SubAP/MALDI (ng) HR and *SubAP/MALDI (ng)* UHR 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 *sub-AP/MALDI (ng)* HR or *sub-AP/MALDI (ng)* UHR sources should be handled with **extreme** care. Touching the optical parts with one's bare hand/fingers, storing, or exposing them to dirty/dusty environments can result in permanent damage to the optical components. To clean the optics, an ACS grade aceton and optical wipes must be used.

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

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MassTech

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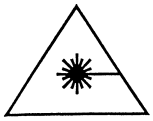
PREFACE

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

This symbol in the manual margin is used to emphasize the presence of 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:

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ATTN: Service Department
6992 Columbia Gateway Drive
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1 INTRODUCTION: SUB-AP/MALDI (NG) HR AND SUB-AP/MALDI (NG) UHR: AN ION SOURCE FOR MASS SPECTROMETERS WITH SUBATMOSPHERIC PRESSURE INTERFACE

Sub-atmospheric Pressure Matrix-Assisted Laser Desorption/Ionization – Sub-AP/MALDI:

The Sub-AP/MALDI source is designed to produce ions of different analytes under low pressure conditions from a mixture of the matrix and the analyte microcrystals; this is done by irradiating these crystals with UV laser pulses. The produced ions are analyzed by the mass spectrometer, which is equipped with subatmospheric pressure interface housing the Sub-AP MALDI source. The mechanism of the Sub-**AP/MALDI** ion production is similar to that of a **conventional (vacuum) MALDI**. The main difference is that the Sub-AP/MALDI produces ions under low pressure conditions **in a separate (external to MS system) low pressure** housing. The main consequences are as follows:

- The Sub-AP/MALDI source is an ionization source, external to mass spectrometer. It is designed to be easily interchangeable with other sources such as the nanoESI, APCI, DART, etc. via MassTech ESI interface.

- The replacement of the target (sample) plates is a simple and relatively quick operation. The MS system is not exposed to atmosphere all the time. The MS system is separated from atmosphere by means of vacuum gate (shutter). The latter is a part of the iFunnel subsystem. The Sub-AP MALDI source is equipped with a small external pump that aids in establishing low pressure after the sample plate is loaded. After about one minute of pumping, the pressure in the source is established at approximately 7 Torr. After that, the shutter can be slowly opened until the pressure readings drop to 1-3 Torr. Then the in-line valve on the hose connecting the source and small external pump must be closed and the small pump must be shut down.

- The Sub-AP/MALDI source is designed as an additional external source to the mass spectrometer. The process of the mass spectra measurement is completely decoupled with the sample ionization process. Thus, the Sub-AP/MALDI inherits all the power of the Thermo instruments: the high mass resolution, 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 Sub-AP/MALDI source, like the conventional MALDI source, produces mostly single-charged ions*.

- The Sub-AP/MALDI is 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 Sub-AP/MALDI source operates under low pressure conditions. The Sub-AP/MALDI and ESI sources (MassTech's nanoESI adapter sold separately) are interchangeable and typically provide complimentary analytical information. The appropriate use of both the ESI and Sub-AP/MALDI sources provides the opportunity to cover a broad range of problems in modern analytical chemistry.

The *Sub-AP/MALDI (ng) HR* and *Sub-AP/MALDI (ng) UHR* sources are designed to provide the laser spot size down to 20 micron (10 micron correspondingly, for UHR) on the sample. This enables "High Resolution," more specifically, higher lateral resolution. By employing the "Zoom Mode", one can create and monitor the 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 Sub-AP/MALDI (ng) source after the Sub-AP/MALDI (ng) source, Target Software, iFunnel software and the Thermo Mass Spectrometer have been properly installed and set-up.

Once the ion source is installed, 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 main power switch and electronic system switch on the mass spectrometers turned "OFF." (The order and timing for turning off main and electronic system rocker switches are described in corresponding Thermo's MS system manuals). Before proceeding, the operator is strongly urged to read the safety procedures in Section 4 of this manual.

1. Prepare the sample(s) according to Section 6 of this manual; the sample preparation procedures are similar to methods used for the conventional (vacuum) MALDI.
2. Load the target plate containing the sample(s) into the target plate holder according to Section 6.1 of this manual. Ensure that the Control unit (the black-anodized aluminum box mounted on two hinges) is closed completely and holding bracket is fully closed.
3. Pump down the ion source to 7 Torr by turning on power on the small vacuum pump and opening the in-line valve on the red vacuum hose. After the pressure reaches approximately 7 Torr (pressure readings are taken from the iFunnel software control program), start to slowly open the vacuum shutter (i.e., by slowly rotating counterclockwise the black knob on the left), watch (after completing 4 and a half knob revolutions) for the pressure inside the box start to slowly decrease, when the pressure starts to decrease, one is advised to proceed opening but with somewhat slower speed. Watch that the pressure readings in the first (low pressure) chamber of mass spectrometer always stays below 1.8 mbar (1.5 Torr). If it starts to exceed this value, then stop the shutter opening and immediately start closing it until the pressure in the first chamber becomes normal. Then, one must observe that in a time interval of 20-30 sec after the moment when the pressure started to decrease from approximately 7 Torr, it reaches eventually the range of 1-3 Torr. Open the shutter completely (approximately 12 full revolutions). Close the in-line valve on a red hose leading to the small pump (the handle bar of

the valve is perpendicular the hose in “Closed” position). Turn off the small pump.

4. Turn the unit “ON” (by pressing the black round button on the front panel of the Control unit), and wait for the controller to initialize (the blue LED inside the Power button first goes from bright to dim and then, in 20-25 sec, turns bright again). Run the Target Software and then iFunnel Software on the PC. Wait until the initialization of Target software is completed and the “Ready” notification is displayed in the status field of the Target Software. Press “Continuous” button on the iFunnel software.

5. Since the Thermo Software (LTQ Tune, Tune, or Orbitrap Fusion etc.) is normally optimized for the electrospray source, the operator must adjust the Tune software’s parameters so that it is optimized for the Sub-AP/MALDI :

Set the software to the following initial settings: Plate Voltage: disregard Injection Time (for C trap): 200-500ms

6. Use Target Software to fire the laser and test the samples. To operate the software in the manual mode (spot-by-spot spectra recording), make sure that the auto sequence toolbar and zoom mode icons are unchecked. Then, choose a desirable spot to analyze (A1 for instance) using Target Software. If necessary, adjust the position of the irradiated area on the target and the laser spot scanning mode using the plate motion options in the Target Software. Choose the option to use the spiral or raster motion in Target Software. Start the laser firing. Now, the operator can repeat the procedure for other spots (a detailed explanation of the automatic operation is included as Section 7.5 of this manual).

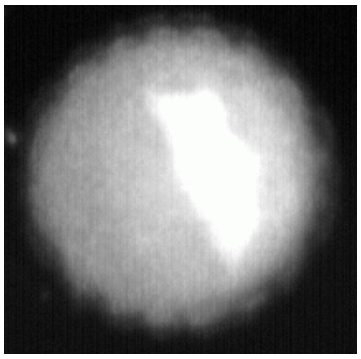


Figure 1. An Ideal Starting Sample Spot

7. When the operator finishes the data acquisition, the operator must complete the following steps: stop the data acquisition (by using the Thermo Software), put the mass spectrometer to the "Standby Mode", and stop both the laser firing and target motion (by using the Target Software "Stop" button  . Then, use the "Eject" button  , located on the right of the control line, wait for the plate to stop, close the shutter (12 clockwise rotations of the black knob on the left side of the iFunnel unit , until it is fully shut and no further rotation is possible); observe the pressure growth inside the source (read pressure in Torr using the iFunnel software), open the holding latch, wait until the Control unit is disengaged (opened) from the funnel part by its own weight; if this does not happen in 5 minutes, then open the vent valve located on the manifold (approximately 2 rotations), open the Control unit almost perpendicularly (for a convenience) with respect to the iFunnel part, and finally gently remove (pull from the holder) the used target plate. The target plate is hold in place by a small magnet in the plate holder.

8. Take the plate containing the new sample to be studied, gently insert it into the plate holder (beware that to insert the plate properly, a truncated plate corner should be in a 10 o'clock position), close the Control unit using the latch on the right side of the iFunnel unit, press the "Eject" button  under Target Software, and go to step 3 to obtain mass spectra from the new sample.

9.

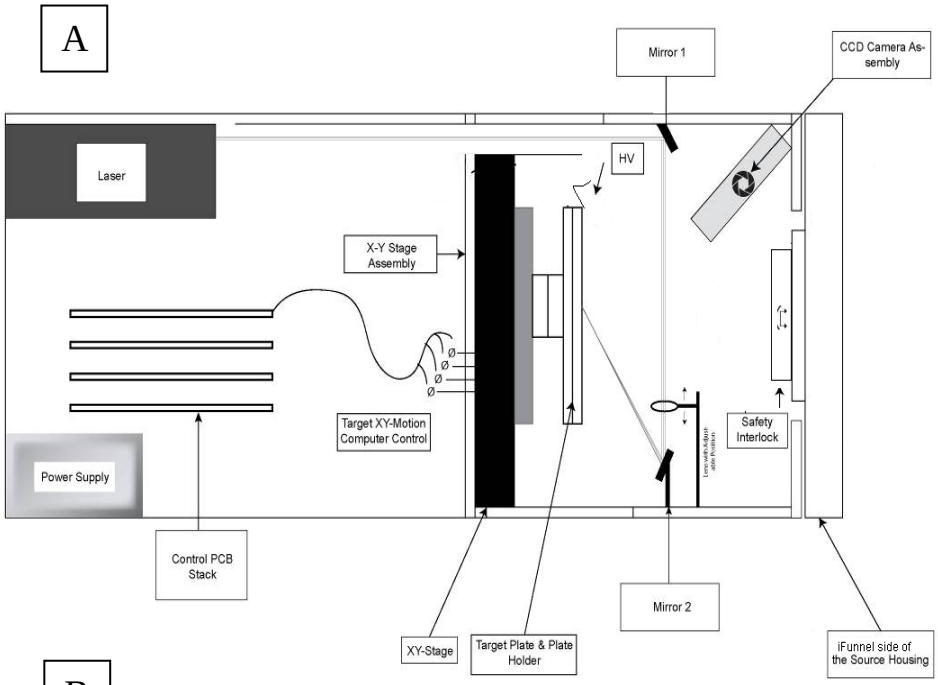
2 SUB-AP/MALDI BASIC PRINCIPLES

This section will focus on the description of the Sub-AP/MALDI process.

Control Unit of the Sub-AP/MALDI Source:

A simplified schematic of the *Control Unit of the Sub-*AP/MALDI *(ng)* source is represented in Fig. 2.1A.

A



B

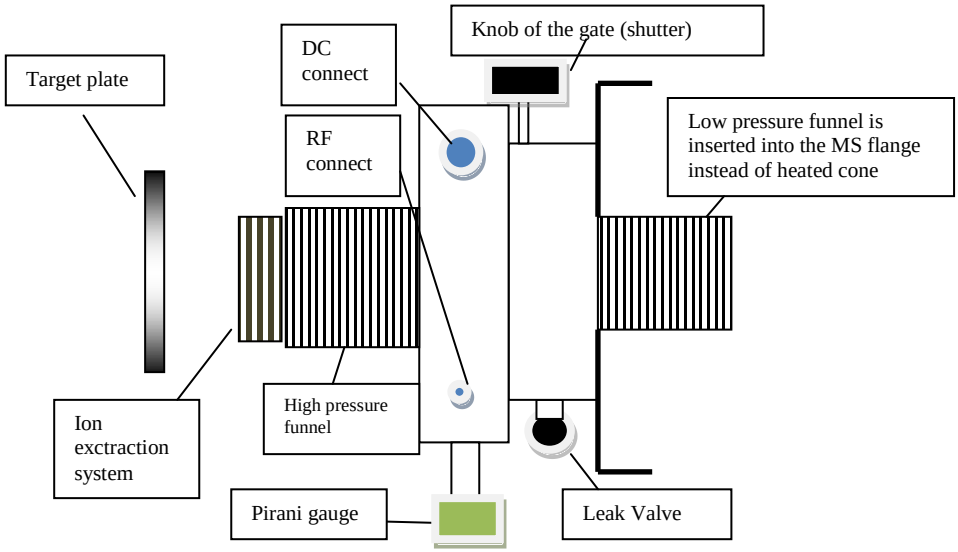


Figure 2.1. Simplified Schematic Diagrams of the Control unit (A) and iFunnel (B) of the Sub-AP/MALDI *Source. Control unit is attached through the hinge to the iFunnel part, the latter is installed into Thermo's MS Instrument.*

The Control unit of Sub-AP/MALDI source is a monolithic source housing. The source is attached to the iFunnel part installed into the mass spectrometer. All ions produced inside the Control unit travel toward the iFunnel and then to the mass spectrometer. The Control unit housing contains the following components: the laser, optics, XY-stages with the target plate holder, and control electronics. The MALDI samples are placed onto the surface of a stainless steel target plate and the latter is inserted into a target plate holder. The high voltage (typically 350-370 V) is applied across sample plate. Slightly lower voltage (approximately 320-290 V) is applied across the extracting electrode of the iFunnel part to assist the transportation of the ions toward the iFunnel interior and then to mass spectrometer.

The sample material deposited on the surface of the target plate is irradiated with the laser light. The high-repetition rate (all-solid-state Nd:YAG laser) has a wavelength 355 nm, is mounted inside a control unit, and has a repetition rate range from 100 to 10,000 Hz for both the Sub-AP/MALDI *(ng) HR* and Sub-AP/MALDI *(ng*) *UHR* (Appendix A contains a list of specifications for the laser).

The CCD camera and imaging optics enable the operator to monitor the target plate motion and sample ablation processes on the computer monitor. Inside the Control unit housing, there is also a source of visible light (LED) to illuminate the target plate surface. The Control unit of Sub-AP/MALDI ion source can be easily opened to replace the target plates. A safety interlock prevents the laser from being switched "ON" or the HV to be applied to a target plate if the source is opened.

The Control Unit communicates with the Target Software via the Ethernet connection from the source to the MS instrument's computer. The Target Software controls the target plate motion and laser firing, which are important features of the Sub-AP/MALDI source.

iFunnel part of the Sub-AP/MALDI Source:

A simplified schematic of the *iFunnel part of the Sub-*AP/MALDI *(ng)* source is represented in Fig. 2.1B.

4 SAFETY PROCEDURES WHILE USING THE SUB-AP/MALDI ION SOURCE

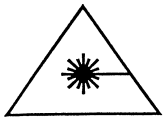
If operated properly, the Sub-AP/MALDI ion source is safe. No special knowledge of laser or electrical safety is necessary to operate the source. However, there are two potentially hazardous factors connected with the Sub-AP/MALDI source installation, operation, and maintenance/ troubleshooting:

1. Invisible coherent UV irradiation 355 nm, up to 10 µJ per pulse
2. High voltage up to 400 V DC

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

4.1 Safety Precautions

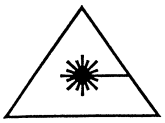
This section describes important precautions that must be observed during the Sub-AP/MALDI (ng) source ***installation/un-installation, operation, and maintenance***. Appropriate precautions can be divided into the following stages:



- **Installing and Uninstalling:** Before the source is installed or uninstalled into the mass spectrometer, the mass spectrometer must be in the "Shutdown" mode. To use the shut down please read Thermo manual for corresponding MS instrument.

> Never switch the power "ON" of the Sub-AP/MALDI(ng) source before the source is completely installed.

As stated earlier, when uninstalling the device, make sure that the MS is in "Shutdown" mode. The operator must also switch the power of the Sub-AP/MALDI (ng) source to the "OFF" mode; then the operator may detach the source or begin any of the disassembling operations. The Sub-AP/MALDI (ng) source safety interlocks the safeguard mode, which prevents the operator from accidental application of the high voltage or laser radiation when the source and control unit are covered in their housing units.



- **Target Plate Loading/Unloading:** The operator needs to open the Sub-AP/MALDI source to load/unload the target plate. For safety purposes, it is mandated that the operator first switch the instrument to either the "Standby" 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(s) as described in Section 6.1 of this manual. If the Control unit of the source is opened, the laser firing and application of high voltage are prohibited; first, by safety interlocks preventing operator to activate the laser ON button and second, by iFunnel software automatically preventing engaging the Continuous mode.

Caution: The target plate and translation stages may be hot!

- **Mass Spectra Recording:** During the data acquisition, the Sub-AP/MALDI source is closed and attached to the MS instrument, which excludes any possibility of high voltage shock or laser radiation exposure. If the shutter is closed (the MS system is isolated from atmosphere) but the source is opened by accident while the laser is "ON" and HV is applied (DC/RF units is in the Continuous mode), the Sub-AP/MALDI source automatically initiates the safety interlocks as well as software interlock to turn the high voltage and laser off.

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.

Remember:

- **Never attempt to open the Control unit during operation**
- **Never switch the power "ON" at the control unit if the Sub-AP/MALDI source is not properly attached to the mass spectrometer.**

4.2 Operator Controls and Indicators

The two figures below illustrate the front and back plate of the Sub-AP/MALDI (ng) Control unit.

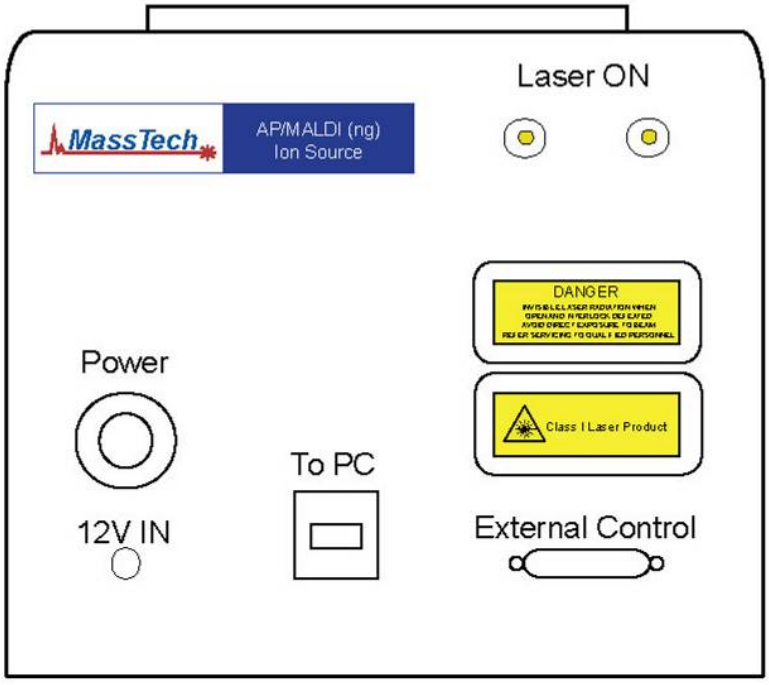


Figure 4.1 The Control Unit's Forepart of the Source

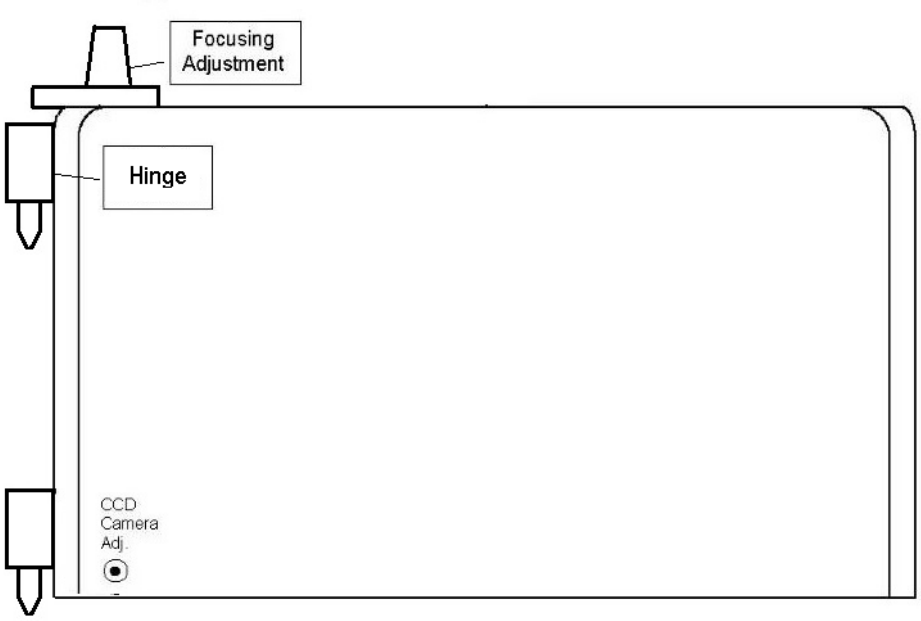


Figure 4.2 The Control Unit's Side A of the Source

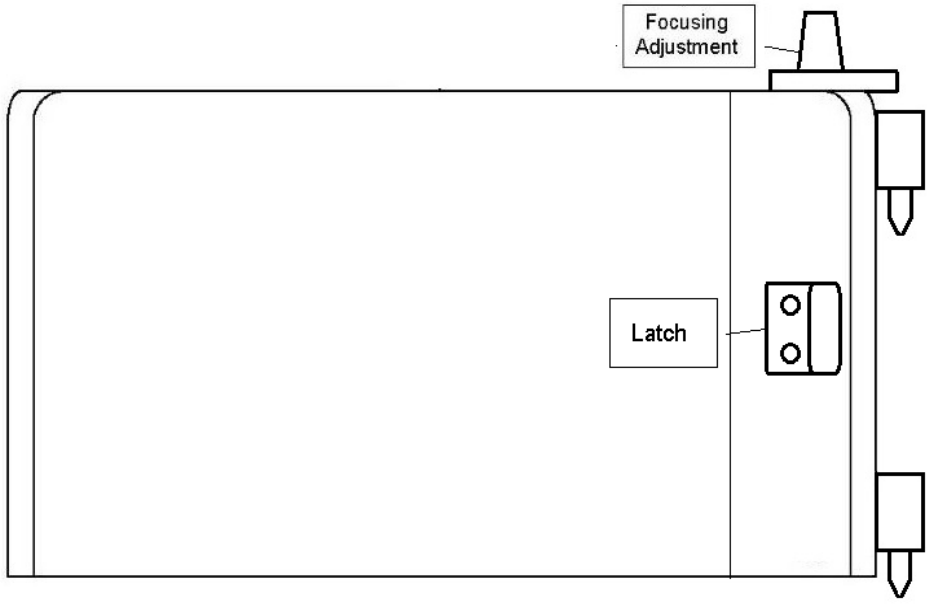


Figure 4.3 The Control Unit's Side B of the Source

5 SOURCE INSTALLATION

5.1 Checking that all Components have been received

Before the operator starts installing the source, the operator must ensure that all necessary parts and accessories have been delivered. Figures 5.1 through 5.4 (below) show these components and introduce some definitions as well as the part names used in the installation explanations.

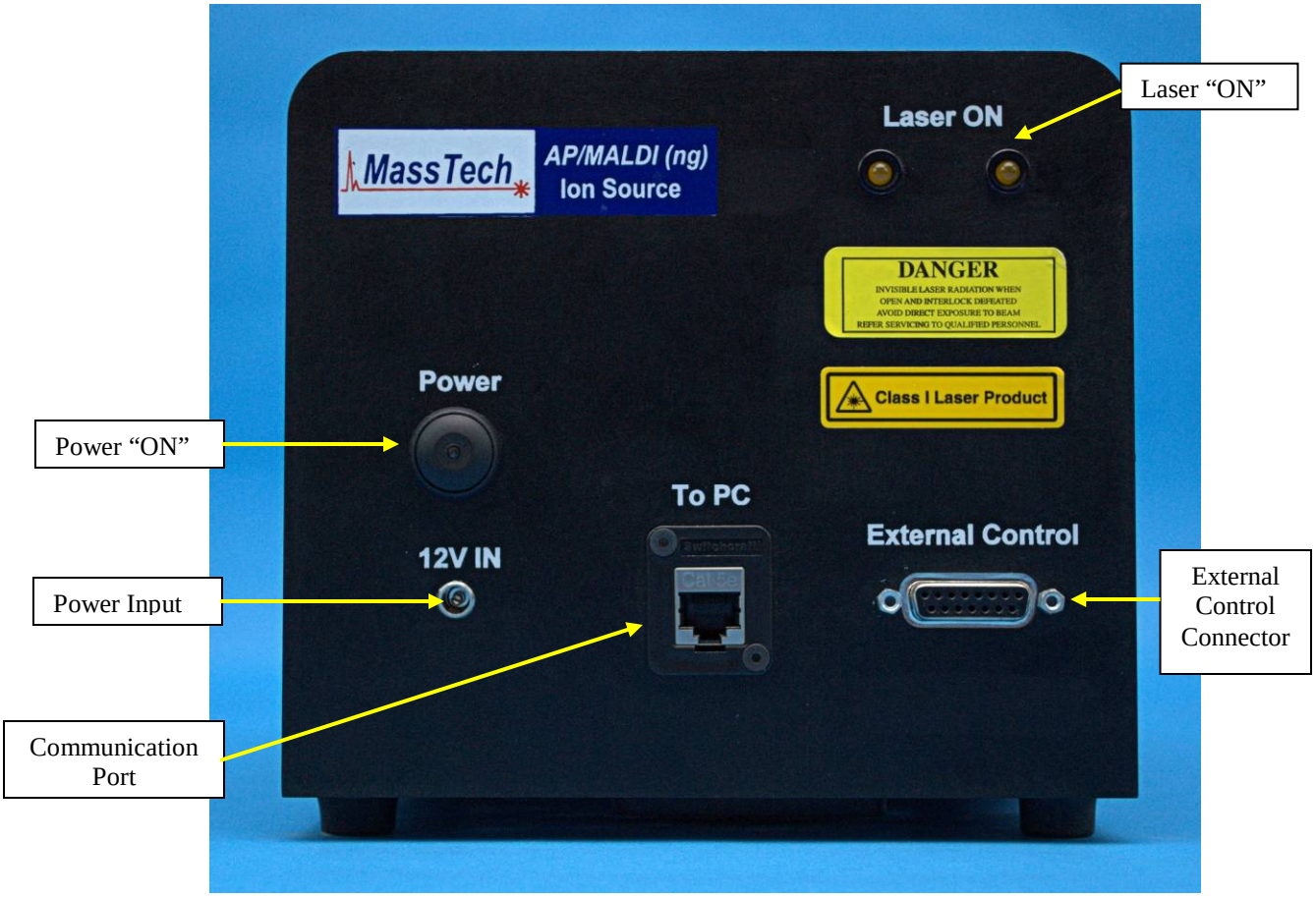


Figure 5.1 Front View of the Control Unit

Figure 5.1b Side/Bottom View of the Control Unit with Serial Number

Figure 5.2. Control unit for the Thermo Mass Spectrometer with inserted Ethernet cable and power cord from 12 V power supply

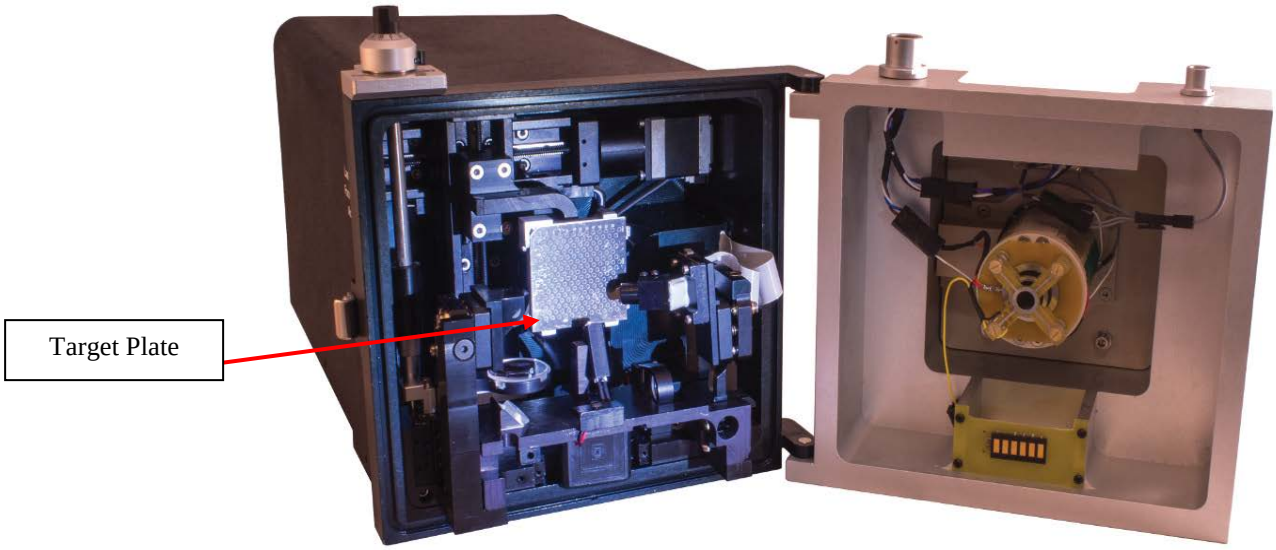


Figure 5.3. Opened Control box of Sub-AP/MALDI (ng) Ion Source mounted on iFunnel part

Figure 5.3 shows the opened Sub-AP/MALDI ion source. It accommodates a 192-spot Opti-TOF MALDI plate loaded on a holder. **The truncated edge of the Opti plate is in the top left corner of the holder**. This Figure also shows that the target plate holder is moved into the "extract/loading" position (the black triangle icon in the Target Software was pressed to move the plate holder into extract position).

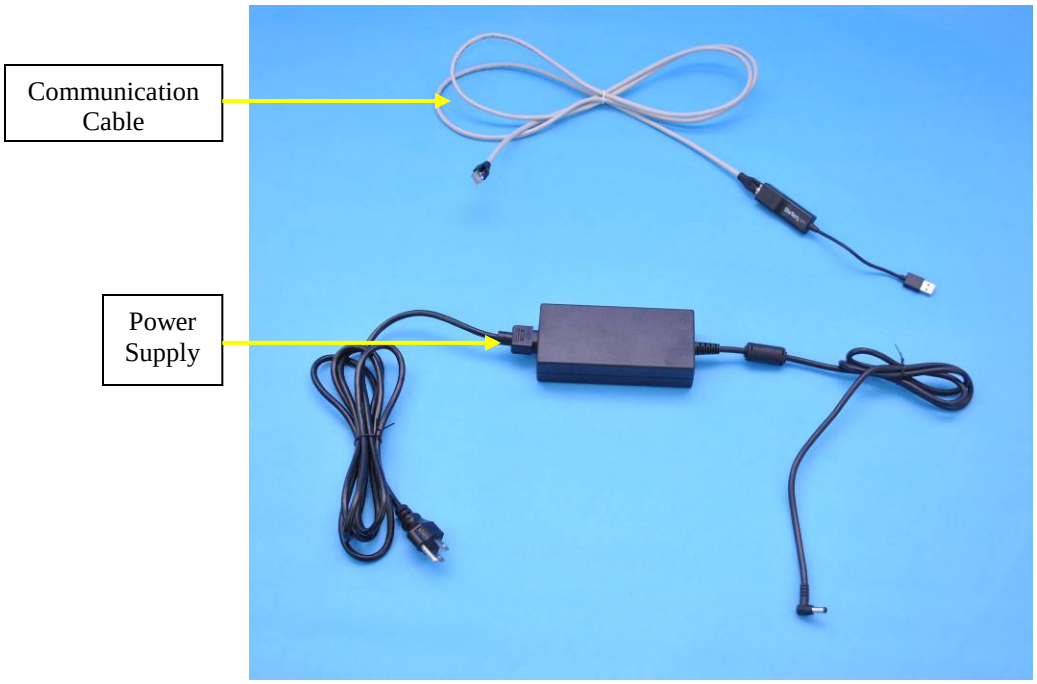


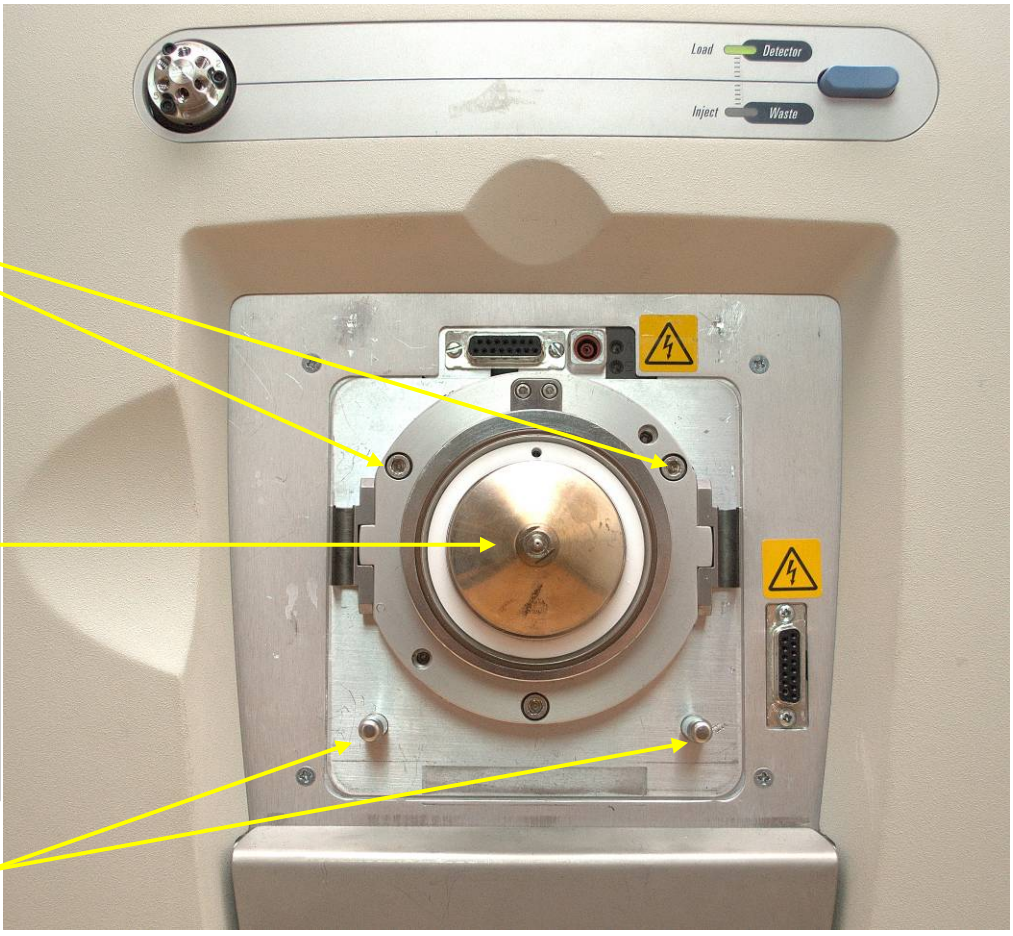
Figure 5.4 Communication Cable (Ethernet) and Power Supply

5.2 Installation of the Source

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

> ***Never*** switch the power "ON" on the rear panel of the Sub-AP/MALDI (ng) Control unit before the source is ***completely installed***.

The ion sweep cone must also be removed (CAUTION – the ion sweep cone may be hot). The mass spectrometer inlet typically looks like Fig. 5.5 when it is ready for the Sub-AP/MALDI (ng) installation.



These two bolts must be unscrewed to install iFunnel

Capillary heating cone (inlet) with Ion Sweep Cone REMOVED. **The capillary heating cone can be removed (pooled out) by hands 30 minutes after the instrument is OFF. Beware, it can be still rather hot! Wait until it cools down.**

Conical Supports

Fig 5.5 The Inlet of the Mass Spectrometer

Fig 5.6 The Ion Sweep Cone Must be Removed Prior to Source Installation

CAUTION: The ion sweep cone may be hot!

Next, insert the iFunnel part into the mass spectrometer while making sure to line up the holes on a bottom of iFunnel with the conical supports (see Fig. 5.5). Use the 2 bolts (M4x22 mm length) to firmly attach the iFunnel to the Mass Spectrometer. Only two upper threaded holes (from which the original bolts were removed) are used for bolting the iFunnel while the bottom bolt is kept untouched. Also, the MassTech's Plug unit (DB-15 plug in a metal shell) should be inserted into the 15-pin connector located above the Inlet of Mass Spectrometer. In Q Exactive, use MassTech's plastic insert to defeat the source interlock. Then mount the Control box on the iFunnel part using the hinges (see 5.3).

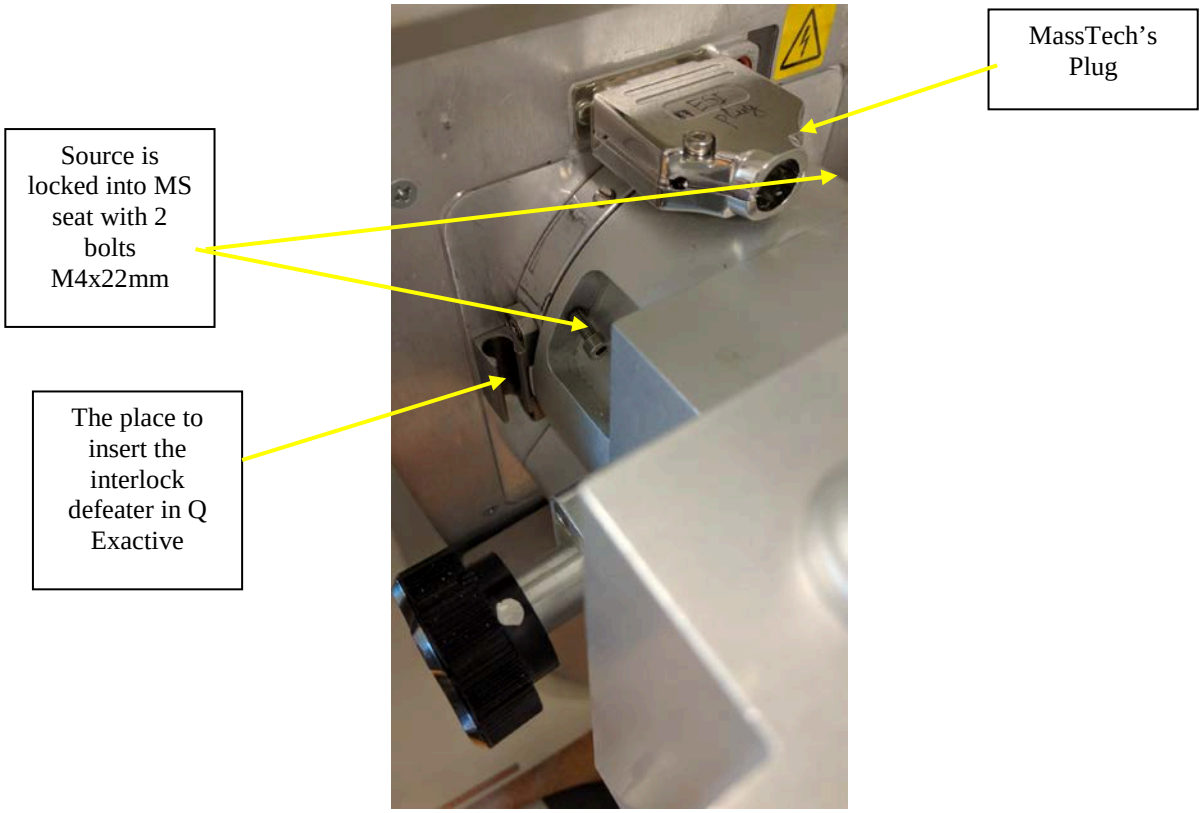


Figure 5.7. Installation Step for the iFunnel part into the Mass Spectrometer. To the left is a knob of manual vacuum shutter (gate).

5.3 Connecting cables

Control Unit cabling:

Ensure that the power on the Control unit is “OFF” until the source is wired to the control unit.

Connect the output power cord from the 12-V AC/DC power supply (provided by MassTech Inc.) and Ethernet cable to the corresponding connectors at the rear plate of the Control unit (see Fig 5.2). The Ethernet cable (Fig. 5.12) should be attached to the Ethernet jack labeled “To PC” and the other end should be connected to USB-Ethernet adapter, which in turn, is connected to the USB port of the user’s computer.

No adjustment is necessary for 12 volt AC/DC converter, it can take power from ~110/~127/~220/~240V AC!

DC RF box cabling:

Connect the AC power cord to the DC/RF box (back panel). Connect DC and RF cords (the RF cable comprises two thin cables terminated with BNC connectors joined in one) to the DC and RF outputs located on the front panel of the DC/RF box. Connect the DB-9 connector of the pressure measurement cable to the 9-pin connector on the top-right corner of the DC/RF box. Connect USB (A to B) cable to the to the front panel of the DC/RF unit (see Fig 5.8). Connect the other end of the USB cable to PC.

Figure 5.8. Connecting cables to DC/RF box

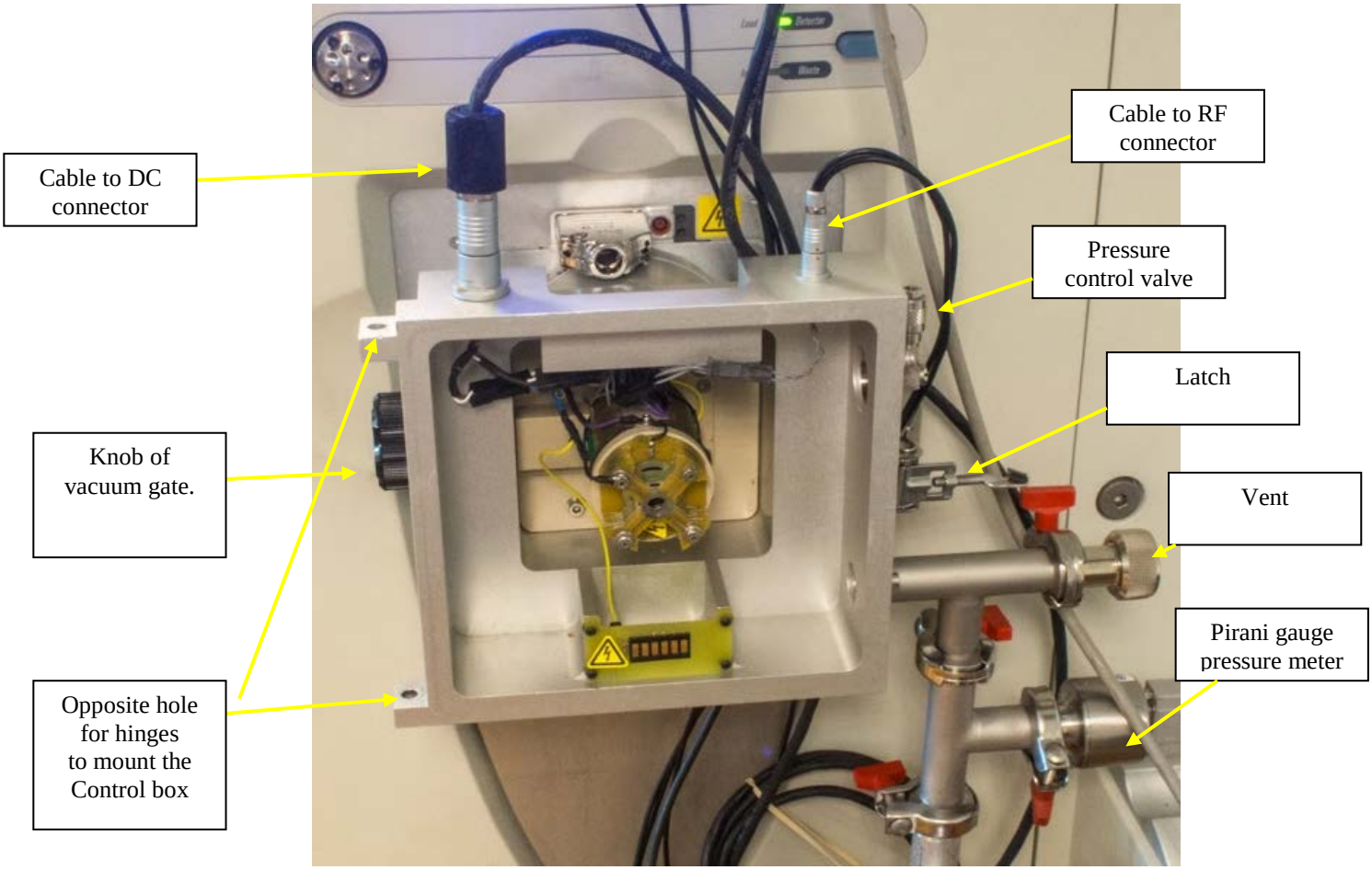


Figure 5.9. Operating position of the iFunnel part in the Mass Spectrometer. To the right is a vacuum manifold with attached Pirani gauge pressure meter and knob providing the air vent into the Sub-AP/MALDI source ***when the shutter (vacuum gate) is closed****. The bottom of manifold is connected via the hose to the vacuum pump. The hose has the in-line manual valve.*

iFunnel and manifold cabling

Connect the DC cable to 9-pin Fisher's DC connector on the top of the iFunnel part. Make sure that red dot on the receptacle is right below that on the jack. Connect the RF cable (the RF cable is terminated with a pair of BNC connectors and single Fisher connector) to the small 3-pin Fisher's RF receptacle on the right side of the iFunnel part (see Fig. 5.9). Make sure that red dot on the receptacle is right below that on the jack. Connect the DB-9 connector of the pressure measurement cable to the 9-pin output of the Pirani gauge attached to the vacuum manifold.

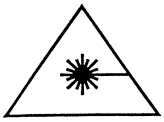
Before Switching on the Power “On"

1. Ensure that the connectors are firmly connected.
2. Now it is safe to turn on the Control unit.

5.4 Fine Adjustments to the Source

The unit comes pre-adjusted, yet if necessary, the fine adjustments to the imaging (e.g. sharpness of an image) and laser spot position can be made.

Adjustment of the laser spot position



This procedure can be safely performed under condition when operator wears special laser glasses blocking 355 nm laser radiation!

It can be done even if the source is unlatched (the source is open). The shutter is closed. One can defeat the laser OFF interlock by shortening with a short piece of thin bare wire the first and third spring electrodes on the interconnect board located beneath the optical bench of the Control box.

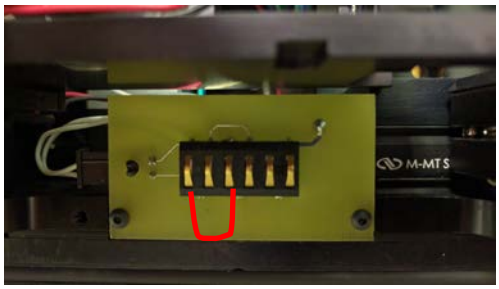


Fig 5.10. Defeating Laser OFF interlock

To make a repositioning of the laser beam on the target, use metric Allen wrench

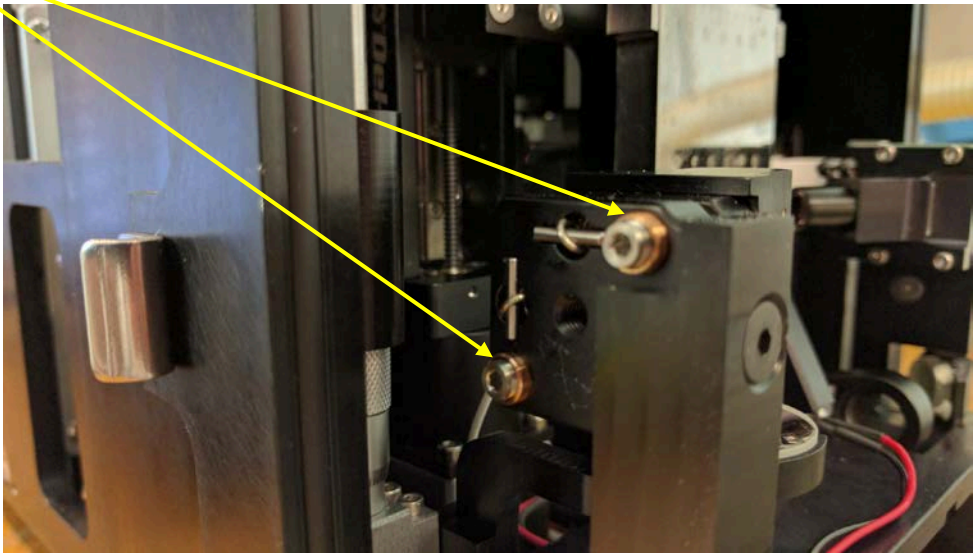


Fig 5.11. Laser spot position adjustment

Be sure that line indicating laser status shows “Ready” status.

Click the black triangle  on the Target Software that turns the laser “ON”. When the laser starts, gently turn the knobs of the mirror holder with metric Allen wrench to reposition the laser beam. The best results are reached when the laser spot coincides with the axis of symmetry of the iFunnel. Typically, it is close (within 0.5 mm) to a geometrical center of a bright spot created on the target by LED. Generally, the axis is pointing to a vertical line that crosses the center of the LED-generated bright spot. *One can order a special jig from MassTech Inc that aids in the laser spot positioning with respect to the iFunnel axis.* One is advised to compare ion signals before and after the laser beam repositioning to be sure that the latter, in fact, improved the ion signal. After the laser beam repositioning is completed, remove the interlock defeater.

Adjustment of the CCD camera field of view position and its focusing.

One does not need to defeat the laser interlock. One needs gently turn the knobs of the CCD camera holder with metric Allen wrench to find the camera position providing sharp images of the target plate.

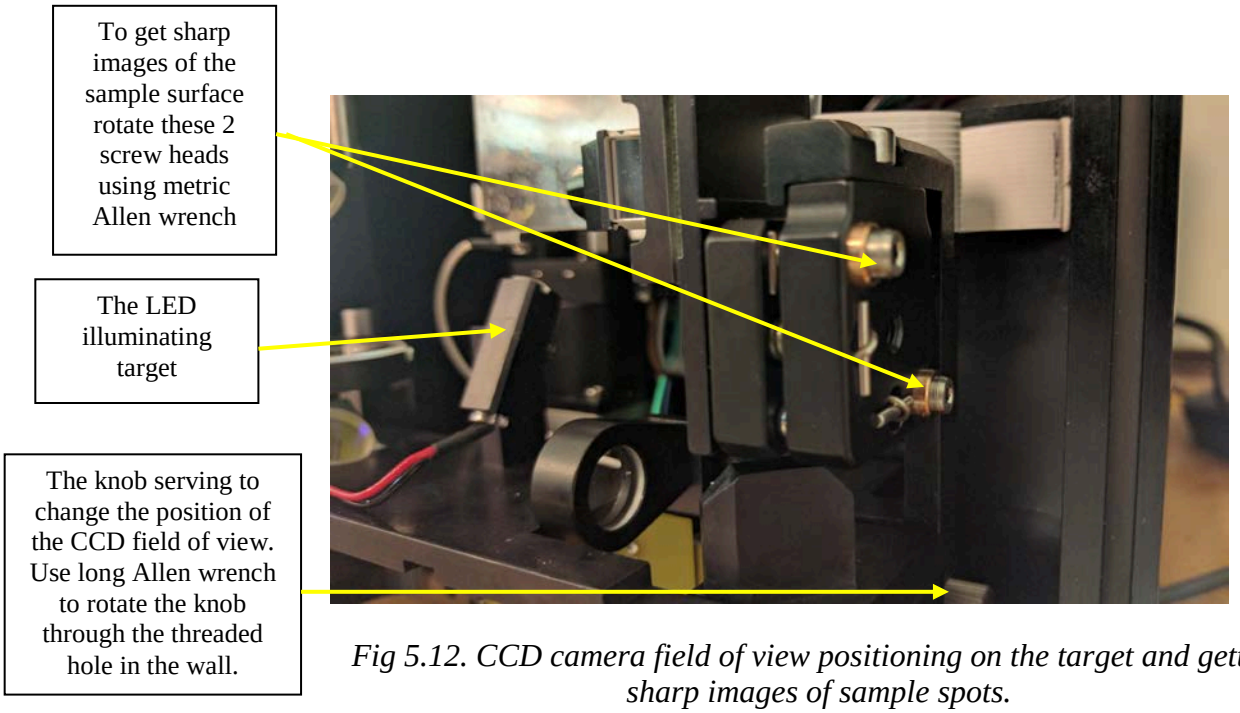


Fig 5.12. CCD camera field of view positioning on the target and getting sharp images of sample spots.

If necessary, the fine adjustments to the CCD positioning and focusing can be made as shown in Fig. 5.12. Pay attention that to access the knob that moves CCD in a horizontal direction (i.e., along the plate) one needs **to remove the airtight screw (standard, nonmetric thread) located on the side of the enclosure wall opposite to the knob. Do not forget to tight the crew back after CCD camera adjustment.**

5.5 Source Removal and Uninstallation

When the operator 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 OFF on the Control and DC/RF units.
3. Then, uninstall the source by reversing the installation procedure as described in Sections 5.2 and 5.3.

6 SAMPLE PREPARATION

The same sample preparation techniques and the same matrix used for the conventional MALDI vacuum can be used for the Sub-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 Sub-AP/MALDI (ng). 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 II, Bradykinin 1-7, $P_{14}R$ 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

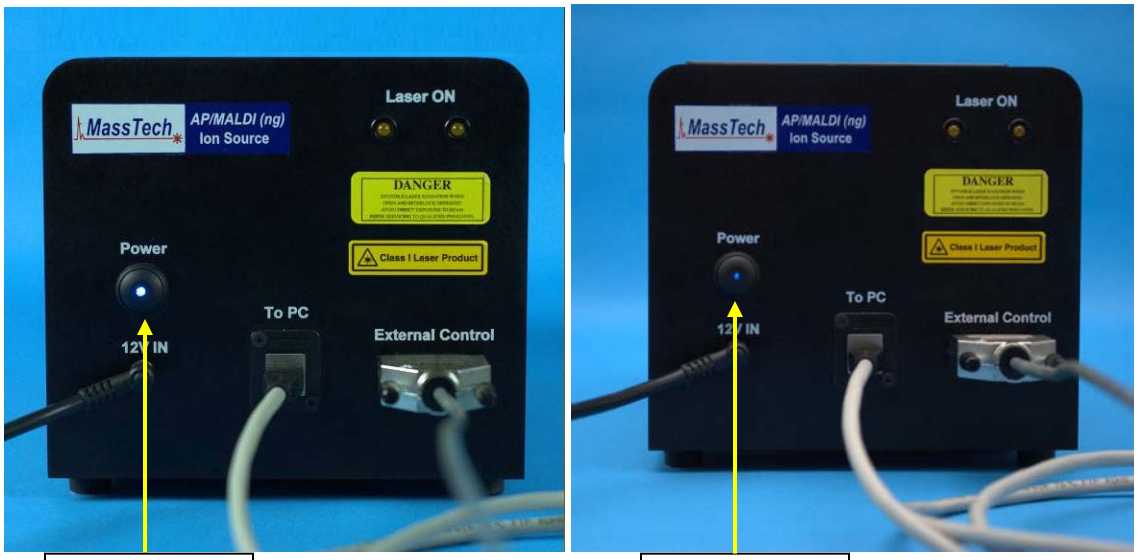


Figure 6.2. Bright and Dim Power LED Lights

The operator needs to first open the Sub-AP/MALDI source to load or unload the target plate. The operator 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" indicators on the front panel dim.*** After that, click on the Eject button.

To open the source, first close the shutter, then open the latch, and watch that the pressure inside the source is steadily growing. Then wait for several minutes. If necessary, open the vent valve. Either way, the source will open by itself when the gas pressure inside it reaches the atmospheric one. Open the source, and take the plate off the plate holder inside the source (Figure 5.3).

Caution – The target plate and translation stages may be hot when unloading!

Mounting the MALDI plate inside the Sub-AP/MALDI source

The source is open. Insert the new target Opti plate (with the prepared sample spots) into the target plate holder. *Attention to a position of the truncated (cut) corner of the plate – this corner should be in 10 o'clock position.* The plate is held in place by a magnet.

Close the Control box of the Sub-AP/MALDI source (see Fig 6.2). Put the arm of the latch onto the hook mounted on the Control box and engage the latch handle. Turn on the small pump, open the in-line valve and watch the gas pressure inside the Control box going down (till approximately 7 Torr). Subsequent steps were described in Chapter 1.1.

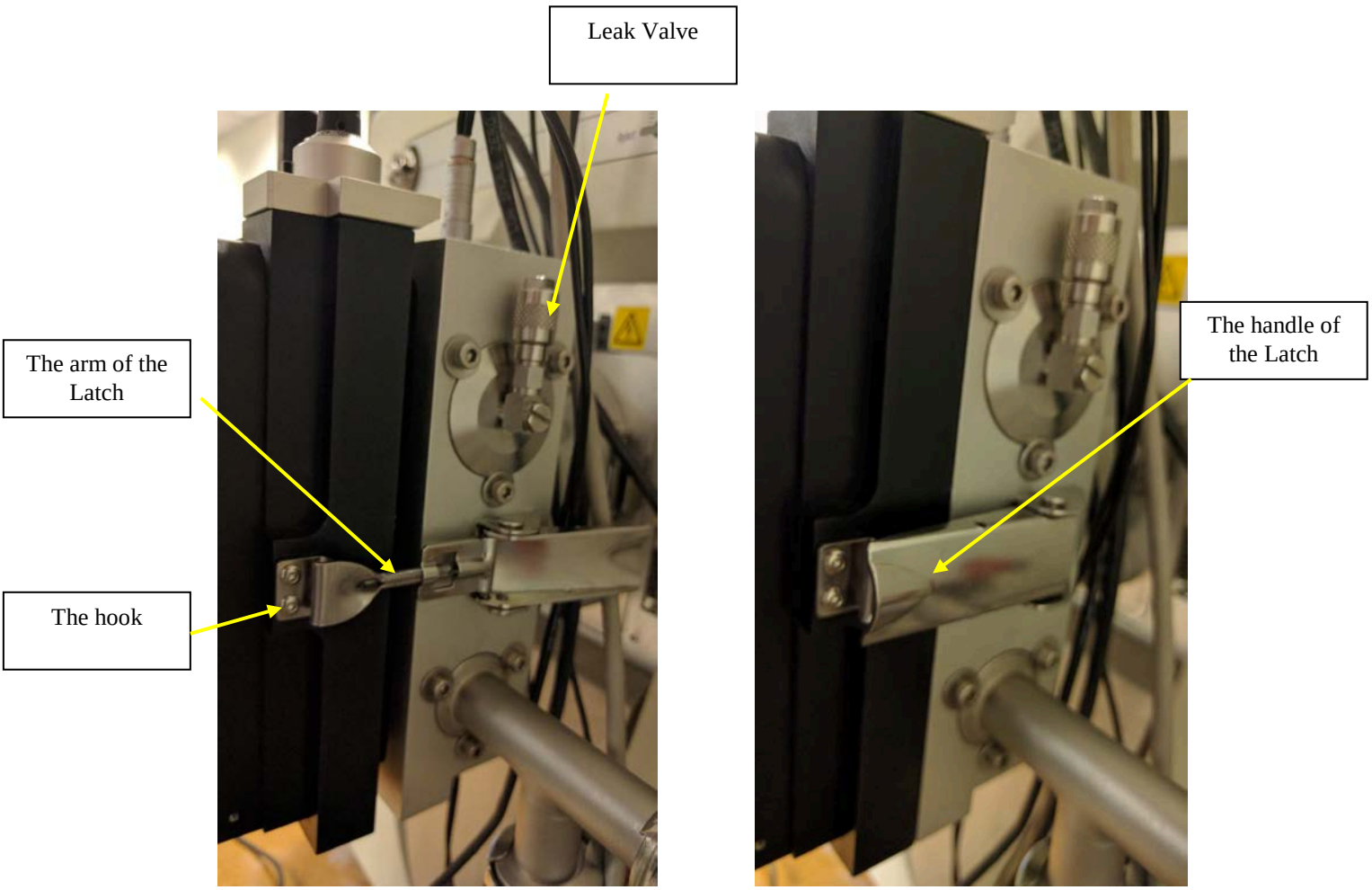


Figure 6.2. Closing the Source

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6 SUB-AP/MALDI SOURCE OPERATION

7.1 Installing the TARGET Software and USB Drivers:

The Target Version 8.0 Software is used to control the Sub-AP/MALDI target motion and laser firing operation.

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

1. Download the Installer Package, which can be found on the Downloads page from apmaldi.com; the Installer Package will be under the Downloads for Sub-AP/MALDI (ng) and Sub-AP/MALDI *(ng)* 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 operator 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 operator 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/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.

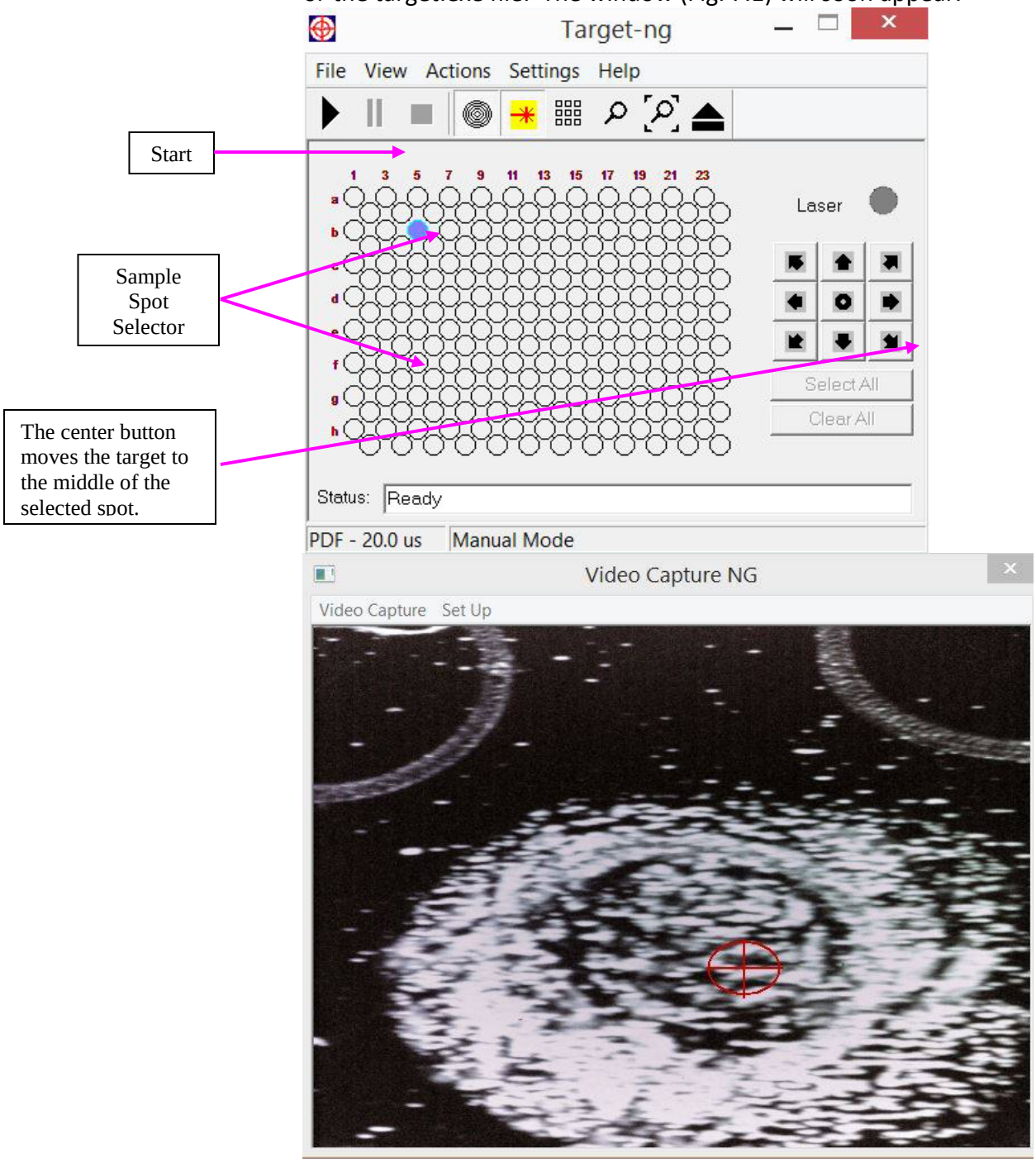


Figure 7.1. The Target Software Started, Properly Initialized, and Ready

At this moment, the initialization of the XY stages will start automatically. If everything has been connected properly, the operator will see the 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 operator 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.

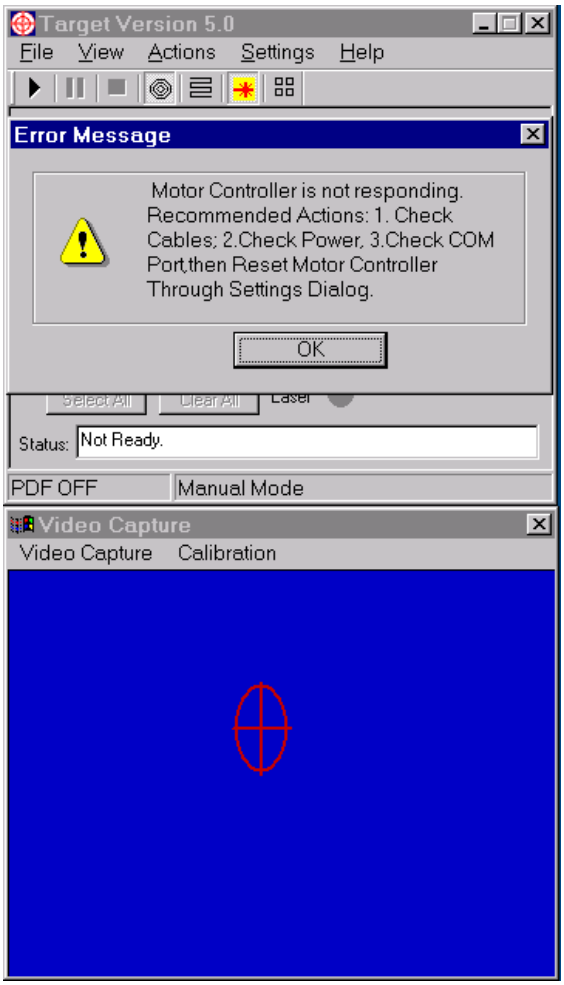


Figure 7.2. Error Message

After the Sub-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, and when closed, make sure the fiber optic is tightly secured to the connector.

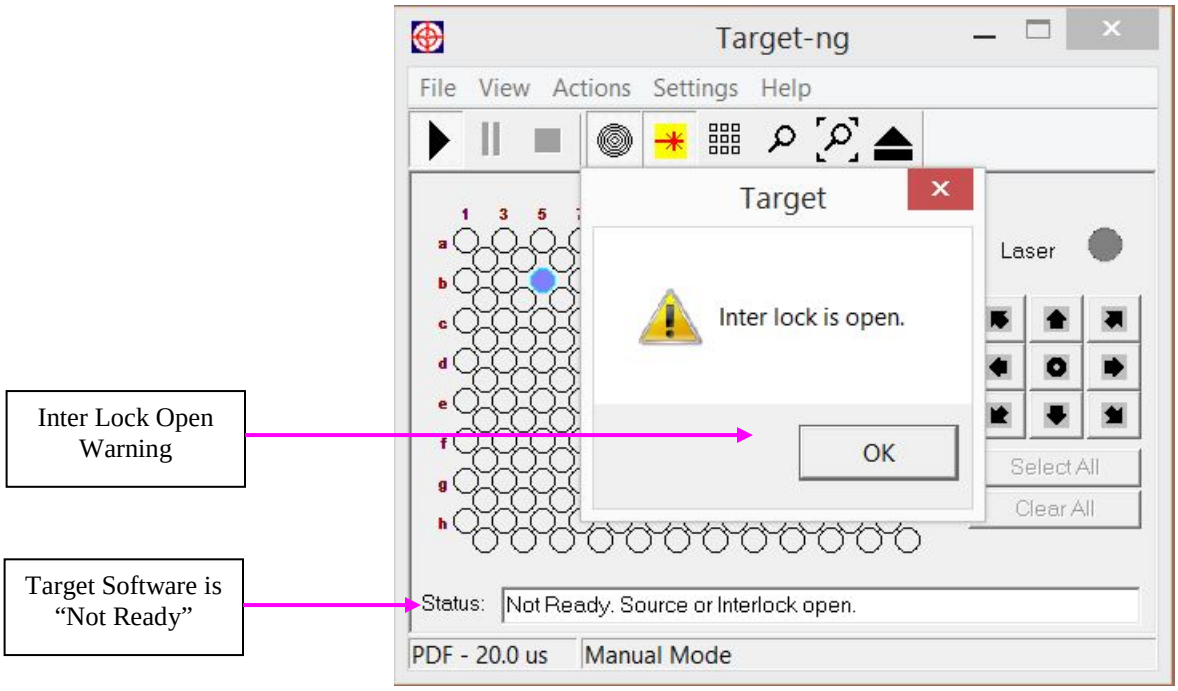


Figure 7.3. Target Software Showing the "Not Ready" State

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 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 do where the laser is firing (Fig. 7.4).

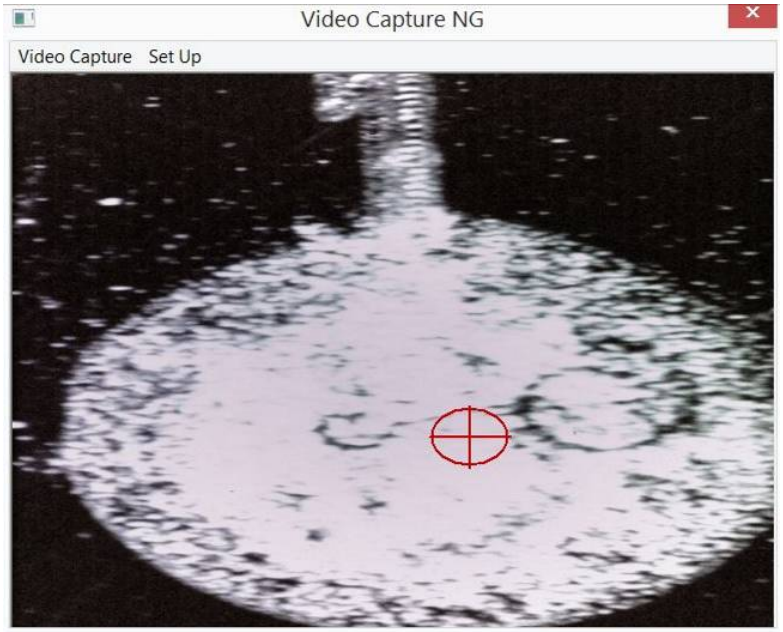


Figure 7.4 Positioning the Red (Laser) Cross Hairs

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

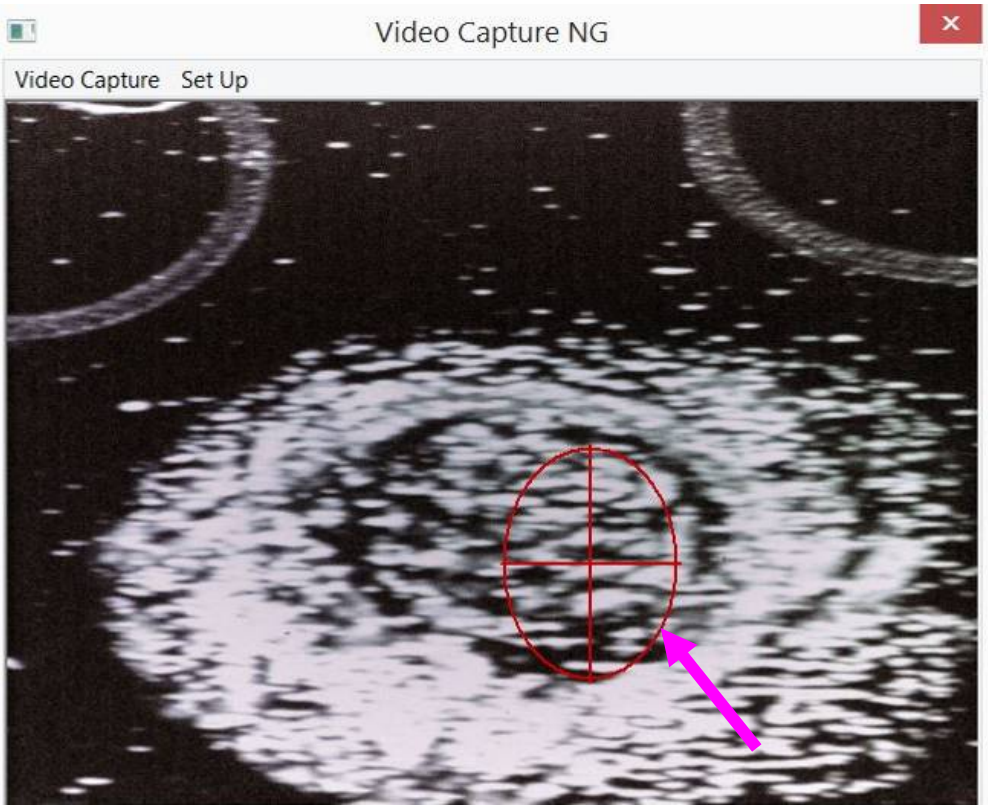


Figure 7.5 Resizing the Crosshair

The laser can be resized by placing the mouse at the center of the red cross hairs, pressing *Shift,* and then 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 needs 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 operator 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).

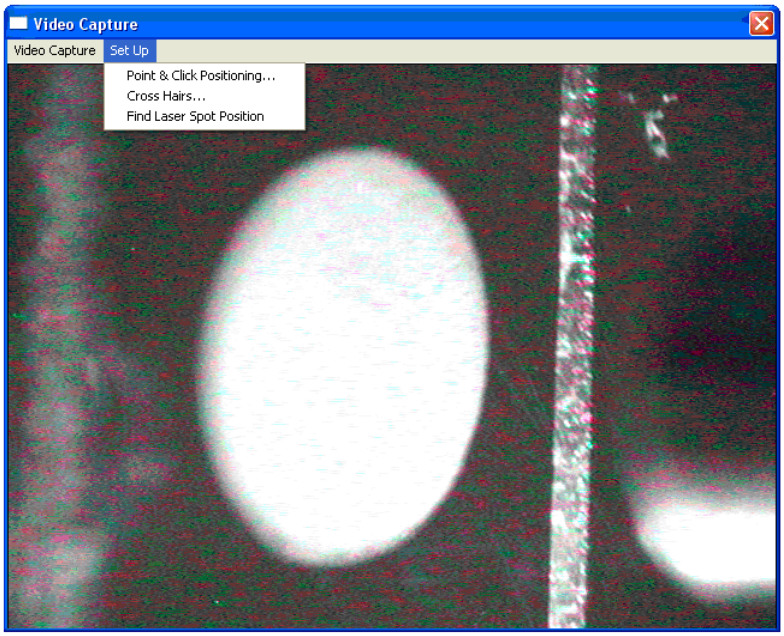


Figure 7.6. Start of the Video Capture Sample Positioning Calibration Procedure

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).

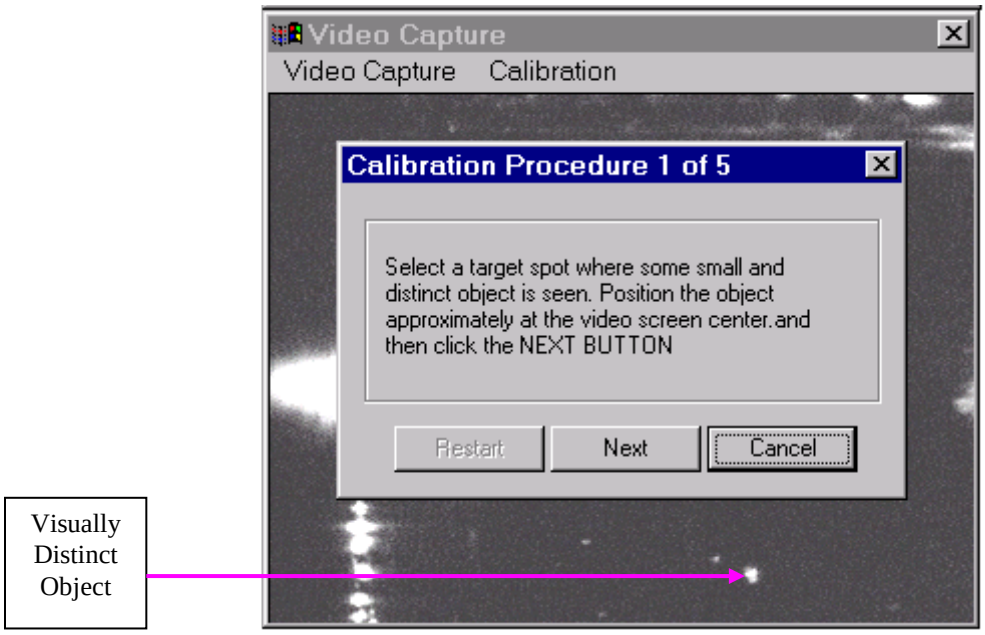


Figure 7.7. Step 1 of the Video Capture Sample Positioning Calibration

In Step 2 Figure 7.8a shows the position of the original green target on the video capture screen. Drag and immediately drop the green target icon to the visually distinct object in the lower right hand quadrant (Fig. 7.8b). Then, click "Next."

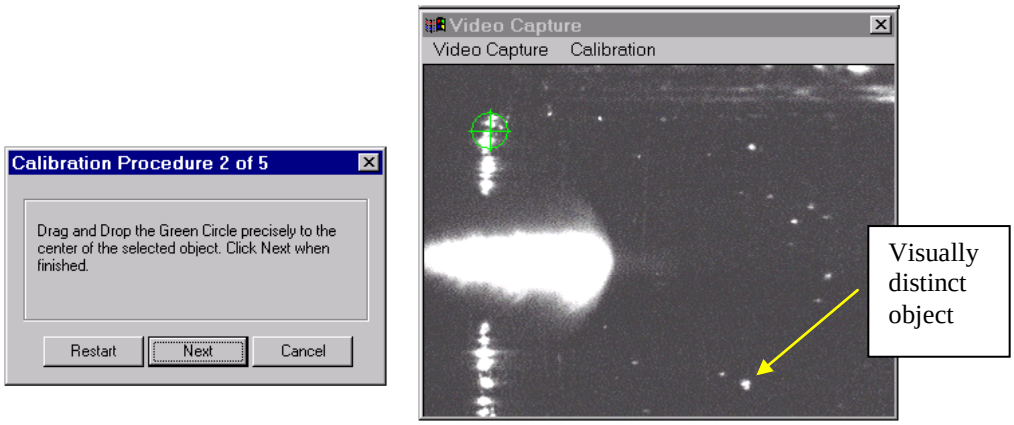


Figure 7.8a. Step 2 of the Video Capture Sample Positioning Calibration showing the position of the original green target.

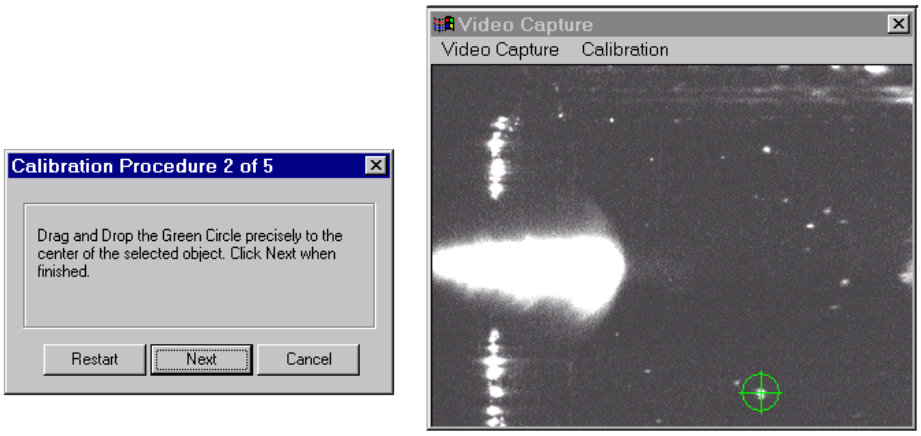


Figure 7.8b. Step 2 of the Video Capture Sample Positioning Calibration showing the green target dragged to a visually distinct object in the lower right hand quadrant.

In Step 3 (Fig. 7.9), the target plate moves horizontally. Watch where the visually distinct object moves to (Fig. 7.9a). When it stops, drag and drop the green target icon to the new position of the visually distinct object (Fig. 7.9b). Then, click "Next."

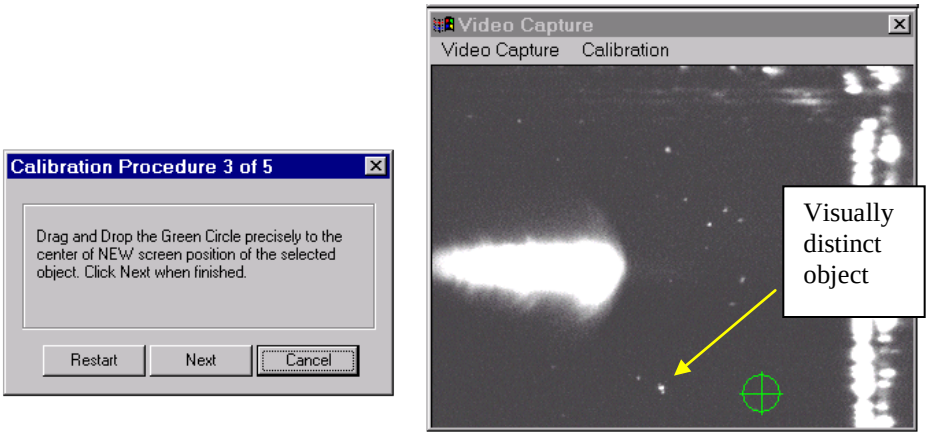


Figure 7-9a. Step 3 of the Video Capture Sample Positioning Calibration showing the visually distinct object moved horizontally away from the green target.

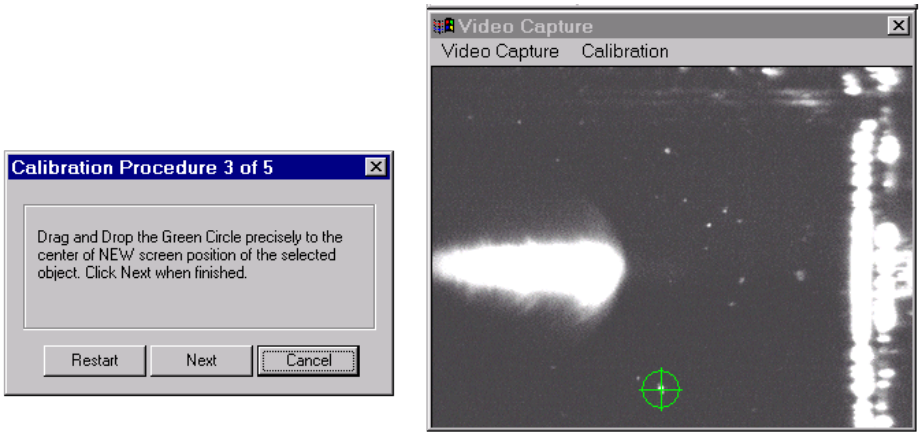


Figure 7-9b. Step 3 of the Video Capture Sample Positioning Calibration showing the green target dragged and dropped back on top of the visually distinct object.

In Step 4 (Fig.7.10), the target plate moves vertically. This time it moves vertically. Watch where the visually distinct object moves to (Fig. 7.10a). Drag and drop the green target icon to the new position of the visually distinct object (Fig. 7.10b); then, click "Next."

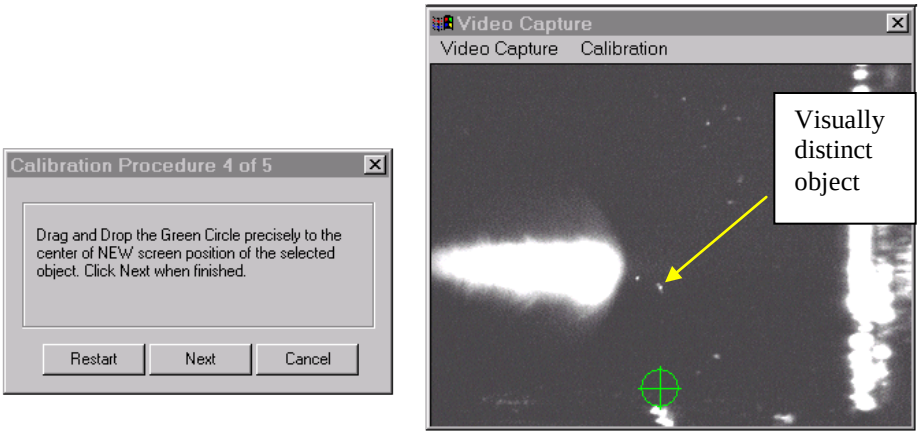


Figure 7-10a. Step 4 of the Video Capture Sample Positioning Calibration showing the visually distinct object moved vertically away from the green target.

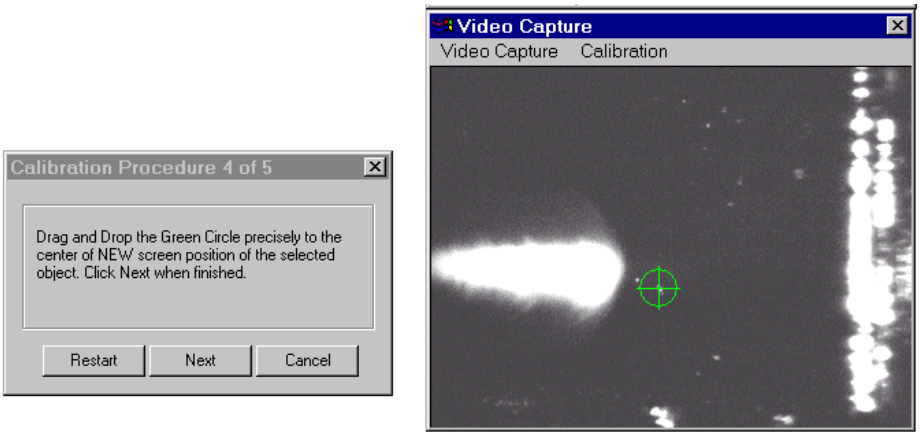


Figure 7-10b. Step 4 of the Video Capture Sample Positioning Calibration showing the green target dragged and dropped back on top of the visually distinct object.

In Step 5 (Fig. 7.11), click “Finish” to accept the new calibration. The “Cancel” button keeps the original calibration and the “Restart” button allows for the operator to recalibrate the system again.

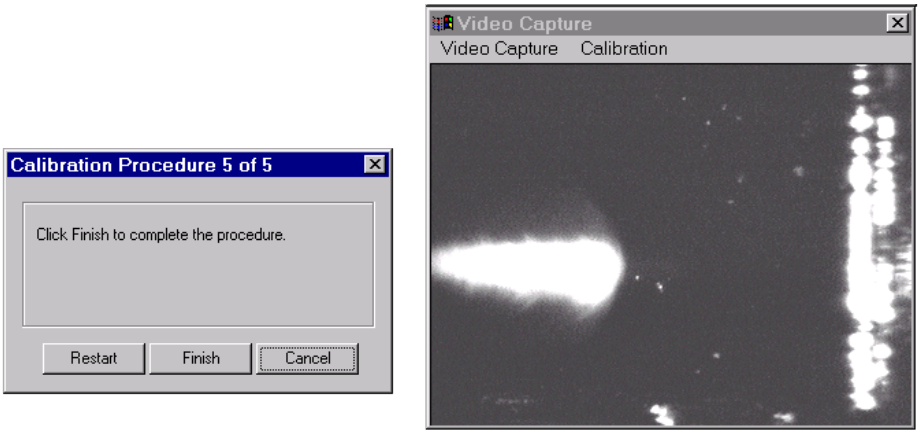


Figure 7-11. Step 5 of the Video Capture Sample Positioning Calibration.

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

7.5 Running the Sub-AP/MALDI on the Thermo MS Instrument

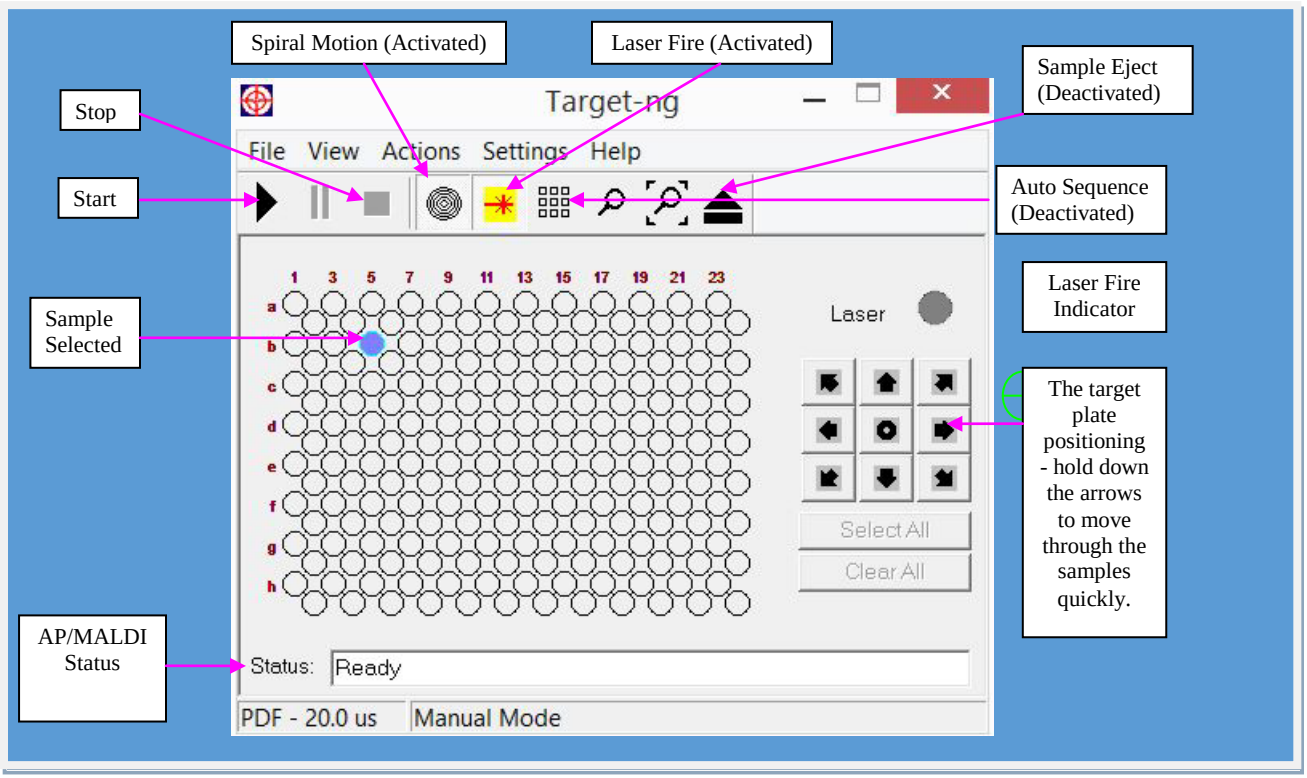


Figure 7.12. Target Software Controls

There are two modes of operations for the Target Software: manual (auto sequence toolbar icon is deactivated) and auto sequence. The operator can 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 operator 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 and Fire/Spiral/Raster Motions) 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 operator can manually shift the spot by clicking on the video capture image. The operator 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 7.16 below.

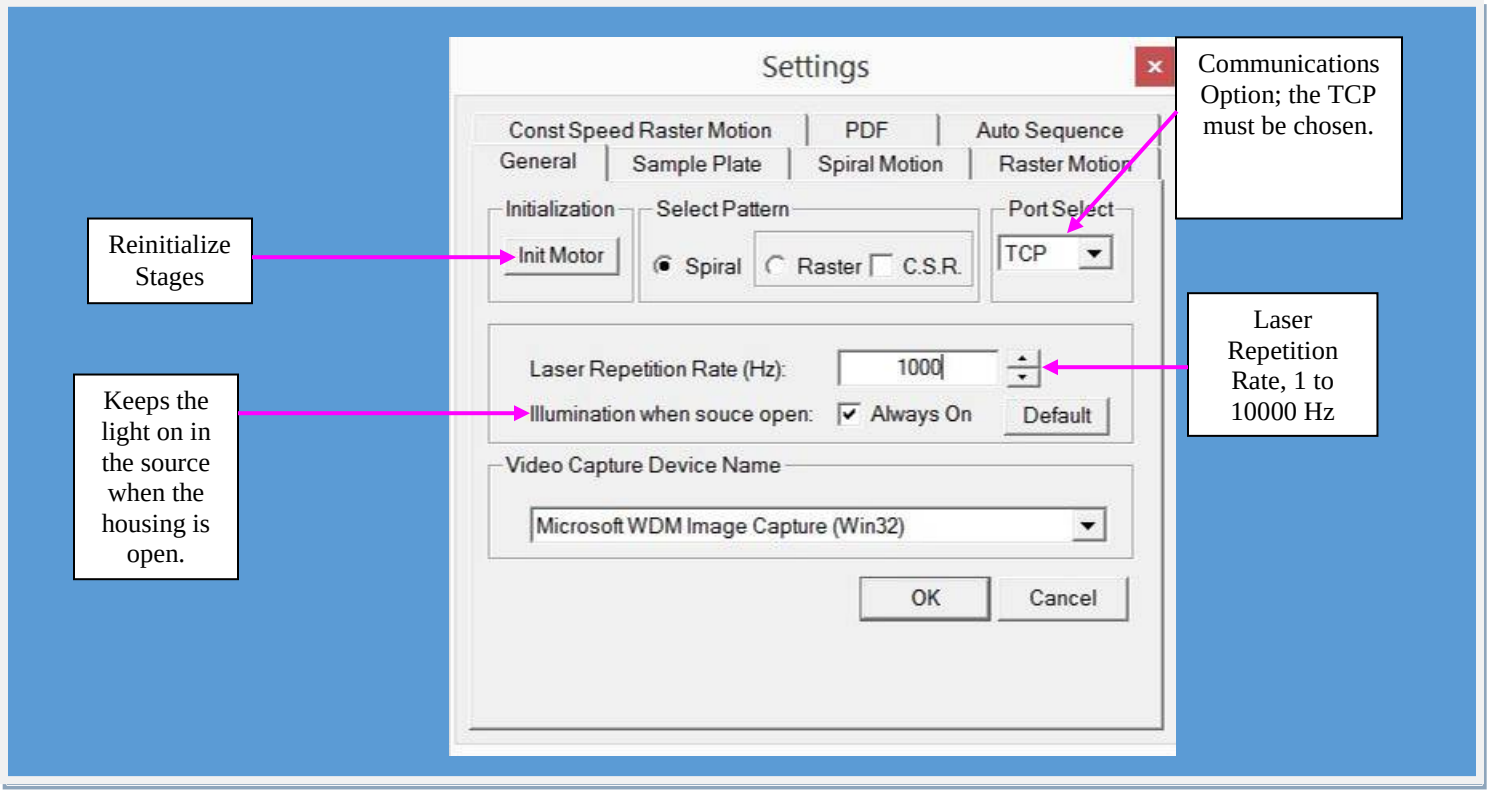


Figure 7.13. General Settings in the Target Software

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 and raster images can all be seen in Fig. 7.13. 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 CSR, use “Zoom Mode” (Section 7.12).

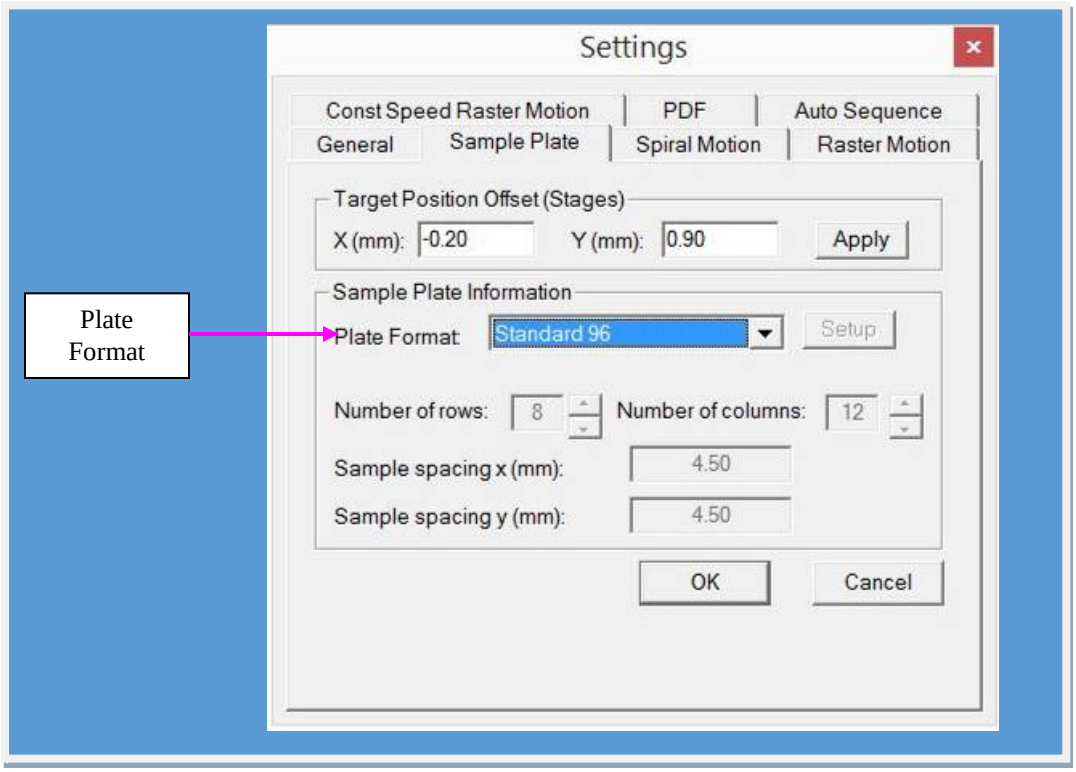


Figure 7.14 Sample Plate Settings in the Target Software

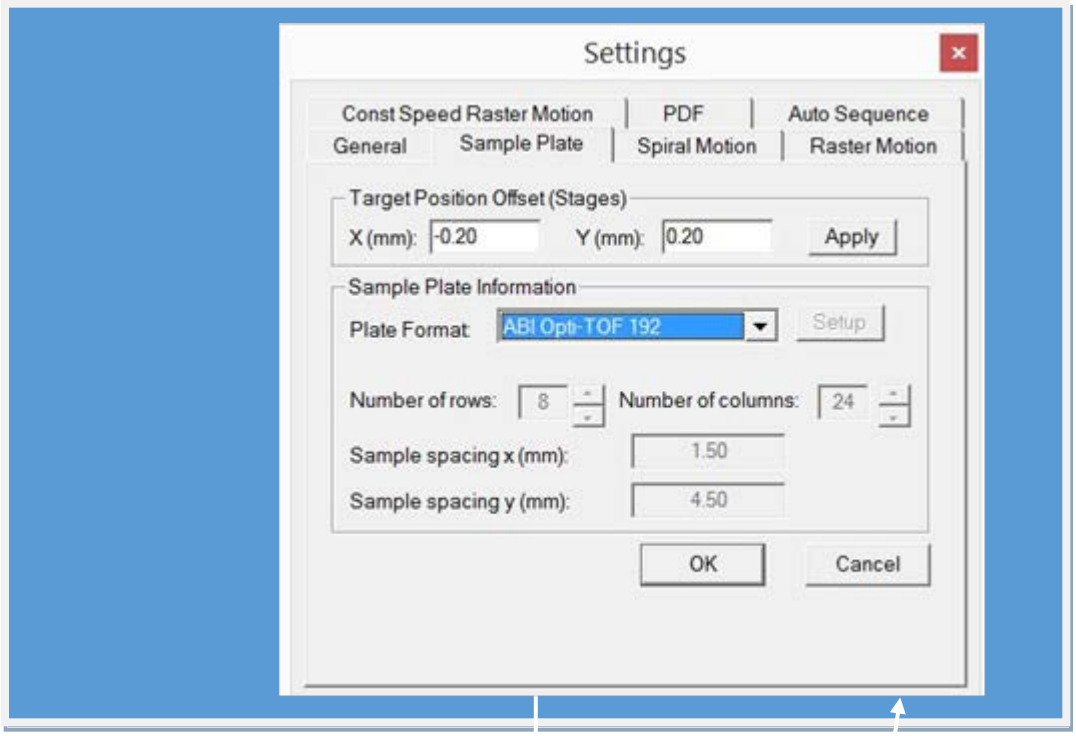


Figure 7.15. Plate Formats Supported by the Target Version 7.0 and the Sub-AP/MALDI Ion Source

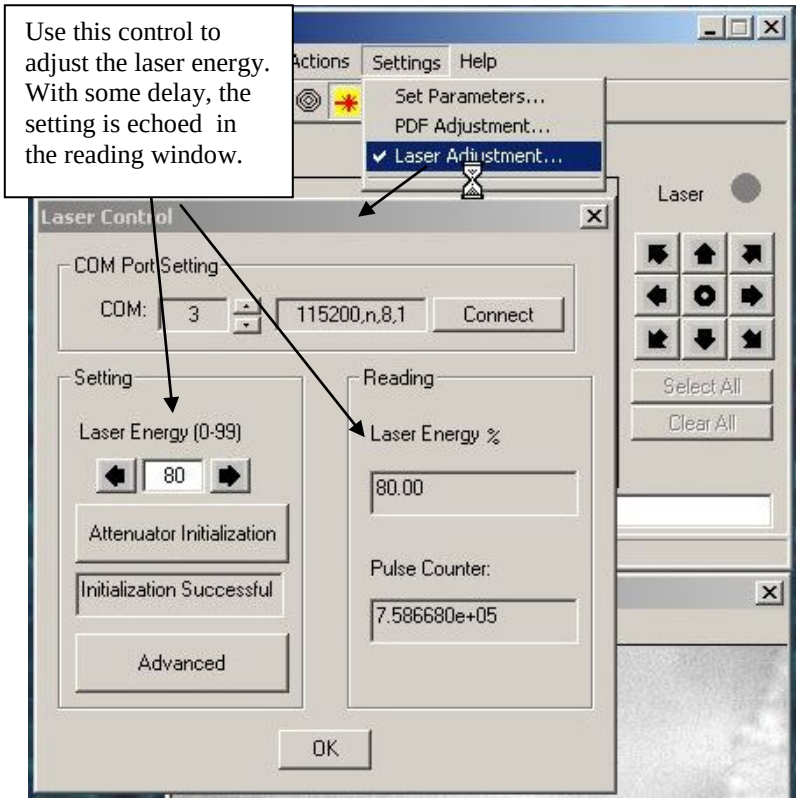


Figure 7.16. Laser Adjustment Dialog

To adjust the laser attenuation, use the dialog box shown in Fig. 7.16 (accessed through the **Settings -> Laser Adjustment** menu item).

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

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

7.7 Setting the Parameters of the Thermo Mass Spectrometers

To run the Sub-AP/MALDI on the MS instrument optimally, the following tuning procedure of the Thermo Xcalibur Program is recommended:

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

- Typical parameters that represent a good starting poins for the Sub-AP/MALDI measurements are shown in Figure 7.17.

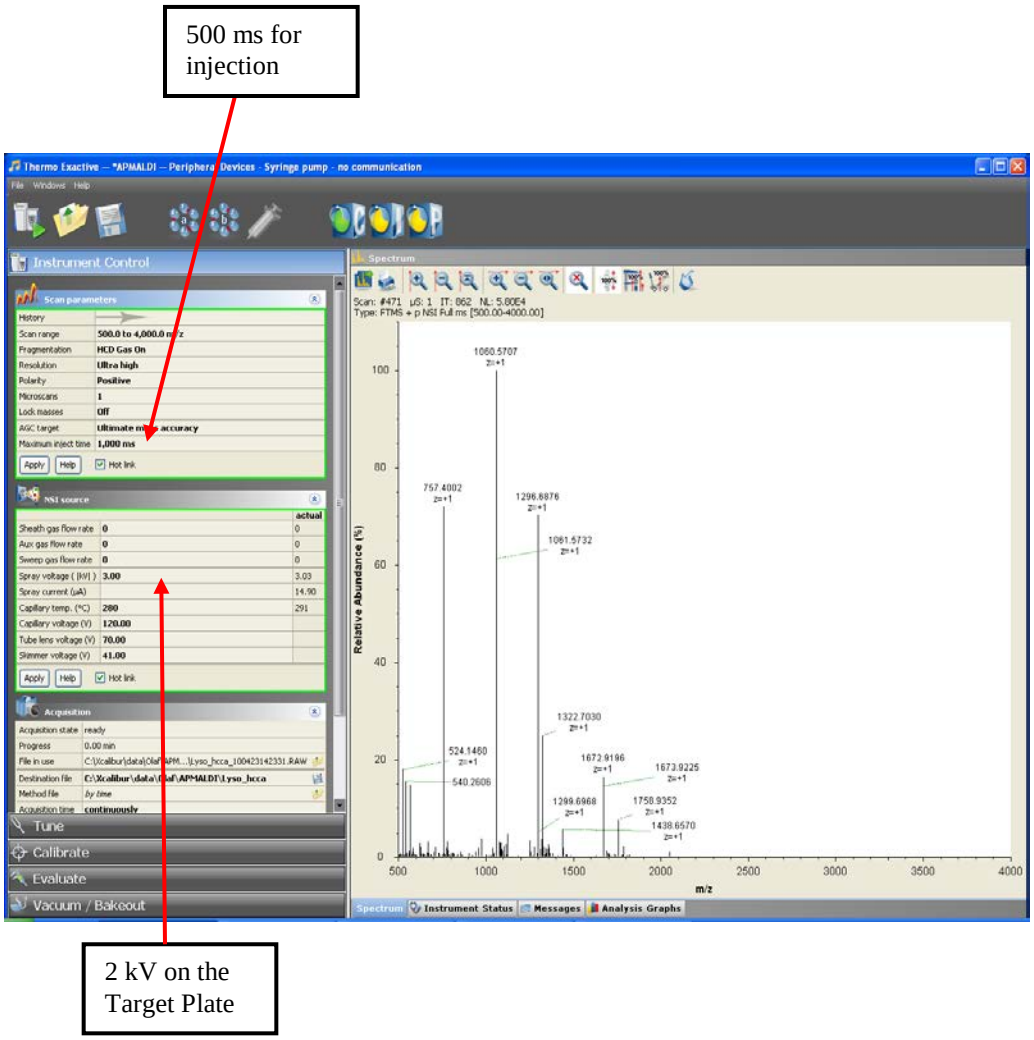


Figure 7.17. Good Parameters for the Capillary HV (2000 V) and Injection (Accumulation) Time (500 ms)

- **Drying gas and nebulizing gas settings**: The AP/MALDI process does not utilize drying or nebulizer gas. The source settings in the XCalibur control software,in most cases, are recognized as the MALDI source. In some Thermo MS systems, if such recognition does not happen, the operator needs to set the ion source as nanoESI.

- Laser pulse energy and frequency may be easily tuned through the Target Software (see Fig. 7.16 and 7.13, correspondingly). Typically, the operator should pay attenuation to the maximum signal, for every matrix type (α-CHC, DHB and so on).
- The final recommendation is how to choose between the 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; however, the manual target motion will produce an ion signal that is unstable over the acquisition time. If the operator needs a long and stable signal, he/she should start the laser firing and then initiate either of the predefined target motion patterns (spiral or raster motion). This mode will enable the operator to continuously expose fresh parts of the sample to laser irradiation. The spiral motion will give the operator a stable AP/MALDI signal for 10-20 seconds; this is sufficient for MS and MS/MS experiments.
- Fig. 7.17 represents a screen copy made during an Sub-AP/MALDI spectrum measurement of CHCA matrix. The operator can easily switch between the *XCalibur* and *TARGET* Programs to operate both the MS and Sub-AP/MALDI source from the same computer. An alternative method for the operator is to run the program on separate computers.

-

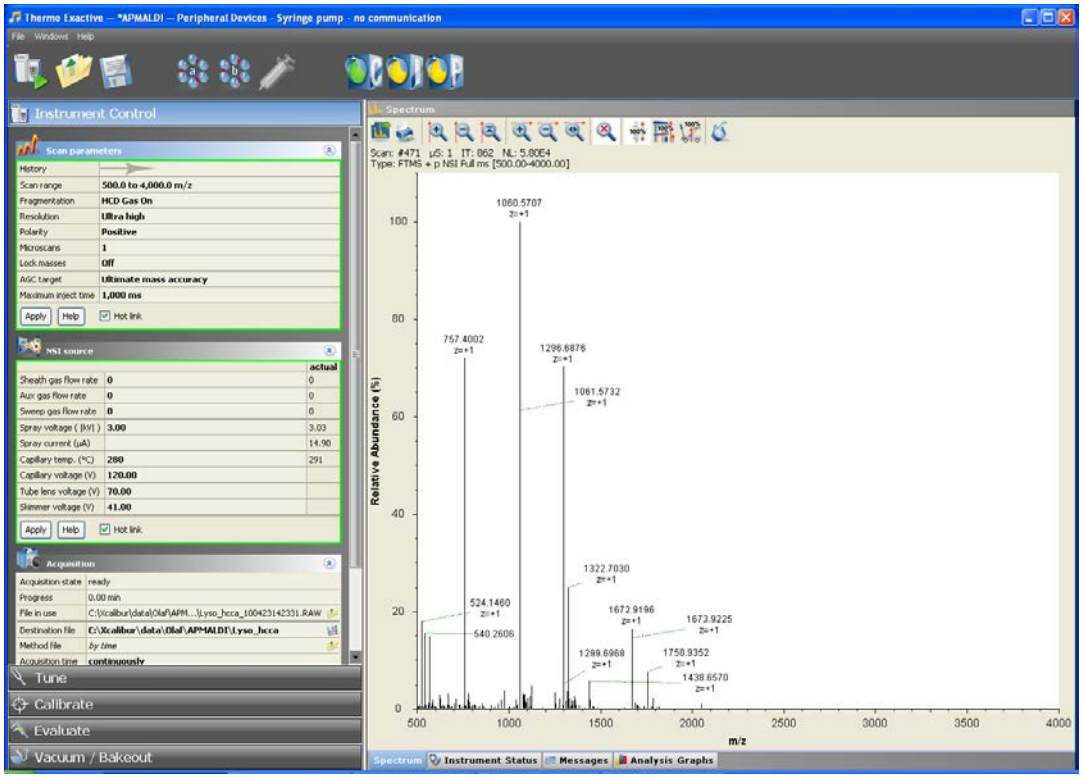


Figure 7.18 Operation of the Target Software

To further enhance the throughput of the Sub-AP/MALDI setup, de-focus the UV laser by readjusting the lens focus by using a micrometer knob on the source's right side. There is a scale on the side of the micrometer vernier (knob) to facilitate this adjustment.

Then increase the laser energy in Target Software so that roughly the same laser fluence (energy/area) is maintained (Figure 7.16). The purpose behind this exercise is to generate more analyte ions per laser shot.

The operator should tune the focusing lens position, laser energy, and frequency while optimizing the MALDI signal intensity for a standard chemical compound (e.g., a tryptic peptide). The Sub-AP/MALDI is sensitive to the laser fluence. If the laser fluence is too high, there can be an increased chemical noise, especially in a low m/z part of the spectral range, 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.

7.6 Sub-AP/MALDI(ng) UHR Operation

1. 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)
2. Spot Shape: For spot shape optimization, both the mirror and the lens may need to be adjusted.
3. Signal: ion counts are typically less than standard Sub-AP/MALDI. The ion count dissipates to zero in 1-2 seconds if the operator remains at the same spot. The spiral motion velocity should be 15 mm/sec. Therefore, by quickly moving the stages/ laser on the sample, on scan obtain similar ion counts.
4. Watch the Injection Time: it may be too high if the operator observes space charge effect (peaks on the MS will appear at higher mass and will be broad).
5. See the Troubleshooting section for fine tune of the laser spot size.

7.7 Manual Mode of Operation

Manual control means that the operator 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 within the Target Software. The spectra acquired will depend on which 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 operator 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 its location in the software).

2. Start the 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).

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.

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" or "Manual Motion Control" arrow buttons in the Target software window while observing the sample on the video capture screen).

7. Save the data acquired (when necessary)by using Xcalibur Software.

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

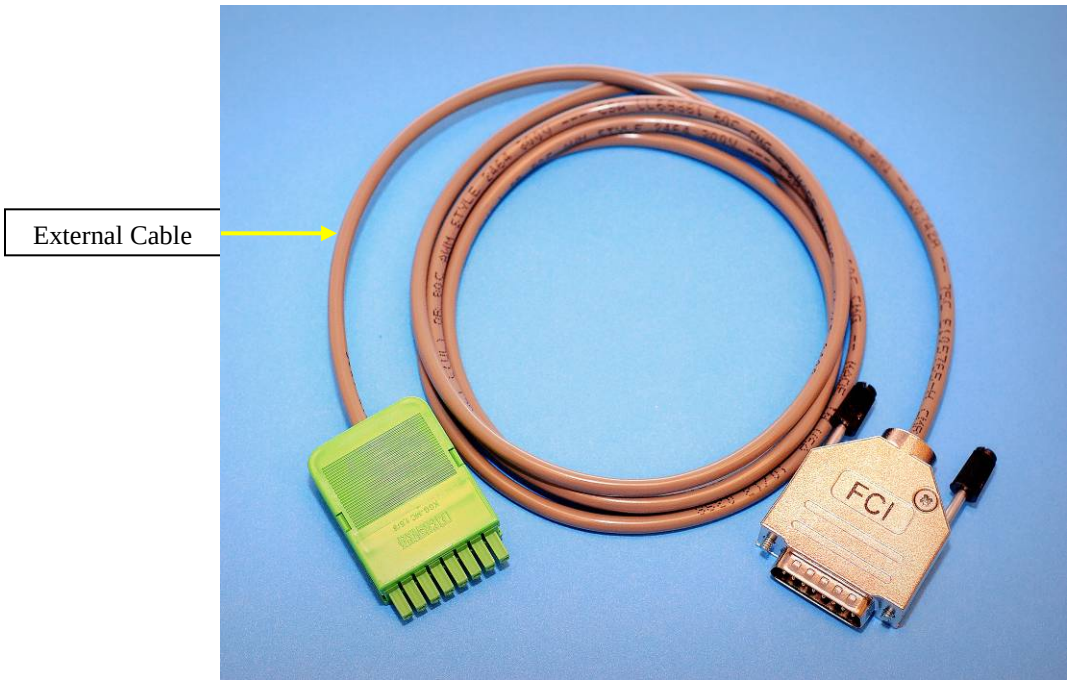
9. Repeat steps 3-8 to acquire one or more spectras from the same (or different) sample.

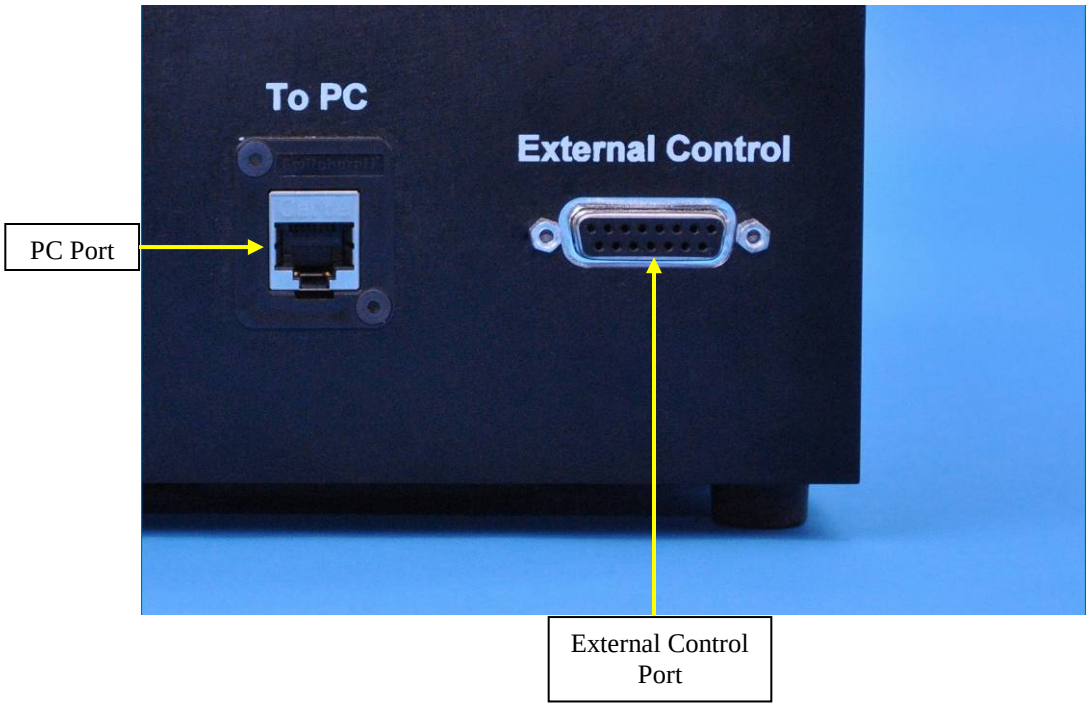
10. Stop data acquisition on the Xcalibur Program.

7.8 Automated Mode of Operation

In the manual mode of operation, described in the previous section, the operator 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 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 Sub-AP/MALDI source operation with the mass spectrometer data acquisition. On the mass spectrometer side, the operator starts the continuous data acquisition/recording. However, on the Sub-AP/MALDI side, the operator 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 operator 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 operator can extract the spectra for the individual samples based on the retention time of the corresponding spectra (during data processing).


External Cable
FCI


To PC
External Control
PC Port
External Control Port

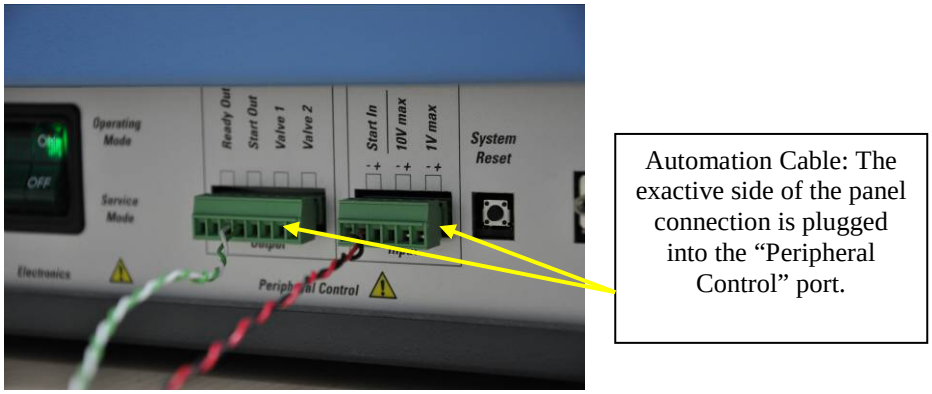


Figure 7.19 External Control Cable and Its Connections

Figure 7.19 shows the external control and its connection to the rare panel of the Sub-AP/MALDI's control unit and to the side panel of exactive. The second automated mode, the external timing, enables the operator 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 Sub-AP/MALDI source through an exchange of the bi-directional "Start" and "Ready" signals. The Sub-AP/MALDI's external control cable connects to the rear panel through using the peripheral control port; the cable and its connection point are shown in the Fig. 7.19.

The selection between the two automation modes is implemented in the "Auto Sequence" tab of the "Settings" dialog box in the Target Software (the dialog is activated through "Settings" menu item); this is shown in Fig. 7.20.

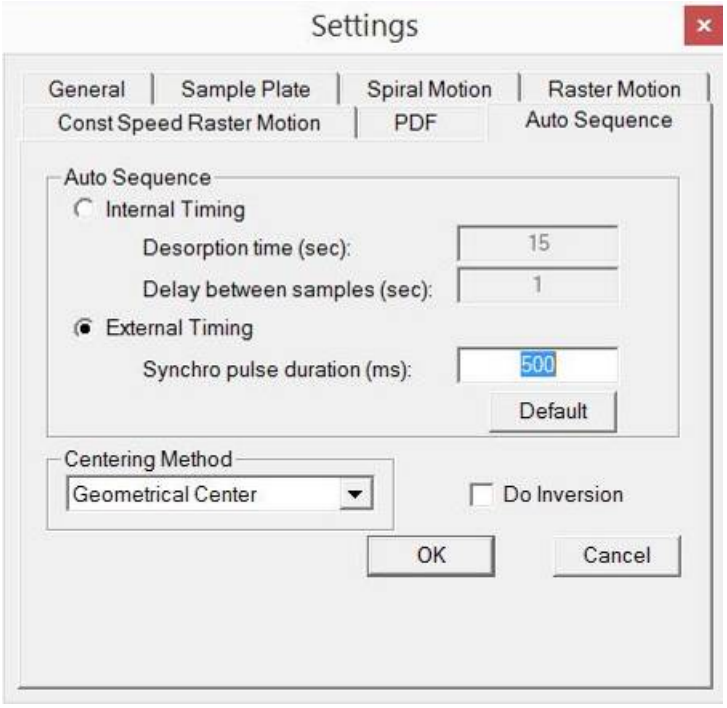


Figure 7.20 Auto Sequence Settings

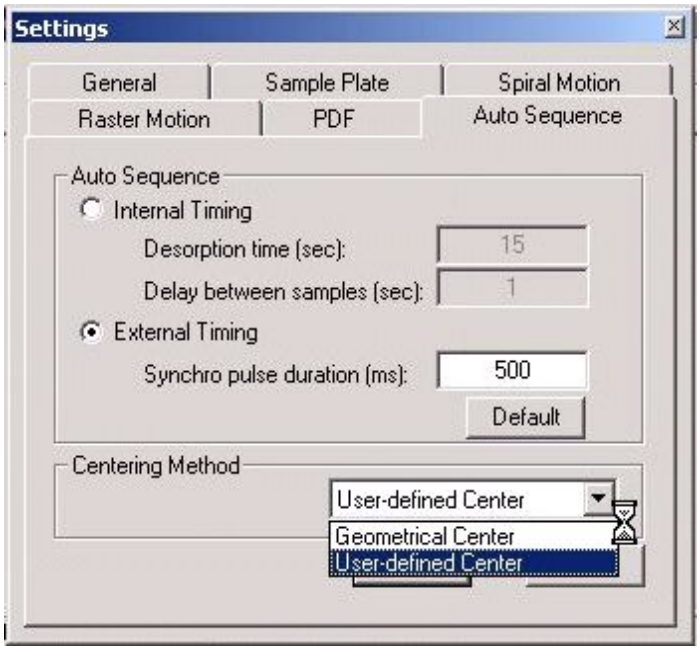


Figure 7.21 Selection of the Sample being Centered

Different modes of the auto sequence mode are available in the software and include geometrical centering (geometry) and user-defined centering (manual), which can be seen in Fig. 7.20. The geometrical centering mode is used when the center of entire sample, which is spotted 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 operator establish the center position of the samples on a target plate. When the operator is manually hand spotting of samples, the user-defined centering mode may be helpful. The user-defined centering mode also allows the operator to tell the Target Software where the center of the sample is for each spot that needs to be analyzed.

The procedure for in the operator to operate the source in the auto sequence mode can be found below:

1. For the external timing mode, connect the "External Control" connector (located on the control unit's rear panel) to the Thermo MS system's Peripheral Controls" port (Fig. 7.19).

2. Check that the "Auto Sequence" button is activated in the main Target Software window (see Fig. 7.12) and that the "External Timing" radio button in the Target's "Settings Dialog" window is activated as well (see Fig. 7.23).

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. 7.20).

    a. For external control, click the "External Timing" radio button; 500 ms in the figure is the appropriate duration of "Start" pulse.

4. Select the desired position(s) on the sample spot selector (map) in the main Target Software window by first using the "Clear All" or "Select All" buttons 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 make multiple selections that can be located at different areas of the target plate, or use the *Shift* button too. 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, the “Start” button continues the data acquisition/recording in Tune-Plus Program of the Xcalibur package. Then, click on the “Play” button in the TARGET Software window to start Sub-AP/MALDI operation.
    a. The data acquisition will continue during the time specified for the spot selected, which is known as the desorption time (Fig. 7.20). When the data acquisition from the first sample is done, the laser firing is automatically stopped (by the software) 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 operator 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 7.11; this can only be used for the Xcalibur Software.**
7. After Xcalibur is properly configured, the operator 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 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.

7.9 The Automated Mode of Operation: Configuring the Xcalibur for the External Timing Mode

I. The Instrument Setup:

1. If the operator is currently running the Tune-Plus Program, close it.
2. Run the Xcalibur/Instrument Setup Program.
3. The operator has the option to create or edit an existing instrument method file. If the operator 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. 7.22 below).

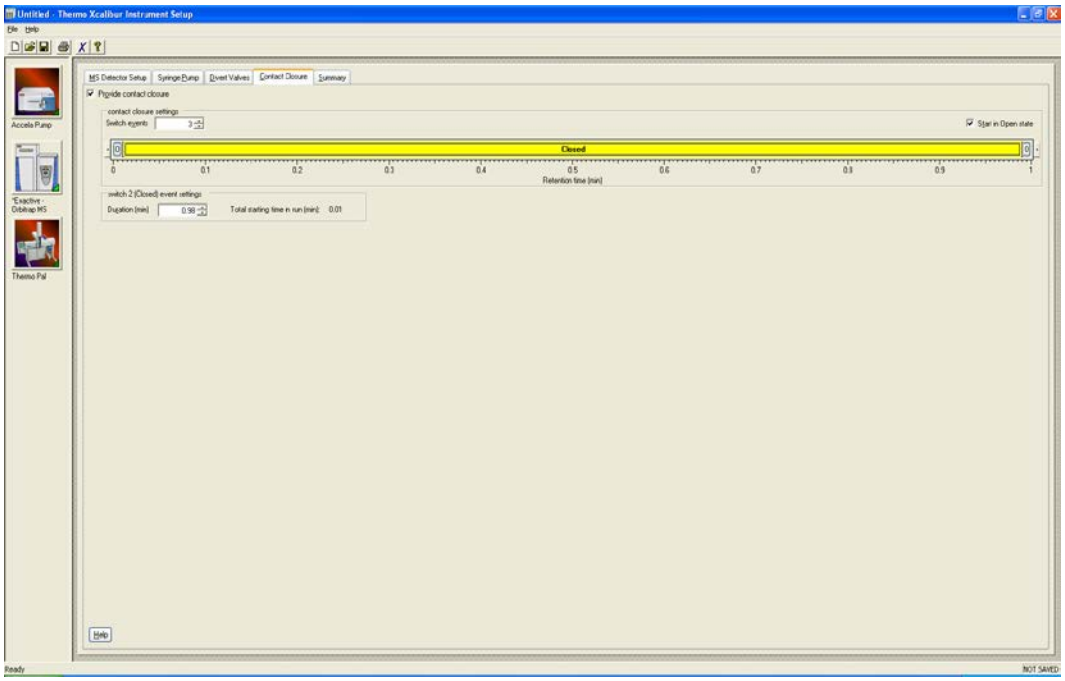


Figure 7.22 Instrument Set Up

Figure 7.22 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. 7.22 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 operator 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. 7.22.

II. The Sequence Setup:

6. Run the Xcalibur/Sequence Setup Program.
7. Create the Xcalibur Sequence or open an existing Xcalibur Sequence file (see the ThermoXcalibur Software Manual for specific details). The number of samples in the sequence setup window table (see Fig. 7.23) should correspond to the total number of samples selected for the analysis.

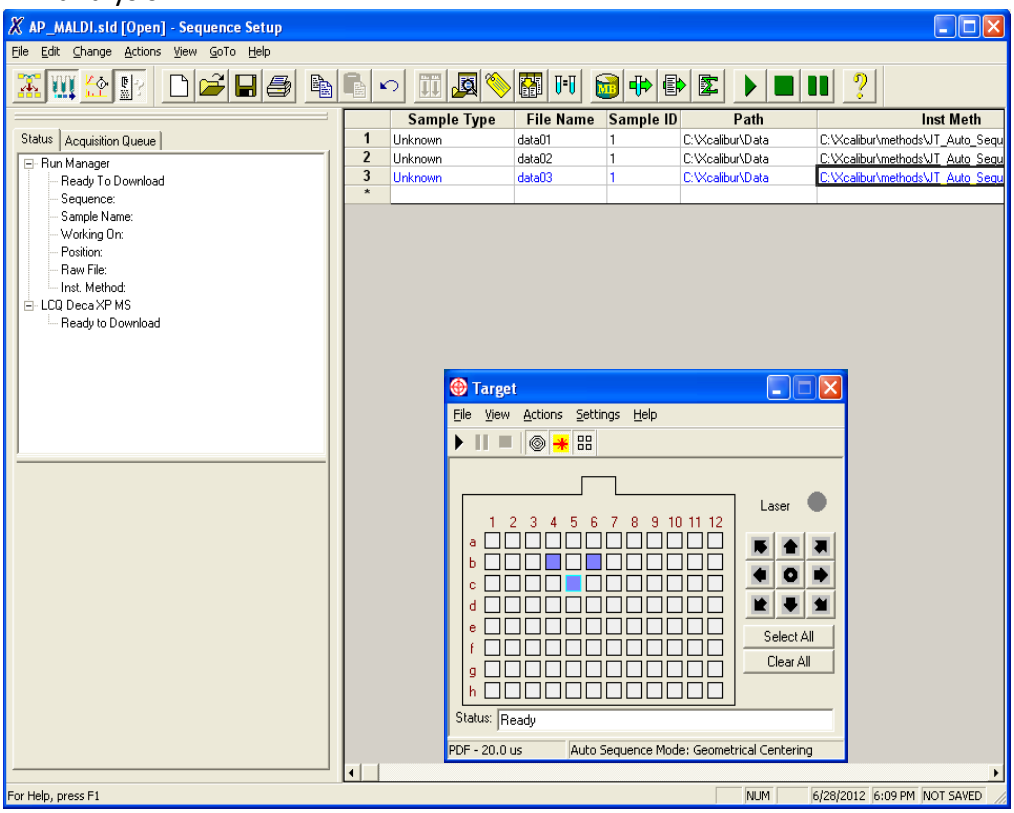


Figure 7.23 Xcalibur/Instrument Setup/Sequence Setup and Target

Figure 7.23 shows the sequence setup that is configured for the external timing and automated Sub-AP/MALDI three 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. 7.23). 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 7.22. 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 7.24 below).

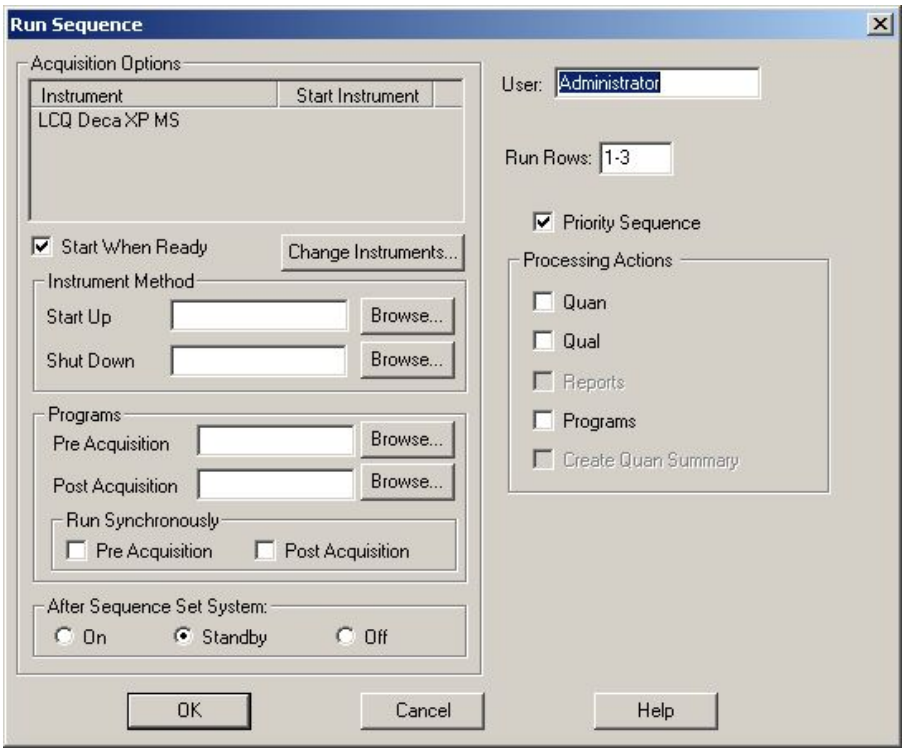


Figure 7.24 Starting the Sequence Run

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. 7.24); 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 operator 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 operator 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. 7.23), 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.

7.10 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 operator 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 operator 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 7.25. Both options have two different scan patterns that are

available for use, which include the flyback and meandering modes displayed in Figure 7.26. 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.

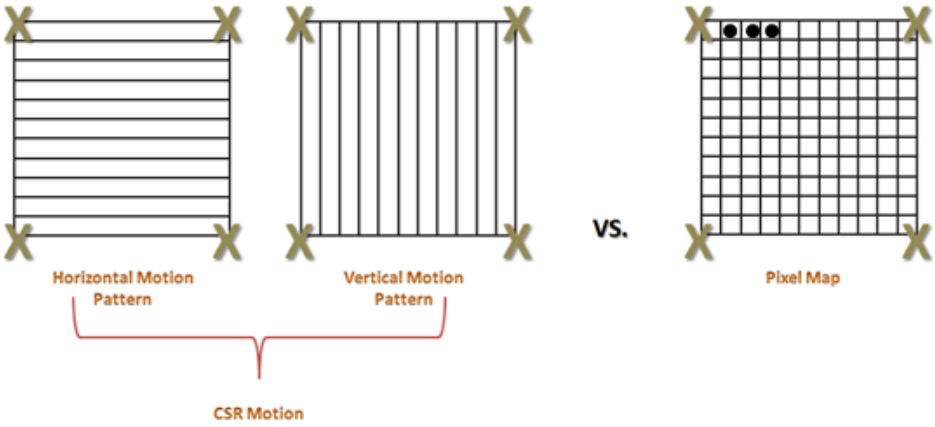


Figure 7.25. CSR Mode vs. Pixel Map Mode

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, the laser only fires within the pixel points.

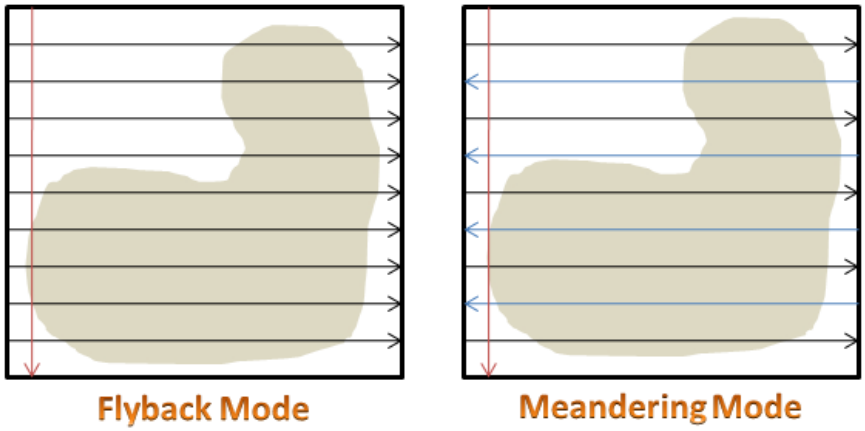


Figure 7.26. Flyback Mode vs. Meandering Mode

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

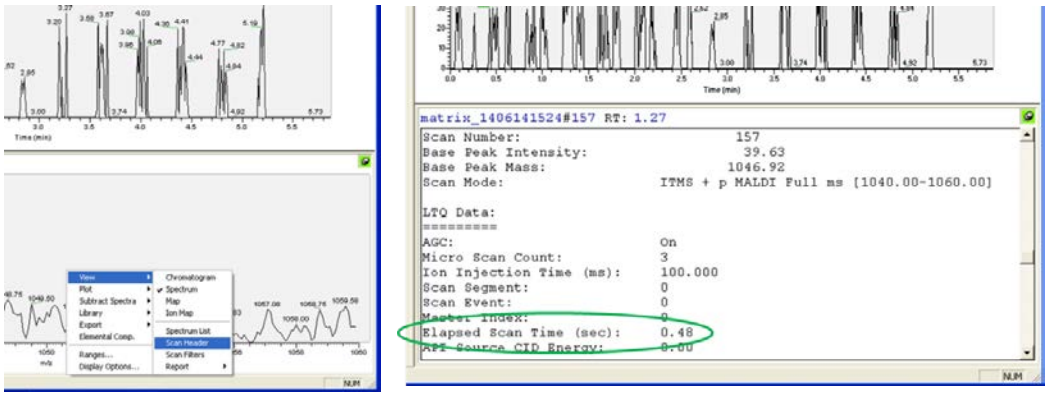


Figure 7.27. Viewing the Scan

    b. Qual Browser- right click to the MS window and select the **View >Scan Header** (the operator needs to look for the "Elapsed Scan Time"-see Figure 7.27).
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.

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 7.28). Each spot should be approximately 1 mm (in distance) from the original point; try to avoid having the samples touch one another.

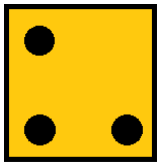


Figure 7.28. End Point Shapes

Creating the Test Data in the CSR Mode

1. Choose the zoom type in the **General** tab (see Figure 7.29):

   a. **Settings>Zoom Parameters>General**

   Zoom Type→CSR

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

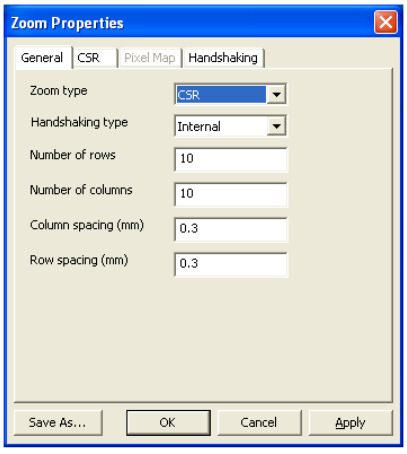


Figure 7.29. CSR –General

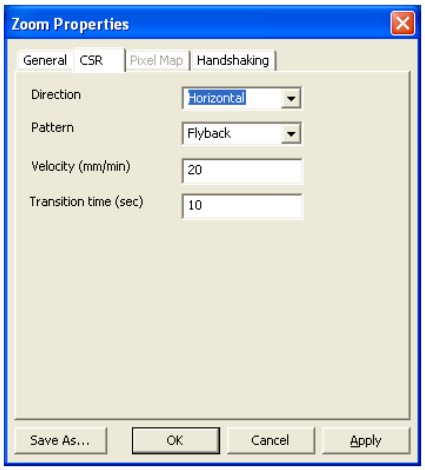


Figure 7.30. CSR Specific Settings

3. Choose the **CSR** tab (see Figure 7.30):

   Direction →Horizontal

   Pattern →Flyback or Meandering

   Velocity (mm/min) →20

   Transition Time (sec) →10

a. The operator 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:

a. Choose the zoom type in the **General** tab (see Figure 7.31): **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 operator must click the **Save As** button in order for the position file to be active; this file is known as the *raster XML file*.

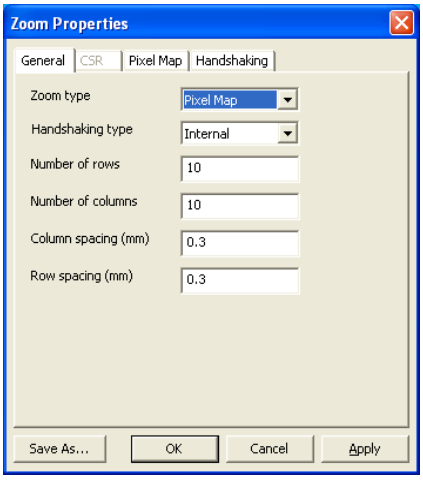


Figure 7.31. Pixel Map General Settings

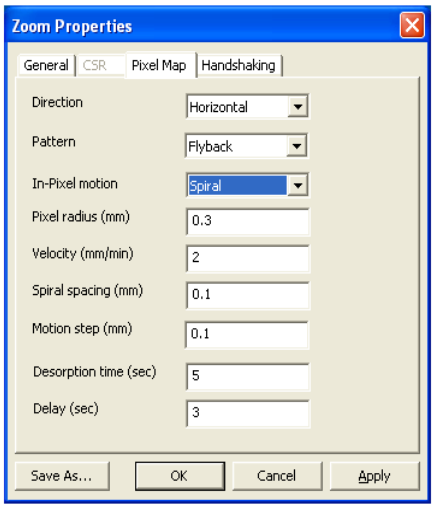


Figure 7.32 Pixel Map Properties

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 7.32 shows a screenshot of the default settings:
    a. The in-pixel motion is typically enabled only when the "pixeled 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.

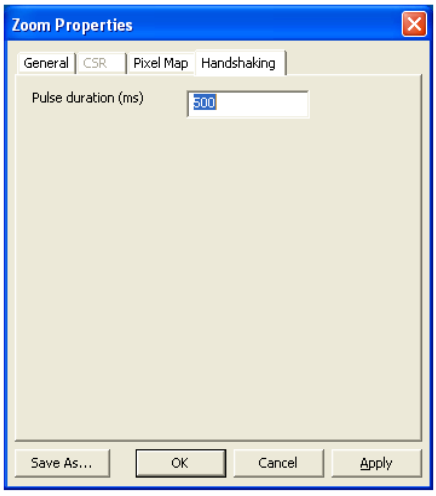


Figure 7.33. Pulse Duration

4. In the **Handshaking** tab, set the **Pulse Duration** to 500 (see Figure 7.33).
    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 operator needs to get the mass spectrometer ready.
3. The Target Software has a calculator that informs the operator 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 7.34):

   In the **Define Scanning** tab (on the mass spectrometer) set the beginning mass range from 1040 – 1060 (**Scan Mode> Define Scan)**

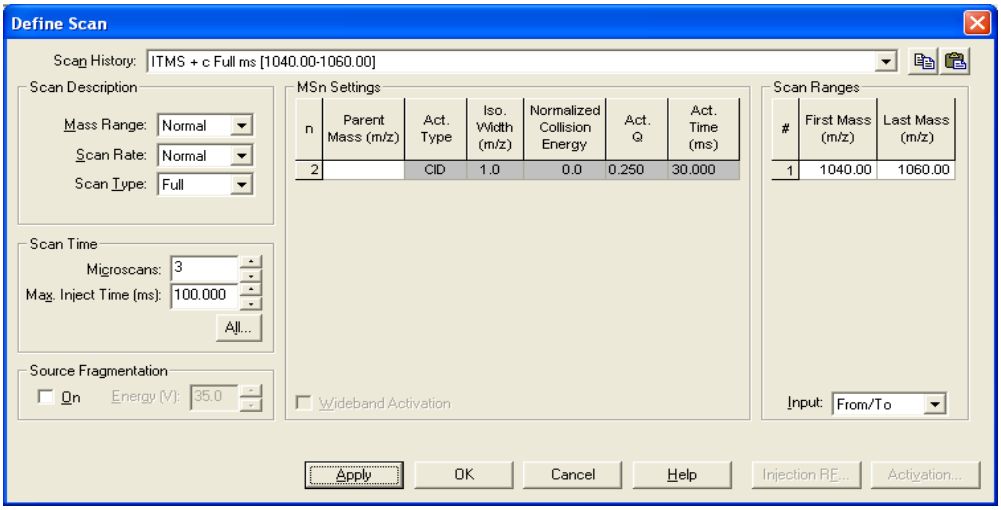


Figure 7.34. Define Scan as shown in XCalibur

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

   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 operator 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 operator needs to ensure identical file names of the raw file and the raster xml file. For example, 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 operator 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:

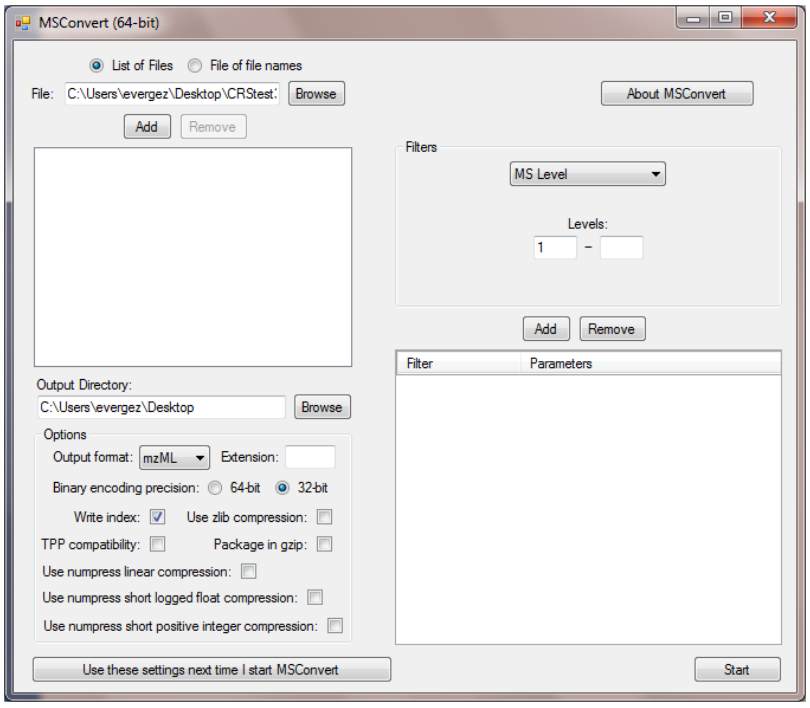


Figure 7.35. MS Convert Settings

- 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 operator should use the settings shown in Figure 7.35 to convert the data file.
2. If successful, the operator 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 operator 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.

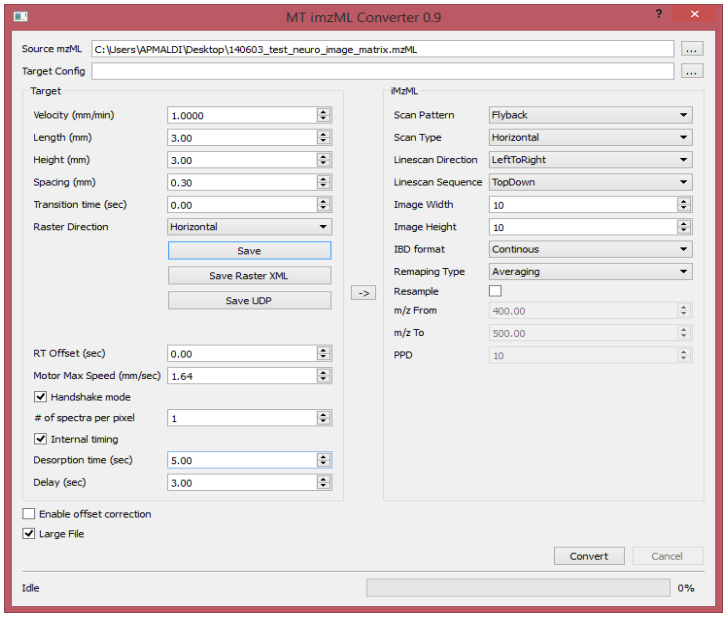


Figure 7.36. The MT imzML Converter 0.9

4. Open the MT imzML Converter- the demo software provided by MassTech. Populate the dialog box with the information as shown in Figure 7.36.
    a. When completed, press the  button to set the figures. Then press the “Convert” button.
5. If successful, the operator will have an imzML data file that is somewhat smaller than the original mzML data file.
6. Next, the operator 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 operator 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.

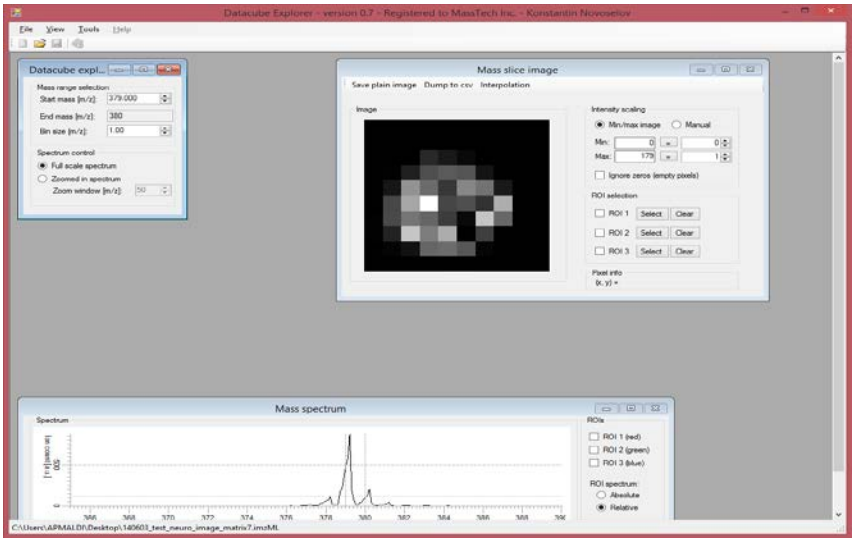


Figure 7.37. Datacube Explorer – Version 0.7

8. By changing the minimum/maximum value and selecting the appropriate m/z interval, the user’s test data should look like in Figure 7.37.
9. Now the zoom mode is ready use. Please contact MassTech Inc. if there are any questions or concerns.

Notes:
- If the operator 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 operator 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 operatorneeds 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

7.11 Running iFunnel software

Turn on the DC RF box. Double click on the iFunnel icon. When the program starts, one must see 2 twinkling green buttons near Tx and Rx. If the source is open (atmospheric pressure inside the source and below pressure reading line, one sees "HV Disabled"), the applications of all the voltages is disabled. If one presses Continuous button, then nothing happens.

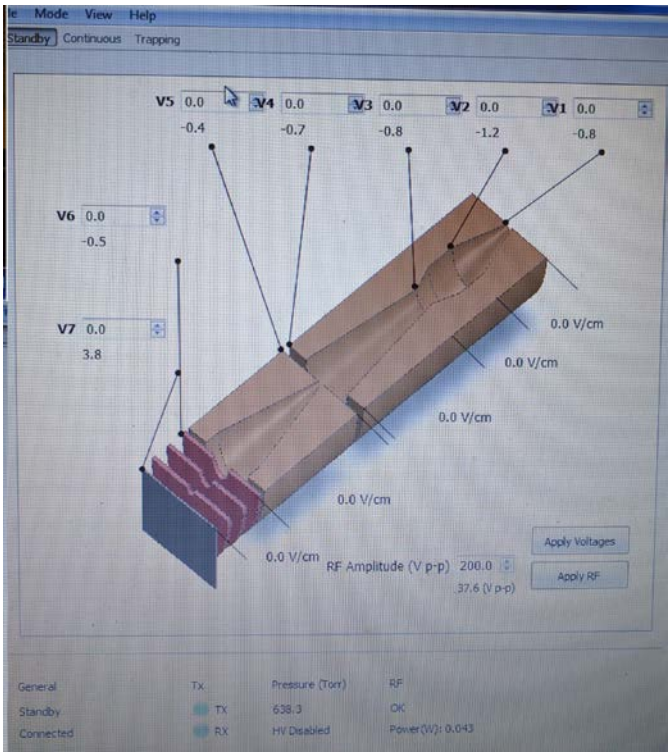


Figure 7.38. Computer screen when the pressure in the source is too high (>20 Torr). Continuous button is disabled and no DC/RF voltages is applied

When the small pump is pumping gas out of the iFunnel, then the pressure goes down and, eventually, the iFunnel Software becomes responsive. Now one may type in some DC and RF values in the corresponding voltage boxes. To apply voltages to the system electrodes one needs to click Apply voltages for DC and Apply RF (for RF) and then click on the "Continuous" button. If one simply clicks the "Continuous" button without typing in, the system chooses the most recently used combination of DC and RF voltages. When operating in the "Continuous" mode, one can change the voltages "on-the-fly" by typing in the new numbers into the small voltage boxes and then click "Apply Voltages" button. The same can be done for the RF voltage.

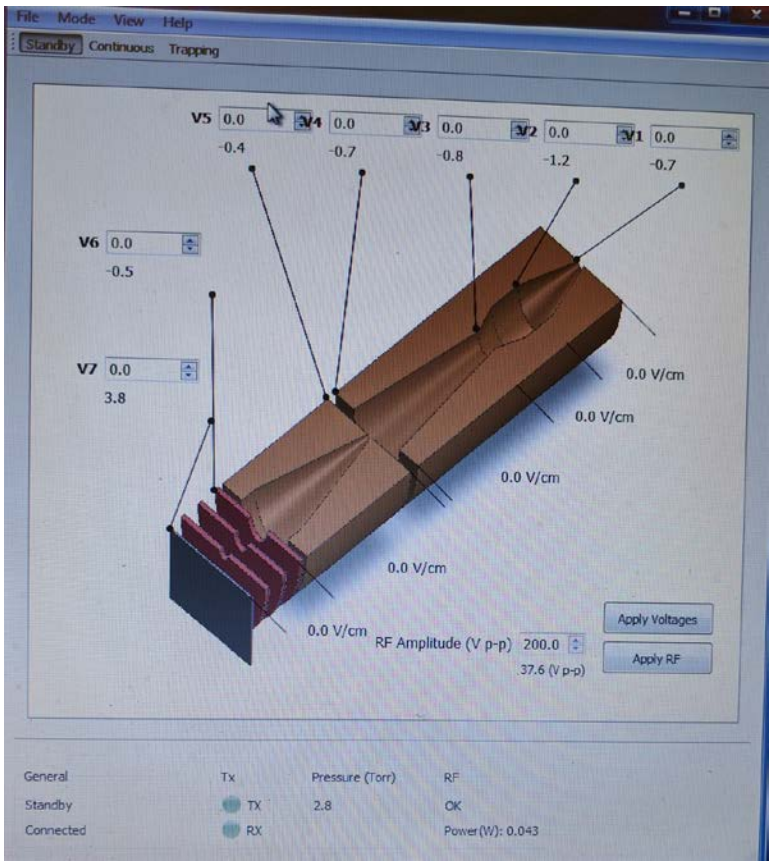


Figure 7.39. Computer screen when the pressure in the source is below 20 Torr. Continuous button is not yet engaged and no DC/RF voltages is applied.

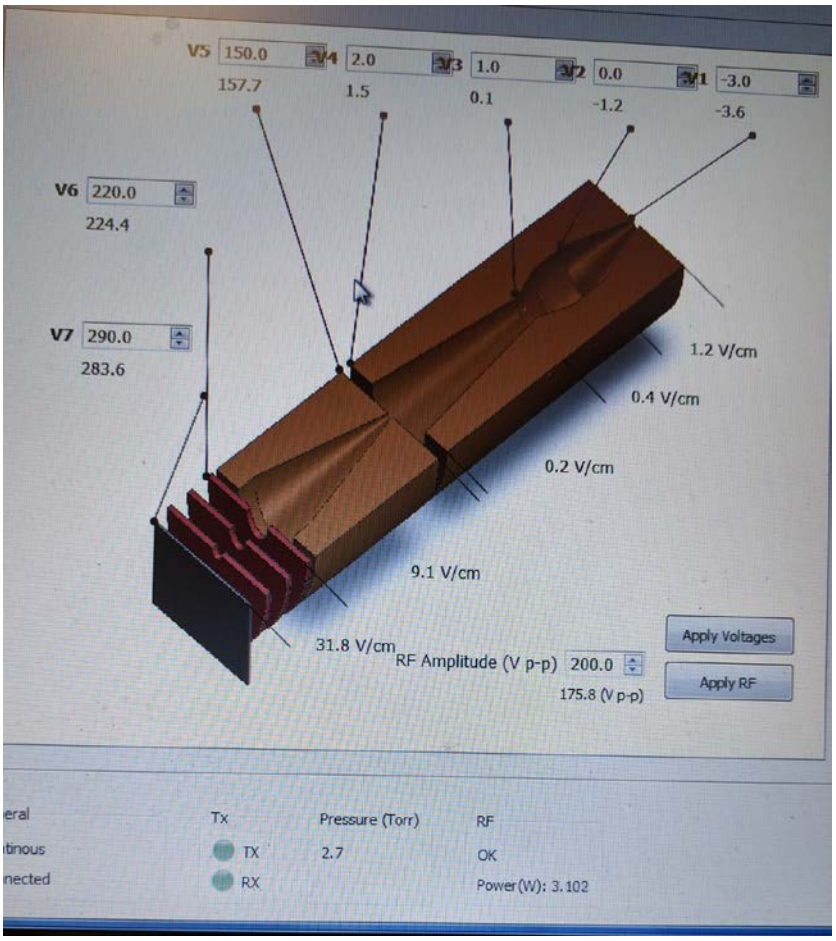


Figure 7.39. Computer screen when the pressure in the source is below 20 Torr. The operator pressed Continuous button and voltages are applied. Below the voltage boxes one can see readback voltages. Along the funnel image, one can see numbers that indicate the electric field strength over funnel sections.

To save current settings for DC and RF one clicks on "File" on the top line, then "Save as", and then types in the file name (see Fig 7.40). To open file with previously saved settings, when operating either in "Standby" or "Continuous" modes, one click on "File", then "Open", and then choose the file to open. One needs to click "Continuous" to start DC/RF box operation with new settings (loading of settings switches the system to Standby mode).

Option "Setting" under the "File" directory also allows one to establish communication with PC. Both Stellaris and RF generator Serial Numbers for any given DC/RF box are written on the internal surface of the DC/RF box top cover. "Trapping mode ejection time" setting is disabled (due to its low efficiency) for QExactive and Velos/LTQ Orbitraps. Though it can be use with stand along Velos or LTQ MS systems. One needs to request this option separately from MassTech Inc when operating stand along LTQ or Velos.

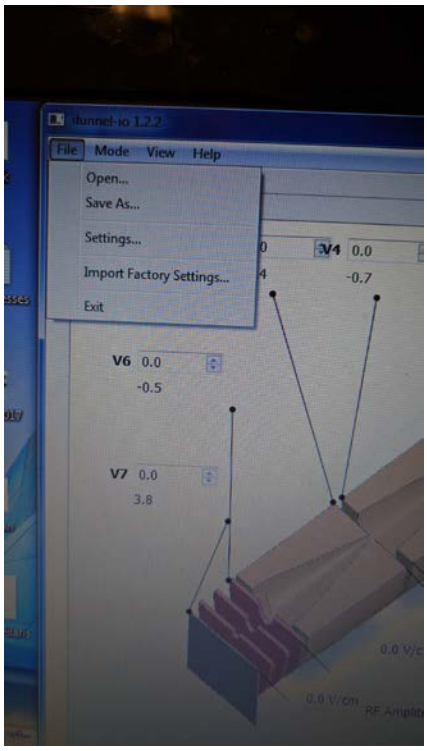


Figure 7.40. When the operator click on "File", several options appear. "Open" allows one to download the set file containing previously recorded voltages. "Save As" allows recording current voltage settings. "Setting" allows one to change RF frequency and establish the DC/RF box communication with PC.

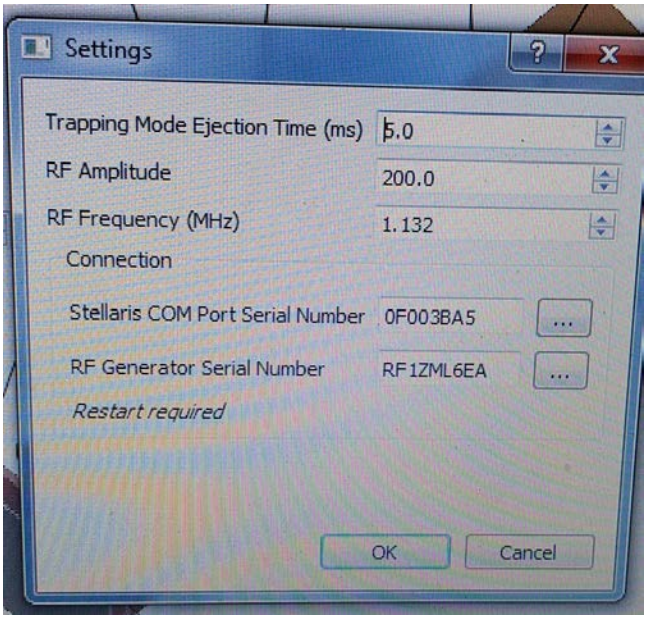


Figure 7.41. Option "Setting" also allows one to establish communication with PC. Stellaris Number and RF generator Serial Number for any given DC/RF box are written on the internal surface of the DC/RF box cover.

8 MAINTENANCE —TROUBLESHOOTING THE SOURCE.

The AP/MALDI ion source does not require regular maintenance.

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(ng) 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.

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 operatorneeds to see if the matrix crystals at that spot are disappearing at maximum (100%) laser level while the laser spot is gradually decreased (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 and tightest laser focusing, then laser power is NOT sufficient.
6. If the laser power is NOT sufficient, the operatorhas three options to fix the problem.

   unit.

i. Try to improve the focus of the laser beam on the target. To do this, adjust the position of the focus micrometer as described in Section 8.2. Make sure the tightest focus is observed within the range of the micrometer dial.

ii. Try to improve the position of the laser beam relative to the MS 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 1: 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 Laser Focus adjusting micrometer on the right side of the source.
2. Set micrometer dial at the maximum (mm) position.
3. Disable the spiral/raster so that the laser light strikes the same spot
4. Start the laser firing at maximum power (not necessarily 99%-See section on AP/MALDI *UHR*)
5. Using the camera, the operator will be able to see how fast the matrix desorbs.
6. If the spot does not desorb quickly, turn micrometer to get dial at down 1 or 2 mm less and repeat the experiment.
7. Once the operator sees that the matrix is desorbed in less than a minute, the focus position may be tuned with a smaller (0.3mm) dial step to get better focusing.

The above focusing procedure may be good enough for general use, typically associated with the HR source (25-100 µm laser spot size). To get smaller laser spot (down to 10 µm) typically required for UHR performance additional tune-up efforts are required as described below.

Goal 2: To focus the laser spot size to about 10 µm size.
Note: a microscope may be necessary to use in this focusing optimization procedure to see the laser burn marks if a laser spot size as small as 10 µm is desirable.

1. Once the alignment procedure described in Section 8.3 below is completed, optimization of the laser focusing requires additional effort to ensure minimum laser spot size.

2. To ensure this, a special matrix solution is prepared (CHCA 4 mg/mL in Acetone). 3 µL of this solution is smeared to an area on a clean AP-MALDI plate: 3 by 20 mm wide. It should dry immediately. You can do this at 50 °C hotplate to quicker evaporation.
   Tip: A Sharpie® permanent ink pen can be used instead of the special matrix solution above in this focusing optimization procedure (it has slightly low laser threshold for ink evaporation so the laser energy should be readjusted at the end of this procedure to match the matrix threshold).
3. Locate portions of the matrix that is uniform and thin.
4. Set Laser Energy to minimum level where laser burn is noticeable.
5. Set up a 3x5 pixel map run with 50 µm spacing in the Target software Zoom mode. Try to move the lens adjustment micrometer by steps of 25 units (250 µm). At each step, run the pixelmap.
6. Observe the change of shape in the laser burn. Note/record the position.
7. Determine the first range of observable burn marks by looking at the best lens positions.
8. Put the lens position at the middle of the first range. Reduce the laser energy to the minimum when the laser burn is still observable. Repeat steps 5-7 to determine a new lens range. Repeat steps 5-7 until the range is made as small as 50 units (500 µm).
9. At this new range of 50 units, move the lens adjustment by steps of 10 units (100 µm).
10. Observe the change of shape in the laser burn. Note/record the position.
11. Reduce the laser energy by 10% and repeat steps 9 and 10.
12. Determine a new range by looking at the best 3 lens positions.
13. At this new range of 30 units, reduce the laser energy again and move the lens adjustment by steps of 5 units (50 µm).
14. Observe the change of shape in the laser burn. Note/record the position.
15. If necessary, repeat steps 12-14. The goal is to find a minimum laser energy and lens position corresponding to the best focusing conditions within 3-5 units (30-50 µm) of the laser lens micrometer. At minimal laser energies, the laser burn spots should look like that at the top picture below.

Tip: Alternatively, the pixelmap run can be done by 10x5 pixels and you can move the lens adjustment at each row.

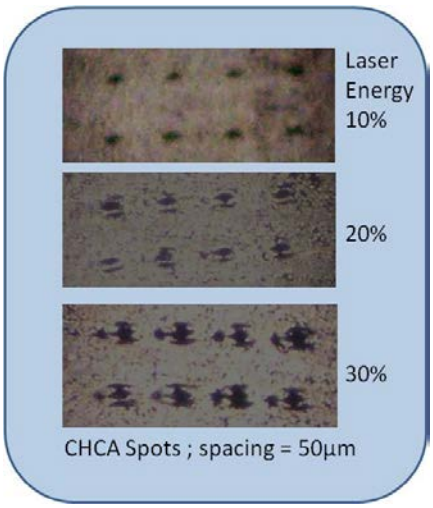


Figure 8.1. Laser burns at the CHCA matrix at various laser energy levels

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 operator 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 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: Spectral Response with High Background and Mass-Shifted Ion Peaks

Sometimes the AP/MALDI allows too many ions into the ion trap so that the space-charge effects can occur in the trap. It may be necessary to reduce the injection time of the ion trap to 100ms or less 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. Moskovets E., Misharin A., Laiko V., Doroshenko V. “A comparative study on the analytical utility of atmospheric and low-pressure MALDI sources for the mass spectrometric characterization of peptides” *Methods*. 2016; **104**:21-32
2. Gillig K.J., Ruotolo B., Stone E.G., Russell D.H., Fuhrer K., Gonin M., Schultz A.J. Coupling high-pressure MALDI with ion mobility/ orthogonal time-of-flight mass spectrometry. *Anal Chem*. 2000; **72**(17):3965-71.

ADDITIONAL REFERENCES

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.

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

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. 2462-2469Miller 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(ng) 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) 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: SPECIFICATIONS FOR SUB-AP/MALDI(NG) HR AND SUB-AP/MALDI(NG) UHR ION SOURCES

Type of ionization	Sub-atmospheric pressure (Sub-AP) matrix-assisted laser/desorption ionization (MALDI)
Laser wavelength	355 nm (Nd:YAG DPSS laser)
Laser repetition rate	1-10 kHz
Laser energy	<3 μJ/pulse
Minimum laser spot size	AP/MALDI(ng) HR: 25-30 μm AP/MALDI(ng) UHR: ~10 μm
Maximum laser spot size	50-100 μm (depending on matrix)
Limit of detection	1 femtomole/sample (Bradykinin) (typically)
Mass range	Limited by mass spectrometer
Available sample plate formats	Sciex Opti-TOF (192 spots) Agilent/MassTech (96 spots) 25-mm wide microscope slides
X stage travel distance	55 mm
Y stage travel distance	40 mm
Accuracy of X-Y stages	<10 μm
Modes of operation	Manual Automated (for multiple spots) Pattern (for MS imaging)
Sample monitoring	CCD camera
Available for MS manufacturers	Agilent Technologies Bruker Daltonics SCIEX Thermo Fisher Scientific
Interchangeability	Interchangeable with ESI, APCI and other AP sources within minutes
Synchronization with MS	LC protocol (Contact Closure)
Control software platform	Windows 7
Control Interface	Ethernet RJ-45 or USB
Dimensions	33cm(L)x17cm(H)x18cm(W)
Weight	6 kg
Power	95-240 VAC, 80 W

APPENDIX B: WARNING AND IDENTIFICATION LABELS

Danger – Laser Inside label (on the front panel)

Laser Class 1 product label (on the front panel)

Hot temperature sign (on the top door)

High Voltage sign (on the top panel)

Attention sign (on the top panel)

Serial number label (at the bottom)

CE sign (at the bottom)