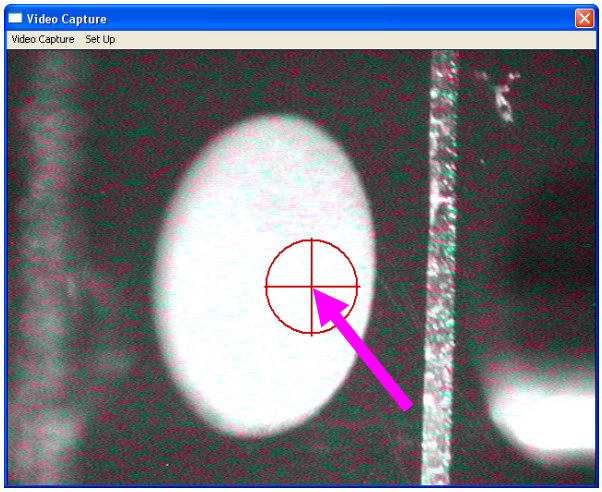



*Figure 7.5 Resizing the Laser*

The laser can be resized by placing the mouse at the center of the red cross hairs, pressing *Shift,* and dragging the mouse to the 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; double-clicking the left mouse button will move the sample to the desired location. Before

this system can be accurately utilized, it must be calibrated. The user can calibrate the positioning of the "point-and-click" under the video capture menu bar. He/she will click the "Set Up" icon and then choose the "Point & Click Positioning" option (Fig. 7.6).

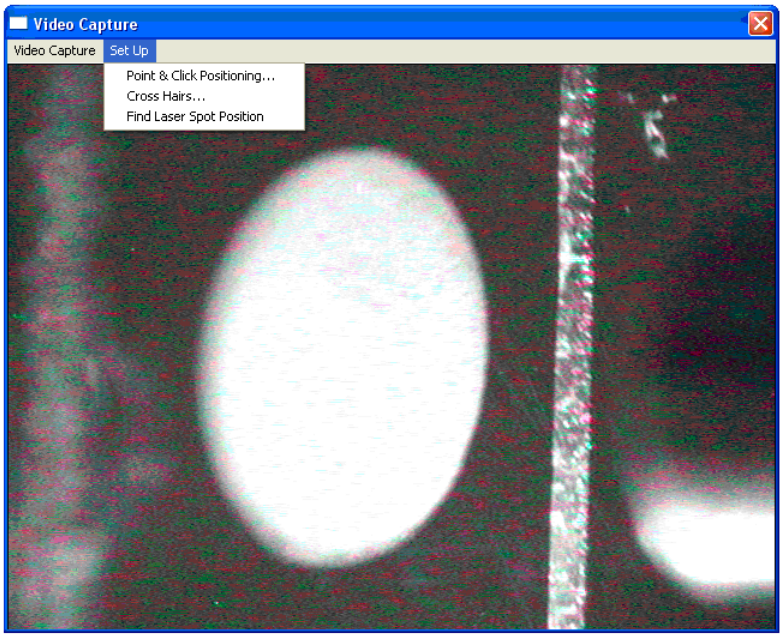



*Figure 7.6 Start of the Video Capture Sample Positioning Calibration Procedure*

Following the calibration, a five-step procedure will be described in the upcoming dialog boxes. The first step is to ensure that there is a distinct visual object on the screen (see Fig. 7.7). A sample plate with an ablated 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).

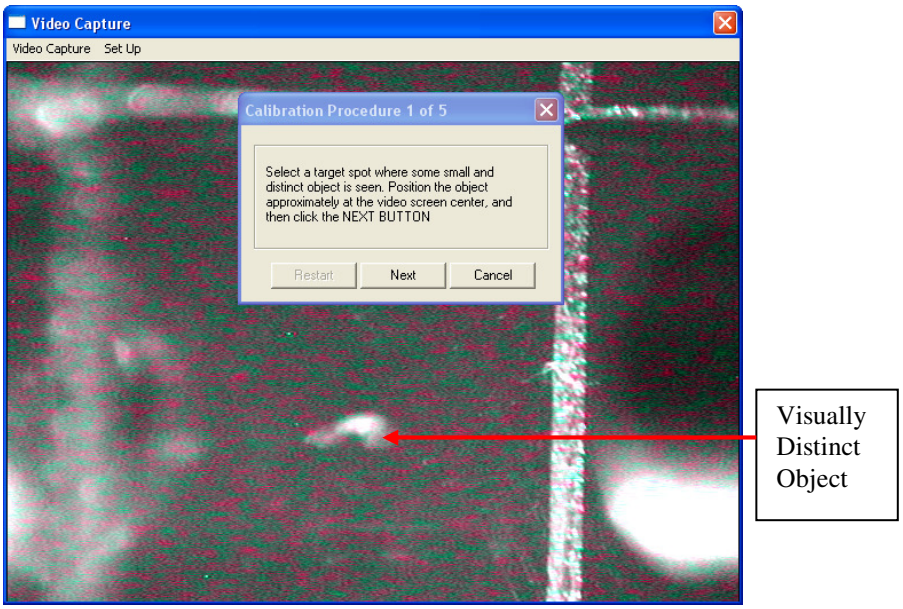



*Figure 7.7 Step 1*

In Step 2 (Fig. 7.8), drag and immediately drop the green target icon to the visually distinct object in the lower right hand quadrant. Then, click "Next."

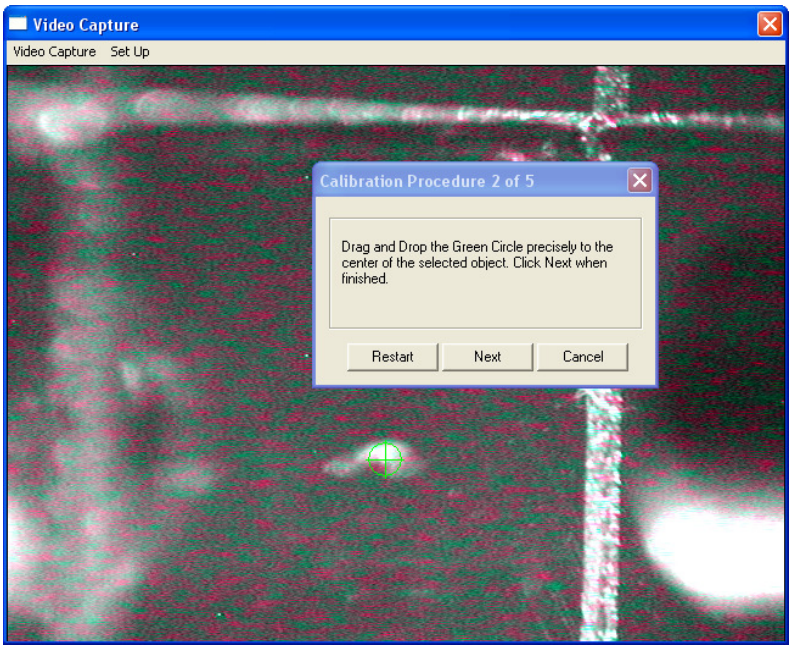



*Figure 7.8a Step 2*

Figure 7.8a shows the position of the original green target on the video capture screen.

In Step 3 (Fig. 7.9), the target plate moves horizontally. Drag and quickly drop the green target icon to the same visually distinct object. Then, click "Next."

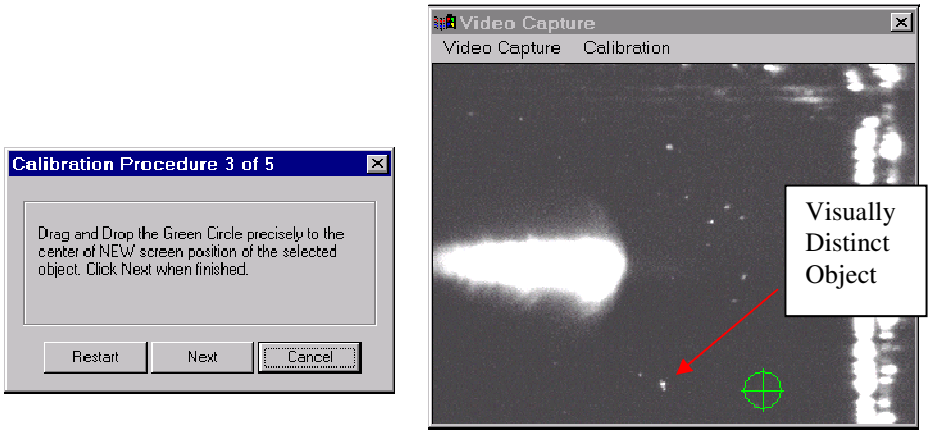



*Figure 7.9a Step 3*

Figure 7.9a shows that the visually distinct object will move *horizontally* (away from the green target).

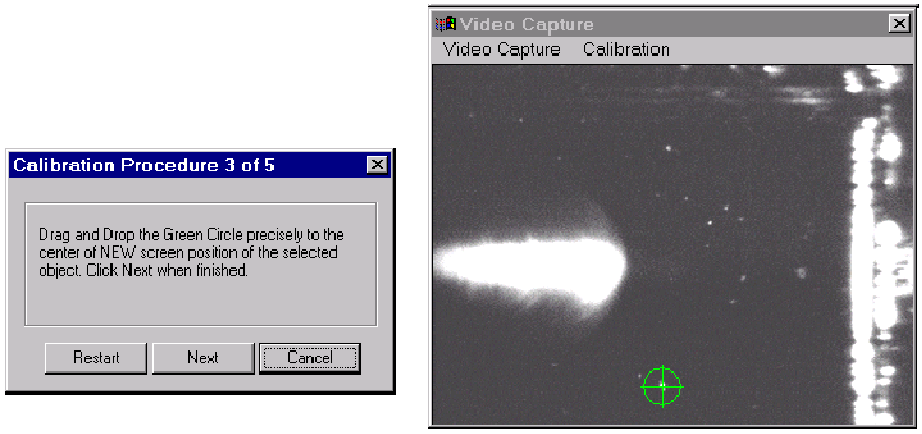



*Figure 7.9b Step*

Figure 7.9b shows the green target dragged and dropped back on top of the visually distinct object.

In Step 4 (Fig.7.10), the target plate moves vertically. Drag and drop the green target icon to the same visually distinct object; then, click "Next."

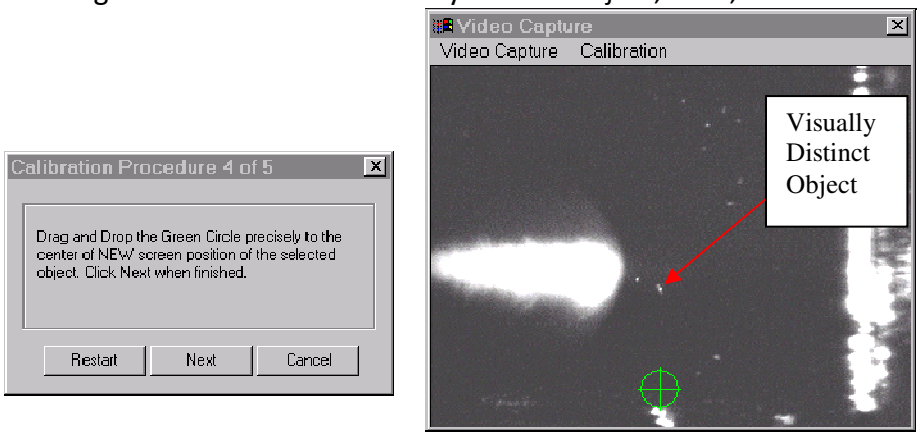



*Figure 7.10a Step 4*

The image above shows that the visually distinct object moved *vertically* (away from the green target).

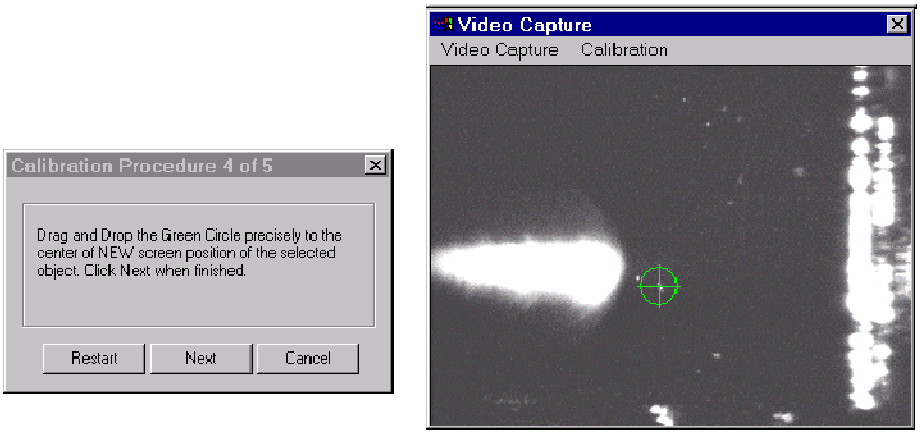



*Figure 7.10b Step 4*

Step B shows 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. The “Cancel” button keeps the original calibration while the “Restart” button allows for the user to recalibrate the system again.

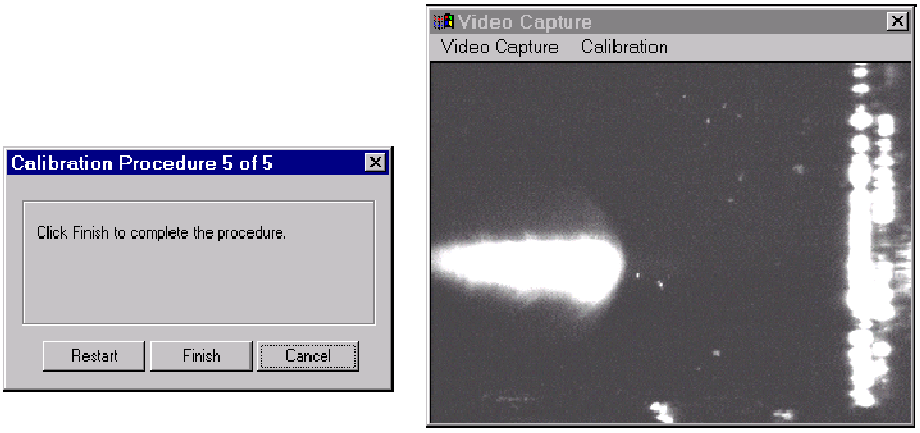



*Figure 7.11 Step 5*

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

### *7.5 Finding the Laser Spot Position*

To get the maximum sensitivity, the user needs to put the laser spot between the capillary inlet of the mass spectrometer and the image of the capillary inlet.

In Step 1, drag and drop the green target icon to the capillary inlet of the mass spectrometer (Fig. 7.12). In Step 2, drag and drop the green target icon to the image of the capillary inlet of the mass spectrometer (Fig. 7.13). Then click “Next.” Now the user should see the red cross hair between the capillary inlet of the mass spectrometer and the image of the capillary inlet.

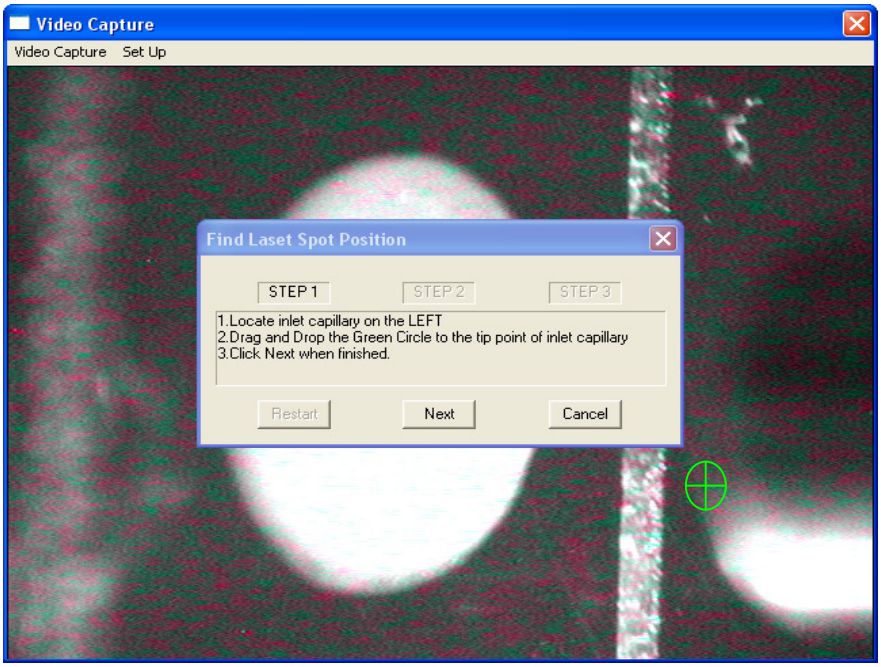



*Figure 7.12 Step 1*

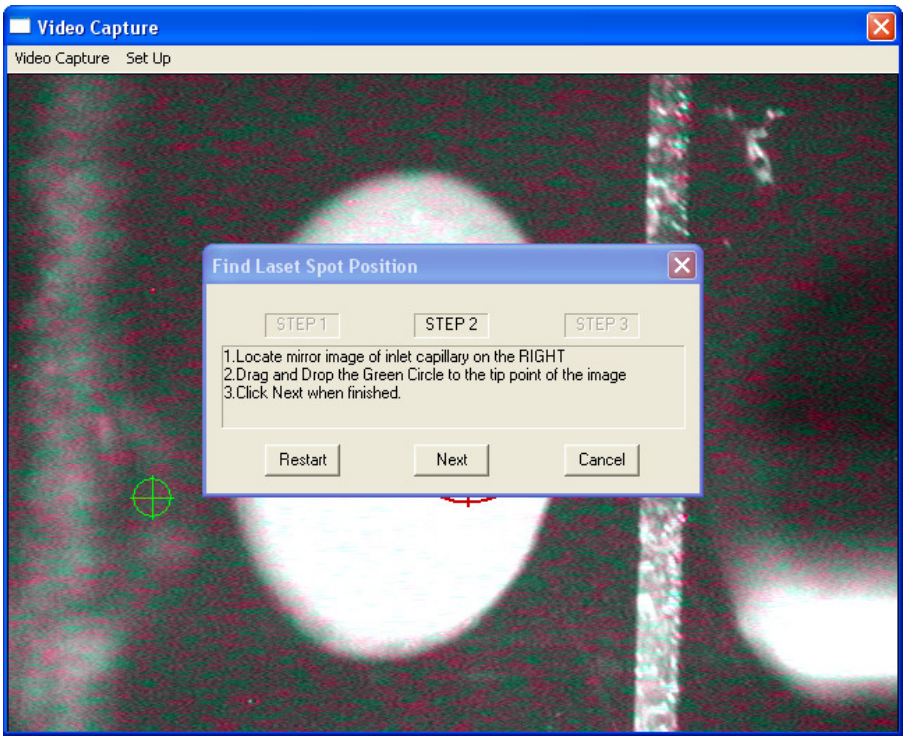



*Figure 7.13 Step 2*

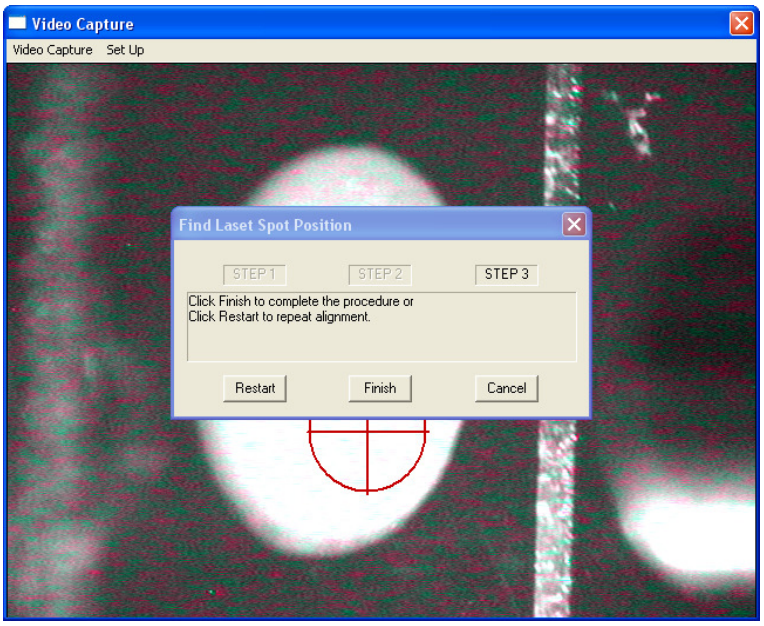



*Figure 7.14 Step 3*

## *7.6 Running the AP/MALDI on the Thermo MS Instrument*

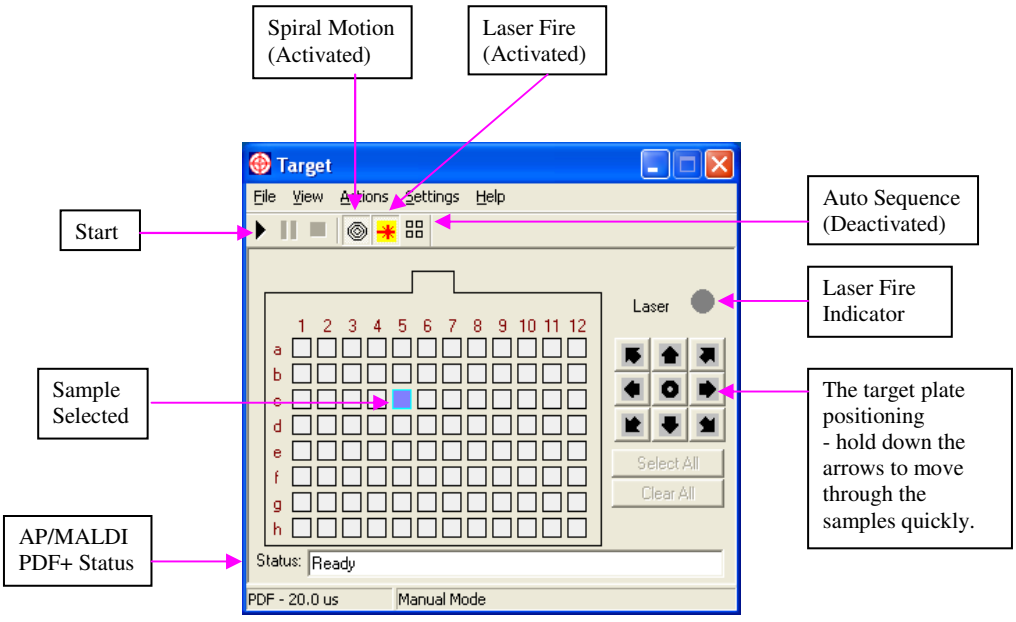



*Figure 7.15 Target Software Controls*

There are two modes of operation for the Target Software: manual (auto sequence toolbar icon is deactivated) and auto sequence. Switch between the modes by pressing the auto sequence toolbar icon  (Fig. 7.12). In the 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. The user can shift the position of the spot by clicking the arrow buttons placed around the center button as shown in Fig. 7.12, or through holding down on the arrow buttons to advance to the next sample faster. Click the center button to restore the central spot position.

In the auto sequence mode, multiple samples can be pre-selected. Use the clear all/select all buttons to select/clear all the spots. To select a continuous series of spots, follow the required procedures:

- Click the first spot.
- Pressing/holding down the *Shift* button, click the last spot.
- To choose selected spots, press *CTRL* and click the spots needed for analyzing.

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

Note, that even after the actions have started (i.e., the “PLAY” button has been pressed), the user can manually shift the spot by clicking on the video capture image. The user can additionally switch the laser to the “ON” or “OFF” mode and start/stop the spiral motion by activating/deactivating the appropriate button(s).

In the auto sequence mode, after the “PLAY” button has been 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. if activated)). After a pre-selected time, all actions stop automatically and the target moves to the next pre-selected spot; the same selected spiraling motion will occur if originally 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) and from the top to bottom rows. Additional time delays can be introduced between the samples and between the rows.

To change the various program parameters such as the: spiral/raster motion properties, laser frequency, auto sequence 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.

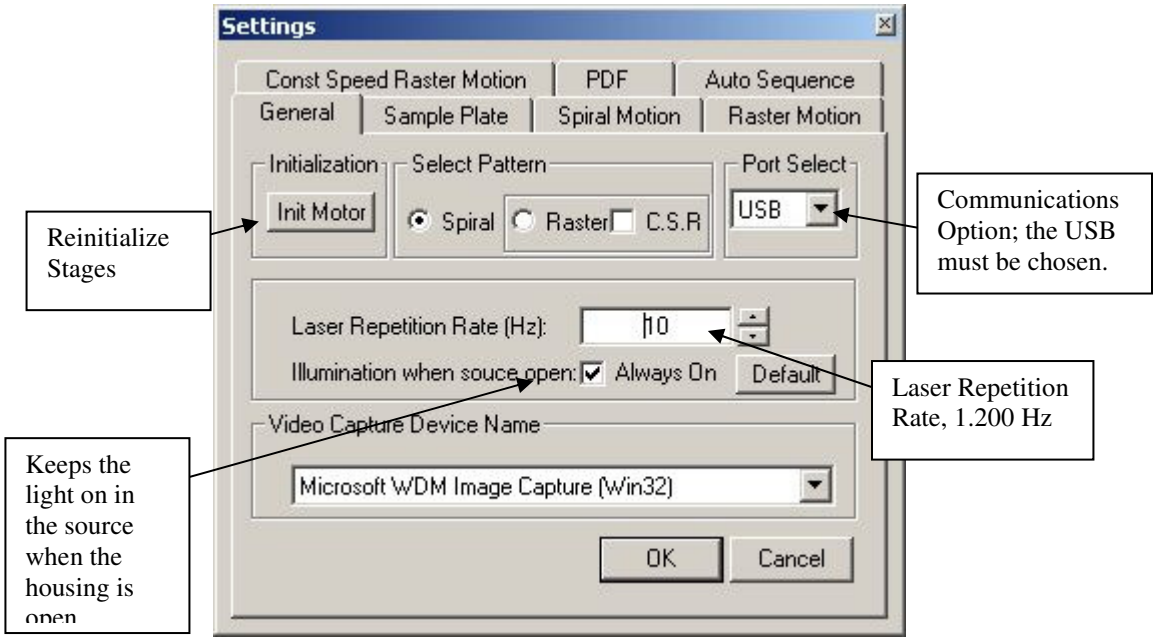



*Figure 7.16 General Settings in the Target Software*

There are three different sample plate patterns that can be selected during the MS data acquisition to desorb the fresh regions of the sample surface by laser radiation. The spiral motion can be seen in Fig. 7.16, while the raster images can be seen in Fig. 7.16 as well. The parameters of the patterned motion can be adjusted using the two separate pages of the setting dialog function: spiral and raster motion, correspondingly. The spiral or raster motions can be used if the acquisition of long enough MS signal is desirable. A relatively weak MS/MS data recording would expected during the signal accumulation if using either motions.

Note: Constant Speed Raster Motion (CSR) tab under the Settings Window is obsolete. Do not use it. To use and Constant Speed Raster Motion, use Zoom Mode (Section 7.12)

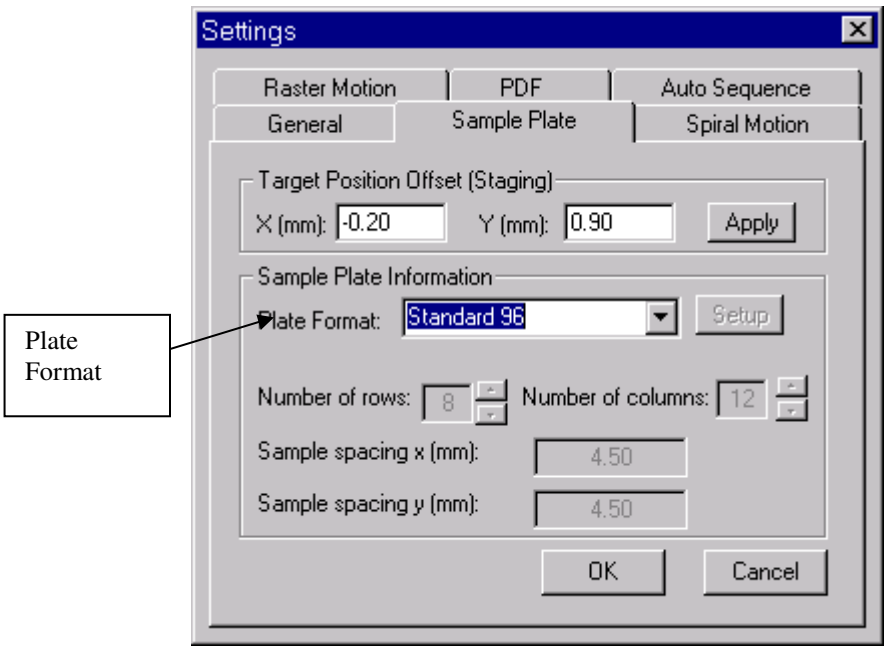



*Figure 7.17 Sample Plate Settings in the Target Software*

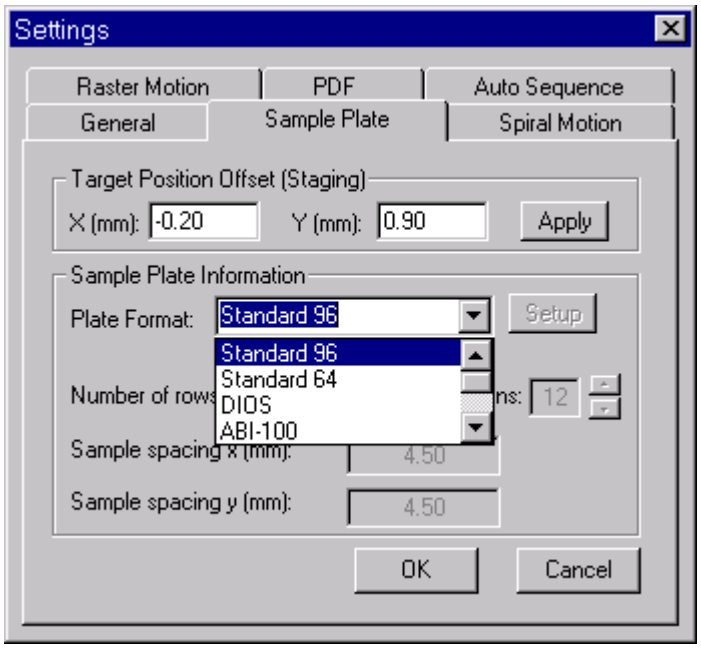



*Figure 7.18 Plate Formats Supported by the Target Version 7.0 and the AP/MALDI Ion Source*

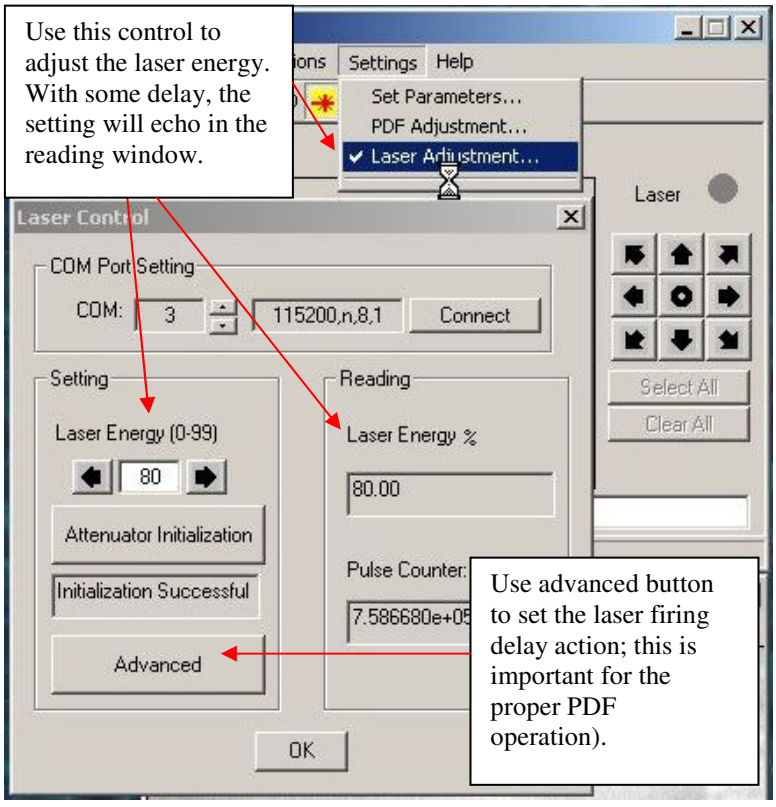



*Figure 7.19 Laser Adjustment Dialog*

To adjust the laser attenuation, use the dialog box shown in Fig. 7.17 (accessed through the **Settings -> Laser Adjustment** menu item).

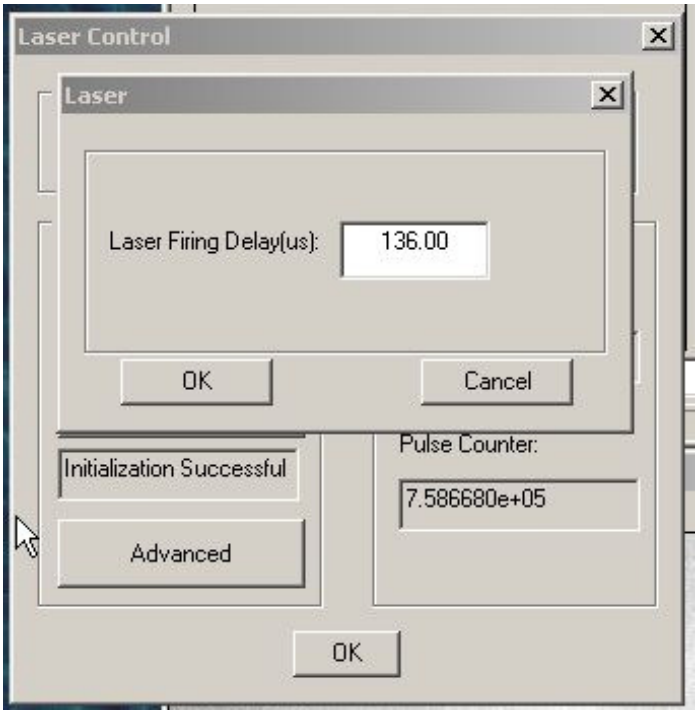



*Figure 7.20 Laser Firing Delay*

The AP/MALDI source is ready for operation after the user has completed the following:

1. Hardware/software installation
2. Ssample preparation
3. Successful run of the Target Software

### *7.6 Setting the Parameters of the Thermo Mass Spectrometers*

To run the AP/MALDI on the MS instrument optimally, the following tuning procedure of the Analyst MS Control Program is recommended:

- Tune the instrument in ESI mode before switching the source to the AP/MALDI mode and save the corresponding tune-file; refer to the MS Operator's Manual.

- Typical parameters that represent a good starting point for the AP/MALDI measurements are shown in Figure 7.18 and 7.19.

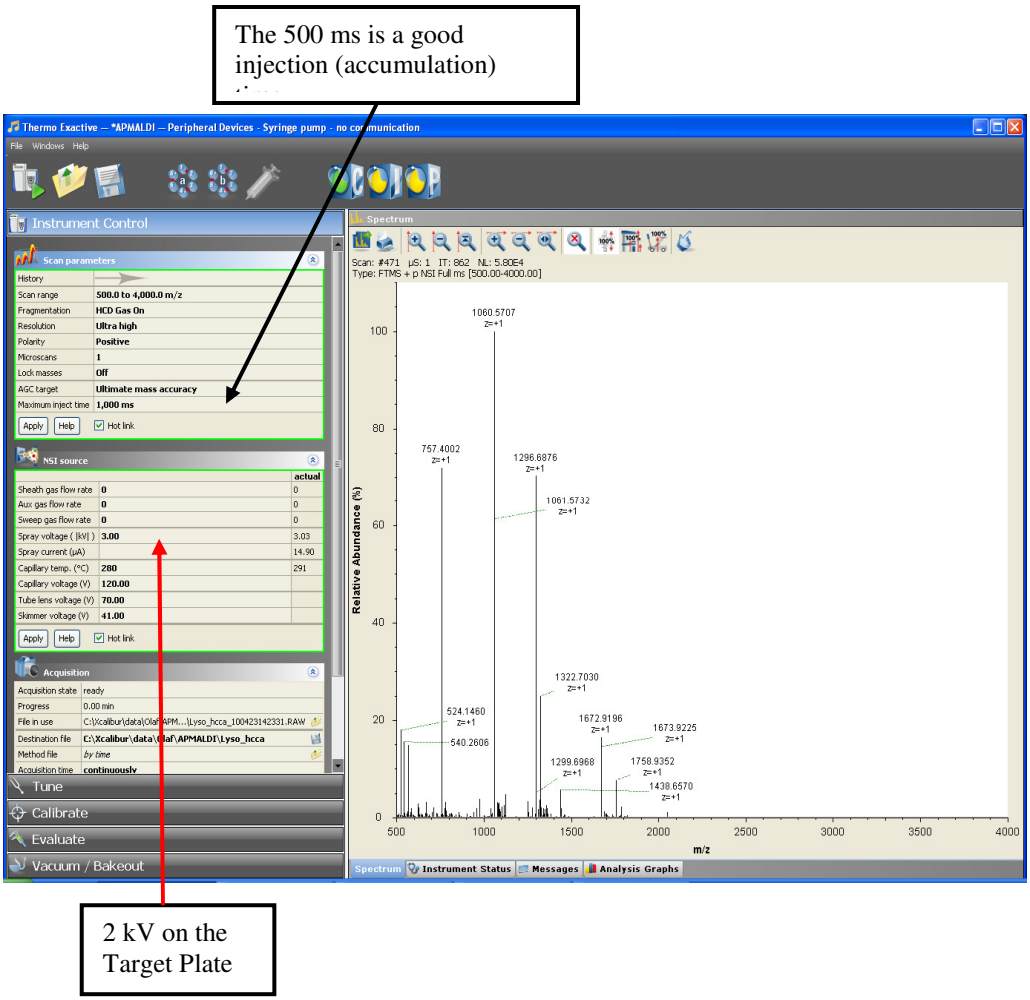



*Figure 7.21 Good Parameters for the Capillary HV (2000 V) and Injection (Accumulation) Time (500 ms)*

- **Drying gas and nebulizing gas settings**: AP/MALDI process does not utilize drying gas or nebulizer gas. The source settings in the XCalibur control software –in most cases recognized as MALDI source- should reflect this. In some Thermo MS systems, if such recognition does not happen, the user needs to set the ion source as nanoESI.
- Laser pulse energy and frequency may be easily tuned through Target software (see Fig. 7.13 and 7.17). 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 seconds. It is sufficient for MS and MS/MS experiments.

- Fig. 7.19 represents a screen copy made during an AP/MALDI spectrum measurement of CHCA matrix. You can easily switch between the *XCalibur* and *TARGET* programs to operate both the MS and AP/MALDI source from the same computer. Or alternatively, separate computers can be used to run *TARGET* software and operate AP/MALDI.

-

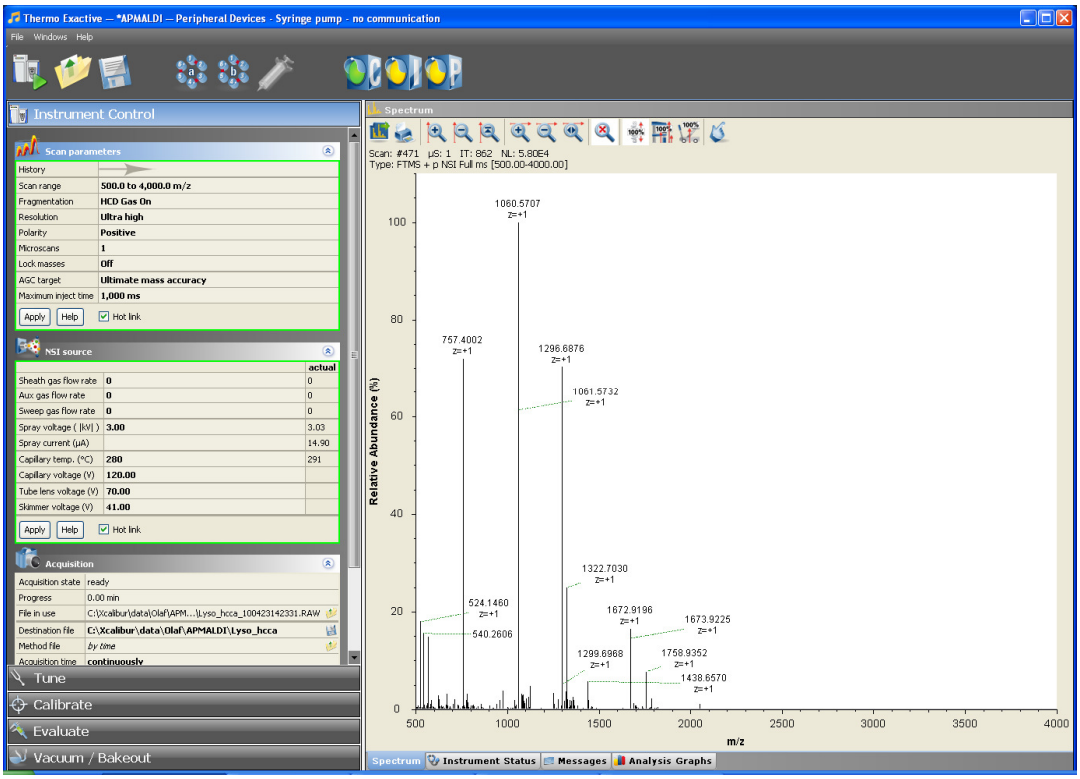



*Figure 7.22 Operation of the Target Software*

### *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, which is located on 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. 7.24. This timed interval is also later displayed in microseconds on the lower left-hand corner of the Target Operating Software.

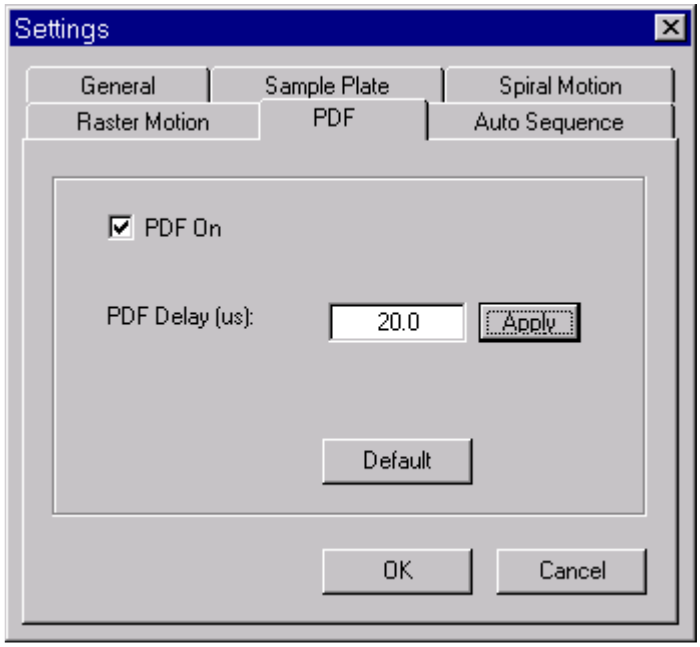



*Figure 7.23 Pulse Delay Adjustment*

To achieve the best performance from applying the PDF technique, the user should first operate the PDF in the "OFF" mode by un-checking the PDF box (Fig. 7.24 shows the PDF box checked in the "ON" mode). A standard peptide of 100 fmol level can be used to tune the AP/MALDI PDF setup (see sample preparation procedures in Section 6 of this manual).

Record the spectrum from classical AP/MALDI operation, making note of the signal level. Change the HV on the tune page of the Xcalibur Software so that the "Capillary Voltage" is increased from 3.0kV to 3.5kV.

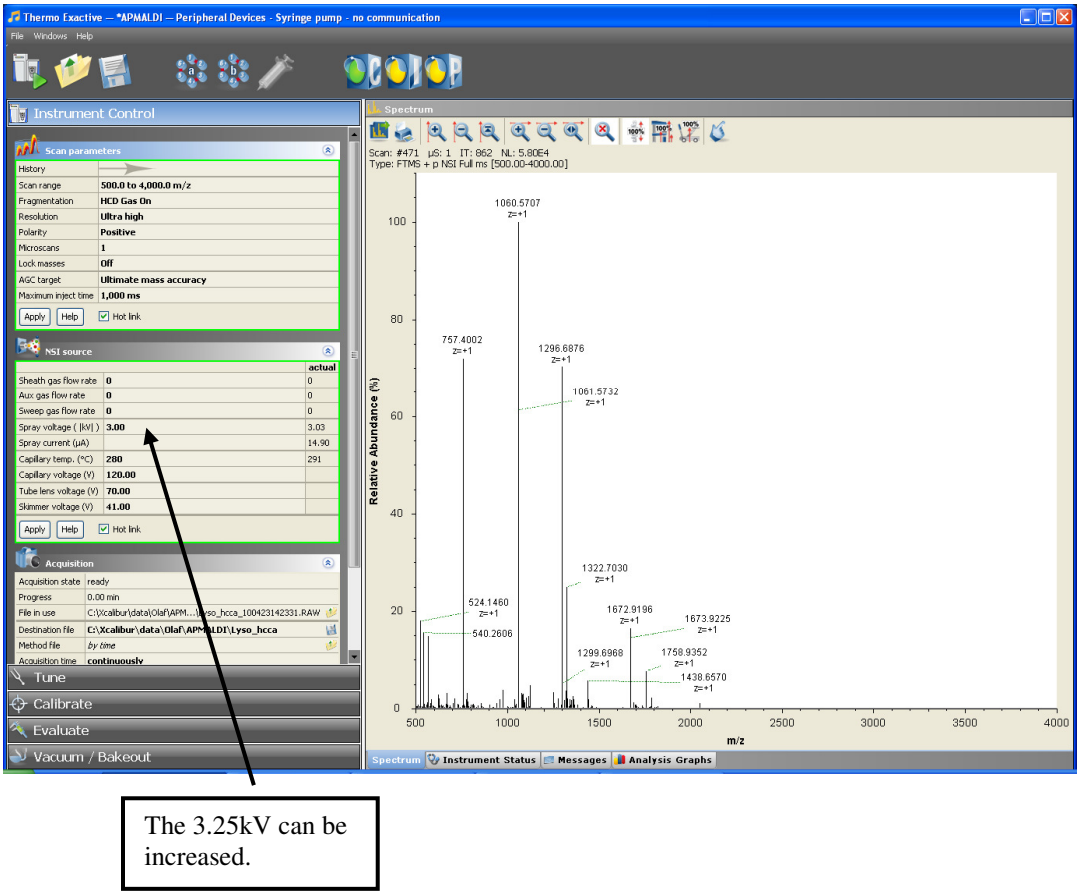



*Figure 7.24 Capillary's High Voltage*

Adjust the pulse delay to around 20 microseconds, and adjust it +/-15 microseconds to determine the time for the highest signal level. The user can confirm that the PDF is operational by switching the pulse delay time to 1 µs, which should show a dramatic drop in the ion signal. This is correct because there is virtually no electric field to transport ions to the MS inlet due to such a short pulse delay time. When the pulse delay is too long (for example >200 µs), the ions will have already entered into the MS inlet. Thus, there is an 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. 7.25). There is a scale on the side of the focusing tube to facilitate this adjustment.

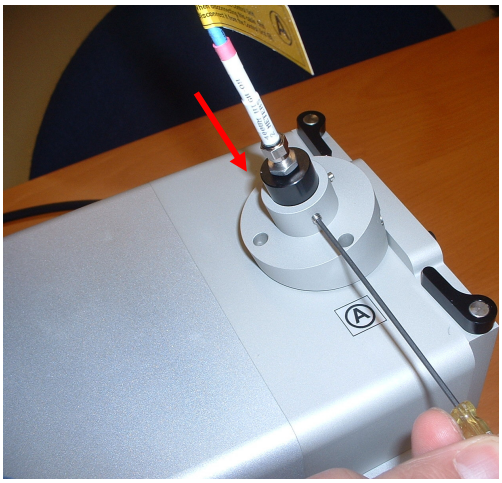


*Figure 7.25 Adjustment the Focusing Tube on the AP/MALDI Ion Source*

Then increase the laser energy in Target Software so that roughly the same laser fluence (energy/area) is maintained (Figure 7.20). The purpose behind this exercise is to generate more analyte ions per laser shot. With the PDF Technology, the ions generated far away from the MS entrance can even be entrained into the MS.

The user should the tune the focusing tube position, laser energy, and frequency while optimizing the signal intensity for a standard chemical. The AP/MALDI is sensitive to the laser fluence. If the fluence is too high, there can be increased chemical noise and poor analyte signal to noise ratio (S/N). If the fluence is too low, the analyte peaks may not be present due to insufficient ionization energy. Carefully adjust the laser energy and 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.

### *7.7 AP/MALDI HR Operation*

1. Energy: at 40 % (depending on the unit) **not to exceed 8 µJ; Energy setting should not exceed 40% (or whatever the value may be)!**
2. Fibers: make sure it is the high energy end that is inserted to laser.
3. Fiber Concentricity: optimize by turning while measuring energy
4. Spot Size: (visible-note the laser spot on CCD image is about 5-10 times bigger than the actual laser beam measured by the burn)
5. Spot Shape: it may need to turn the fiber at the source end
6. Signal: ion counts are typically less than standard AP/MALDI but
7. Watch the Injection Time: it may be too high if you observe space charge effect.

The ion count dissipates to zero in 1-2 seconds if you remain at the same spot. Spiral motion velocity should be 40 mm/sec.

### *7.9 Manual Mode of Operation*

Manual control means that the user controls the data acquisition in an interactive real-time manner. Most of the acquisition parameters can be accessed and changed during the data acquisition using the Xcalibur Tune Plus Program and Target features. The data acquisition in *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 parameters. Some of the samples and parameters include the following:

1. Laser frequency
2. Laser energy
3. Speed of the target plate accessible via Target Software
4. The amount of voltages on the capillary
5. Octopole and ion optics voltages that area accessible via Xcalibur

Saving the spectral data is the responsibility of the user and is done using appropriate Xcalibur functions.

<u>The procedure for operating in the manual mode consists of several basic steps:</u>

1. Deactivate the “Auto Sequence” button in the Target Software window (See Fig. 7.15 for the location of the auto sequence button).

2. Start data acquisition using the Xcalibur Software (refer to the previous Setting Parameters Section (Section 7.5) in this manual or the Thermo’s Xcalibur Software Manual for more details).

3. Set desired Target settings (using the settings dialog window).

    3.1. Set the desired laser energy and repetition rate.
    3.2. Activate the “Laser Fire” button and “Spiral Motion” button (if desired).

3.3. Activate the 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 the AP/MALDI operation and the PDF (if activated).

6. Adjust the desired laser energy using the micrometer knob on the control unit front panel, or position the laser spot on the sample.

    6.1. Use 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 Xcalibur Software.

8. Press the “Stop” button in the Target Software window to stop the AP/MALDI operation.

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

10. Stop data acquisition on the Xcalibur Program.

### *7.10 Automated Mode of Operation*

In the manual mode of operation described in the previous section, the user acquires the spectra of different target spots (samples) one by one. The automated mode of operation enables unattended recording of multiple spectra for several samples as a batch. The Target source control software provides two modes, the internal timing and automate mode, of the data acquisition. Since the procedures of the configuring and running automation modes are similar to a family of various MS instruments of the Thermo Electron, Inc. (LCQ, LTQ, LTQ-FT or LTQ-Orbitrap, exactive), it is described below for LCQ instrument.

The first mode, that is called internal timing mode; there is no synchronization of the AP/MALDI source operation with the mass spectrometer data acquisition. On the mass spectrometer side, the user starts the continuous data acquisition/recording. However, on the 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 Exactive manually. A major disadvantage of this regime is that the spectra for the different samples are recorded in a single spectrum under the same file name. The user can extract the spectra for the individual samples based on the retention time of the corresponding spectra (during data processing).

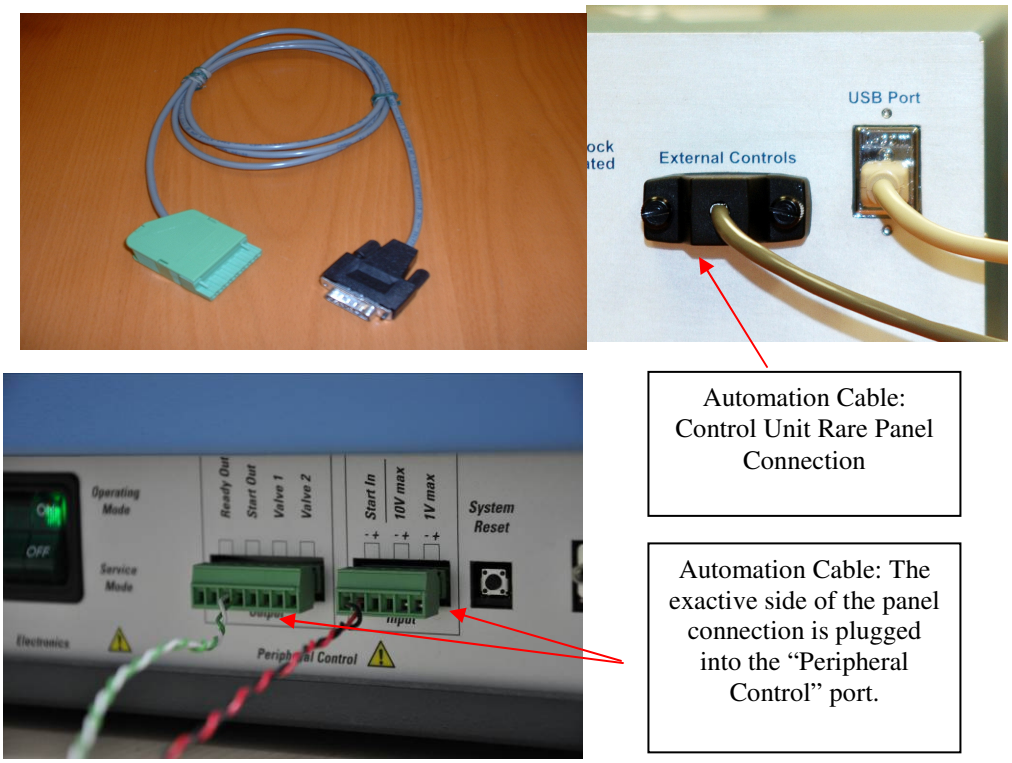



*Figure 7.26 External Control Cable*

Figure 7.26 shows the external control and its connection to the rare panel of the AP/MALDI's control unit and to the side panel of exactive.

The second automated mode, the external timing, enables the user to record the spectra of various individual samples; the recorded spectra are in separate files under separate user-defined names. The exactive communicates with the AP/MALDI source through exchange of the bi-directional "Start" and "Ready" signals. The AP/MALDI's external control cable connects to the rear panel through using the peripheral control port; the cable and its connection are shown in the Fig. 7.26.

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); this is shown in Fig. 7.27.

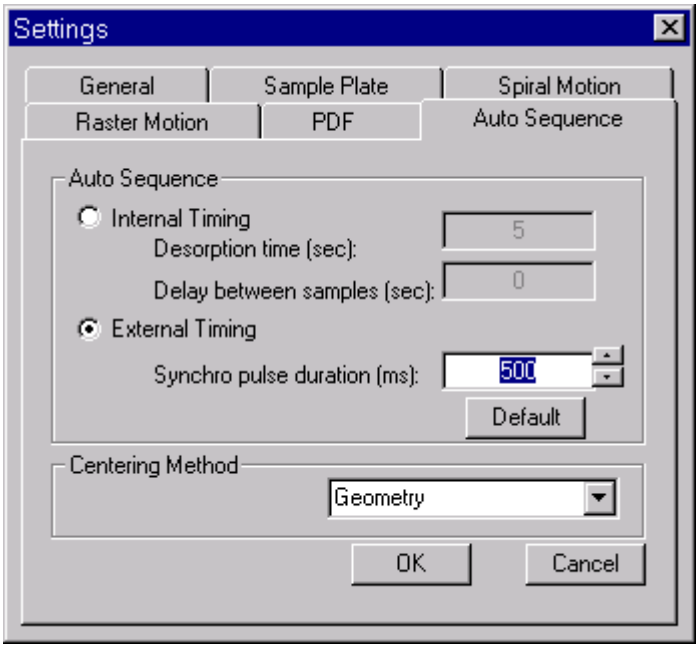



*Figure 7.27 Auto Sequence Settings*

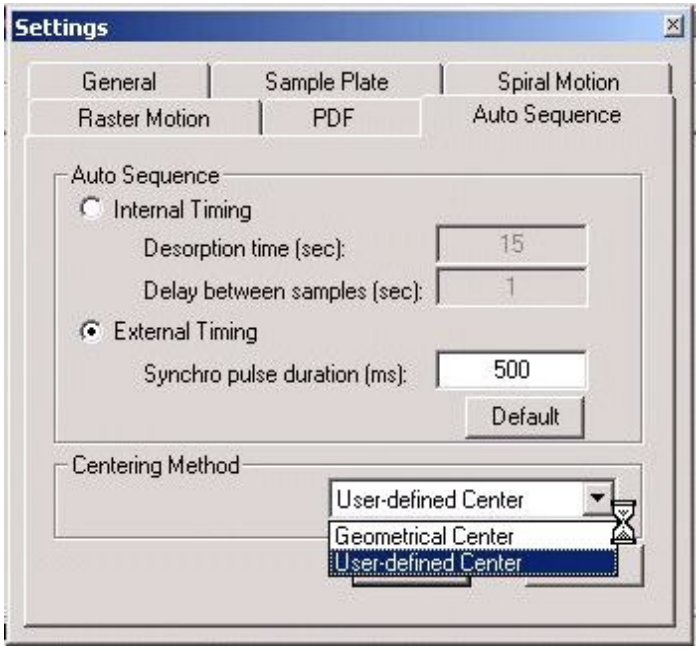



*Figure 7.28 Selection of the Sample being Centered*

Different modes of auto sequence mode are available in the software and include the following: geometrical centering (Geometry) and user-defined centering (manual), which can be seen in Fig. 7.28. The geometrical centering mode is used when the center of entire sample, which isspotted on

the target plate, is precisely in the same spot in each sample cell. This is useful for the automated mode, which controls the spotters. The user-defined centering helps the user establish the center position of the samples on a target plate. When the user is manually hand spotting of samples, the user-defined centering mode may be helpful. The user-defined centering mode also allows the user to tell the Target Software where the center of the sample is for each spot that needs to be analyzed.

Following is the procedure for operating in the auto sequence mode:

1. For external timing mode, connect the "External Control" connector (located on the control unit's rear panel) to the exactive's "Peripheral Controls" port (Fig. 7.27).

2. Check that the "Auto Sequence" button is activated in the main Target Software window (see Fig. 7.15) and that the "external timing" radio button in the Target's "Settings Dialog" window is activated as well (see Fig. 7.29).

3. Make sure that the "Auto Sequence" button is activated in the main Target Software window (see Fig. 7.15). For the internal timing mode, click the "Internal Timing" radio button in the Target's "Settings Dialog" window (see Fig. 7.29).

    a. For external control, click the "External Timing" radio button. 500 ms 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 while simultaneously pressing the Shift or Ctrl keyboard buttons and clicking on the sample map
    a. Selecting the sample spots is similar to using the mouse for selecting files in the Windows operation system. Use the Ctrl button to making multiple selections of different that can be located at different areas of the target plate, or use the Shift button to . The selected samples will be executed in order from left-to-right 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 the Xcalibur package. Then click on the "Play" button in the TARGET Software window to start AP/MALDI (PDF) operation.

a. The data acquisition will continue during the time specified for the spot selected, which is known as the desorption time (Fig. 7.29). 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, the user will need to first configure the Xcalibur Software, which includes both the instrument and sequence setup. The Tune-Plus Program cannot be used for automated data acquisition in the external timing mode. **The procedure for the tuning of the automated analysis is described in the chapter 7.10; this can only be used for the Xcalibur Software.**
7. After Xcalibur is properly configured, the user needs to start the sequence run and then wait for the following status message to appear: "Waiting for contact closure" and then click on the "PLAY" button in the Target Software window to start the AP/MALDI (PDF) operation.
    a. The data acquisition will continue during the time specified for the segment in Xcalibur Software. When the data acquisition from the first sample is done, the laser firing stops until the target moves to the next sample spot. This 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.

### *7.11 The Automated Mode of Operation: Configuring the Xcalibur for the External Timing Mode*

**I. The Instrument Setup**:

1. If the user is currently running the Tune-Plus Program, close it.
2. Run the Xcalibur/Instrument Setup Program.
3. The user has the option to create or edit an existing instrument method file. If the user chooses to select an existing file, he/she must open an instrument method file that is appropriate for the user's experiment (see Thermo *Xcalibur* Software Manual for details). Then, click on the contact closure tab (see Fig. 7.29 below).

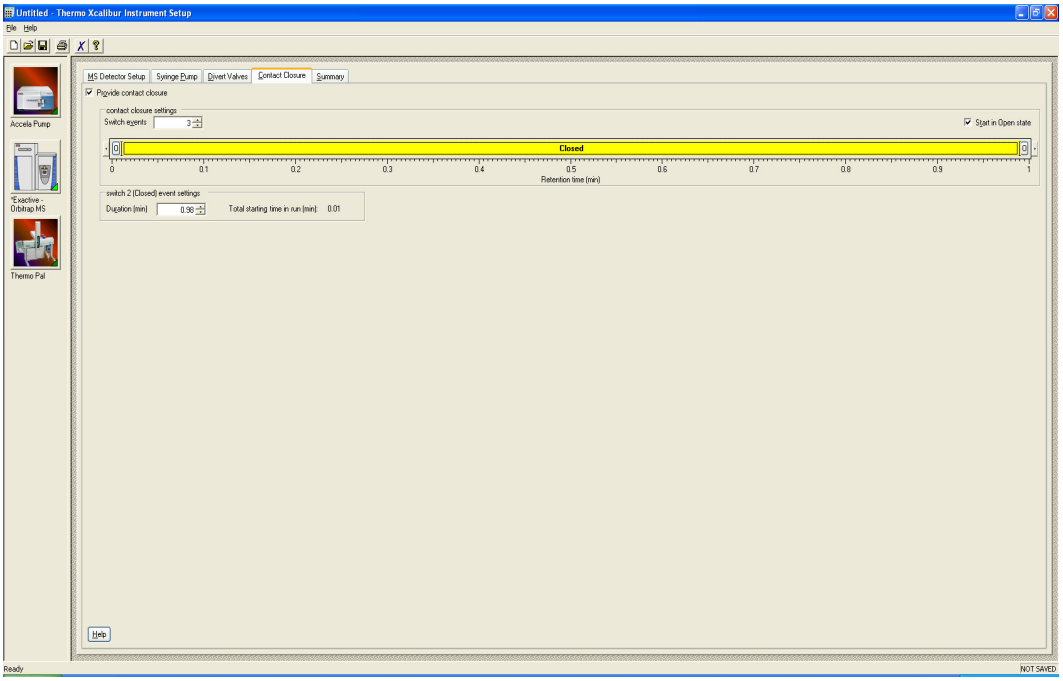



*Figure 7.29 Instrument Set Up*

Figure 7.24 shows the contact closer when it is configured for the external timing and automated AP/MALDI data recording.

4. Make changes to this page so it looks exactly like that shown in Fig. 7.26 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

5. Save the settings to the instrument method file and close the Instrument Setup Program. These settings ensure that the signals on contacts 1-2 of the "Peripheral Control" connector control the laser firing by the *TARGET* Software. They will be used later during data acquisition. If the user can prepare several different instrument methods for the different types of experiments and experiment durations, he/she must make sure that the contact closure profile for every method looks like it is shown in Fig. 730.

**II. The Sequence Setup:**

6. Run the Xcalibur/Sequence Setup Program.
7. Create Xcalibur Sequence or open an Xcalibur Sequence file (see the ThermoXcalibur Software Manual for specific details). The number of samples in the sequence setup window table (see Fig. 7.31) should correspond to the total number of samples selected for the analysis.

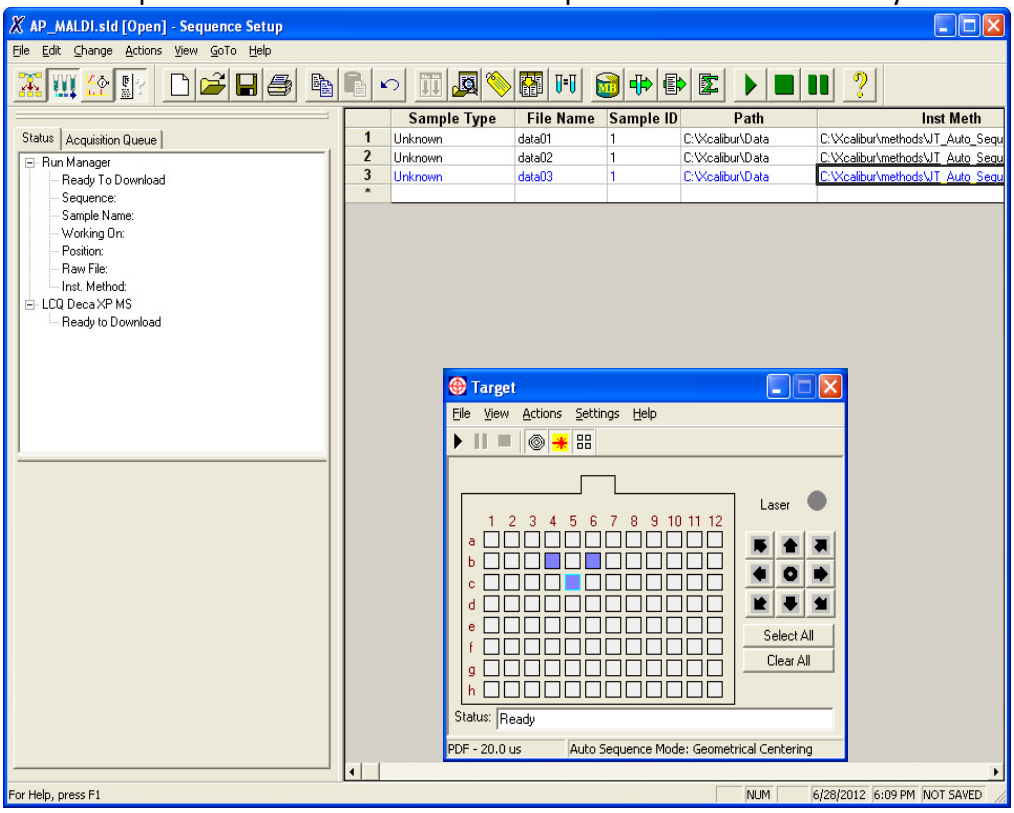



*Figure 7.30 Xcalibur/Instrument Setup/Sequence Setup and Target*

Figure 7.30 shows the sequence setup that is configured for the external timing and automated AP/MALDI 3 sample data recording process.

The lines in the  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. 7.27).  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  table are saved with contact closure settings, which is described in the previous step above in Figure 7.30.  The files where the acquired data will be saved are shown in the file name column.

**III. Data Acquisition:**

8. Go to the Actions/Run Sequence menu to open the run sequence dialog window (see Figure 7.31 below).

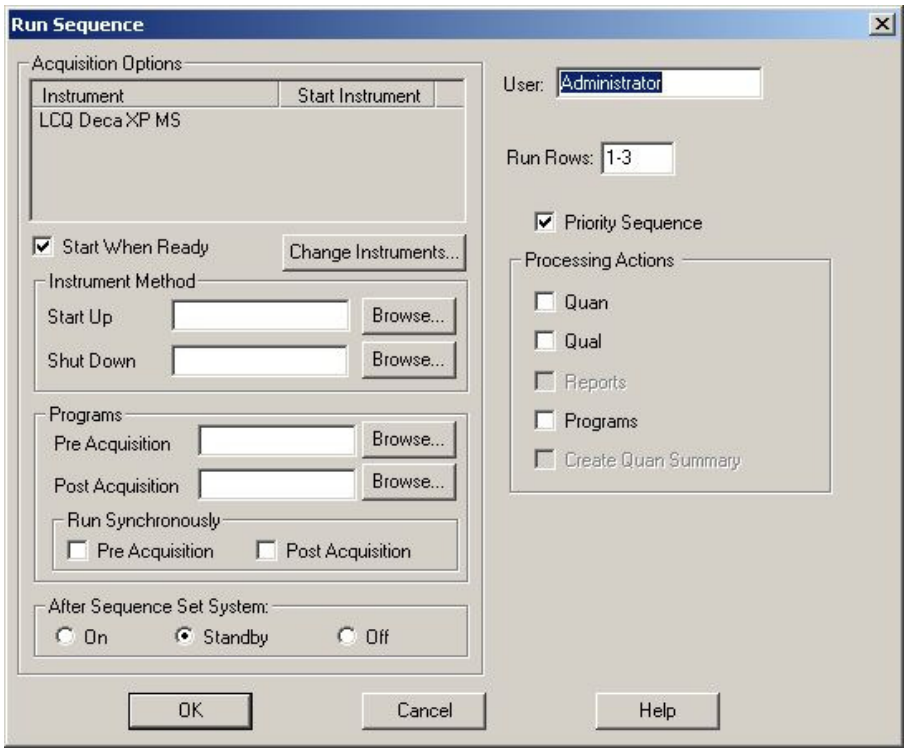



*Figure 7.31 Starting the Sequence Run*

9. Make sure that exactive is **not** chosen as the start instrument and that the “Start When Ready” check box is checked,like in the example above.
    a. Leave the “Start Up” and “Shut Down” fields empty..
10. Describe all rows, e.g., 1-20, in the “Run Rows:” field.
    a. Check the “Priority Sequence” check box to run the sequence immediately.  Click
11. “Change Instruments…” button (see Fig. 7.32); the dialog “Change Instruments In Use” will immediately start.

a. The first column should contain only one instrument, the LCQ.
b. The column "In Use" for the MS instrument must contain "Yes"; the column "Start Instrument" **must be empty**. If it is not, then highlight and delete "Yes" mark in "Start Instrument" column (by pressing "Del" keyboard key). Finally, click the OK button to run the sequence.

The user should refer to the "Run Sequence Help" if he/she has any problems changing the settings. The settings in the run window ensure that the exactive is properly triggered by the AP/MALDI hardware.

12. In the Status page of the Xcalibur/Sequence Setup Program window, the user will see a downloading message at the exactive status line and then a waiting for the contact closure message.
13. After the waiting for the contact closure message is displayed in the sequence setup window (see Fig. 7.30), press the PLAY button in the Target Software window to start AP/MALDI operation.
    a. The running message will be displayed in the exactive status line, which later will be replaced by the waiting for the 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, however, is shown by a blinking color.

## *7.12 Zoom Mode Operation*

**Introduction**

A new feature, the AP-MALDI zoom mode, is introduced in Version 7 of the Target Software. The goal of the software is to:

1. Give the user more flexibility and options to run both the Target and MS Software together.
2. Create position information with the necessary details to create a map of ion signals.
3. Export the position information for the post processing of the data.

The description of the test procedure (in the Motion Modes section below) introduces the user to the capabilities of the AP-MALDI zoom mode.

**Motion Modes: CSR and Pixel Map**

The two different options that can be used for the AP-MALDI zoom mode include the constant speed raster motion (CSR) and pixel map, which can be seen in Figure 7.12.1. Both options have two different scan patterns that are

available for use, which include the flyback and meandering modes displayed in Figure 7.12.2. The flyback mode includes line scans that move in the same direction (horizontally and vertically), while the meandering mode's line scans move in alternating directions so that the total scan time is minimal.

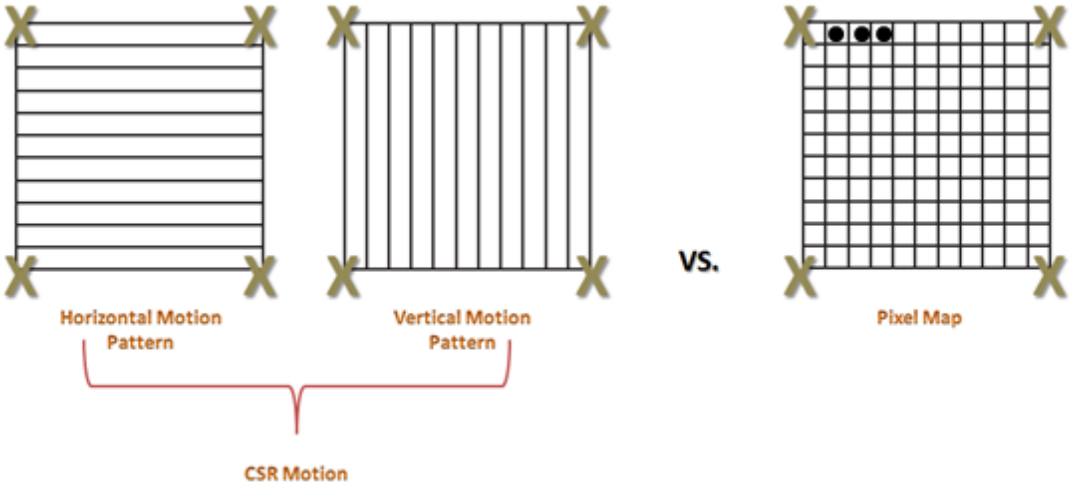
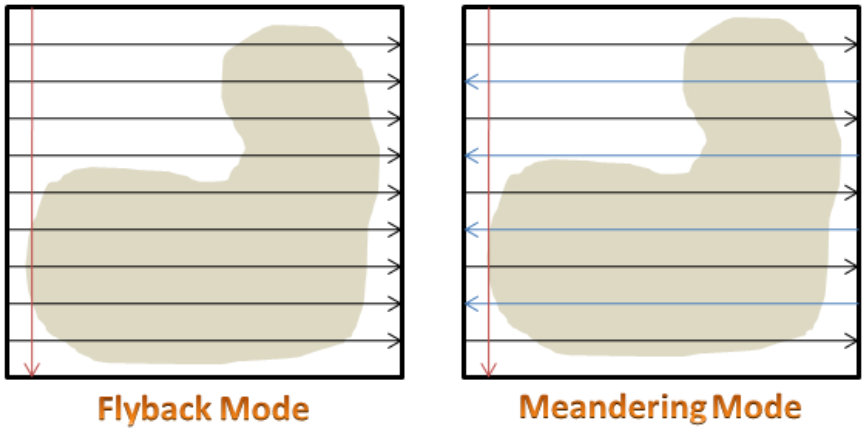



*Figure 7.12.1 CSR Mode vs. Pixel Map Mode*

Note: In the CSR mode, the laser fires constantly as the stage moves (except during transitions from one row/column) to the next. In the pixel map




mode,tThe laser only fires within the pixel points.

*Figure 7.12.2 Flyback Mode vs. Meandering Mode*

Based on the specific needs of the sample and available instrumentation, the user needs to decide which zoom mode should to be used. When making this decision, the user should consider the following preliminaries:

1. If the number of pixels are larger than the number of data files that the MS Software (e.g. XCalibur, Analyst, MassHunter) can handle, the CSR mode should be used. Each line will be a separate file.
2. The availability of the handshaking option (available in the Thermo Q Exactive Instrument Software) enables the use of one file for all the pixels. The use of such instruments creates a very efficient setup for the pixel map mode.
3. The scan time cannot be too long, as each pixel needs at least one mass spectrum per scan. If there is no mass spectrum available for a given pixel, the result of the scan will show an empty pixel (i.e. the number of scans is greater than the number of pixels). The amount of time the laser will fire To tune the pixel, the overall scan time for each MS scan needs to be known and should be minimized. Finding out how long the scan time is, depends on the MS software; e.g. for the XCalibur Program the overall scan time is displayed in two places:
    a. During the Acquisition - the scan is usually displayed as the ST in the LTQ tune window.
    b. Qual Browser- right click to the MS window and select the **View >Scan Header** (the user needs to look for the "Elapsed Scan Time"-see Figure 7.12.3).
4. If the sample area is more than 10 mm X 10mm, using the meandering mode is advised; the flyback mode can require a long transition time.

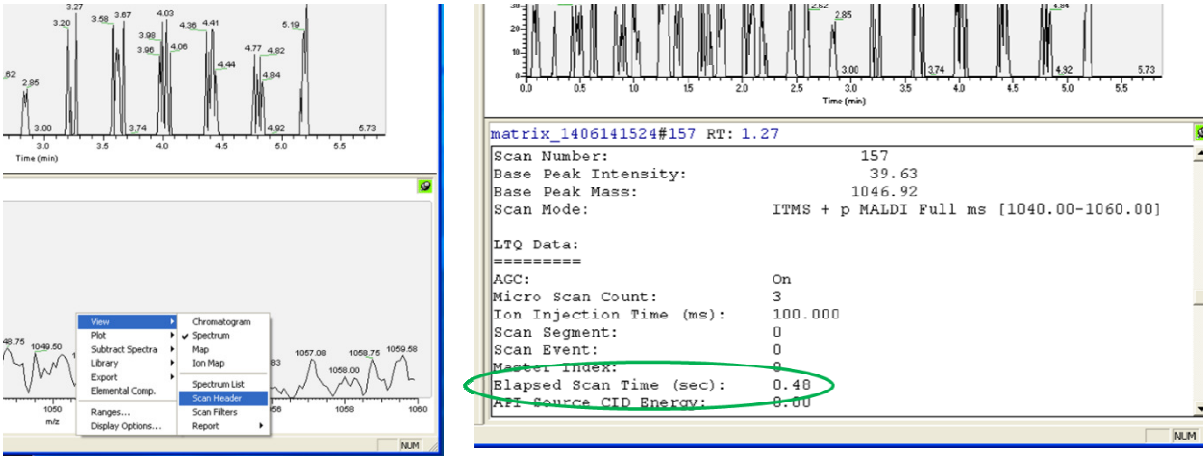



*Figure 7.12.3 Viewing the Scan*

**Test Sample Preparations**

1. On a cleaned AP/MALDI sample plate, spot a 0.5 µL of Angiotensin II solution (FW: 1046.18- or any other sample that is available – the suggested amount is 100 fmol/µl) near the top left hand corner of the plate. The solution must be pre-mixed with a matrix that consist of 1 mg/ml CHCA in 70%Acetonitrile and 0.1%TFA.
2. Spot the solution two more times so that the spots form a right triangle; more specifically, the spots will act as the endpoints (see Figure 1). Each spot should be approximately 1 mm (in distance) from the original point; try to avoid having the samples touch one another.

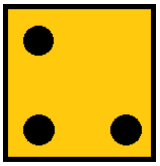


*Figure 4 End Point Shapes*

**Creating the Test Data in the CSR Mode**

1. Choose the zoom type in the **General** tab (see Figure 7.12.5):
   a. **Settings>Zoom**

      **Parameters>General**

      **Zoom Type→**CSR

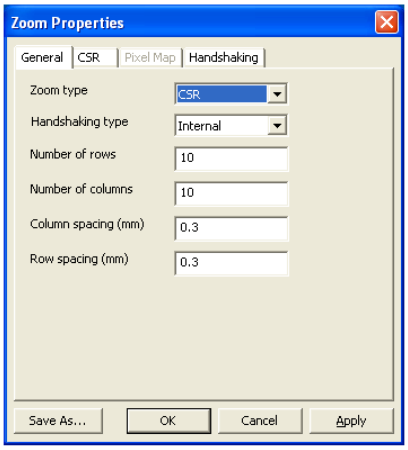



*Figure 7.12.5 CSR –General*

2. When completed, press **Apply**.

3. Choose the **CSR** tab (see Figure 7.12. 6):

   **Direction** →Horizontal

   **Pattern** →Flyback or Meandering

   **Velocity (mm/min)** →20

   **Transition Time (sec)** →10

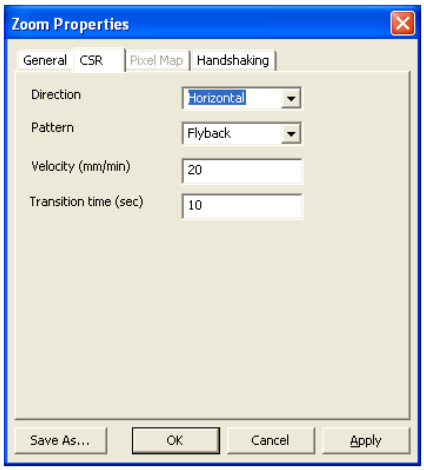



*Figure 7.12.6 CSR Specific*

a. The user must click the **Save As** button in order for the position file to be active; this file is known as the raster XML file.

b. When the file has been named, click the **Save** button.

**Creating the Test Data in the Pixel Map Mode:**

1. Choose the zoom type in the **General** tab (see Figure 7.12.7):

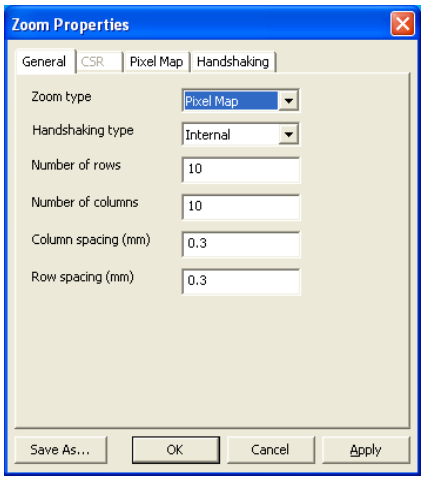



*Figure 7.12.7 Pixel Map General Settings*

a. **Settings>Zoom Parameters> General/ Zoom Type**→Pixel Map
**Handshaking Type** →Internal

b. The remaining properties should be specified to the user's needs. In this case,
the number of columns and rows were 10, while the spacing used was 0.3mm.

c. The user must click the **Save As** button in order for the position file to be active; this file is known as the *raster XML file*.

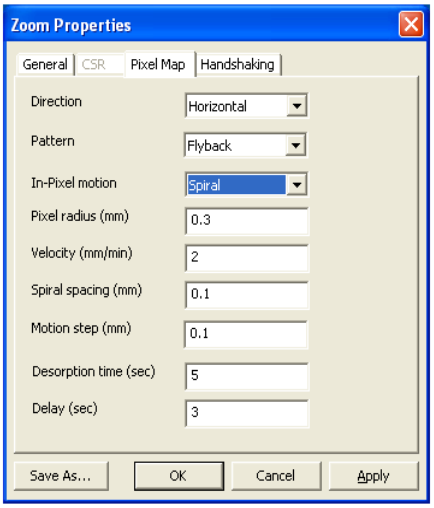



*Figure 7.12.8 Pixel Map Properties*

2. When completed press the **Apply** button and then immediately following, press **OK**.

3. Choose the necessary properties for the data in the **Pixel Map** tab; the properties should be specified to the user's needs. Figure 8 shows a screenshot of the default settings:

a. The in-pixel motion is typically enabled only when the "pixed area" is larger than the spot size.

b. The desorption time refers to the laser 'on' duration.

c. The delay time refers to the delay between the laser on.

Note: The handshaking action is needed only for the external synchronization.

4. In the **Handshaking** tab, set the **Pulse Duration** to 500 (see Figure 9).

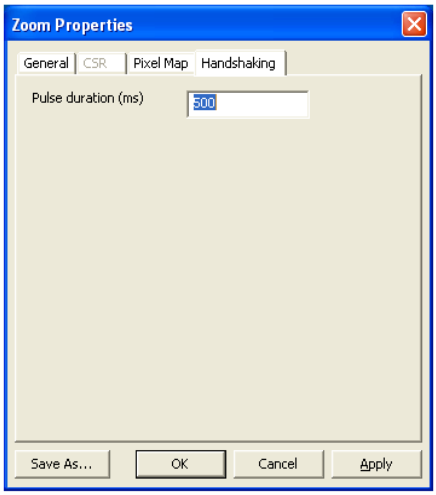



*Figure 7.12.9 Pulse Duration*

   a. Then, press the **Apply** and then **OK** button once all the zoom properties are set. **Pulse Duration (ms)** → 500.
   b. It is highly recommended to use the 500(ms) for the **Pulse Duration**.
5. Then press **Apply** and **OK**.

**Acquiring the Data**

1. In the Target program, this button  is used to enable the zoom mode. This  button, on the other hand, is used to scan the total area of the sample.
2. Now, the user needs to get the mass spectrometer ready.
3. The Target Software has a calculator that informs the user of the total acquisition time (found at the bottom of the CSR tab; the MS should scan for that period. Once finished, press **OK** and close the dialog box.

4. Set the scanning to a narrow mass range(see Figure 7.12.10):
   In the **Define Scanning** tab (on the mass spectrometer) set the beginning mass range from 1040 - 1060.

a. **Scan Mode> Define Scan**

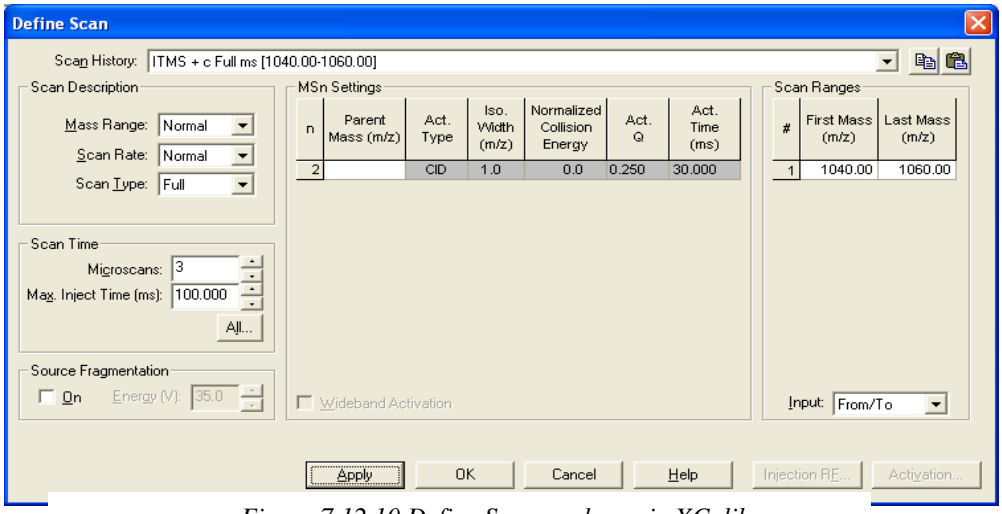



*Figure 7.12.10 Define Scan as shown in XCalibur*

5. ext, press **Apply** and immediately following that action, push the **OK** button before closing the window.

   a. The number of minutes for the MS data acquired is the same, or slightly more to the time calculated by the Target Software.

6. Turn on the laser.

7. Start the scan by pressing start 

on the Target Program, which activates the laser and sample motion scan. The filename needs to be the same as the user's raster XML file.

8. Next, acquire the data using the MS immediately following the start from the Target Software. (If MS software has the option, contact closure can be used to synchronize Target and MS Start) When acquiring the data, the user needs to make sure that only the single scans save and that no summation is used.

**Processing the Test Data with Thermo Image Quest (Thermo .raw files only)**
Thermo ImageQuest (version 1.1.0 or later) can create images directly from the .raw files and the raster XML file (see above) created under Target software.
In order to ensure ImageQuest matching the raw file with raster xml file, the user needs to ensure identical file names of the raw file and the raster xml

file. e.g. if the raw file name is 'mydata1234.raw' , then the raster xml file name should be 'mydata1234.xml'. When ImageQuest asks for 'MALDIPos not found' prompt, the user just should click 'no' and your image should appear instantly.
Note: when saving raster xml, make sure to click apply before saving.

**Processing the Test Data with demo software (any format files that can be converted to mzML)**

*Note: The steps outlined here are for demonstration purposes only. They involve freely available software that is not supported by MassTech. There may be alternative ways of doing similar tasks by other commercially available software.*

There are few major steps needed to create the images from the gathered MS data:

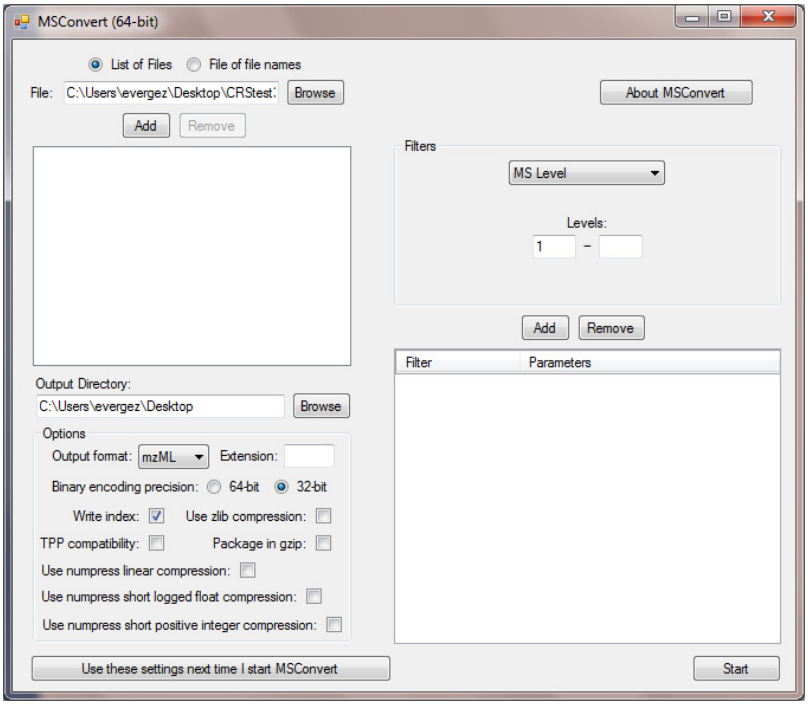



*Figure 7.12.11 MS Convert Settings*

- Converting the raw data file to the mzML format.
- Converting the mzML formatted data file to an imzML data file with the help of position information.
- Create digital images from the imzML data file.

1. The native data from the mass spectrometer in order to create a universal mzML; this allows for the data file to be properly formatted. Use the Proteowizard Software, which is available at the following

website: **proteowizard.sourceforge.net**. The MS Component will be used, which can be found directly on the website's download page..

a. Download the MS Convert (if not installed on the user's computer).
b. Launch the MS Convert and load the *Xcalibur RAW file* into the browser.
c. The user should use the settings shown in Figure 7.12.11 to convert the data file.

2. If successful, the user should have an mzML data file that should be somewhat larger than the original MS data file (e.g. Thermo, AB Sciex, Agilent). If unsuccessful, the user will get either an error message or a file with no data in it. Check the settings if there is an issue. In some cases, the data files may be too big (especially Windows XP created files; they cannot be larger than 2 GB) or corrupt.
3. Next, find the Raster XML file created by the Target Software and the mzML file; make a copy of the files and place them in a folder that is easily accessible.

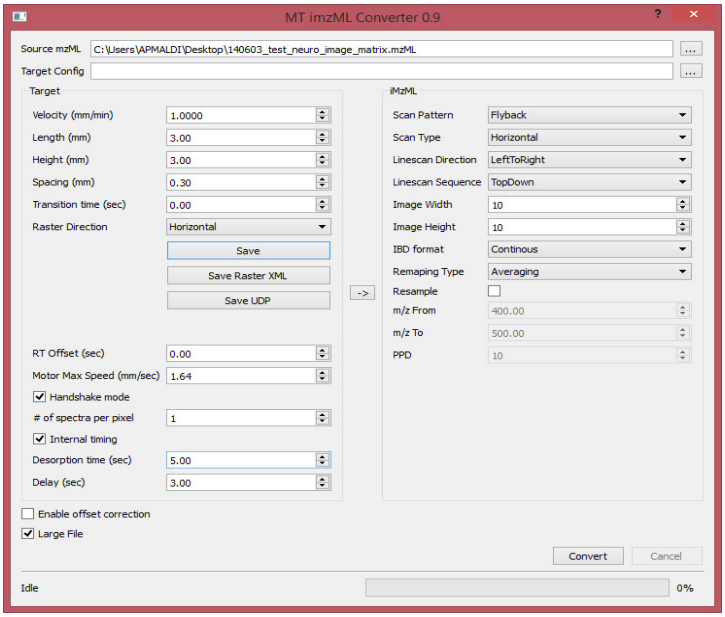



*Figure 7.12.12 The MT imzML Converter 0.9*

4. Open the MT imzML Converter- the demo software provided by MassTech. Populate the dialog box with the information as shown in Figure 7.12.12.

a. When completed, press the 

button to set the figures. Then press the "Convert" button.

5. If successful, the user will have an imzML data file that is somewhat smaller than the original mzML data file.
6. Next, the user will be able to open the imzML data file with the appropriate software. Here the Datacube Explorer by FOM Institute-AMOLF. **http://www.amolf.nl/download/datacubeexplorer/** is shown.

7. When the file is open, the default settings will point to the lowest m/z available in the user's MS data file. Also, the user needs verify that there is a decent number of ion counts appearing at the MS display; MassTech recommends x amount of ions to be present.

8. By changing the minimum/maximum value and selecting the

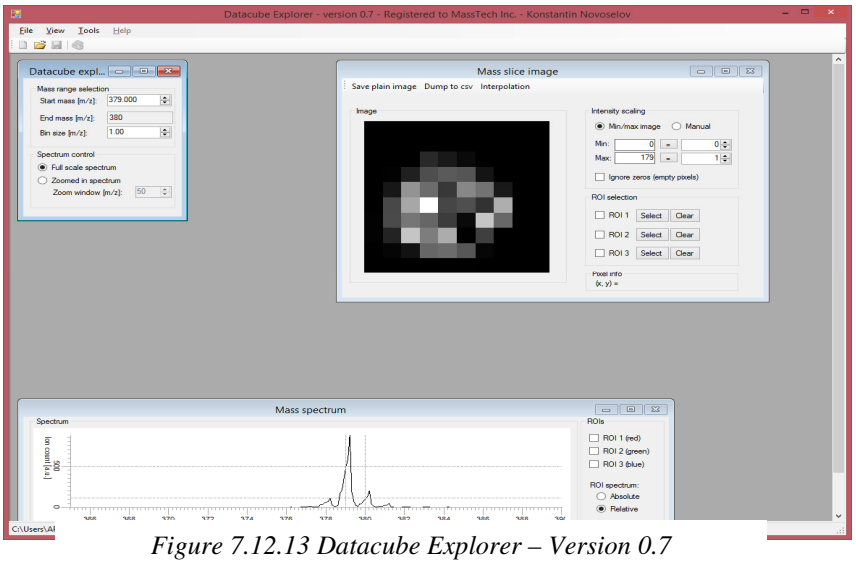



*Figure 7.12.13 Datacube Explorer – Version 0.7*

appropriate m/z interval, the user's test data should look like in Figure 7.12.13.

9. Now the zoom mode is ready use. Please contact MassTech Inc. if there are any questions or concerns.

Notes:
- If the user needs an area larger than 30x30 mm, please inform MassTech; the limit may be stretched based on the sample plate geometry.
- Verify that the cross hair is located where the laser beam hits the sample.
- MassTech recommends the scanning narrow mass for specific applications: for example, if the user is interested in m/z 750, the scan range should only be 740-760; define scanning is based on the sample. MassTech used 1040 – 1060 because the analyte was at 1046; the user's analyte may be different.
- Due to the nature of the CSR motion, once scanning on a row starts, the process is unable to be stopped. Therefore, if the user needs to stop a run, he/she should click on "Stop" button and wait until the current row is finished.
- The Laser energy is dependent upon the sample; if the laser is too high then the sample erodes while on the target plate.
- Please do not hesitate to call or e-mail for more information

# 8 MAINTENANCE —TROUBLESHOOTING THE SOURCE.

The AP/MALDI ion source does not require regular maintenance, except for cleaning of the optical fiber cable ends every six weeks to avoid deposit accumulation (section 8.5 of this manual describes a cleaning procedure).
*Note : optical fibers that come with AP-MALDI HR cannot be cleaned!*
Please refer to Section 5.3 of this manual for the instructions regarding connecting and disconnecting the optical fiber. It is strongly recommended that the user follows the troubleshooting procedures that are described below.

**DO NOT ATTEMPT services or repairs that are not covered in the Troubleshooting Section. For services and repairs beyond those specifically provided in the Troubleshooting Section, contact the manufacturer:**

**MassTech Inc.**
**ATTN: Service Department**
**6992 Columbia Gateway Dr.**
**Suite 160**
**Columbia, MD, 21046**
**(443)539-1758**

The AP/MALDI PDF source comes supplied, completely tuned, and ready for operation. However, there are several reasons why the MS signal might decrease significantly or even disappear at times. The following sections describe possible symptoms with the machine's remedies.

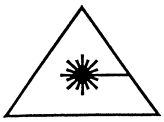
**Remember: any contamination of the optical fiber's opened ends results in irreversible fiber damage during the source operation.**

The user must put the protective plastic caps back on the optical fiber ends immediately after it disconnected from the control unit. If by accident, the user touches/contaminates) the opened ends of the fiber, he/she needs to immediately clean it according to the procedure in Section 8.5.

## *8.1 PROBLEM: Insufficient Ion Production - Lack of the 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 for this test).

2. Set the laser energy to full power (e.g., 90-100%).
3. Fire the laser and watch the computer's video capture screen.
4. If there is a blinking spot on the computer's video capture screen, the user needs to see if the matrix crystals at that spot are disappearing (for CHCA matrix without the beam attenuation, the crystals should disappear in 5-15 seconds). If they disappear, then laser power is sufficient.
5. If the laser crystals do not disappear in 5-15 seconds at the blinking spot, then laser power is NOT sufficient.
6. If the laser power is NOT sufficient, the user has three options to fix the problem.
   i. Try another optical fiber (one spare was shipped with the unit).



**IMPORTANT**: If the user chooses to replace the optical fiber, turn the power to the "OFF" mode on the control unit.

   ii. Try to improve the focus of the laser beam on the target. To do this, adjust the position of the source fiber connector with respect to the housing source as described in Section 8.2

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

* If these actions do not help, call MassTech Inc. for assistance and ask to speak with the Service Department.

### *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.

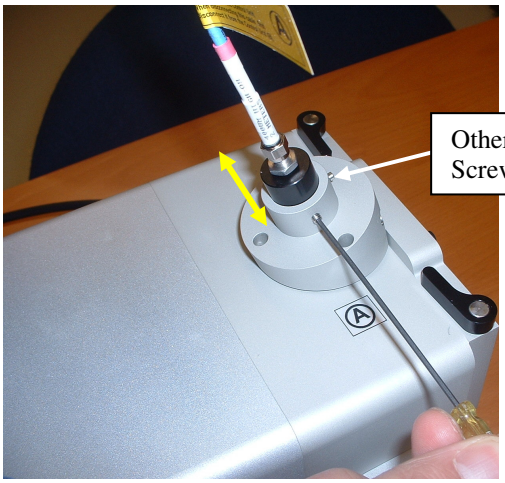



*Figure 8.1 Adjusting the Laser Focusing*

The laser can be focused 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. (not necessarily 99%-See section on AP/MALDI *HR*)

6. Using the camera, the user 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 the user sees 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 Properly 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 MS instrument and AP/MALDI PDF source switched to the "ON" mode.

**Step 1**. First, the user needs to determine if the laser spot is properly aligned or misaligned.

a. Prepare several target spots with 1-2 µL of undiluted matrix (it could be either a pure matrix solution or matrix/any analyte mixture).
b. After drying, insert the target plate into the source, close it, switch it the "ON" mode (if it was not switched before) and run the Target Software (if this was not done already).
c. Choose any empty (blank) target position. The picture on the video capture screen should look as follows (Fig. 8.2):

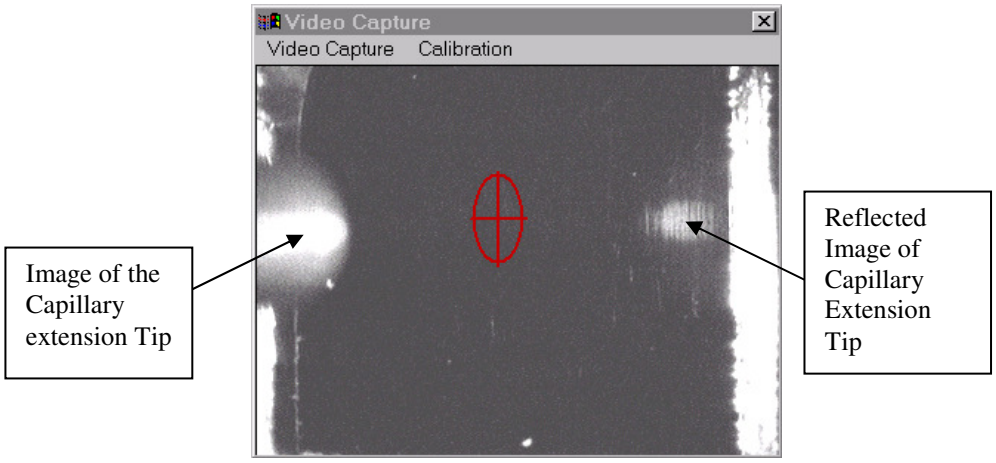



*Figure 8.2 Blank Sample Spot on the Video Capture Screen*

Both the capillary extension tip image and its reflection are not well focused; to ensure that the user identifies the images correctly, 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.

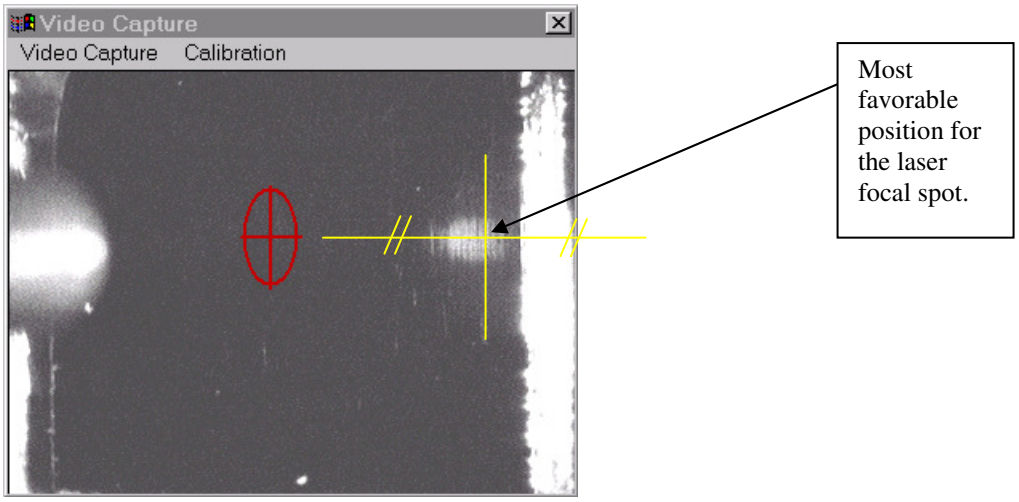



*Figure 8.3 Ideal Laser Positioning*

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). In order to determine the real position of each sample spot, move the target plate to a position where a matrix was deposited (see Figure 8.4).

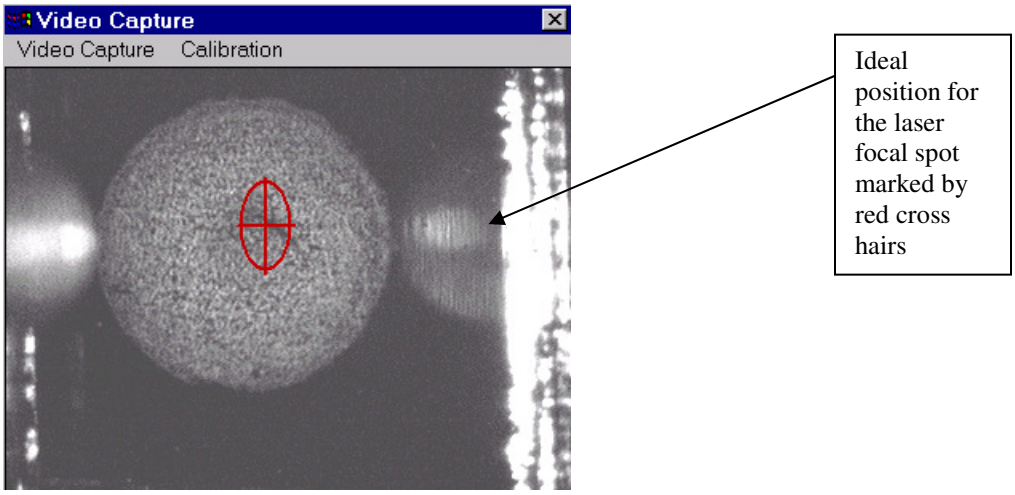



*Figure 8.4 Image of the Matrix Crystals*

**Step 2.** Switch the laser to the "ON" mode and turn the spiral/raster motion to the "OFF" position. Set the maximum laser through Target Software so that the matrix crystal's evaporation (at the place where the laser beam is focused) can be seen (Figure 8.4).

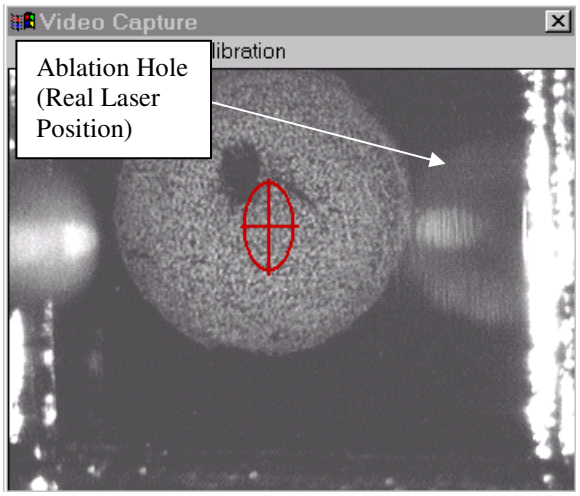



*Figure 8.5 Ablation of the Matrix Sample Away from the Ideal Laser Position*

By comparing the location of the ablation hole with the ideal position shown by the red cross-hairs, the user can see that the laser focus is close to its ideal position. However, the laser post is slightly higher and to the left. The deviation of the focal points shown in Figures 8.4 and 8.5 are acceptable, especially with the PDF activated, but the source sensitivity can be improved by fine-tuning.

**Step 3.** Continue with the same spot. Turn the laser to the maximum power (minimum attenuation) by pressing the "ON" position. Using a hexagonal screwdriver, turn the three screws (see Fig. 8.6).

The user needs to look at the video capture screen for the corresponding motion of the laser focal spot. The objective is to move that spot as close as possible to its ideal position which lies at the middle of the imaginary line that connects the capillary extension tip (see Fig. 8.3). The position in Fig. 8.7 below shows a good alignment.

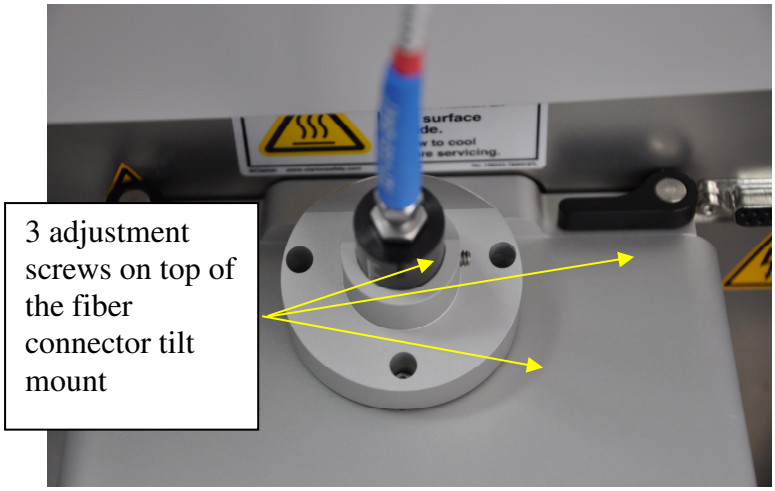



*Figure 8.6 Adjustment of the Laser Position*

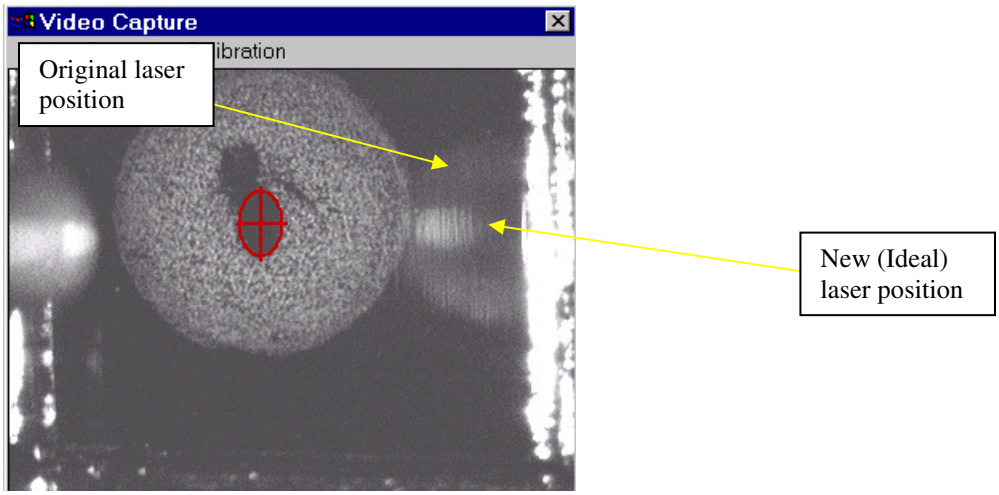



*Figure 8.7 Good Alignment of the Laser Position*

Now the user can set the best laser energy that appropriate for his/her matrix. Alternatively, the position of the laser focal spot can be adjusted by rotating the tuning screws (Figure 8.6) based on the quality of the MS signal by a trial-and-error method.

### *8.4 PROBLEM: the Ion Transport into the MS Instrument is Clogged/Blocked*

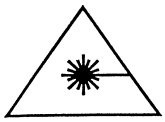
Goal: To determine if the ion transport path to the MS is blocked.

1. To test for a clogged ion transport (in the MS instrument) prepare several target spots with a dense α -CHCA matrix. A α-CHCA provides the brightest fluorescence and the lowest pulse energy necessary.
2. Set the attenuation to full laser power.
3. Fire the laser and watch the computer screen for a larger beam.
4. If the user can see a blinking spot on the computer screen, he/she needs to check if the matrix crystals at that spot are disappearing. For CHCA matrix without the beam attenuation the crystals should disappear within 5-15 seconds. If they disappear, then:
    a. Ensure that the MS interlock is operating properly.
    b. Ensure that the MS Control Program is configured as described in this manual.
    c. Ensure that the probe preparation & matrix material are being used properly.
    d. 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.
    e. Finally, ensure that the MS instrument operates properly with the electrospray instrument attaches. The problem may be with the Exactive instrument rather than the source.

### *8.5 PROBLEM: The Optical Fiber Ends need to be Cleaned*

* **Note**: This is not to be used for the AP-MALDI HR's fiber ends; only use with the AP-MALDI PDF+ units.

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.*** **<u>One</u>** spare optical fiber cable has been shipped with the user's source.



**IMPORTANT:** Whenever the optical fiber is being detached from or connected to the control unit/housing source area, **MAKE SURE the power switch on the control unit is turned "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 <u>must be worn</u> at all times.**

1. Prior to cleaning the fibers, it is recommended to inspect the fiber ends for damage or burn areas using a microscope.
   The inspection of the fiber should reveal a uniform, blush, smooth, and shiny surface. This may includeminor scratches, inclusions, or dust particles. After the 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. This must be done while 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 has the same process from the first method, except that the tissue strip is 2-3 cm wide and is fixed to the desk edge by adhesive tape. The other end will need to be 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 the 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 drop the cleaning solvent 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 the buyer's shipment. To use this product:

i. Press the optical fiber end onto the Fiberclean tape and rub in figure 8 motions (with the user's finger tips).

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 the AP/MALDI PDF allows many more ions into the ion trap than does classical AP/MALDI, it is important to be aware the 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 that the trap does not become saturated.

An alternative way to solve this problem would be to adjust the laser energy 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:** msms@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.**
**Attn: Service Dept.**
**6992 Columbia Gateway Dr.**
**Suite 160**
**Columbia, MD, 21046**

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 LASER SPECIFICATIONS

### High repetition rate all-solid-state Nd:YAG Laser Specifications

| | |
|---|---|
| **Part Number** | SES-100 for AP/MALDI *PDF+*<br>SES-1000 for AP/MALDI *HR* |
| **Wavelength** | 355 nm |
| **Repetition Rate** | software controlled<br>Up to 200 Hz for AP/MALDI *PDF+*<br>500 Hz- 1 kHz for AP/MALDI *HR* |
| **Pulse Width, FWHM** | 3-5 nsec |
| **Pulse Energy** | 60 μJ for AP/MALDI *PDF+*<br>8 μJ for AP/MALDI *HR* |
| **Build-in attenuator** | 0-100% in 2-3% increments (software controlled) |
| **Lifetime** | > 1 billion pulses |

# APPENDIX B 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