

**Agilent G1974A
PDF-MALDI Source for
LC/MSD Trap and TOF
Systems**

Installation Guide



Agilent Technologies

Notices

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WARNING

A **WARNING** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a **WARNING** notice until the indicated conditions are fully understood and met.

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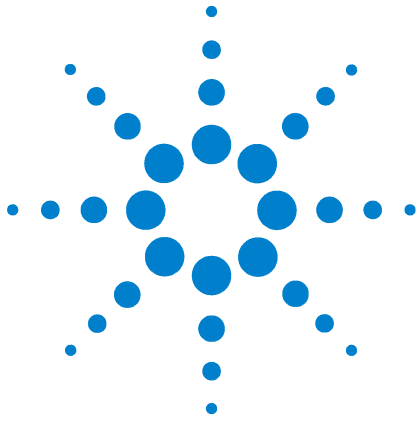
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This chapter contains instructions for the initial installation, configuration, and verification of the PDF-MALDI Ion Source on the LC/MSD SL Trap, XCT Trap, and XCT Plus Trap systems. For subsequent installations, you can skip steps in the procedure as noted.

Installation

This step describes how to install the PDF-MALDI Ion Source on LC/MSD SL Trap, XCT Trap, and XCT Plus Trap systems. It also describes how to install the Target 5.0 software on LC/MSD SL, LC/MSD XCT, and LC/MSD XCT Plus system control computers.

Step 1. Prepare to install the Source

You do this only the first time the Source is installed. If this is a subsequent installation, skip to “[Step 5. Install the Source](#)” on page 19.

- 1 Check the calibration of the LC/MSD Ion Trap. All the tuning mass ions must have the correct mass assignments and acceptable peak widths. You should also check the electron multiplier voltage (EMV).
 - **For LC/MSD SL Trap systems** - If the accumulation time is greater than 0.2 ms with an ICC setting of 50,000, clean the capillary, skimmer 1, and octopole 1. After cleaning the ion optics components, the detector voltage should be recalibrated. Rerun the scan calibration to confirm that the mass assignments and peak widths are correct.
 - **For LC/MSD XCT Trap systems** - If the accumulation time is greater than 0.2 ms with a smart ICC setting of 125,000, clean the capillary, skimmer 1, and octopole 1. After cleaning the ion optics components, the detector voltage should be recalibrated. Rerun the scan calibration to confirm that the mass assignments and peak widths are correct.
 - **For LC/MSD XCT Plus Trap systems** - If the accumulation time is greater than 0.2 ms with a smart ICC setting of 500,000, clean the capillary, skimmer 1, and octopole 1. After cleaning the ion optics components, the detector voltage should be recalibrated. Rerun the scan calibration to confirm that the mass assignments and peak widths are correct.
 - **For All LC/MSD Trap systems** - The vacuum and helium conditions are correct. The high vacuum pressure reading should be less than 2.0×10^{-5} mtorr or 2.5×10^{-5} mbar (depending on the pressure units you use). The helium differential pressure reading should be 0.6×10^{-5} mbar less than the high vacuum reading. If the helium pressure is not set correctly, you will experience a loss of sensitivity.

After you clean the system, check the mass calibration again for proper mass assignments, accumulation times, and EMV settings.

2 Check that you have received all components:

- Control Unit - [Figure 1](#).
- PDF-MALDI Ion Source shown from various angles. - See [Figures 2](#) and [3](#).
- Capillary Extension - [Figure 4](#).
- Heating Sleeve - [Figure 4](#).
- Centering Bushing - [Figure 4](#).
- Wave Disk Spring - [Figure 4](#).
- APPI internal cable - [Figure 5](#).
- USB cable to connect Control Unit to computer - [Figure 6](#).
- cable to connect Control Unit to Source - [Figure 7](#).
- Laser Synchronization cable - [Figure 8](#).
- video cable for CDD camera - [Figure 9](#).
- video capture board components - [Figures 10](#), [11](#), and [12](#).
- UV-grade fiber optic cable with SMA-connectors - [Figure 13](#).
- Laser Alignment Tool - [Figure 14](#)



Figure 1 Control Unit

1 Installation for LC/MSD Trap Systems

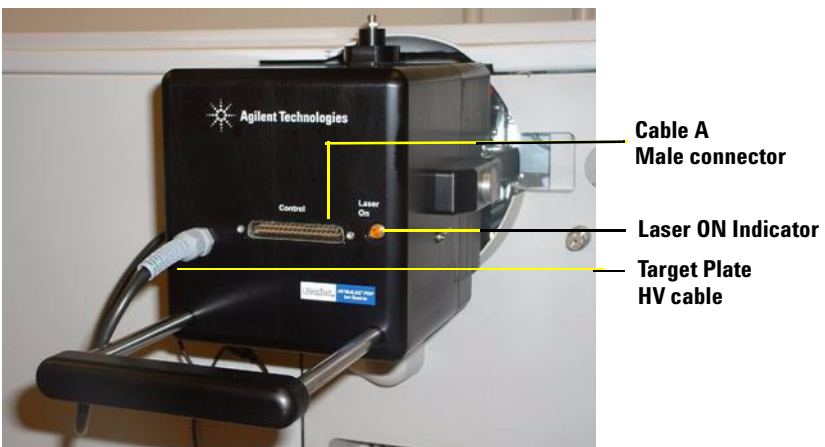


Figure 2 PDF-MALDI Ion Source

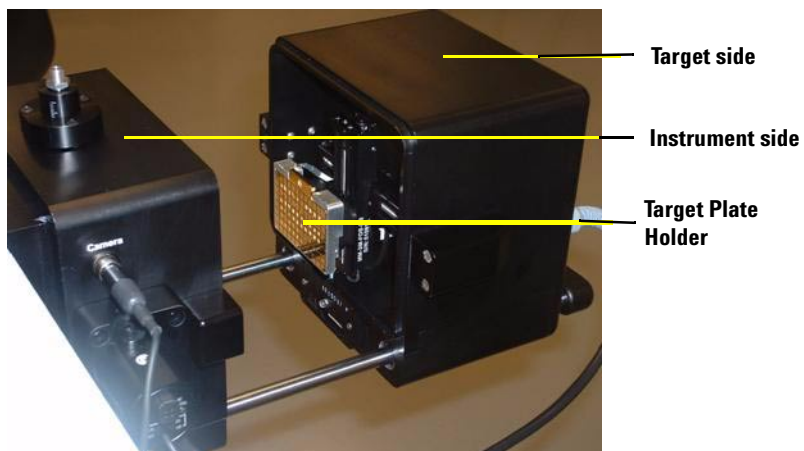


Figure 3 PDF-MALDI Ion Source open to show the 96-spot target plate

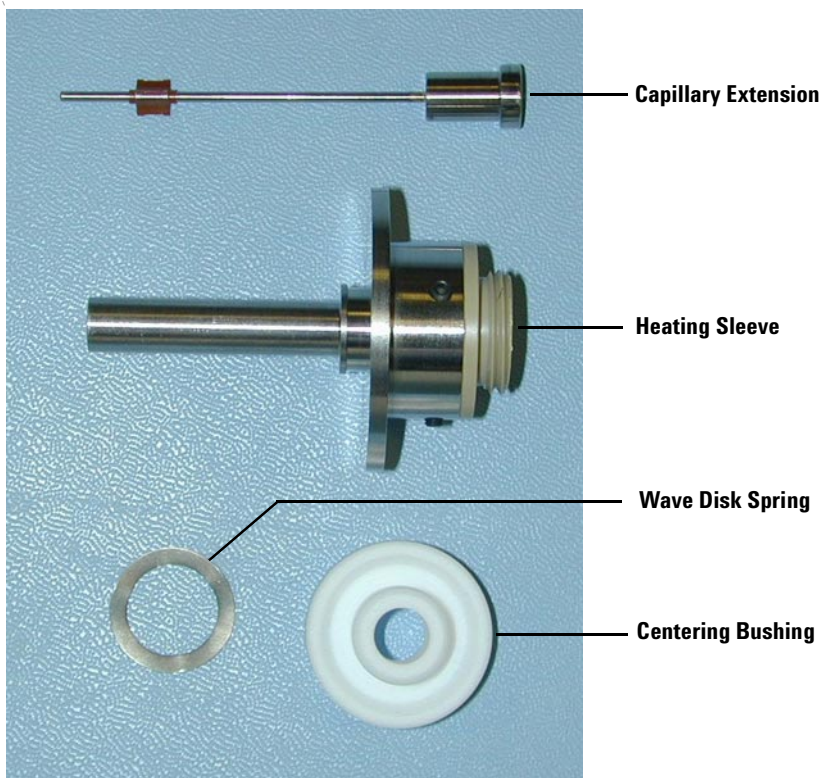


Figure 4 Capillary Extension components

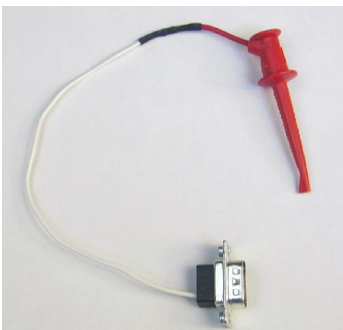


Figure 5 APPI Internal cable

1 Installation for LC/MSD Trap Systems

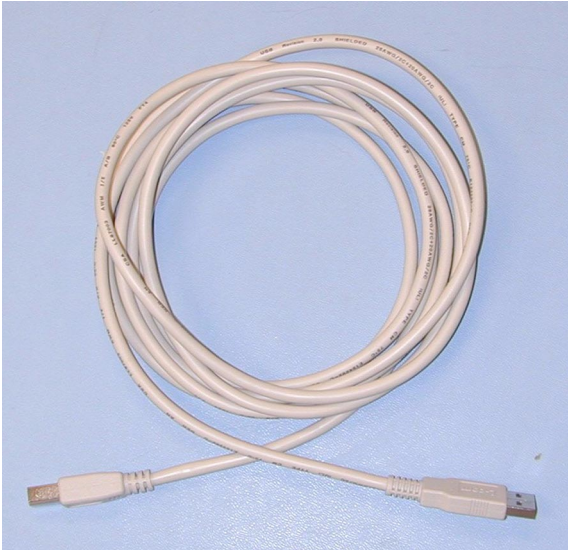


Figure 6 USB cable for Connecting Control Computer to Target Stage/Laser Control Unit



Figure 7 Cable for connecting Control unit to Source

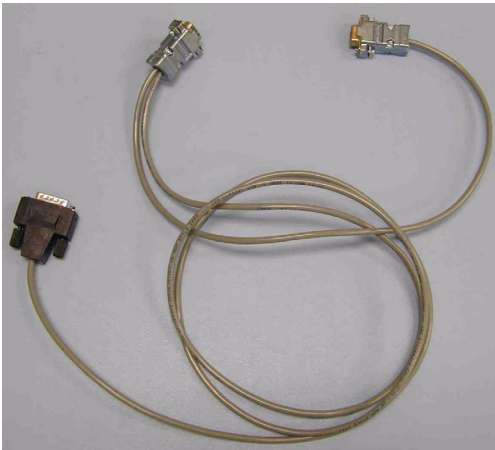


Figure 8 Laser Synchronization cable



Figure 9 Video cable for CCD Video camera



Figure 10 Video Capture Board box

1 Installation for LC/MSD Trap Systems

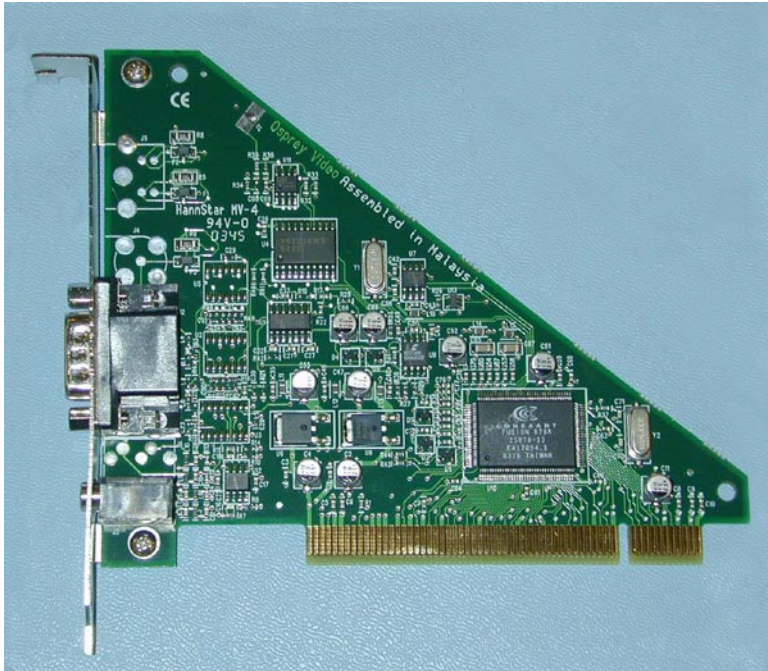


Figure 11 Video Capture Board

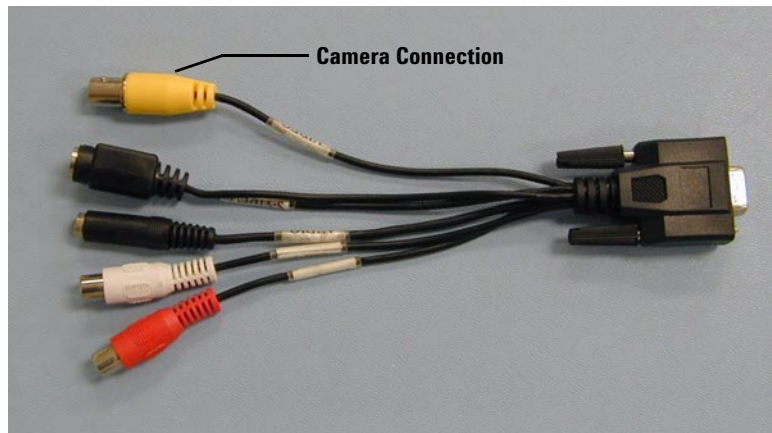


Figure 12 Video Chapter Board cable

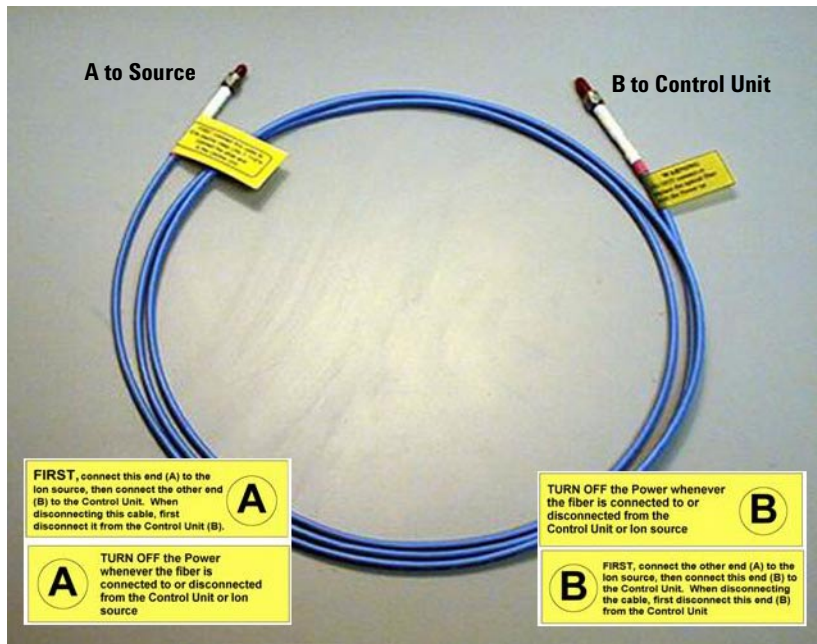


Figure 13 UV-grade fiber optic cable with SMA-connectors. Connectors are labeled on both sides and are covered with protective plastic caps. The shipment includes one spare fiber optic cable (not shown here).



Figure 14 Laser Alignment Tool

Step 2. Connect the APPI Internal cable

In this step, you connect the APPI Internal cable (p/n G2440-60083). You do this only the first time the Source is installed. If this is a subsequent installation, skip to “[Step 5. Install the Source](#)” on page 19.

WARNING

This step should only be performed by an Agilent service technician.

- 1 Remove the electronics tub cover. This will turn off the power to the electronics tub.
- 2 Use a pair of needle-nose pliers to remove the sheet metal punch-out located on the hole labeled **AUX** on the side of sheet metal.

You can also fasten a bolt to remove the metal punch-out. See [Figure 15](#).

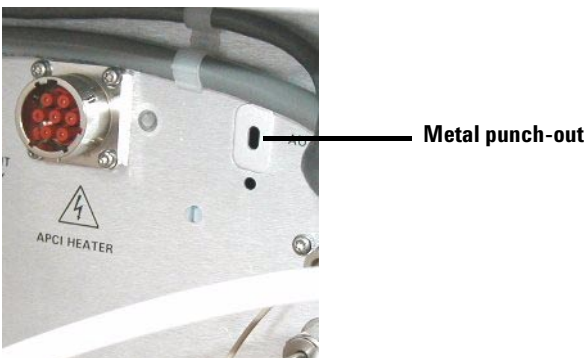


Figure 15 Sheet metal punch-out

- 3 From inside of the electronics tub, insert the 9-pin end of the APPI Connector into the hole labeled **AUX Port** and fasten the connector to the sheet metal using the two screws provided in the kit.

- Carefully fasten the other end of the cable (red clip) to the port that is labeled **X303** on the GELV board (Lens Voltage Board). See [Figure 16](#).

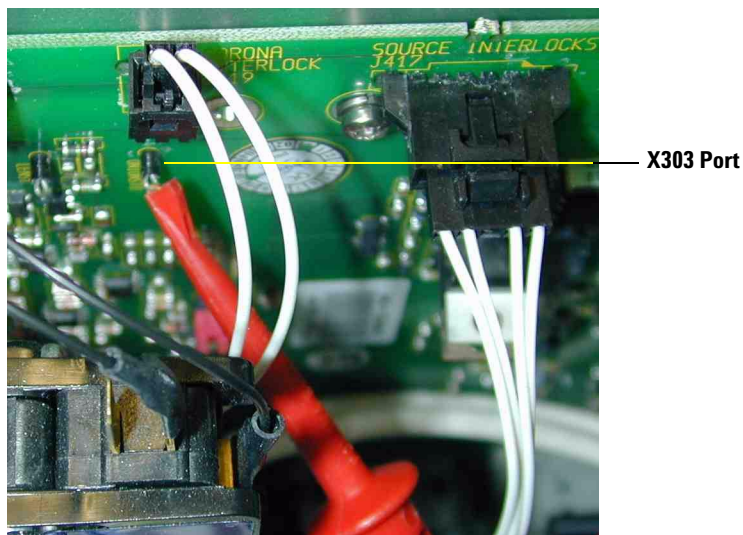


Figure 16 Connect the APPI cable to the X303 GELV board

Step 3. Install the Video Capture Board software

Install the Osprey drivers

- Using Windows Explorer, locate and access the CD-ROM drive containing the Osprey Installation CD-ROM.
- Navigate to the WIN2000 directory or to WinXP_VfW depending on the operating system you are using.
- Double-click **SETUP.EXE**.

The Osprey Multimedia Capture Driver window displays. See [Figure 17](#).

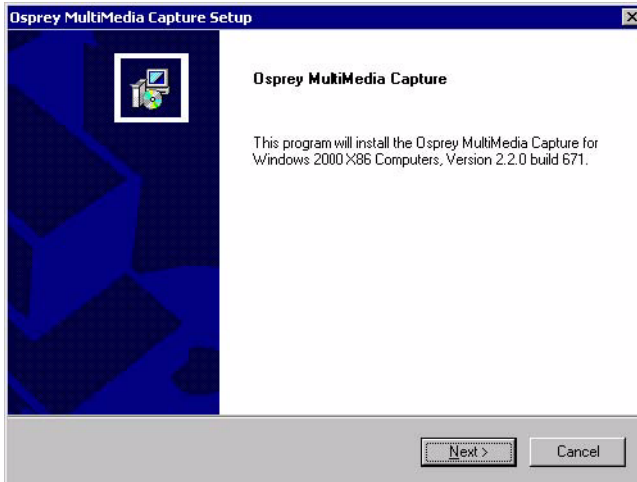


Figure 17 Video Capture Driver window

4 Click Next.

The Information window displays. See Figure 18.

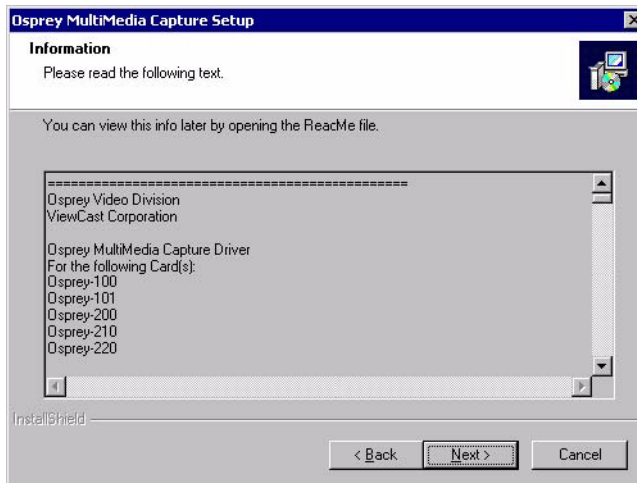


Figure 18 Information window

5 Click Next.

The Software License Agreement window displays. See [Figure 19](#).

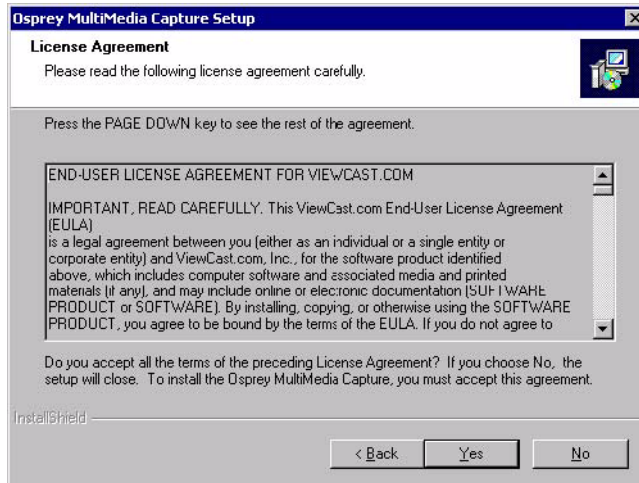


Figure 19 Software License Agreement window

6 Click Yes to accept the End User Software Agreement. If you do not wish to accept the agreement, click No to terminate the installation routine.

The Select Components window displays. See [Figure 20](#).

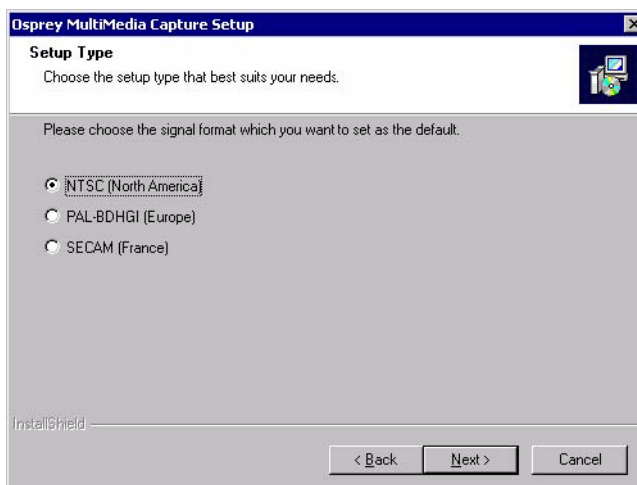


Figure 20 Select Components window

- 7 To set this default, select the video signal standard NTSC and click **Next**. See Video Standard for more information about signal formats.

The Choose Destination Location window displays. See [Figure 21](#).

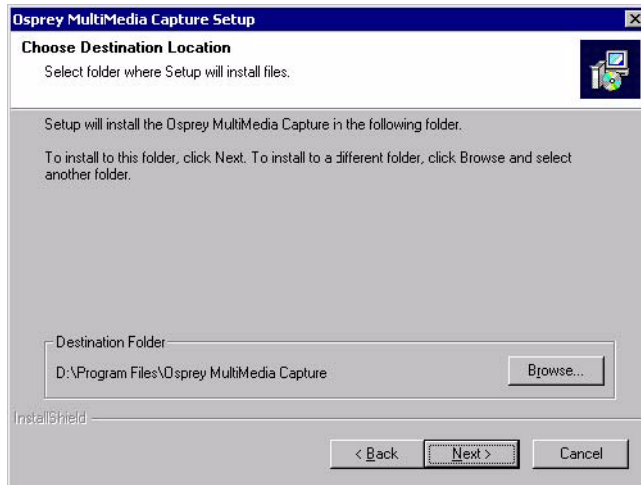


Figure 21 Choose Destination Location window

- 8 If you wish to change the destination location for the files, click **Browse**. Click **Next**.

The Select Program Folder window displays. See [Figure 22](#).

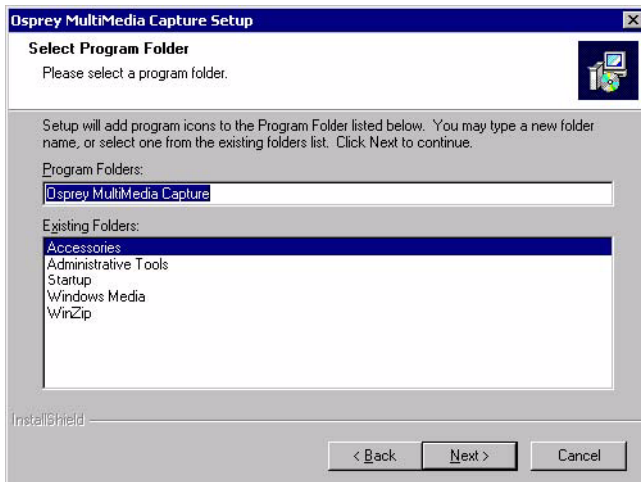


Figure 22 Select Program Folder window

- 9 Click **Next**. If you wish to change the program folder, type the new name in the Program Folders field.

The Start Copying Files window displays. See [Figure 23](#).

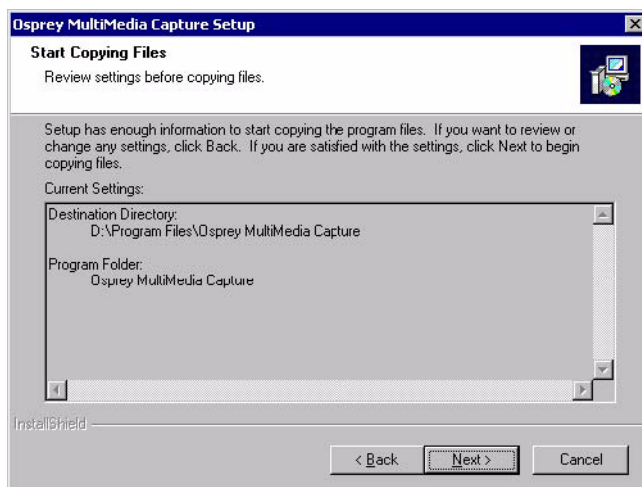


Figure 23 Start Copying Files window

- 10 Click **Next**.

The Ligos® Indeo® Information window displays. See [Figure 24](#).

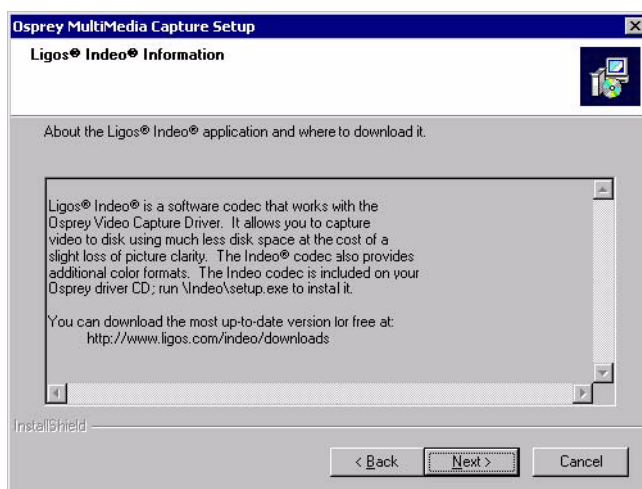


Figure 24 Ligos Indeo Information window

- 11 Click **Next**. If there are any settings to be changed prior to installation, click **Back** to return to the previous windows.

The Digital Signature Not Found window displays. See [Figure 25](#).

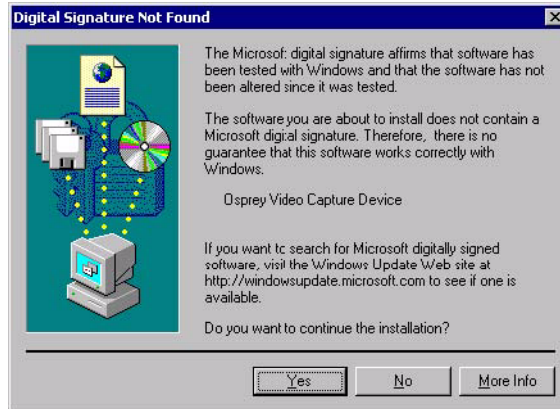


Figure 25 Digital Signature Not Found window

NOTE

Windows 2000 recognizes the audio and video portions of the Osprey cards as separate items.

- 12 Click **Yes** to continue the installation process.

The Osprey Technologies Special Offers Shortcut window displays. See [Figure 26](#).



Figure 26 Osprey Technologies Special Offers Shortcut window

- 13 Install a shortcut.

If you would like to install a shortcut on your desktop,

Click **Yes** and a shortcut is created on the desktop.

If you would not like to install a shortcut on your desktop,

Click **No**. A **Special Offers** link is also available on the **Programs** menu.

An Information window displays. See [Figure 27](#).

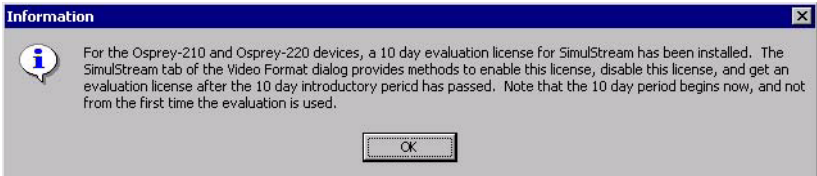


Figure 27 Information window

14 Click **OK** to continue the installation.

The Product Registration window displays. See [Figure 28](#).



Figure 28 Product Registration window

15 Register your Osprey Multimedia Capture card.

If you want to register your Osprey Multimedia Capture card,

Click **Yes**. A browser window opens.

If you do not want to register your Osprey Multimedia Capture card,

Click **No**. A product registration link is also available on the Programs menu or on the Osprey Video web site (<http://www.ospreyvideo.com/>)

The Setup Complete window displays. See [Figure 29](#).

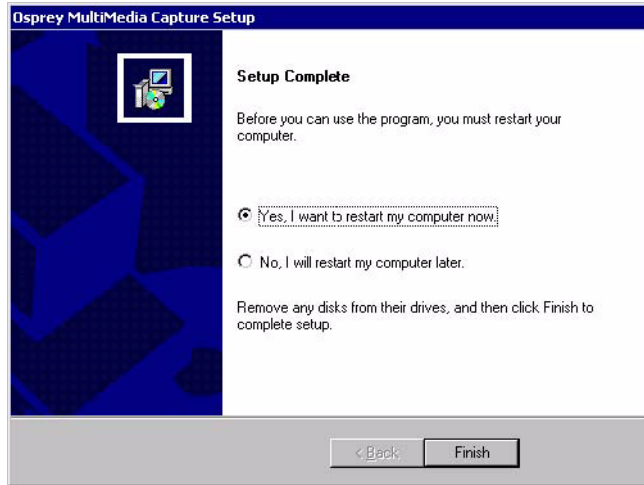


Figure 29 Setup Complete window

16 Click **Finish** to complete the installation. When prompted to restart the computer, click **No**, and proceed to the next step.

Step 4. Install the Video Capture Board hardware

CAUTION

Computer cards are sensitive to electrostatic discharge. Slight discharges from clothing or even from the normal work environment can adversely affect these cards. Follow these simple guidelines, to minimize the chance of damaging your Osprey card.

- Handle cards only by the non-conducting edges.
- Do not touch the card components or any other metal parts.
- Wear a grounding strap while handling the cards (if in a high static area).
- Provide a continuous path to ground by leaving the power cord plugged into a grounded power outlet.
- Ensure that the workstation is powered OFF before installing any components.

NOTE

If you are not familiar with how to install a PCI bus card, refer to your system's documentation for more complete, step-by-step instructions.

Use the following steps to install the Osprey card:

- 1 Power down the computer. Make sure that the computer's power switch is turned OFF. Read the Caution note above for grounding precautions.
- 2 Remove the computer's cover.
- 3 Locate an empty PCI slot.
- 4 Remove the slot-cover screw from the empty PCI slot's cover, set the screw aside, and remove the slot cover.
- 5 Remove the card from its anti-static bag.
- 6 Install the Osprey card into the empty slot.

NOTE

Be sure that the card is seated firmly and evenly in the slot.

- 7 Secure the backpanel of the card with the slot's cover screw.
- 8 Replace the computer cover.
- 9 Turn the computer **ON**.

Once your computer has booted and you have logged on, two windows display regarding “Digital Signature Not Found” for the Video Capture Board and the audio capture portion of the board. See [Figure 25](#) for an example of these Warning windows.

- 10 Click **Yes** to the “Digital Signature Not Found” message to continue with the installation.

Step 5. Install the Source

In this step, you attach the PDF-MALDI Source to the LC/MSD SL Trap, XCT Trap, or XCT Plus Trap. If you are installing the Source for the first time, you may skip instruction 1 in this procedure. You should have already confirmed that the LC/MSD Ion Trap meets or has met these requirements.

WARNING

Never switch the power ON at the rear panel of the PDF-MALDI Control Unit until the Source is completely installed, fiber optic cable properly connected at both ends, and the Control connector properly connected to the PDF-MALDI Source.

The Agilent 1100 Series LC/MSD Trap Control software must be in the Shutdown state.

The Spray Shield and the Capillary Cap may be very hot. Allow them to cool before handling.

- 1 Check the calibration of the LC/MSD Ion Trap. All the tuning mass ions must have the correct mass assignments and acceptable peak widths. You should also check the electron multiplier voltage (EMV).
 - **For LC/MSD SL Trap systems** - If the accumulation time is greater than 0.2 ms with an ICC setting of 50,000, clean the capillary, skimmer 1, and octopole 1. After cleaning the ion optics components, the detector voltage should be recalibrated. Rerun the scan calibration to confirm that the mass assignments and peak widths are correct.
 - **For LC/MSD XCT Trap systems** - If the accumulation time is greater than 0.2 ms with a smart ICC setting of 125,000, clean the capillary, skimmer 1, and octopole 1. After cleaning the ion optics components, the detector voltage should be recalibrated. Rerun the scan calibration to confirm that the mass assignments and peak widths are correct.
 - **For LC/MSD XCT Plus Trap systems** - If the accumulation time is greater than 0.2 ms with a smart ICC setting of 500,000, clean the capillary, skimmer 1, and octopole 1. After cleaning the ion optics components, the detector voltage should be recalibrated. Rerun the scan calibration to confirm that the mass assignments and peak widths are correct.
 - **For All LC/MSD Trap systems** - The vacuum and helium conditions are correct. The high vacuum pressure reading should be less than 2.0×10^{-5} mtorr or 2.5×10^{-5} mbar (depending on the pressure units you use). The helium differential pressure reading should be 0.6×10^{-5} mbar less than the high vacuum reading. If the helium pressure is not set correctly, you will experience a loss of sensitivity.

After you clean the system, check the mass calibration again for proper mass assignments, accumulation times, and EMV settings.

- 2 Confirm that the system is in the Shutdown state before proceeding.
- 3 Disconnect the nebulizing gas tube and the sample line from the ESI Source.
- 4 Remove the ESI Source:
 - a Loosen the latch on the spray chamber.
 - b Swing the Source open.
 - c Lift the ESI Source off the hinge. The contact pin must clear the PTFE end plate mount.
- 5 Unscrew the Spray Shield and Capillary Cap. Gently pull them off.
- 6 Place the ESI Source and its components in a safe, dust-free environment.
- 7 Gently install the Capillary Extension on top of the LC/MSD glass capillary. See [Figure 30](#).

You will feel a slight resistance at first as the gold-plated spring inside of the Capillary Extension is compressed. Be careful not to apply excessive force.

WARNING

Do not twist the Capillary Extension onto the glass capillary. It can damage the glass capillary.



Figure 30 Install the Capillary Extension to the LC/MSD

- 8** Carefully slide the Heating Sleeve over the Capillary Extension, and screw it into the end plate (finger tight). See [Figure 31](#).



Figure 31 Install the Heating Sleeve over the Capillary Extension

- 9** Place the Wave Disk Spring over the Heating Sleeve. See [Figure 32](#).



Figure 32 Install the Wave Disk Spring

- 10 Slide the white PTFE Centering Bushing over the Heating Sleeve. See [Figure 33](#).



Figure 33 Slide the PTFE Centering Bushing over the Wave Disk Spring to finish the Capillary Extension Assembly

- 11 For ease of installation, remove the side cover door. This will also prevent accidental damage to the CCD camera. Align the interface Flange prongs with the hinge on the Inlet Flange. See [Figure 34](#). The CCD camera should be almost touching the instrument cabinet.

CAUTION

Do not tap the Capillary Extension assembly.

- 12 Carefully slide the prongs into the hinge and pivot the Source until the Interface Flange rests against the Inlet Flange. Do not damage the capillary extension.

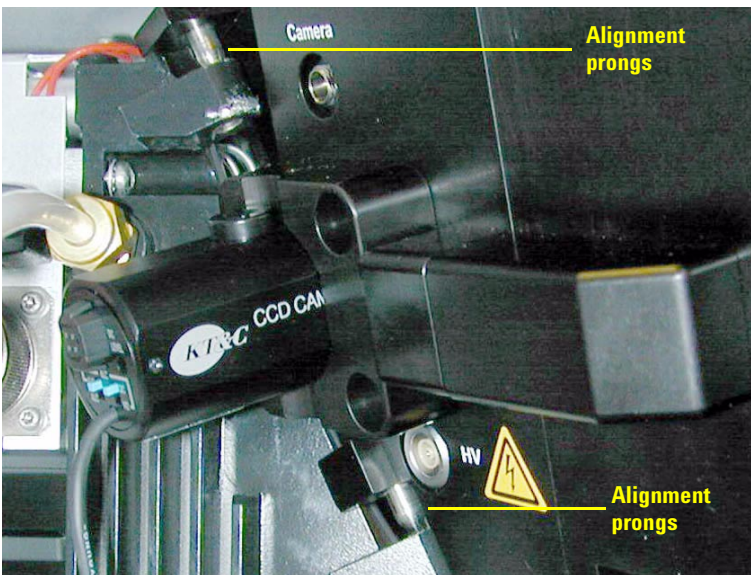


Figure 34 Slide the prongs into the hinge

- 13 Secure the desolvation assembly latch on the PDF-MALDI Source to a very tight fit. See [Figure 35](#).

NOTE

If the desolvation assembly latch is not tight, the PDF-MALDI Source will not be aligned properly resulting in a significant loss of sensitivity. To be sure, rotate the latch clockwise one turn at a time until the latch cannot be closed.

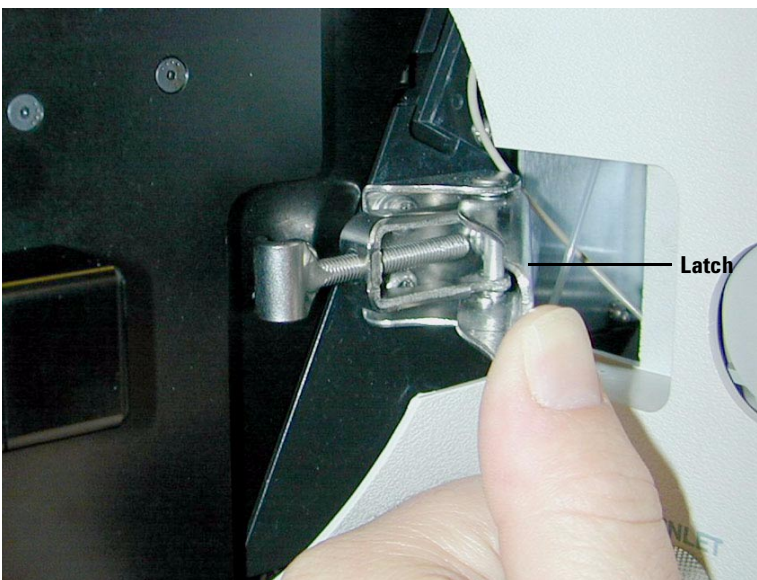


Figure 35 Secure both halves of the Source, and anchor it by latching it to the LC/MSD instrument

Step 6. Attach cables to the Control Unit and the Source

- 1 Check that the power on the Control Unit is turned OFF.
- 2 Connect cables to the back of the Control Unit.
 - a Attach the power cord to the power receptacle.
 - b Connect one end of the USB cable of the **Target Stage/Laser Control Box** to the port labeled **USB**. Please use [Figure 36](#) for a reference picture.
 - c Attach the 15-pin end of the Laser Synchronization cable to the **External Controls** port.

No adjustment is necessary for $\sim 110/\sim 127/\sim 220/\sim 240$ V AC.

1 Installation for LC/MSD Trap Systems

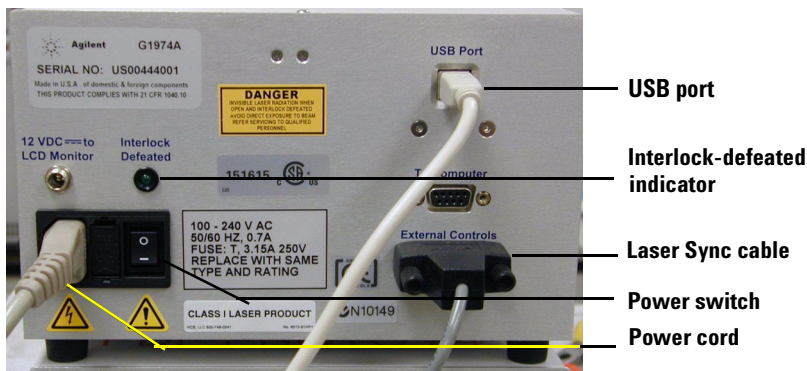


Figure 36 Rear panel of the Control Unit

- 3 Connect the other end of USB cable to an open USB port on the LC/MSD instrument control computer. See [Figure 37](#).

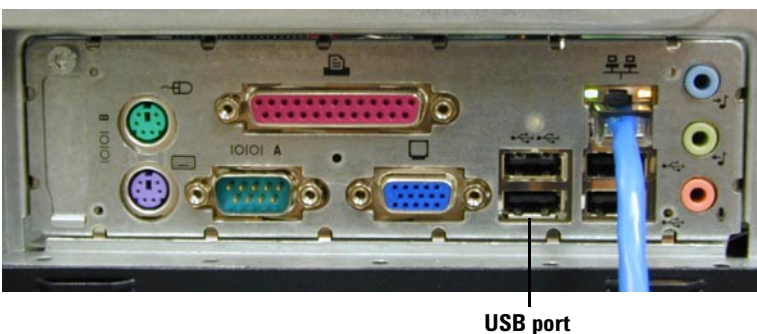


Figure 37 USB connector on the back panel of a computer

- 4 Connect the other ends of the Laser Synchronization cable to the side of the LC/MSD Trap.
 - a Attach the 9-pin connector with two cables present to the **Remote** port on the LC/MSD Trap Processor Board. See [Figure 38](#).
 - b Attach the 9-pin connector with one cable present to the **AUX** port on the LC/MSD Trap. See [Figure 39](#).

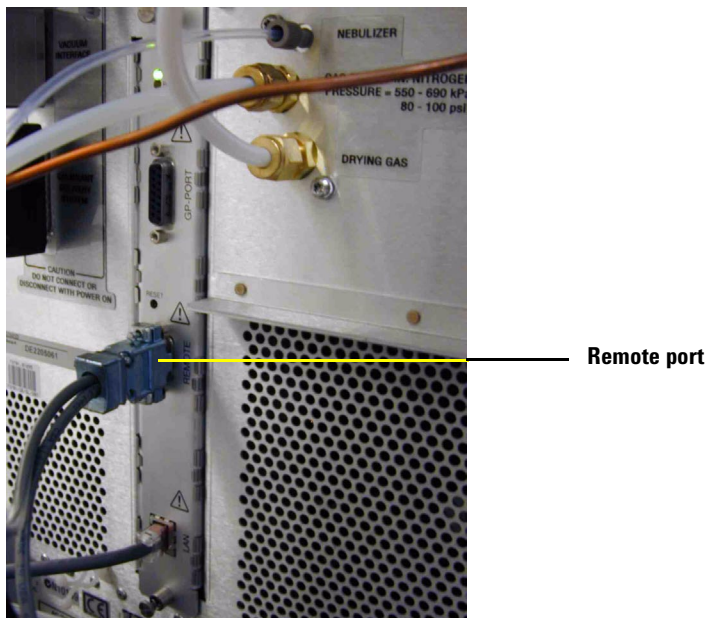


Figure 38 Connect the Laser Synchronization cable to the Remote port

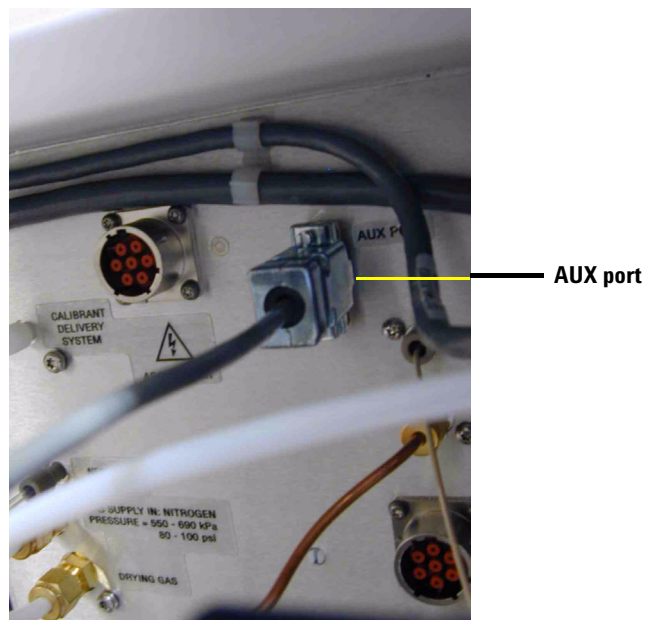


Figure 39 Connect the video cable to the LCD cable

1 Installation for LC/MSD Trap Systems

- 5 Connect one end of the video camera cable to the yellow RCA connector on the Video Capture Board cable. See [Figure 40](#).



Figure 40 Connect the video camera cable to the Video Capture Board connector

- 6 Connect the Video cable to the Camera Video cable. See [Figure 41](#).



Figure 41 Connect the video cable to the camera video cable

- 7 Attach the Stage Control cable to the Source Flange. See [Figure 42](#).



Figure 42 Connect Stage Control cable to Source Flange

8 Connect Stage Control cable to Control unit. See [Figure 43](#).



Figure 43 Connect Stage Control cable to Control unit

9 Connect the Target Stage HV cable to the HV connection on the Source body.

- 10 Connect the end of the fiber optic cable labeled “A” to the PDF-MALDI Source Flange. Tighten the nut using only your fingers (“finger-tight”). See [Figure 44](#).

WARNING

Be careful not to turn the connector.



Figure 44 Connect fiber optic cable to Source Flange

- 11 Connect the end of the fiber optic cable labeled “B” to the Control Unit. See [Figure 45](#).



Figure 45 Connect fiber optic cable to Control Unit

- 12 Prepare to turn on the power:
 - a Check that the HV connector is firmly connected.
 - b Check that both ends of the fiber optic cable are firmly connected.

WARNING

Do not turn on the Control Unit until after the USB drivers are loaded.

When complete, your LC/MSD Trap system with PDF-MALDI Source will look like [Figure 46](#).

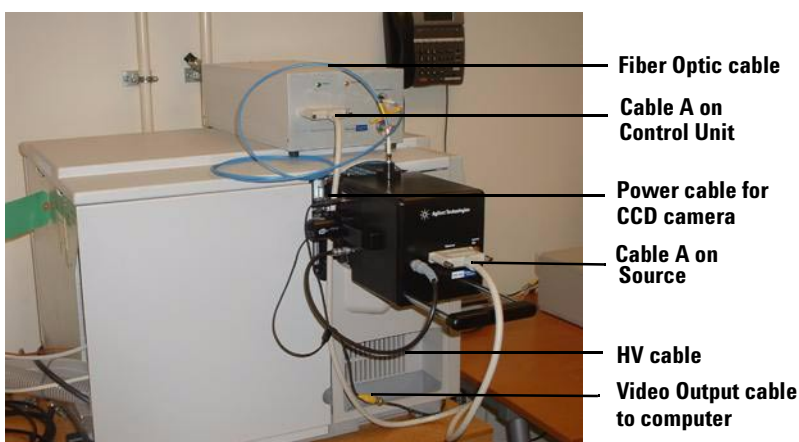


Figure 46 PDF-MALDI Source installed on LC/MSD Trap

Step 7. Install the Target software

This step describes how to install the Target software. You only need to do this the first time the Source is installed.

- 1 If you are installing the Target 5.0 software on a Windows 2000 or Windows XP Professional system, check to be sure that you have Administrator privileges.
- 2 Insert the Target 5.0 software CD into the CDROM drive. The disk should start automatically and install the Target 5.0 software. If it does not start automatically, browse to the **Setup** directory and run the `Setup.exe` program. When the program starts, click the Next button to proceed with the installation. See [Figure 47](#).

1 Installation for LC/MSD Trap Systems



Figure 47 Target setup Welcome window

- 3 Choose a location and folder name for the Target software. By default, the folder is **C:\Program Files\MassTech\AP MALDI PDF**. See [Figure 48](#).

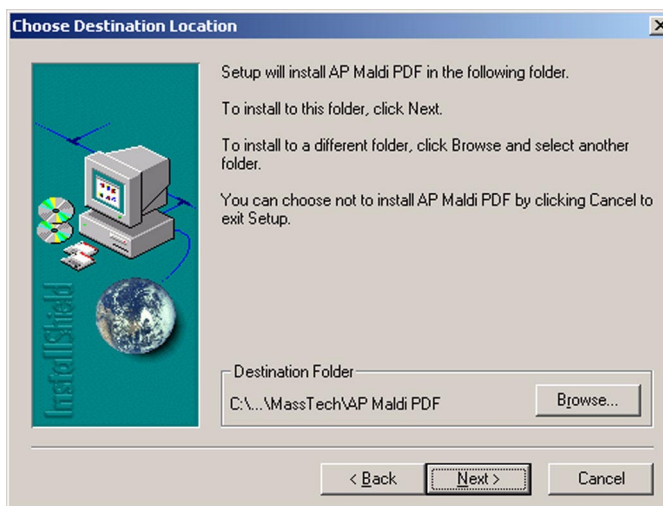


Figure 48 Choose destination location for Target software

- 4 Click Next in the next window to install the files required to control the PDF-MALDI Source. See [Figure 49](#)



Figure 49 Load Target software control components

- 5 Click Next in the next window to create the program file folder. See [Figure 50](#).

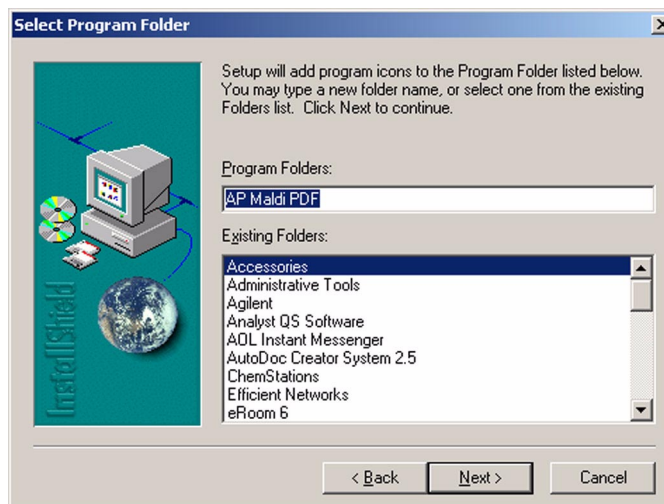


Figure 50 Select program folder for Target software

- 6 After the installation has completed, a window displays confirming that the installation has completed successfully. See [Figure 51](#).

1 Installation for LC/MSD Trap Systems

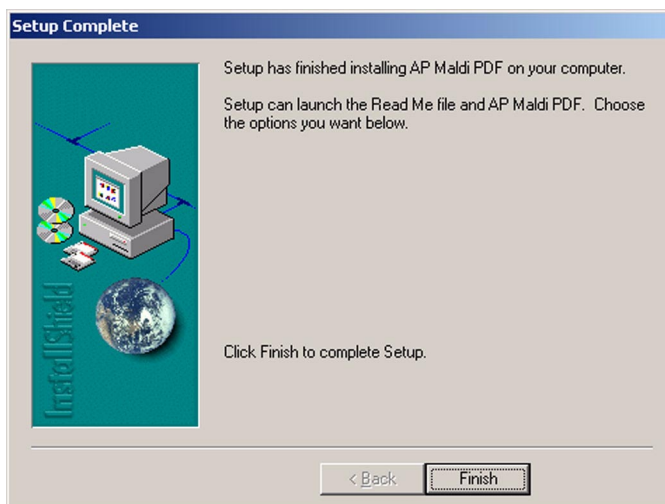


Figure 51 Target software setup completed window

Step 8. Load the PDF-MALDI USB Drivers

- 1 If the PDF-MALDI Software CDROM is not in the CDROM drive, load the CDROM into the CDROM drive of the computer.
- 2 Turn on the PDF Control Unit. The first time the PDF-MALDI source is turned on, the LC/MSD TOF Control computer will detect the source as a new USB device. See [Figure 52](#).



Figure 52 Found new hardware

- 3 Then, the Windows Found New Hardware Wizard will appear. See [Figure 53](#).



Figure 53 Found New Hardware Wizard

- 4 Click the Next Button.
- 5 Then the Wizard will prompt you to search for a suitable driver. See [Figure 54](#).



Figure 54 Search for a suitable driver

- 6 Select only the CD-ROM drives as locations to search for the driver. Then click Next. See Figure 55.

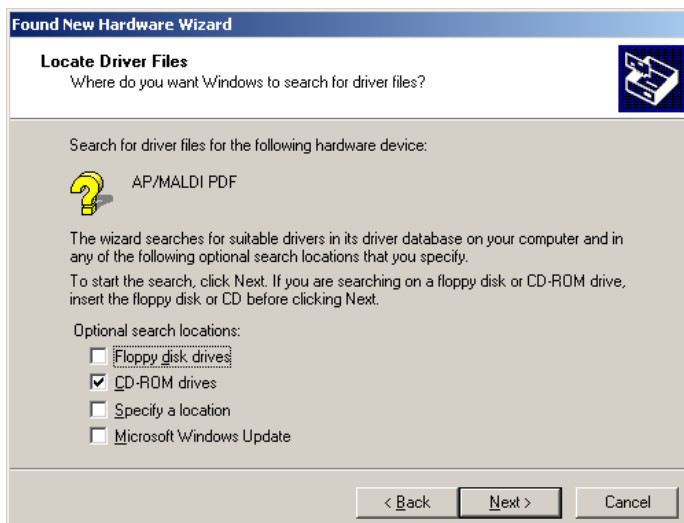


Figure 55 Select only the CD-ROM drives

- 7 The wizard should find the correct USB driver for the PDF-MALDI source. See Figure 56 for the correct driver name.

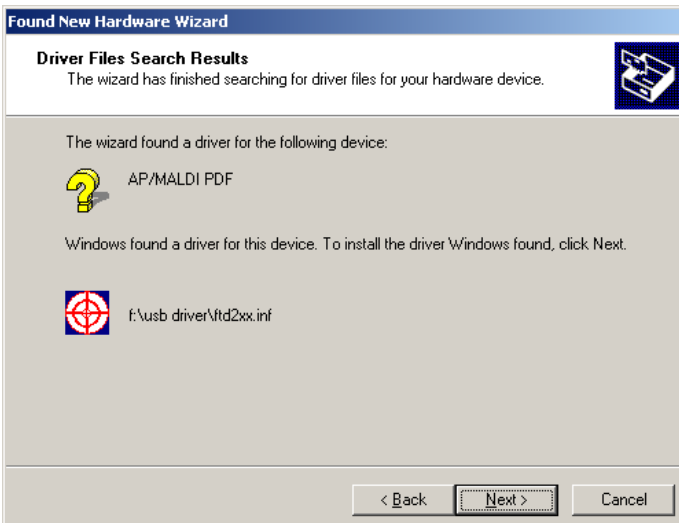


Figure 56 Correct driver found

- 8 Click the Next button to load the driver.
- 9 After the driver has loaded correctly, a message will appear confirming that the driver has loaded correctly. See [Figure 57](#).

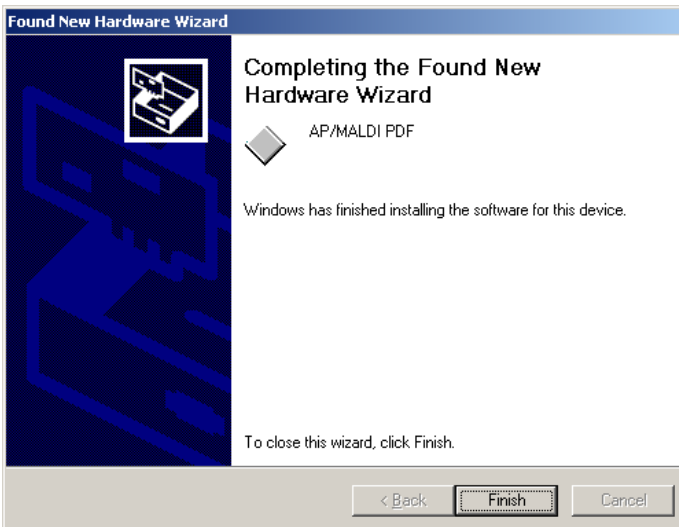


Figure 57 USB driver loaded successfully

Configuration and verification

This step describes how to configure your system and to verify the installation after you have installed all the necessary hardware and software.

NOTE

The initial verification should be performed by a qualified Agilent service representative. The following procedure can be used as a troubleshooting tool or to check for sensitivity during normal use.

Step 1. Program the default methods

The methods for these systems should be used as templates for developing other methods. Keep in mind that Agilent has already optimized these methods for PDF-MALDI so further optimization should not be necessary.

MS method for LC/MSD Trap SL, XCT, or XCT Plus

Mode	Scan mode	Ultra scan (XCT and XCT Plus); or standard normal (SL)
	Segment end time	1.5 min
Tune	Source	AP-MALDI (positive)
	Vcap	3000 V
	Drying gas flow	5.0 L/min
	Dry gas temperature	325°C
	Skim1	40 V
	Capillary exit	200 V
	Oct 1 DC	12.0 V
	Oct 2 DC	3.2
	Trap drive	110 V
	Oct RF	200 V
	Lens 1	-5 V
	Lens 2	-60 V
	Block voltages	Mark the check box for Follow values on Page Tune Expert.

ICC values	Target	200,000 for XCT; 500,000 for XCT Plus; 50,000 for SL
	Maximum accumulation time	535 ms (corresponds to 6 laser shots)
	Scan range	400-2200 <i>m/z</i>
	Averages	8
	Save the method as PEP_PMF.m .	

MS/MS method for LC/MSD Trap SL, XCT, or XCT Plus

Mode	Scan mode	Ultra scan (XCT and XCT Plus); or standard normal (SL)
	Segments	Segment 1 end time 0.25 min; Segment 2 end time 7 min
Tune	Source	AP-MALDI (positive)
	Vcap	3000 V
	Drying gas flow	5.0 L/min
	Dry gas temperature	325°C
	Skim1	40 V
	Capillary exit	200 V
	Oct 1 DC	12.0 V
	Oct 2 DC	3.2 V
	Trap drive	110 V
	Oct RF	200 V
	Lens 1	-5 V
	Lens 2	-60 V
	Block voltages	Mark the check box for Follow values on Page Tune/Expert .
ICC values	Target	200,000 for XCT; 500,000 for XCT Plus; 50,000 for SL
	Maximum accumulation time	535 ms (corresponds to 6 laser shots)
	MS scan range	400-2200 <i>m/z</i>

Averages Segment 1 uses 4 averages;
Segment 2 uses 8 averages

MS/MS fragmentation conditions

Auto MS(n) 2, enabled for Segment 2 only
Fragmentation amplitude 1.50 V
Precursor selection Include 400-2200 m/z
Number of precursor ions 3
SPS Enabled
Threshold absolute 200 counts
Threshold relative 2%
SmartFrag On, 30-200%
Active exclusion On, exclude after 1 spectrum,
release after 20 min

Advanced Auto MS(n) parameters

MS(n) averages 16
Isolation width 4.0
Use values from MS scan Yes
Use ultra scan Yes

To activate the DataAnalysis Visual Basic script

- 1 In **MSD Trap Control**, open the LC/MSD Trap method you created to do the PDF-MALDI MS/MS analysis of peptides.
- 2 Select **Method > Add DataAnalysis Method Part**.
- 3 Select the DataAnalysis method **Example DA Methods with Scripts\Auto_MIS_MgfExport.ms**.
- 4 Select **Method > Save entire Method As** to save the LC/MS/MS method with the DataAnalysis part attached.

NOTE

For more information on DataAnalysis script, refer to pages 57–62 of the *PDF-MALDI User's Guide*.

Save the method as **PEP_MIS.m**.

Step 2. Use the Alignment Tool

NOTE

Use this procedure *only* for the G1974A PDF-MALDI Source.

- 1 In the LC/MSD Trap Control software, set the drying gas flow to 0.5 L/min., drying gas temperature to 0°C, and turn off the capillary high voltage by clearing the HV check box in the MSD Trap Control screen.
- 2 Click the **Target** software icon on the desktop to start the PDF-MALDI Target software. Wait until initialization finishes and the **Ready** status message displays.

CAUTION

Do *not* open the Source while the software is initializing. The plate transport mechanism locates its reference points by moving to the extremes of its travel. Opening the Source during this time may jam the mechanism or damage the hardware.

- 3 In the PDF-MALDI Target software, click spot D6 to select it. Deselect the push button for Spiral Motion. See [Figure 58](#).

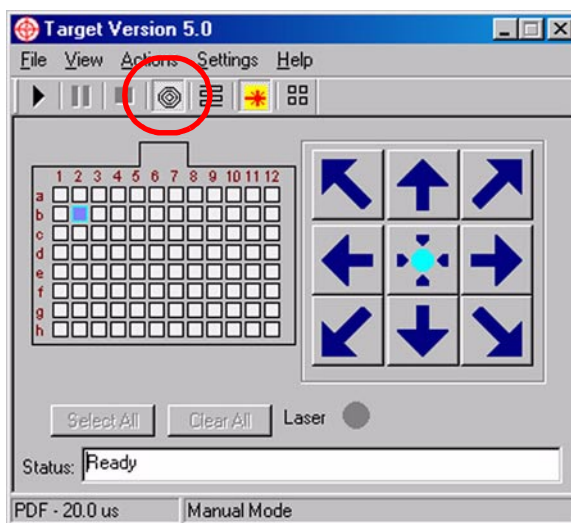


Figure 58 Deselect the Spiral Motion button circled in red

- 4 Affix a yellow label to the center of the back side of a MALDI plate, as shown on [Figure 59](#). Be careful to select the back of the plate rather than the front side since any adhesive residue from the label can contaminate the surface of the plate and render it unusable.

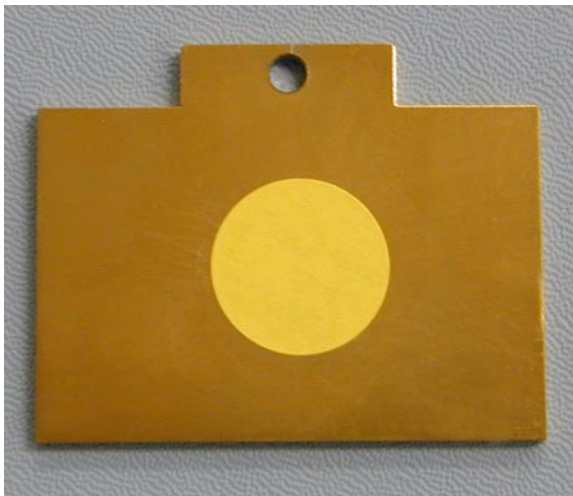


Figure 59 Target plate with yellow label at its center

- 5 Open the PDF-MALDI Target Flange, and insert the plate in the plate holder with the yellow label facing up.

WARNING

Although the Source voltages are OFF when the Ion Source is open, the Capillary Extension will be hot, and heated drying gas is still flowing around the Capillary Extension. The Laser Alignment tool will be heated by the drying gas flow.

- 6 Carefully insert the Laser Alignment fixture on the Capillary Extension. Make sure the spring loaded Alignment fixture can move freely on the Capillary Extension by pushing it gently all the way in against the tip of the Capillary Extension. See [Figure 60](#).

CAUTION

Be careful not to apply any radial force to the Capillary Extension to avoid bending it off center.

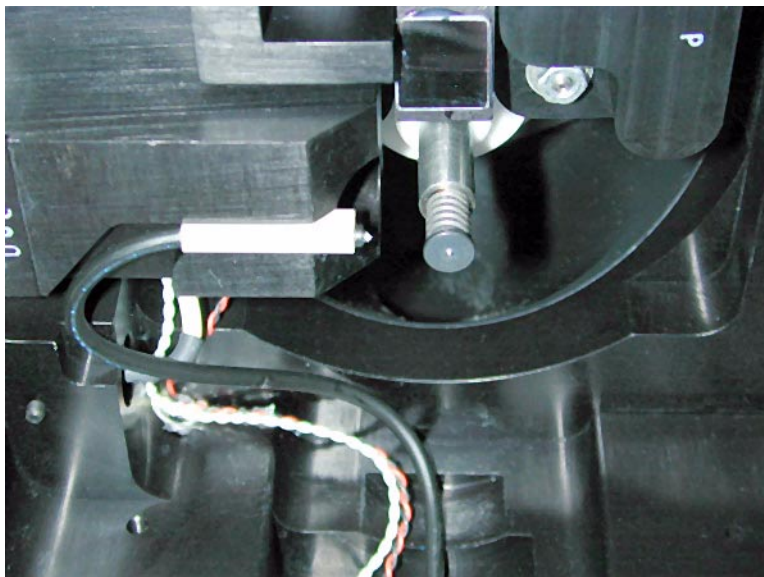


Figure 60 Install the Laser Alignment tool carefully

- 7 Gently close the Source completely, then re-open it.
- 8 Remove the Alignment tool and re-close the Source.
- 9 A small dimple left by the capillary Alignment Tool should be visible on the plate video image. See the example in [Figure 61](#).

NOTE

You may have to decrease the video image brightness in order to see the dimple clearly.

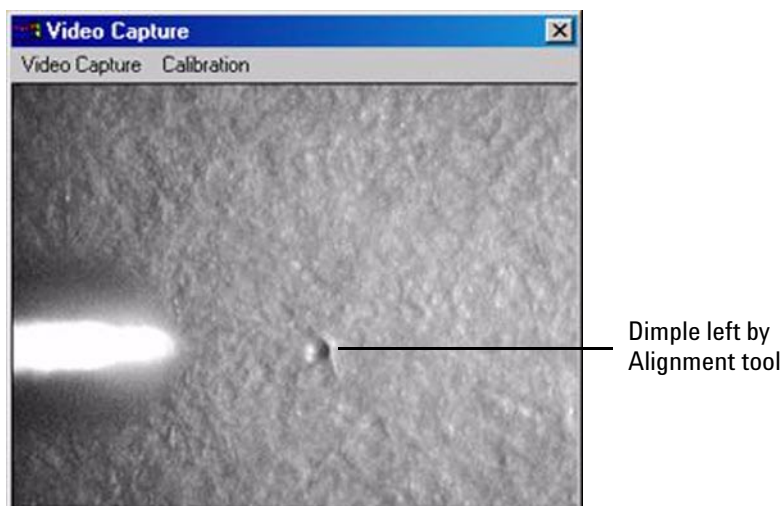



Figure 61 Dimple in paper target plate

- 10 Select the **Play (Start)**  button to turn on the laser. If the laser optics are properly aligned, the dimple should be in the center of the laser spot, and the laser beam should create a small fluorescent ellipse centered on the dimple. See the example in [Figure 62](#). If the laser is not properly centered on the dimple, the laser spot must be repositioned.

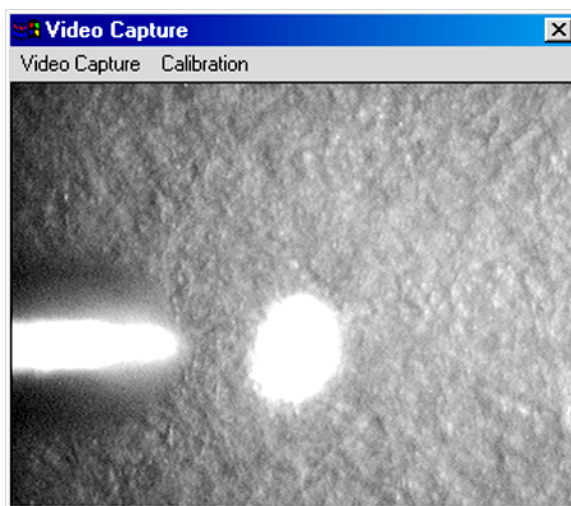


Figure 62 Properly aligned laser spot

- 11 Use the following procedure to properly align the laser.
- Typically, only two of the setscrews need to be turned to adjust the laser spot.
- Using a 2.5 mm hex driver, carefully adjust the two screws indicated in [Figure 63](#).
 - Turn the screws very slowly because the adjustment is very sensitive.
 - Watch the computer monitor screen while adjusting the location of the laser spot until it covers the dimple. The dimple should be in the center of the laser spot.
 - Once adjusted, the laser position should resemble [Figure 62](#) from the previous step in this procedure.

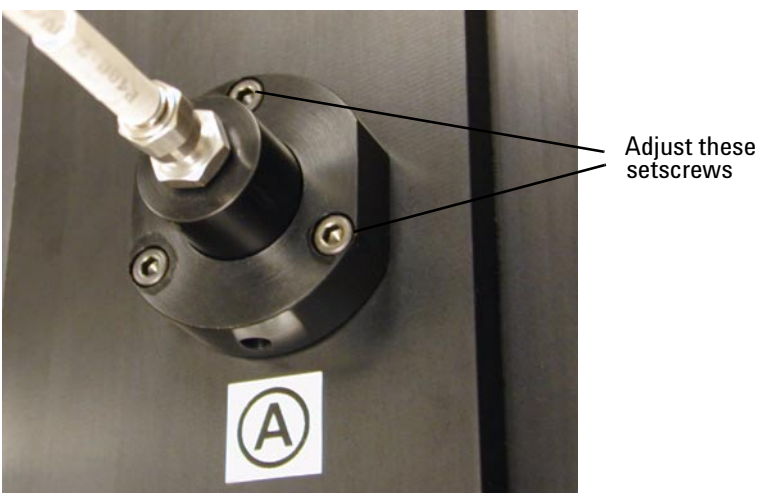


Figure 63 Turn the setscrews to adjust the laser spot location

- 12 Remove the MALDI plate and carefully remove fluorescent label. Remove any adhesive residue from the label by wiping the plate surface with a tissue wetted with isopropanol.

WARNING

The MALDI target plate will be warm to the touch.

- 13 The laser alignment procedure is now complete.

NOTE

If the laser spot appear diffuse, please refer to the Troubleshooting section in chapter 3 for refocusing the laser spot.

Step 3. Verify the hardware installation

- 1 In the LC/MSD Trap Control software:
 - **For LC/MSD Control software revision 4.1** - Select the Source type as **Online Nano ESI**.
 - **For LC/MSD Trap Control software revisions 4.2 and later** - Select the Source type as **AP-MALDI**.

NOTE

You can change the Source configuration only if the instrument is in the Shutdown state.

- 2 Click **Options > Vacuum System** in the LC/MSD Trap Control window to check the vacuum.
 - a The foreline pressure should read between 3.5 and 4.5 mBar (2.5 – 3.5 Torr) when the capillary extension forms a proper seal with the glass capillary.
 - b If the pressure in the instrument is too high, re-install the Capillary Extension and push it in more tightly.
- 3 Close the Target Plate Flange if it is open. Click **Operate** to check that the high voltage safety interlock is working properly. A check should appear automatically in the **HV** check box under **Source**. Open and close the Source. When properly adjusted, the **HV** check disappears when the Source is opened, and it appears when the Source is closed.

WARNING

To avoid electrical shock, clear the HV check box whenever the Target Flange is open. Never defeat the safety interlock. Always click Standby to turn off the high voltage before you open the Source.

Step 4. Check the Target Plate alignment

In this step, you prepare the Target Plate to center the Matrix spot. Use the G2037A matrix undiluted.

- 1 Prepare a 1:5 dilution of the G2037A Matrix. Take 10 µl of Matrix and add 40 µl of Matrix Solution solvent.
- 2 Using an Agilent Target Plate (p/n G1972-60025), spot 1 µl of the diluted Matrix mixture on one corner of the plate. For example, at A1.
- 3 Open the PDF-MALDI Source. See [Figure 64](#).



Figure 64 Open the PDF-MALDI Source

- 4 Insert the Target Plate with the prepared sample spots into the Target Plate Holder. The plate is held in place by a magnet. See [Figure 65](#).

NOTE

If you open the Source while the LC/MSD Trap is recording the spectrum (HV is ON) or the laser is firing, the PDF-MALDI Source safety interlocks automatically switch the High Voltage and the Laser OFF.

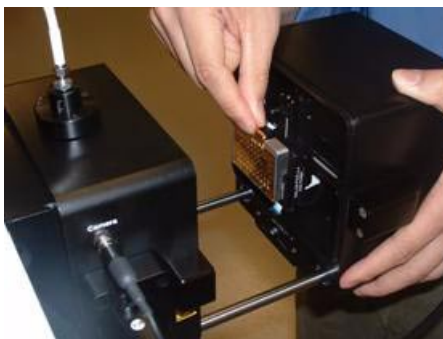


Figure 65 Inserting the Target Plate

- 5 Close and lock the Source.

When the Source is closed, you should hear a “click” sound indicating that the two halves of the Source are locked together.

When the software is running and the Target Plate is loaded properly, you will see the following video capture image. See [Figure 66](#).

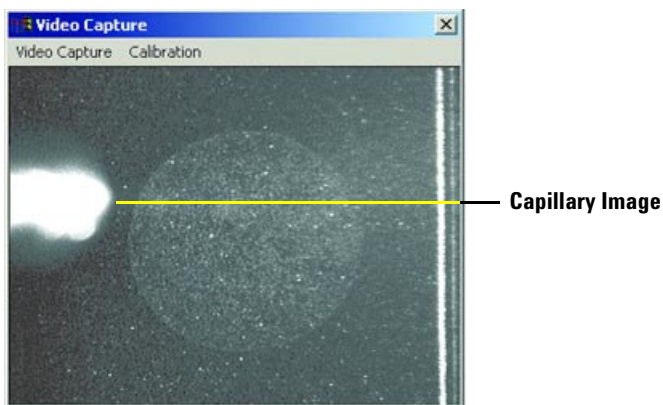


Figure 66 Typical sample spot

Step 5. Center the matrix spot

NOTE

Do these steps only if you plan to use the Geometry setting for Autosequence runs with the Target software.

- 1 In the Target software, select the Settings pull-down menu, and click on the Spiral Motion tab. Copy the settings from [Figure 67](#) and enter them into the appropriate places in your software.

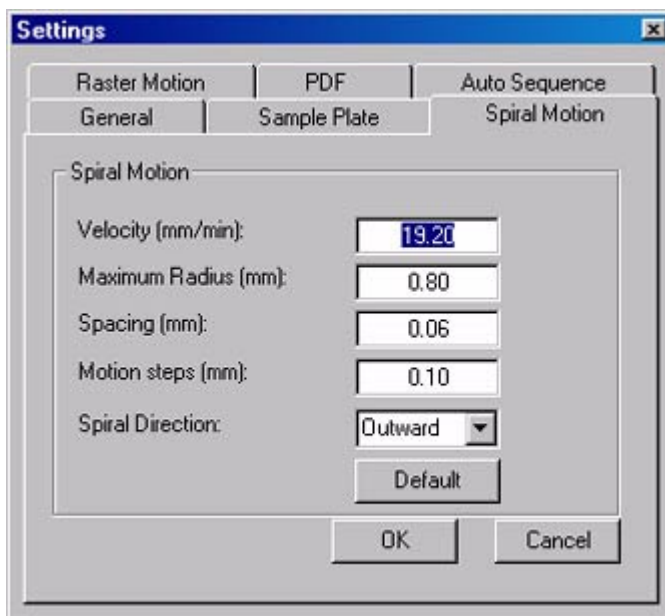


Figure 67 Spiral motion setting for Target Plate

2 Next, click on the Sample Plate tab. See Figure 68.

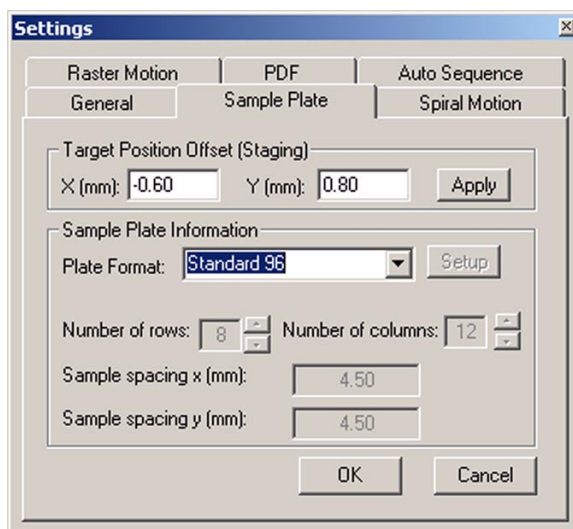


Figure 68 Sample Plate Settings tab

- 3 Center the Matrix spot:
 - a Move the **Settings** window to a position on the screen where you can view the **Video Capture** window and the **Sample Plate** settings window at the same time.
 - b In the **Target Position Offset (Staging)** field, enter values that approximate the center of the Target Plate.
 - c After you enter a value, click **Apply** to see if the plate moves in the proper direction
 - d You will have obtained the best position when it requires an equal number of manual movements to reach either side of the plate or the top and bottom of the plate.

Step 6. Calibrate the Video Capture sample-positioning system

A “point-and-click” system using the Video Capture imaging system was developed for changing the position of a sample that you want to irradiate with the laser. With this system, you can use the mouse pointer to choose a desired location on the ample image, then double-click the left mouse button to move the sample to a new location. You must calibrate this system before you can use it.

- 1 To calibrate the position of the spot using the “point-and-click” positioning system, go to **Calibration** on the Video Capture menu bar and choose **Start**.

After you begin calibration, a five-step procedure is described in dialog boxes.

- 2 Ensure that there is a distinct visual object in the lower right-hand quadrant of the screen. See example in [Figure 69](#).

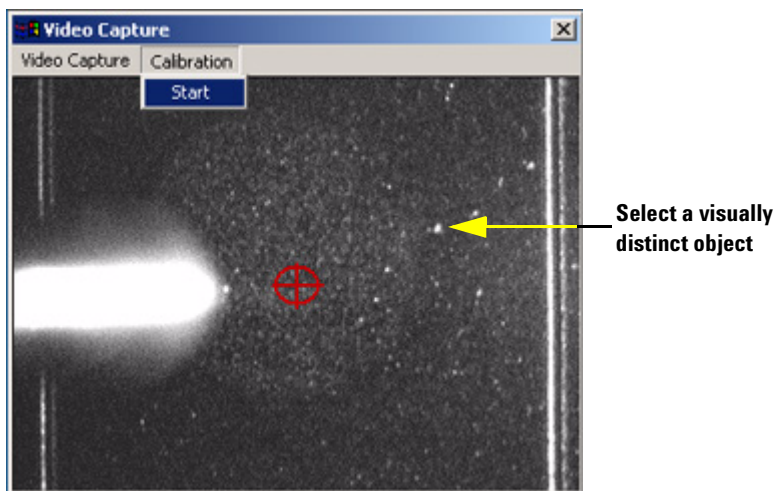


Figure 69 Start Video Capture sample-positioning Calibration Procedure by selecting visually distinct object

You can use a sample plate with ablated Matrix or dilute Matrix. If there is no distinct visual object, select another spot or prepare a new sample. Advance through the calibration procedure by clicking **Next**. See [Figure 70](#).

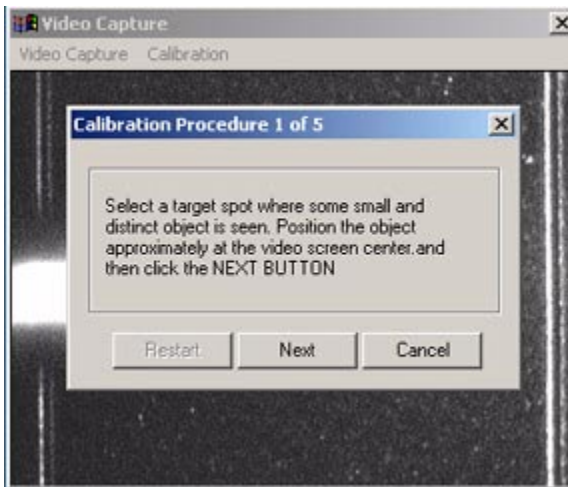


Figure 70 Step 1 dialog box of the calibration procedure

- 3 Locate the visually distinct object in the right-hand quadrant, then drag and drop the green target icon over the object. Click **Next**. See Figures 71 and 72.

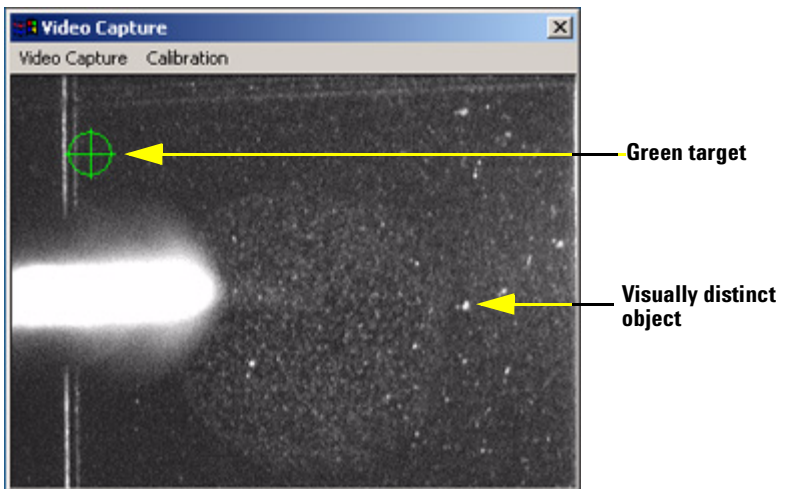


Figure 71 Video Capture screen showing green target and the visually distinct object that was selected

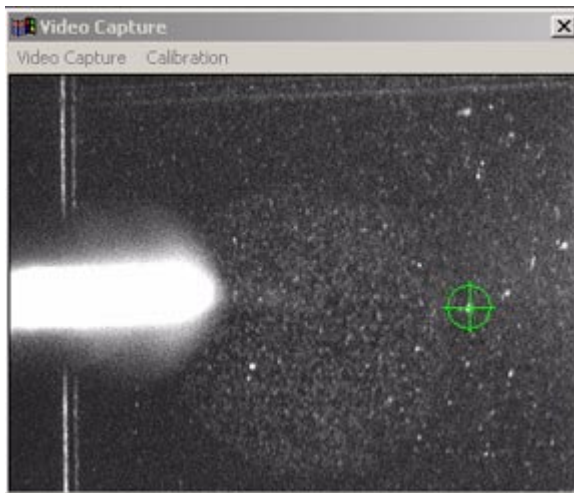


Figure 72 Video Capture screen showing green target that was dragged over the visually distinct object in step 2

- 4 In step 3 of this calibration procedure, the Target Plate moves horizontally (see [Figure 73](#)). Watch to see where the visually distinct object moves, then drag and drop the green target icon over the same object. See [Figure 74](#). Click **Next**.

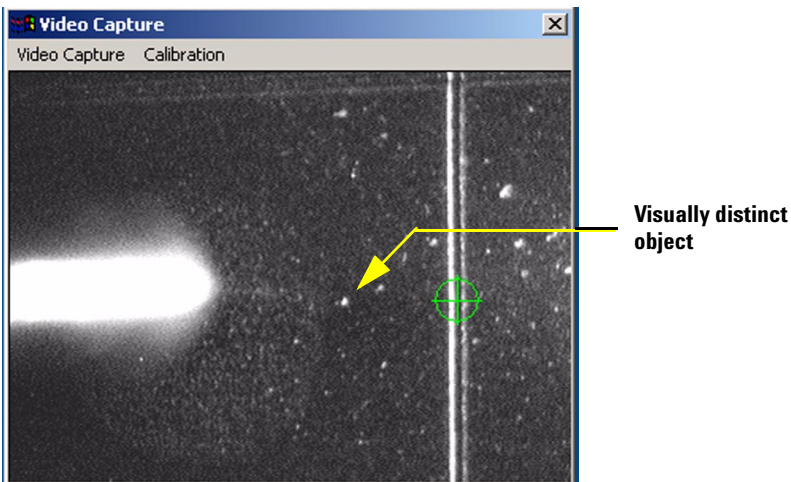


Figure 73 Video capture screen showing the location of the visually distinct object after step 2

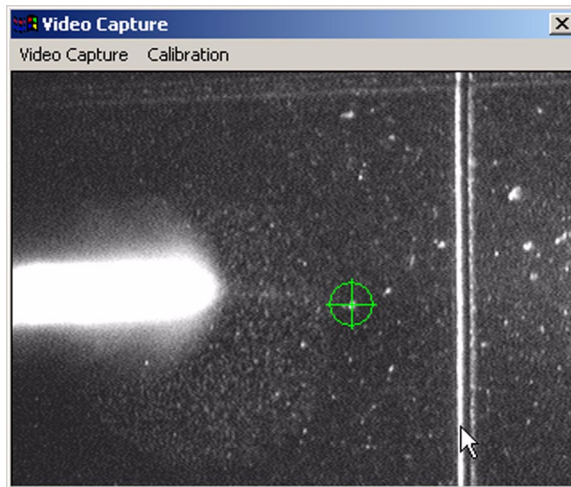


Figure 74 Video Capture screen showing green target that was dragged over the visually distinct object in step 3

- 5 In step 4 of this calibration procedure, the Target Plate moves again, vertically (see [Figure 75](#)). Watch to where it moves, then drag and drop the green target icon over the same object. See [Figure 76](#). Click **Next**.

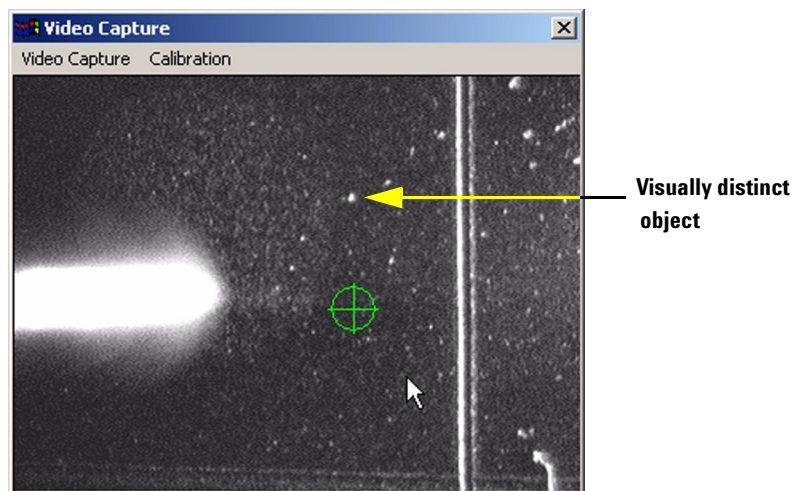


Figure 75 Video capture screen showing the location of the visually distinct object after step 4

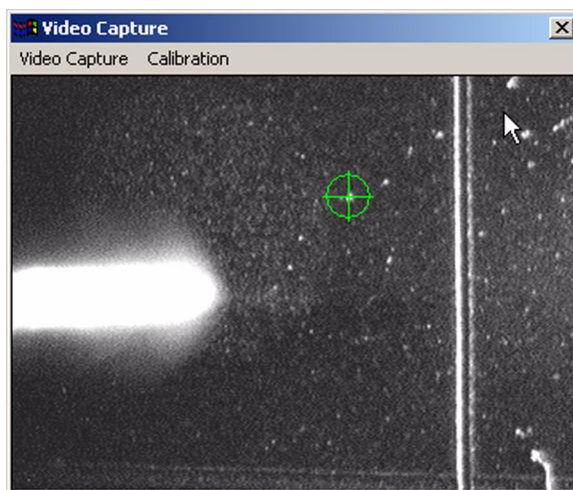


Figure 76 Video Capture screen showing green target that was dragged over the visually distinct object in step 4

- 6 In step 5 of this calibration procedure, click **Finish** to accept the new calibration. See [Figure 77](#).

You can click **Cancel** to retain the original calibration, or you can click **Restart** to start over and recalibrate the system again.

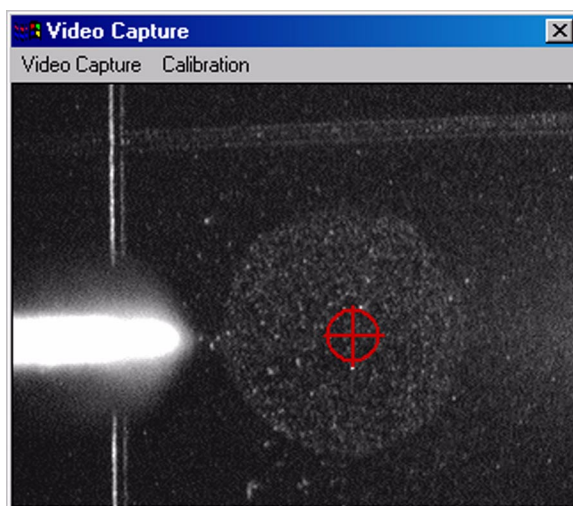


Figure 77 Video Capture screen after calibration was completed in step 5

- 7 To confirm that the “point-and-click” sample-positioning system is working, double-click the left mouse button on any location visible in the video capture window. The Target Plate should move to the location you selected.

Step 7. Verify the installation

This step should be performed by a qualified Agilent representative. These steps are only done the first time the Source is installed.

The verification test checks that the PDF-MALDI Source is properly installed and adjusted, and determines the sensitivity of the PDF-MALDI Source to the Agilent LC/MSD Ion Trap system.

CAUTION

You must use Agilent Target Plates (p/n G1972-60025) to successfully complete verification.

- 1 Clean the Target Plate:
 - a Submerge plates in acetone and sonicate for about 3 minutes.
 - b Submerge plates in deionized water and methanol (1:1, v/v) with 50 mM ammonium formate (about 3-4 g/L). Sonicate for about 3 minutes.
 - c Submerge plates in deionized water and sonicate for about 3 minutes.
 - d Submerge plates in methanol and sonicate for about 3 minutes.
 - e Heat the plate in PDF-MALDI Source for 20 minutes or with a heat gun to completely dry the plate. You can also dry the Target Plate with a heat gun or in a clean oven at 105 – 120°C. If you do so, cool the Target Plate to room temperature before you spot the sample.

CAUTION

Do not use glassware such as beakers or flasks when cleaning the Target Plate. The glassware will add sodium to the Target Plate which will suppress ionization of the sample.

Do not use organic solvents (methanol, acetonitrile, isopropanol, acetone, or ethanol) in excess of 60% in the final matrix/sample mixture. You can overspread the matrix/sample deposit.

Do not use acetic acid in excess of 2%. High concentrations can cause unfavorable crystallization.

1 Installation for LC/MSD Trap Systems

- 2 Prepare the BSA tryptic digest sample dilution.
 - a Dilute the BSA sample (p/n G1990-85000) with 500 µl of a water:acetonitrile solution (85:15) containing 0.1% formic acid.
 - b Add 10 µl of Matrix stock solution to a 500 µl centrifuge vial.
 - c Add 30 µl methanol to the vial, and vortex briefly to mix.
 - d Add 39.2 µl of Matrix dilution solution (water:isopropanol:acetic acid, 75:24:1), and vortex briefly to mix.
 - e Add 0.8 µl BSA sample that you prepared in step 2a and vortex thoroughly.

NOTE

For all solutions, you should use the highest quality solvents and acids that are available. You must mix the sample and Matrix thoroughly for each dilution step. Use a vortex mixer and centrifuge for faster results.

- 3 Spot the plates.
 - a Spot 3 to 5 target locations with 0.5 µl of the dilution mixture at room temperature. Try to use the center of the target location.
 - b Allow the Target Plate to air dry.

NOTE

Remember to clean the Target Plate before using it. Clean the plate using plastic beakers only, and be sure to dry the plate thoroughly. Allow the plate to cool before spotting the sample.

- 4 In the Ion Trap Control software, load the method, **PEP_MIS.m**, that was created in “[Step 1. Program the default methods](#)” on page 38.
- 5 Load the Target Plate.
- 6 Turn on the LC/MSD Trap.

- 7 To run the verification manually:
 - a In the Ion Trap software, create a directory and use the **Prefix/Counter** option to create file names for the verification test.
 - b In the Target software, select a location where you spotted a sample on the Target Plate.
 - c In the Target software, click **Play** button.
 - d In the LC/MSD Trap Control software, click **Run Method**.
 - e Select the next sample location using the Target software.
 - f Repeat steps **c** through **e** until data has been acquired from all of the sample locations.
- 8 To run the verification with autosequence:
 - a Follow steps 1, 2, and 3 of the procedure "To acquire data automatically" on pages 6-9 of the *PDF-MALDI User's Guide* (p/n G1974-90005) to configure the LC/MSD Trap Control software for remote control operation.
 - b Follow steps 1 through 11 of the procedure "To acquire data automatically" on pages 11-13 of the *PDF-MALDI User's Guide* (p/n G1974-90005) to configure the Target software for automated data acquisition. Be sure to select the **Spiral Motion** option in the Target software so the laser will fire on different regions of the sample spots during data acquisition.

NOTE

The laser may require attenuation. Increase laser intensity by turning the laser attenuation knob counterclockwise starting from 2 in 0.25 increments until you get the results that you want.

The laser intensity should generate a clean, isotopically resolved mass spectrum. Deterioration of the resolution means that the trap is over filled and the laser intensity is too high. Real ICC charge in excess of 100,000 can also mean that the laser intensity is too high.

- 9 Determine the signal-to-noise (s/n) ratio by running DataAnalysis.
 - a Load a data file acquired into the DataAnalysis software.
 - b Create an average spectrum of the entire MS-only segment by right-clicking and dragging your cursor across the

segment. The selected averaged spectrum will appear in the Mass Spectrum View window.

- c In the Mass Spectrum View window, right-click and select **Copy to Compound Mass Spectrum** from the drop-down menu.
 - d Click **Window** in the task bar, and select Mass List from the drop-down menu. The Mass List is displayed on the right side of your monitor screen.
 - e In the Mass List find the intensity (abundance) and signal-to-noise ratio for the 927 ion.
 - f Check that the minimum intensity for the 927 ion is 250,000 or above with a s/n of 50 or above.
- 10 Compare the MS-only spectrum for 5 femtomole BSA digest to [Figure 78](#). They should be similar. If not, refer to Chapter 7, “Troubleshooting” in the *PDF-MALDI User’s Guide*.
 - 11 In a similar way, compare the MS/MS spectra for the 927, 1305, 1479, and 1639 ions. Refer to [Figures 79-82](#).

If the verification test fails, refer to Chapter 7, “Troubleshooting” the *PDF-MALDI User’s Guide*.

Using the Mascot Database search

If you want to search the spectrum using the Mascot Database search, refer to page 55-62 of the *PDF-MALDI User’s Guide*.

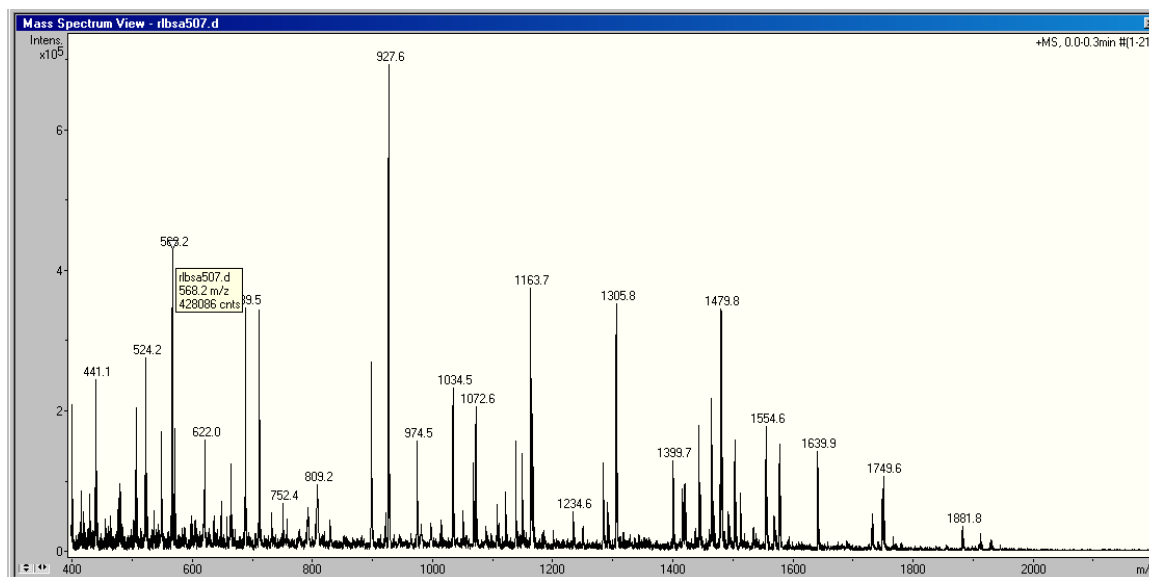


Figure 78 5 femtomole BSA MS only spectrum

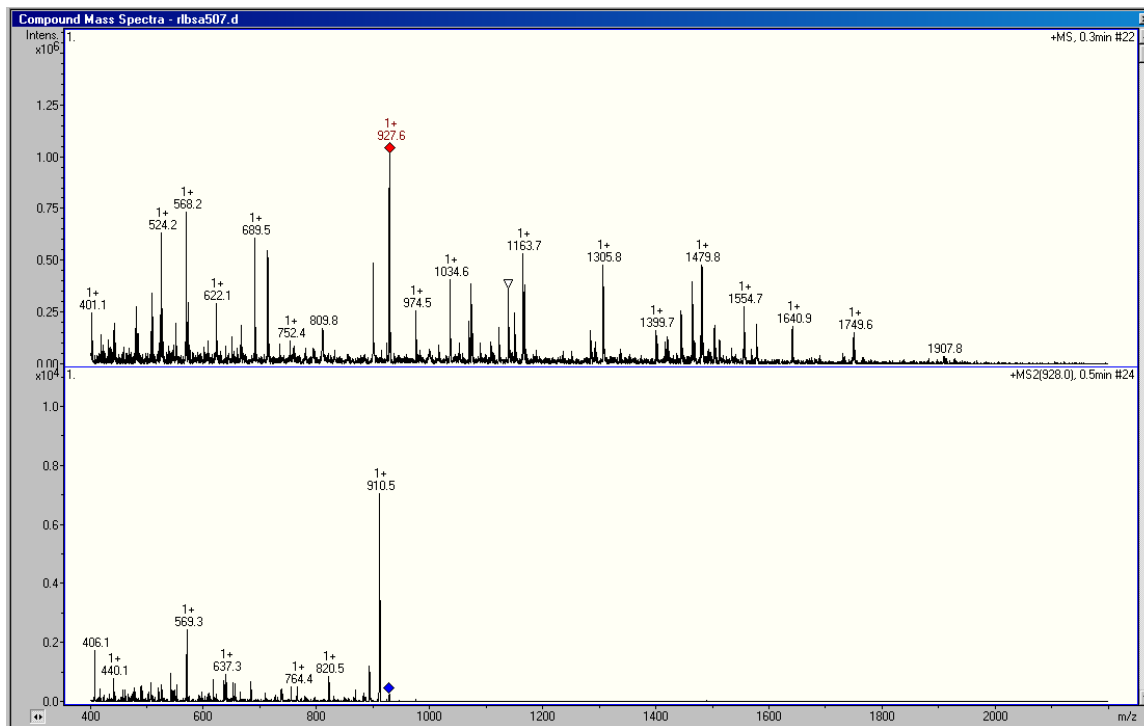


Figure 79 5 femtomole BSA - MS/MS spectrum of the 927 ion

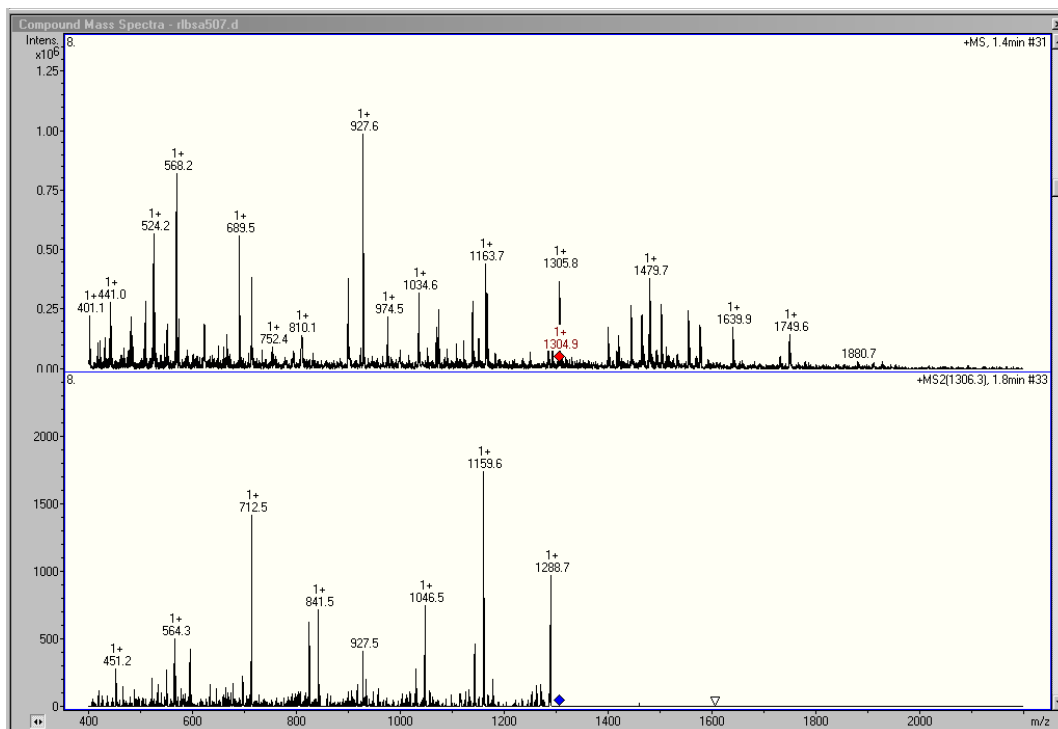


Figure 80 5 femtomole BSA - MS/MS spectrum of the 1305 ion

1 Installation for LC/MSD Trap Systems

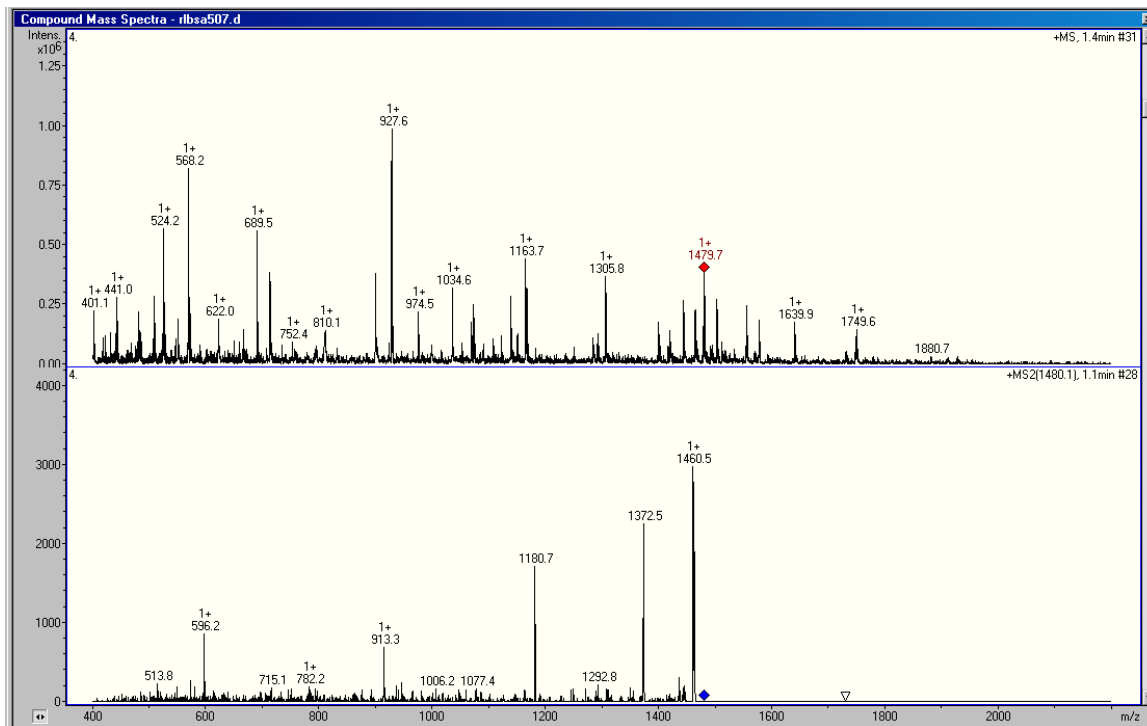


Figure 81 5 femtomole BSA - MS/MS spectrum of the 1479 ion

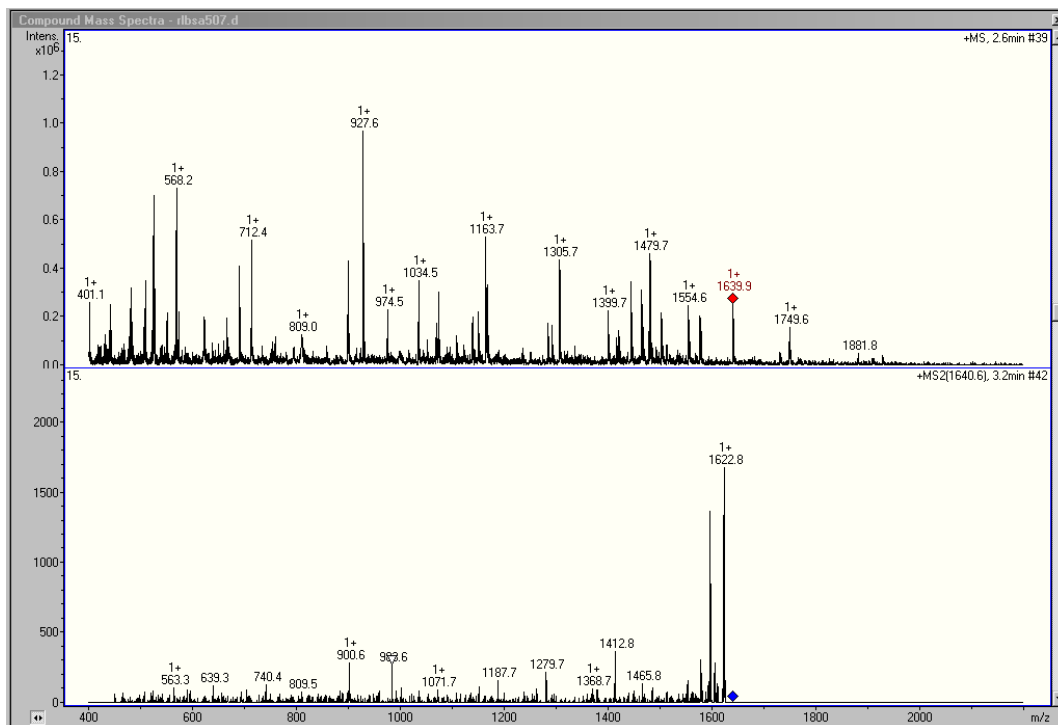
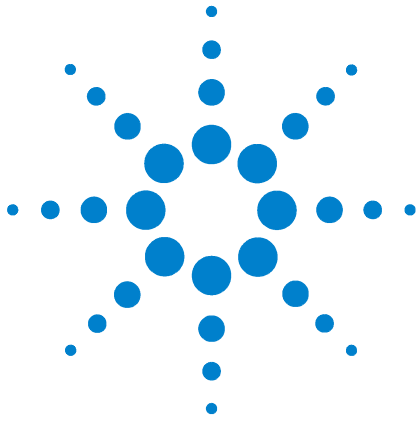


Figure 82 5 femtomole BSA - MS/MS spectrum of the 1639 ion



2 Installation for LC/MSD TOF Systems

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This chapter contains instructions for the initial installation, configuration, and verification of the PDF-MALDI Ion Source on the LC/MSD TOF systems. For subsequent installations, you can skip steps in the procedure as noted.



Installation

This step describes how to install the PDF-MALDI Ion Source on LC/MSD TOF systems. It also describes how to install the Target 5.0 software on LC/MSD TOF system control computers.

Step 1. Prepare to install the Source

You do this only the first time the Source is installed. If this is a subsequent installation, skip to “[Step 4. Install the Source](#)” on page 76.

- 1 Using the Dual Electrospray source, the LC/MSD TOF should be autotuned before installing the PDF-MALDI Source. All the tuning mass ions must have the correct mass assignments and acceptable resolution.
- 2 Check that you have received all components:
 - Control Unit - [Figure 83](#).
 - PDF-MALDI Ion Source shown from various angles. - See [Figures 84](#) and [85](#).
 - Capillary Extension - [Figure 86](#).
 - Heating Sleeve - [Figure 86](#).
 - Centering Bushing - [Figure 86](#).
 - Wave Disk Spring - [Figure 86](#).
 - USB cable to connect Control Unit to computer - [Figure 87](#).
 - cable to connect Control Unit to Source - [Figure 88](#).
 - Laser Synchronization cable - [Figure 89](#).
 - video cable for CDD camera - [Figure 90](#).
 - Video Capture Board components - [Figures 91](#), [92](#), and [93](#).
 - UV-grade fiber optic cable with SMA-connectors - [Figure 94](#).
 - Laser Alignment Tool - [Figure 95](#).



Figure 83 Control Unit

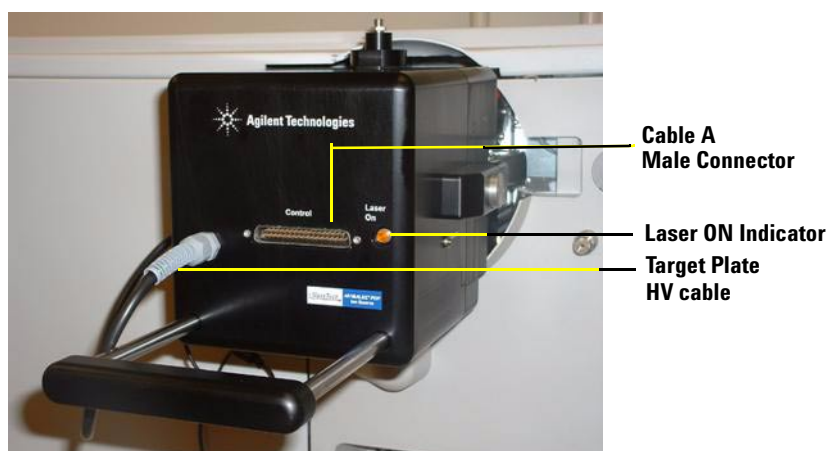


Figure 84 PDF-MALDI Ion Source

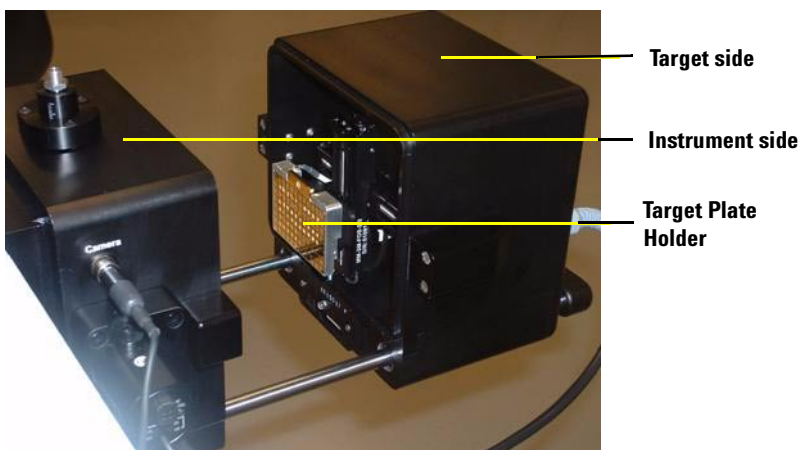


Figure 85 PDF-MALDI Ion Source open to show the 96-spot target plate

2 Installation for LC/MSD TOF Systems

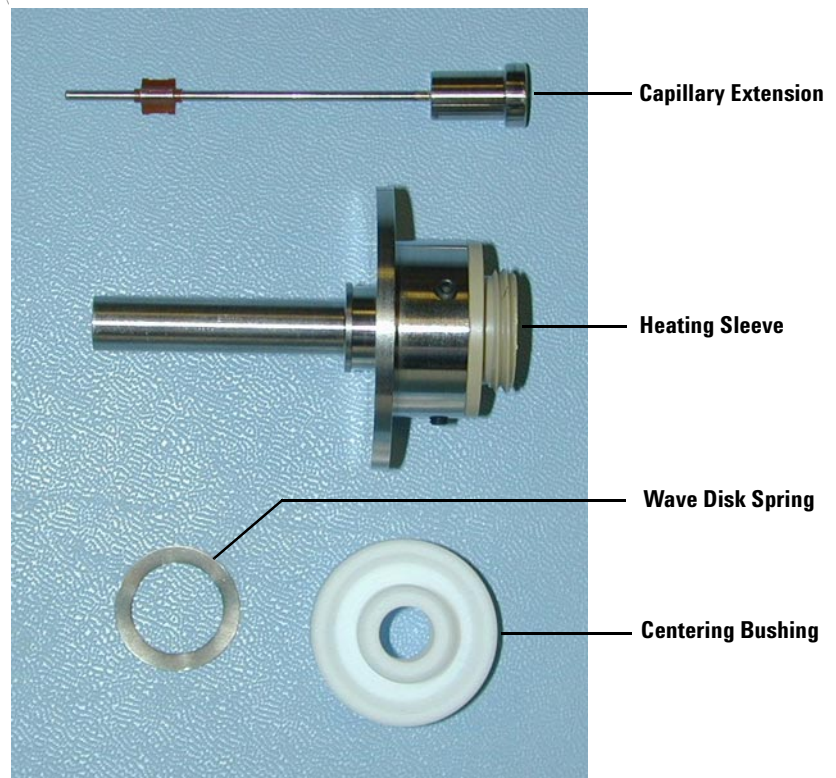


Figure 86 Capillary Extension components

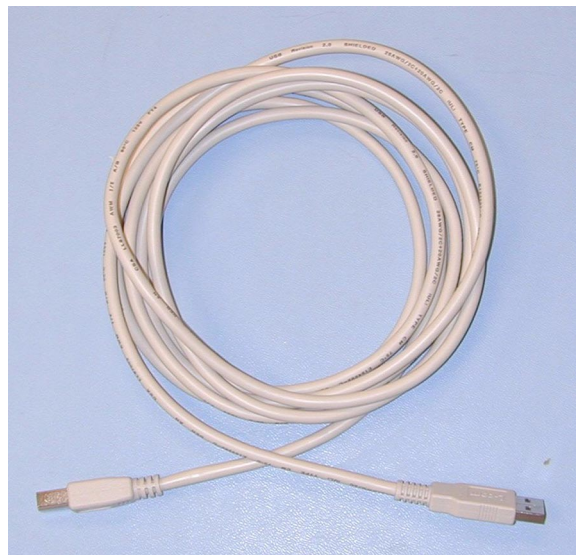


Figure 87 USB cable for Connecting Control Computer to Target Stage/Laser Control Unit



Figure 88 Cable for connecting Control unit to Source

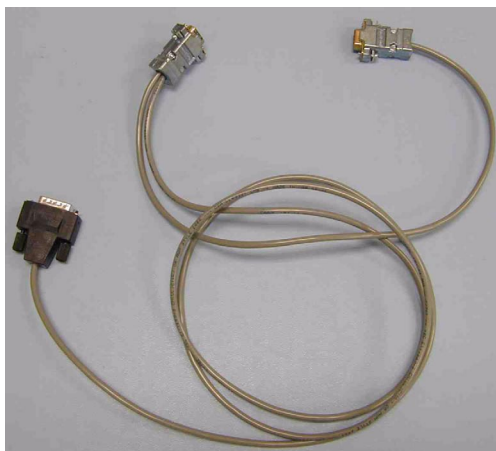


Figure 89 Laser Synchronization cable



Figure 90 Video cable for CCD Video camera

2 Installation for LC/MSD TOF Systems



Figure 91 Video Capture Board box

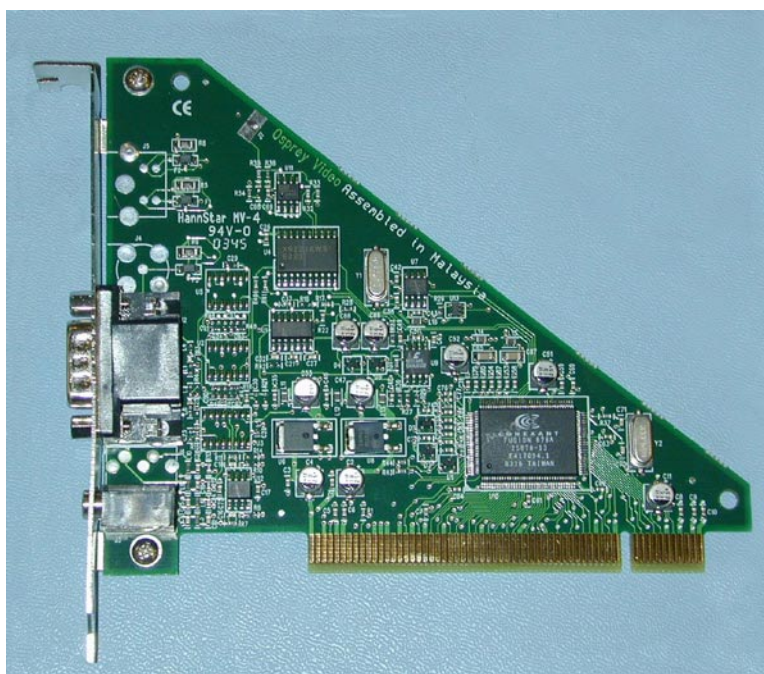


Figure 92 Video Capture Board

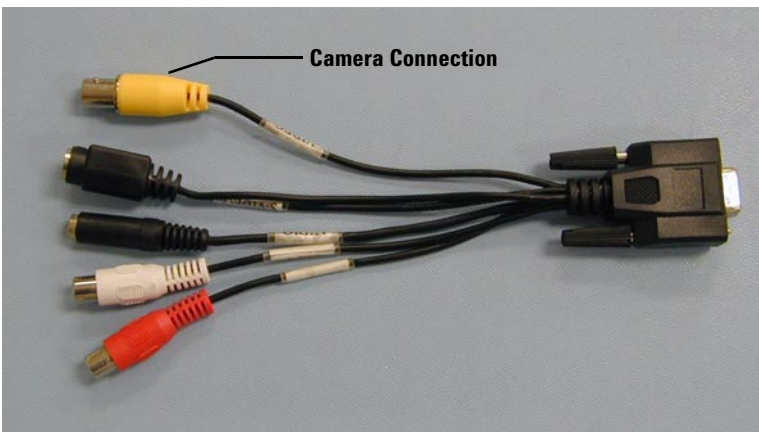


Figure 93 Video Capture Board cable

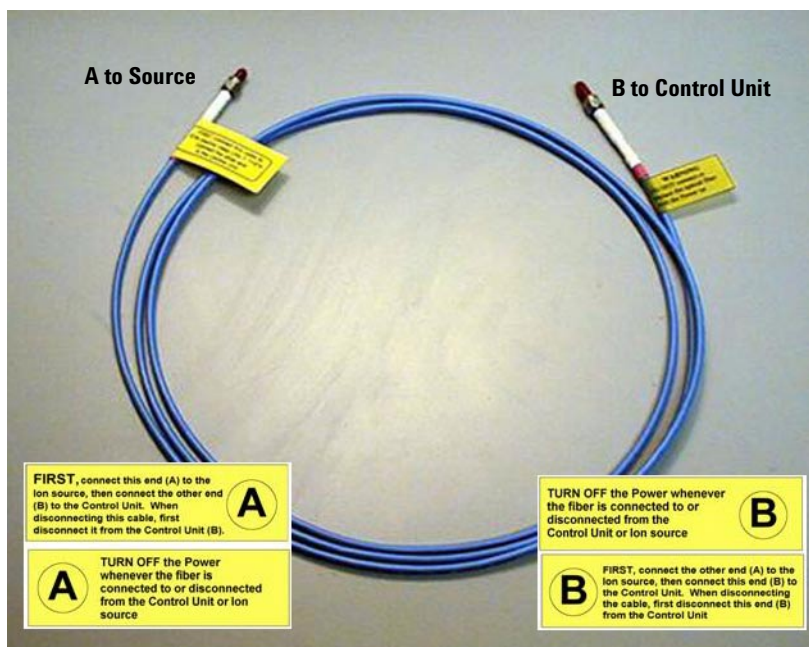


Figure 94 UV-grade fiber optic cable with SMA-connectors. Connectors are labeled on both sides and are covered with protective plastic caps. The shipment includes one spare fiber optic cable (not shown here).



Figure 95 Laser Alignment Tool

Step 2. Install the Video Capture Board software

Install the Osprey drivers

- 1 Using Windows Explorer, locate and access the CD-ROM drive containing the Osprey Installation CD-ROM.
- 2 Navigate to the WIN2000 directory.
- 3 Double-click **SETUP.EXE**.

The Osprey Multimedia Capture Driver window displays. See [Figure 96](#).

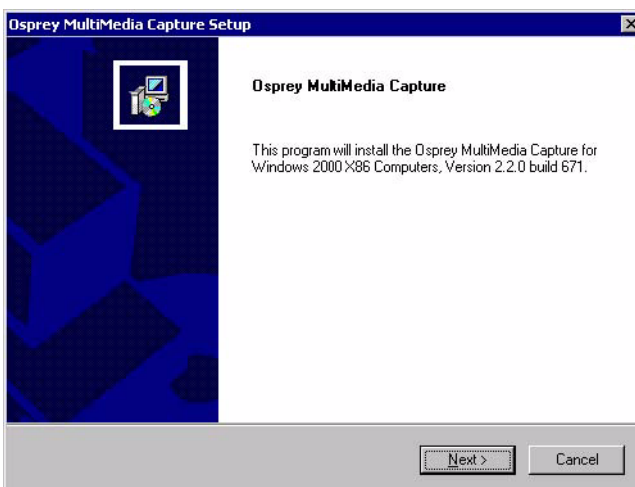


Figure 96 Video Capture Driver window

4 Click **Next**.

The Information window displays. See [Figure 97](#).

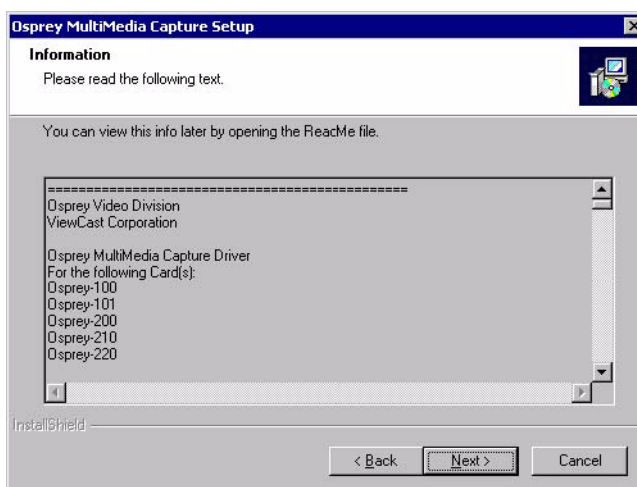


Figure 97 Information window

5 Click **Next**.

The Software License Agreement window displays. See [Figure 98](#).

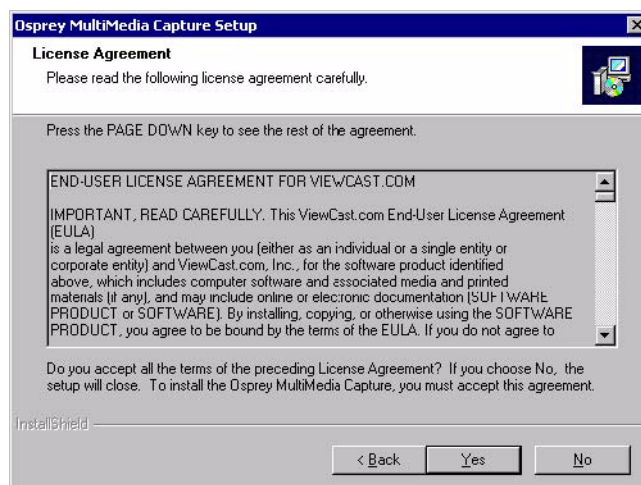


Figure 98 Software License Agreement window

6 Click **Yes** to accept the End User Software Agreement. If you do not wish to accept the agreement, click **No** to terminate the installation routine.

The Select Components window displays. See [Figure 99](#).

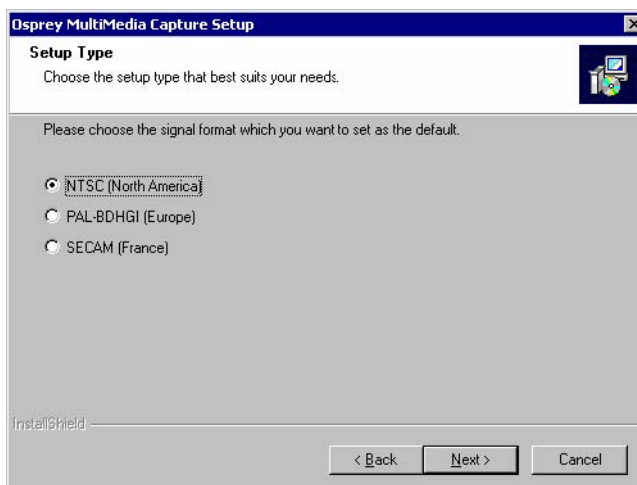


Figure 99 Select Components window

- 7 To set this default, select the video signal standard NTSC and click **Next**. See Video Standard for more information about signal formats.

The Choose Destination Location window displays. See [Figure 100](#).

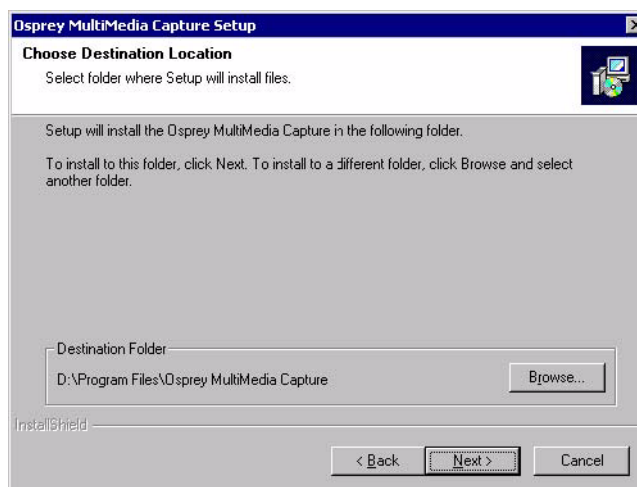


Figure 100 Choose Destination Location window

- 8 If you wish to change the destination location for the files, click **Browse**. Click **Next**.

The Select Program Folder window displays. See [Figure 101](#).

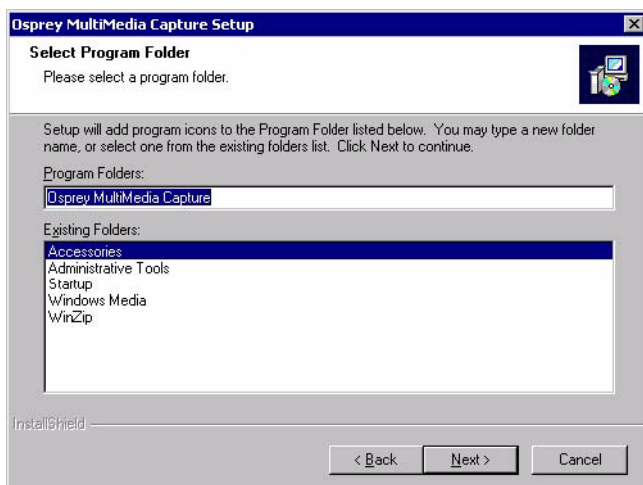


Figure 101 Select Program Folder window

- 9 Click **Next**. If you wish to change the program folder, type the new name in the Program Folders field.

The Start Copying Files window displays. See [Figure 102](#).

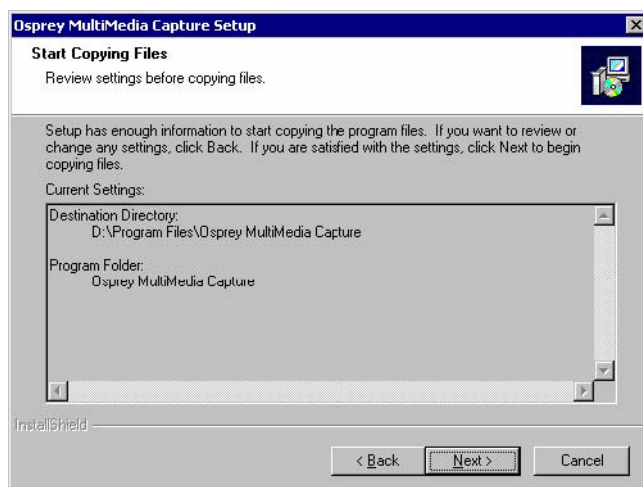


Figure 102 Start Copying Files window

10 Click **Next**.

The Ligos® Indeo® Information window displays. See [Figure 103](#).

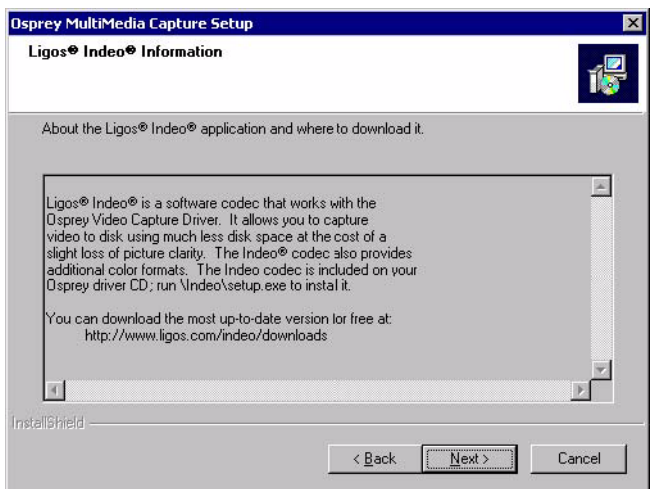


Figure 103 Ligos Indeo Information window

11 Click **Next**. If there are any settings to be changed prior to installation, click **Back** to return to the previous windows.

The Digital Signature Not Found window displays. See [Figure 104](#).



Figure 104 Digital Signature Not Found window

NOTE

Windows 2000 recognizes the audio and video portions of the Osprey cards as separate items.

12 Click **Yes** to continue the installation process.

The Osprey Technologies Special Offers Shortcut window displays. See [Figure 105](#).

**Figure 105** Osprey Technologies Special Offers Shortcut window**13** Install a shortcut.

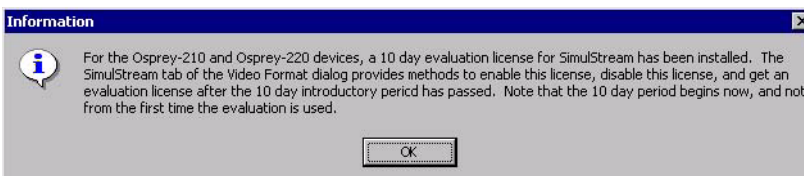
If you would like to install a shortcut on your desktop,

Click **Yes** and a shortcut is created on the desktop.

If you would not like to install a shortcut on your desktop,

Click **No**. A **Special Offers** link is also available on the **Programs** menu.

An Information window displays. See [Figure 106](#).

**Figure 106** Information window**14** Click **OK** to continue the installation.

The Product Registration window displays. See [Figure 107](#).

**Figure 107** Product Registration window

15 Register your Osprey Multimedia Capture card.

If you want to register your Osprey Multimedia Capture card,

Click **Yes**. A browser window opens.

If you do not want to register your Osprey Multimedia Capture card,

Click **No**. A product registration link is also available on the Programs menu or on the Osprey Video web site (<http://www.ospreyvideo.com/>)

The Setup Complete window displays. See [Figure 108](#).

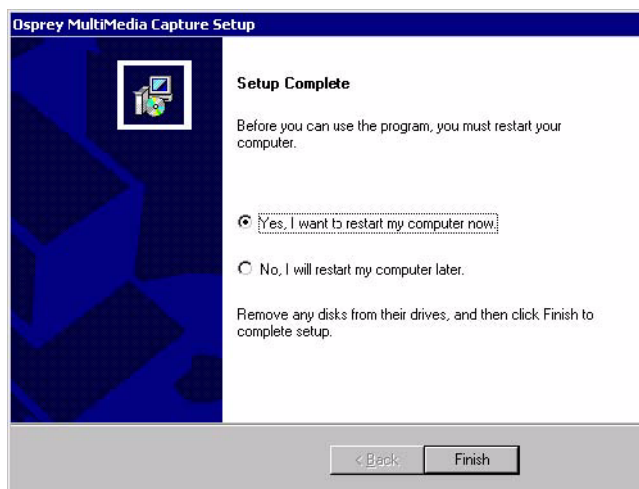


Figure 108 Setup Complete window

16 Click **Finish** to complete the installation. When prompted to restart the computer, click **No**, and proceed to the next step.

Step 3. Install the Video Capture Board hardware

CAUTION

Computer cards are sensitive to electrostatic discharge. Slight discharges from clothing or even from the normal work environment can adversely affect these cards. Follow these simple guidelines, to minimize the chance of damaging your Osprey card.

- Handle cards only by the non-conducting edges.
- Do not touch the card components or any other metal parts.
- Wear a grounding strap while handling the cards (if in a high static area).
- Provide a continuous path to ground by leaving the power cord plugged into a grounded power outlet.
- Ensure that the workstation is powered OFF before installing any components.

NOTE

If you are not familiar with how to install a PCI bus card, refer to your system's documentation for more complete, step-by-step instructions.

Use the following steps to install the Osprey card:

- 1 Power down the computer. Make sure that the computer's power switch is turned OFF. Read the Caution note above for grounding precautions.
- 2 Remove the computer's cover.
- 3 Locate an empty PCI slot.
- 4 Remove the slot-cover screw from the empty PCI slot's cover, set the screw aside, and remove the slot cover.
- 5 Remove the card from its anti-static bag.
- 6 Install the Osprey card into the empty slot.

NOTE

Be sure that the card is seated firmly and evenly in the slot.

- 7 Secure the backpanel of the card with the slot's cover screw.
- 8 Replace the computer cover.
- 9 Turn the computer **ON**.

Once your computer has booted and you have logged on, two windows display regarding “Digital Signature Not Found” for the Video Capture Board and the audio capture portion of the board. See [Figure 104](#) for an example of these Warning windows.

- 10 Click **Yes** to the “Digital Signature Not Found” message to continue the installation.

Step 4. Install the Source

In this step, you attach the PDF-MALDI Source to the LC/MSD TOF. If you are installing the Source for the first time, you may skip instruction 1 in this procedure. You should have already confirmed that LC/MSD TOF meets or has met these requirements.

WARNING

Never switch the power ON at the rear panel of the PDF-MALDI Control Unit until the Source is completely installed, fiber optic cable properly connected at both ends, and the Control connector properly connected to the PDF-MALDI Source.

The Agilent 1100 Series LC/MSD TOF software must be in the Shutdown state.

The Spray Shield and the Capillary Cap may be very hot. Allow them to cool before handling.

- 1 Using the Dual Electrospray source, the LC/MSD TOF should be autotuned before installing the PDF-MALDI Source. All the tuning mass ions must have the correct mass assignments and acceptable resolution.
- 2 If the TOF software application is open, shut down the user interface. Using the system launcher, shut down the TOF software engines.
- 3 From the **Windows** start button, go to **Programs > Agilent > TOF Software > Tools** and launch Instrument Configuration.
- 4 In the TOF Software Instrument Manager, remove the device type **Agilent 1100 Series System Access** in the **Device Type** list.
- 5 Click **OK**.
- 6 Disconnect the HPLC nebulizing gas line, the HPLC sample introduction line, the Reference Mass Introduction Line, and the Reference Nebulizer Gas line. You will need to plug the Reference Gas line at the side of the instrument. See [Figure 109](#).

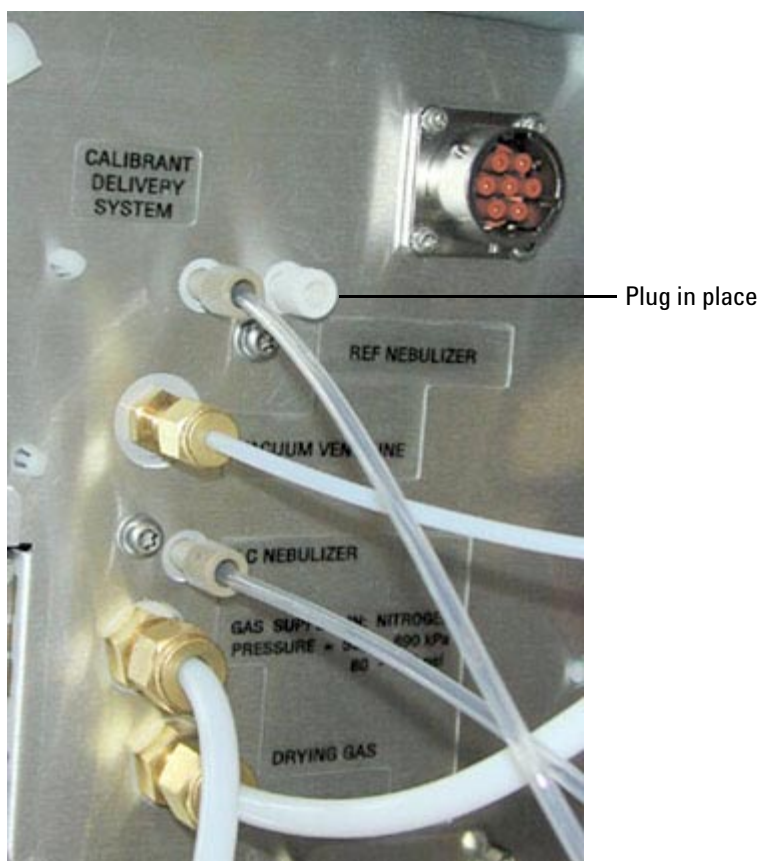


Figure 109 Plug the Reference Gas supply line

- 7 Remove the ESI Source:
 - a Loosen the latch on the spray chamber.
 - b Swing the Source open.
 - c Lift the ESI Source off the hinge. The contact pin must clear the PTFE end plate mount.
- 8 Unscrew the Spray Shield and Capillary Cap. Gently pull them off.
- 9 Place the ESI Source and its components in a safe, dust-free environment.
- 10 Gently install the Capillary Extension on top of the LC/MSD glass capillary. See [Figure 110](#).

You will feel a slight resistance at first as the gold-plated spring inside of the Capillary Extension is compressed. Be careful not to apply excessive force.

WARNING

Do not twist the Capillary Extension onto the glass capillary. It can damage the glass capillary.



Figure 110 Install the Capillary Extension to the LC/MSD TOF

- 11 Carefully slide the Heating Sleeve over the Capillary Extension, and screw it into the end plate (finger tight). See [Figure 111](#).



Figure 111 Install the Heating Sleeve over the Capillary Extension

- 12 Place the Wave Disk Spring over the Heating Sleeve. See [Figure 112](#).



Figure 112 Install the Wave Disk Spring

- 13** Slide the white PTFE Centering Bushing over the Heating Sleeve. See [Figure 113](#).



Figure 113 Slide the PTFE Centering Bushing over the Wave Disk Spring to finish the Capillary Extension Assembly

- 14** For ease of installation, remove the side cover door. This will also prevent accidental damage to the CCD camera. Align the interface Flange prongs with the hinge on the Inlet Flange. See [Figure 114](#). The CCD camera should be almost touching the instrument cabinet.

CAUTION

Do not tap the Capillary Extension assembly.

- 15 Carefully slide the prongs into the hinge and pivot the Source until the Interface Flange rests against the Inlet Flange. Do not damage the capillary extension.

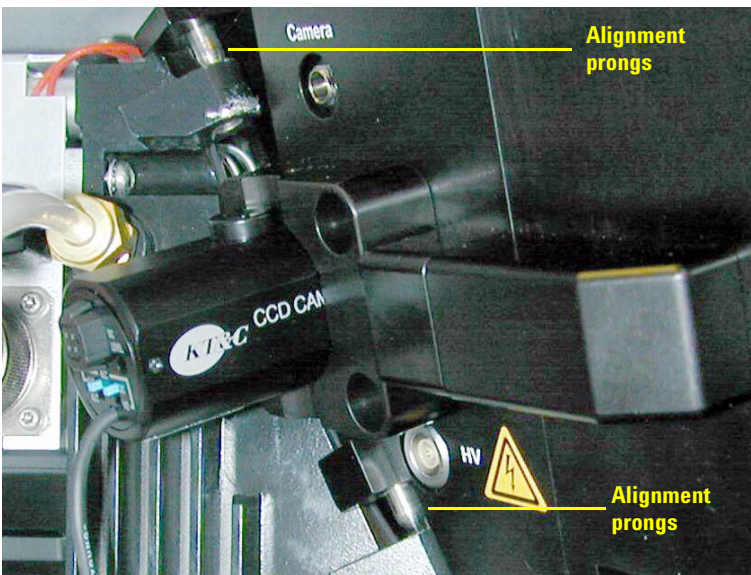


Figure 114 Slide the prongs into the hinge

- 16 Secure the desolvation assembly latch on the PDF-MALDI Source to a very tight fit. See [Figure 115](#).

NOTE

If the desolvation assembly latch is not tight, the PDF-MALDI Source will not be aligned properly resulting in a significant loss of sensitivity. To be sure, rotate the latch clockwise one turn at a time until the latch cannot be closed.

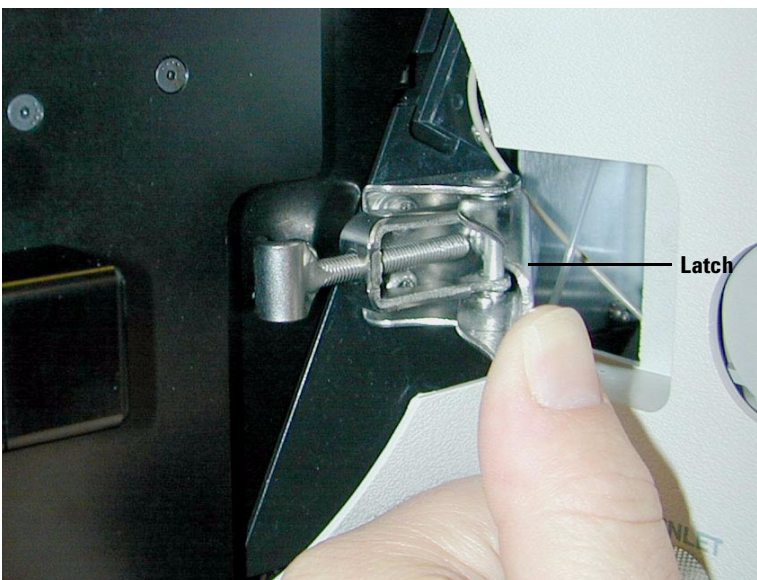


Figure 115 Secure both halves of the Source, and anchor it by latching it to the LC/MSD instrument

Step 5. Attach cables to the Control Unit and the Source

- 1 Check that the power on the Control Unit is turned OFF.
- 2 Connect cables to the back of the Control Unit.
 - a Attach the power cord to the power receptacle.
 - b Connect one end of the USB cable of the **Target Stage/Laser Control Box** to the port labeled **USB**. Please use [Figure 116](#) for a reference picture.
 - c Attach the 15-pin end of the Laser Synchronization cable to the **External Controls** port.

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

2 Installation for LC/MSD TOF Systems

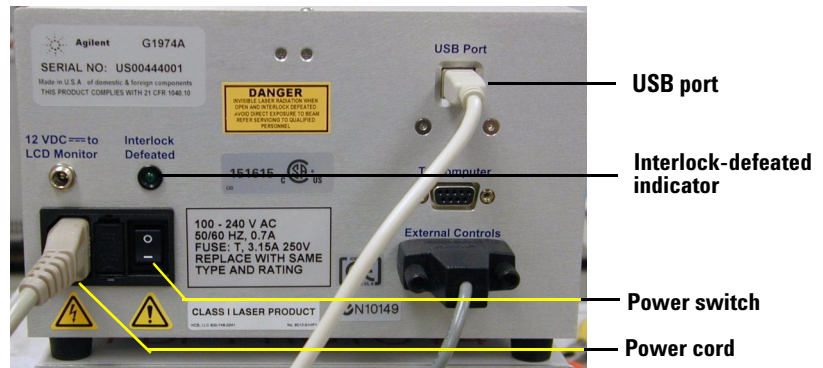


Figure 116 Rear panel of the Control Unit

- 3 Connect the other end of USB cable to an open USB port on the LC/MSD instrument control computer. See [Figure 117](#).

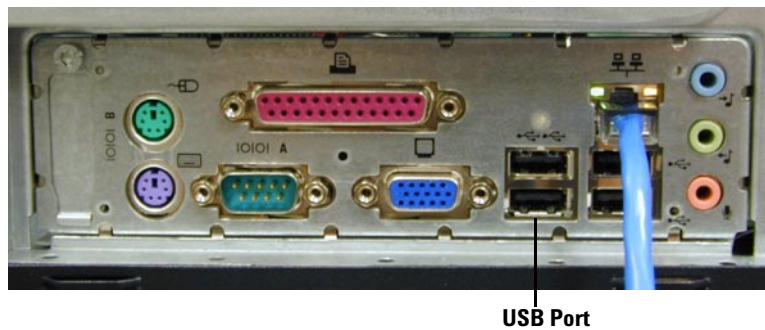


Figure 117 USB connector on the back panel of a computer

- 4 Connect the other ends of the Laser Synchronization cable to the side of the LC/MSD TOF.
 - a Attach the 9-pin connector with two cables present to the **Remote** port on the LC/MSD TOF Smart Card. See [Figure 118](#).

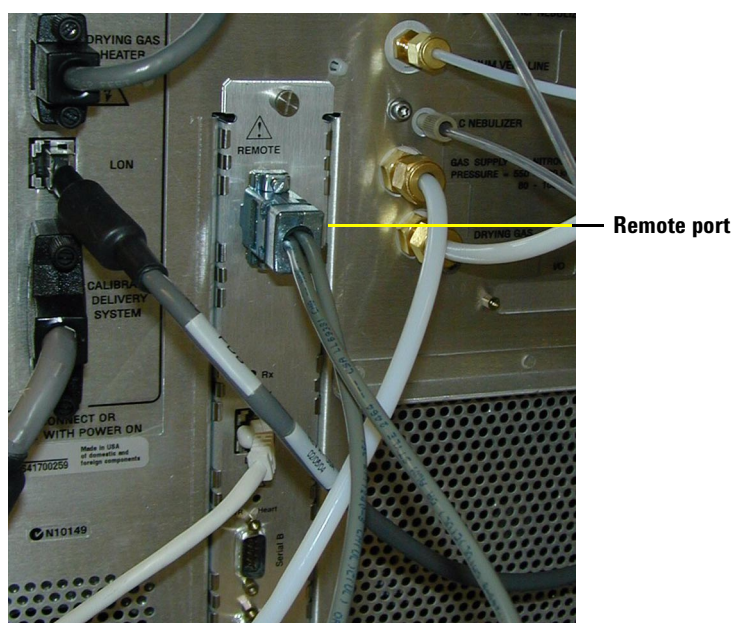


Figure 118 Connect the Laser Synchronization cable to the Remote port

- 5 Connect one end of the video camera cable to the yellow RCA connector on the Video Capture Board cable. See [Figure 119](#).



Figure 119 Connect the video camera cable to the Video Capture Board connector

- 6 Connect the Video cable to the Camera Video cable. See [Figure 120](#).



Figure 120 Connect the video cable to the camera video cable

- 7 Attach the Stage Control cable to the Source Flange. See [Figure 121](#).



Figure 121 Connect Stage Control cable to Source Flange

- 8 Connect Stage Control cable to Control unit. See [Figure 122](#).



Figure 122 Connect Stage Control cable to Control unit

- 9 Connect the Target Stage HV cable to the HV connection on the Source body.
- 10 Connect the end of the fiber optic cable labeled “A” to the PDF-MALDI Source Flange. Tighten the nut using only your fingers (“finger-tight”). See [Figure 123](#).

WARNING

Be careful not to turn the connector.



Figure 123 Connect fiber optic cable to Source Flange

- 11 Connect the end of the fiber optic cable labeled “B” to the Control Unit. See [Figure 124](#).



Figure 124 Connect fiber optic cable to Control Unit

- 12 Prepare to turn on the power:
 - a Check that the HV connector is firmly connected.
 - b Check that both ends of the fiber optic cable are firmly connected.

WARNING

Do not turn on the Control Unit until after the USB drivers are loaded.

When complete, your LC/MSD TOF with PDF-MALDI Source will look like [Figure 125](#).

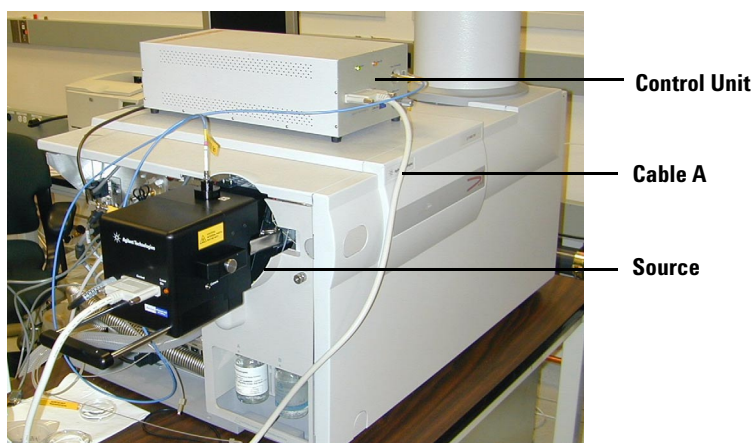


Figure 125 PDF-MALDI Source installed on LC/MSD TOF

Step 6. Install the Target software

This step describes how to install the Target software. You only need to do this the first time the Source is installed.

- 1 If you are installing the Target 5.0 software on a Windows 2000 system, check to be sure that you have Administrator privileges.
- 2 Insert the Target 5.0 software CD into the CDROM drive. The disk should start automatically and install the Target 5.0 software. If it does not start automatically, browse to the **Setup** directory and run the `Setup.exe` program. When the program starts, click the Next button to proceed with the installation. See [Figure 126](#).

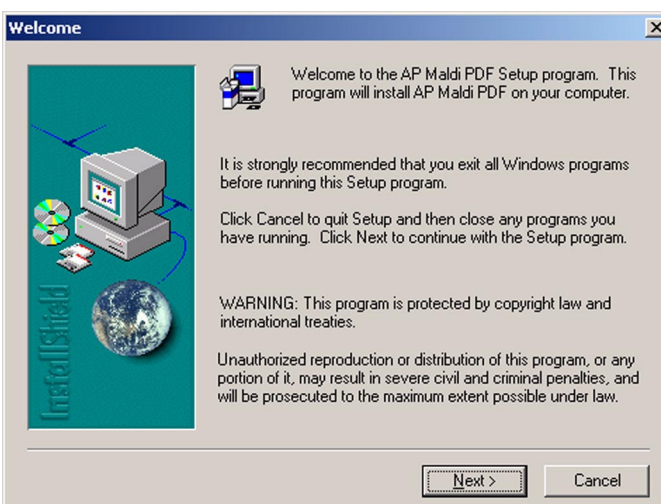


Figure 126 Target setup Welcome window

- 3 Choose a location and folder name for the Target software. By default, the folder is **C:\Program Files\MassTech\AP MALDI PDF**. See [Figure 127](#).

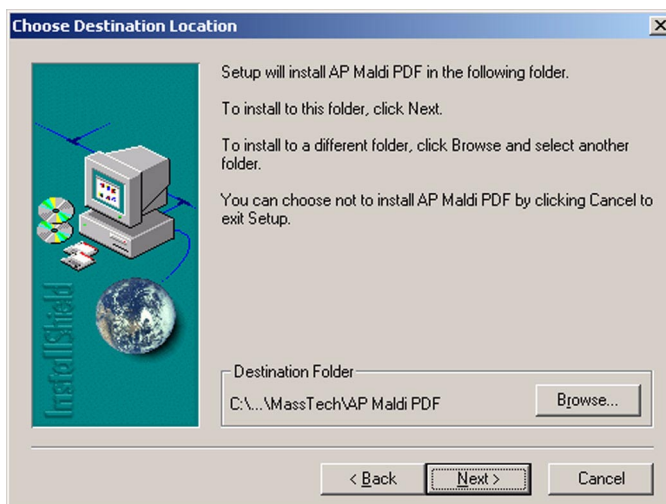


Figure 127 Choose destination location for Target software

- 4 Click Next in the next window to install the files required to control the PDF-MALDI Source. See [Figure 128](#)



Figure 128 Load Target software control components

- 5 Click Next in the next window to create the program file folder. See [Figure 129](#).

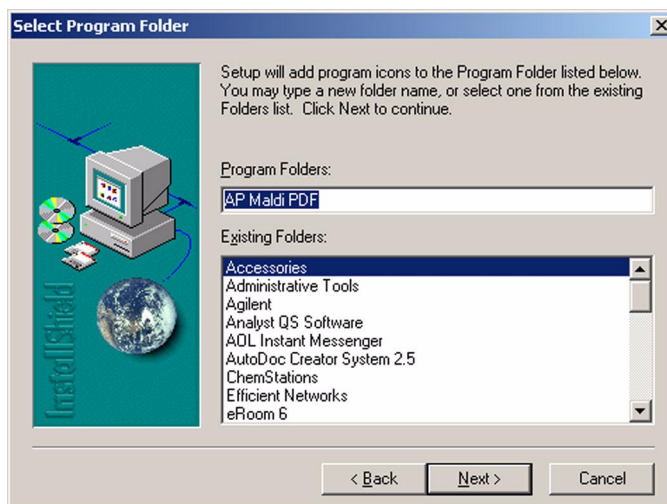


Figure 129 Select program folder for Target software

- 6 After the installation has completed, a window displays confirming that the installation has completed successfully. See [Figure 130](#).

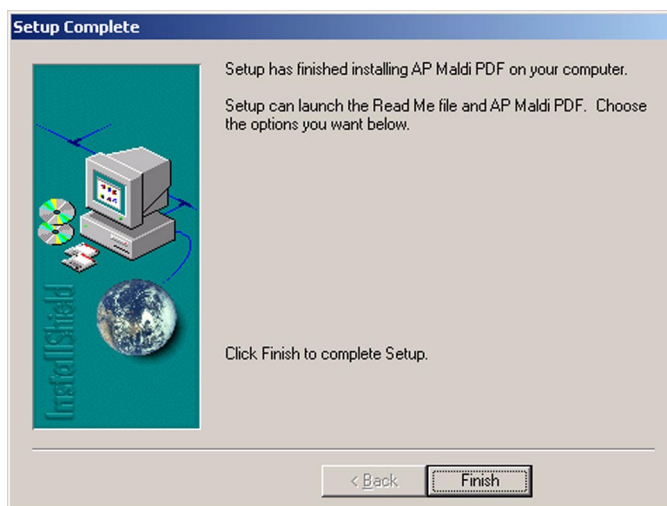


Figure 130 Target software setup completion window

Step 7. Loading the PDF-MALDI USB Drivers

- 1 If the PDF-MALDI Software CDROM is not in the CDROM driver, load the CDROM into the CDROM drive of the computer.
- 2 Turn on the PDF Control Unit. The first time the PDF-MALDI source is turned on, the LC/MSD TOF Control computer will detect the source as a new USB device. See [Figure 131](#).



Figure 131 Found new hardware

The Found New Hardware Wizard window will appear. See [Figure 132](#).

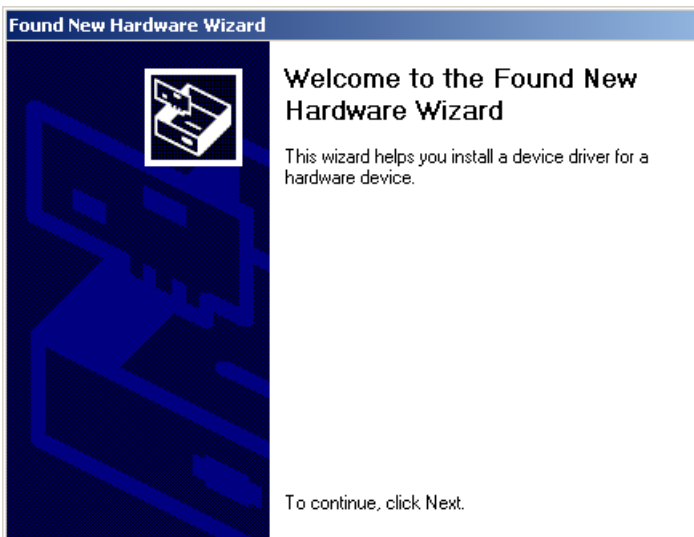


Figure 132 Found New Hardware Wizard

- 3 Click the Next Button.
- 4 Then the Wizard will prompt you to search for a suitable driver. See [Figure 133](#).



Figure 133 Search for a suitable driver

- 5 Select only the CD-ROM drives as locations to search for the driver. Then click Next. See [Figure 134](#).

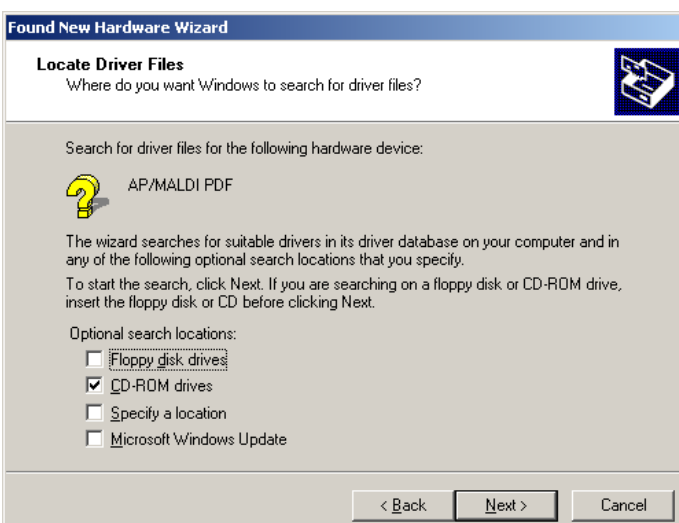


Figure 134 Select only the CD-ROM drives

- 6 The wizard should find the correct USB driver for the PDF-MALDI source. See [Figure 135](#) for the correct driver name.

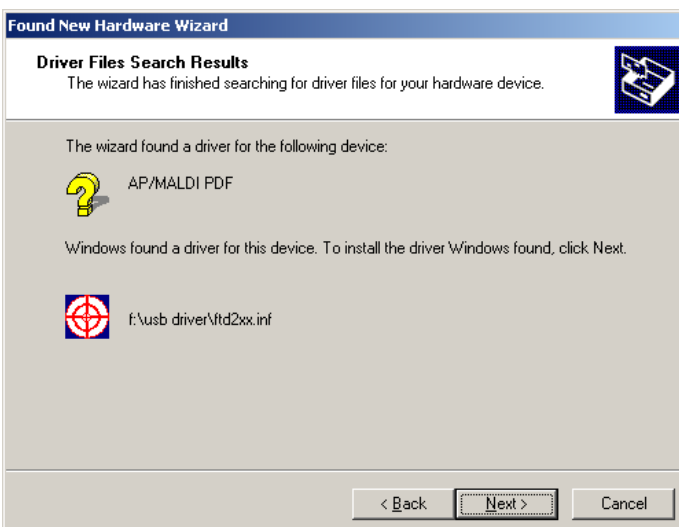


Figure 135 Correct driver found

- 7 Click the Next button to load the driver.
- 8 After the driver has loaded correctly, a message will appear confirming that the driver has loaded correctly. See [Figure 136](#).



Figure 136 USB driver loaded successfully

Configuration and verification

This step describes how to configure your system and verify the installation after installing all of the hardware and software.

NOTE

The initial verification should be performed by a qualified Agilent service representative. The following procedure can be used as a troubleshooting tool or to check for sensitivity during normal use.

Step 1. Program the default method

This method should be used as a template for developing other PDF-MALDI methods. Keep in mind that Agilent has already optimized this method for PDF-MALDI so further optimization should not be necessary.

MS method for LC/MSD TOF

Data	Stop time	1.00 min
	Storage mode	Profile
	Transients/Scan	40,000
	Min Range	300 <i>m/z</i>
	Max Range	3200 <i>m/z</i>
	Abs. Centroid Threshold	5000 counts
	Rel. Centroid Threshold	0.010 (% counts)
	MS Parameters	Fragmentor
Skimmer		60 V
Oct RF V		300 V
Ion Source	Source	MALDI
	Ion Polarity	Positive
	Gas Temp	325°C
	Drying Gas	5.0 L/min
Reference Masses	Clear check boxes for Enable Reference Mass Correction and Use Bottle A .	

NOTE

Save this method as **PDF-MALDI default.m**.

Step 2. Use the Alignment Tool

NOTE

Use this procedure *only* for the G1974A PDF-MALDI Source.

- 1 In the LC/MSD TOF software, set the drying gas flow to 3.5 L/min., drying gas temperature to 0°C, and turn off the capillary high voltage by setting the capillary voltage to 0 V for LC/MSD TOF.
- 2 In the PDF-MALDI Target software, click spot D6 to select it. Deselect the push button for Spiral Motion. See [Figure 137](#).

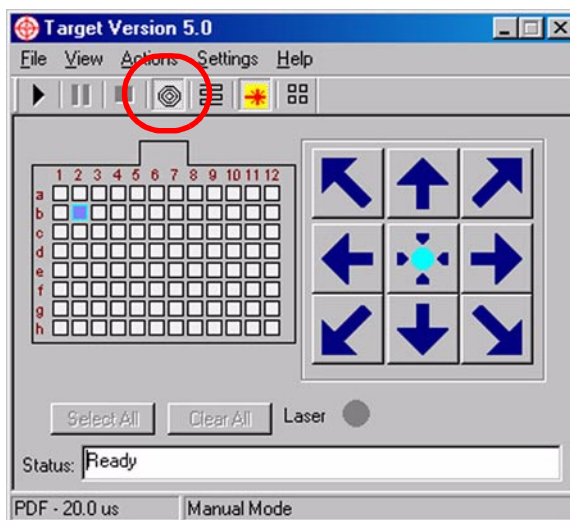


Figure 137 Deselect the Spiral Motion button circled in red

- 3 Affix a yellow label to the center of the back side of a MALDI plate, as shown on [Figure 138](#). Be careful to select the back of the plate rather than the front side since any adhesive residue from the label can contaminate the surface of the plate and render it unusable.

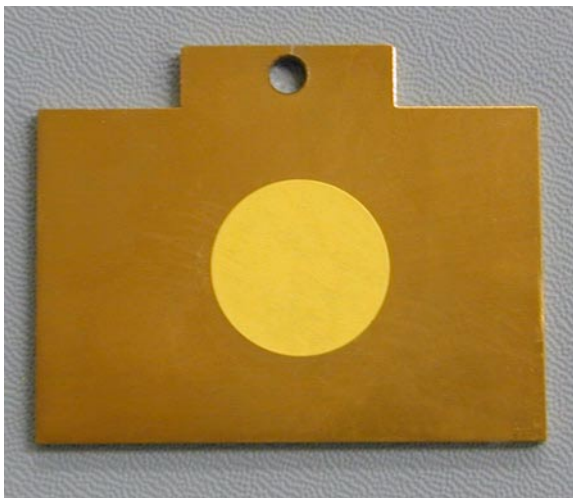


Figure 138 Target plate with yellow label at its center

- 4 Open the AP-MALDI Target Flange, and insert the plate in the plate holder with the yellow label facing up.

WARNING

Although the Source voltages are OFF when the Ion Source is open, the Capillary Extension will be hot, and heated drying gas is still flowing around the Capillary Extension. The Laser Alignment tool will be heated by the drying gas flow.

- 5 Carefully insert the Laser Alignment fixture on the Capillary Extension. Make sure the spring loaded Alignment fixture can move freely on the Capillary Extension by pushing it gently all the way in against the tip of the Capillary Extension. See [Figure 139](#).

CAUTION

Be careful not to apply any radial force to the Capillary Extension to avoid bending it off center.

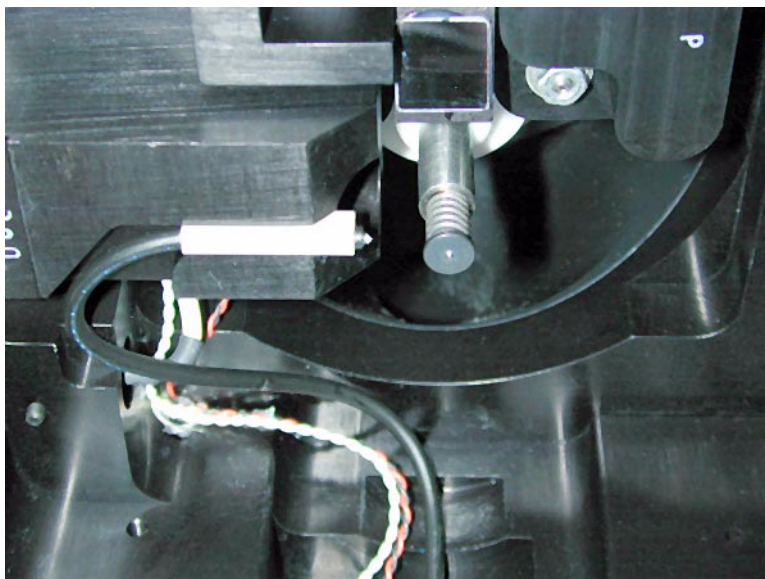


Figure 139 Install the Laser Alignment tool carefully

- 6 Gently close the Source completely, then re-open it.
- 7 Remove the Alignment tool and re-close the Source.
- 8 A small dimple left by the capillary Alignment Tool should be visible on the plate video image. See the example in [Figure 140](#).

NOTE

You may have to decrease the video image brightness in order to see the dimple clearly.

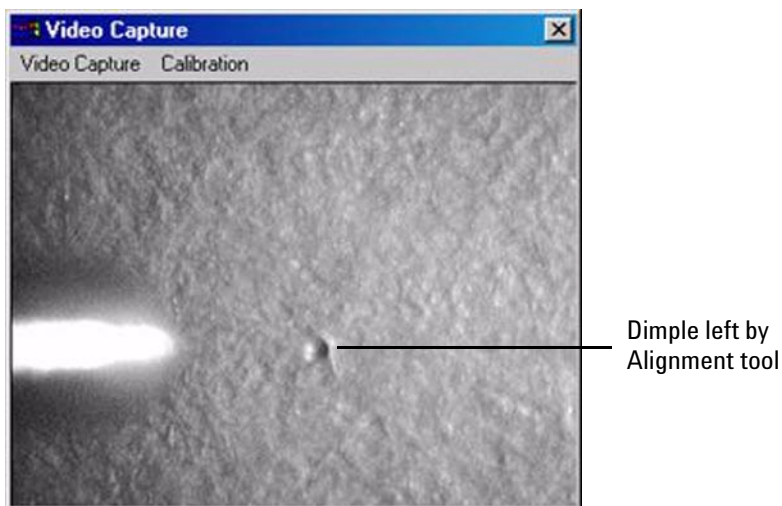



Figure 140 Dimple in paper target plate

- 9 Select the **Play** (Start) button  to turn on the laser. If the laser optics are properly aligned, the dimple should be in the center of the laser spot. See the example in [Figure 141](#). If the laser is not properly centered on the dimple, the laser spot must be repositioned.

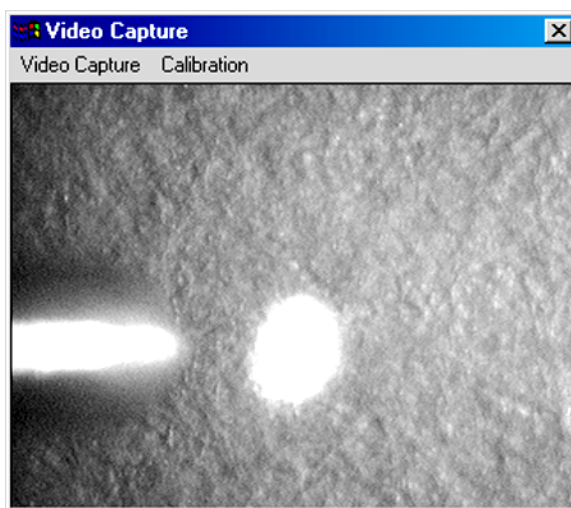


Figure 141 Properly aligned laser spot

- 10 Use the following procedure to properly align the laser. Typically, only two of the setscrews need to be turned to adjust the laser spot.
- Using a 2.5 mm hex driver, carefully adjust the two screws indicated in [Figure 142](#).
 - Turn the screws very slowly because the adjustment is very sensitive.
 - While watching the computer monitor screen, adjust the location of the laser spot until it covers the dimple.
 - Once adjusted, the laser position should resemble [Figure 141](#) from the previous step in this procedure.

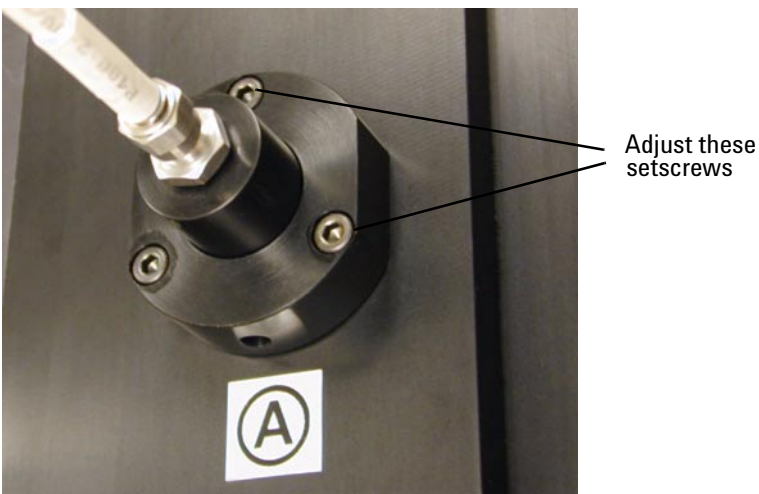


Figure 142 Turn the setscrews to adjust the laser spot location

- 11 Remove the MALDI plate and carefully remove fluorescent label. Remove any adhesive residue from the label by wiping the plate surface with a tissue wetted with isopropanol.

WARNING

The MALDI target plate will be warm to the touch.

- 12 Return the drying gas to its normal value of 5 L/min. at 325°C and allow at least 10 minutes for it to reach proper temperature equilibrium before running a sample. Remember to set the Capillary Voltage back to 3000 V in the TOF control software.

- 13 The laser alignment procedure is now complete.

NOTE

If the laser spot appear diffuse, please refer to the Troubleshooting section in chapter 3 for refocusing the laser spot.

Step 3. Verify the hardware installation

If the Target software is not open, click the **Target** software icon on the desktop to start the PDF-MALDI Target software. Wait until initialization finishes and the **Ready** status message displays.

CAUTION

Do *not* open the Source while the software is initializing. The plate transport mechanism locates its reference points by moving to the extremes of its travel. Opening the Source during this time may jam the mechanism or damage the hardware.

Step 4. Check the Target Plate alignment

In this step, you prepare the Target Plate to center the Matrix spot. Use the G2037A matrix undiluted.

- 1 Prepare a 1:5 dilution of the G2037A Matrix. Take 10 μl of Matrix and add 40 μl of Matrix Solution solvent.
- 2 Using an Agilent Target Plate (p/n G1972-60025), spot 1 μl of the diluted Matrix mixture on one corner of the plate. For example, at A1.
- 3 Open the PDF-MALDI Source. See [Figure 143](#).



Figure 143 Open the PDF-MALDI Source

- 4 Insert the Target Plate with the prepared sample spots into the Target Plate Holder. The plate is held in place by a magnet. See [Figure 144](#).

NOTE

If you open the Source while the LC/MSD TOF is recording the spectrum (HV is ON) or the laser is firing, the PDF-MALDI Source safety interlocks automatically switch the High Voltage and the Laser OFF.

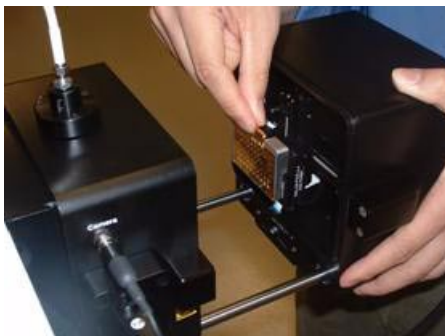


Figure 144 Inserting the Target Plate

5 Close and lock the Source.

When the Source is closed, you should hear a “click” sound indicating that the two halves of the Source are locked together.

When the software is running and the Target Plate is loaded properly, you will see the following video capture image. See [Figure 145](#).

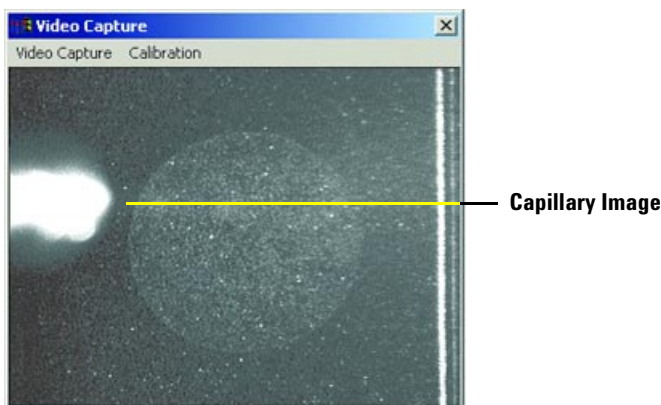


Figure 145 Typical sample spot

Step 5. Center the matrix spot

NOTE

Do these steps only if you plan to use the Geometry setting for Autosequence runs with the Target software

- 1 In the Target software, select the Settings pull-down menu, and click on the Spiral Motion tab. Copy the settings from

Figure 146 and enter them into the appropriate places in your software.

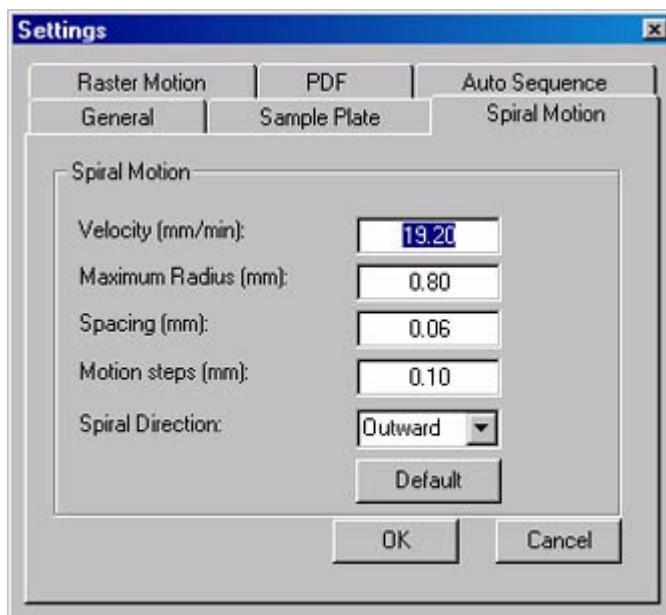


Figure 146 Spiral motion setting for Target Plate

2 Next, click on the Sample Plate tab. See Figure 147.

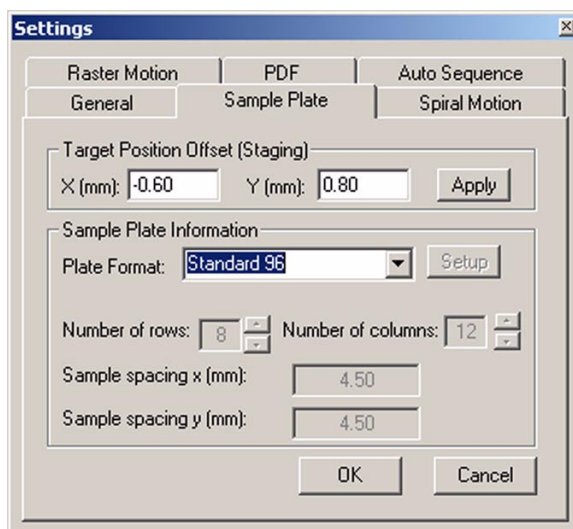


Figure 147 Sample Plate Settings tab

- 3 Center the Matrix spot:
 - a Move the **Settings** window to a position on the screen where you can view the **Video Capture** window and the **Sample Plate** settings window at the same time.
 - b In the Target Position Offset (Staging) step, enter values that approximate the center of the Target Plate.
 - c After you enter a value, click **Apply** to move the plate in the desired direction.
 - d You will have obtained the best position when it requires an equal number of manual movements to reach either side of the plate or the top and bottom of the plate.

Step 6. Calibrate the Video Capture sample-positioning system

A “point-and-click” system using the Video Capture imaging system was developed for changing the position of a sample that you want to irradiate with the laser. With this system, you can use the mouse pointer to choose a desired location on the ample image, then double-click the left mouse button to move the sample to a new location. You must calibrate this system before you can use it.

- 1 To calibrate the position of the spot using the “point-and-click” positioning system, go to **Calibration** on the Video Capture menu bar and choose **Start**. See [Figure 148](#).

A five-step calibration procedure is described in dialog boxes.

- 2 Ensure that there is a distinct visual object in the right-hand quadrant of the screen. See example in [Figure 148](#).

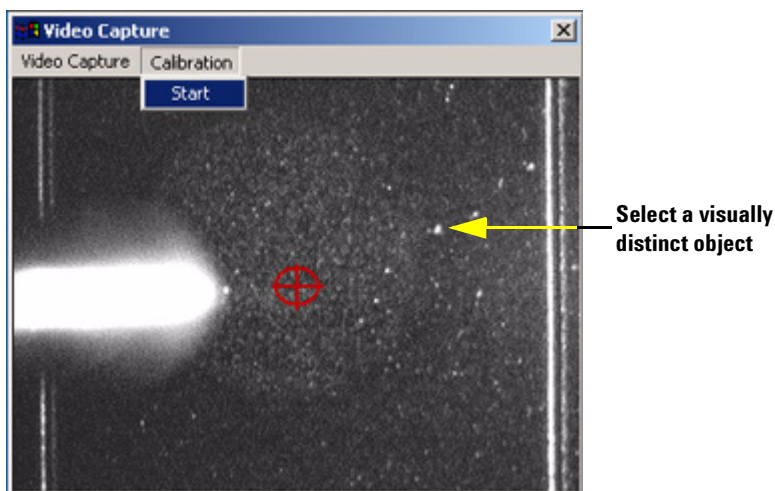


Figure 148 Start Video Capture sample-positioning Calibration Procedure by selecting visually distinct object

You can use a sample plate with ablated Matrix or dilute Matrix. If there is no distinct visual object, select another spot or prepare a new sample. Advance through the calibration procedure clicking **Next**. See [Figure 149](#).

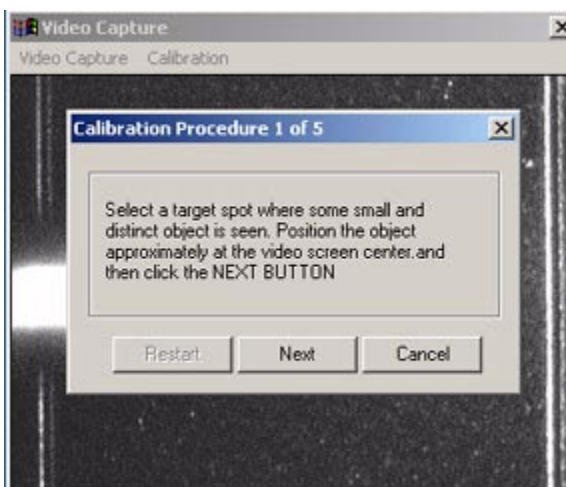


Figure 149 Step 1 dialog box of the calibration procedure

- 3 Locate the visually distinct object in the right-hand quadrant, then drag and drop the green target icon over the object. Click **Next**. See Figures 150 and 151.

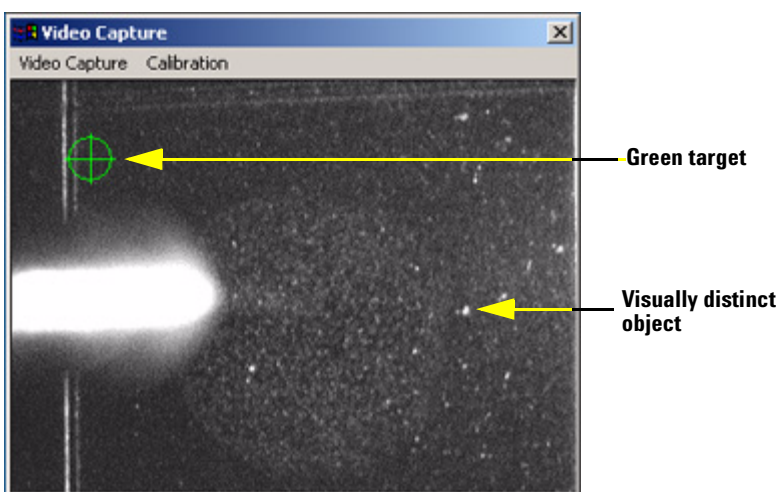


Figure 150 Video Capture screen showing green target and the visually distinct object that was selected

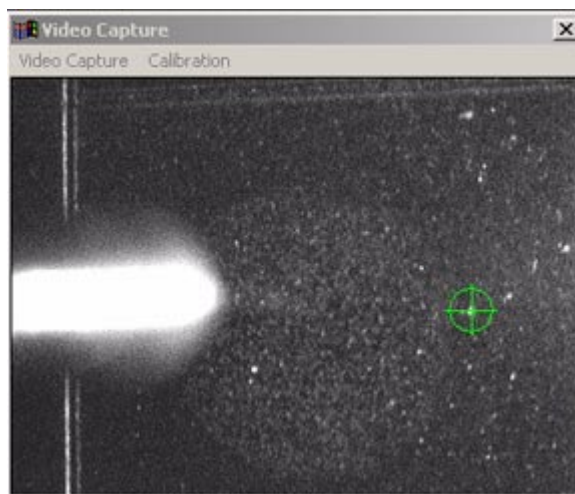


Figure 151 Video Capture screen showing green target that was dragged over the visually distinct object in step 2

- 4 In step 3 of this calibration procedure, the Target Plate moves horizontally (see [Figure 152](#)). Watch to see where the visually distinct object moves, then drag and drop the green target icon over the same object. See [Figure 153](#). Click **Next**.

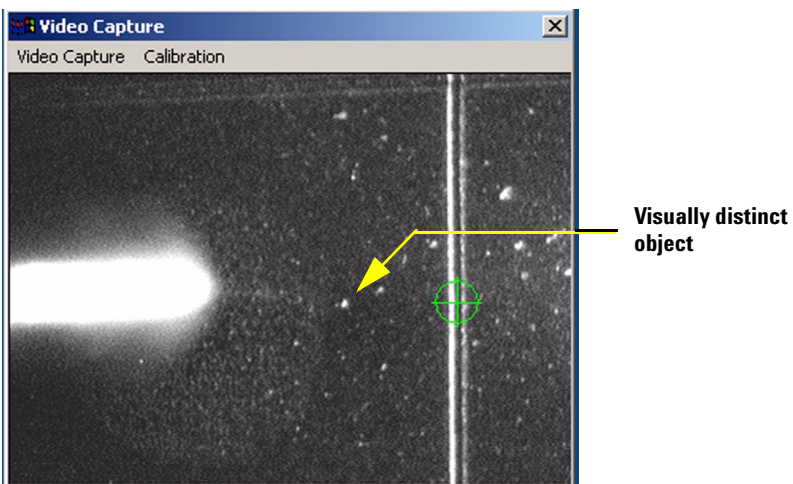


Figure 152 Video capture screen showing the location of the visually distinct object after step 2

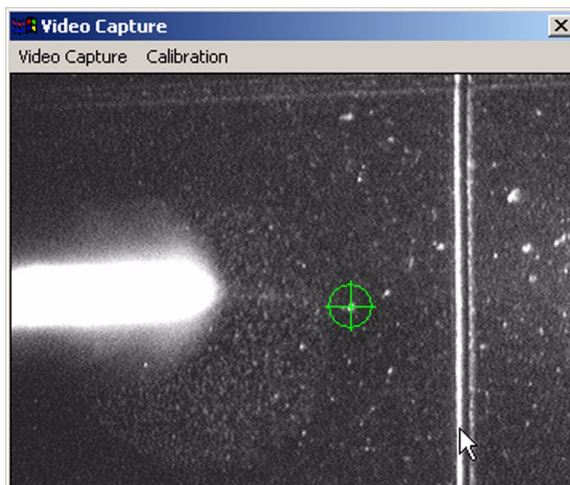


Figure 153 Video Capture screen showing green target that was dragged over the visually distinct object in step 3

- 5 In step 4 of this calibration procedure, the Target Plate moves again, vertically (see [Figure 154](#)). Watch to see where the visually distinct object moves, then drag and drop the green target icon over the same object. See [Figure 155](#). Click **Next**.

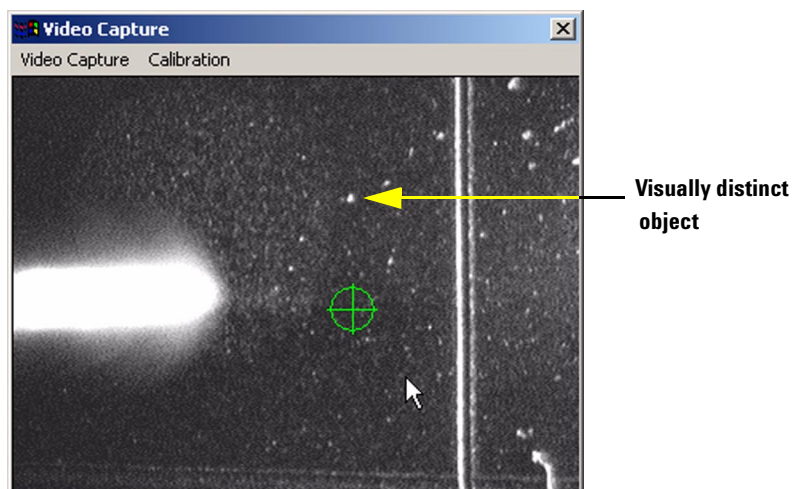


Figure 154 Video capture screen showing the location of the visually distinct object after step 4

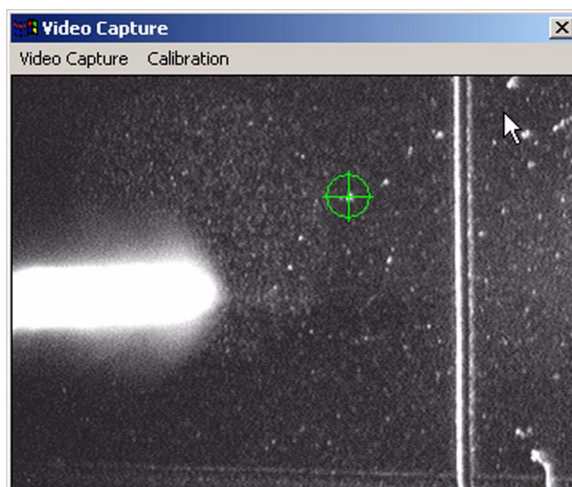


Figure 155 Video Capture screen showing green target that was dragged over the visually distinct object in step 4

- 6 In step 5 of the calibration procedure, click **Finish** to accept the new calibration. See [Figure 156](#).

You can click **Cancel** to retain the original calibration, or you can click **Restart** to start over and recalibrate the system again.

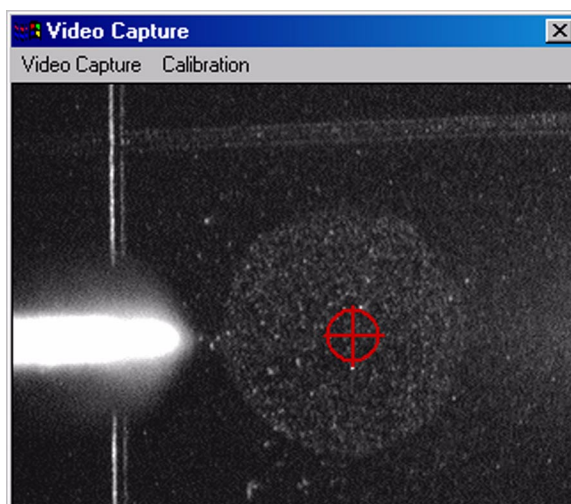


Figure 156 Video Capture screen after calibration was completed in step 5

- 7 To confirm that the “point-and-click” sample positioning is working, double-click the left mouse button on any location visible in the video capture window. The Target Plate should move to the location you selected.

Step 7. Verify the installation

This step should be performed by a qualified Agilent representative. These steps are only done the first time the Source is installed.

The verification test checks that the PDF-MALDI Source is properly installed and adjusted, and determines the sensitivity of the PDF-MALDI Source to the Agilent LC/MSD TOF system.

CAUTION

You must use Agilent Target Plates (p/n G1972-60025) to successfully complete verification.

- 1 Clean the Target Plate:
 - a Submerge plates in acetone and sonicate for about 3 minutes.
 - b Submerge plates in deionized water and methanol (1:1, v/v) with 50 mM ammonium formate (about 3-4 g/L). Sonicate for about 3 minutes.
 - c Submerge plates in deionized water and sonicate for about 3 minutes.
 - d Submerge plates in methanol and sonicate for about 3 minutes.
 - e Heat the plate in PDF-MALDI Source for 20 minutes or with a heat gun to completely dry the plate. You can also dry the Target Plate with a heat gun or in a clean oven at 105 – 120°C. If you do so, cool the Target Plate to room temperature before you spot the sample.

CAUTION

Do not use glassware such as beakers or flasks when cleaning the Target Plate. The glassware will add sodium to the Target Plate which will suppress ionization of the sample.

Do not use organic solvents (methanol, acetonitrile, isopropanol, acetone, or ethanol) in excess of 60% in the final matrix/sample mixture. You can overspread the matrix/sample deposit.

Do not use acetic acid in excess of 2%. High concentrations can cause unfavorable crystallization.

- 2 Prepare the BSA tryptic digest sample dilution.
 - a Dilute the BSA sample (p/n G1990-85000) with 500 µl of a water/acetonitrile solution (85/15) containing 0.1% formic acid.
 - b Add 10 µl of Matrix stock solution to a 500 µl centrifuge vial.
 - c Add 30 µl methanol to the vial, and vortex briefly to mix.
 - d Add 36.8 µl of Matrix dilution solution (water:isopropanol:acetic acid, 75:24:1), and vortex briefly to mix.
 - e Add 3.2 µl BSA sample that you prepared in step 2a and vortex thoroughly.

NOTE


For all solutions, you should use the highest quality solvents and acids that are available. You must mix the sample and Matrix thoroughly for each dilution step. Use a vortex mixer and centrifuge for faster results.

- 3 Spot the plates.
 - a Spot 3 to 5 target locations with 0.5 µl of the dilution mixture at room temperature. Try to use the center of the target location. This will spot 20 femtomoles of BSA on the Target Plate.
 - b Allow the Target Plate to air dry.

NOTE

Remember to clean the Target Plate before using it. Clean the plate using plastic beakers only, and be sure to dry the plate thoroughly. Allow the plate to cool before spotting the sample.

- 4 In the TOF software, load the method, **PDF-MALDI default.m**, that was created in [Step 1. Program the default method \(page 93\)](#).
- 5 Load the Target Plate.
- 6 Turn ON the LC/MSD TOF.

- 7 To run the verification manually:
 - a In the AnalystQS software, create a project by selecting from the menu: **Tools > Project > Create**. Enter the project name as PDF-MALDI checkout.
 - b In the Target software, select a location where you spotted a sample on the Target Plate.
 - c In the TOF software, select the **Method** pane. Then select the **Sample** tab.
 - d Enter a sample name and a data file name.
 - e Select the project PDF-MALDI checkout.
 - f Set the **Run** type to **Manual Start**.
 - g In the Target software, click the **Play** button.
 - h Click the **Start Sample Run button**  in the TOF software.
 - i Select the next sample location using the Target software.
 - j Repeat steps **c** through **h** until data has been acquired from all of the sample locations.

NOTE

The laser may require attenuation. Increase laser intensity by turning the laser attenuation knob counterclockwise starting from 3 in 0.25 increments until you get the results that you want. The laser intensity should generate a clean, isotopically resolved mass spectrum.

- 8 To run the verification with autosequence, follow the steps on pages 9 - 13 of the procedure "To acquire data automatically" of the *PDF-MALDI User's Guide* (p/n G1974-90005).
- 9 Determine the signal height by running DataAnalysis.
 - a Load the data file acquired onto the DataAnalysis software.
 - b Highlight the entire TIC trace, then right-click to select **Show Spectrum**.
 - c In the **Spectrum** pane, right-click to select **List Data**.
 - d Check that the minimum peak intensity (abundance) for the 927 peak is above 150 intensity counts.
 - e Compare the acquired spectra to [Figure 157](#) and [158](#).

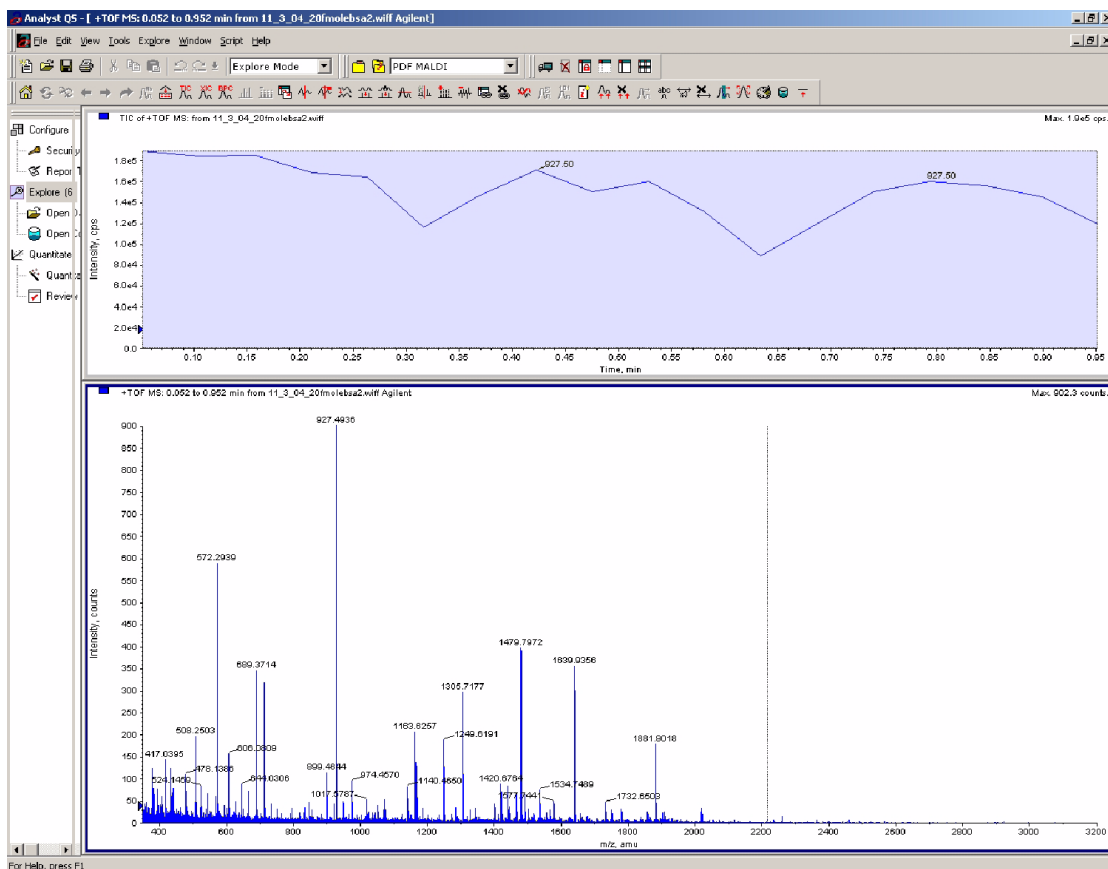


Figure 157 20 fmole BSA spectrum (unzoomed)

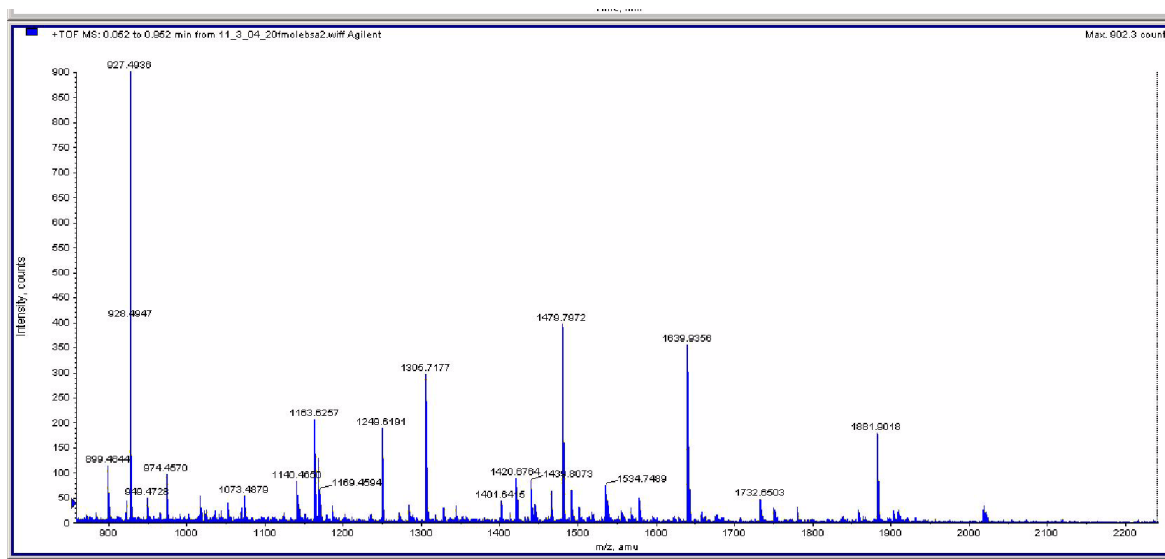
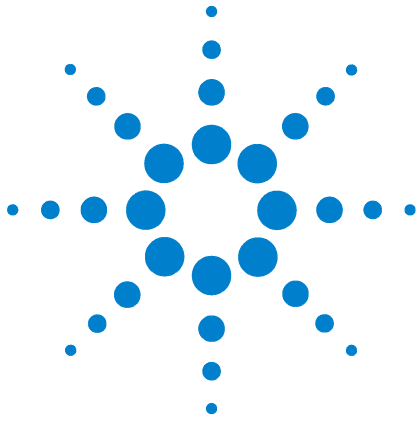


Figure 158 20 fmole BSA spectrum (zoomed)

If the verification test fails, refer to Chapter 7, “Troubleshooting” the *PDF-MALDI User’s Guide*.

Using the Mascot Database search

If you want to search the spectrum using the Mascot Database search, refer to page 55-62 of the *PDF-MALDI User’s Guide*.



3 Reference

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This chapter contains information about the default methods. If the default PDF-MALDI methods are not present on the system, use the following settings to create the default methods.



PDF-MALDI Method Settings for LC/MSD Trap SL, XCT, or XCT Plus

Default setting for an MS method

Mode	Scan mode:	Ultra scan (XCT and XCT Plus); or standard normal (SL)
	Segment end time:	1.5 min
Tune	Source:	AP-MALDI (positive)
	Vcap:	3000 V
	Drying gas flow:	5.0 L/min
	Dry gas temperature:	325°C
	Skim1:	40 V
	Capillary exit:	200 V
	Oct 1 DC:	12.0 V
	Oct 2 DC:	3.2 V
	Trap drive:	110 V
	Oct RF:	200 V
	Lens 1:	- 5 V
	Lens 2:	- 60 V
	Block voltages:	Mark the check box for Follow values on Page Tune Expert.
	ICC Values	Target:
Maximum accumulation time:		535 ms (corresponding to 6 laser shots)
Scan range:		400-2200 <i>m/z</i>
Averages:		8

Processing script for auto_PMF_MgfExport.MS

This processing script may have to be programmed into the LC/MSD Trap software DataAnalysis.

```
' Auto_PMF_MgfExport.MS
' Agilent 5/2004. This Script is used to generate an average mass
' spectrum from AutoMS(1) data, deconvolute that data, and then
' export it to a *.pmf data file name containing the same filename
' as the original *.d file and located in the same *.d folder.
```

```
Dim DataFilePath, DataFileName, SubExt, TargetPath
Dim fso
Set fso=CreateObject("Scripting.FileSystemObject")

DataFilePath = Analysis.Path
DataFileName = Analysis.Name

SubExt = Len(DataFileName) - 2

TargetPath = DataFilePath + "\" + Mid(DataFileName, 1, SubExt) & ".mgf"

' Check if export *.MGF file already exists and delete if true
if fso.FolderExists(TargetPath) then
    fso.deletefolder TargetPath
end if

' Carry out the data processing of a protein digest
Analysis.ClearResults
Analysis.Chromatograms.Clear
Analysis.Compounds.Clear
Analysis.Spectra.MassListClear

Analysis.Chromatograms.Add daTIC, daAllMS, "", daBoth
Analysis.Chromatograms(1).ClearRangeSelections
Analysis.Chromatograms(1).AddRangeSelection 0.0, 1.5, 0, 0
Analysis.Chromatograms(1).AverageMassSpectrum false, true
Analysis.Spectra(1).Deconvolute

'export to *.d\datafilename.mgf
Analysis.Spectra(1).Export TargetPath, damgf

' Save processed results
Analysis.save

form.close
```

Default settings for an MS/MS method

Mode	Scan mode:	Ultra scan (XCT and XCT Plus); or standard normal (SL)
	Segments:	Segment 1 end time 0.25 min; Segment 2 end time 7 min
Tune	Source:	AP-MALDI (positive)
	Vcap:	3000 V
	Drying gas flow:	5.0 L/min
	Dry gas temperature:	325°C
	Skim1:	40 V
	Capillary exit:	200 V
	Oct 1 DC:	12.0 V
	Oct 2 DC:	3.2 V
	Trap drive:	110 V
	Oct RF:	200 V
	Lens 1:	- 5 V
	Lens 2:	- 60 V
	Block voltages:	Mark the check box for Follow values on Page Tune Expert.
ICC values	Target:	200,000 for XCT; 500,000 for XCT Plus; 50,000 for SL
	Maximum accumulation time:	535 ms (corresponding to 6 laser shots)
	Scan range:	400-2200 <i>m/z</i>
	Averages:	Segment 1 uses 4 averages; Segment 2 uses 8 averages
MS/MS fragmentation conditions	Auto MS(n):	2, enabled for Segment 2 only
	Fragmentation amplitude:	1.50 V
	Precursor selection:	Include 400-2200 <i>m/z</i>

	Number of precursor ions:	3
	SPS:	Enabled
	Threshold absolute:	200 counts
	Threshold relative:	2%
	SmartFrag:	On, 30–200%
	Active exclusion:	On, exclude after 1 spectrum release after 20 min
Advanced Auto MS(n) parameters	MS(n) averages:	16
	Isolation width:	4.0
	Use values from MS scan:	Yes
	Use ultra scan:	Yes

Processing script for auto_MIS_MgfExport

This processing script may have to be programmed into the LC/MSD Trap software DataAnalysis.

```
' Auto_MIS_MgfExport.MS
' Agilent 2003. This Script is used to generate compound mass
' spectra from AutoMS(n) data, deconvolute that data, and then
' export it to a *.mgf data file name containing the same filename
' as the original *.d file and located in the same *.d folder.
```

```
Dim DataFilePath, DataFileName, SubExt, TargetPath
Dim fso
Set fso=CreateObject("Scripting.FileSystemObject")

DataFilePath = Analysis.Path
DataFileName = Analysis.Name

SubExt = Len(DataFileName) - 2

TargetPath = DataFilePath + "\" + Mid(DataFileName, 1, SubExt) & ".mgf"

' Check if export *.MGF file already exists and delete if true
if fso.FolderExists(TargetPath) then
    fso.deletefolder TargetPath
end if

' Carry out the data processing of a protein digest
Analysis.ClearResults
Analysis.Chromatograms.Clear
Analysis.Compounds.Clear
Analysis.Chromatograms.Add daTIC, daAll, "", daBoth
Analysis.Chromatograms.Add daTIC, daAllMSn, "", daBoth
Analysis.Chromatograms.Add daBPC, daAllMS, "", daBoth
Analysis.FindAutoMSn
Analysis.Compounds.Deconvolute

'export to *.d\datafilename.mgf
Analysis.Compouds.Export TargetPath, damgf

' Save processed results
Analysis.save

form.close
```

Default PDF-MALDI method settings for LC/MSD TOF

Starting with the method **Default.m**, save the method as PDF-MALDI Default.M.

Data	Stop time:	1.00 min
	Storage mode:	Profile
	Transients/Scan:	40,000
	Min Range:	300 <i>m/z</i>
	Max Range:	3200 <i>m/z</i>
	Abs. Centroid: Threshold:	5000 counts
	Rel. Centroid Threshold:	0.010 (% counts)
MS Parameters	Ion Polarity:	Positive
	Fragmentor:	300 V
	Skimmer:	60 V
	Oct RF V:	300 V
Ion Source	Source:	MALDI
	Polarity:	Positive
	Gas Temp.:	325°C
	Drying Gas:	5.0 L/min
Reference Masses	Clear check boxes for Enable Reference Mass Correction and Use Bottle A .	

Troubleshooting

To refocus the laser spot

NOTE

Use this procedure *only* for the G1974A PDF-MALDI Source.

If a laser spot appears to be "comet like", i.e. a very bright spot with a tail, very diffuse or unfocused, you may have to refocus the laser spot. If the system has been in use for several weeks or months, the fiber optic cable may be dirty. Check the ends of the fiber optic cable for burn marks. If the ends of the fiber optic cable are burned, clean the ends of the fiber optic cable using fiber cleaning tape and isopropanol, or simply replace the cable.

If you replace the fiber optic cable with a cable known to be good or with a new fiber optic cable, and the laser spot continues to be diffuse or unfocused, use the following steps to refocus the laser spot. After refocusing the laser, you will have to repeat the alignment steps.

- 1 This procedure assumes that a plate with a fluorescent label has been installed in the Source as outlined in **step 1** (see [page 41](#) for Trap systems and [page 95](#) for TOF).
- 2 Loosen the setscrew on the fiber cable connector to allow vertical positioning of the barrel. See [Figure 159](#).



Figure 159 Loosen three setscrews to adjust fiber cable connector

- 3 Move the barrel up and down to minimize the size of the laser spot. The laser spot should be very tight with very little

diffusion along the edges of the spot. If the spot is diffuse, the system will lose sensitivity. See [Figure 160](#).

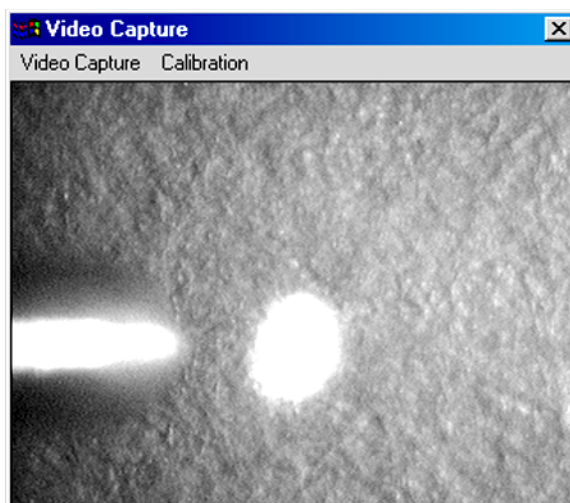


Figure 160 Example of a laser spot on the Laser Alignment tool (on paper)

- 4 After the laser spot is refocused, check laser alignment.

To disassemble and uninstall the Source

Follow these steps if you need to remove the PDF-MALDI ion Source to put another device on the LC/MSD.

- 1 Set the LC/MSD instrument to Standby or OFF mode.
- 2 Turn off the power on the Control Unit
- 3 Disassemble the Source by reversing the installation procedure just described in the previous steps.

WARNING

Before you disconnect the fiber optic cable, turn off the power to the Control Unit. Otherwise, any laser fire can emit visible laser radiation from the ends of the fiber optic cable.

NOTE

For additional troubleshooting information, see “Troubleshooting” on page 87 of the *PDF-MALDI Source for LC/MSD Trap and TOF systems User’s Guide*.

3 Reference

www.agilent.com

In This Book

The Installation Guide contains instructions to install and verify the PDF-MALDI Source for LC/MSD Trap and TOF Systems.

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