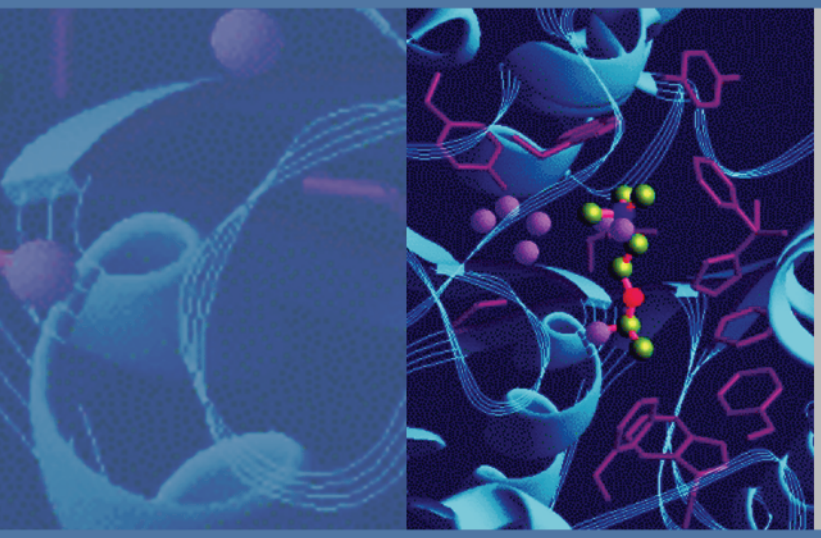

MALDI Source

Hardware Manual

97155-97013 Revision A January 2008

Thermo SCIENTIFIC

Release history: Revision A released January 2008.

Software Version: Xcalibur 2.0.7 or higher, MALDI Source 2.5.0, LTQ XL Series 2.5

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EMC Directive 204/108/EC

EMC compliance has been evaluated by TUV Rheinland of North America.

EN 55011: 1998, A1: 1999, A2: 2002
EN 61000-3-2: 2000
EN 61000-3-3: 1995, A1: 2001
EN 61326-1: 1998, A2: 2001, A3: 2003
EN 61000-4-2: 2001
FCC Class A, CFR 47 Part 15: 2005
EN 61000-4-3: 2002
EN 61000-4-4: 1995, A1: 2000, A2: 2001
EN 61000-4-5: 2001
EN 61000-4-6: 2003
EN 61000-4-11: 2001
CISPR 11: 1998, A1:1999, A2:2002

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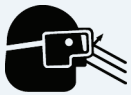
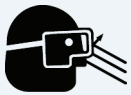
CAUTION Symbol	CAUTION	VORSICHT	ATTENTION	PRECAUCION	AVVERTENZA
	**Electric Shock:** This instrument uses high voltages that can cause personal injury. Before servicing, shut down the instrument and disconnect the instrument from line power. Keep the top cover on while operating the instrument. Do not remove protective covers from PCBs.	**Elektroschock:** In diesem Gerät werden Hochspannungen verwendet, die Verletzungen verursachen können. Vor Wartungsarbeiten muß das Gerät abgeschaltet und vom Netz getrennt werden. Betreiben Sie Wartungsarbeiten nicht mit abgenommenem Deckel. Nehmen Sie die Schutzabdeckung von Leiterplatten nicht ab.	**Choc électrique:** L'instrument utilise des tensions capables d'infliger des blessures corprelles. L'instrument doit être arrêté et débranché de la source de courant avant tout intervention. Ne pas utiliser l'instrument sans son couvercle. Ne pas elensver les étuis protecteurs des cartes de circuits imprimés.	**Descarga eléctrica:** Este instrumento utiliza altas tensiones, capaces de producir lesiones personales. Antes de dar servicio de mantenimiento al instrumento, éste debera apagarse y desconectarse de la línea de alimentacion eléctrica. No opere el instrumento sin sus cubiertas exteriores quitadas. No remueva las cubiertas protectoras de las tarjetas de circuito impreso.	**Shock da folgorazione.** L'apparecchio è alimentato da corrente ad alta tensione che puo provocare lesioni fisiche. Prima di effettuare qualsiasi intervento di manutenzione occorre spegnere ed isolare l'apparecchio dalla linea elettrica. Non attivare lo strumento senza lo schermo superiore. Non togliere i coperchi a protezione dalle schede di circuito stampato (PCB).
	**Chemical:** This instrument might contain hazardous chemicals. Wear gloves when handling toxic, carcinogenic, mutagenic, or corrosive or irritant chemicals. Use approved containers and proper procedures to dispose waste oil.	**Chemikalien:** Dieses Gerät kann gefährliche Chemikalien enthalten. Tragen Sie Schutzhandschuhe beim Umgang mit toxischen, karzinogenen, mutagenen oder ätzenden/reizenden Chemikalien. Entsorgen Sie verbrauchtes Öl entsprechend den Vorschriften in den vorgeschriebenen Behältern.	**Chimique:** Des produits chemiques dangereux peuven se trouver dans l'instrument. Proted dos gants pour manipuler tous produits chemiques toxiques, cancérigènes, mutagènes, ou corrosifs/irritants. Utiliser des récipients et des procédures homologuées pour se débarrasser des déchets d'huile.	**Química:** El instrumento puede contener productos quimicos peligrosos. Utilice guantes al manejar productos quimicos tóxicos, carcinogenos, mutagenos o corrosivos/irritantes. Utilice recipientes y procedimientos aprobados para deshacerse del aceite usado.	**Prodotti chimici.** Possibile presenza di sostanze chimiche pericolose nell'apparecchio. Indossare dei guanti per maneggiare prodotti chimici tossici, cancerogeni, mutageni, o corrosivi/irritanti. Utilizzare contenitori aprovo e seguire la procedura indicata per lo smaltimento dei residui di olio.
	**Heat:** Before servicing the instrument, allow any heated components to cool.	**Hitze:** Warten Sie erhitzte Komponenten erst nachdem diese sich abgekühlt haben.	**Haute Temperature:** Permettre aux composants chauffés de refroidir avant tout intervention.	**Altas temperaturas:** Permita que lop componentes se enfríen, ante de efectuar servicio de mantenimiento.	**Calore.** Attendere che i componenti riscaldati si raffreddino prima di effetturare l'intervento di manutenzione.
	**Fire:** Use care when operating the system in the presence of flammable gases.	**Feuer:** Beachten Sie die einschlägigen VorsichtsmaBnahmen, wenn Sie das System in Gegenwart von entzündbaren Gasen betreiben.	**Incendie:** Agir avec précaution lors de l'utilisation du système en présence de gaz inflammables.	**Fuego:** Tenga cuidado al operar el sistema en presencia de gases inflamables.	**Incendio.** Adottare le dovute precauzioni quando si usa il sistema in presenza di gas infiammabili.
	**Eye Hazard:** Eye damage could occur from splattered chemicals or flying particles. Wear safety glasses when handling chemicals or servicing the instrument.	**Verletzungsgefahr der Augen:** Verspritzte Chemikalien oder kleine Partikel können Augenverletzungen verursachen. Tragen Sie beim Umgang mit Chemikalien oder bei der Wartung des Gerätes eine Schutzbrille.	**Danger pour les yeux:** Dex projections chimiques, liquides, ou solides peuvent être dangereuses pour les yeux. Porter des lunettes de protection lors de toute manipulationde produit chimique ou pour toute intervention sur l'instrument.	**Peligro par los ojos:** Las salicaduras de productos químicos o particulas que salten bruscamente pueden causar lesiones en los ojos. Utilice anteojos protectores al mnipular productos químicos o al darle servicio de mantenimiento al instrumento.	**Pericolo per la vista.** Gli schizzi di prodotti chimici o delle particelle presenti nell'aria potrebbero causare danni alla vista. Indossare occhiali protettivi quando si maneggiano prodotti chimici o si effettuano interventi di manutenzione sull'apparecchio.
	**General Hazard:** A hazard is present that is not included in the above categories. Also, this symbol appears on the instrument to refer the user to instructions in this manual.	**Allgemeine Gefahr:** Es besteht eine weitere Gefahr, die nicht in den vorstehenden Kategorien beschrieben ist. Dieses Symbol wird im Handbuch auBerdem dazu verwendet, um den Benutzer auf Anweisungen hinzuweisen.	**Danger général:** Indique la présence d;un risque n'appartenant pas aux catégories citées plus haut. Ce symbole figure également sur l'instrument pour renvoyer l'utilisateur aux instructions du présent manuel.	**Peligro general:** Significa que existe un peligro no incluido en las categorias anteriores. Este simbolo también se utiliza en el instrumento par referir al usuario a las instrucciones contenidas en este manual.	**Pericolo generico.** Pericolo non compreso tra le precedenti categorie. Questo simbolo è utilizzato inoltre sull'apparecchio per segnalare all'utente di consultare le istruzioni descritte nel presente manuale.
	When the safety of a procedure is questionable, contact your local Technical Support organization for Thermo Fisher Scientific San Jose Products.	Wenn Sie sich über die Sicherheit eines Verfahrens im unklaren sind, setzen Sie sich, bevor Sie fortfahren, mit Ihrer lokalen technischen Unterstützungsorganisation für Thermo Fisher Scientific San Jose Produkte in Verbindung.	Si la sûreté d'un procédure est incertaine, avant de continuer, contacter le plus proche Service Clientèle pour les produits de Thermo Fisher Scientific San Jose.	Cuando la certidumbre acerca de un procedimiento sea dudosa, antes de proseguir, pongase en contacto con la Oficina de Asistencia Tecnica local para los productos de Thermo Fisher Scientific San Jose.	Quando e in dubbio la misura di sicurezza per una procedura, prima di continuare, si prega di mettersi in contatto con il Servizio di Assistenza Tecnica locale per i prodotti di Thermo Fisher Scientific San Jose.

CAUTION Symbol	CAUTION	危険警告	危險警告
	**Electric Shock:** This instrument uses high voltages that can cause personal injury. Before servicing, shut down the instrument and disconnect the instrument from line power. Keep the top cover on while operating the instrument. Do not remove protective covers from PCBs.	**電撃：**この計測器は高電圧を使用し、人体に危害を与える可能性があります。保守・修理は、必ず操業を停止し、電源を切ってから実施して下さい。上部カバーを外したままで 計測器を使用しないで下さい。プリント配線板の保護カバーは外さないで下さい。	電擊：儀器設備使用會造成人身傷害的高伏電壓。在維修之前，必須先關儀器設備並切除電源。務必要在頂蓋蓋上的情況下操作儀器。請勿拆除PCB保護蓋。
	**Chemical:** This instrument might contain hazardous chemicals. Wear gloves when handling toxic, carcinogenic, mutagenic, or corrosive or irritant chemicals. Use approved containers and proper procedures to dispose waste oil.	**化学物質：**危険な化学物質が計測器中に存在している可能性があります。毒性、発がん性、突然変異性、腐食・刺激性などのある薬品を取り扱う際は、手袋を着用して下さい。廃油の処分には、規定の容器と手順を使用して下さい。	化學品：儀器設備中可能存在有危險性的化學物品。接觸毒性致癌、誘變或腐蝕／刺激性化學品時，請配帶手套。處置廢油時，請使用經過許可的容器和程序。
	**Heat:** Before servicing the instrument, allow any heated components to cool.	**熱：**熱くなった部品は冷えるのを待ってから保守・修理を行って下さい。	高温：請先等高温零件冷卻之後再進行維修。
	**Fire:** Use care when operating the system in the presence of flammable gases.	**火災：**可燃性のガスが存在する場所でシステムを操作する場合は、充分な注意を払って下さい。	火災：在有易燃氣體的場地操作該系統時，請務必小心謹慎。
	**Eye Hazard:** Eye damage could occur from splattered chemicals or flying particles. Wear safety glasses when handling chemicals or servicing the instrument.	**眼に対する危険：**化学物質や微粒子が飛散して眼を傷つける危険性があります。化学物質の取り扱い、あるいは計測器の保守・修理に際しては防護眼鏡を着用して下さい。	眼睛傷害危險：飛濺的化學品或顆粒可能造成眼睛傷害。處理化學品或維儀器設備時請佩戴安全眼鏡。
	**General Hazard:** A hazard is present that is not included in the above categories. Also, this symbol appears on the instrument to refer the user to instructions in this manual.	**一般的な危険：**この標識は上記以外のタイプの危険が存在することを示します。また、計測器にこの標識がついている場合は、本マニュアル中の指示を参照して下さい。	一般性危險：說明未包括在上述類別中的其他危險。此外，儀器設備上使用這個標誌，以指示用戶本使用手冊中的說明。
	When the safety of a procedure is questionable, contact your local Technical Support organization for Thermo Fisher Scientific San Jose Products.	**安全を確保する手順がよくわからない時は、作業を一時中止し、お近くのサーモエレクトロンサンローゼプロダクトのテクニカールサポートセンターごご連絡ください。**	如对安全程序有疑问，请在操作之前与当地的菲尼根技术服务中心联系。

Contents

Preface

About This Guide

Welcome to the Thermo Scientific MALDI source and LTQ™ XL mass spectrometer.

This manual contains a description of the modes of operation and principle hardware components of the MALDI source. In addition, this manual provides step-by-step instructions for cleaning and maintaining your MALDI source.

Related Documentation

In addition to this guide, Thermo Fisher Scientific provides the following documentation for the LTQ Series ion trap mass spectrometers:

- A printed copy of the *Safety and Regulatory Guide*
- Electronic copies of the documents listed in Table 1 as PDF files that you can access from the data system computer

The *Safety and Regulatory Guide* contains important safety information about Thermo Scientific mass spectrometry and liquid chromatography systems. This document is shipped with every Thermo Scientific mass spectrometer and liquid chromatography device.

Table 1. MALDI LTQ XL MS documentation

Model	Related Documents
LTQ XL	*LTQ XL Series Getting Connected Guide*
	LTQ XL Series Preinstallation Requirements Guide
MALDI	*MALDI Source Getting Started Guide*

To access the manuals for the mass spectrometer, from the Windows™ XP taskbar, choose **Start > All Programs > Xcalibur > Manuals >** *Brand name of mass spectrometer* and then click the listing for the PDF that you want to view. The software also provides Help. To access the Help, choose **Help** from the menu bar or click the  button on the toolbar.

Safety and Special Notices

Make sure you follow the precautionary statements presented in this manual. The safety and other special notices appear in boxes.

Safety and special notices include the following:

CAUTION Highlights hazards to humans, property, or the environment. Each CAUTION notice is accompanied by an appropriate CAUTION symbol.

IMPORTANT Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or may contain information that is critical for optimal performance of the system.

Note Highlights information of general interest.

Tip Highlights helpful information that can make a task easier.

Safety Information About the MALDI Source

CAUTION Failure to understand and comply with laser cautions and operating instructions can result in property damage, or serious or fatal injuries to personnel.

Contacting Us

There are several ways to contact Thermo Fisher Scientific.

❖ To contact Technical Support

Phone	800-685-9535
Fax	561-688-8736
E-mail	TechSupport.C+MS@thermofisher.com
Knowledge base	www.thermokb.com

Find software updates and utilities to download at www.mssupport.thermo.com.

❖ To contact Customer Service for ordering information

Phone	800-532-4752
Fax	561-688-8731
Web site	www.thermo.com/finnigan

❖ To suggest changes to documentation or to Help

- Fill out a reader survey online at www.thermo.com/lcms-techpubs.
- Send an e-mail message to the Technical Publications Editor at techpubs.finnigan-lcms@thermofisher.com.

Introduction

This chapter describes the MALDI source, its basic components, and some of the advantages and disadvantages of matrix-assisted laser desorption ionization (MALDI) relative to electrospray ionization (ESI).

Contents

MALDI Overview

The MALDI source is part of the Thermo Scientific family of mass spectrometer (MS) ion sources. The MALDI source is an optional source that is attached to the LTQ XL MS detector, in place of the Ion MAX™ API source.

In a typical MALDI analysis, you dissolve a sample in a solution containing a large excess of a matrix material with strong absorbency in the ultraviolet band. A few microliters of this solution are evaporated onto a sample plate that is then placed in an evacuated chamber. A UV laser vaporizes the sample crystals, carrying the analyte molecules into the vapor phase. Various charge transfer processes ionize the sample molecules, and an electrical potential draws the sample ions into the LTQ XL MS detector for analysis.

The MALDI modules are identified in Figure 1 and listed in Table 1.

Figure 1. MALDI LTQ XL system modules

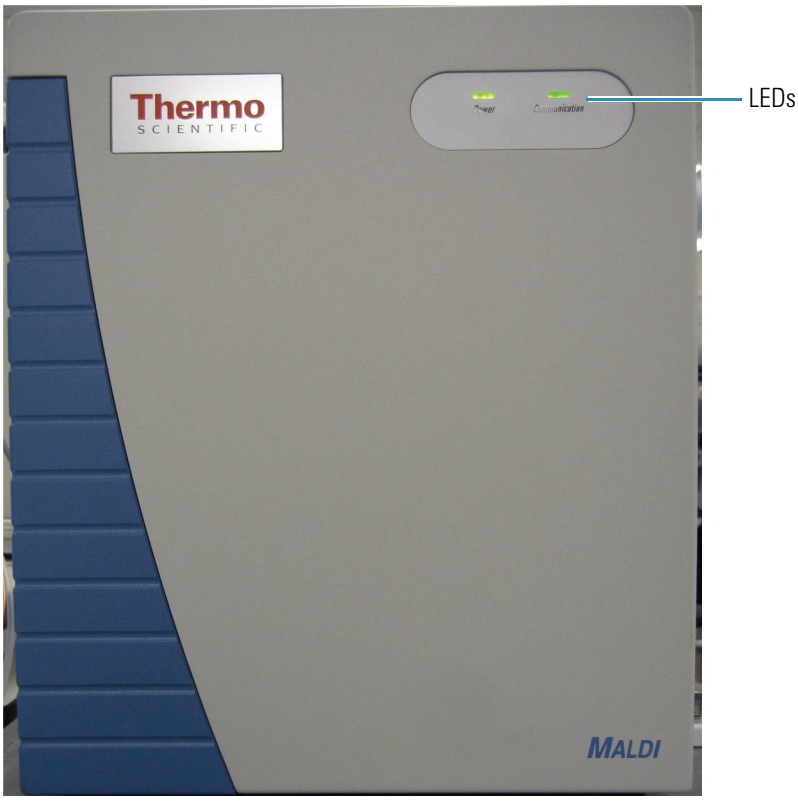
Table 1. MALDI LTQ XL system modules

Module	Function
Control module	Controls the MALDI source. See MALDI Control Module.
Optics module	Contains the charge-coupled device (CCD) camera and laser. See MALDI Optics Module.
Sample module	Manipulates the sample. See MALDI Sample Module.

MALDI Control Module

The MALDI control module (Figure 2) contains the electronics to control the MALDI source and interface with the instrument's workstation.

Figure 2. Front panel of the MALDI control module



The MALDI control module has two LEDs shown in Figure 3 and described in Table 2.

Figure 3. Control module LEDs

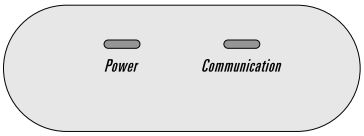


Table 2. MALDI control module LEDs

LED	State	Meaning
Power	Green	The power is on.
	Off	The power is off.
Communication	Green	The MALDI control module is communicating normally with the LTQ XL MS detector.
	Flashing green	The MALDI control module is not communicating correctly with the LTQ XL MS detector.
	Off	The MALDI control module is not communicating with the LTQ XL MS detector.

MALDI Optics Module

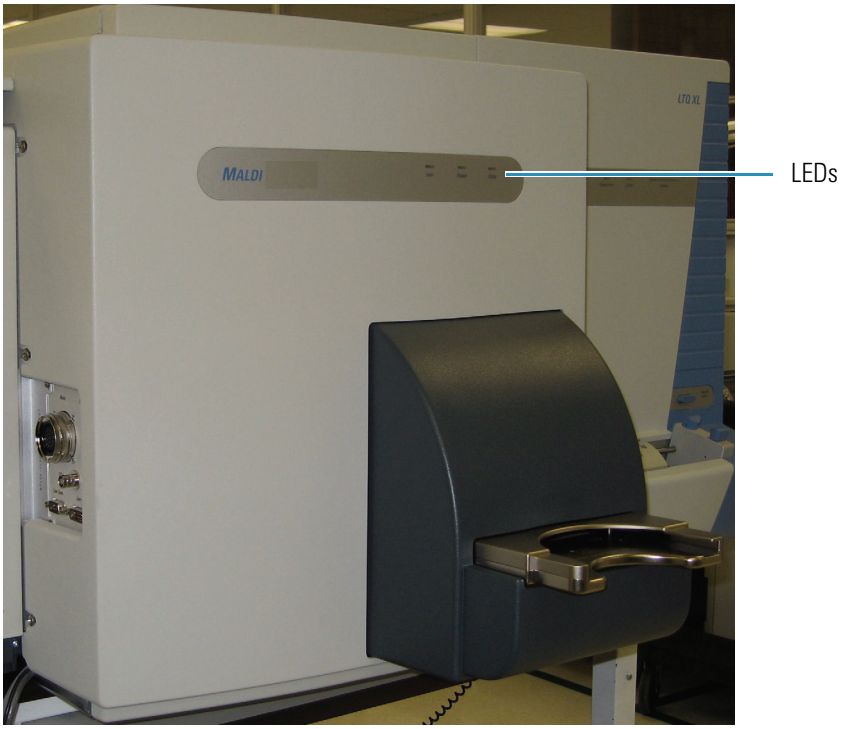
The MALDI optics module (Figure 4) is attached to the left side of the LTQ XL MS and contains the optics that image the sample and direct the laser beam from the laser to the sample. These optical components include a charge-coupled device (CCD) camera and the laser.

Figure 4. MALDI optics module

MALDI Sample Module

The MALDI sample module (Figure 5) contains all the components that manipulate the sample.

Figure 5. MALDI sample module



The sample module mounts directly to the manifold of the LTQ XL MS detector. Major components of the sample module include the following:

- A load lock that cycles sample plates from atmospheric pressure to vacuum
- An evacuated sample chamber
- An XYZ mechanism that moves the sample plate to present the sample wells to the laser
- Ion transfer optics that carry sample ions into the LTQ XL MS detector

The sample module has three LEDs as show in Figure 6 and described in Table 3.

Figure 6. MALDI sample module LEDs

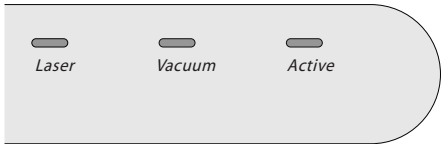


Table 3. Sample module LEDs

LED	State	Meaning
Laser	Flashing red	The laser is firing.
	Off	The laser is not firing.
Vacuum	Green	The vacuum levels are normal in both the sample chamber and the load lock.
	Off	The vacuum is broken in one or both chambers.
Active	Flashing green	The sample plate is in motion.
	Off	The sample plate is stationary.

How do ESI and MALDI Compare?

The following topics describe the benefits of using the MALDI and ESI methods. See Table 4 for a comparison of these methods.

Using the MALDI Method

MALDI analysis has undergone recent changes that reduce or eliminate many previous shortcomings, such as difficulty with coupling MALDI with chromatographic separation as in LC/ESI. Recent improvements to the method include offline HPLC for sample preparation, which offers some advantages over inline separation. Similarly, the adaptation of the MALDI source to the LTQ XL™ mass spectrometer means that there is no longer any limitation to use MALDI for MS^n analysis.

MALDI is characterized by speed, simplicity, and ease of automation. A MALDI sample plate can process 96 or 384 samples much more rapidly than an LC/ESI/MS. If you use offline HPLC when preparing the samples, then the stable samples deposited on the plate can be analyzed without the time constraints of a continuous solvent flow through an LC. MALDI deals with mixtures quite well, so chromatographic separation might not be necessary. MALDI is also less vulnerable than ESI to residual salts in the sample.

Using the ESI Method

ESI offers a lower practical mass limit. You can use ESI inline with an LC system when precise sample retention times are important. ESI tends to produce multiple charges, which offset MALDI higher mass ranges by lowering *m/z* ratios. Multiple charging also makes MS/MS analysis easier and more informative.

The limitations of ESI are complementary to MALDI strengths. ESI is sensitive to residual salts in the sample which often makes more elaborate sample preparation necessary. Chromatographic separation, essential for ESI with complex mixtures, leads to longer run

times and additional equipment costs that might be unnecessary with a MALDI source. And multiple charging, while often an advantage for ESI, can lead to confusing spectra in the presence of several charge states.

Using Both the MALDI and ESI Methods

Today MALDI and ESI are complementary methods, best used in conjunction with one another. Several means of combining the methods can prove effective, such as splitting an LC output between an ESI source and a MALDI sample plate and then using the plate as a stable method of reanalyzing selected peaks from the ESI run.

Table 4. Comparison of MALDI with ESI

	Strengths	Limitations
MALDI	• Simple to use • Fast analysis • fmol to amol sensitivity • If offline HPLC is used, allows time-independent analysis of separated fractions • Salt tolerance up to mmol concentrations • Suitable for complex mixtures	• Matrix interference at *m/z* ratios below 600 • Produces mostly singly charged ions
ESI	• Inline LC can give retention time data as well as MS data • fmol sensitivity • No matrix interference at low mass • Multiple charging gives smaller *m/z* ratios and improved MS/MS	• Poor salt tolerance • Chromatographic separation required for mixtures • Multiple charging can complicate spectra

Functional Description

This chapter describes the MALDI source assembly components and the software that handles the tasks associated with sample plates and data acquisition.

Contents

Sample Module

The MALDI sample module is a precision ion source that has the following internal components:

- Load Lock and Sample Chamber
- Ion Transfer Optics
- Vacuum System

Load Lock and Sample Chamber

The load lock and the sample chamber are parts of the sample module. In the sample chamber, the sample plate mounts on a platform that moves in two dimensions to position each sample well for the laser. This platform is part of the XYZ mechanism. Spring tension clamps hold the sample plate onto the XYZ mechanism.

The movement of the XYZ mechanism is guided by two precision vacuum-rated stepper motors, each of which drives a stainless steel linear actuator. The movement of the stage is bounded by optical interrupt limit switches at the beginning and end of each actuator's range of motion.

You control the movement of the XYZ mechanism through the MALDI software. The software uses a pixel map based on the image from the camera to provide point- and click-on positioning for the sample plate.

Note If the XYZ mechanism in your MALDI source is defective, contact Thermo Fisher Scientific Technical Support or a field service engineer.

Ion Transfer Optics

The MALDI source contains a quadrupole ion guide and a DC extraction field that channels ions from the sample plate into the ion optics of the LTQ XL MS detector. This quadrupole and DC extraction field assembly (Q00) provides no mass selection—it is only a field to regulate ions in the detector.

Note If the ion transfer optics assembly in the MALDI source is defective, contact Thermo Scientific Technical Support or a field service engineer.

CAUTION Failure to understand and comply with laser cautions and operating instructions can result in property damage, or serious or fatal injuries to personnel.

Vacuum System

The MALDI source uses the pumps that are specific to the LTQ XL MS detector to pump down the load lock and the sample chamber. A turbomolecular pump with a Holweck stage, backed up by an oil-sealed rotary vane rough pump, evacuates the LTQ XL ion path and, by extension, the MALDI sample chamber that is contiguous with it. A second rotary vane pump evacuates the load lock. Vacuum pressure sensors measure the vacuum level in both chambers, and the sensors feed data back to the LTQ XL vacuum controls.

When you place a sample plate on the load lock and you click the Insert Plate button to load the sample plate, the sample plate is moved into the load lock chamber, the door closes, and the vacuum system engages automatically to pump down the load lock. When the load lock chamber pressure is less than 120 mTorr, the sample plate moves from the load lock chamber to the sample chamber. (See Loading Sample Plates for information about loading a plate.)

When you click the Eject Sample Plate button, a vent valve automatically restores the load lock chamber to atmospheric pressure within about 40 seconds.

Optics Module

The optics module contains the following components:

- Camera
- Laser

Camera

The MALDI source uses a charge-coupled device (CCD) camera to image the target crystals. The camera uses the same optical axis as the laser beam, picking up the image of the sample plate from the dichroic mirror that guides the laser.

Light for the camera is provided by a fiber optic strand that carries light from an LED through the ion transfer quadrupole and into the sample chamber. See Figure 4 on page 4 for more information about the camera and the optical illuminator.

The MALDI software uses optical recognition routines on the camera image to identify the type of sample plate (see Sample Plates) and to identify the sample plate calibration targets on the corners of each plate. These calibration targets orient the sample plate and create a digital map of the plate for navigation.

Laser

The MALDI source uses a nitrogen gas laser (337.7 nm) with a frequency of 60 Hz. The laser is housed in the MALDI optics module (see Figure 4 on page 4).

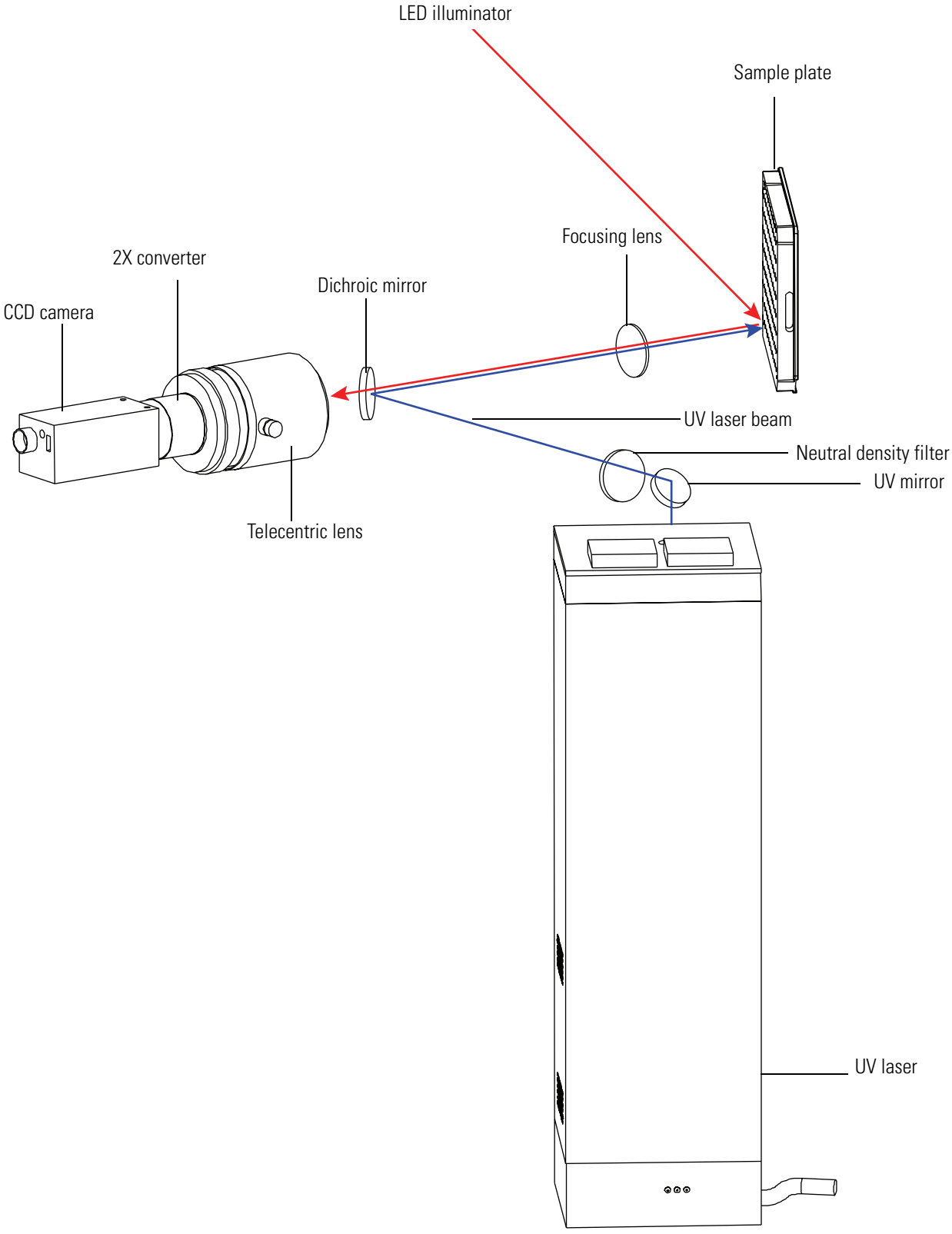
This section contains the following topics:

- Laser Energy Path
- Laser Performance

Laser Energy Path

Figure 7 shows the path of the laser energy through the MALDI optics module.

Figure 7. Laser energy path in the MALDI optics module

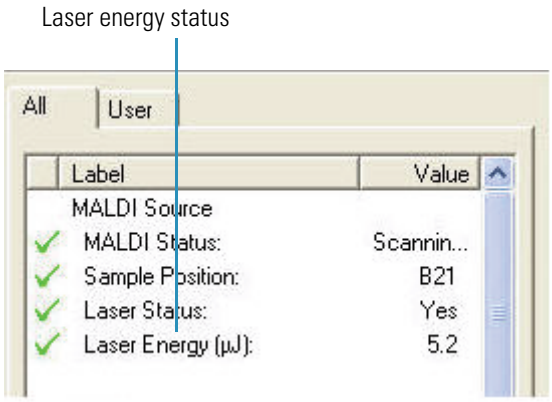


Laser Performance

In addition to alignment, the other factor that influences the laser energy delivered to the sample is the output power of the laser itself.

The laser energy is displayed in the MALDI Source section of the Status View in the Tune Plus window (Figure 8). A green check mark indicates that the laser energy is acceptable.

Figure 8. MALDI laser energy status in Tune Plus



The MALDI laser degrades with time. If the value is outside the set value, the MALDI system places an "X" by the laser energy in the status window to indicate that the laser is outside acceptable tolerances.

Note If you notice a steady drop in laser energy over time, contact Thermo Fisher Scientific Technical Support to determine whether replacing the laser is necessary and, if so, to schedule a visit by a field service engineer.

Sample and Data Automation

The LTQ XL MS detector workstation runs software that controls the MALDI source. All commands pass from the instrument workstation through the LTQ XL MS detector to the MALDI control module. The control software also has several powerful features to automate the processes of handling the sample plates and acquiring data:

- Crystal Positioning System (CPS)
- Automatic Spectrum Filter (ASF)
- Automatic Gain Control (AGC)
- Point and Click Crystal Selection
- Automatic Plate Recognition
- Automatic Plate Calibration
- Automatic Video Calibration

Crystal Positioning System (CPS)

MALDI processes the image gathered by the camera into a pixel map and automatically identifies matrix crystals on the image. In MALDI you can select the pattern that you want to use when the laser is fired. The MALDI Crystal Positioning System (CPS) automatically recognizes and navigates from crystal to crystal.

Automatic Spectrum Filter (ASF)

You can instruct MALDI to recognize acceptable spectra, defined as having a total ion current above a set threshold value over a designated mass range. By setting the mass range to exclude most of the matrix background and choosing a suitable threshold level, you can cause the system to recognize useful crystals and continue collecting spectral data from those crystals. Likewise, the system disregards junk data and continues on when a selected crystal proves to have little or no sample.

ASF increases the speed of analyses and reduces wasted data. It also improves the signal-to-noise ratio of the final spectrum by filtering out bad data before performing spectrum averaging.

Note If the plate is moving but no spectrum is shown within 30 seconds, then you might have set the threshold too high.

Automatic Gain Control (AGC)

The AGC feature allows the software to determine the number of laser shots on a given crystal based on the signal strength measured in a given mass range: a low signal results in more data acquisition shots, while a high signal allows fewer shots.

Point and Click Crystal Selection

When you set the Plate Motion to Manual in the MALDI software, you can use the mouse to select the desired crystal in the camera image.

Automatic Plate Recognition

MALDI uses patterns etched in the surface of the sample plate to identify the plate (see "Sample Plates" on page 15). The software then automatically uses this information to map the plate for navigation.

Automatic Plate Calibration

Accurate positioning of the sample plate is critical to MALDI. The laser and camera must be accurately aligned to allow for precise crystal selection. The sample well must be directly centered on the ion transfer quadrupole for efficient collection of the sample ions into the MALDI LTQ XL MS detector.

Each sample plate is marked with four crosses, one at each corner, three of which are used to map and position the sample plate. MALDI can then find and identify these crosses.

Automatic Video Calibration

MALDI automatically calibrates the video display, both to center the image and to measure the distance represented by each pixel in the pixel map. This information, combined with the sample plate calibration, allows MALDI to move the sample plate accurately to present any point to the camera and laser.

MALDI Source Status Information

Tune Plus for the MALDI source includes information about the MALDI source in addition to standard information for the LTQ XL (see Figure 8 on page 13). This information includes the following:

- The current state of the MALDI source: Scanning, Standby, Off.
- The location of the sample well that is being scanned.
- The status of the laser: Yes (on), Standby, or Off.
- The current laser energy rating.

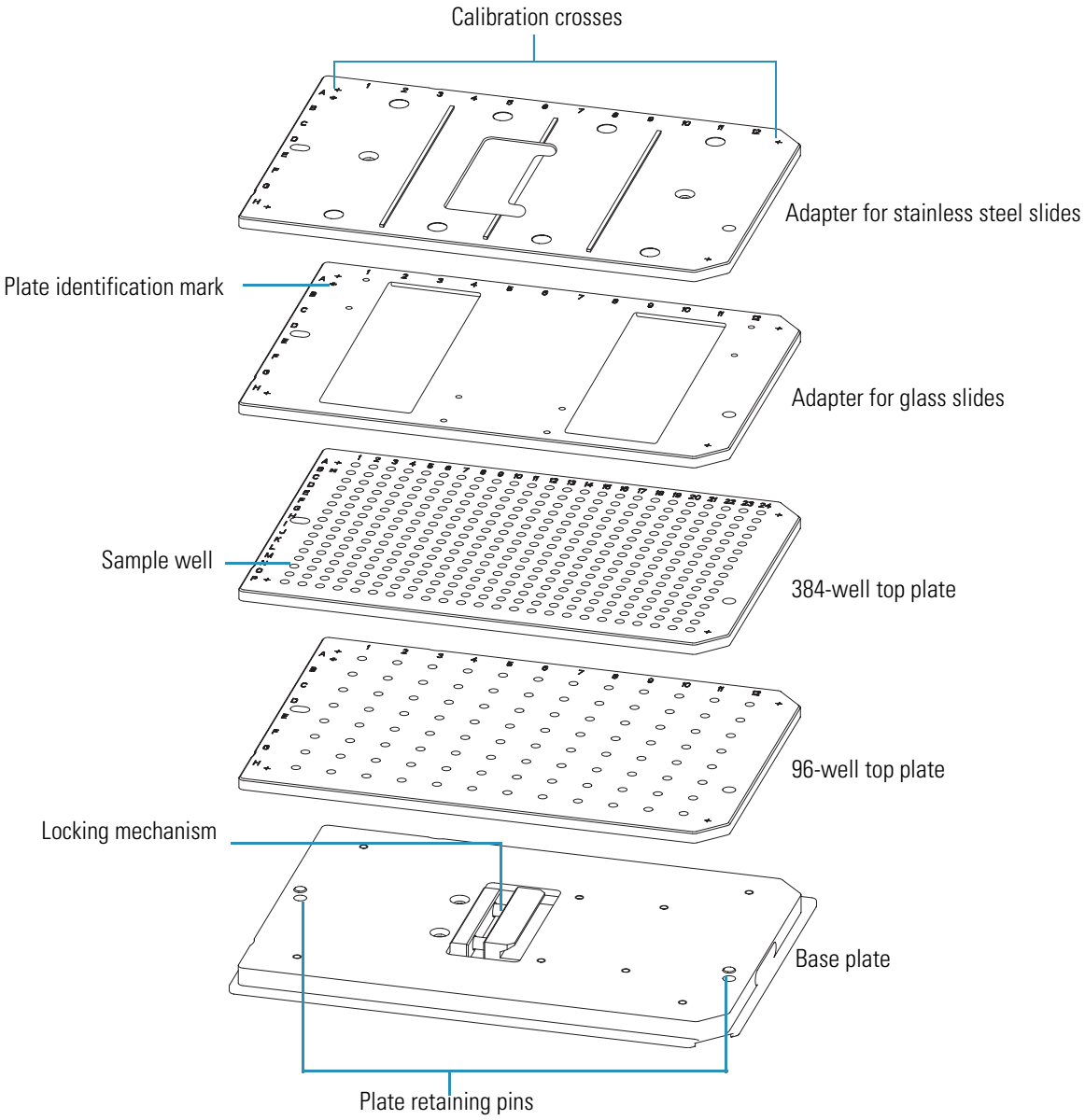
Sample Plates

The sample plates used in the MALDI source (Figure 9) are a two-piece construction: a base and a stainless steel top plate that contains the samples. Four types of top plates are available:

- 96 sample wells
- 384 sample wells
- Adapter for four stainless steel slides (for tissue imaging)
- Adapter for two glass slides (for tissue imaging)

The MALDI control software automatically identifies which sample plate is being used, and the sample plate type diagnostics provide information about the plate. For more information, see "Sample Plate Type Determination" on page 45.

Figure 9. MALDI source sample plates



The base and top plate are specifically made for use with the MALDI source. Plates from other instruments or other manufacturers do not have the proper dimensions or optical identifiers and cannot be used in the MALDI source.

CAUTION Always use approved sample plates from Thermo Fisher Scientific with the MALDI source. Unapproved plates can jam and cause significant damage to the MALDI LTQ XL system.

The stainless steel sample plate has a polished mirror finish to minimize roughness and potential cross-contamination. Always clean the sample plate carefully, as described in the *LTQ XL Series Getting Started Guide*.

CAUTION Improper cleaning of the sample plate can potentially etch and leave contaminants on the surface or scratches on the plate. Scratches reduce the useful life of the plate and can harbor impurities.

Loading Sample Plates

You load the sample plate by sliding the plate into the loading slot where clamps lock the sample plate to the pickup stage. The pickup stage accepts the plate in only one orientation. A sensor in the load lock interface verifies that there is a sample plate on the pickup stage. The pickup stage then retracts with the plate, rotates it to the vertical position, and seals the load lock chamber.

The load lock is cycled from atmospheric pressure to main chamber vacuum pressure by means of the rough vacuum pump. When the pressure in the load lock reaches 120 mTorr, the pickup stage transfers the sample plate through the automated gate valve between the load lock and the sample chamber. The plate is then handed off to the XYZ mechanism (see "Load Lock and Sample Chamber" on page 9). Optical interrupt limit sensors at the top and bottom of the load lock define the upper and lower limits of the pickup stage's movement.

Daily Operation

For optimal MALDI operation, perform the procedures in this chapter each day before and after operating the source. Use these daily operations to help you detect, fix, and prevent problems that could reduce the sensitivity of the LTQ XL MS.

Contents

For regular maintenance procedures, see "Maintenance" on page 25.

Operational Guidelines

To run the mass spectrometer in the MALDI mode, read through these operational guidelines before you set the MALDI parameters and the mass spectrometer parameters to acquire data:

- Controlling the Motion of the Target Plate
- Adding Microscans
- Handling Sample Plates

Controlling the Motion of the Target Plate

As described in the previous chapter, you can control the motion of the target plate either automatically (for example, spiral motion) or manually. In order to decide which mode to use, consider that the rate of sample degradation depends on the matrix, the sample preparation technique, and the laser energy. You can manually shift the target plate to another position within the same spot, but this requires constant monitoring of the signal level.

Adding Microscans

The number of microscans depends on the type of experiment that you are performing. If you are working with relatively concentrated samples (15 to 100 fmol of sample per sample spot), 1 to 3 microscans are usually sufficient. For 10 fmol or less, up to 5 microscans might be

needed, and if you are working with 1 fmol or less, then you might need 10 microscans or more. A scan with a higher number of microscans provides more signal averaging but takes longer to acquire.

If your analyte concentration is too low, you might need to increase the number of scans averaged to get a good signal-to-noise ratio in your data.

Handling Sample Plates

Observe the following guidelines when handling the sample plate:

- Avoid scratching or denting the plate in any way. Even very small scratches can harbor contaminants.
- Always handle the sample plate with lint-free, powder-free gloves.
- Never touch the top surface of the plate, even with gloves.
- Use only high-purity solvents: HPLC grade or better.
- Avoid the use of anything abrasive on the plate, including abrasive cleaners.
- Avoid caustic materials such as strong acids or bases, as they can etch the surface of the plate.
- Avoid exposing the plate to high heat. (Gentle warming to dry the plate is acceptable.)
- Store the plate in a desiccator or under vacuum when not in use.
- Protect the plate from dust.
- If a plate has been stored for an extended period, repeat the cleaning procedure described in the *LTQ XL Series Getting Started Guide* immediately before using the plate, even if the plate was cleaned before being stored.

Turning the MALDI LTQ XL System Off or On

After you have completed your work for the day, place the MALDI LTQ XL system into standby mode which turns the MALDI source off. Do the reverse to resume your work.

❖ To turn the LTQ XL into standby mode

1. On the LTQ XL workstation choose **Start > Programs > Xcalibur > LTQ XL Tune**.
2. In Tune Plus, click **On/Standby** to put the instrument into standby mode.

   The MALDI source is automatically turned off.

❖ **To turn on the LTQ XL**

1. On the LTQ XL workstation choose **Start > Programs > Xcalibur > LTQ XL Tune**.
2. In the Tune Plus window, click **On/Standby**  to turn on the LTQ XL MS.

   The MALDI source is automatically put into standby mode.

Preparing the MALDI Source for Use

Before running samples on the MALDI source, ensure that the LTQ XL MS and the MALDI source are in the On mode and perform the following routine checks:

- Checking System Vacuum Levels
- Checking the Laser
- Calibrating the Sample Plate Position

Note The MALDI source is an integral part of the LTQ XL MS system. Refer to the documentation for your LTQ XL MS system for pre-operation tasks specific to the LTQ XL MS detector.

If you are running samples for the first time on the MALDI source, or after moving the instrument to a new location, or after an extended downtime, refer to the *LTQ XL Series Getting Started Guide*.

Checking System Vacuum Levels

For proper performance, your MALDI source must operate at the proper vacuum levels. Operation of the system with poor vacuum levels can cause reduced mass resolution, breakdowns in the multipole RF circuit, tuning problems, and reduced lifetime of the electron multiplier in the LTQ XL MS detector. To check your system for air leaks, check the system vacuum levels before you begin your first acquisition.

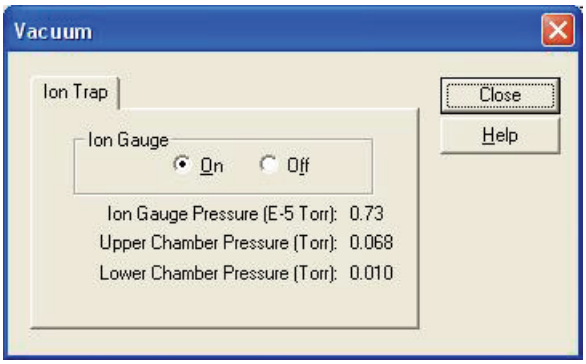
Note You can occasionally detect major air leaks by listening for a rush of air or a hissing sound somewhere on the instrument. Causes for a major leak include a loose or disconnected fitting, an O-ring that is not properly seated, or an open valve.

❖ **To check the current pressures in the load lock (lower chamber) and the sample chamber (upper chamber)**

1. On the LTQ XL workstation choose **Start > Programs > Xcalibur™ > LTQ Tune**.
2. In the Tune Plus window, click **On/Standby**  to put the instrument into standby mode.
3. Choose **Setup > Vacuum**.

The Vacuum dialog box opens (Figure 10).

Figure 10. Vacuum dialog box



4. If the pressure in the sample chamber is higher than 1 x 10^{-2} Torr, and you have restarted the system within the last 30 to 60 minutes, wait an additional 10 minutes and recheck the pressure.

5. If the pressure decreases with time, check the pressure periodically until it is below 1 x 10^{-2} Torr.

   If the pressure remains high, your system might have an air leak. If you suspect an air leak:

   a. Shut down the system. See the LTQ XL MS documentation for your system for instructions.

   b. Make a visual inspection of the vacuum system and vacuum lines for leaks.

   c. Check each fitting and flange on the system for tightness, and tighten the fittings or flanges that are loose. Do not tighten fittings indiscriminately. Pay special attention to fittings that have been changed recently or to fittings that have been subjected to heating and cooling.

   d. Make sure that the seals of the vacuum manifold are properly seated.

Checking the Laser

Many analytical difficulties can be traced back to problems with the laser. A slight misalignment of the beam can dramatically affect results. The laser energy can drop slightly in power thereafter so that the laser no longer generates ions efficiently. For these reasons, check the laser status before using the instrument.

To check the laser status, check in the Status View of Tune Plus for the green status indicators: Laser Status and Laser Energy (Figure 8 on page 13). If the laser appears to be not working correctly, refer to "Laser Optics Evaluation" on page 35 for diagnostic tests to evaluate the laser performance.

Calibrating the Sample Plate Position

The MALDI source automatically calibrates the sample plate each time a plate is inserted into the source. If an automatic plate calibration fails, see "Sample Plate Calibration Fails" on page 68.

Maintenance

This chapter describes the basic maintenance tasks that help to keep your MALDI source working properly.

Contents

Checking the Laser Position

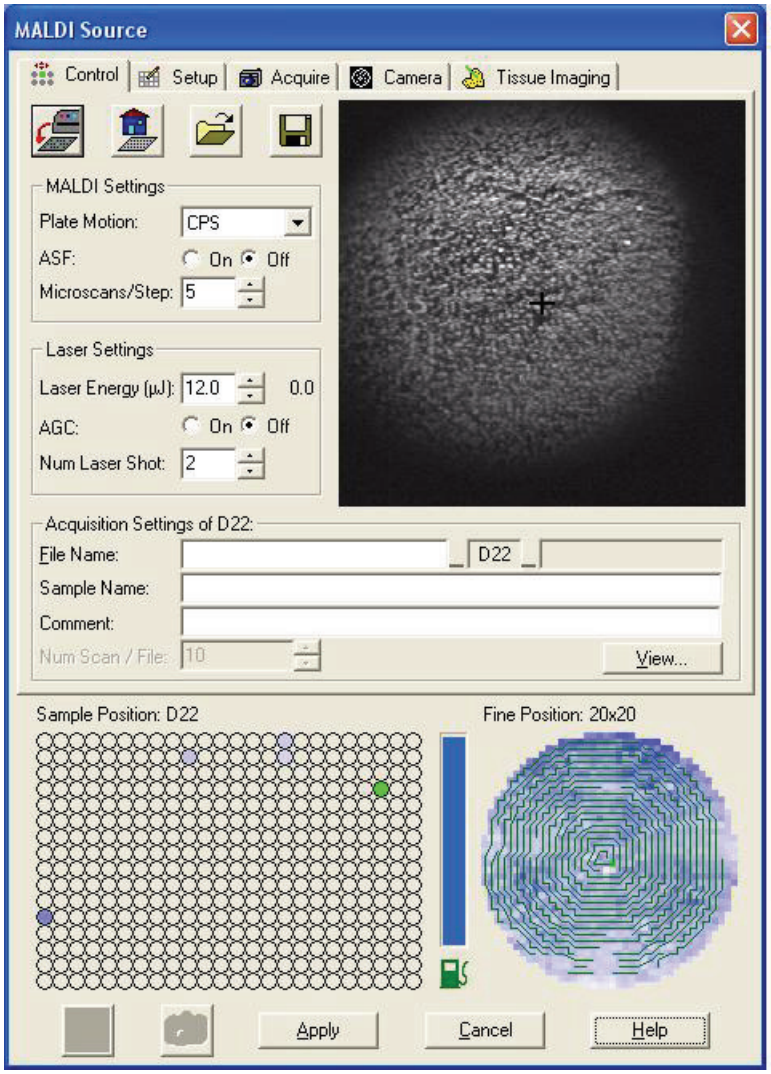
In order to guide the laser accurately to an analyte crystal, you must center the laser beam on the optical axis of the camera and on the hole in the ion transfer quadrupole.

❖ **To align the laser with the optical axis of the camera**

1. Prepare a sample plate according to your standard procedure. Apply a thin, homogenous, matrix-only layer to sample well A1 (and other wells as needed).
2. Slide the sample plate into the loading slot.
3. In the Tune Plus window, choose **Setup > MALDI Source**.

   The MALDI Source dialog box opens with the Control tab displayed (Figure 11).

Figure 11. MALDI Source dialog box

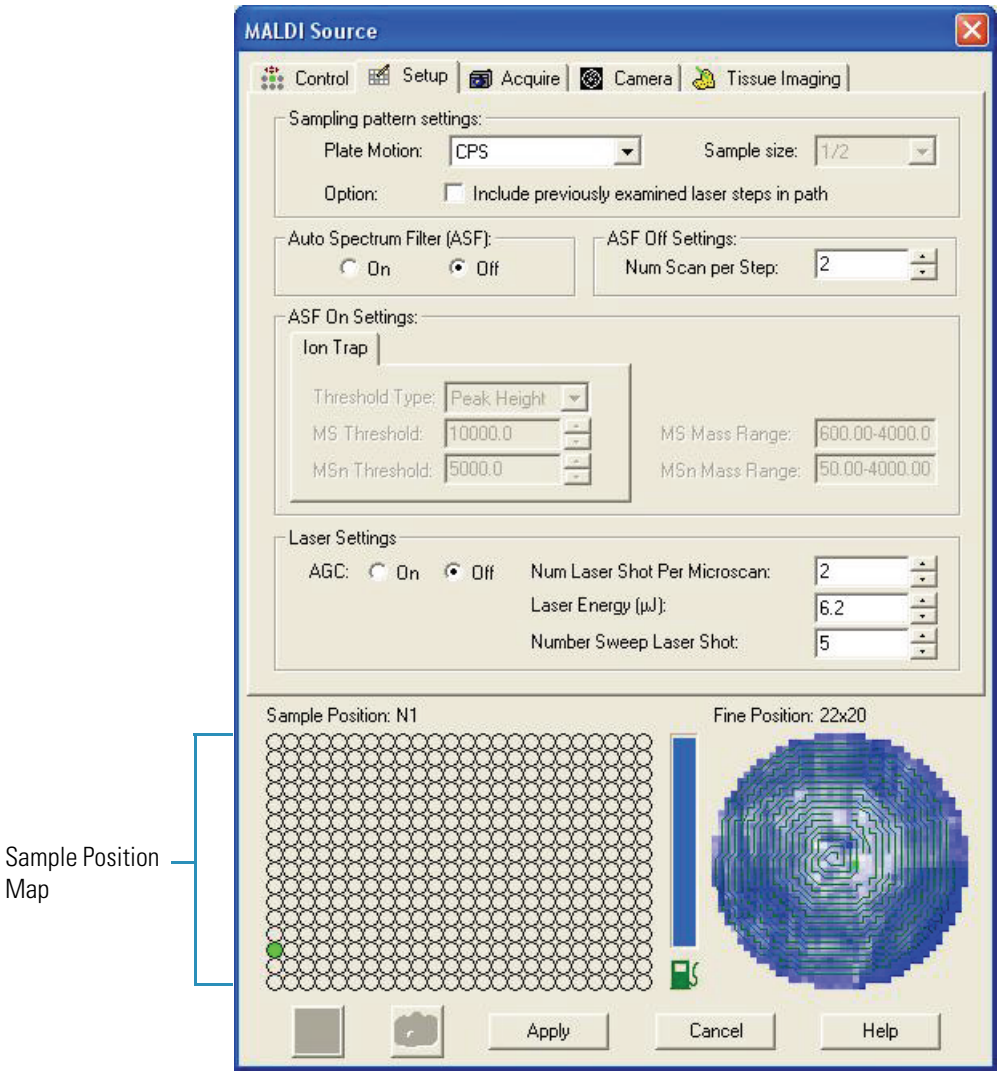


Note: The Tissue Imaging tab is only displayed when you have the tissue imaging license installed.

4. Click the **Setup** tab.

   The Setup page opens (Figure 12).

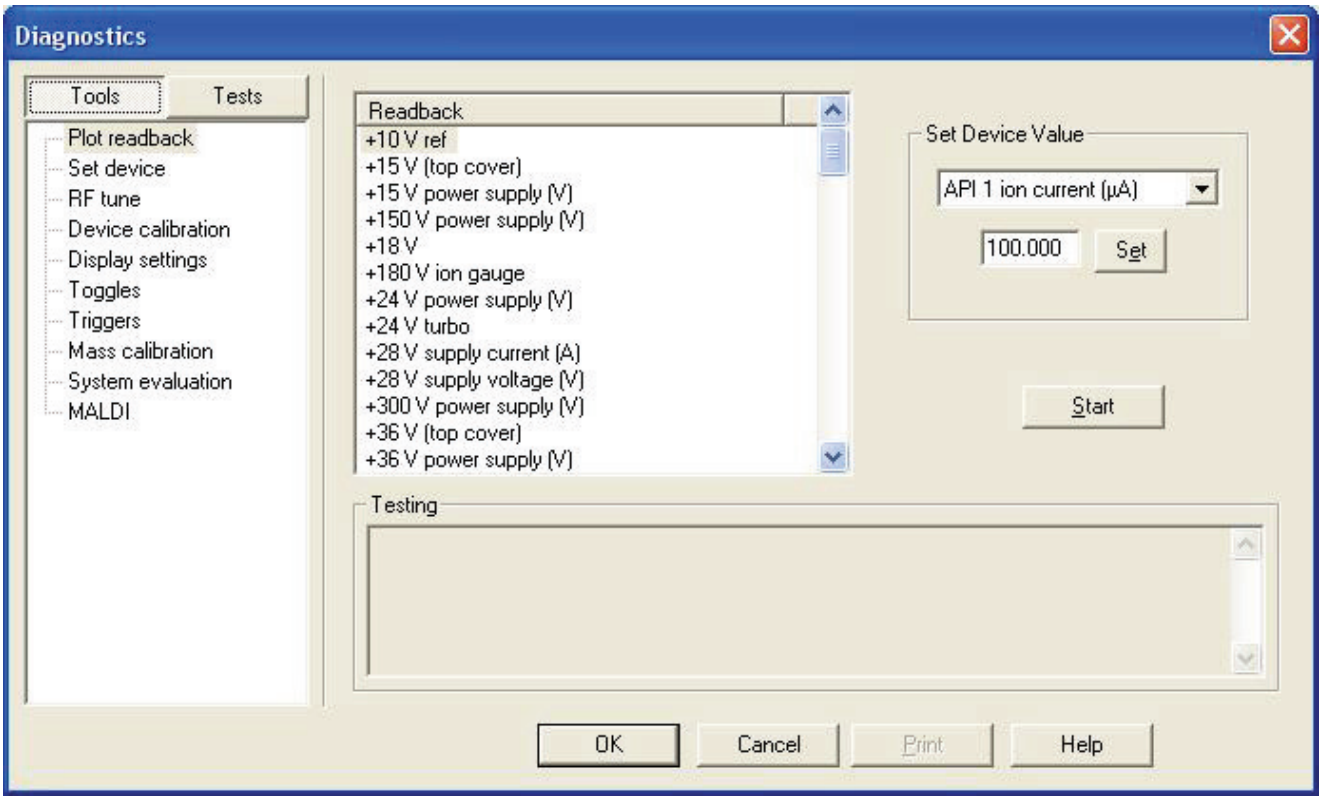
Figure 12. Setup page



5. In the Sample Position Map (Figure 12), point and click to position the sample well with the thin layer of matrix in the center of the camera display.
6. In the Laser Settings area, enter **100** in the Laser Energy box, to open the attenuator fully and click **Apply**.
7. In Tune Plus, choose **Diagnostics > Diagnostics**.

   The Diagnostics dialog box opens (Figure 13).

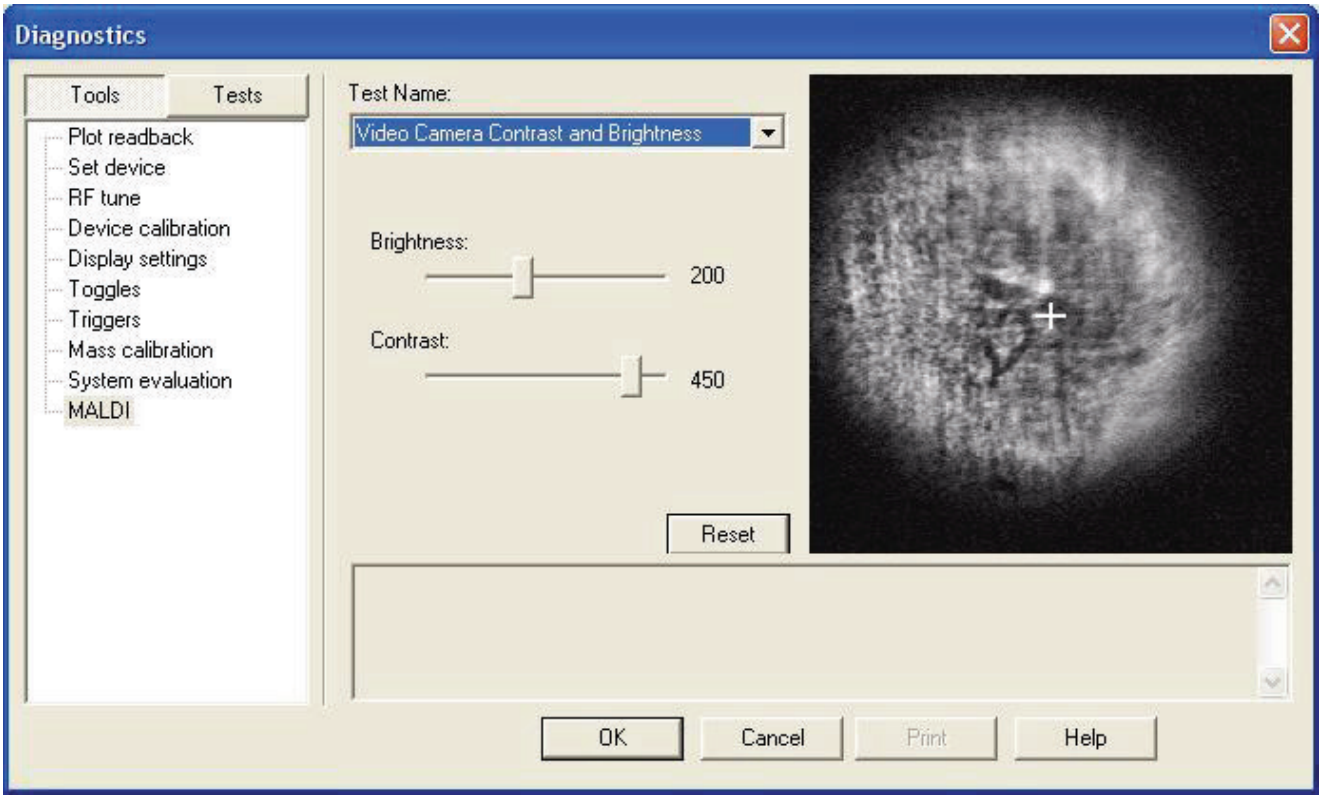
Figure 13. Diagnostics dialog box



8. In the Diagnostics dialog box, from the Tools list, select **MALDI**.

   MALDI tests are displayed with the Video Camera Contrast and Brightness test shown by default (Figure 14).

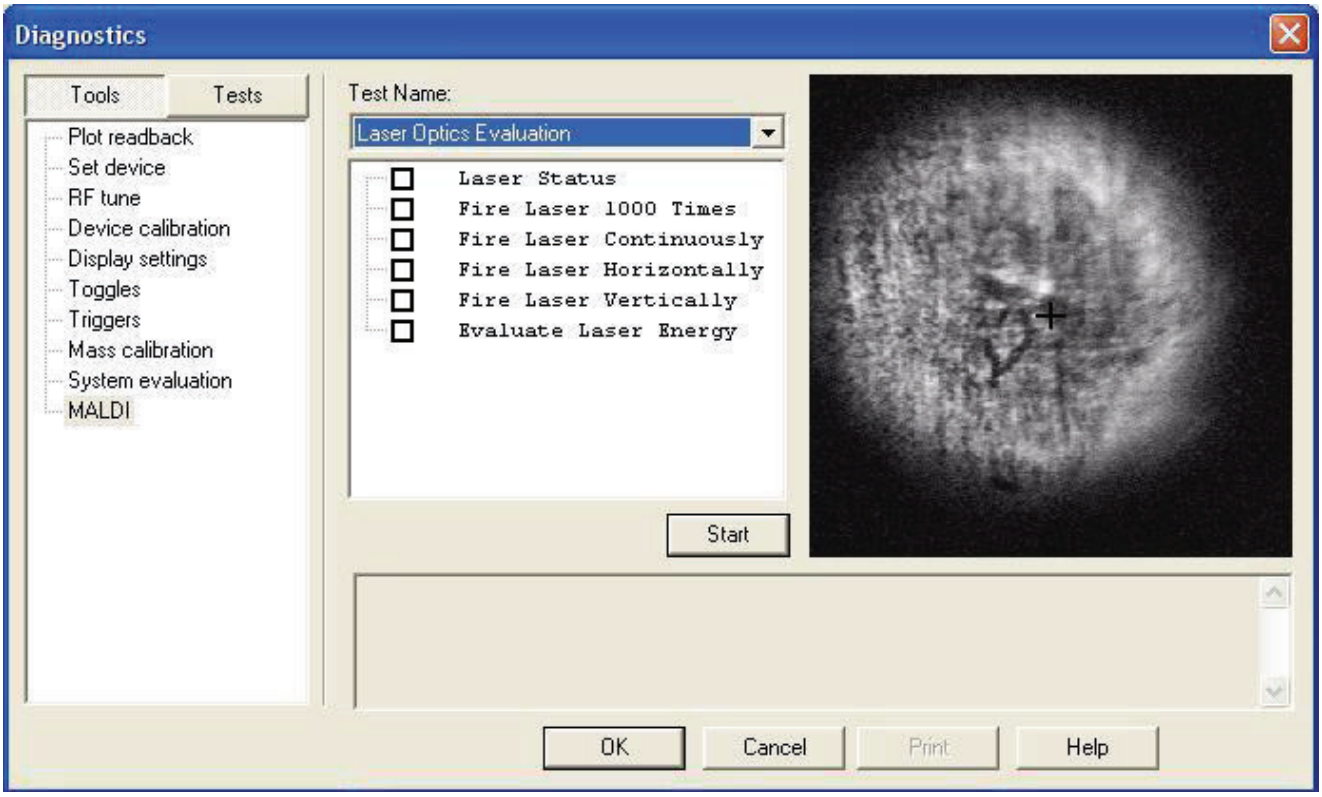
Figure 14. Video Camera Contrast and Brightness test



9. From the Test Name list, select **Laser Optics Evaluation**.

   The Laser Optics Evaluation test list opens (Figure 15).

Figure 15. Laser Optics Evaluation test list



For more information about the other MALDI diagnostic tests, refer to "Diagnostics" on page 33.

10. In the Laser Optics Evaluation test list, select the **Fire Laser Continuously** check box and click **Start**.

11. Observe the camera display for a dark spot that appears and grows where the laser is burning the matrix. That spot will center on the cross in the center of the camera display. If not, then adjust the laser position.

    a. To access the laser adjustment knobs in the optics module, see "Accessing the Camera and Laser" on page 77.

    b. Use the two adjustment knobs to position the laser spot on the cross.

    c. As the matrix becomes depleted in any given area, click the mouse on the camera display to move to a new spot.

    Note If you must take a break for more than a few seconds from adjusting the laser position, click **Stop** in the Diagnostics dialog box to stop the laser from firing. To resume, click **Start** at any time.

12. When the laser is centered in the camera display, click **Stop** in the Diagnostics dialog box, and close the dialog box.

13. Remove the sample plate and store it in a dry, dark location.
14. Reinstall the optics module cover and the LTQ XL top cover.

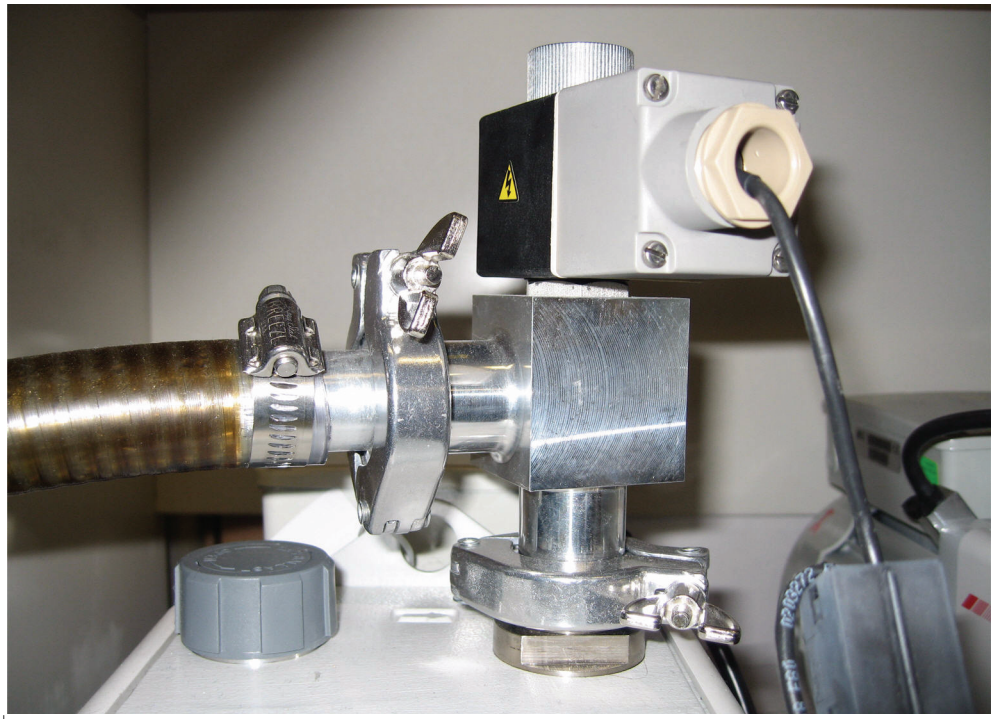
Replacing the Solenoid Vacuum Valve

The mechanical pump connected to the MALDI sample module and the NW25 solenoid vacuum valve control the evacuation and venting of the load lock chamber.

The solenoid vacuum valve (Figure 16) has two Kwik-Flange™ (KF) fittings. One fitting is inline with the body of the valve (the "inline fitting"), and the other is placed at 90 degrees to it (the "orthogonal fitting"). Each fitting is sealed by a center-ring assembly and clamped to a mated fitting on the vacuum line.

During normal operations, the solenoid might fail. If the solenoid vacuum valve fails, replace the vacuum valve.

Figure 16. Solenoid vacuum valve and fittings



❖ To replace the vacuum valve

1. With the load lock chamber at atmospheric pressure, unplug the vacuum valve cable from the port labeled Vacuum Valve on the back of the MALDI control module.
2. Remove the hinged clamps from the KF fittings on the vacuum valve.
3. Inspect the O-ring portion of the centering ring to ensure that it is free of any cuts, nicks, or imperfections that might cause it to leak. If it is defective, replace the centering ring.

4. Use the hinged clamps to secure the KF fittings on the vacuum valve.
   - Clamp the solenoid vacuum valve to the pump inlet fitting.
   - Clamp the vacuum line from the load lock chamber to the orthogonal fitting of the solenoid vacuum valve.
5. Connect the solenoid cable from the replacement valve to the port labeled Vacuum Valve on the back of the MALDI control module.

Diagnostics

This chapter describes how to use the MALDI diagnostics to diagnose, evaluate, tune, and calibrate the MALDI LTQ XL system.

Contents

Overview of the Diagnostic System

The diagnostic system is available through the Diagnostics menu in Tune Plus.

❖ **To diagnose and resolve instrument problems using Tune Plus**

1. In Tune Plus, choose **Diagnostics > Diagnostics**.

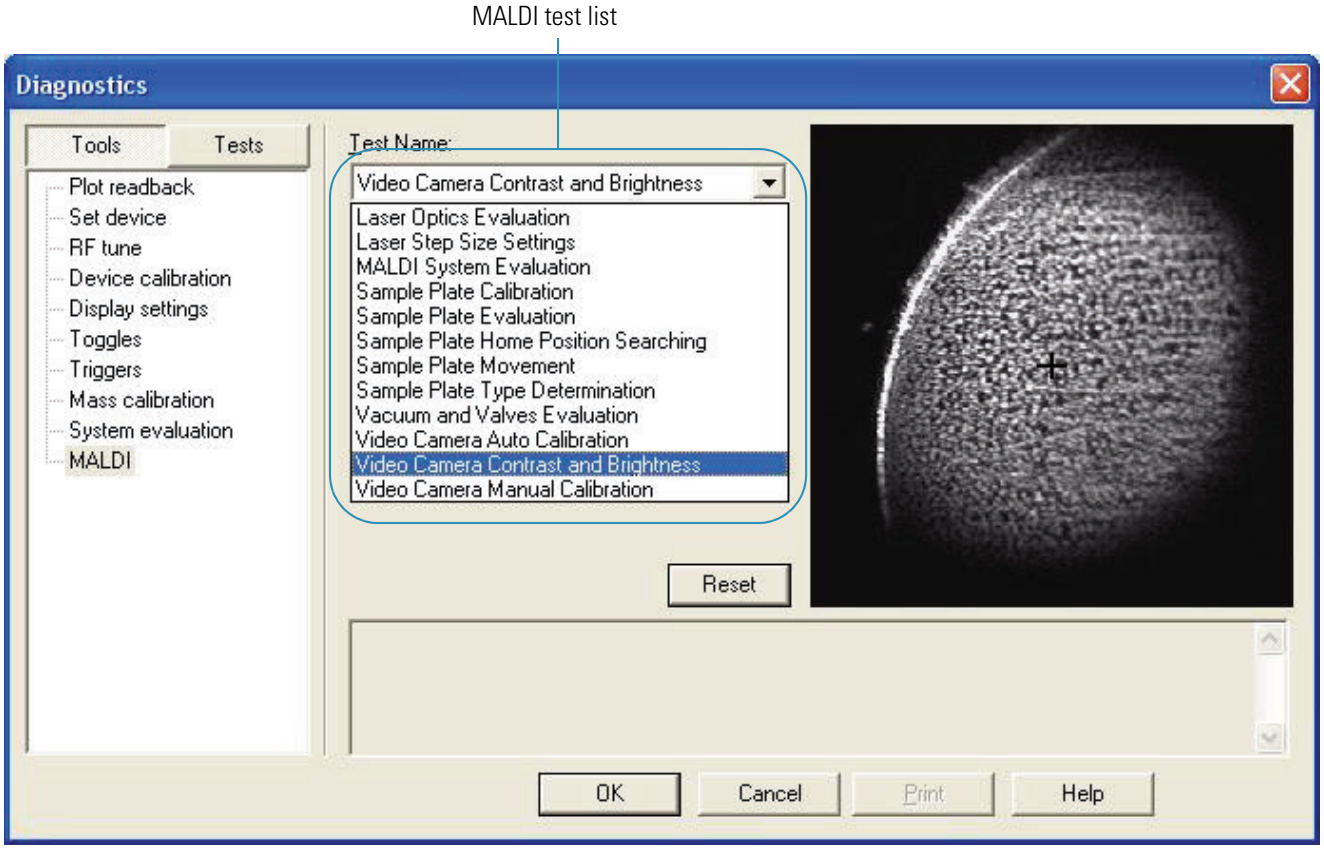
   The Diagnostics dialog box opens (Figure 13 on page 28).

2. In the Diagnostics dialog box, from the Tools list, select **MALDI**.

   MALDI tests are displayed with the Video Camera Contrast and Brightness test shown by default (Figure 15 on page 30.)

   Figure 17 shows the expanded MALDI test list.

Figure 17. MALDI test list



3. From the Diagnostics dialog box, select a diagnostic tool from the Test Name list.

   For more information about each diagnostic option, see the following topics:

Laser Optics Evaluation	Page 35
Laser Step Size Settings	Not implemented
MALDI System Evaluation	Page 36
Sample Plate Calibration	Page 38
Sample Plate Evaluation	Page 39
Sample Plate Home Position Searching	Page 41
Sample Plate Movement	Page 42
Sample Plate Type Determination	Page 45
Vacuum and Valves Evaluation	Page 47
Video Camera Auto Calibration	Page 48
Video Camera Manual Calibration	Page 49
Video Camera Contrast and Brightness	Page 51

Laser Optics Evaluation

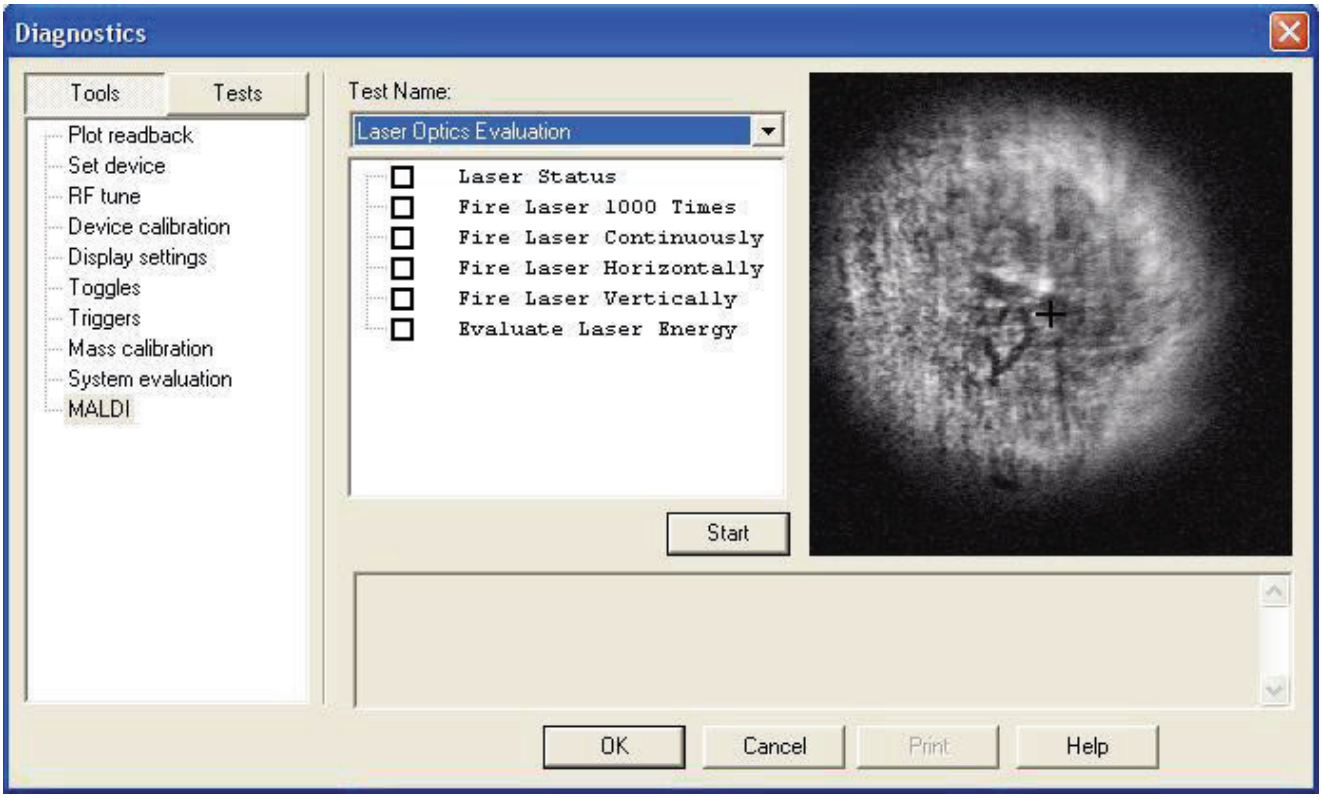
The Laser Optics Evaluation test contains diagnostic tools for checking the function of the laser.

❖ To check the function of the laser

1. In the Diagnostics dialog box, select **Laser Options Evaluation** from the Test Name list.

   The Laser Optics Evaluation test list opens (Figure 18).

Figure 18. Laser Optics Evaluation test list



2. Select from the following tests:

   - Laser Status: Displays information about the laser status including the laser shot counter and the laser energy.
   - Fire Laser 1000 times: Signals the MALDI optics module to fire 1000 laser pulses. Use this diagnostic routine to verify the laser function and observe the matrix burning in the camera display.
   - Fire Laser Continuously: Signals the MALDI optics module to fire the laser continuously until you click Stop. Use this diagnostic routine to verify the laser function and observe the matrix burning in the camera display.

- Fire Laser Horizontally: Signals the MALDI optics module to repeat a sequence of firing the laser 20 times and then moving the sample plate100 µm in the X direction. Use this diagnostic routine to verify the laser function and observe the matrix burning in the camera display. The laser stops automatically when it passes the edge of the sample spot.
- Fire Laser Vertically: Signals the MALDI optics module to repeat a sequence of firing the laser 20 times and then moving the sample plate 100 µm in the Y direction. Use this diagnostic routine to verify the laser function and observe the matrix burning in the camera display. The laser stops automatically when it passes the edge of the sample spot.
- Evaluate Laser Energy: Displays the current energy reading for the laser.

3. Click **Start**.

   If the tests indicate any problems with the laser alignment, you must manually align the laser.

MALDI System Evaluation

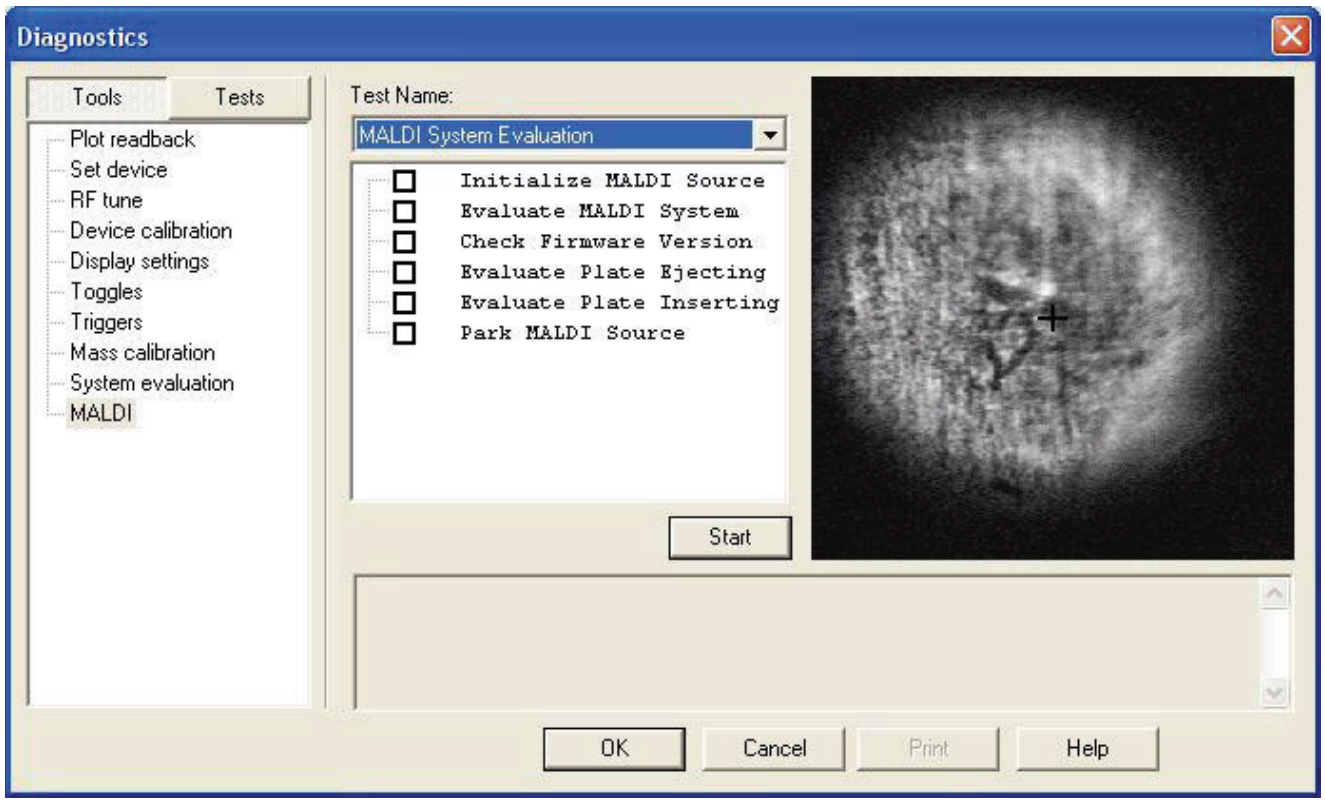
The MALDI System Evaluation diagnostics provide tools for checking the functions of the hardware and firmware in the MALDI source. These tools test the firmware version and check the functions of the mechanical systems for handling the sample plate.

❖ To check functions of the hardware and firmware in the MALDI source

1. In the Diagnostics dialog box, select **MALDI System Evaluation** from the Test Name list.

   The MALDI System Evaluation test list opens (Figure 19).

Figure 19. MALDI System Evaluation test list



2. Select from the following options:

   - Initialize MALDI Source: Signals the source to move the XYZ mechanism and pickup tray between their upper and lower limit switches. It also signals the source to pump down the sample chamber if the chamber is already closed.

     Note If for any reason the MALDI source becomes unstable and is not responding, run the Initialize MALDI Source diagnostic.

   - Evaluate MALDI System: Returns information on current instrument conditions, the results of the last source initialization, any current errors or warnings, and the firmware version.
   - Check Firmware Version: Returns the version number of the currently installed firmware.
   - Evaluate Plate Ejecting: Signals the MALDI source to eject a sample plate and reports on any failures if this cannot be done.
   - Evaluate Plate Inserting: Signals the MALDI source to accept a sample plate, and reports on any failures if this cannot be done.
   - Park MALDI Source: Locks down the MALDI source so that it can be moved or exchanged for the API source.

3. Click **Start**.

Working With Sample Plates

This section describes how to work with sample plates and contains the following procedures:

- Sample Plate Calibration
- Sample Plate Evaluation
- Sample Plate Home Position Searching
- Sample Plate Movement
- Sample Plate Type Determination

Sample Plate Calibration

The Sample Plate Calibration diagnostic menu activates or deactivates the processes that the MALDI source uses for position calibration of the sample plate. Positional calibration is a process of locating the calibration marks on the plate and giving the source the positional information. The MALDI source runs this process automatically each time a sample plate is inserted into the source (see Figure 20).

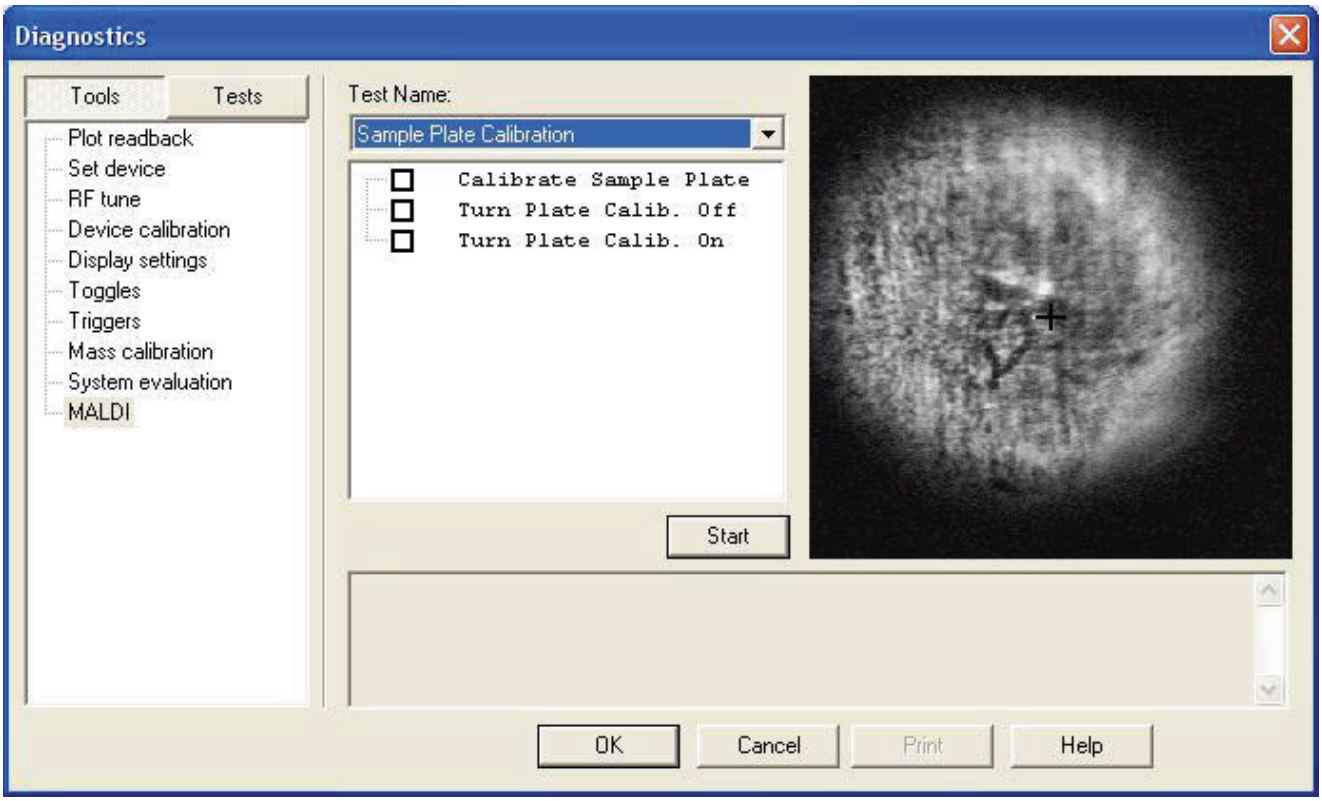
Without calibration data (or if calibration is turned off), the source relies on purely mechanical information (the distance the plate is moved and the standard dimensions of the plate) to position the sample plate. This method introduces a small amount of error, so calibration is important for accurate work.

❖ To activate or deactive processes used for position calibration of the sample plate

1. From the Diagnostics dialog box, select **Sample Plate Calibration** from the Test Name list.

   The Sample Plate Calibration test list opens (Figure 20).

Figure 20. Sample Plate Calibration test list



2. Select from the following options:
   - Calibrate Sample Plate: Begins the positional calibration process.
   - Turn Plate Calib. Off: Disables the most recently acquired calibration data and tells the source to rely on mechanical information to position the sample plate.
   - Turn Plate Calib. On: Signals the source to use the most recently acquired calibration data. This option is automatically enabled after successful calibration.
3. Click **Start**.

Sample Plate Evaluation

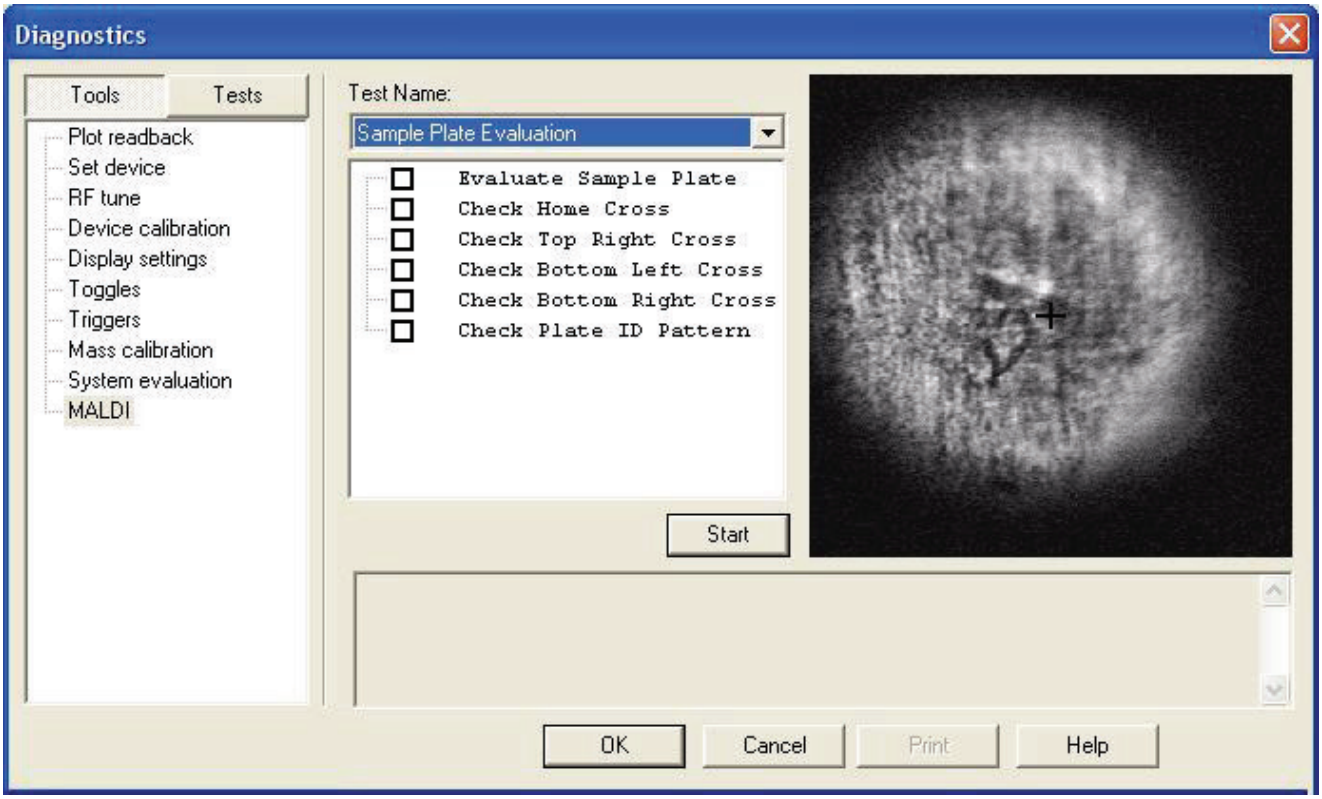
The Sample Plate Evaluation diagnostic menu contains tools for checking the calibration and identification marks on the sample plate.

❖ To check the calibration and identification marks on the sample plate

1. From the Diagnostics dialog box, select **Sample Plate Evaluation** from the Test Name list.

   The Sample Plate Evaluation test list opens (Figure 21).

Figure 21. Sample Plate Evaluation test list



2. Select from the following tests:

   - Evaluate Sample Plate: Begins the process of checking each of the calibration and identification marks on the sample plate. This diagnostic routine performs all of the other diagnostics in this list.
   - Check Home Cross: Signals the MALDI source to locate the calibration cross on the upper-left corner of the sample plate and verify that it is readable.
   - Check Top Right Cross: Signals the MALDI source to locate and verify the calibration cross on the upper-right corner of the sample plate.
   - Check Bottom Left Cross: Signals the MALDI source to locate and verify the calibration cross on the lower-left corner of the sample plate.
   - Check Bottom Right Cross: Signals the MALDI source to locate and verify the calibration cross on the lower-right corner of the sample plate.
   - Check Plate ID Pattern: Signals the MALDI source to locate and verify the identification mark that identifies the sample plate.

3. Click **Start**.

Sample Plate Home Position Searching

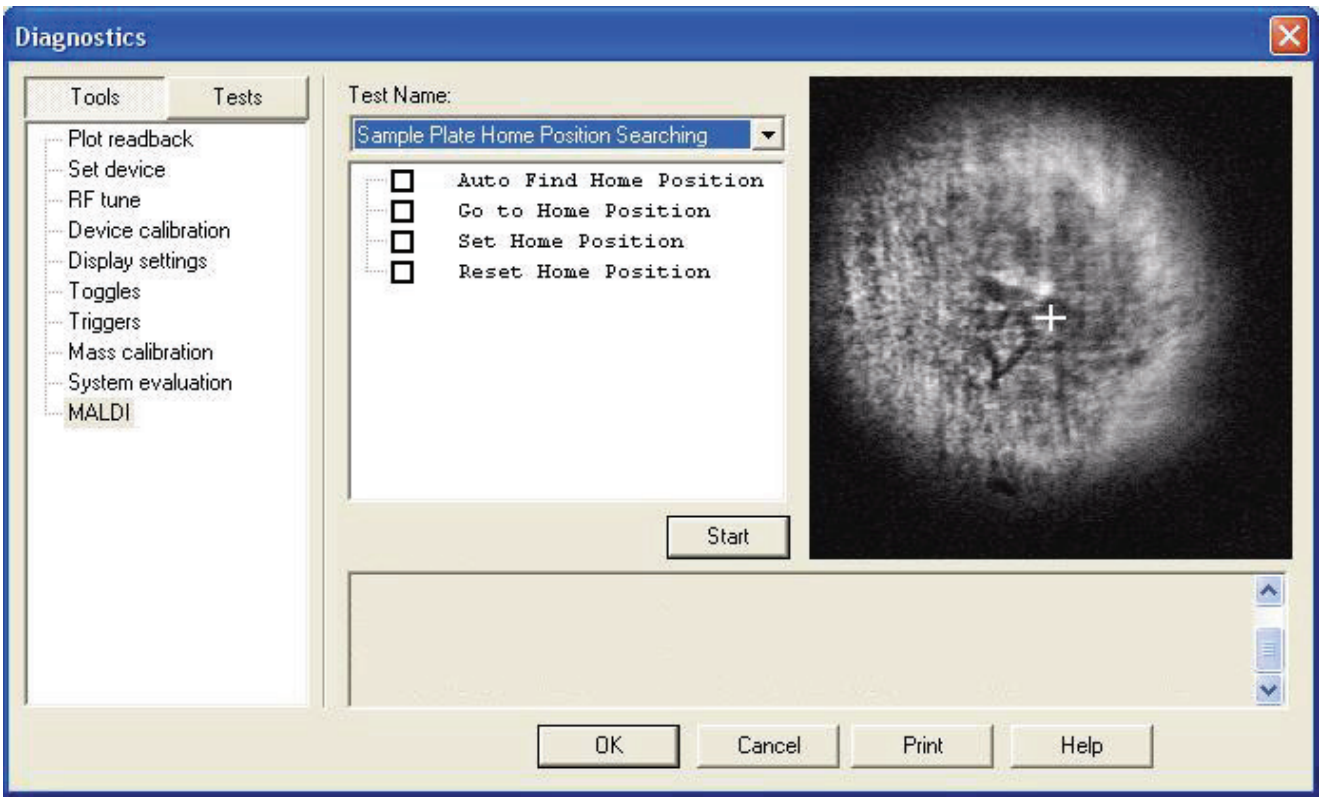
The Sample Plate Home Position Searching diagnostic menu contains tools to locate the home position either automatically or manually.

❖ To locate the sample plate home position

1. From the Diagnostics dialog box, select **Sample Plate Home Position Searching** from the Test Name list.

   The Sample Plate Home Position Searching test list opens (Figure 22).

Figure 22. Sample Plate Home Position Searching test list



2. Select from the following options:

   - Auto Find Home Position: Signals the source to locate the home cross in the upper-left corner of the sample plate, bring the home cross to the center of the camera field, and set that position as "home."

     The Auto Find Home Position command is the best way to locate the home calibration cross and set the home position. If that command fails, then the most likely cause is one of the following:

     – The camera is out of alignment. In this case, run a full video calibration as described in "Running a Full Video Calibration" on page 53.

- The camera aperture is too small. In this case the camera display appears dark. You might need to adjust the aperture, as described in "Fine Position Map is Misaligned" on page 62.
- The camera aperture is too large. In this case the camera display appears too bright. You might need to adjust the aperture, also described in "Fine Position Map is Misaligned" on page 62.
- The camera is not focusing properly. You might need to adjust the focus, as described in "Camera Focus is Out of Adjustment" on page 61.
- The optical illuminator is not putting enough light on the sample plate because the illuminator LED is bad, the illuminator fiber is broken, or something is blocking the light.

  Note If the optical illuminator in your MALDI source is defective, contact Thermo Fisher Scientific Technical Support or a field service engineer.

- Something is obscuring the identifier mark on the sample plate.

  If you are unable to rule out or repair all of these causes, you can move the plate manually using the Go to Home Position command. You may also move the sample plate using the mouse. Click any point in the camera display to signal the MALDI source to center the camera display on that point. However, these are stopgap measures only, until you identify and repair the problem that is preventing the Auto Find Home Position command from working properly.

- Go to Home Position: Moves the sample plate to the last set "home" position.
- Set Home Position: Sets the home position manually, wherever the camera display is centered.

  Note The Set Home Position command cannot be undone. Once set, the only way to reset the home position is to run this command again or use the **Auto Find Home Position** command.

- Reset Home Position: Resets the home position to the manufacturing default location.

3. Click **Start**.

Sample Plate Movement

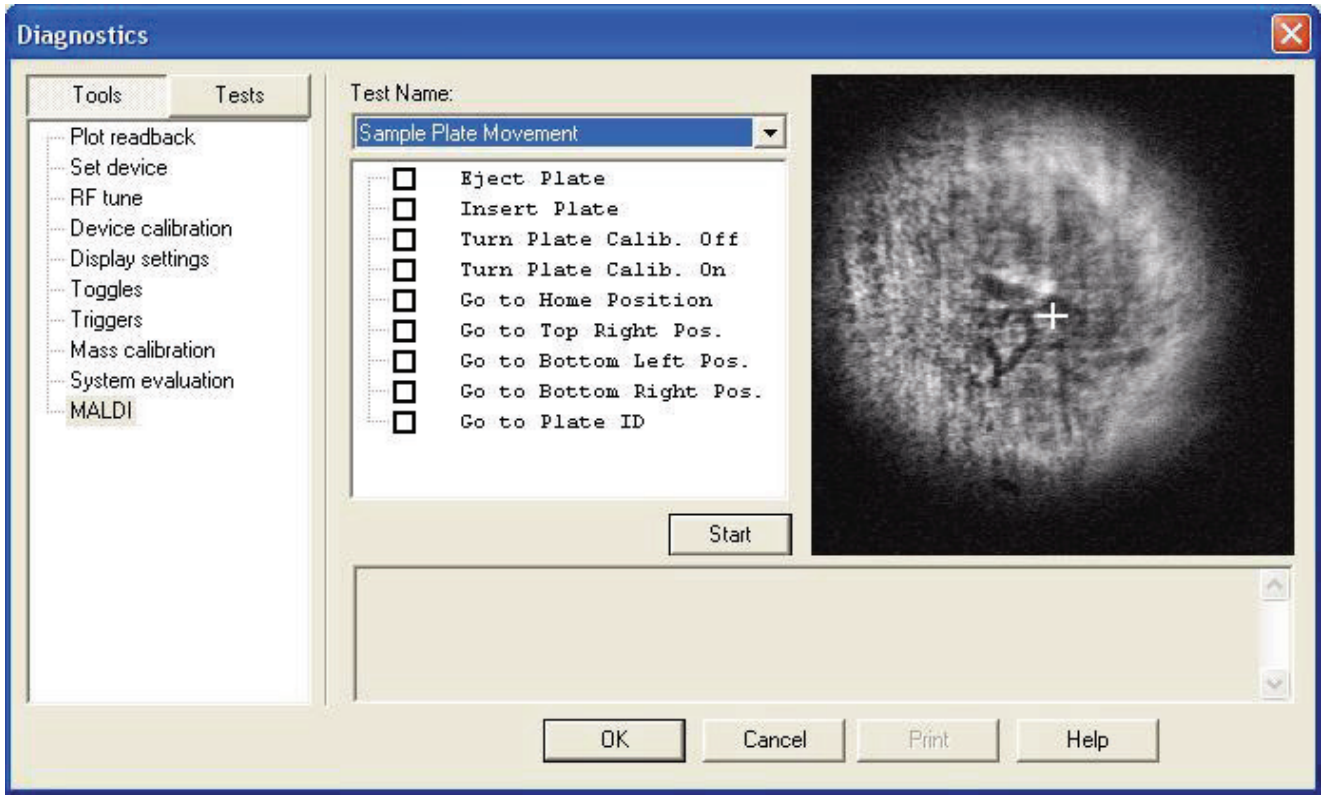
The Sample Plate Movement list contains tools for testing the movement of the XYZ mechanism as it holds the sample plate. These commands also help you check the accuracy of the plate calibration information.

❖ To test the movement of the XYZ mechanism

1. From the Diagnostics dialog box, select **Sample Plate Movement** from the Test Name list.

   The Sample Plate Movement test list opens (Figure 23).

Figure 23. Sample Plate Movement test list



2. Select from the following diagnostic tools:

   - Eject Plate: Signals the MALDI source to eject a sample plate and reports on any failures if this cannot be done.
   - Insert Plate: Signals the MALDI source to accept a sample plate and reports on any failures if this cannot be done.
   - Turn Plate Calib. Off: Disables the most recently acquired calibration data and tells the source to rely on mechanical information to position the sample plate.
   - Turn Plate Calib. On: Signals the source to use the most recently acquired calibration data. This option is automatically enabled at the end of any successful calibration.
   - Go to Home Position: Moves the sample plate to the last set home position.
   - Go to Top Right Pos.: Moves the sample plate to place the upper-right calibration cross in the camera display.

- Go to Bottom Left Pos.: Moves the sample plate to place the lower-left calibration cross in the camera display.
- Go to Bottom Right Pos.: Moves the sample plate to place the lower-right calibration cross in the camera display.
- Go to Plate ID: Moves the sample plate to place the plate identification pattern in the camera display.

3. Click **Start**.

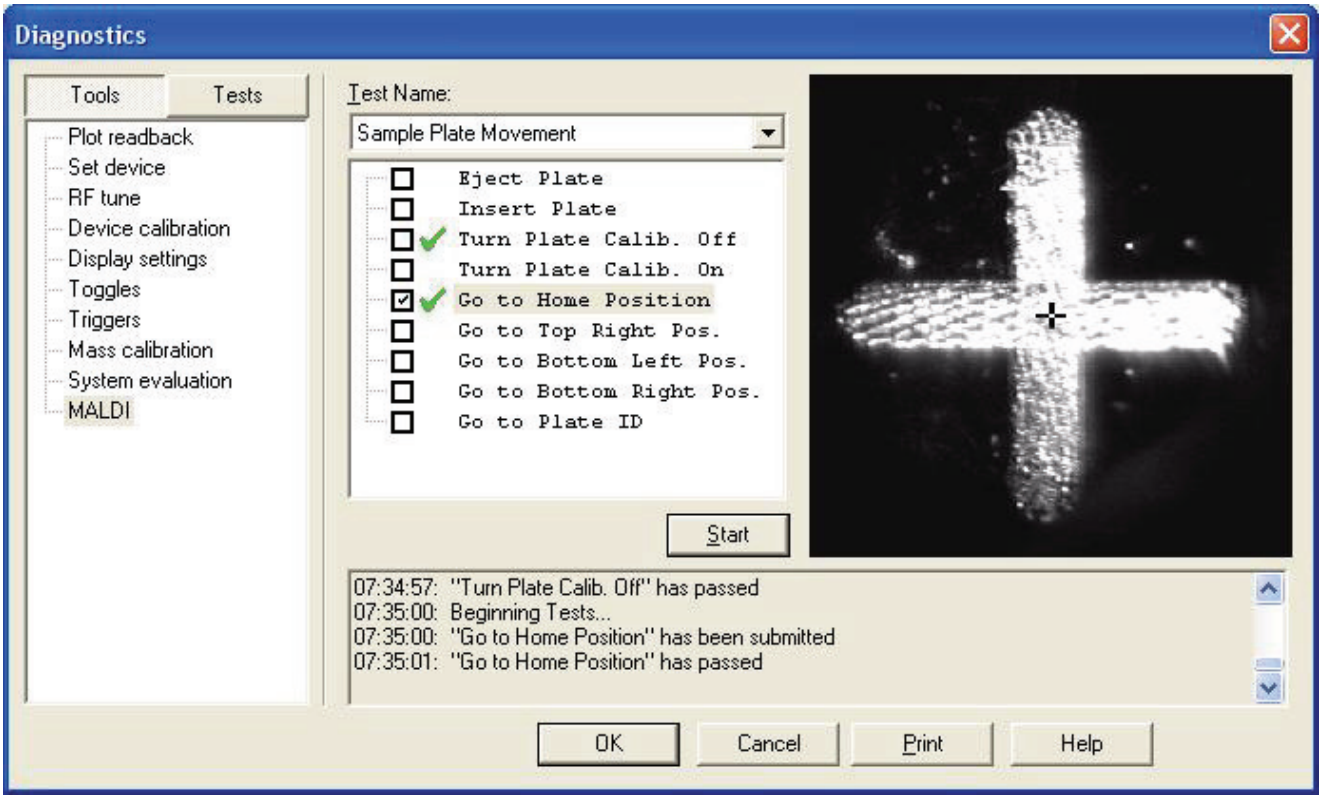
❖ To check the accuracy of the plate calibration

1. From the Diagnostics dialog box, select **Sample Plate Movement** from the Test Name list.

   The Sample Plate Movement test list opens (Figure 23 on page 43).

2. Select **Turn Plate Calib. Off**.
3. Click **Start**.
4. From the Sample Plate Movement test list, select **Go to Home Position**.
5. Click **Start**.
6. Verify that the calibration cross on the sample plate is centered beneath the cross on the camera display (Figure 24).

Figure 24. Calibration cross and sample plate cross



7. Repeat Step 2 for the Top Right, Bottom Left, and Bottom Right position commands.
8. From the Sample Plate Movement test list, select **Turn Plate Calib. On**.
9. Click **Start**.

If all four calibration crosses are properly centered, then the plate is properly calibrated. If not, then recalibrate the plate.

Sample Plate Type Determination

Use the Sample Plate Type Determination diagnostic menu to set the sample plate type manually. The plate type is critical for properly mapping the plate. Use the Autoconfig Plate Type command in most cases. Use the manual set commands to set the plate type only when the automatic command fails.

Note If you insert a tissue imaging plate without the tissue imaging license installed, the plate type defaults to a Thermo 96 Well Plate. You can use this menu to set the plate type to a tissue imaging plate, but the Tissue Imaging tab is not shown in the MALDI source dialog box.

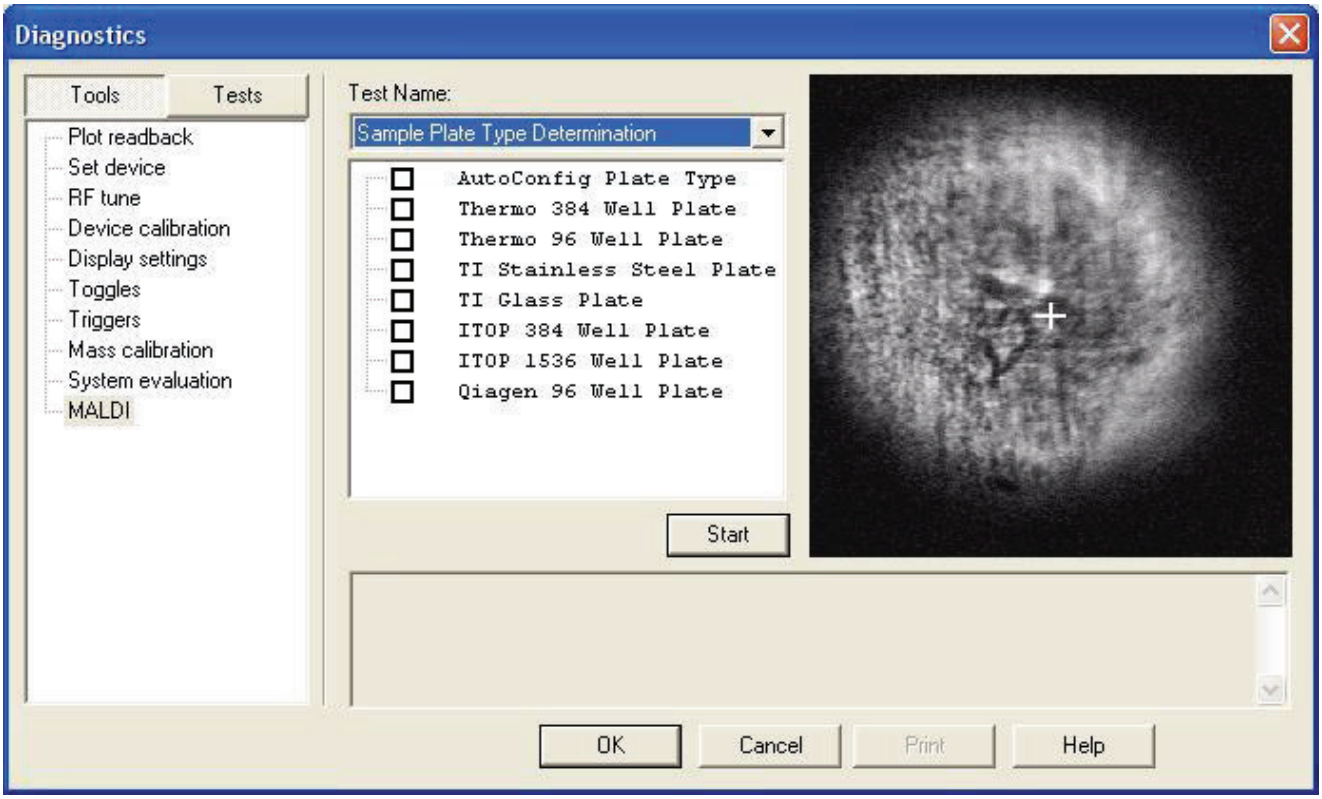
If the diagnostics cannot determine the sample plate type, the plate type defaults to a Thermo 96 Well Plate.

❖ To set the sample plate type

1. From the Diagnostics dialog box, select **Sample Plate Type Determination** from the Test Name list.

   The Sample Plate Type Determination test list opens (Figure 25).

Figure 25. Sample Plate Type Determination test list



2. Select from the following options:

   - Autoconfig Plate Type: Signals the MALDI source to automatically locate the plate identification pattern and determine the type of plate.
   - Thermo 384 Well Plate: Manually sets the plate type to a Thermo Scientific 384-well plate.
   - Thermo 96 Well Plate: Manually sets the plate type to a Thermo Scientific 96-well plate.
   - TI Stainless Steel Plate: Manually sets the plate type to a Thermo Scientific steel slide tissue imaging plate.
   - TI Glass Plate: Manually sets the plate type to a Thermo Scientific glass slide tissue imaging plate.
   - ITOP 384 Well Plate

- ITOP 96 Well Plate
- Qiagen™ 96 Well Plate

3. Click **Start**.

Vacuum and Valves Evaluation

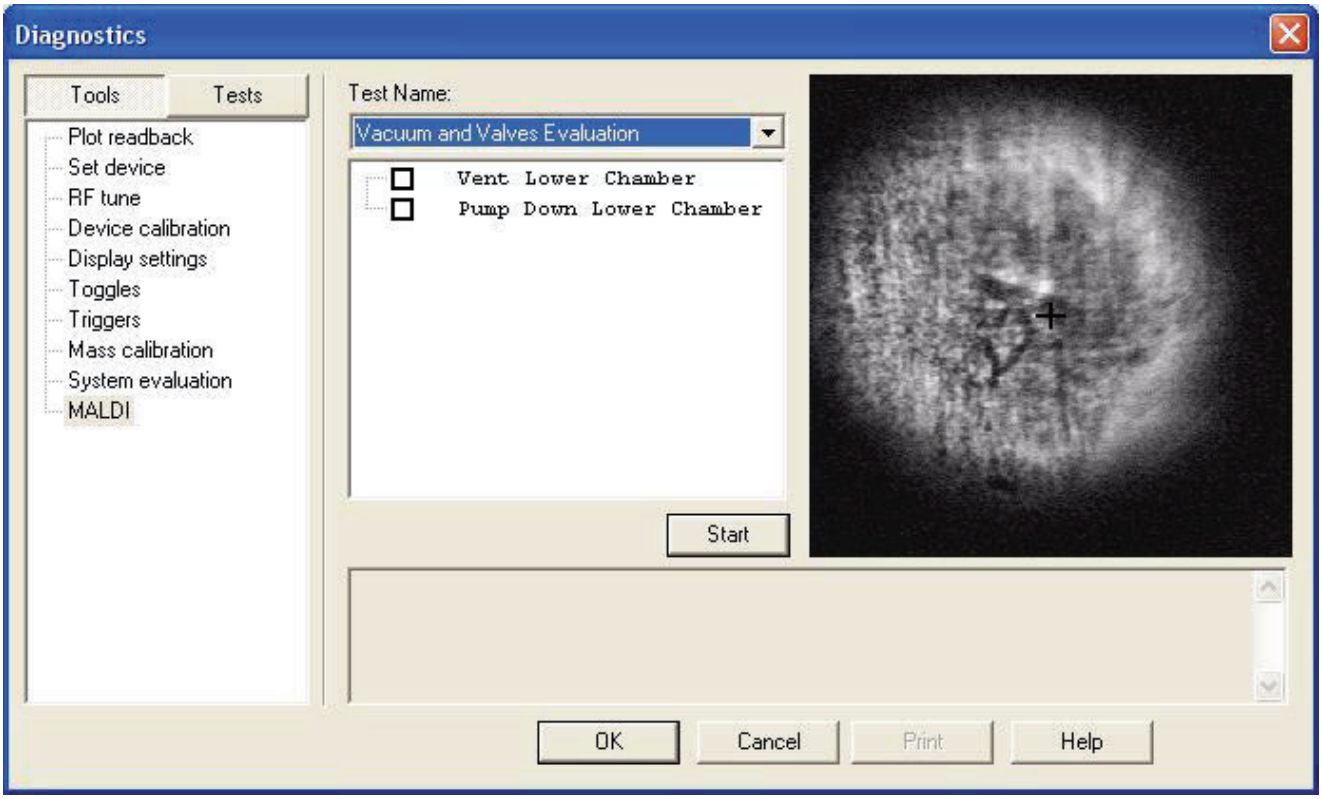
The Vacuum and Valves Evaluation diagnostic menu provides tools for checking the function of the vent valve and vacuum system that serve the lower chamber of the MALDI source. Use these commands if there is a malfunction in the process of inserting or ejecting a sample plate, or to check for a vent valve failure.

❖ To evaluate the function of the vent valve and vacuum system

1. From the Diagnostics dialog box, select **Vacuum and Valves Evaluation** from the Test Name list.

   The Vacuum and Valves Evaluation test list opens (Figure 26).

Figure 26. Vacuum and Valves Evaluation test list



2. Select from the following diagnostic tools:
   - Vent Lower Chamber: Signals the MALDI source to open the vent valve in the load lock and vent the chamber to atmosphere. The vacuum in the upper chamber, which contains the XYZ mechanisms, is not affected.
   - Pump Down Lower Chamber: Signals the MALDI source to pump the load lock down to approximately 100 mTorr.
3. Click **Start**.

Tuning the Video Camera

This section provides the following procedures for tuning the video camera:

- Video Camera Auto Calibration
- Video Camera Manual Calibration
- Video Camera Contrast and Brightness

Note In order to run the following tests, you must have a sample plate loaded in the MALDI source.

Video Camera Auto Calibration

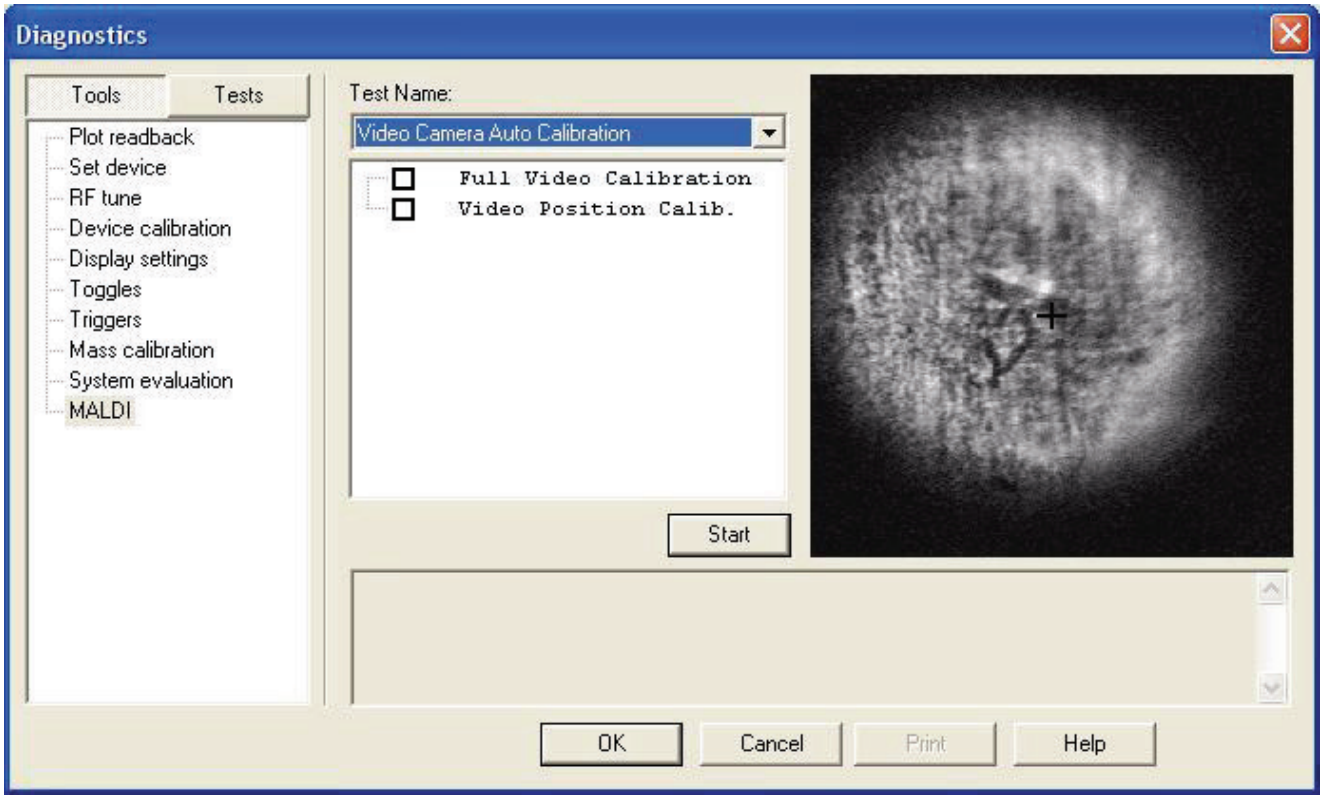
Use the Video Camera Auto Calibration diagnostic menu to calibrate the camera automatically. There are two components to this process: position calibration, in which the camera is centered on the calibration cross on the sample plate, and dimension calibration in which the system measures the relationship of a pixel of movement on the camera display to the actual movement of the XYZ mechanism.

❖ To calibrate the camera automatically

1. From the Diagnostics dialog box, select **Video Camera Auto Calibration** from the Test Name list.

   The Video Camera Auto Calibration test list opens (Figure 27).

Figure 27. Video Camera Auto Calibration test list



2. Select from the following diagnostic tools:

   - Full Video Calibration: Signals the MALDI source to calibrate the sample plate and the camera. Use this routine when both the sample plate and the camera are out of calibration.
   - Video Position Calibration: Signals the MALDI source to run a video calibration only. In a video calibration, the XYZ mechanism positions the sample plate so that the Home cross in the upper-left corner is in the field of the camera. The MALDI optical recognition software then directs the XYZ mechanism to move so as to center the Home cross in the camera's field of view. The system designates the X-Y coordinates so defined as Home. Use this routine when the sample plate is calibrated but the camera is not.

3. Click **Start**.

Video Camera Manual Calibration

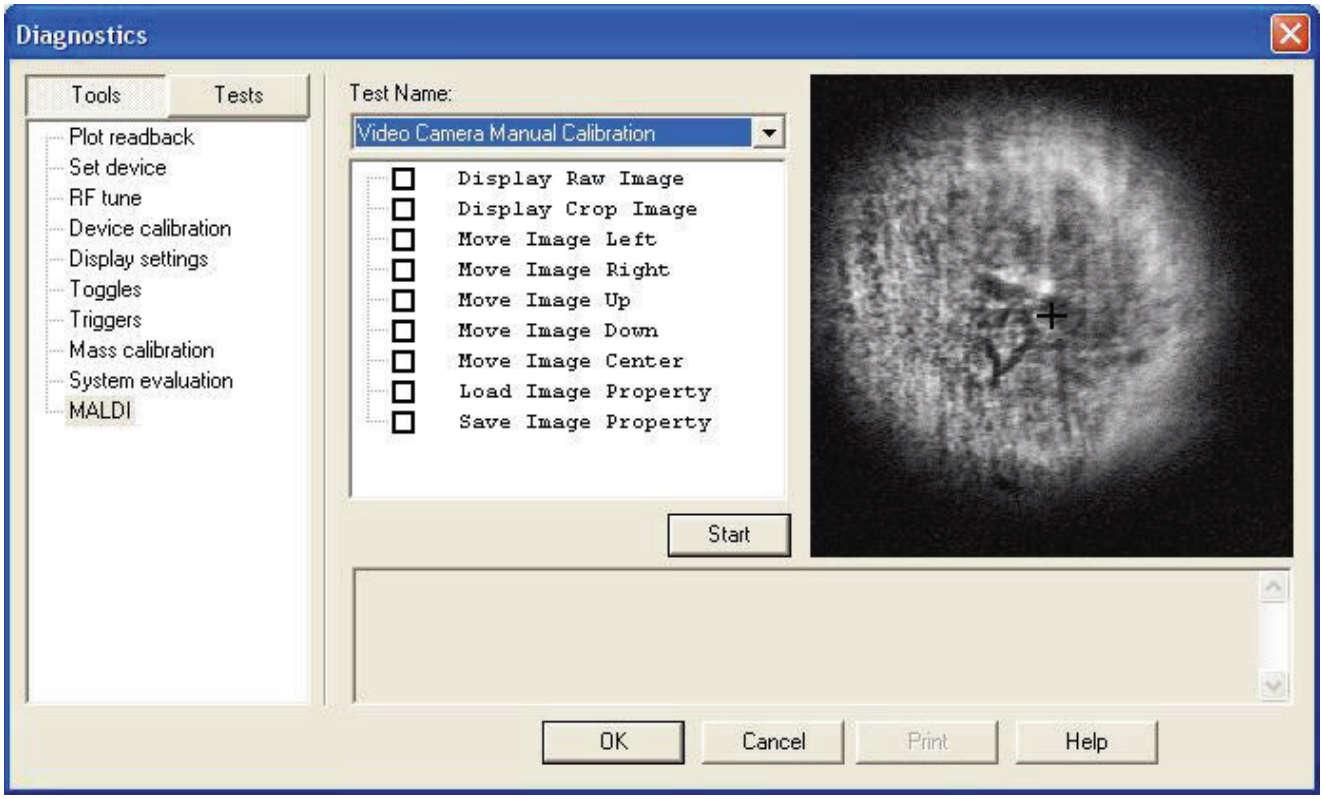
Use the Video Camera Manual Calibration diagnostic menu to calibrate the camera manually. This process makes certain that the camera is centered on the calibration cross on the sample plate.

❖ To calibrate the camera manually

1. From the Diagnostics dialog box, select **Video Camera Manual Calibration** from the Test Name list.

   The Video Camera Manual Calibration test list opens (Figure 28).

Figure 28. Video Camera Manual Calibration test list



2. Select from the following diagnostic tools:

   - Display Raw Image: Places the raw, unprocessed camera feed in the video display box. The raw image is uncorrected for the oblique viewing angle and contains edge images such as portions of the ion transfer quadrupole. A rectangle superimposed on the raw image defines the field that is used to produce the cropped image. When properly positioned, the raw image is centered in this rectangle.
   - Display Crop Image: Places the cropped and processed camera feed in the video display box. This image is digitally processed based on the last saved parameters for the crop rectangle to refine the field of view and correct for the oblique angle. The images then appear as they would looking straight down on the sample plate.

3. Use the following options only on the raw image. After you run these commands and save the resulting changes, you must recalibrate the camera display for position and dimension.

   - Move Image Left: Shifts the crop rectangle to the left.

- Move Image Right: Shifts the crop rectangle to the right.
- Move Image Up: Shifts the crop rectangle upward.
- Move Image Down: Shifts the crop rectangle downward.
- Move Image Center: Centers the crop rectangle in the raw image.
- Load Image Property: Undoes any changes to the crop rectangle and reloads the last saved values.
- Save Image Property: Saves the current parameters for the crop rectangle.

4. Click **Start**.

Video Camera Contrast and Brightness

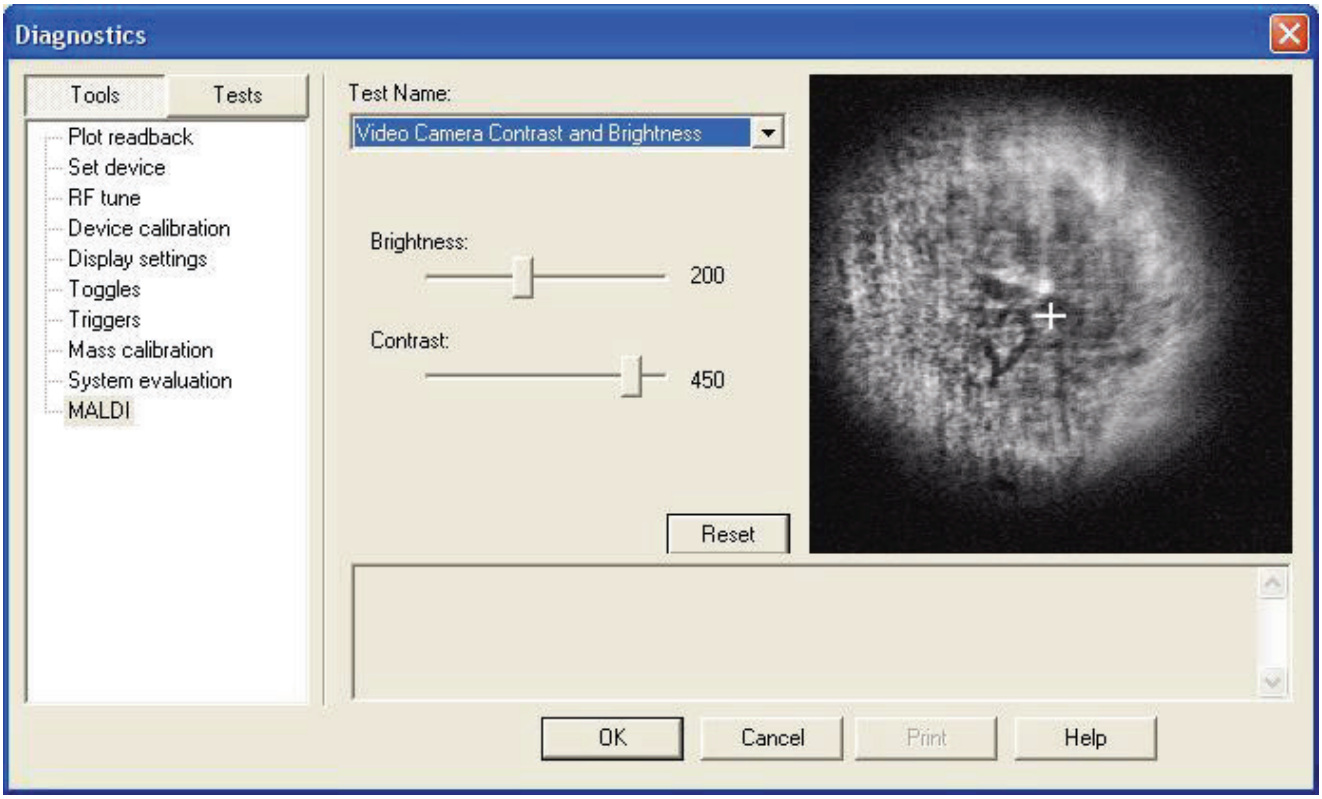
The Video Camera Contrast and Brightness diagnostic menu provides two sliding bars to adjust the appearance of the camera display.

❖ To adjust camera contrast and brightness

1. From the Diagnostics dialog box, select **Video Camera Contrast and Brightness** from the Test Name list.

   The Video Camera Contrast and Brightness test opens (Figure 29).

Figure 29. Video Camera Contrast and Brightness test



2. Use the Brightness and Contrast slider bars to adjust the camera image.
3. If needed, click **Reset** to restore the factory default values for brightness and contrast.

Preparing a Thin-Layer Matrix Sample

Some of the procedures that follow require a special sample consisting of a thin, even layer of matrix with no included analytes.

❖ To prepare a thin-layer matrix sample

1. Prepare 1mL of a saturated solution of any standard MALDI matrix material (for example, α-cyano-4-hydroxycinammic acid) in 100% HPLC-grade acetone.
2. Spot at least 10µL of the saturated solution onto at least a clean A1 position sample well on a MALDI sample plate.

Note The matrix solution may spread out beyond the edges of the sample well. This is acceptable, but the solution must not be allowed to touch any other well that contains a sample. To prevent spreading into other wells, spot the saturated solution in a sample well that has at least one clean well adjacent to it on each side, for a 96-well sample plate. For a 384-well sample plate, select a sample well that has at least two clean wells in all directions.

When preparing a sample plate for a full video calibration, coat the entire sample area of the plate with matrix. (The sample area is the sample wells and the area around them.) Avoid applying matrix to the calibration crosses and the plate identification mark.

3. Allow the matrix to dry normally. Do not heat the sample plate or place it in vacuum to accelerate the drying process.

Running a Full Video Calibration

Use full video calibration when both the sample plate and the camera are out of calibration.

Before performing this procedure, prepare a thin-layer matrix sample plate, with the matrix deposited over the entire sample area of the sample plate. See "Preparing a Thin-Layer Matrix Sample" on page 52.

Note The sample area is the sample wells and the area around them. Avoid applying matrix to the calibration crosses and the plate identification mark.

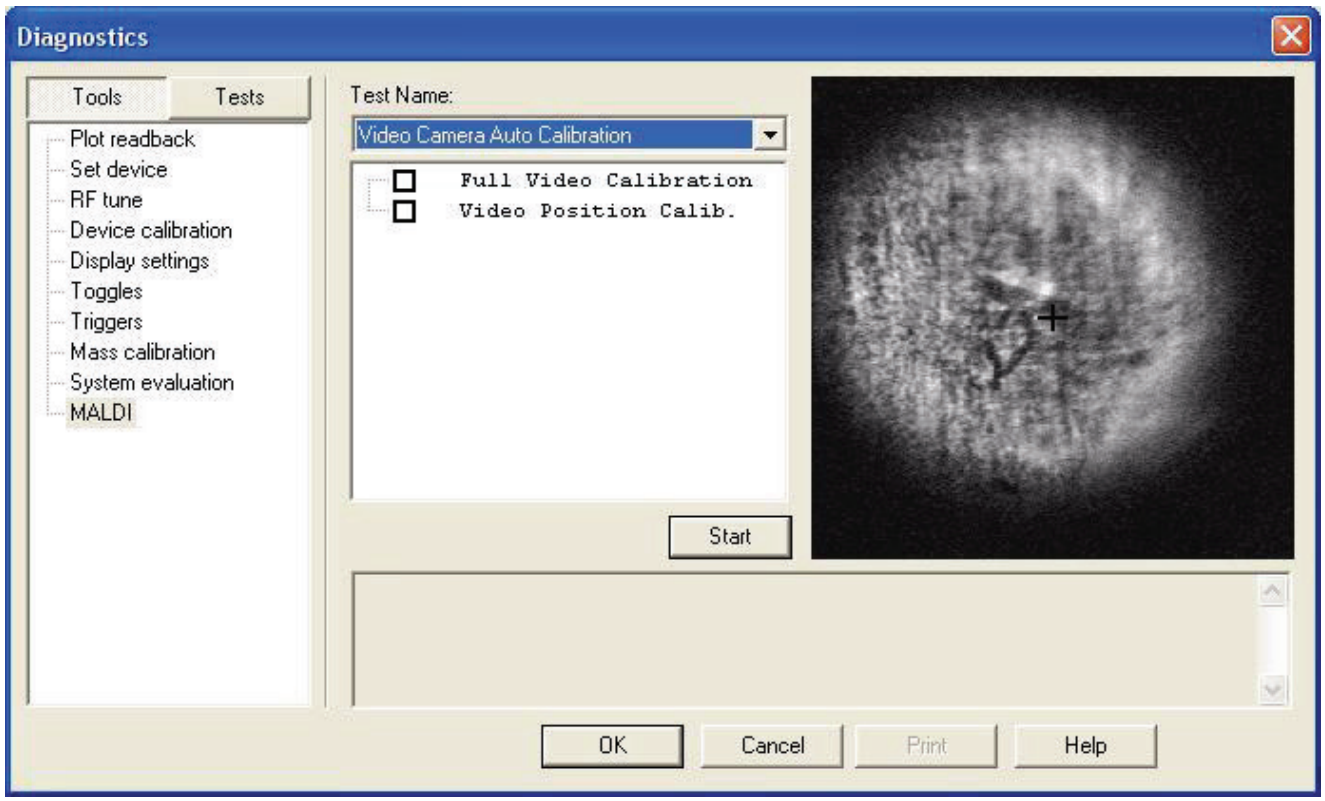
❖ To perform a full video calibration

1. Load the thin-layer matrix sample plate into the instrument.
2. To access the camera adjustment knobs in the optics module, see "Accessing the Camera and Laser" on page 77.
3. To run the diagnostic test, open the Tune Plus window.
   a. Choose **Diagnostics > Diagnostics.**

      The Diagnostics dialog box opens (Figure 13 on page 28).
   b. In the Tools area, click **MALDI**.
   c. From the Test Name list, select **Video Camera Auto Calibration**.

      The Video Camera Auto Calibration test list opens (Figure 30).

Figure 30. Video Camera Auto Calibration test list



d. Select **Full Video Calibration**.

e. Click **Start**. The system responds with the message:

If your sample plate is well calibrated, you don't need to run the procedure. Run video position calibration procedure. Are you sure you want to calibrate video system?

f. Click **OK**. The system responds with the message:

Do you have a special sample plate for video calibration?

g. Click **OK**. The system responds with the message:

Focus the camera to the optical hole to make a well-defined circle.

4. If needed, adjust the camera aperture and focus until the CD camera display shows a bright, well-defined oval.

Note The aperture control is the smaller knurled ring behind the focus adjustment. Rotate this smaller ring clockwise to close the aperture and counterclockwise to open the aperture.

The focus adjustment is the large knurled ring at the front of the camera. The focus adjustment slides in and out along the barrel of the camera.

5. In the Diagnostics dialog box, click **OK**. The system responds with the message:

Focus the camera back to the sample plate, and secure the camera.

6. Carefully adjust the zoom and aperture of the camera until a sharp, well-defined cross is visible in the camera display.
7. Click **OK**. The MALDI source automatically calibrates the sample plate. The system responds with the message:

   Are you sure you want to overwrite the previous saved image property with the current one?

8. Click **OK**.
9. Eject the thin-layer matrix sample plate from the instrument.
10. Reinstall the optics module cover and the LTQ XL top cover.

Troubleshooting

This chapter offers some examples of possible instrument problems, along with approaches to solving them.

Contents

Camera Image Not Centered

If you go to the home cross on your sample plate and the image of the cross is not centered in the camera display (for example, one of the arms of the cross is cut off short), then the cropping of the video image is off.

❖ To fix the cropping

1. In Tune Plus, choose **Diagnostics > Diagnostics**

   The Diagnostics dialog box opens (Figure 13 on page 28).

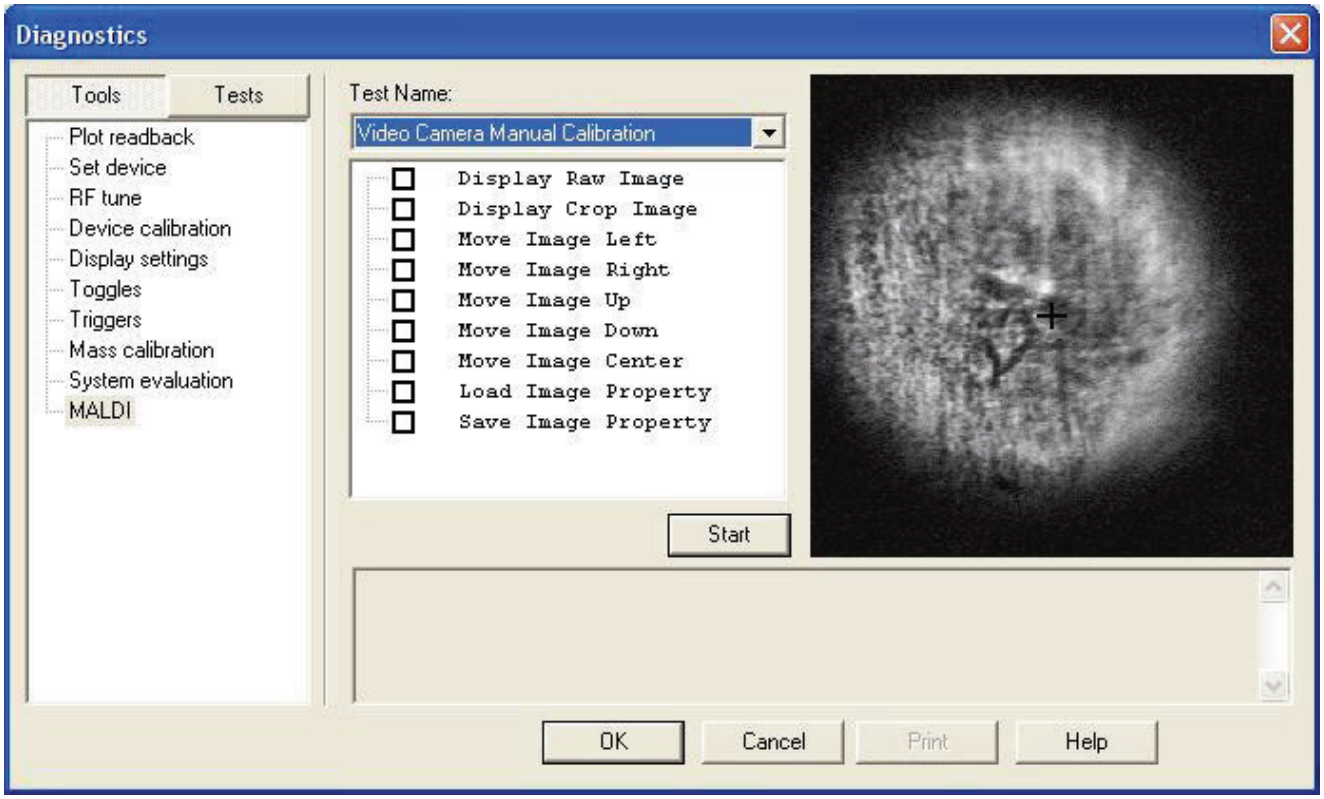
2. In the Tools area, click **MALDI**.

   MALDI tests are displayed with the Video Camera Contrast and Brightness test shown by default (Figure 15 on page 30).

3. In the Diagnostics dialog box, select **Video Camera Manual Calibration** from the Test Name list.

   The Video Camera Manual Calibration test list opens (Figure 31).

Figure 31. Video Camera Manual Calibration test list



4. Select the **Display Raw Image** check box, and click **Start** to change the camera display to the unprocessed video image.

5. Adjust the crop rectangle using the Move Image control. Use the following diagnostic tools to reposition the image:

   - Move Image Left: Moves the rectangle to the left.
   - Move Image Right: Moves the rectangle to the right.
   - Move Image Up: Moves the rectangle up.
   - Move Image Down: Moves the rectangle down.
   - Move Image Center: Moves the rectangle on the raw image.

6. Click **Start**.

7. When the crop rectangle is in the desired position, select the **Display Crop Image** check box.
8. Click **Start** to change the camera display to the regular cropped video image.
   - If the calibration cross is now fully visible and centered, then stop.
   - If the cross is still off center, repeat this procedure starting at Step 5.
   - If you cannot center the cross after several attempts, contact Thermo Fisher Scientific Technical Support.

No Video Display from the Camera

If no image appears in the camera display in the MALDI Source dialog box, then follow these instructions.

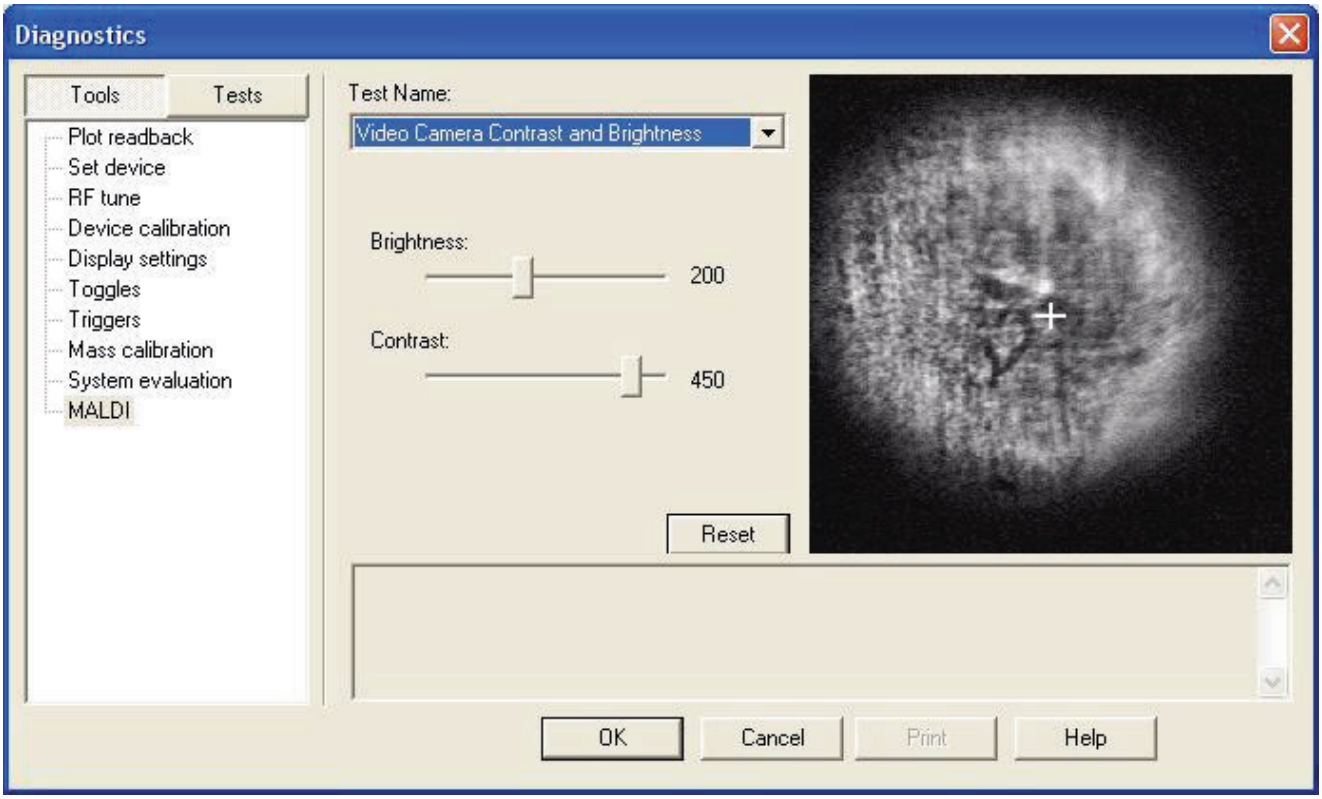
❖ To diagnose why no image appears in the camera display

1. Check the Power LED on the MALDI control module (see Table 2 on page 3).
   a. If the LED is off, then check the power connectors and make certain that the source is powered on.
   b. If bringing up the power to the MALDI source restores the camera image, then stop.
   c. If the Power LED is already green, or if restoring power does not restore the camera image, then go to step 2.
2. Check the camera aperture:
   a. To access the camera adjustment knobs in the optics module, see "Accessing the Camera and Laser" on page 77.
   b. Watch the camera display as you adjust the camera aperture. The aperture is the rotating knurled ring behind the zoom control on the camera barrel.
   c. When the camera image is clearly visible, or when adjustment fails to make it visible, reinstall the RF shielding cover.
3. Check contrast and brightness in the video display.

   The Diagnostics dialog box opens (Figure 13 on page 28).
4. In the Tools area, click **MALDI**.

   MALDI tests are displayed with the Video Camera Contrast and Brightness test shown by default (Figure 32).

Figure 32. Video Camera Contrast and Brightness test



a. Use your mouse to adjust the Brightness and Contrast slider bars.

- If these adjustments restore the camera display, then click **OK**, close the dialog box, and stop.
- If the adjustments do not restore the camera display, contact Thermo Fisher Scientific Technical Support.

5. Reinstall the optics module cover and the LTQ XL top cover.

Sample Appears Too Dark or Too Light

When the sample you are viewing appears to be too dark or too bright, you can adjust the amount of light on the sample.

❖ To adjust the light

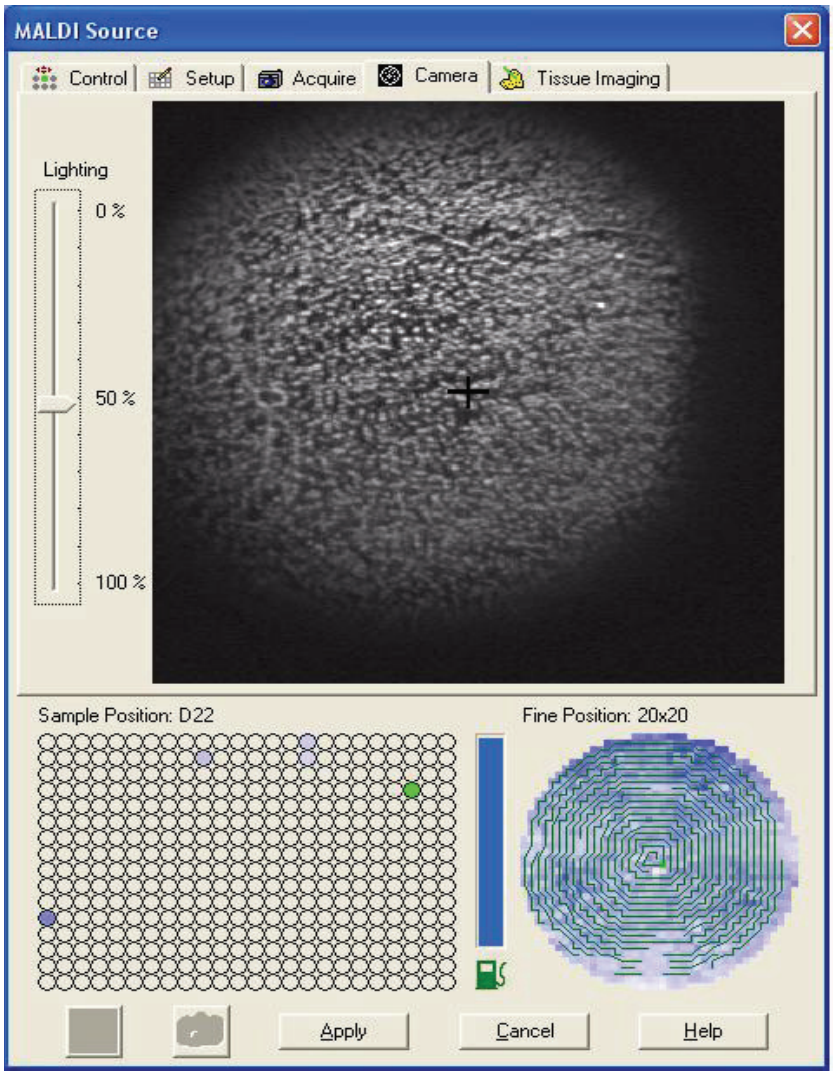
1. In the Tune Plus window, choose **Setup > MALDI Source**.

   The MALDI Source dialog box opens with the Control tab displayed (Figure 11 on page 26).

2. Click the **Camera** tab.

The Camera page opens (Figure 33).

Figure 33. Camera page



3. Use your mouse to adjust the Lighting slider bar until the sample you are viewing appears to have the correct amount of light.
4. Click **Apply**.

Note This setting is saved for future scans until you change it.

Camera Focus is Out of Adjustment

❖ **To adjust the camera focus**

1. To access the camera in the optics module, see "Accessing the Camera and Laser" on page 77.

2. Watch the camera display as you adjust the camera focus, which is the zoom control on the camera barrel. The zoom adjustment is the large knurled ring at the front of the camera. The zoom adjustment slides in and out along the barrel of the camera.
   - If adjusting the camera focus restored the image, then stop.
   - If adjusting the camera focus fails to restore the image, then contact Thermo Fisher Scientific Technical Support.
3. Reinstall the optics module cover and the LTQ XL top cover.

Fine Position Map is Misaligned

If the fine position sample spot map in the MALDI Source dialog box shows a division line or a cross down the center, then the aperture opening of the camera might be too wide. An excessively bright image in the camera display might also indicate that the aperture opening is too wide.

If the aperture is open too wide, it reduces the ability of the optical software to recognize and map the sample spot. An aperture that is too wide also limits the functions of the MALDI Crystal Positioning System (CPS).

❖ **To adjust the camera aperture**

1. To access the camera in the optics module, see "Accessing the Camera and Laser" on page 77.
2. Watch the camera display as you adjust the camera aperture, which is the rotating knurled ring behind the zoom control on the camera barrel. Adjust the aperture until the camera display is in sharp contrast, but not too bright.
3. When the camera image is clear and sharp, or when adjustment fails to make the image clear, replace the angled cover on the side of the optical module and tighten the thumbscrews.
4. Reset the fine position sample spot map by clicking any other spot in the sample map. Click on the original spot to return to the original position.
   - If adjusting the camera aperture restores the fine position sample spot map, then stop.
   - If adjusting the camera aperture fails to restore the fine position sample spot map, then contact Thermo Fisher Scientific Technical Support.
5. Reinstall the optics module cover and the LTQ XL top cover.

No Ions Detected

Use the following procedures if the sample is visible in the camera, but when you activate the laser to collect ions for analysis, no ions are detected.

- Determine if the Laser is Firing
- Check the Ion Transfer Quadrupole
- Run a Multipole Calibration Check

Determine if the Laser is Firing

❖ To determine if the laser is firing

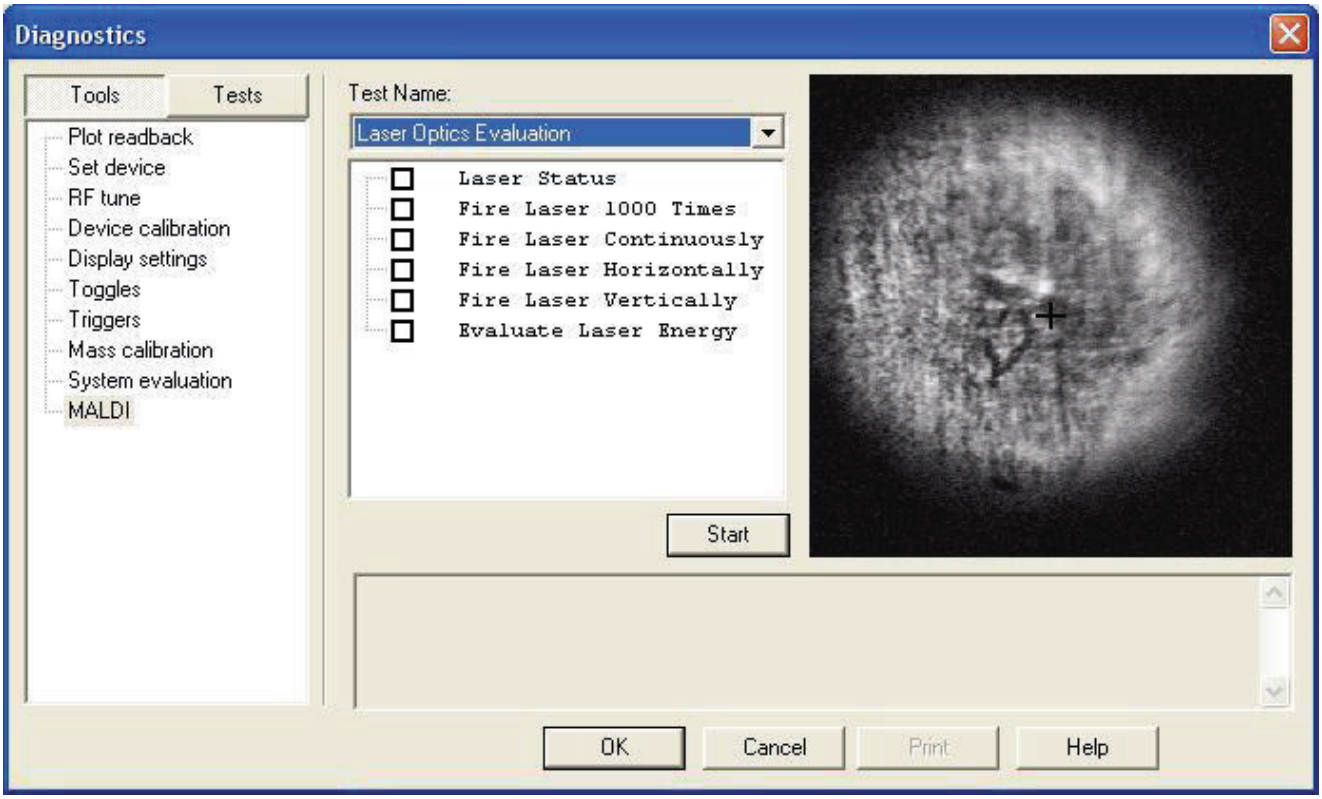
1. When you activate the laser, verify that the Laser LED on the MALDI sample module is flashing red.
2. Verify that the laser is reaching the sample.
   a. Prepare a thin-layer matrix sample.
   b. Load the sample plate into the MALDI source.
   c. Use the Sample Position Map in the MALDI Source dialog box to position the sample well with the thin-layer matrix sample in the camera display.
   d. In Tune Plus, choose **Diagnostics > Diagnostics**.

      The Diagnostics dialog box opens (Figure 13 on page 28).
   e. In the Diagnostics dialog box, from the Tools list, select **MALDI**.

      MALDI tests are displayed with the Video Camera Contrast and Brightness test shown by default (Figure 15 on page 30.)
   f. In the Diagnostics dialog box, select **Laser Optics Evaluation** from the Test Name list.

      The Laser Optics Evaluation test list opens (Figure 34).

Figure 34. Laser Optics Evaluation test list



g. From the Laser Optics Evaluation test list, select **Fire Laser Continuously**.

h. Click **Start** to begin firing the laser.

i. Watch the camera display. If the laser reaches the sample, then a dark spot appears and grows at the center of the display, as the laser burns away the matrix.

- If the laser is reaching the sample, click **Stop** to halt the laser firing. Go to the next section, "Check the Ion Transfer Quadrupole."
- If the laser status indicators are green, but the laser is not reaching the sample, then there might be a problem with the optical elements in the optical module. Contact Thermo Fisher Scientific Technical Support.
- If the spot does not burn, run the Evaluate Laser Energy test to determine if enough laser energy is being produced.

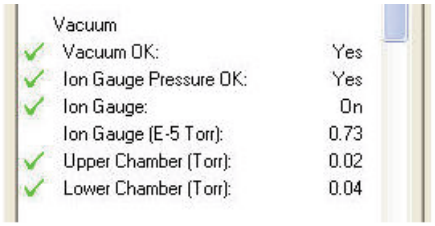
Check the Ion Transfer Quadrupole

If the laser is firing and reaching the sample, then the cause for ion loss could be a lack of pressure in the vicinity of the ion transfer quadrupole.

❖ **To check the pressure at the ion transfer quadrupole (and adjust it if necessary)**

1. In the Vacuum section of the Status View in the Tune Plus window (Figure 35), check the vacuum status indicators:
   - The Vacuum OK and Upper Chamber (Torr) indicators must both be checked green.
   - The Upper Chamber pressure should be 75 mTorr.

Figure 35. Vacuum status in Tune Plus



2. If the Upper Chamber pressure is less than 75 mTorr, verify that the nitrogen tank or supply line is delivering gas at 100 ± 20 psi (690 ± 140 kPa).
3. If the nitrogen tank or supply line is not delivering nitrogen at the proper pressure, adjust the pressure or replace the tank.
   - If adjusting the nitrogen flow brings the pressure in the sample chamber to 70 mTorr, then go to step 4.
   - If adjusting the pressure or replacing the nitrogen tank does not bring the pressure to 70 mTorr, then contact Thermo Fisher Scientific Technical Support.
4. Fire the laser for data collection to see if ions are now coming across.
   - If adjusting the pressure restores normal ion flow, then stop.
   - If the ion flow is still abnormal, then contact Thermo Fisher Scientific Technical Support.

Run a Multipole Calibration Check

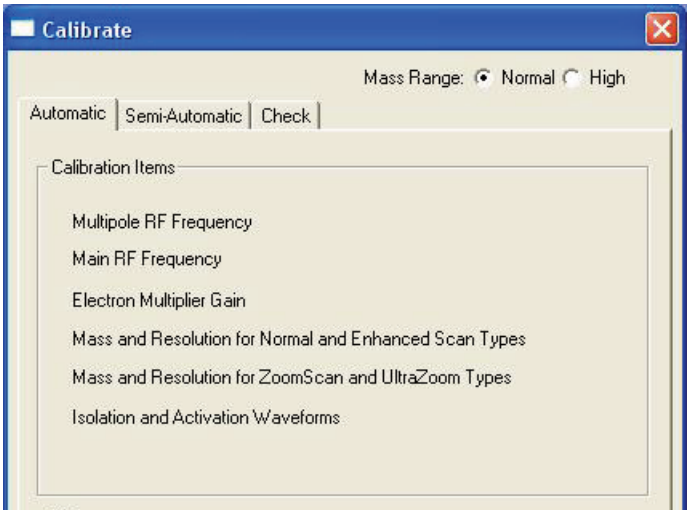
The multipole calibration check can help determine if the multipole has been tuned to the wrong frequency, which can compromise ion transmission.

❖ **To run a multipole calibration check**

1. In Tune Plus, choose **Control > Calibrate**.

   The Calibrate dialog box opens (Figure 36).

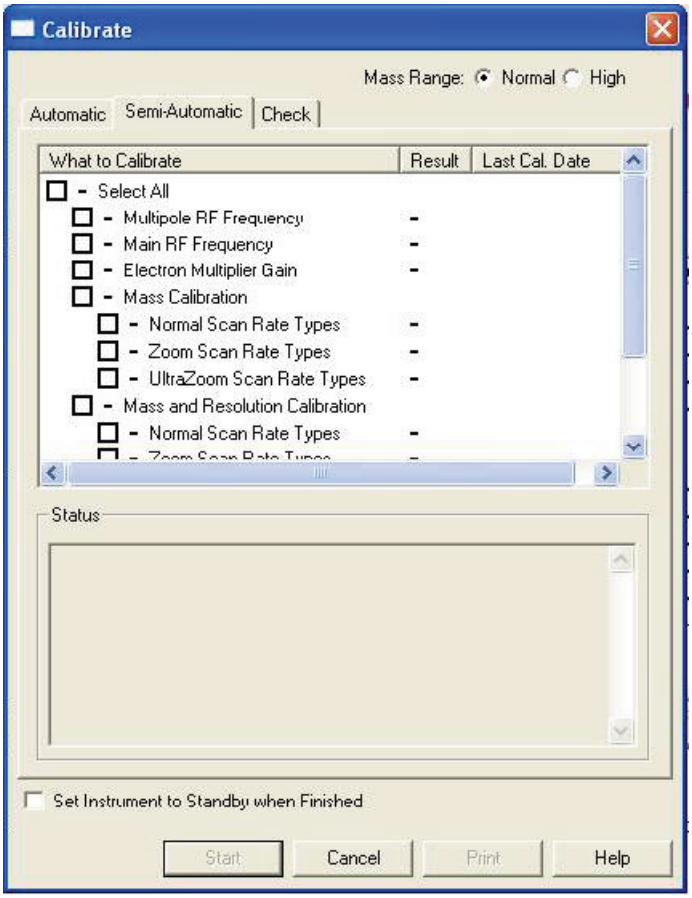
Figure 36. Calibrate dialog box



2. In the Calibrate dialog box, select the **Semi-Automatic** tab.

   The Semi-Automatic page opens (Figure 37).

Figure 37. Semi-automatic page



3. From the What to Calibrate list, select the **Multipole RF Frequency** and **Main RF Frequency** check boxes.
4. Click **Start** to begin the calibration sequence.
   - If both the multipole RF frequency calibration and the main RF frequency calibration pass, then the multipole is tuned correctly.
   - If either calibration fails, then contact Thermo Fisher Scientific Technical Support.

Sample Plate Jams

A grinding noise coming from the MALDI source usually indicates that a sample plate has become jammed somewhere in the XYZ mechanism. If this situation occurs, then turn off the LTQ XL MS and contact Thermo Fisher Scientific Technical Support.

CAUTION Always use approved sample plates from Thermo Fisher Scientific with the MALDI source. Unapproved plates can jam and cause significant damage to the MALDI LTQ XL system.

To prevent sample plates from jamming, follow the sample plate assembly instructions and observe the warnings in "Working With Sample Plates" in the *MALDI Source Getting Started Guide*.

Sample Plate Does Not Load Correctly

Occasionally, a sample plate can fail to load properly and either becomes "lost" to the data system or is ejected. To resolve this problem, you must reset the MALDI source.

❖ To reset the MALDI source

1. In Tune Plus, choose **Diagnostics > Diagnostics**.

   The Diagnostics dialog box opens (Figure 13 on page 28).

2. In the Diagnostics dialog box, from the Tools list, select **MALDI**.

   MALDI tests are displayed with the Video Camera Contrast and Brightness test shown by default (Figure 29 on page 52.)

3. In the Diagnostics dialog box, select **MALDI System Evaluation** from the Test Name list.

   The MALDI System Evaluation test list opens (Figure 19 on page 37).

4. From the MALDI System Evaluation test list, select **Initialize MALDI Source**.

The LTQ XL resets the MALDI source. The sample plate can be ejected or the MALDI source can recognize the plate and you can proceed.

CAUTION If the sample plate is not ejected or if there is a grinding noise coming from the MALDI source, see "Sample Plate Jams" on page 67.

Sample Plate Calibration Fails

The MALDI source runs through a plate calibration procedure each time you insert a sample plate.

❖ To diagnose why a plate fails calibration

Note You must have a valid home position saved in order to perform this procedure. You might also need to run a full video calibration, in which case you need a second sample plate in addition to the one that failed calibration. See "Tuning the Video Camera" on page 48.

1. Check for sample or matrix material that might be obscuring the calibration marks.
   a. In Tune Plus, choose **Diagnostics > Diagnostics**.

      The Diagnostics dialog box opens (Figure 13 on page 28).

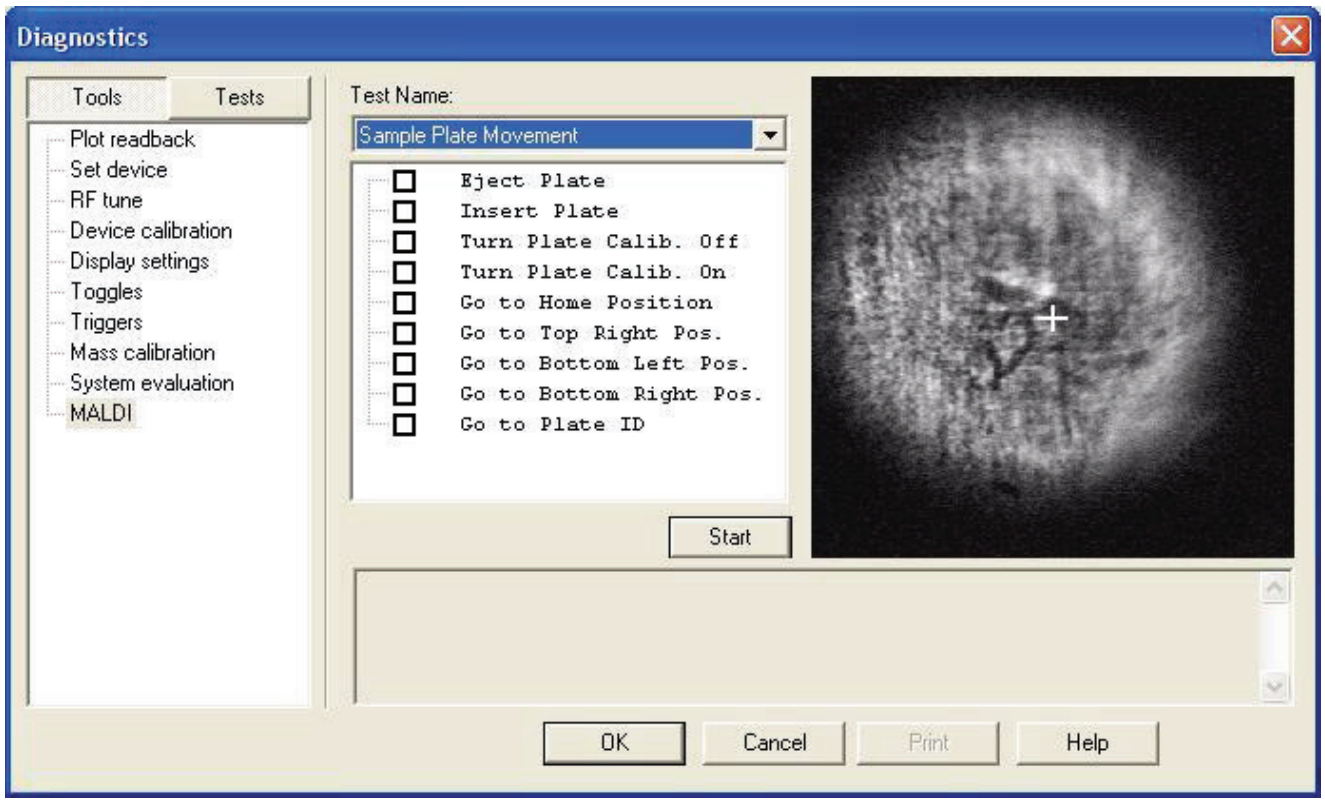
   b. In the Diagnostics dialog box, from the Tools list, select **MALDI**.

      MALDI tests are displayed with the Video Camera Contrast and Brightness test shown by default (Figure 15 on page 30.)

   c. From the Diagnostics dialog box, select **Sample Plate Movement** from the Test Name list.

      The Sample Plate Movement test list opens (Figure 38).

Figure 38. Sample Plate Movement test list



d. From the Sample Plate Movement diagnostic test list, select the **Go to Home Position**.

e. Click **Start** to move the home cross to the center of the camera display.

f. Use the camera display to check for matrix material obscuring any part of the cross.

g. Repeat steps c and d, and select the **Go To Top Right Pos.** check box to center the upper-right cross in the camera display.

h. Repeat steps c and d, and select the **Go To Bottom Left Pos.** check box to center the lower-left cross in the camera display.

i. Repeat steps c and d, and select the **Go To Bottom Right Pos.** check box to center the lower-right cross in the camera display.

j. Repeat steps c and d, and select the **Go To Plate ID** check box to center the plate identification mark in the camera display.

k. If any matrix material obscures any of the calibration marks, then eject the sample plate and carefully wipe the calibration marks with a soft optical wipe moistened with methanol.

Note Observe all of the normal precautions in handling the sample plate. See "Handling Sample Plates" on page 20 for details.

l. Insert the sample plate again and allow the instrument to run through the plate calibration procedure.

- If removing the obstructing matrix material results in a successful plate calibration, then stop.
- If there is no matrix material obscuring the calibration marks, or if removing the matrix material does not result in a successful plate calibration, then go to step 2.

2. Eject the sample plate from the instrument, and load the thin-layer matrix sample plate.
3. Perform a full video calibration procedure. See "Running a Full Video Calibration" on page 53.
4. Eject the thin-layer matrix sample plate from the instrument, and load the original sample plate.
   - If the original sample plate passes calibration, then stop.
   - If the original sample plate still fails calibration, then go to step 5.
5. Check for a defective sample plate.
   a. In Tune Plus, choose **Diagnostics > Diagnostics**.

      The Diagnostics dialog box opens (Figure 13 on page 28).

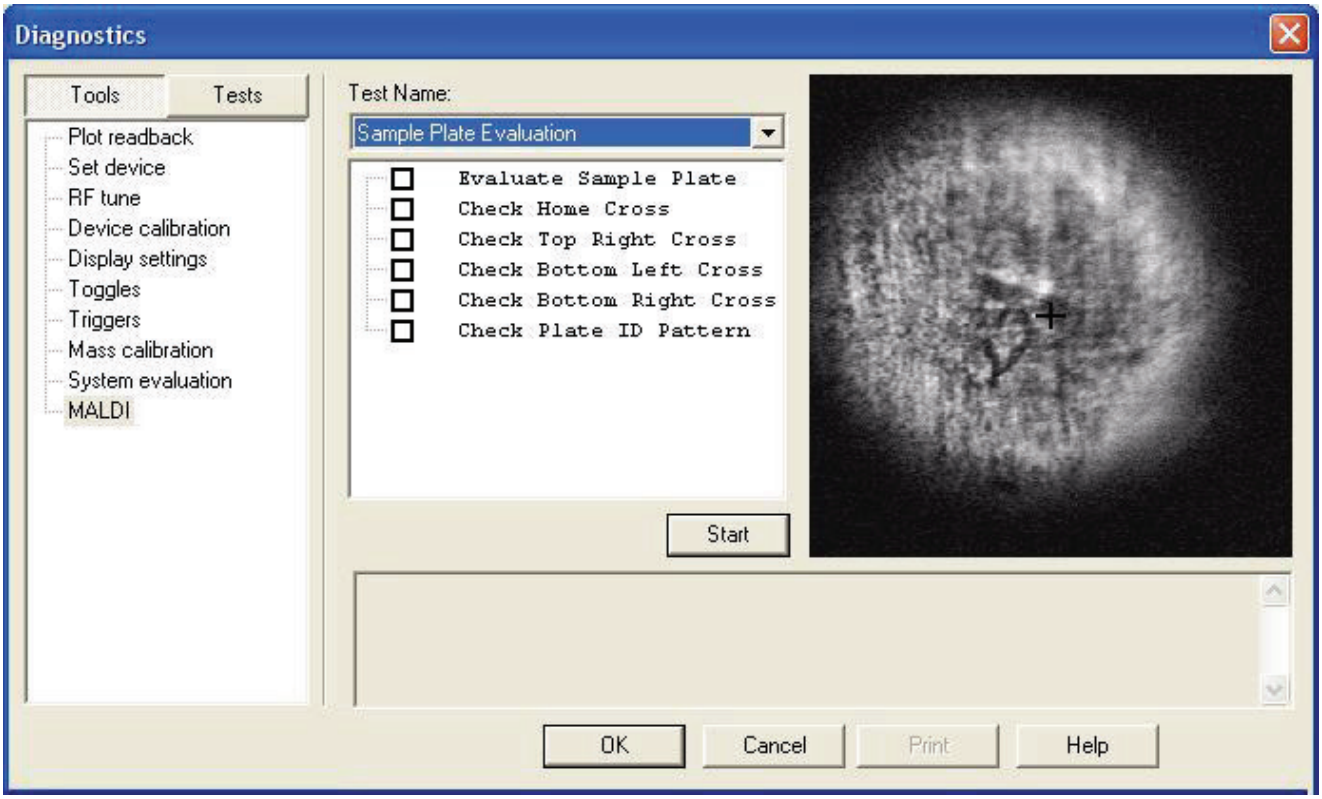
   b. In the Diagnostics dialog box, from the Tools list, select **MALDI**.

      MALDI tests are displayed with the Video Camera Contrast and Brightness test shown by default (Figure 15 on page 30.)

   c. From the Diagnostics dialog box, select **Sample Plate Evaluation** from the Test Name list.

      The Sample Plate Evaluation test list opens (Figure 39).

Figure 39. Sample Plate Evaluation test list



d. From the Sample Plate Evaluation test list, select **Evaluate Sample Plate.**

e. Click **Start**.

f. The status box in the Sample Plate Evaluation dialog box tells you whether the plate passes or fails.

- If the sample plate fails, then respot the samples on a new plate.
- If the sample plate passes the evaluation but does not pass calibration, then contact Thermo Fisher Scientific Technical Support.

Instrument Calibration Fails

❖ **To diagnose why the instrument fails a mass calibration**

1. Eject the sample plate with the calibration samples. Insert the special calibration plate that shipped with your MALDI source.
2. Run a multipole RF frequency calibration and a main RF frequency calibration.
   a. In Tune Plus, choose **Control > Calibrate.**

      The Calibrate dialog box opens (Figure 36 on page 66).

   b. In the Calibrate dialog box, select the **Semi-Automatic** tab.

The Semi-Automatic page opens (Figure 37 on page 66).

c. From the What to Calibrate list, select the **Multipole RF Frequency** and **Main RF Frequency** check boxes.

d. Click **Start** to begin the calibration sequence.

- If both the multipole RF frequency calibration and the main RF frequency calibration pass, then go to step 3.
- If either calibration fails, then contact Thermo Fisher Scientific Technical Support.

3. Prepare a new calibration sample using fresh solvents and freshly-prepared matrix solution.
4. Prepare sample spots according to the standard calibration procedure. Refer to the *LTQ XL Series Getting Connected Guide* for instructions.
5. In the Vacuum section of the Status View in the Tune Plus window (Figure 35 on page 65) check the Vacuum OK and Upper Chamber (Torr) status indicators. Both must be checked green, with the upper chamber pressure reading 170 millitorr.

   a. If the pressure status indicators are satisfactory, then go to step 6.

   b. If either pressure indicator is marked red, then adjust the vacuum. If the vacuum level is too low, then verify that the nitrogen sheath gas is flowing properly. If the vacuum is too high, then check your system for leaks.

6. Insert the sample plate with prepared calibration spots into the MALDI source.
7. Tune the laser energy.

   a. In the MALDI Source dialog box (see Figure 11 on page 26), Control page, adjust the value of the Laser Energy in the Laser Settings area to improve the signal according to the guidelines in Table 40.

Figure 40. Guidelines for adjusting MALDI laser energy

Problem	Solution
No signal	Increase laser energy.
Low signal	Increase laser energy.
High baseline	Decrease laser energy.
High signal, low baseline, and poor mass resolution	Decrease laser energy in small increments.
High chemical noise	Decrease laser energy.
Peak broadening and shifting to higher mass values (space charge effects)	Decrease laser energy.

b. Make initial adjustments in increments (or decrements) of 10, and then make finer adjustments in smaller increments. After each adjustment, fire the laser once and check the mass spectrum in the Tune Plus window.

- When you are satisfied with the mass spectrum, go to step 8.
- If you cannot obtain a satisfactory mass spectrum by adjusting the laser energy, then contact Thermo Fisher Scientific Technical Support.

8. On the Semi-automatic page (Figure 37 on page 66), select the **Electron Multiplier Gain** check box.
9. Click **Start** to begin the calibration sequence. Wait until the electron multiplier gain calibration has finished before proceeding.
   - If the calibration succeeds, then go to step 10.
   - If the calibration fails, then go to step 20.
10. On the Semi-automatic page (Figure 37 on page 66), select the **Normal Scan Rate Types** check boxes under both Mass Calibration and Mass and Resolution Calibration.
11. Click **Start** to begin the calibration sequence. Wait until the calibrations have finished before proceeding.
    - If both calibrations succeed, then go to step 12.
    - If either calibration fails, then go to step 20.
12. On the Semi-automatic page (Figure 37 on page 66), select the **Zoom Scan Rate Types** check boxes under both Mass Calibration and Mass and Resolution Calibration.
13. Click **Start** to begin the calibration sequence. Wait until the calibrations have finished before proceeding.
    - If both calibrations succeed, then go to step 14.
    - If either calibration fails, then go to step 20.
14. On the Semi-automatic page (Figure 37 on page 66), select the **Isolation Waveforms** check box.
15. Click **Start** to begin the calibration sequence. Wait until the isolation waveform calibration has finished before proceeding.
    - If the calibration succeeds, then go to step 16.
    - If the calibration fails, then go to step 20.
16. On the Semi-automatic page (Figure 37 on page 66), select the **Ultrazoom Scan Rate Types** check boxes under the Mass Calibration and Mass and Resolution Calibration lists.
17. Click **Start** to begin the calibration sequence. Wait until the calibrations have finished before proceeding.

- If both calibrations succeed, then go to step 18.
- If either calibration fails, then go to step 20.

18. On the Semi-automatic page (Figure 37 on page 66), select the **Activation Waveforms** check box.
19. Click **Start** to begin the calibration sequence. Wait until the activation waveform calibration has finished before proceeding.
    - If the calibration succeeds, then stop. Your instrument is calibrated.
    - If the calibration fails, then go to step 20.
20. If one or more of these calibrations fail, then run them again. Repeat a calibration up to three times if necessary. If a calibration fails three times, then contact Thermo Fisher Scientific Technical Support.

Increase in System Noise

If the system begins producing unusually high levels of noise, first determine whether it is electronic noise or chemical noise.

Electronic Noise

This type of noise results from random electronic signals in or near your equipment. It is characterized by large signal spikes at random points in a mass spectrum.

❖ **To determine if the noise is coming from the LTQ XL MS**

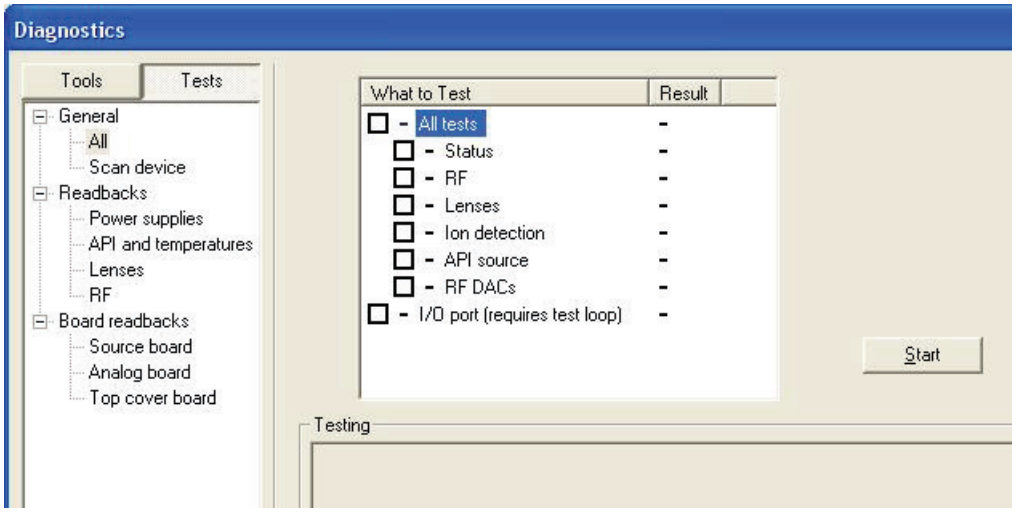
1. In Tune Plus, choose **Diagnostics > Diagnostics**.

   The Diagnostics dialog box opens (Figure 13 on page 28).

2. In the Diagnostics dialog box, click **Tests**.

   The list of system-level tests is displayed, with the General/All tests displayed (Figure 41).

Figure 41. Tests list



3. Click **All tests**.
4. Click **Start**.

   If the tests indicate that there is a hardware problem in the LTQ XL system, contact Thermo Fisher Scientific Technical Support.

Chemical Noise

This type of noise usually results from excess matrix, or from contaminants in the matrix, in the sample, or on the sample plate. Chemical noise has these characteristics:

- A continuous series of small peaks spaced 1 amu apart over large areas of a mass spectrum.
- Increasing intensity toward the low-mass end of the spectrum.
- Distinct clusters of larger peaks throughout the mass spectrum (these larger peaks result from matrix clusters).

Chemical noise is a complex problem. Some contributing factors are as follows:

- Excess matrix relative to the analyte. If possible, increase the proportion of analyte in your sample's spots to reduce chemical noise.
- High levels of alkali metal ions in the sample, particularly sodium and potassium.
- Excessive laser energy.
- Contaminants on the sample plate.

If chemical noise persists, contact Thermo Fisher Scientific Technical Support.

It is best to take a preventive approach to chemical noise. To minimize this problem, observe the following precautions:

- Clean the sample plate regularly. Refer to the *LTQ XL Series Getting Started Guide* for information about cleaning the sample plate.
- Take all recommended precautions when cleaning or handling the sample plate. See "Handling Sample Plates" on page 20.
- Develop regular, reproducible procedures for preparing sample and matrix solutions, and for spotting samples on the sample plate.
- If you prepare matrix solutions in bulk, then develop a protocol for storing these solutions and disposing of any unused portions on a regular basis.

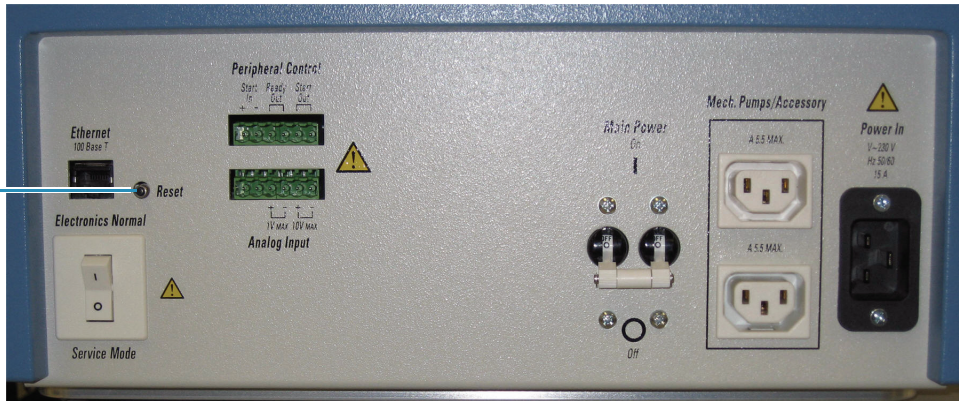
Control Module is Not Communicating

If you cannot send commands to the instrument, or if the Communication LED on the MALDI control module is not green, then the control module is not communicating with the instrument.

❖ **To diagnose why the control module is not in sync with the instrument**

1. Verify the connections on all cables that connect the MALDI control module with the LTQ XL MS detector.
   - If securing the cables restores communication, then stop.
   - If securing the cables does not restore communication, then go to step 2.
2. Reboot the LTQ XL MS and the MALDI source by pressing the Reset button located on the left side of the LTQ XL power entry panel (Figure 42).

Figure 42. LTQ XL MS reset button



   - If resetting the instrument restores communication, then stop.
   - If resetting the instrument does not restore communication, then contact Thermo Fisher Scientific Technical Support.

Accessing the Camera and Laser

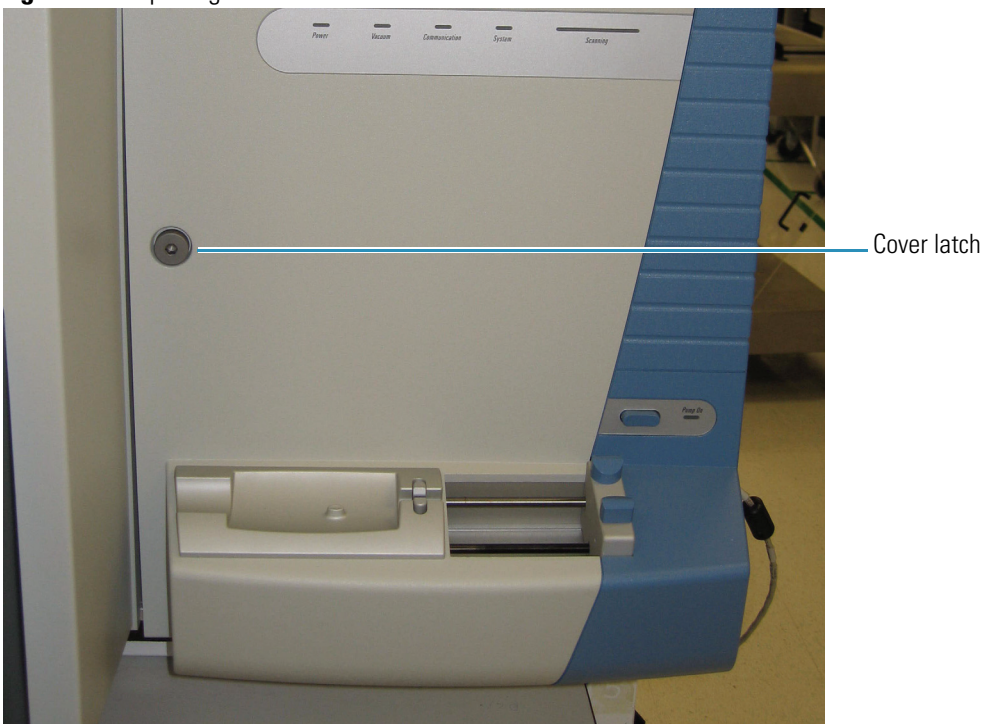
To make fine adjustments to the camera or laser, you must open the optics module.

CAUTION Proceed with CAUTION when accessing the optics module. Delicate and critical components will be exposed.

❖ To access the camera or laser

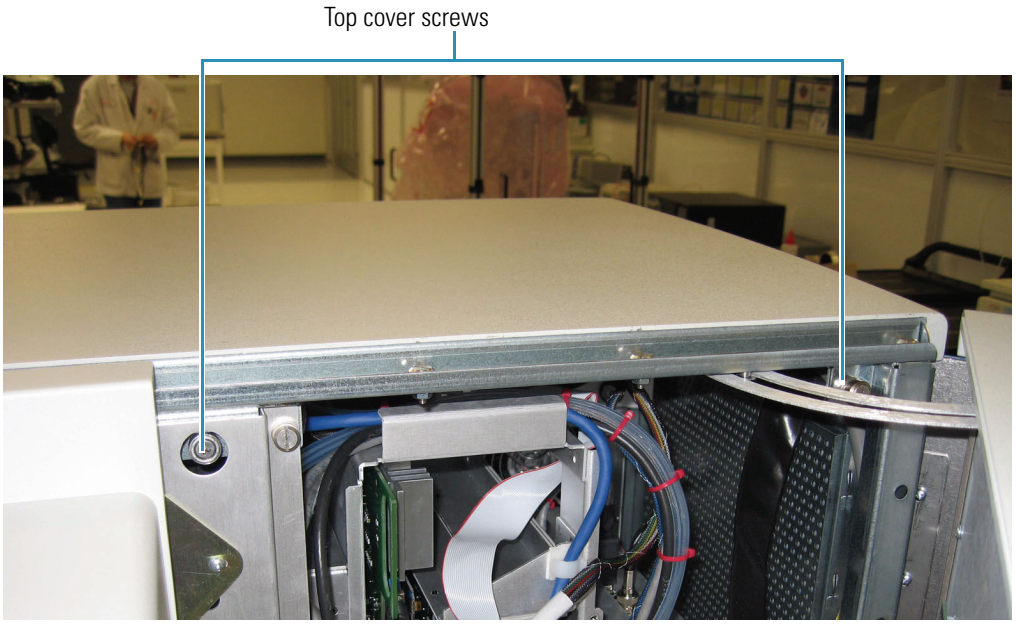
1. Use a 1/4-in. hex wrench to loosen the cover latch (Figure 43).

Figure 43. Opening the front cover



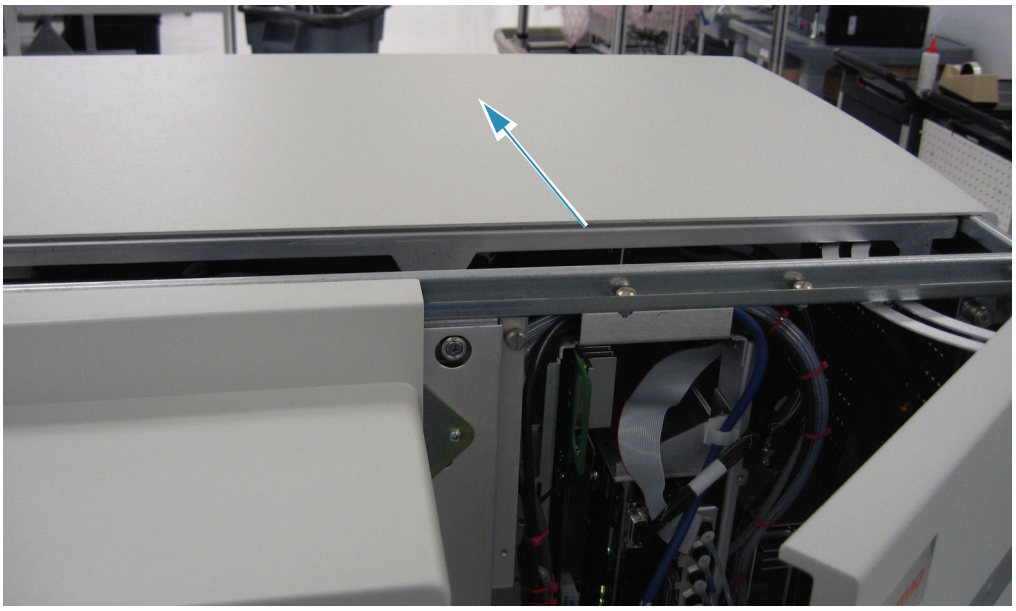
2. Open the front cover and loosen the two Phillips head captive screws that hold the top cover on (Figure 44).

Figure 44. Top cover screws



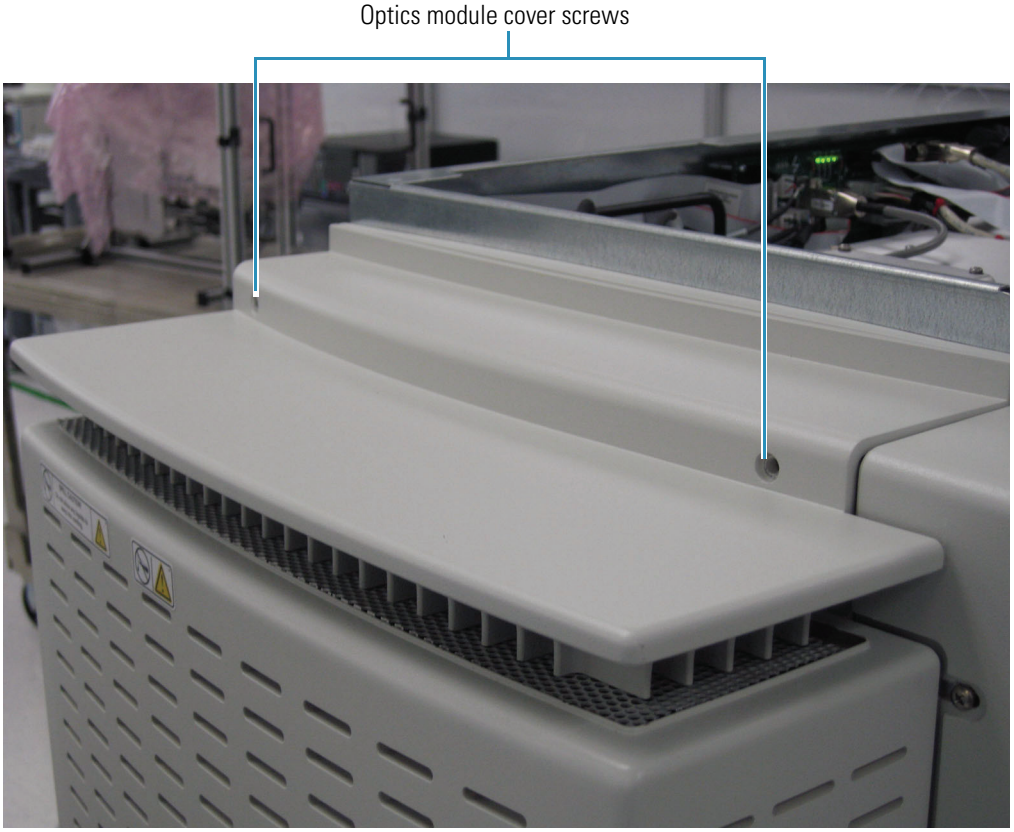
3. Remove the top cover by sliding it back and off (Figure 45).

Figure 45. Removing the top cover



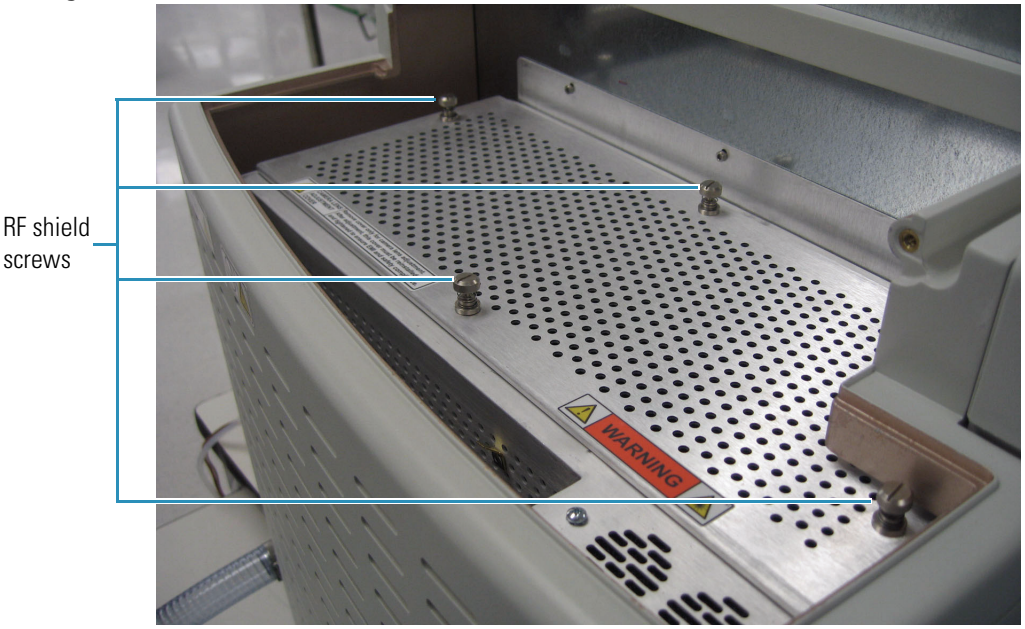
4. Use a #2 Phillips screwdriver to remove the two screws that secure the cover of the optics module (Figure 46).

Figure 46. Removing the screws from the optics module cover



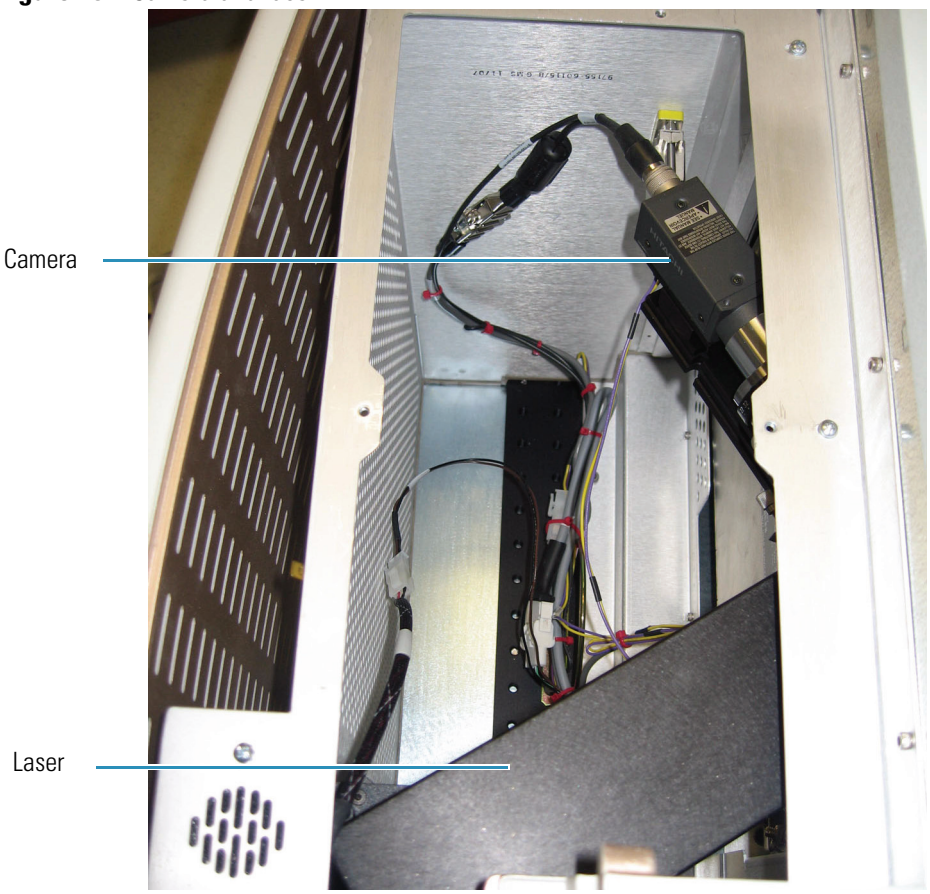
5. Remove the optics module cover (Figure 47).
6. Loosen the four captive screws that secure the RF shield.

Figure 47. RF shield screws



7. Remove the RF shield to access the laser and camera (Figure 48).

Figure 48. Camera and laser



Replaceable Parts and Accessories

This chapter provides information about the replaceable parts, chemicals, and supplies for your LTQ XL MALDI source.

Contents

Replaceable Parts

MALDI Vacuum Solenoid Assembly (P/N 97155-60088)

Accessories

ProteoMass™ MALDI Calibration kit available from Thermo Fisher Scientific (P/N HAZMAT-01-0033) or Sigma-Aldrich (P/N MSCAL4, call 1-800-325-5832).

Tissue Imaging Kit (P/N 97155-62124) containing tissue imaging slides, a software CD, an adapter for stainless steel slides, an adapter for glass slides, a base plate, a scanner, and a frame to hold the sample plate for scanning on the scanner.

Sample Plate Kit (P/N 97155-62033) which contains a 96- and 384-well sample plate and a base plate.

API kit (P/N 97155-62123) which contains the additional parts required to convert a MALDI LTQ XL system to an LTQ XL API system.

MALDI Accessory Kit (P/N 97155-62025), which contains laser protective eyewear, tools, swabs, gloves, and other items

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