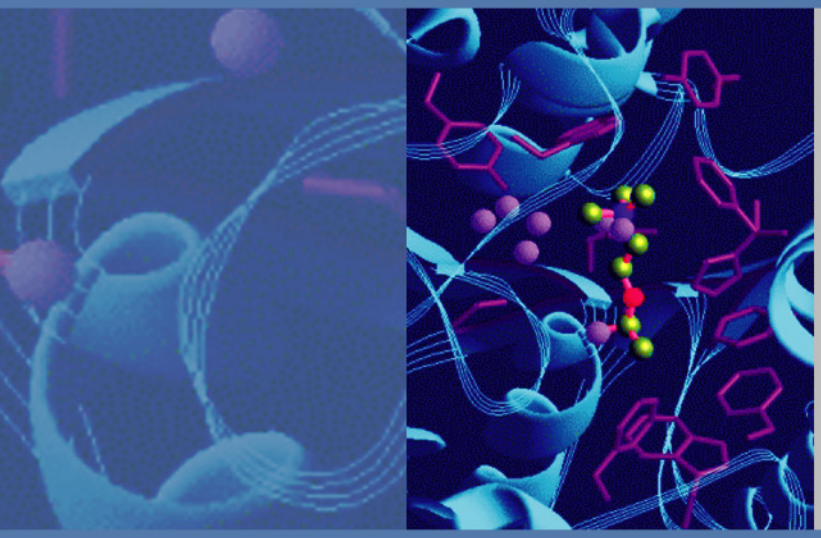

Nanospray Ion Source

User Guide

97055-97046 Revision C September 2010

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Release history: Revision A September 2005, Revision B February 2009, Revision C September 2010

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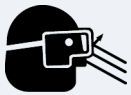
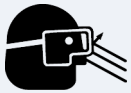
CAUTION Symbol	CAUTION	VORSICHT	ATTENTION	PRECAUCION	AVVERTENZA
	**Electric Shock:** This instrument uses high voltages that can cause personal injury. Before servicing, shut down the instrument and disconnect the instrument from line power. Keep the top cover on while operating the instrument. Do not 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 corporelles. 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 enlever 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 chimiques dangereux peuvent se trouver dans l'instrument. Portez des gants pour manipuler tous produits chimiques 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:** Des projections chimiques, liquides, ou solides peuvent être dangereuses pour les yeux. Porter des lunettes de protection lors de toute manipulation de 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'une 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。	高溫：請先等高溫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。處理化學品或維修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

This manual provides you with information on how to install, operate, and troubleshoot the Thermo Scientific NSI source.

The NSI source can be used on a TSQ™ Series, LCQ™ Series, or LTQ™ Series mass spectrometer.

Note Thermo Fisher Scientific has discontinued the NSI-2 dynamic nanospray and packed-tip probes. For information about installing the NSI-1 dynamic nanospray probe, refer to the *Dynamic Nanospray Probe (NSI-1) Installation Guide*.

Related Documentation

In addition to this guide, Thermo Fisher Scientific provides the following documents for the NSI source:

- *Dynamic Nanospray Probe (NSI-1) Installation Guide*
- *Dynamic Nanospray Probe (NSI-2) Quick Reference Guide*

The software also provides Help.

Safety and Special Notices

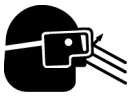
Make sure you follow the precautionary statements presented in this guide. The safety and other special notices appear in boxes.

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.

CAUTION Highlights electric shock hazards to humans. Each electric shock notice is accompanied by the international high voltage symbol.

CAUTION Highlights hot surface hazards to humans. Each hot surface notice is accompanied by the international hot surface symbol.



CAUTION Highlights the necessity to wear protective eyewear. Each protective eyewear notice is accompanied by the international protective eyewear symbol.

CAUTION Highlights sharp object hazards to humans. Each sharp object notice is accompanied by a sharp object symbol.

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

IMPORTANT Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might 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.

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There are several ways to contact Thermo Fisher Scientific for the information you need.

❖ To contact Technical Support

Phone	800-532-4752
Fax	561-688-8736
E-mail	us.techsupport.analyze@thermofisher.com
Knowledge base	www.thermokb.com

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

❖ To contact Customer Service for ordering information

Phone	800-532-4752
Fax	561-688-8731
E-mail	us.customer-support.analyze@thermofisher.com
Web site	www.thermo.com/ms

❖ To copy manuals from the Internet

Go to mssupport.thermo.com and click **Customer Manuals** in the left margin of the window.

❖ To suggest changes to documentation or to Help

- Fill out a reader survey online at http://www.surveymonkey.com/s/PQM6P62.
- Send an e-mail message to the Technical Publications Editor at techpubs-lcms@thermofisher.com.

Introduction

The nanospray ionization (NSI) source is a universal ion source that can be used on a TSQ™ Series mass spectrometer, an LCQ™ Series mass spectrometer, or an LTQ™ Series mass spectrometer.

The Thermo Scientific NSI source generates gas phase ions from sample solutions for analysis by a mass spectrometer. The effectiveness of the ionization process depends upon the concentration and ionization efficiency of the analyte. The Thermo Scientific NSI source is an improvement over conventional electrospray ion sources because nanospray ionization allows you to minimize the flow rate, which increases the signal response for dilute samples by increasing the effective analyte concentration at the emitter tip.

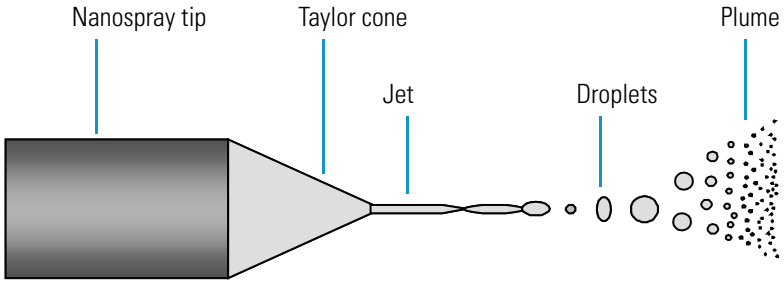
The NSI system gives you considerable flexibility in choosing the type of experiment required for a specific analysis. Nanospray operation can be performed in either of two modes: static nanospray or dynamic nanospray. Thermo Scientific offers two types of NSI probe for the dynamic nanospray mode: the dynamic NSI probe, which is used with a capillary column, and the packed-tip NSI probe, which is used with a PicoFrit™ column.

Contents

Advantages of Nanospray

Electrospray ionization (ESI) has evolved as a leading technique for generating intact, gas phase ions from thermally labile, polar analytes in solution. In this technique, ionization is induced by the use of an *emitter* (a capillary tube or needle) at a controlled distance from a counter electrode. Dc voltage is applied, either to the needle or to the solvent, to produce a strong electrical field at the emitter tip. The electric field interacts with ions in solution as they leave the tip. This interaction results in electrohydrodynamic disintegration of the fluid, generation of droplets, and formation of an aerosol jet. See Figure 1.

Figure 1. Electrohydrodynamic disintegration of the fluid



Conventional ESI employs flow rates from 1 µL/min to 1 mL/min. Due to the high volume of liquid exiting the emitter, a drying gas, thermal heating, or both are often required to expedite desolvation and droplet shrinkage. Nanospray ionization, which is a form of ESI that employs low flow rates from 10 to 1000 nL/min, generally does not require a drying gas or thermal heating. In addition, nanospray ionization is more tolerant to a wider range of liquid compositions including pure water.

As the flow rate is lowered, a lower volume of mobile phase passes through the emitter, producing smaller aerosol droplets. This makes nanospray ionization more effective than conventional ESI at concentrating the analyte at the emitter tip, producing significant increases in sensitivity demonstrated by the signal response of the mass spectrometer.

Components of the Nanospray System

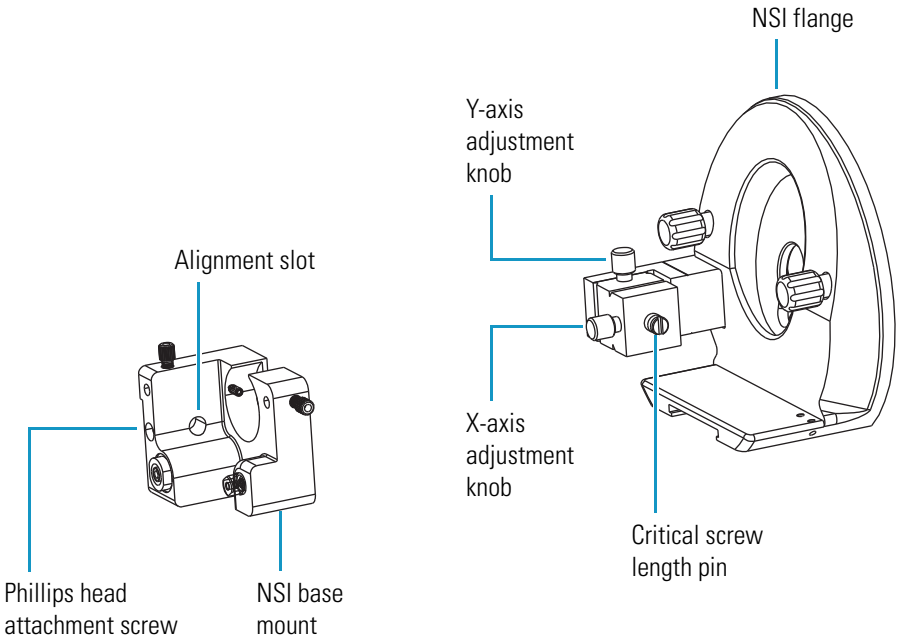
The Nanospray system that mounts to your mass spectrometer consists of these components:

- NSI Source
- Extension Assembly
- Imaging System

NSI Source

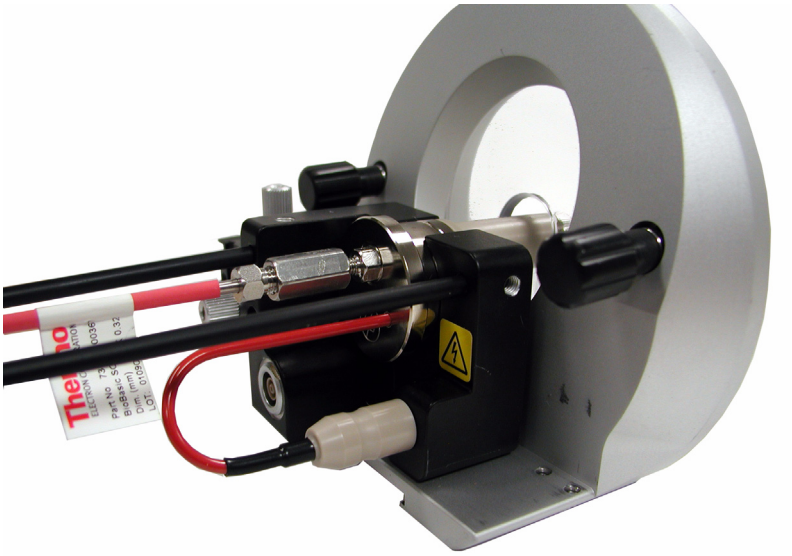
The NSI source assembly consists of the NSI flange, NSI base, and NSI probe. The NSI flange and NSI base are shown in Figure 2.

Figure 2. NSI base mount and NSI flange



The flange contains the XYZ stage, which is used to make fine adjustments to the position of the NSI probe. The NSI base mounts to the NSI flange and holds the NSI probe. A dynamic NSI source is shown in Figure 3.

Figure 3. NSI source with (discontinued) dynamic NSI probe and column supports



Thermo Scientific offers three types of NSI probes:

- Static NSI Probe
- Dynamic NSI Probe
- Packed-Tip NSI Probe

Static NSI Probe

The *static NSI probe*, shown in Figure 4, can perform a continuous analysis of small sample (analyte) solution volumes over an extended period of time. The static nanospray mode is particularly useful for obtaining detailed structural information using MS/MS for purified samples or those present in a simple mixture. For instructions on assembling the static NSI probe, refer to "Assembling the Static NSI Probe" on page 44.

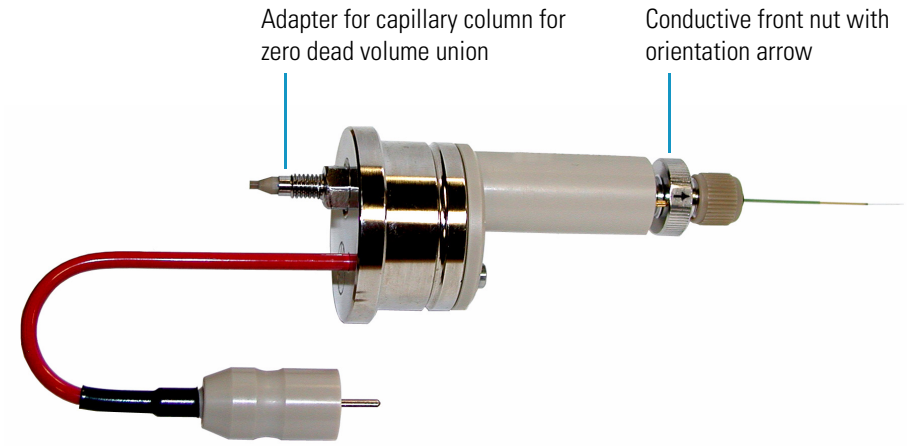
Figure 4. Static nanospray probe

Dynamic NSI Probe

The *dynamic NSI probe*, shown in Figure 5, uses a capillary column and a solvent delivery system to perform chromatographic separations on mixtures prior to analysis by the mass spectrometer. For instructions on assembling the dynamic NSI probe, refer to "Assembling the Dynamic NSI Probe" on page 49.

Note Thermo Fisher Scientific has discontinued the NSI II dynamic nanospray probe. For information about ordering and installing the original dynamic nanospray probe, refer to the *Dynamic Nanospray Probe I Installation and Operation Guide*.

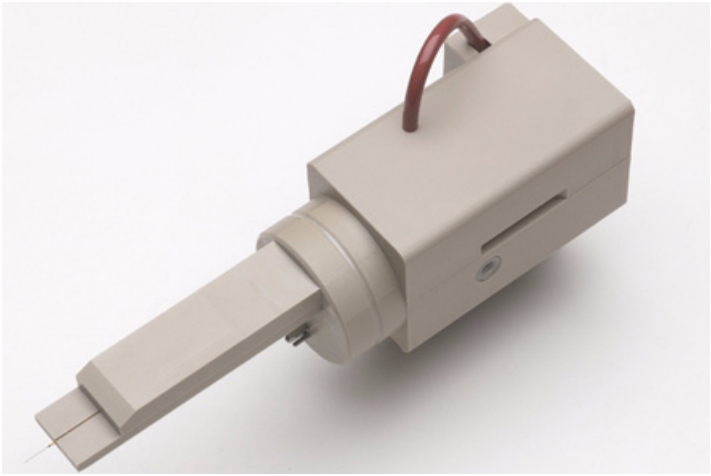
Figure 5. Dynamic nanospray probe (discontinued version)



Packed-Tip NSI Probe

The *packed-tip NSI probe* shown in Figure 6 uses PicoFrit® columns, which are emitters packed with HPLC column material. The PicoFrit column is an integral separation and spray device, which circumvents the junction issues encountered when using a capillary column and a fused silica emitter. For instructions on installing a PicoFrit column, refer to "Replacing the PicoFrit Column" on page 55.

Figure 6. Packed-tip dynamic nanospray probe



Extension Assembly

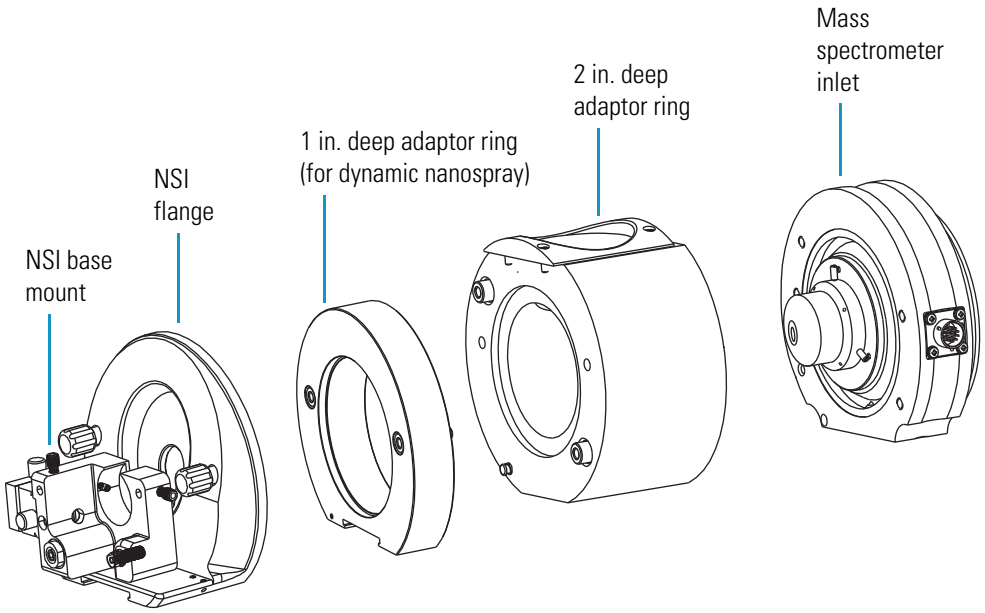
In most cases, the NSI source does not mount directly to the mass spectrometer, but instead mounts to an extension assembly. The extension assembly is mounted directly to the inlet of the mass spectrometer.

Depending on the geometry of the mass spectrometer's inlet, the extension assembly includes either a 2-in. deep adapter ring or an Ion MAX adapter. In addition, a 1 in. deep adapter ring is required for the proper spacing of the dynamic NSI probe and might be required for the proper spacing of the packed-tip NSI probe.

Mass spectrometers that support the NSI source can be categorized into two groups based on their inlet geometries:

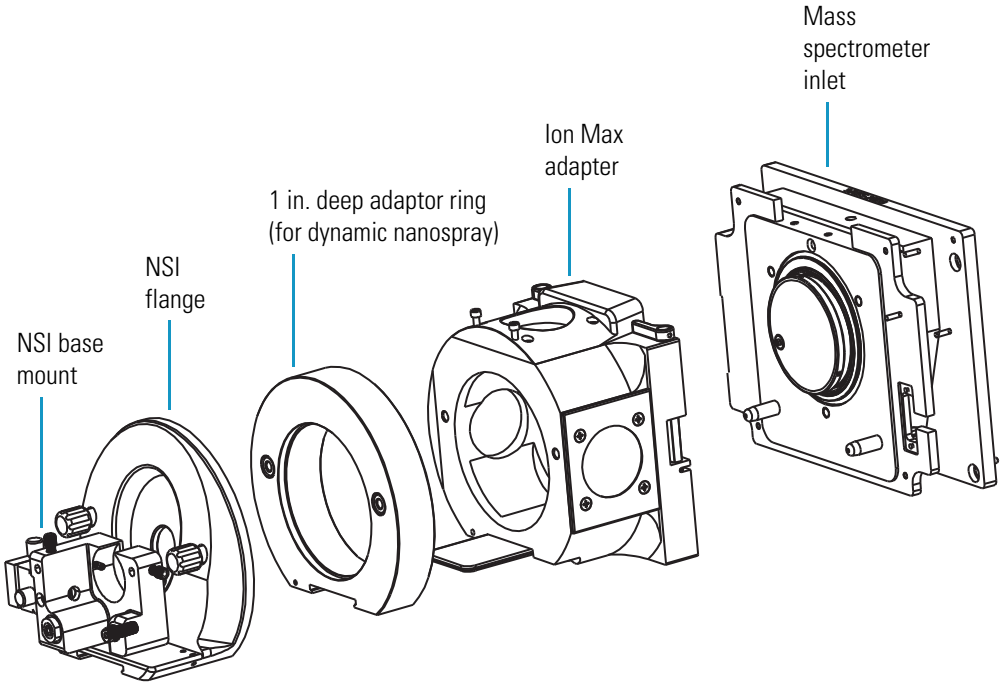
- LCQ Deca XP and LCQ Advantage XP mass spectrometers have a protruding inlet with a circular base. The adapter system for these mass spectrometers is shown in Figure 7.

  Figure 7. LCQ Deca XP or LCQ Advantage XP mass spectrometer adapter system



- Mass spectrometers with an Ion MAX ion source have a protruding inlet with a square base. This category of mass spectrometers includes the LCQ Deca XP MAX, the LTQ Series, and the TSQ Series. The adapter system for these mass spectrometers is shown in Figure 8.

Figure 8. LCQ Deca XP MAX, LTQ Series, or TSQ Series mass spectrometer adapter system

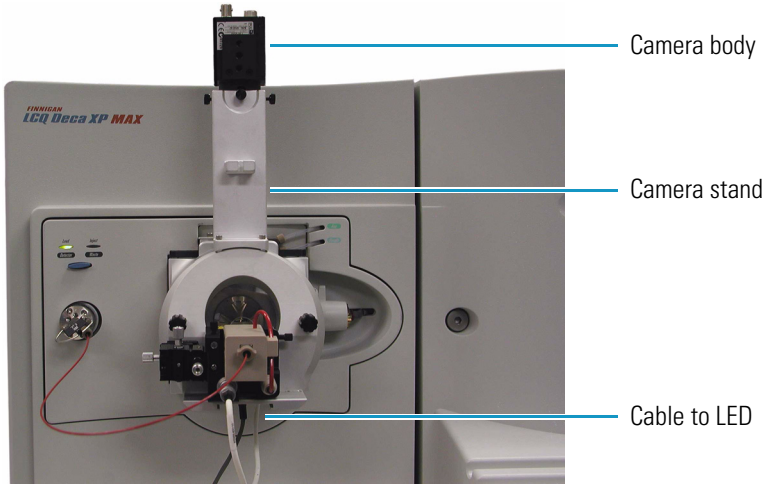


See Chapter 3 for information on installing the appropriate extension assembly for your mass spectrometer.

Imaging System

The imaging system facilitates the positioning of the emitter and allows you to troubleshoot emitter problems. The imaging system mounts to the nanospray adapter for your mass spectrometer. See Figure 9. For instructions on installing the imaging system, refer to Chapter 4.

Figure 9. Imaging system mounted to mass spectrometer



Working with Microfluidic Components and Microflow LC

The Thermo Scientific NSI source connects to a solvent delivery system and an injection valve (the Divert/Inject valve on the front of the mass spectrometer or the injection valve of an autosampler or manual injector) through narrow bore tubing. Plumbing of narrow bore tubing with micron sized inner diameters, low dead volume connections, and fused silica capillary tubing is referred to in this manual as microfluidics. Assembling the plumbing for a Thermo Scientific NSI source is no more difficult than assembling traditional HPLC plumbing, but it is helpful to keep several key factors in mind when working with microfluidics. These factors are discussed in this chapter.

Performing chromatography with a microflow LC requires a capillary column or a PicoFrit column and a capillary pump or a conventional LC pump used in combination with a flow splitter. Instructions for working with PicoFrit columns, connecting a flow splitter, and determining the column flow rate are included in this chapter, along with suggestions for maintaining a stable nanospray.

Contents

Preparing the Microfluidic Worksite

Proper preparation of the worksite for the handling of microfluidic components is critical to the successful operation of your NSI/MS system. Inadequate worksite preparation and a lack of cleanliness can result in clogged tubing or compromised emitters, causing experimental failure. The extra effort required to maintain a clean and dedicated workbench for the preparation and handling of microfluidics quickly translates to reduced maintenance and superior nanospray performance.

Use a stereoscope or microscope to check the microfluidic components of your system prior to assembling them. A robust, high-quality lab stereoscope, with magnification between 10× to 30×, is an essential part of the microfluidics worksite. An example of such a stereoscope is the Leica™ Zoom 2000 (Model No. Z30V, VWR Cat. No. 41433-030 / Leica No13312594F).

Ensure that the air in your laboratory does not contain excessive dust, smoke, or other particulate matter. Dust can contaminate or clog columns and emitters and can also form an insulating layer on the electronics of your mass spectrometer. When columns or emitters become blocked, overheating and short-circuiting of the electronic components of your mass spectrometer, as well as sample loss can occur. For reference, the air should contain fewer than 100 000 particles of size exceeding 5 µm per cubic foot.

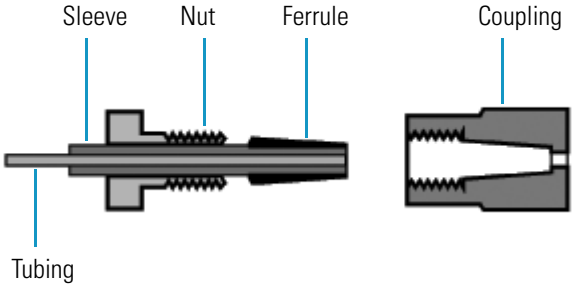
Working with Microscale Fittings and Tubing

The nanospray ion source components are assembled with various types of plastic couplings. MicroTight™ fittings and tubing couplings use fine-pitch threads that are more prone to cross threading than are the coarser threads of conventional HPLC fittings. Alignment is important, so ensure that the coupling axis coincides with the tubing axis. For the same tightening torque, fine threads exert more clamping force than coarse threads, enough to distort ferrules and partially collapse tubing. As a basic rule, initially tighten a coupling connection finger tight. Then, tighten it no more than an additional 1/4 turn.

Note See "Cutting Tubing" on page 13 for recommendations regarding techniques for cutting tubing. Ensure that the ends are cut squarely and that the edges of the cut are smooth.

Capillary tubing (fused silica tubing) is assembled with a tubing sleeve, a nut, and a ferrule, as shown in Figure 10. The capillary tubing is inserted into the coupling sleeve, and then the coupling nut and the ferrule are slid onto the sleeve. The ends of the tubing, sleeve, and ferrule must be flush as they are inserted into the bottom of the coupling, and then held in place while the nut is threaded into the coupling finger tight.

Figure 10. Coupling of capillary tubing



Disconnecting and then reconnecting coupling unions involving fused silica tubing and tubing sleeves is not recommended. Once you disconnect a coupling joint, you compromise the sleeve and ferrule component for further use. Discard or replace used fittings; do not reuse them.

Prior to making connections, clean the coupling components by flushing them with a high quality solution made from LC-MS grade solvents. It is sometimes helpful to assemble the microfluidic components as you flush the system with water. Begin the assembly of the microfluidic components with the fittings connected to the LC pump. Using this procedure, micro particulates are flushed out before they enter the narrow bore section of the system. In addition, flushing with water as you make the microfluidic connections can help you identify potential problems before you start an experiment.

IMPORTANT Do not filter solvents. Use LC-MS grade solvents instead of filtered solvents. Using filtered solvents in the MS detector can introduce contamination into the system.

For optimum performance, use LC-MS grade methanol, acetonitrile, water, and isopropyl alcohol when operating and maintaining the NSI source and your mass spectrometer. Store and handle solvents in accordance with recognized standard laboratory safety procedures. To reduce potential clogging problems caused by particulates, centrifuge samples prior to their injection into the microfluidic system.

Note

- Some solvent impurities are transparent to certain detectors. For example, spectroscopic grade solvents that are suitable for UV detectors might contain contaminants that interfere with the performance of the MS detector. For operation of your MS detector, use only LC-MS grade solvents.
- Thermo Fisher recommends that only LC-MS grade solvents from the following manufacturers be used: Merck, Mallinckrodt, or Burdick & Jackson.

Assembling the microfluidic components of an NSI source involves the use of tubing of various diameters. Be careful not to mix tubing of differing inner diameters because this can lead to bubble formation and flow disturbances. Maintaining consistent tubing ID reduces excess dead volume and unnecessary pressure differentials.

Excess tubing length, the major contributor to dead volume, can significantly increase experimental run times. Table 1 correlates tubing diameter with volume, allowing you to calculate the total dead volume for a given system.

Table 1. Correlation between tubing IDs and volume

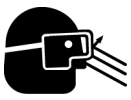
Tubing ID inches	Tubing ID micrometers	Volume µL/inch	Volume µL/cm
0.001	25	0.013	0.005
0.002	51	0.051	0.020
0.003	76	0.116	0.046
0.005	127	0.322	0.127
0.006	152	0.463	0.182
0.010	254	1.287	0.507
0.015	381	2.896	1.140
0.018	457	4.170	1.642
0.028	711	10.90	3.973

Use Table 1 to estimate the excess experimental run time caused by excess tubing. Determine the internal volume for your tubing from the table. To calculate the additional run time due to this tubing, divide its volume by your column flow rate. For example, if you use 10 cm of 76 µm ID tubing to connect your injection valve to your capillary column and you set your column flow rate (using a flow splitter) to 0.1 µL/min, it takes approximately 4.6 minutes for the injected sample to reach the column inlet. Each additional centimeter of tubing adds an additional 0.46 minutes to your run time.

Cutting Tubing and Emitter Tips

In microfluidics, you work with a variety of tubing and emitter types. These topics describe how to cut tubing and emitter tips:

- Cutting Tubing
- Cutting Emitters



CAUTION Wear ANSI approved safety glasses and non-powdered gloves when handling fused silica tubing and emitters.

Cutting Tubing

The technique used to cut tubing is critical for the tubing to exhibit proper flow characteristics. The following procedure describes the recommended technique for cutting tubing.

❖ To cut tubing

1. Place the tubing adjacent to a ruler. Then, mark the necessary length with a felt tip pen.

   You can prevent the formation of particulates and contamination of the tubing end during the cutting procedure by working on a clean, hard-surfaced table. A quick wipe of the table with alcohol or another volatile solvent will eliminate most microparticles.

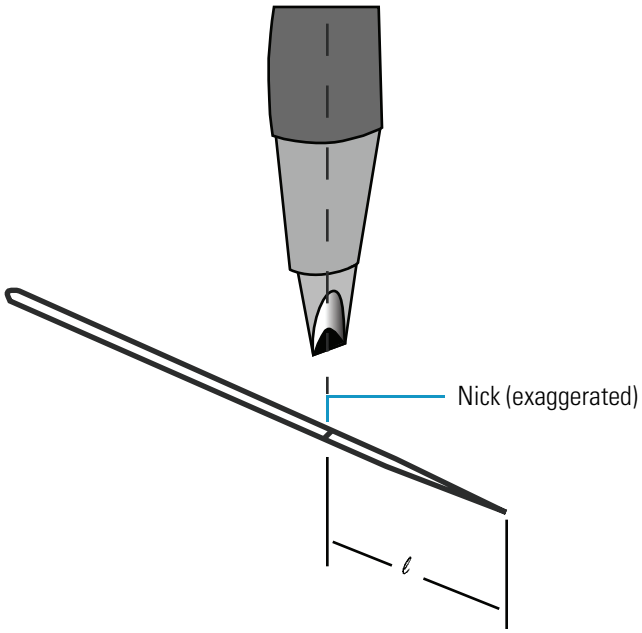
   Note Do **not** use lateral motion to score the tubing surface. Be careful not to force the blade through the tubing.

2. Holding the diamond-edged scribe blade (P/N 00725-00082) at right angles to the tubing axis and to the work surface, apply a light, gentle pressure to the tubing surface to create a nick.

   A freestanding 4× magnifier can be useful for orienting the scribe properly on the tubing. Light scoring is sufficient to create a nick in the surface of the polyimide coating.

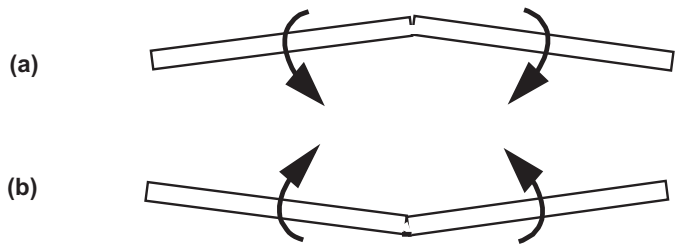
   Figure 11 shows an emitter tip being cut.

   Figure 11. An emitter tip being cut to specified length

3. Keeping your fingers away from the ends, take the tubing in both hands, flex it briefly about 20° away from the nick (see Figure 12a), and then bend it briskly toward the nick to snap it (see Figure 12b).

Figure 12. Snapping the tubing for a square end



4. Examine the snapped-off end at a magnification of 10× to 30× to ensure that the cut is square and smooth. If the cut is not square or smooth discard the piece of tubing or cut its end again.

Tip Thermo Fisher Scientific recommends that you practice several cuts on a piece of straight fused silica tubing prior to cutting costly emitters.

A flat, smooth cut is essential to the integrity of a low dead volume connection. Small leaks can result from improperly cut tubing. Such leaks are usually invisible and are only detected by inconsistent system performance, for example, unreliable spray.

IMPORTANT Clogged emitter tips can also lead to long run times and inconsistent system performance.

Plastic tubing used in the experimental setup is cut with a conventional guillotine-type cutter or a sharp razor blade. Thin-walled tubing tends to collapse in guillotine-type cutters and special care has to be taken to avoid the deformation of the tubing end.

Certain types of tubing require variations of the described basic technique. For example, PicoTips™ of heavy glass or quartz need a somewhat deeper nick, which can be accomplished by a slight rocking of the scribe in a plane at right angles to the tip. If overdone, however, this rocking motion can produce unwanted fine particles.

Cutting Emitters

Cut the emitter to a suitable length before assembling the NSI probe. For the packed-tip NSI probe, the appropriate emitter length is 10.5 cm. For the static NSI probe, the appropriate emitter length (for offline PicoTips) is 2.7 cm. Order pre-cut emitters for the dynamic NSI probe from New Objective, Inc. The distal end of these emitters is polished, guaranteeing better performance.

A cleanly cut, square end is essential to the stable performance of the emitter. If the end of the emitter is not cut squarely, it will not seat properly in the associated fittings and couplings. If not cut cleanly, the ragged end might introduce particles capable of clogging the tip of the emitter. To avoid extra column effects associated with poorly seated fittings and clogging caused by particulate contamination, inspect the cut end of the emitter at a magnification of 10× to 30× with a robust, high-quality lab stereoscope.

If you detect particulate contamination, you can clean the emitter with water or other solvents before you assemble the dynamic NSI probe. However, washing offline PicoTips, which are used for static nanospray, is not recommended because the same capillary action that allows you to load samples into the tip of the emitter will also draw the wash solvent into the capillary. This trapped solvent will act as a diluent when you load samples.

IMPORTANT Be careful not to touch the open ends of emitter tips with any surface while handling them.

Selecting an Emitter

Nanospray emitters are divided into two groups depending on their use: static (offline) emitters and dynamic (online) emitters. A range of emitter tip IDs is available for both groups. The following topics describe the variables that affect your choice of emitter ID:

- Static Nanospray Emitters
- Dynamic Nanospray Emitters

Static Nanospray Emitters

Static nanospray permits continuous analysis of small sample (analyte) solution volumes over an extended period of time. This method is particularly useful for obtaining detailed structural information using MS/MS for purified samples or those present in a simple mixture. A small aliquot of sample solution, usually less than 1 µL in volume, is loaded into a metal-coated pulled glass capillary with a tip internal diameter (ID) ranging from 1 to 4 µm. Voltage is applied to the metal coating on the tip. Simultaneously, an air-filled syringe provides a constant backpressure to initiate and maintain electrospray. After electrospray has commenced, the electric field and solvent properties provide a self-regulated flow rate.

To adjust the flow rate, you modify the parameters that affect the strength of the electric field. These parameters include the emitter tip ID, the spray voltage applied to the emitter or liquid junction, and the distance between the emitter and ion transfer tube (the counter electrode). If you change the parameters that affect the electric field strength, you change the flow rate and therefore alter the stability of the nanospray. Adjusting the spray voltage and optimizing the emitter position are described in the Tuning chapter of this manual that applies to your mass spectrometer.

PicoTip™ emitters supplied by New Objective Inc. are used with the static NSI probe. Refer to Table 2 to select the appropriate emitter ID for your application. The supplier provided flow rate ranges are based on the generation of a stable electrospray plume. Because the generation of a stable electrospray plume also depends on the applied voltage, the solvent composition, and the ion source, your results might differ.

Table 2. Effective flow rate range for PicoTip emitters

Emitter ID (µm)	Flow rate (nL/min)
1	20 to 80
2	20 to 80
4	40 to 100

Dynamic Nanospray Emitters

For dynamic nanospray, the appropriate emitter tip ID depends on the flow rate. The optimal flow rate for an application is typically determined by the capillary column that is required to provide an adequate separation. The dynamic NSI probe uses capillary columns with IDs ranging from 100 to 500 µm and supporting flow rates of 0.1 to 3 µL/min.

Refer to Table 3 to select the appropriate emitter ID for your application. The supplier provided flow rate ranges are based on the generation of a stable electrospray plume. Because the generation of a stable electrospray plume also depends on the applied voltage, the solvent composition, and the ion source, your results might differ.

The packed-tip NSI probe uses a PicoFrit column, which is an integrated column, frit, and spray tip. The optimal flow rate range for the 75 µm ID PicoFrit column is 200 to 300 nL/min. In addition to a capillary column, the dynamic NSI probe uses a pre-cut, 5 cm SilicaTip™ emitter with a polished distal end. You can order these emitters from New Objective, Inc.

Table 3. Effective flow rate range SilicaTip emitters

Emitter ID (µm)	Flow rate (nL/min)
5	20 to 100
8	100 to 300
10	100 to 400
15	150 to 400
30	300 to 1000

Working with PicoFrit Columns

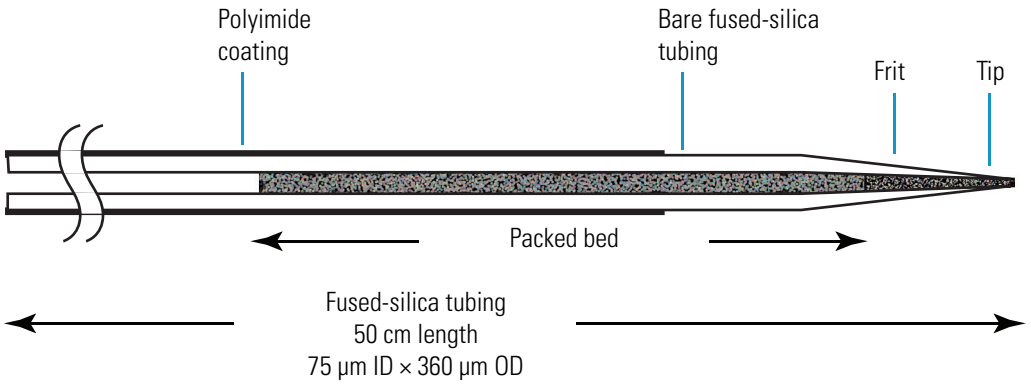
The packed-tip dynamic NSI probe is shipped with a PicoFrit column, which allows the ionization of molecules directly from the column outlet. The proximity of the column outlet to the MS interface minimizes post column losses and increases sensitivity.

Note For proper handling and use of PicoFrit columns, refer to their package inserts.

PicoFrit columns are fabricated from 360 µm OD, polyimide-coated, fused-silica tubing. One end of the column, referred to here as the tip end, has a specially tapered tip with an integral high-porosity frit. Behind the frit is the packed chromatography bed. There is no frit at the back end of the bed, only unpacked fused-silica tubing. Mobile phase flow must always be directed toward the tip. Reversing the flow can result in partial or complete unpacking of the chromatography bed.

Figure 13 shows a schematic of a PicoFrit column.

Figure 13. PicoFrit column (schematic)



PicoFrit columns are shipped in lengths of 50 cm fused-silica tubing. The ends of the column are protected by a piece of clear FEP tubing forming a loop. The PicoFrit column is stored in solvent, which keeps the distal end and the tapered tip end of the column at the same pressure during storage and shipping, and prevents the column from drying out.

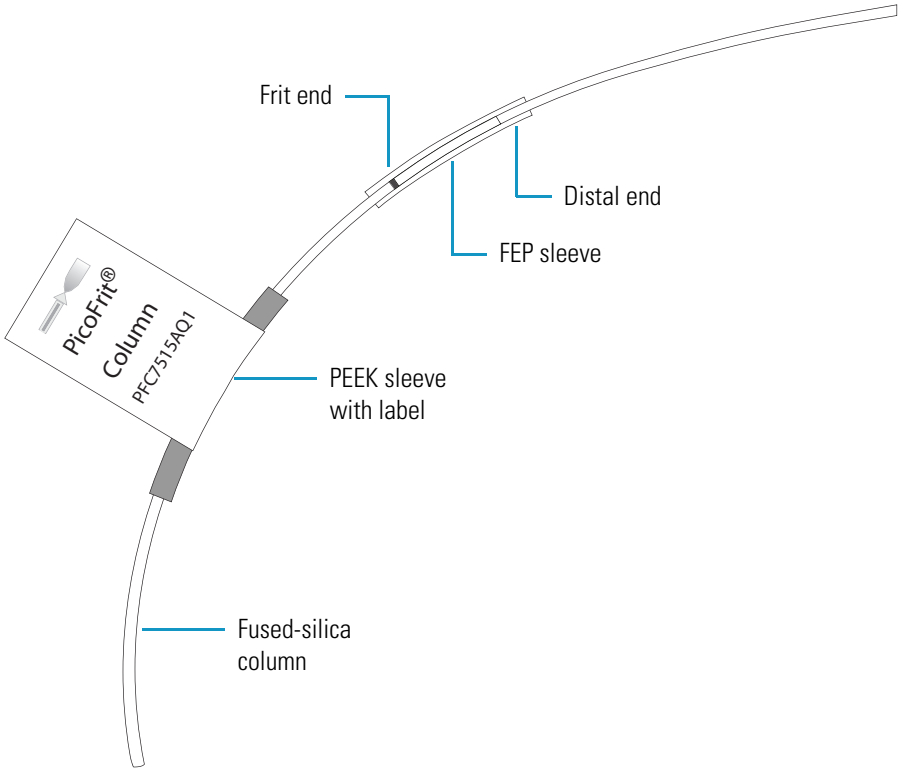
IMPORTANT Do **not** remove the column ends from the loop until the PicoFrit column is ready for installation and conditioning.

For a mass spectrometer and NSI source, the PicoFrit columns need to be cut to 10.5 cm.

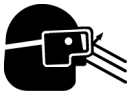
❖ To cut a PicoFrit column and connect it to a packed-tip NSI probe

1. Carefully remove the PicoFrit column from its shipping box.
2. Locate the arrow on the PicoFrit label that points toward the tip end to identify the distal end of the column (opposite side of the arrow pointer). See Figure 14.

Figure 14. PicoFrit column as shipped



3. Remove the distal end of the column from the loop by pulling it free from the sleeve or by cleaving the fused silica tubing near the distal end. Leave the tapered tip end of the column in the FEP sleeve until the column is ready for mounting on the packed-tip NSI probe.
4. Connect the distal end of the column to the nano LC (LC pump with flow splitter) and condition the column with 50:50 organic / aqueous solvent for several minutes. This repacks the column bed, which might have become loose during shipping and handling.
5. Place a mark 10.5 cm from the tip end of the column on the polyimide coating, and then cleave the PicoFrit column as described in "Cutting Tubing and Emitter Tips" on page 12.



CAUTION Wear ANSI approved safety glasses and non-powdered gloves when handling and cutting glass tubing.

6. Remove the tip end of the PicoFrit column from the FEP sleeve by sliding the PEEK sleeve with the label towards the tip end until it butts up against the FEP sleeve (see Figure 3 in the package insert).
7. Point the tip vertically towards the ground and carefully push the PEEK sleeve against the FEP sleeve until the FEP sleeve falls off. It might take a lot of force to move the FEP sleeve 3 to 5 mm.

Note Do not let the PEEK sleeve slide off the end of the tip, but instead remove it by sliding it off the distal end of the column.

8. Attach the PicoFrit column to the packed-tip NSI probe as described in "Replacing the PicoFrit Column" on page 55.
9. Mount the packed-tip probe into the base mount of the NSI source.
10. Ensure that your mass spectrometer is in the Standby mode, and then reconnect the NSI source to the mass spectrometer as described in "Connecting the NSI Source to the Extension Assembly" on page 61.

Controlling the Column Flow Rate

To perform high-sensitivity analyses of mixtures, install either the dynamic NSI probe or the packed-tip NSI probe onto your mass spectrometer. The dynamic NSI probe uses either a capillary or nano LC column. The packed-tip NSI probe uses a PicoFrit column.

Conventional HPLC systems with analytical packed columns of internal diameters between 3 to 4.6 mm employ flow rates from a hundred microliters per minute to several milliliters per minute. Most commercially available chromatography equipment, including the LC pump, has been designed to operate at those flow rates. Capillary and nano-LC performs best at a flow rate of about 1 to 10 µL/min and below, which is 100 to 1000 times lower than conventional LC flow rates. Table 4 compares flow rates and column ID of various HPLC techniques to illustrate the difference in flow rate requirements.

Table 4. Comparison of flow rates and column internal diameters for various HPLC techniques (adapted from Chervet et al, *Anal.Chem.,* 68, pp. 1507–1512 (1996)).

HPLC Technique	Flow Rate	Column ID
Conventional	0.5 to 2 mL/min	3.2 to 4.6 mm
Microbore	0.1 to 0.5 mL/min	1.5 to 3.2 mm
Micro	10 to 100 µL/min	0.5 to 1.5 mm
Capillary	1 to 10 µL/min	150 to 500 µm
Nano	10 to 1000 nL/min	10 to 150 µm

Some LC pumps can form reproducible solvent gradients at flow rates as low as 50 to 200 µL/min, but few LC pumps can produce flow rates under solvent gradient conditions in the low µL/min range. To couple a standard LC pump to your dynamic NSI/MS system, you must split the solvent flow delivered by the LC pump into two solvent streams with one stream flowing through the capillary LC column as the other stream is diverted to waste.

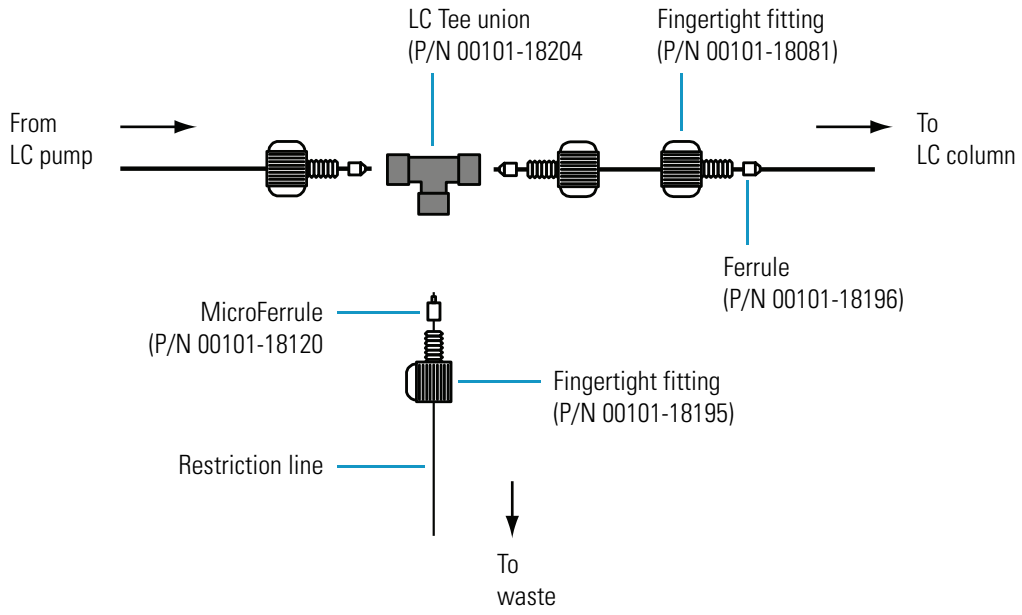
These topics describe how to control the column flow rate:

- Splitting Flow from an LC Pump
- Adjusting the Column Flow Rate
- Measuring the Column Flow Rate

Splitting Flow from an LC Pump

You split the flow from an LC pump with a simple Tee union. The tubing and fitting components of the flow splitter are illustrated in Figure 15.

Figure 15. Flow splitting components



As illustrated in Figure 15, a flow splitter divides the solvent flow delivered by an LC pump into two flow streams. The bleed-off or restriction line carries the majority of flow to waste. The rest of the flow passes through the LC-tee union towards the column. The restriction line is a piece of narrow-bore fused-silica tubing (for example, 50 µm ID with 360 µm OD, P/N 00106-10512) that is cut to a specific length to provide a defined *leak* for excess solvent bleed-off to waste. The flow rate of the solvent shunted to the LC column section of the flow spitting assembly is referred to as the *column flow rate*.

Adjusting the Column Flow Rate

The column flow rate is adjusted by increasing or decreasing the length of the restriction line. To decrease the column flow rate, you decrease the length of the restriction line. To increase the column flow rate, you increase the length of the restriction line.

The effect of adjusting the length of the restriction line on the column flow rate is shown in Figure 16 and Figure 17. For a 150 mm × 180 µm ID column, reducing the length of the restriction line from 50 to 30 cm reduces the column flow rate from 2.8 to 1.7 µL/min. For a 150 mm × 320 µm ID column, reducing the length of the restriction line from 25 to 10 cm reduces the column flow rate from to 5.3 to 2.8 µL/min.

Figure 16. Effect of restriction line length on the flow rate for a 150 mm × 180 µm ID column

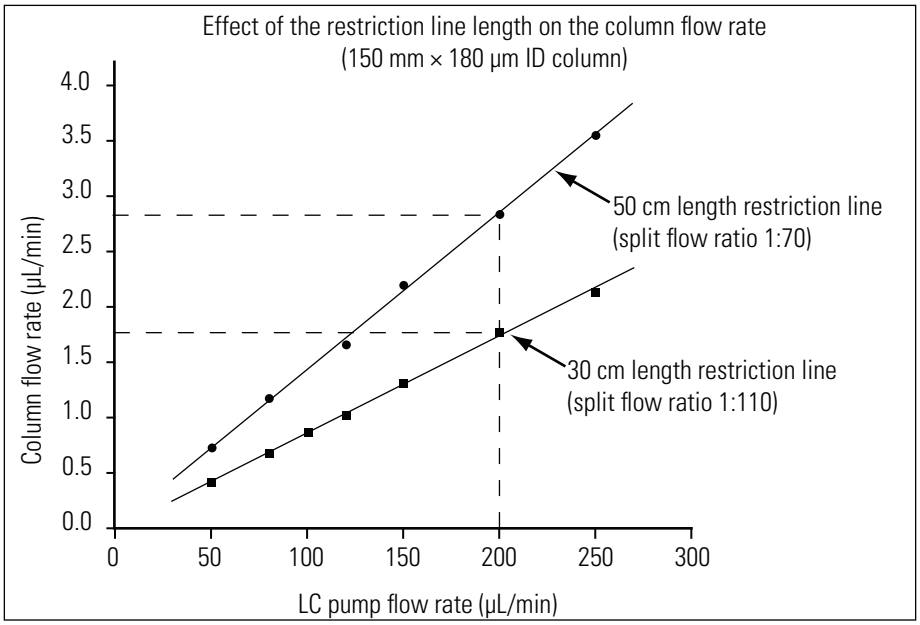
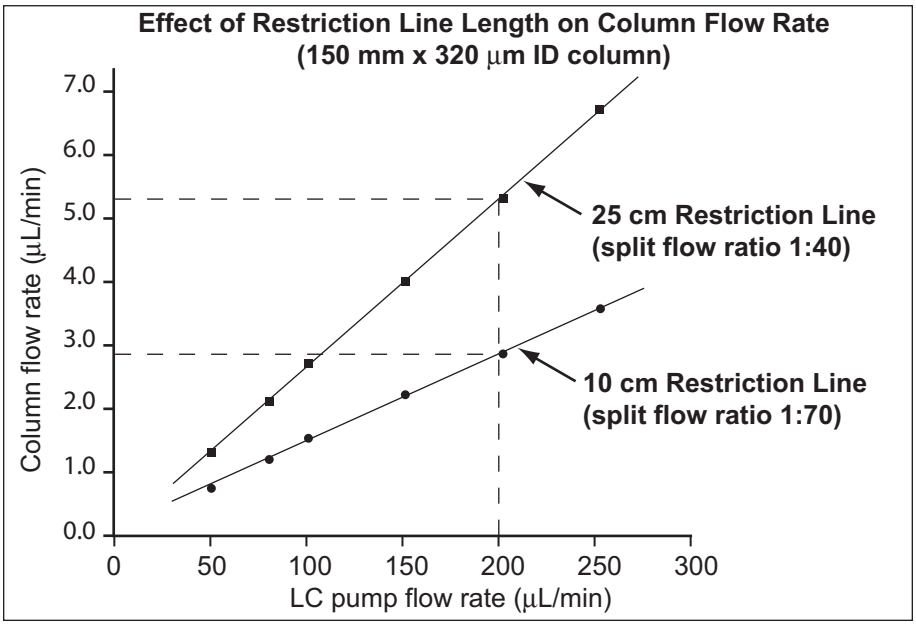


Figure 17. Effect of restriction line length on the flow rate for a 150 mm × 320 µm ID column



The values listed in Table 5 were experimentally determined for the columns supplied by Thermo Scientific. Other microscale columns might require different operating conditions.

Table 5. Operating guidelines for capillary columns

HPLC Parameter	Packed Column (1)	Packed Column (2)	PicoFrit Column (3)
Column ID	320 µm	180 µm	75 µm
Flow Rate	3 to 5 µL/min	0.9 to 1.5 µL/min	200 to 300 nL/min
Max Sample Mass	570 ng	180 ng	50 ng
Max Peak Volume	550 nL	175 nL	30 nL

Note It is often useful to start working with larger ID capillary columns before turning to more demanding nanoscale experiments.

❖ To reduce the column flow rate to a suitable level

1. See Table 5 to determine the optimum flow rate for the ID of your LC column. For example, Table 5 recommends the following:
   - A flow rate range of 0.9 to 1.5 µL/min for a 150 mm × 180 µm ID column
   - A flow rate range of 3.0 to 5.0 µL/min for a 150 mm × 320 µm ID column
2. Set your LC pump to the lowest stable flow rate for your application. To maintain a stable flow rate, most LC pumps require a higher setting for gradient pump programs than for isocratic pump programs. Setting the LC pump to deliver mobile phase at its lowest possible stable flow rate will allow you to maintain a lower split flow ratio.

3. Determine the length of restriction tubing that will allow you to set a stable LC pump flow rate and that will produce the column flow rate determined in step 1. To determine the appropriate length of restriction tubing for the 150 mm × 180 µm ID and the 150 mm × 320 µm ID columns that are provided in the NanoFlow solution kit, see Figure 16 or Figure 17, respectively.

   For example, you can determine from Figure 16 that the split flow ratios for the 50 cm and the 30 cm restriction lines are approximately 1:70 and 1:110, respectively, for the 150 mm × 180 µm ID column. To achieve a column flow rate of 1 µL/min, the flow rate for the LC pump must be set to 70 µL/min for the 50 cm restriction line or to 110 µL/min for the 30 cm restriction line. If your LC pump can maintain a stable flow at both of these flow rates, select the restriction line length that yields the lowest split flow ratio.

4. Cut a fused silica line (P/N 00106-10512) to the length determined in step 3 and assemble the flow splitter as described in "Splitting Flow from an LC Pump" on page 20.

5. Set the LC pump flow rate. Then, measure the actual column flow rate as described in the next topic, "Measuring the Column Flow Rate" on page 23.

6. Make fine adjustments to the LC pump flow rate to reach the optimum column flow rate. Several iterations of LC pump flow rate changes might be necessary to reach the desired column flow rate.

You can construct a graph similar to the ones in Figure 16 and Figure 17 for quick adjustment of the column flow rate.

Tip Low split ratios are preferable. Splitting down from 8 µL/min to 50 nL/min (1:160) is preferable to splitting down from 500 µL/min to 500 nL/min (1:1000). If large split ratios are used, adjustment of the LC pump flow rate is less effective for fine-tuning the column flow rate.

Measuring the Column Flow Rate

To make a direct measurement of the column flow rate, you need a disposable micropipette (1 to 5 µL, Fisher Scientific, Cat No 21-164-2A) or a similar piece of volumetric glassware and a stopwatch.

IMPORTANT When using packed-tip columns, the outlet of the column is the emitter, so take special care when using the following procedure to avoid damaging or clogging the tip.

❖ To determine the column flow rate

1. Butt the micropipette directly to the outlet of the HPLC column. Then, allow the micropipette to fill to the graduated mark.

2. Note the length of time required to fill the micropipette.

3. Divide the volume collected in the micropipette by the measured time to obtain the flow rate through the HPLC column.

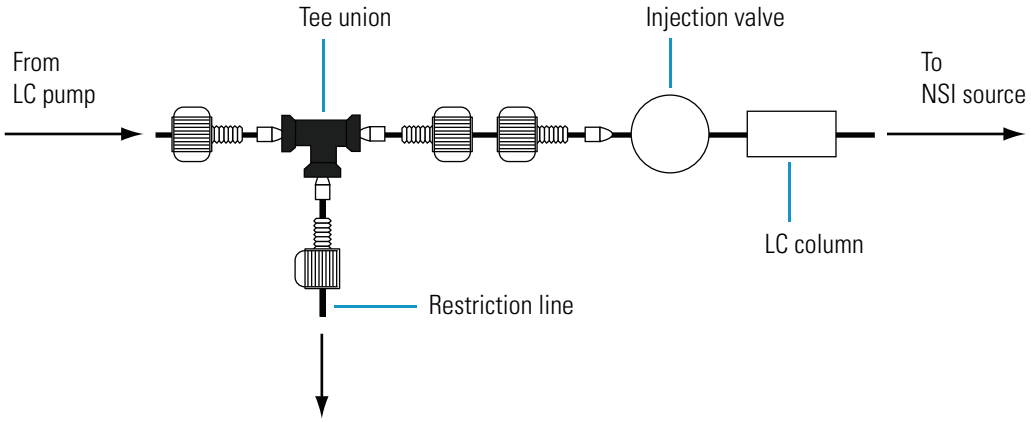
Tip You can also determine the column flow rate indirectly by performing a loop injection experiment. For information on determining the column flow rate by performing a loop injection experiment, contact your local Thermo Fisher Scientific service engineer.

Configuring the System for Microscale LC/MS Experiments

Successful experimentation with microscale LC/MS requires the adaptation of all system hardware components as well as their operating parameters to the microscale range. To obtain the best chromatographic performance with a microscale LC/MS system, the injected sample volume, sample concentration, or both have to correspond with the requirements of the separation column, as shown in Table 5.

For small amounts of sample or if diluted solutions need to be concentrated onto the microscale column, place the injection valve between the flow splitter and the column. See Figure 18. This configuration allows all of the injected sample plug to reach the LC column and is preferred for systems capable of injecting low micro to nanoliter sample volumes.

Figure 18. Injection valve between the flow splitter and the LC column



Effect of Solvents on Maintaining a Stable Nanospray

The optimum spray voltage and flow rate for an experiment are affected by the properties of the solvents used. Solvents with higher surface tension require greater electrical fields to start the nanospray. Table 6 displays the surface tension of three typical solvents used in LC/MS. If you are running a gradient pump program, you need to consider the fact that the surface tension of the liquid exiting the emitter near the beginning of the gradient, where the solvent is mostly aqueous, differs from that near the end of the gradient, where the solvent is mostly organic.

Table 6. Surface tension of common solvents used in LC/MS

Solvent	Surface Tension (N/m)
Acetonitrile	0.0226
Methanol	0.030
Water	0.073

Since solvent needs to be differentially removed from the analyte by evaporation, use only volatile additives in the solvent matrix. As with ESI, avoid non-volatile salts and buffers. They can clog the emitter. If salts are required in the solvent matrix, it is better to use ammonium rather than sodium or potassium salts.

Installing the Nanospray Extension Assembly

This chapter describes how to install the interface components required to provide the appropriate distance between the emitter tip of the NSI probe and the inlet of the mass spectrometer. These components include the Ion MAX adapter, the 2-inch deep adapter ring for dynamic nanospray, and the 1-inch deep adapter ring.

The nanospray extension assembly is the mounting surface for the imaging system and the NSI source.

Contents

To prepare your mass spectrometer for the installation of the imaging system and the NSI source, follow these procedures:

1. Preparing for Nanospray Installation
2. Installing the Components of the Extension Assembly

Preparing for Nanospray Installation

These topics describe how to prepare the mass spectrometer for installation of the Thermo Scientific nanospray ion source:

- Nanospray Power Requirements
- Removing the API Source

Nanospray Power Requirements

The Thermo Scientific NSI source (including its associated components) operates within the range of 100 V to 240 V ac, at 50 to 60 Hz, and requires a power outlet in addition to that needed for the mass spectrometer. Therefore, you need two power outlets to operate both the NSI source and the mass spectrometer.

Removing the API Source

Before you can install the new NSI source, you must remove the current API source from your mass spectrometer.

IMPORTANT Place your LC/MS system in the Standby mode before you remove the API source.

❖ **To remove the API source, do one of the following**

- If your mass spectrometer is already configured with a previous version of the nanospray ion source, put your system in the Standby mode. Then, follow the instructions provided in the manual that was shipped with your original NSI source to remove it. Do not remove the 2-inch Nanospray Adapter Ring or the imaging system.

  The original NSI source includes an NSI flange, NSI body, and NSI probe. You can use the NSI flange that shipped with the original version of the nanospray system after you remove its ZDV arm. You cannot use the NSI body with the new NSI probes.

- If your mass spectrometer is configured with an ESI or APCI ion source, remove the ion source by following the instructions provided in the Hardware manual for your mass spectrometer.

- If your mass spectrometer is configured with a Ion MAX™ ion source, follow the instructions provided in the *Ion Max API Source Hardware Manual* to remove it.

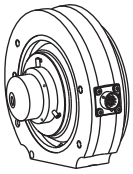
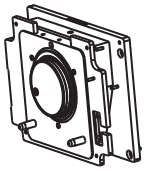
Installing the Components of the Extension Assembly

The extension assembly, which mounts directly to the mass spectrometer, positions the NSI probe at an appropriate distance from the inlet of the mass spectrometer. The components of the extension assembly depend on the geometry of the mass spectrometer inlet and the type of NSI probe used. To determine the components of the extension assembly for your mass spectrometer and NSI probe, see Table 7.

This section contains the following procedures:

- Installing the Ion MAX Adapter
- Installing the Two-Inch Deep Adapter Ring
- Installing the One-Inch Deep Adapter Ring for Dynamic Nanospray

Table 7. Components of extension assembly

Inlet Geometry	Extension Assembly Components for the Static NSI Probe	Extension Assembly Components for the Dynamic NSI Probe and the Packed-Tip NSI Probe
Protruding inlet with a circular base 	2-inch adapter ring	2-inch adapter ring 1-inch adapter ring
Protruding inlet with a square base 	Ion MAX adapter	Ion MAX adapter 1-inch adapter ring

To install the appropriate extension assembly for your mass spectrometer, do one of the following:

- If you have a mass spectrometer with an Ion MAX ion source, go to "Installing the Ion MAX Adapter," below.
- If your mass spectrometer has a protruding inlet with a circular base, go to "Installing the Two-Inch Deep Adapter Ring" on page 31. This category includes the LCQ Advantage, LCQ Deca XP, and LCQ Deca XP Plus mass spectrometers.
- If you have already installed the 2-inch Nanospray Adapter Ring for the previous version of the nanospray ion source and you are preparing to install the either the new dynamic NSI probe or the new packed-tip NSI probe, go to "Installing the One-Inch Deep Adapter Ring for Dynamic Nanospray" on page 32.

Installing the Ion MAX Adapter

❖ **To install the Ion MAX adapter on your mass spectrometer**

1. If you have not already done so, remove the Ion MAX ion source as described in the *Ion Max API Source Hardware Manual*.
2. Carefully align the two guide pin holes on the back of the Ion MAX adapter (see Figure 19) with the guide pins on the mass spectrometer (see Figure 20).

Figure 19. Ion MAX adapter, showing ion source guide pin holes

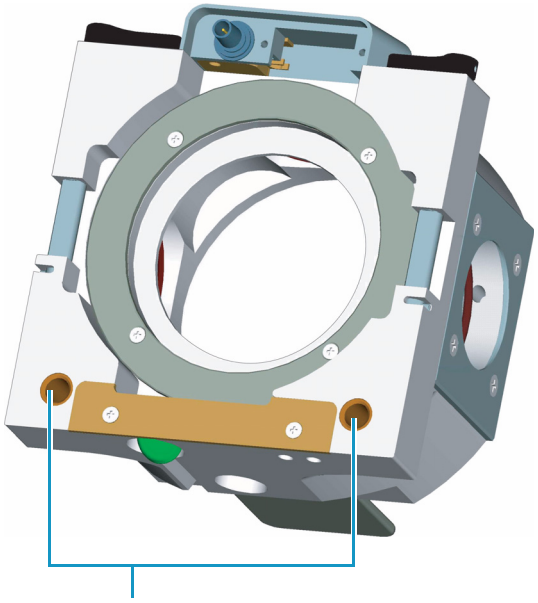
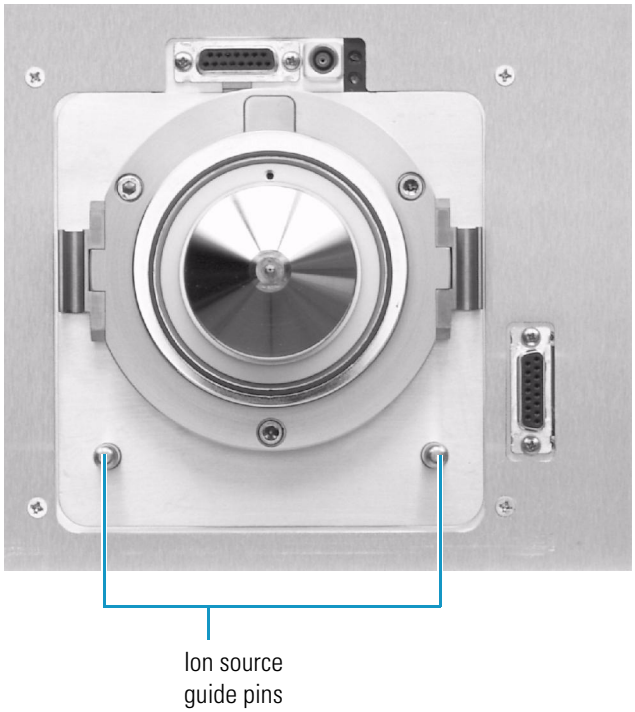


Figure 20. Mass spectrometer, showing ion source guide pins



3. Ensure that the Ion MAX adapter locking levers are open (pointing away from the mass spectrometer), and gently push the Ion MAX adapter onto the mass spectrometer.

4. Rotate the Ion MAX adapter locking levers 90 degrees to lock the nanospray extension assembly onto the mass spectrometer.
5. Depending on the type of NSI probe you are installing, do one of the following:
   - If you are installing a dynamic NSI probe or a packed-tip NSI probe, go to "Installing the One-Inch Deep Adapter Ring for Dynamic Nanospray" on page 32.
   - If you are installing a static NSI probe, you have completed the installation of the extension assembly and are now ready to install the imaging system. Proceed to Chapter 4.

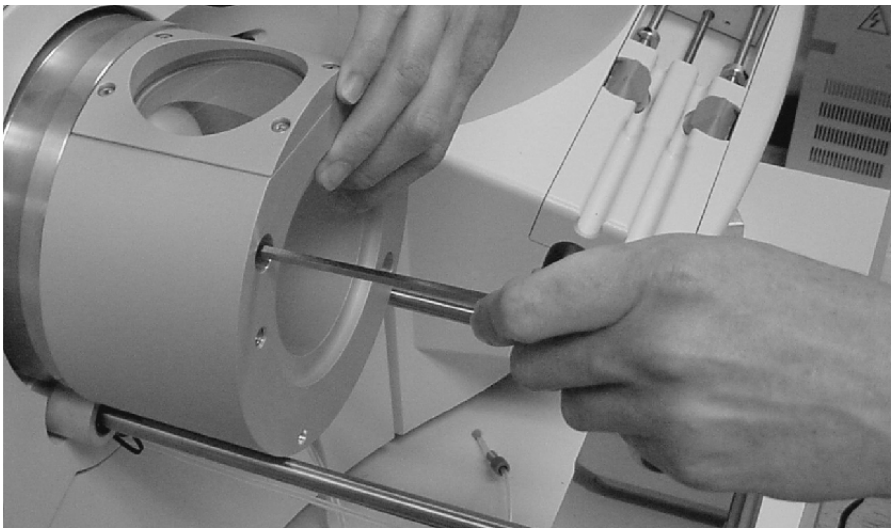
Installing the Two-Inch Deep Adapter Ring

Because the LCQ Advantage, LCQ Deca XP, and LCQ Deca XP Plus mass spectrometers have a protruding inlet with a circular base, they require a 2-inch deep adapter ring.

❖ To install the 2-inch deep adapter ring

1. If you have not already done so, remove the conventional API source as described in the Hardware manual for your mass spectrometer.
2. With the viewing window on the nanospray adapter ring facing upwards, align its extension screws with the threaded holes (4 o'clock and 10 o'clock) on the API spray shield.
3. Use a 3/16-in. Allen wrench to tighten the extension screws to the API spray shield. See Figure 21.

Figure 21. Tightening the extension screws on the 2-inch adapter ring



4. Depending on the type of NSI probe you are installing, do one of the following:
   - If you are installing a dynamic NSI probe or a packed-tip NSI probe, go to "Installing the One-Inch Deep Adapter Ring for Dynamic Nanospray" on page 32.
   - If you are installing a static NSI probe, you have completed the installation of the extension assembly and are now ready to install the imaging system. Proceed to Chapter 4.

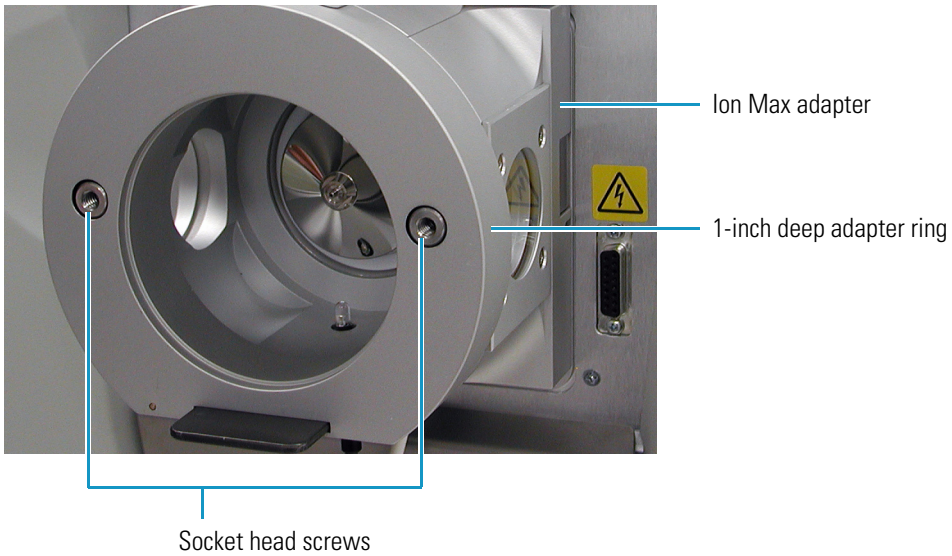
Installing the One-Inch Deep Adapter Ring for Dynamic Nanospray

If you are installing a dynamic NSI probe or a packed-tip NSI probe, you need to install the 1-inch deep adapter ring.

❖ To install the 1-inch deep adapter ring

1. Do one of the following:
   - For mass spectrometers with an Ion MAX adapter, go to step 2.
   - For mass spectrometers with the two-inch adapter ring, go to step 3.
2. With the heads of the two socket head screws facing away from the mass spectrometer, slide the alignment slot in the bottom of the one-inch adapter ring over the alignment tab of the Ion MAX adapter. Then, tighten the socket head screws to the Ion MAX adapter with a 4 mm Allen wrench. See Figure 22.

Figure 22. LTQ Series mass spectrometer with the Ion MAX adapter and the one-inch adapter ring installed



You have completed the installation of the extension assembly for your NSI source. Go to Chapter 4.

3. With the heads of the two socket head screws facing away from the mass spectrometer, align the socket head screws of the one-inch adapter ring with the mounting holes on the two-inch adapter ring. Then, use a 4 mm Allen wrench to tighten the socket head screws to the two-inch adapter ring.

   You have completed the installation of the extension assembly for your new NSI source. If this is your first nanospray system, proceed to Chapter 4. If you have already installed the imaging system, proceed to Chapter 5.

Installing the Imaging System

The Thermo Scientific NSI source comes equipped with an imaging system to facilitate positioning of the emitter and to troubleshoot emitter problems.

Contents

Imaging System Components

The major components of the imaging system are as follows:

- CCD camera
- Camera lens system
- Lens extension tube set
- Osprey video capture card and software
- Camera stand
- LED light source

Some of the components have been pre-assembled. The LED light source is positioned below the NSI source to backlight the nanospray droplets. The camera is attached to the video capture card to display the probe and the emitter tip on the computer workstation.

Before you can install the imaging system, you must install the adapter for the NSI source as described in Chapter 3.

Imaging System Installation

To install the imaging system, follow these procedures:

1. Assembling the CCD Camera and Optics
2. Installing the Camera Assembly
3. Installing the Video Capture Card
4. Adjusting the Imaging System

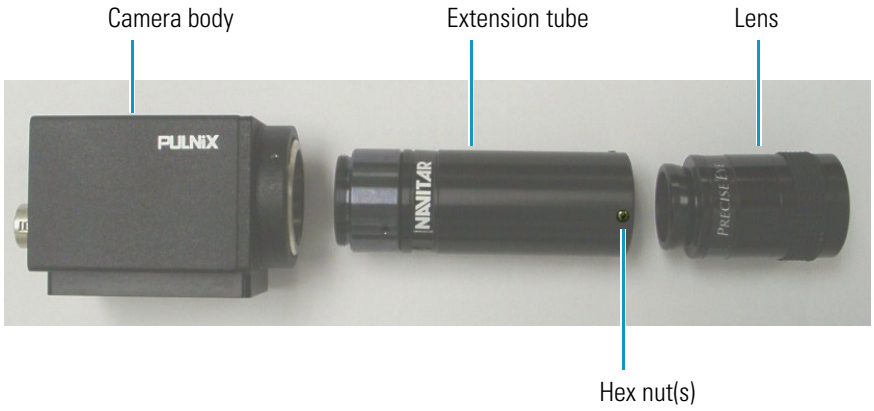
Assembling the CCD Camera and Optics

The CCD camera and optics are shipped unassembled. Before you mount the camera to your mass spectrometer, you need to assemble it.

❖ To assemble the CCD camera and optics system

1. Carefully remove the CCD camera and optics from their shipping container and place the components on a clean surface.
2. Remove the lens cap from the camera.
3. Screw the lens extension tube set to the camera.
4. Attach the lens system to the extension tube by inserting the lens into the extension tube and tightening the three hex nuts. See Figure 23.

Figure 23. Imaging system camera assembly



The CCD camera and optics system is now assembled and is ready to be installed on the camera stand as described in the next section, “Installing the Camera Assembly.” .

Installing the Camera Assembly

The CCD camera and the LED light source are installed on the nanospray extension assembly. If the nanospray extension assembly is not already mounted on your mass spectrometer, refer to the installation instructions in Chapter 3 before proceeding with the following procedure.

❖ To mount the camera and the LED light source on the nanospray extension assembly

1. Attach the camera stand to the nanospray extension assembly as follows:

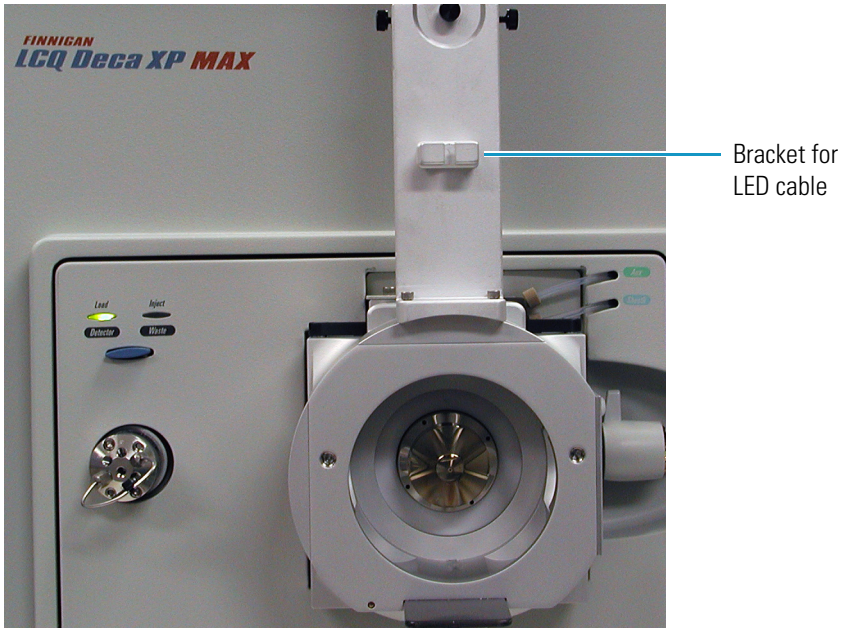
   - On a LCQ Advantage, LCQ Deca XP, or LCQ Deca XP Plus mass spectrometer, mount the camera stand on the top of the nanospray adapter ring as shown in Figure 24.

     Figure 24. Camera stand mounted to the 2-inch deep adapter ring

- On a mass spectrometer that was shipped with an Ion MAX ion source (with the exception of a TSQ Series mass spectrometer) mount the camera stand as shown in Figure 25.

Figure 25. Camera stand mounted to an Ion MAX adapter



- On a TSQ Series mass spectrometer, mount the camera stand on the left side of the Ion MAX adapter.

2. Install the LED by screwing the LED retainer clockwise into the bottom of the Ion MAX adapter or the nanospray adapter ring. See Figure 26 and Figure 27.

Figure 26. 12 V dc Power supply and LED with cable

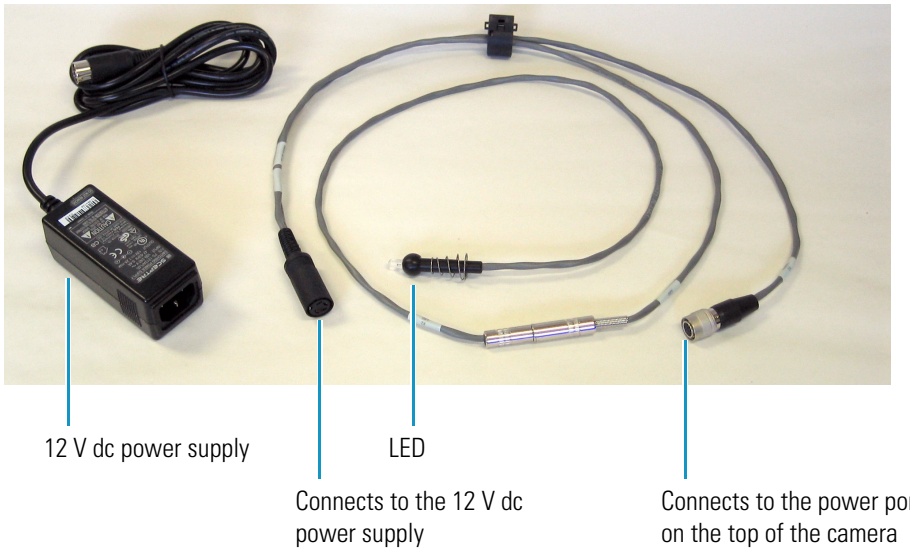


Figure 27. Installation of the LED into the bottom of the Ion MAX adapter

3. Route the LED cable through the bracket in the camera stand.

Installing the Video Capture Card

You need to install a video capture card into the PC so that the image can be processed by the computer and displayed on the monitor. This section contains a general procedure for installing the video capture card. Refer to Appendix B, "Installing the Osprey MultiMedia Capture Device, Software, and Drivers." for a more detailed installation procedure.

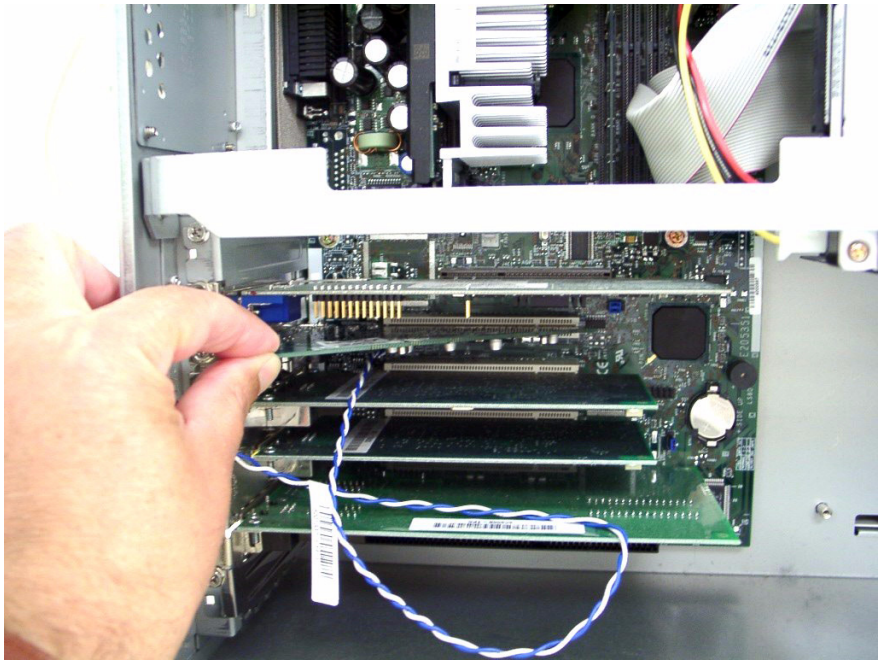
❖ To install the Osprey™ MultiMedia Capture Card

1. Turn off and unplug the computer.

IMPORTANT Wear a grounding strap when installing the video capture card.

2. Remove the computer cover and insert the video capture card into a vacant PCI slot. See Figure 28.

Figure 28. Installation of the video capture card into the computer



3. Reinstall the computer cover.
4. Boot up the computer.
5. Cancel out of the Microsoft™ Windows™ New Hardware Found wizard (use the Osprey MultiMedia Capture Driver installation CD instead).
6. Install the appropriate video capture card driver from the installation CD provided by Osprey.

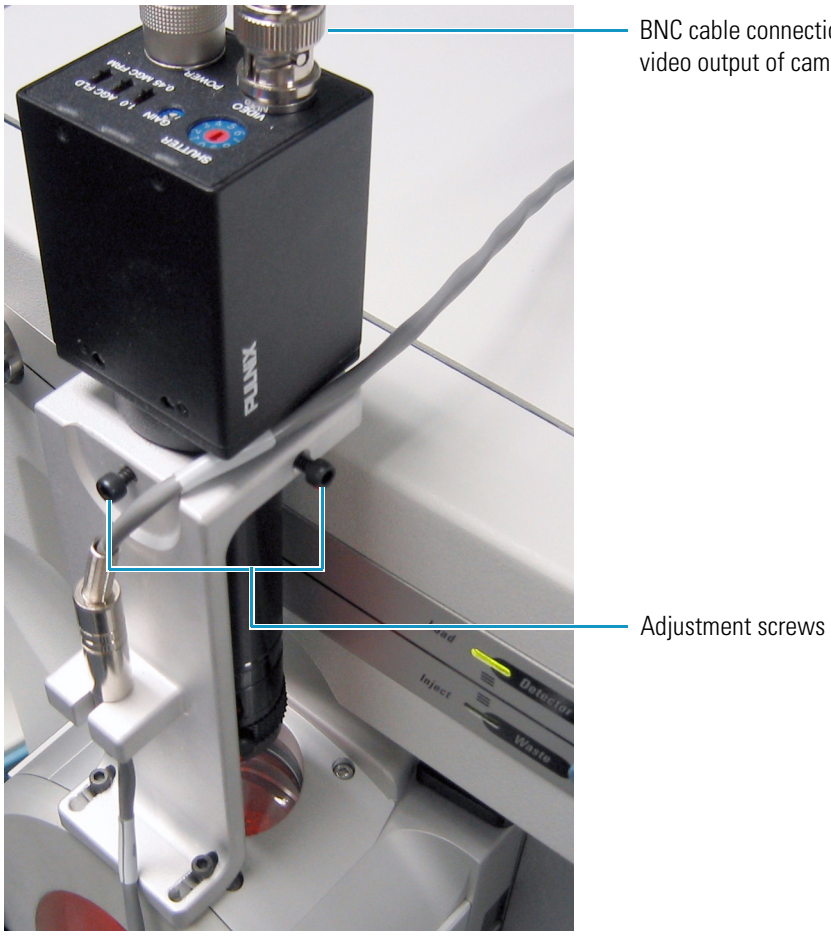
Adjusting the Imaging System

The imaging system needs to be adjusted before initial use of your nanospray ion source.

❖ To adjust the imaging system

1. Place the camera in the camera stand as illustrated in Figure 29.

   Figure 29. Camera mounted in camera stand, showing the camera adjustment screws



2. Connect one end of the BNC cable to the Video port on the top of the camera. Connect the other end of the BNC cable to the video capture card.
3. Connect the LED cable to the Power port on the top of the camera.
4. Connect the LED cable to the 12 V dc power supply. See Figure 26 on page 38.
5. Ensure the proper operation of the video capture card by starting the multimedia software application on your computer. From the Start menu, choose **Start > Programs > Osprey MultiMedia Capture > VidCap32** to display the VidCap window. See Figure 30.

Figure 30. Illustration of the proper LED positioning

6. With the camera stand adjustment screws fully retracted, position the camera to achieve the best view of the ion transfer tube orifice.
7. Adjust the position of the LED light so that the back side of the inlet cone is illuminated (Figure 30).
8. Once you have achieved good camera positioning, tighten the adjustment screws on the camera stand.

Note During nanospray operation and for troubleshooting purposes, adjusting the focal length of the camera in a counterclockwise direction towards infinite focal length gives a clear and focused picture of the spray tip pointing towards the inlet tube.

The above steps are basic to focusing the camera. Practice is required to develop a sense of how camera movement affects the position of the image on the monitor.

The installation of the imaging system is complete. Go to Chapter 5 to continue the installation of your new NSI probe.

Assembling the NSI Probe

Thermo Scientific offers three types of interchangeable nanospray probes: the static NSI probe, the dynamic NSI probe, and the packed-tip NSI probe.

Note Thermo Fisher Scientific has discontinued the NSI-2 dynamic nanospray and packed-tip probes. For information about ordering and installing the original dynamic nanospray probe, refer to the *Dynamic Nanospray Probe (NSI-1) Installation*.

For instructions on assembling your new NSI probe, refer to one of the sections in this chapter.

Contents

Static NSI Probe

This section contains the following topics:

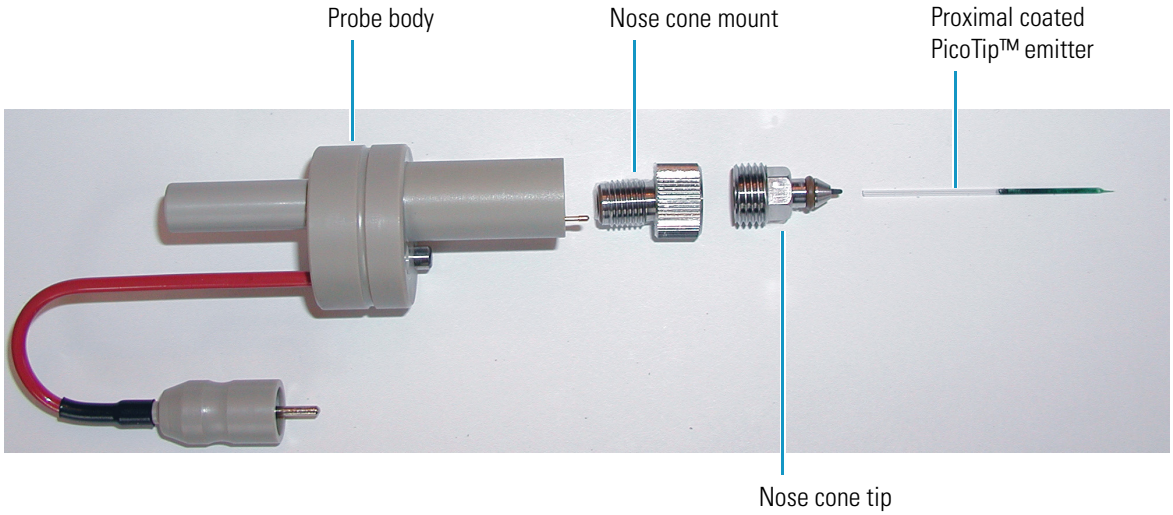
Static NSI Probe Components

The static NSI probe contains an emitter that is pulled from a glass or quartz capillary to a tip of 1 to 2 µm ID and is coated with gold/palladium to enable electrical contact. The O-ring in the center of the nose cone tip assembly holds the body of the static nanospray emitter in place.

The components of the static NSI probe are shown in Figure 31.

CAUTION Take care to avoid touching the emitter tip. It is sharp enough to puncture the skin.

Figure 31. Static NSI probe components



Assembling the Static NSI Probe

To assemble the static NSI probe, follow these procedures:

1. Cutting the Static NSI Emitter
2. Inserting the Static Emitter into the Nose Cone Tip
3. Loading Sample into the Static Emitter
4. Connecting the Nose Cone Tip Mount Assembly to the Probe Body

Cutting the Static NSI Emitter

Using a high-quality diamond chip or sapphire cutting tool, cut the static NSI emitter to a length of about 27 mm. You need to make a square cut or the emitter will not seat properly in the associated fittings and couplings. You also need to make a clean cut because loose particles will quickly clog the tip of the emitter. Refer to "Cutting Tubing and Emitter Tips" on page 12 for recommendations.

CAUTION Avoid touching the emitter tip. It is sharp enough to puncture the skin.

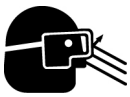
IMPORTANT It is very easy to damage or break the tip of the emitter. Avoid touching any hard surface with the emitter tip.

Note Purchase PicoTip™ emitters for static nanospray (also referred to as offline nanospray) from New Objective, Inc.

Inserting the Static Emitter into the Nose Cone Tip

❖ To insert the static emitter into the nose cone tip

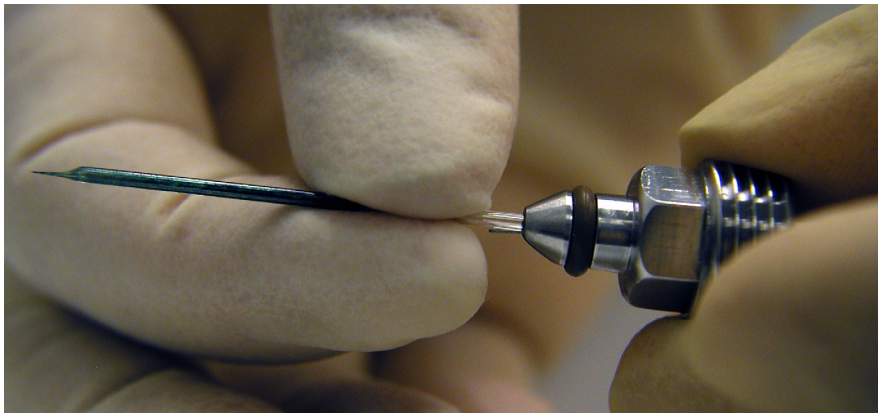
1. Before you handle the emitter, make sure that you are wearing safety glasses and non-powdered gloves.



CAUTION Wear ANSI approved safety glasses and non-powdered gloves when handling emitters.

2. With one hand holding the nose cone tip, use the other hand to guide the flat end of the emitter through the hole in the center of the nose cone. See Figure 32.

Figure 32. Guiding the flat end of the emitter through the hole in the center of the nose cone tip



3. If you cannot fit the flat end of the emitter through the tip of the nose cone, remove the emitter and use a flat head screwdriver to adjust the collar inside the nose cone tip as follows (see Figure 33 and Figure 34):

   - To tighten the emitter, adjust the collar clockwise to compress the O-ring.
   - To loosen the emitter, adjust the collar counterclockwise to decompress the O-ring.

Figure 33. Collar inside the nose cone tip assembly

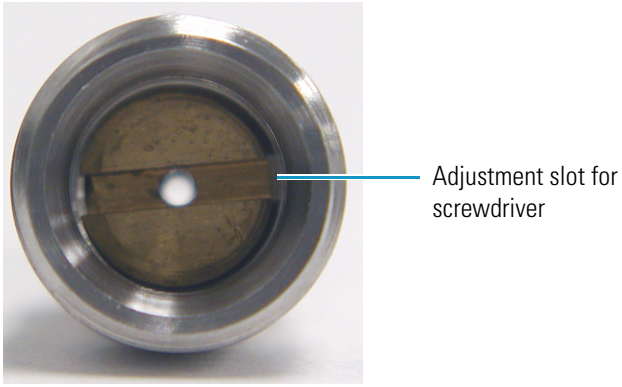
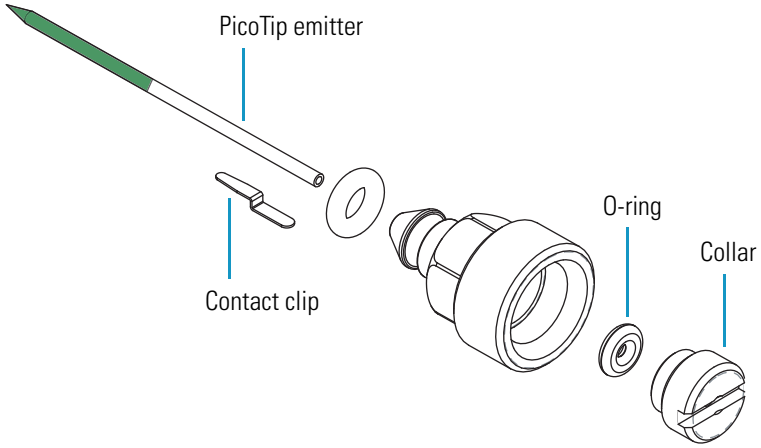


Figure 34. Nose cone tip assembly (exploded view)



4. Using tweezers, gently push the emitter further into the nose cone tip until 5 to 8 mm of the emitter protrudes through the end of the tip. Ensure that the metal contact clip touches the coating on the emitter.

IMPORTANT To avoid removing the coating from the emitter, do **not** slide the emitter in and out of the nose cone tip mount assembly.

Loading Sample into the Static Emitter

Use a micropipette equipped with a gel-loading tip to transfer 1 to 5 µL of bubble-free sample to the static nanospray emitter. Thermo Fisher Scientific recommends gel loading tips with a fine, 350 µm OD tip for this process (Eppendorf/Brinkman Catalog number 22 35 465-6).

To tune the mass spectrometer with the NSI source, use a tuning solution that has a sample concentration of 1 to 5 pmol/µL. For instructions on preparing an appropriate tuning solution for your NSI/MS system, refer to the *Getting Started Guide* or Appendix C, "Tuning Solutions for the NSI Mode."

IMPORTANT High concentrations of the tuning solution can contaminate the system. Tuning solutions prepared for a conventional ESI source must be diluted 100-fold before being used with the NSI source.

Note For best results, centrifuge samples before loading them into the emitter to remove any particulates from the supernatant.

❖ To load sample into the static emitter

1. Insert the pipette tip into the flat end of the static NSI emitter. Push the pipette tip into the emitter until you feel resistance.
2. To avoid forming air bubbles, slowly deliver 1 to 5 µL of liquid as you gradually remove the pipette tip from the emitter.

Although you cannot push the tip of a gel loading pipette into the tip of an emitter, capillary action will cause the sample to fill the tip of the emitter within a few minutes.

3. Gently shake the emitter to remove any air bubbles that might have formed.

Connecting the Nose Cone Tip Mount Assembly to the Probe Body

The nose cone tip mount assembly consists of the nose cone tip assembly and the nose cone mount assembly. The nose cone tip assembly contains the loaded emitter. The nose cone mount assembly consists of a nut, a locking ring, and an O-ring.

❖ To connect the nose cone tip mount assembly to the probe body

1. Connect the nose cone tip assembly, which is holding the loaded emitter, to the nose cone mount assembly as follows:
   a. Align the flat end of the emitter with the hole in the center of the nose cone mounting nut, and then insert the emitter into the hole.
   b. Screw the locking ring of the nose cone mount assembly onto the nose cone tip assembly until it is finger tight.
2. Holding the locking ring, screw the nose cone tip mount assembly into the body of the probe until you feel resistance. See Figure 35 and Figure 36.

   If you continue tightening the nose cone tip mounting assembly beyond the point of resistance, the nose cone tip will begin to detach from the nose cone mounting nut. If this happens, remove the nose cone tip mounting assembly from the probe and retighten the locking ring. Then, being careful not to overtighten the connection, reconnect the nose cone tip mounting assembly to the probe

Figure 35. Nose cone detached from the body of the static NSI probe

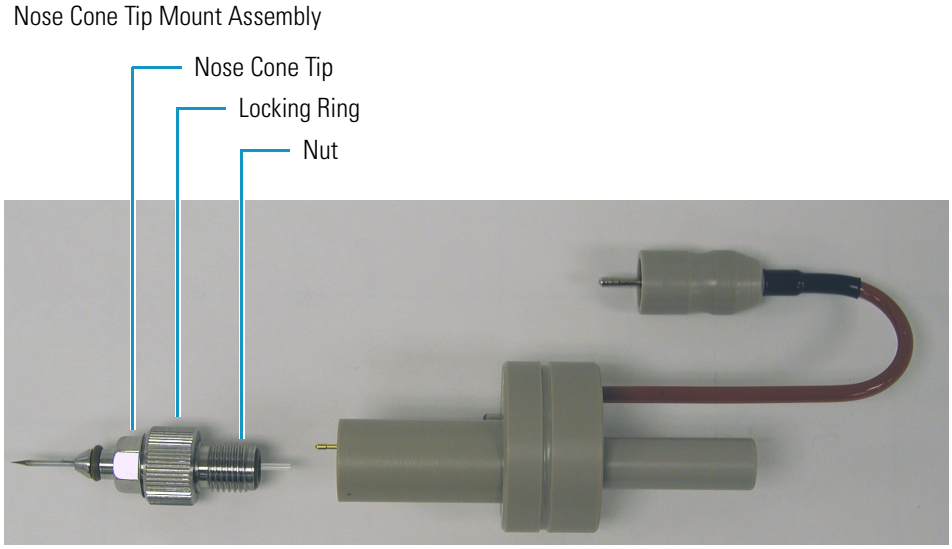
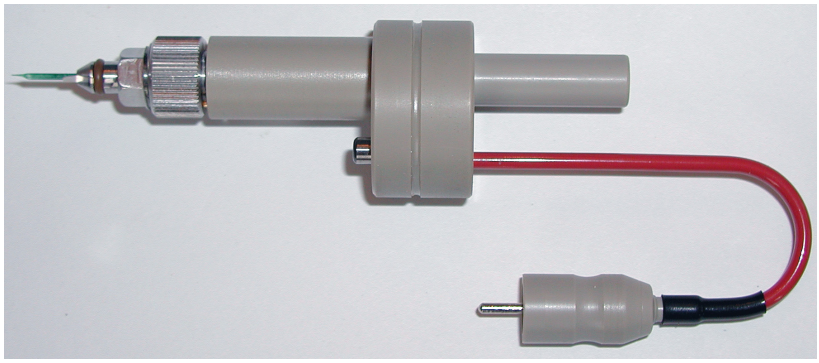


Figure 36. Static NSI probe assembled



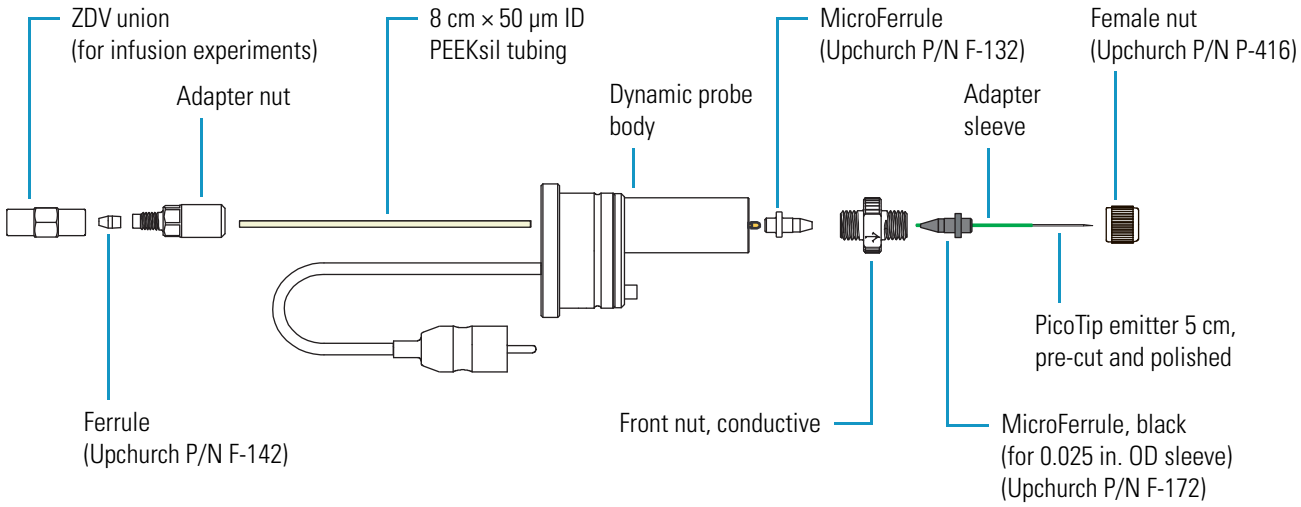
Dynamic NSI Probe

This section contains the following topics:

Dynamic NSI Probe Components

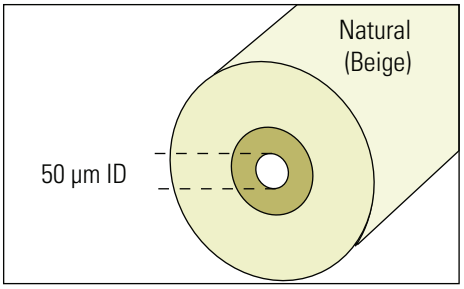
The components of the (discontinued) dynamic NSI probe are shown in Figure 37.

Figure 37. Components of the dynamic NSI probe required to install emitter



IMPORTANT To avoid performance failures, use these recommended parts. Order pre-cut, 8 cm length, 50 µm ID, PEEKsil tubing (natural) from Upchurch Scientific. See Figure 38. Order pre-cut and polished, 5 cm length, PicoTip emitters from New Objective, Inc. The performance of the dynamic nanospray probe depends on the tolerances of these components.

Figure 38. PEEKsil tubing with 50 µm ID



Assembling the Dynamic NSI Probe

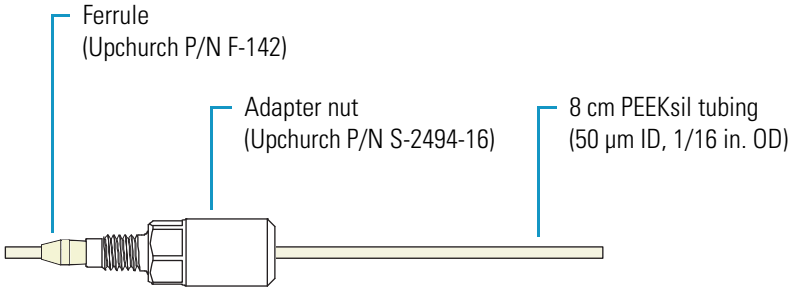
❖ **To assemble the (discontinued) dynamic NSI probe**

1. Connect a capillary column or a ZDV union to the PEEKsil tubing:

   a. Slide the adapter nut and a ferrule onto the precut, 8 cm length of 50 µm ID PEEKsil tubing (Upchurch P/N 6508-1). See Figure 39.

   Note PEEKsil tubing is color coded. Use natural (beige), 50 µm ID PEEKsil tubing with the dynamic nanospray probe. See Figure 38.

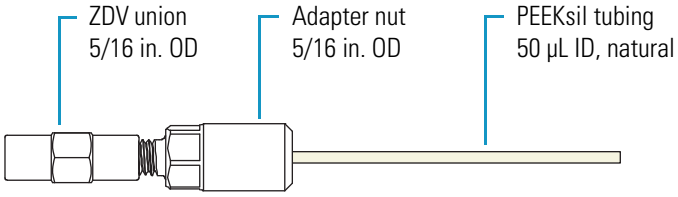
   Figure 39. PEEKsil tubing with adapter nut and ferrule

   b. Depending on the type of experiment, do one of the following:

   - For an LC/MS experiment, screw the adapter nut into one end of a capillary column. Hold the tubing against the bottom of the receiving port as you screw the adapter nut into the column end finger tight. Using a 5/16-in. open-end wrench and a 1/4-in. open-end wrench, tighten the fittings by an additional 1/4 turn to swage the ferrule onto the PEEKsil tubing.
   - For a tuning experiment, screw the adapter nut into one end of a ZDV union. Hold the tubing against the bottom of the receiving port as you screw the adapter nut into the union finger tight. Using two 5/16-in. open-end wrenches, tighten the fittings by an additional 1/4 turn to swage the ferrule onto the PEEKsil tubing. See Figure 40.

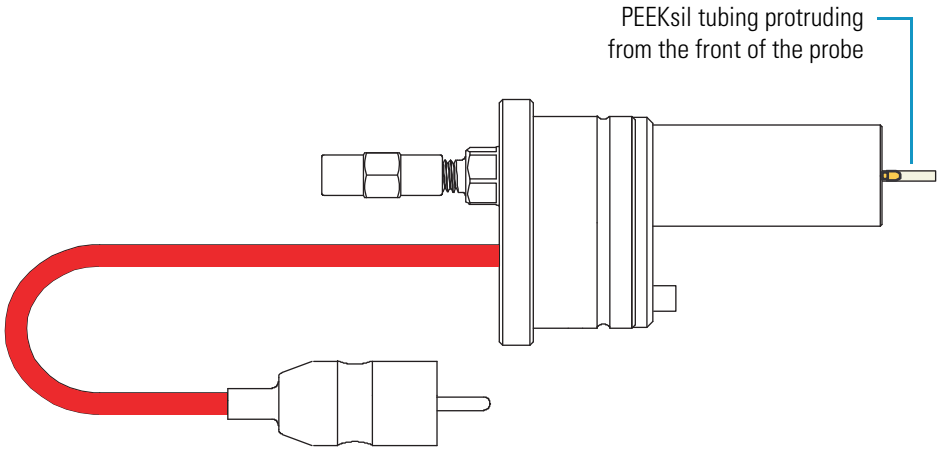
Figure 40. Connecting the ZDV union



2. Connect the PEEKsil tubing to the dynamic nanospray probe:

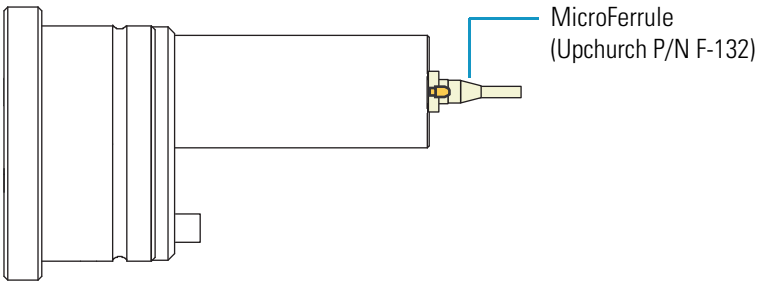
   a. Insert the free end of the PEEKsil tubing through the hole in the back of the dynamic nanospray probe. See Figure 41.

   Figure 41. Inserting the PEEKsil tubing through the back of the probe



   b. Slide the MicroFerrule over the end of the PEEKsil tubing that is protruding from the front of the probe. See Figure 42.

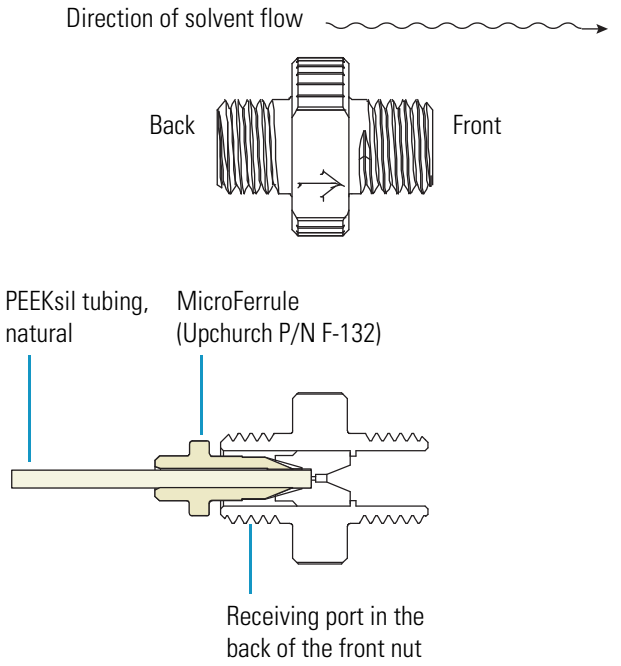
   Figure 42. Slipping the Microferrule onto the PEEKsil tubing



   IMPORTANT The front nut is unidirectional. The arrow etched on the front nut points in the direction of the solvent flow. See Figure 43.

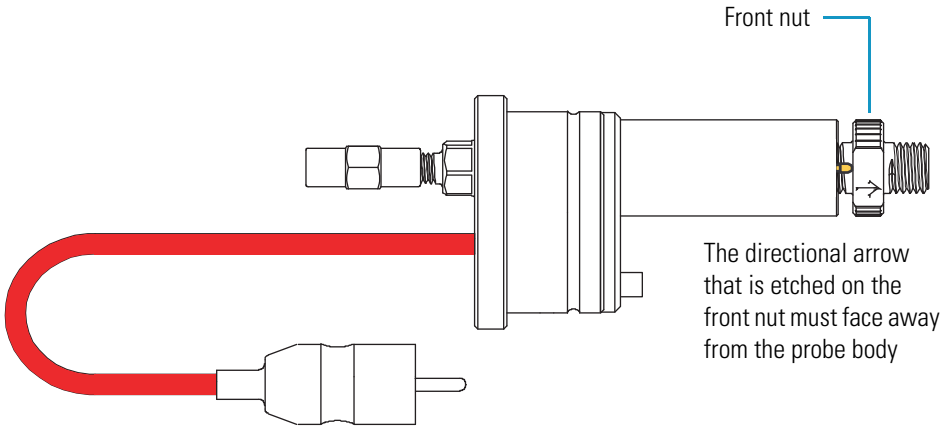
   c. As you gently press the PEEKsil tubing against the receiving port in the back of the front nut, screw the front nut into the probe until finger tight. Then, confirm the tightness of the connection by gently pulling on the capillary column or the ZDV union. See Figure 43 and Figure 44.

Figure 43. Internal view of the front nut, showing the orientation of the front nut and the PEEKsil tubing connection



The directional arrow that is etched on the front nut must face away from the probe body. See Figure 44.

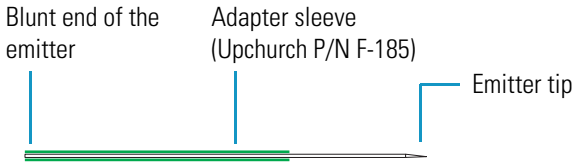
Figure 44. Screwing the front nut onto the front of the probe



3. Connect a PicoTip emitter to the front of the probe as follows:

   a. Taking care to avoid touching the emitter tip, thread the blunt end (distal end) of the PicoTip emitter through the adapter sleeve. Using a magnifier, ensure that the end of the sleeve is flush with the blunt end of the emitter. See Figure 45.

   Figure 45. Sleeved PicoTip emitter

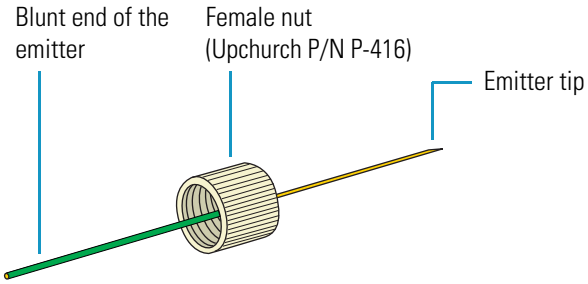


CAUTION Do not touch the emitter tip. It is sharp enough to puncture skin.

IMPORTANT Do **not** touch the emitter tip. Doing so will damage it.

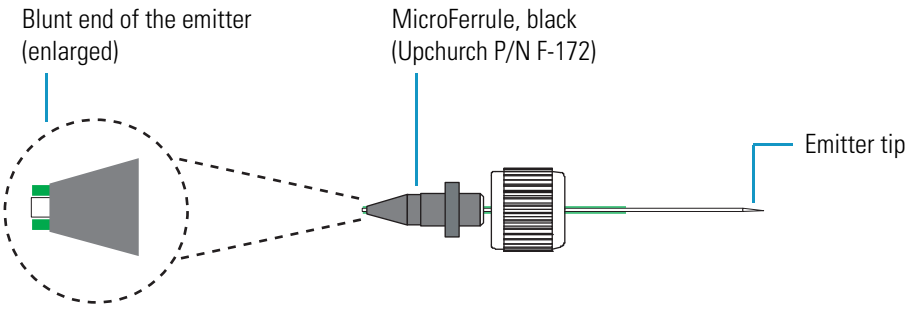
   b. Holding the female nut with its interior threads facing away from the emitter tip, slide the nut over the blunt end of the sleeved emitter. See Figure 46.

   Figure 46. Sliding the female nut over the sleeved emitter



   c. To ensure proper seating, slide the blunt end of the sleeved emitter through the black MicroFerrule (Upchurch P/N F-172) until it protrudes slightly (approximately 1 to 2 mm) from the front of the MicroFerrule. See Figure 47.

   Figure 47. Sliding the Microferrule over the blunt end of the emitter



d. Gently pressing the sleeved emitter against the bottom of the receiving port in the front side of the front nut, screw the female nut onto the front nut until finger tight. Be careful not to overtighten the fitting.

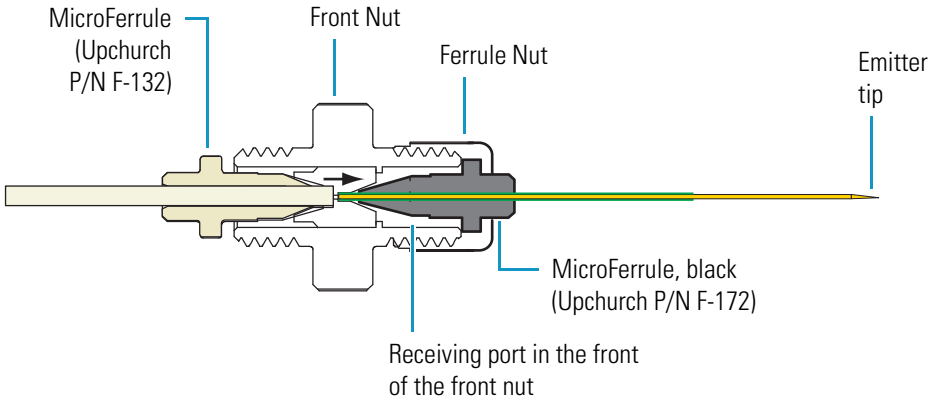
e. Unscrew the female nut and verify the following:

- The black MicroFerrule is swaged onto the sleeved emitter.
- The end of the sleeved emitter protrudes from the tip of the black MicroFerrule.
- The blunt end of the emitter is flush with the sleeve.

IMPORTANT It is extremely important that the PEEKsil tubing and the sleeved emitter are seated properly in the receiving ports of the front nut. See Figure 48.

If these components are not seated properly, you will see band broadening in your chromatograms and a loss in sensitivity.

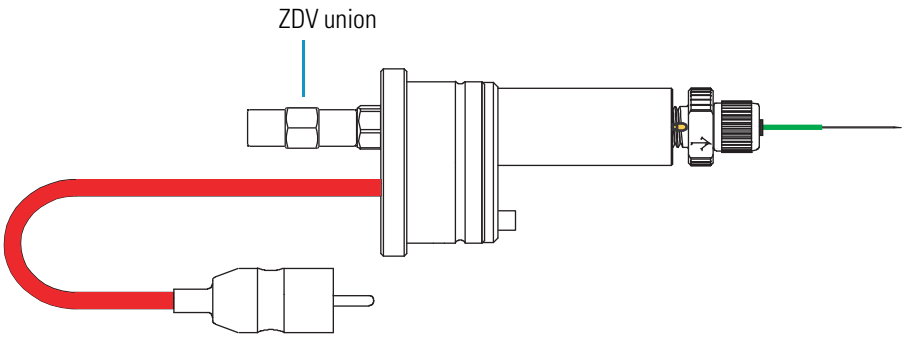
Figure 48. Front nut, showing the PEEKsil and the emitter connections



f. Reconnect the emitter to the probe. Pressing the sleeved emitter against the receiving port in the front of the front nut, screw the female nut onto the front nut.

Figure 49 shows the dynamic nanospray probe assembled for a tuning experiment.

Figure 49. Dynamic nanospray probe, assembled for a tuning experiment



Packed-Tip NSI Probe

This section describes the components of the packed-tip NSI probe and how to replace the PicoFrit column. Occasionally, the PEEKsil tubing portion of the liquid junction becomes clogged. For instructions on replacing the PEEKsil tubing, refer to "Replacing the PEEKsil Tubing of the Packed-Tip NSI Probe" on page 115.

This section contains the following topics:

Packed-Tip NSI Probe Components

The components of the packed-tip NSI probe are shown in Figure 50 and Figure 51.

Figure 50. Components of the packed-tip NSI probe

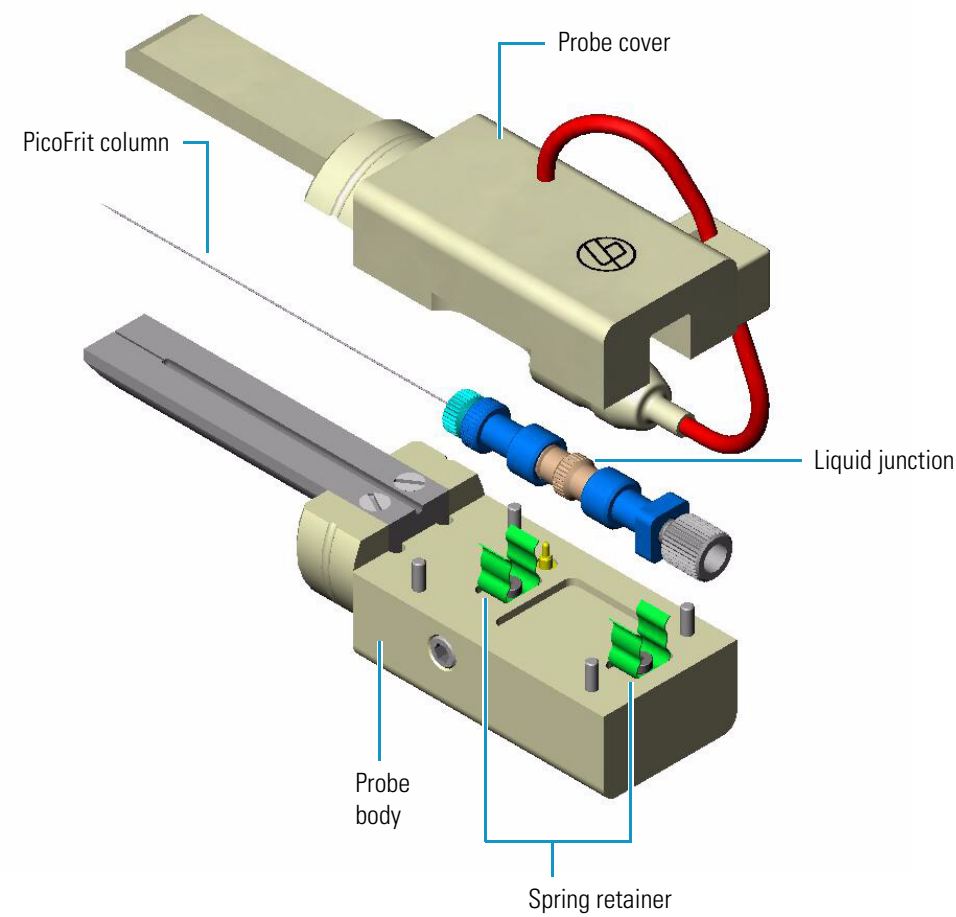
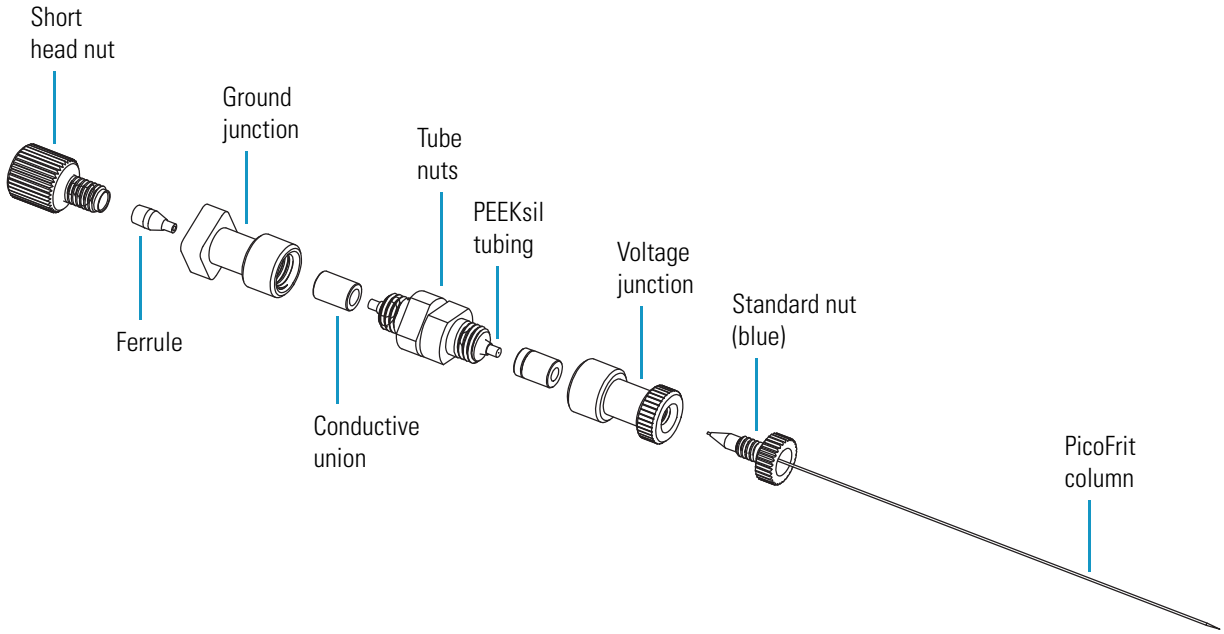


Figure 51. Liquid junction of the packed-tip NSI probe

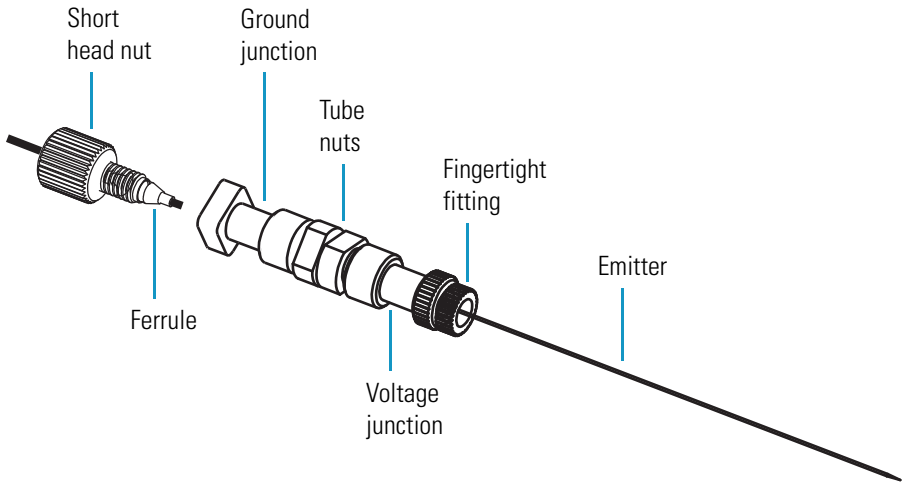


Replacing the PicoFrit Column

❖ **To replace the PicoFrit column of the packed-tip NSI probe**

1. Put your LC/NSI/MS system in the Standby mode and ensure that the solvent flow from the LC pump is Off.
2. Loosen, and then remove the short head nut on the back of the probe to disconnect the plumbing between the packed-tip NSI probe and the LC system or the syringe pump.
3. Detach the NSI source from the mass spectrometer.
4. Detach the packed-tip probe from the NSI source.
5. Pull the probe cover off the probe body. See Figure 50 on page 54.
6. Pull the liquid junction out of the spring retainer.
7. Unscrew the fingertight fitting that connects the PicoFrit column to the liquid junction. Then, dispose of the used PicoFrit column. See Figure 52.

Figure 52. Liquid junction of packed-tip NSI probe



8. Using the fingertight fitting, connect a new emitter to the voltage junction of the liquid junction.
9. Reinsert the liquid junction into the spring retainer.
10. Reattach the probe cover to the probe body.

Figure 53 shows the assembled packed-tip NSI probe.

Figure 53. Packed-tip NSI probe assembled

Installing the NSI Source

The NSI source includes the NSI flange, the NSI base mount, and the NSI probe. Thermo Fisher Scientific offers three types of interchangeable NSI probes: the static NSI probe, the dynamic NSI probe, and the packed-tip NSI probe. For instructions on assembling your NSI probe, refer to Chapter 5.

CAUTION Do not modify the protective glass shield or any other part of the NSI source. By making modifications, you could potentially expose yourself to dangerously high voltages!

Contents

To install your new NSI source, follow the procedures that apply to your nanospray system:

1. Assembling the NSI Source
2. Connecting the NSI Source to the Extension Assembly
3. Aligning the Emitter Tip Position
4. Completing the Plumbing Connections

Assembling the NSI Source

The NSI source includes an NSI flange, an NSI base mount, and an NSI probe.

Note If you are using the previous version of the NSI flange, use a Phillips head screwdriver to remove its ZDV union.

To assemble your NSI Source, follow these procedures:

1. Attaching the NSI Base Mount to the NSI Flange
2. Inserting the NSI Probe into the NSI Base Mount and NSI Flange Assembly

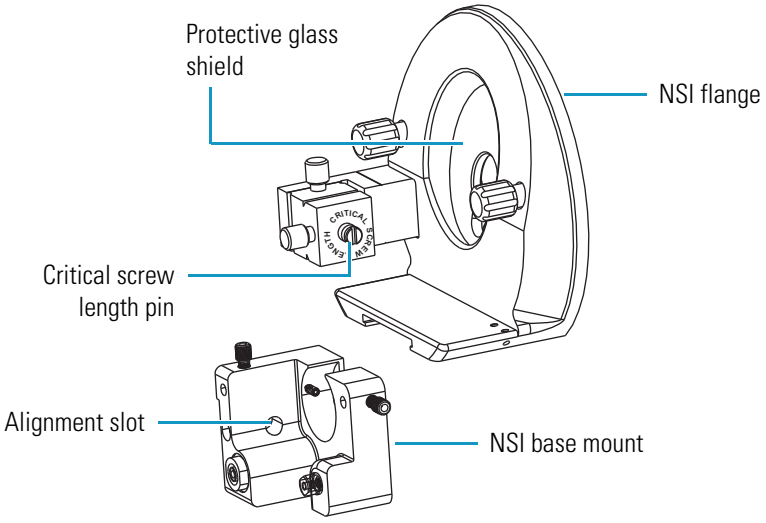
Attaching the NSI Base Mount to the NSI Flange

When you initially install your NSI source, you need to attach the NSI base mount to the NSI flange.

❖ **To attach the NSI base mount to the NSI flange**

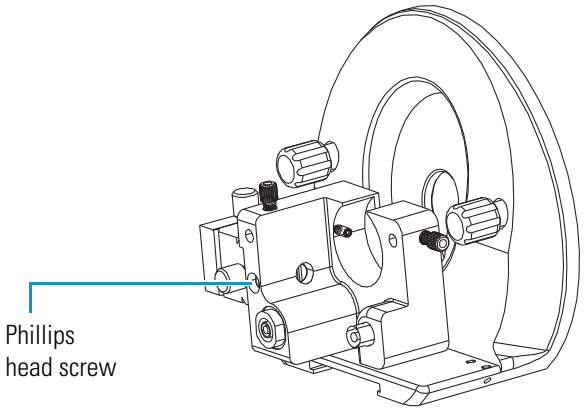
1. Slide the alignment slot in the NSI base mount over the critical screw length pin on the NSI flange. See Figure 54.

   Figure 54. Critical screw length pin and alignment slot in the NSI base mount



2. Using a #1 Phillips head screwdriver, tighten the screw that secures the NSI base mount to the NSI flange.

Figure 55. View of Phillips head screw



Inserting the NSI Probe into the NSI Base Mount and NSI Flange Assembly

❖ **To install the NSI probe into the NSI base mount**

1. Slide the NSI probe into the NSI base mount until it snaps into place.
2. For capillary columns that need additional support, connect the column support assembly to the NSI base mount:

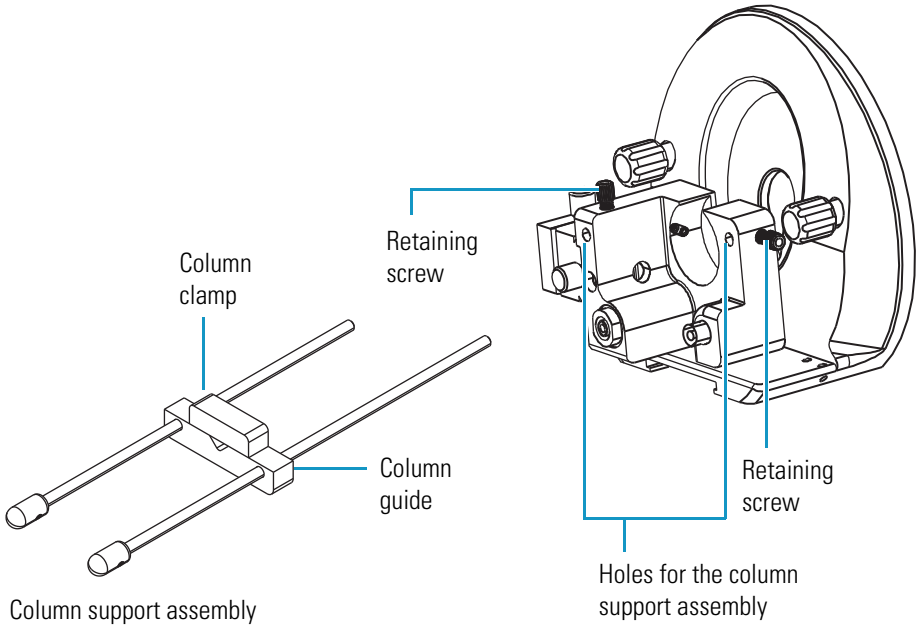
   Note The column support assembly is shipped in pieces. The assembly consists of two rods, a column guide, a column clamp, and two retaining screws. An additional column guide and column clamp are provided.

   a. Assemble the column support assembly if you have not already done so by slipping the rods through the holes in the column guide. Do not connect the column clamp to the column guide.

      You connect the column clamp to the column guide after you align the capillary column with the notch in the center of the column guide.

   b. Loosen the two column support retaining screws on the NSI base mount. See Figure 56.

Figure 56. Holes for column support assembly in the NSI base mount



c. Insert the rods of the column support assembly into the holes in the NSI base mount as far as they will go. Then, tighten the retaining screws.

d. Ensure that the capillary column is supported by the notch in the center of the sliding column guide. Then, connect the column clamp to the column guide.

3. Connect the high voltage cable of the NSI probe to the high voltage port on the NSI base mount by inserting the pin at the end of the high voltage cable into the high voltage port on the NSI base mount. Then, slip the high voltage shield that surrounds the pin over the port.

Figure 57 shows the dynamic NSI probe, Figure 58 shows the packed-tip NSI probe, and Figure 59 shows the static NSI probe.

Figure 57. NSI source with dynamic NSI probe

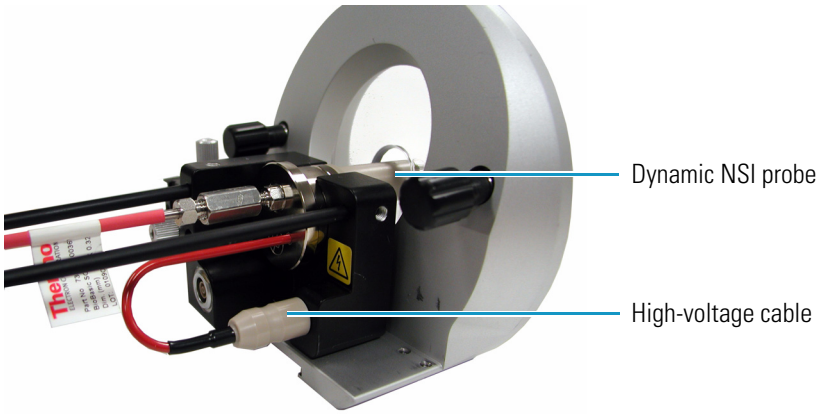


Figure 58. NSI source with packed-tip NSI probe

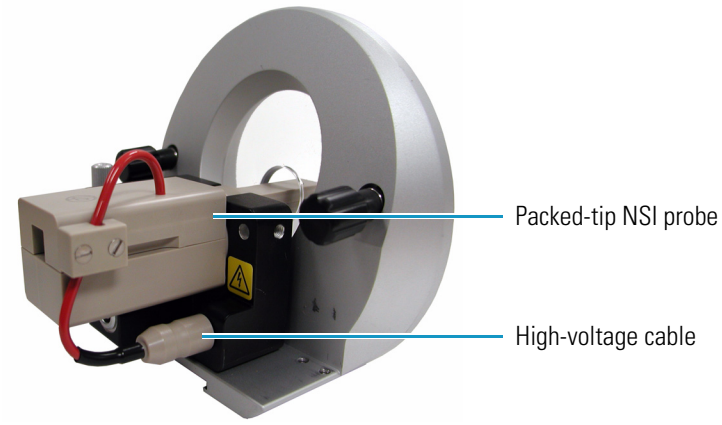
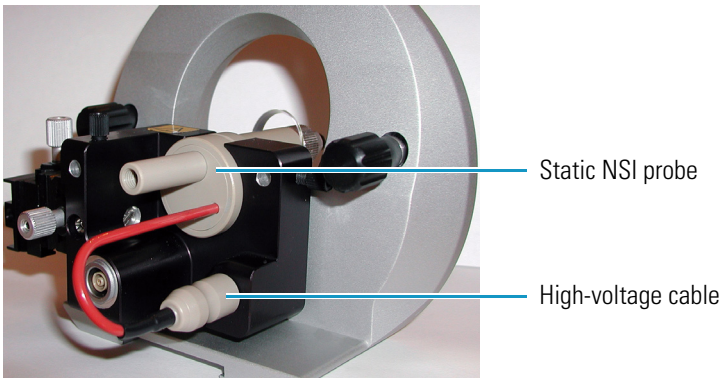


Figure 59. NSI source with static NSI probe

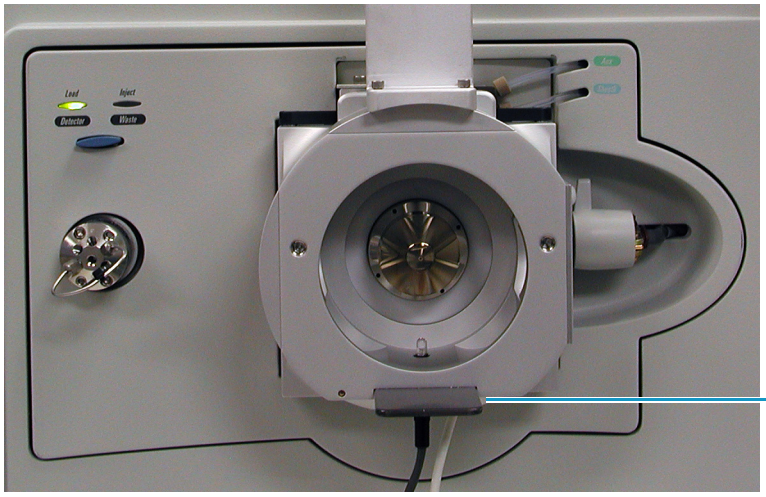


Connecting the NSI Source to the Extension Assembly

❖ **To connect the NSI source to the extension assembly**

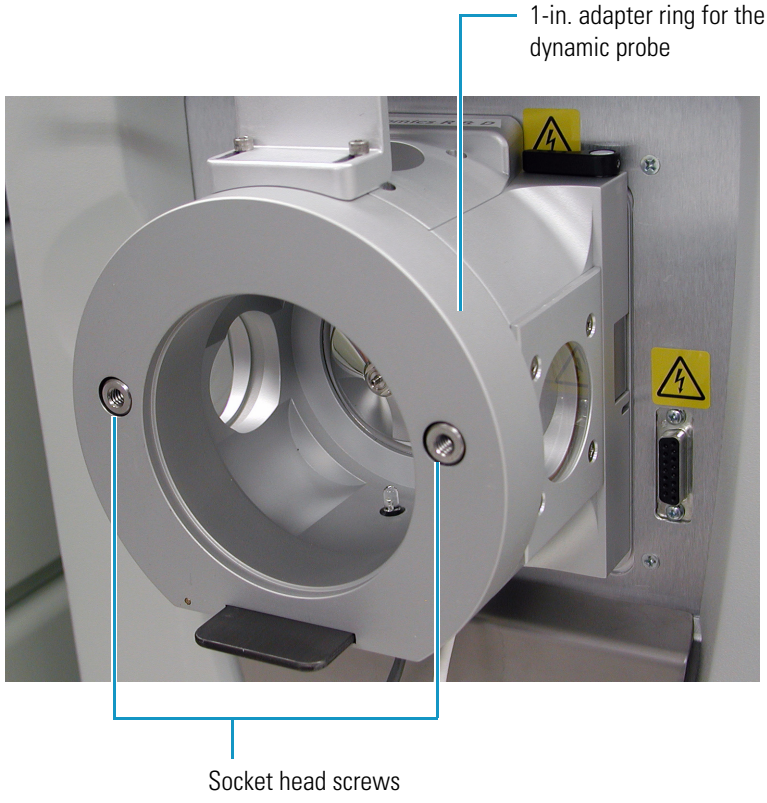
1. If the NSI source contains either a dynamic NSI probe or a packed-tip NSI probe, install the 1-in. deep adapter ring if it is not already installed as follows:
   a. Align the alignment slot on the 1 in. deep adapter ring with the alignment tab on the NSI extension assembly (see Figure 60). Then, slide the 1-in. deep adapter ring onto the extension assembly.

Figure 60. Extension assembly mounted on the mass spectrometer (front view)



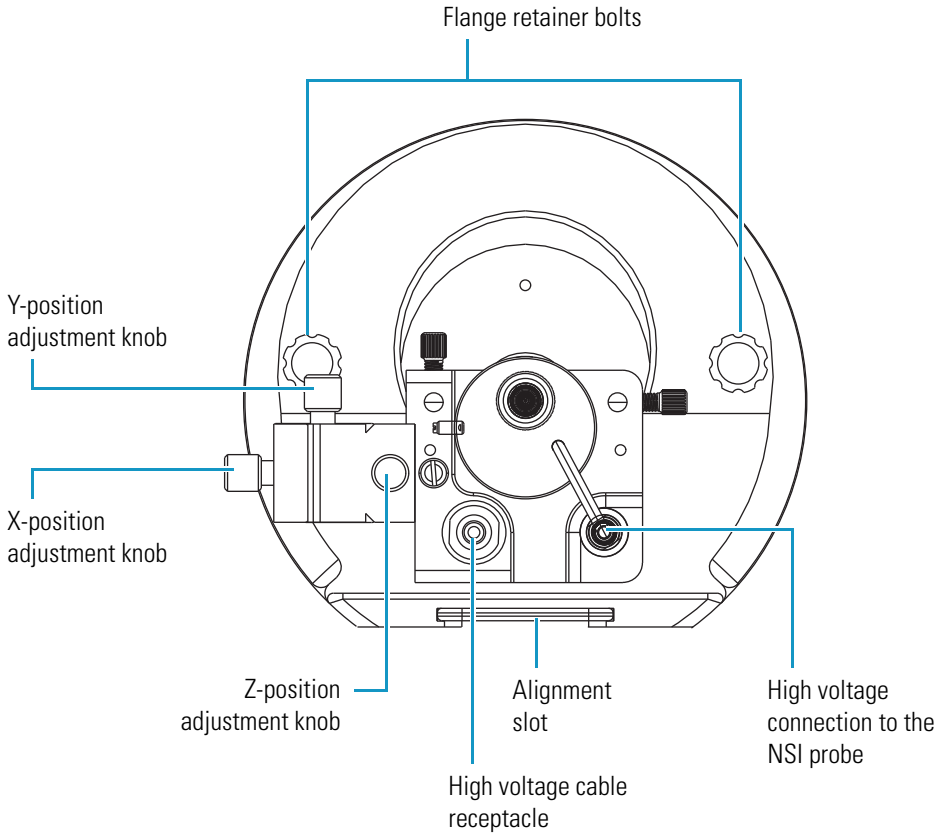
b. Secure the 1 in. deep adapter ring to the extension assembly by tightening the socket head screws. See Figure 61.

Figure 61. LTQ mass spectrometer shown with extension assembly and 1 in. deep adapter ring



2. Ensure that the XYZ stage of the NSI source is fully retracted. If necessary, turn the Z position adjustment knob (see Figure 62) counterclockwise to retract the stage.

Figure 62. NSI source with dynamic NSI probe

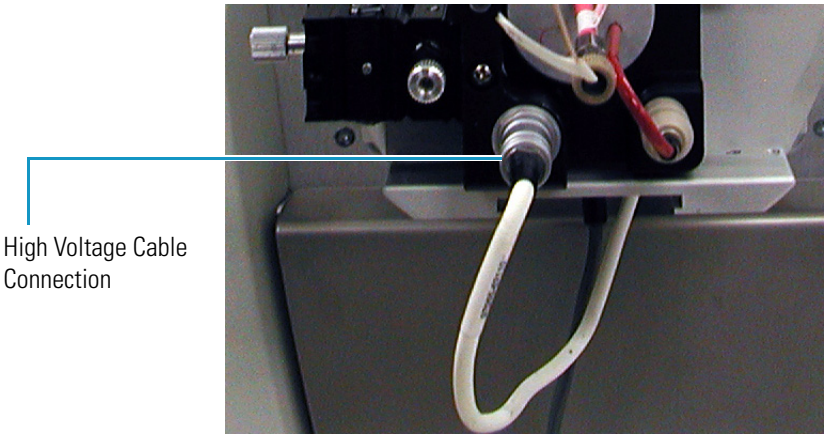


3. Align the NSI source alignment slot with the alignment tab on the NSI extension assembly. Then, slide the NSI source onto the extension assembly.
4. Tighten the two flange retainer bolts to secure the NSI source to the NSI extension assembly.

CAUTION AVOID ELECTRICAL SHOCK. Make certain that all electrical voltages are at ground potential before handling the high voltage cable.

5. Connect the high voltage cable from the Ion MAX adapter to the high voltage cable connector on the NSI base mount. Turn the locking ring on the cable clockwise to secure the connection. See Figure 63.

Figure 63. High voltage connection between Ion MAX adapter and the NSI base mount



Aligning the Emitter Tip Position

After you install the probe, align the position of the emitter tip with the center of the ion transfer tube.

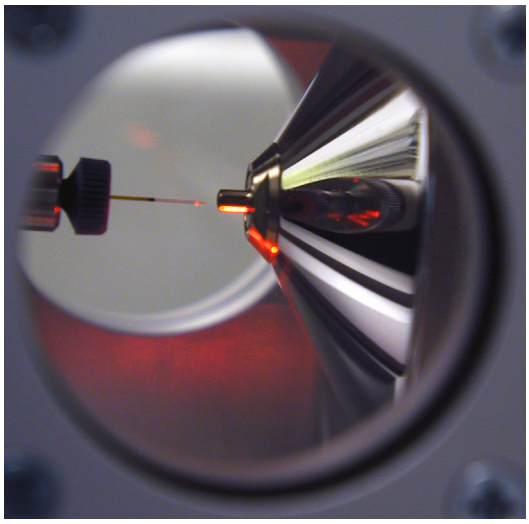
❖ **To align the position of the emitter tip**

1. Use the X and Y adjustment knobs on the XYZ stage to align the emitter tip with the center of the ion transfer tube. See Figure 62 on page 63.

   Spray quality can be affected if the emitter is more than 1 mm (within 1 mm tolerance) from the center of the ion transfer tube.

2. Use the Z adjustment knob to position the emitter approximately 2 mm away from the ion transfer tube. See Figure 62 on page 63 and Figure 64.

   Figure 64. View through the window of the Ion MAX adapter, showing the Z-axis distance between the emitter and the ion transfer tube



Completing the Plumbing Connections

Refer to the following sections of this manual for instructions on connecting the NSI probe to syringe pump on the front of your mass spectrometer or to your LC system:

- If you are preparing to tune your NSI/MS system or operate the static NSI probe, refer to Chapter 7.
- If you are preparing to perform an LC/NSI/MS experiment, refer to "Configuring the System for Microscale LC/MS Experiments" on page 24.

Setting Up for Tuning the Mass Spectrometer

Tune your NSI/MS system to maximize its ion signal intensity. Because tuning optimizes ion transport, which is affected by the ion source conditions, it is important to tune the mass spectrometer with the NSI source connected. You can tune your mass spectrometer with any of the three interchangeable NSI probes.

If you are using the static NSI probe, the setup for tuning and general operation are the same. In both cases, you first fill the static emitter with solution. You then reassemble the NSI probe, reattach the NSI source to the mass spectrometer, and reconnect the gas inlet of the NSI probe to the syringe pump. Before you begin your sample analysis or the automated tuning procedure, you optimize the position of the emitter tip.

If you want to tune your NSI/MS system with either the dynamic NSI probe or the packed-tip NSI probe, you need to remove the capillary column or the packed-tip emitter from the probe, and then attach the probe to the syringe pump.

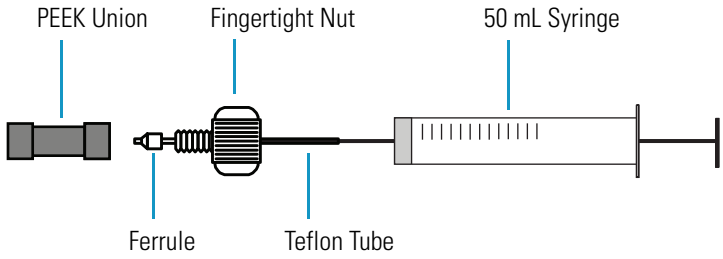
After you set up your NSI/MS for tuning as described in this chapter, proceed to the tuning chapter in this manual that applies to your mass spectrometer.

Contents

Creating a Syringe Adapter

The syringe adapter consists of a piece of 0.007 in. ID Teflon™ tubing and a fingertight fitting (see Figure 65).

Figure 65. Syringe assembly



❖ To create a syringe adapter

1. Cut a 4 to 5 cm length of 0.007 in. ID Teflon tubing.
2. Insert the syringe needle into the Teflon tubing until the end of the needle is flush with the end of the tubing. The needle serves to prevent the tubing from collapsing when you swage on the ferrule.
3. Slip a fingertight nut and ferrule onto the Teflon tubing. Insert the tubing and fittings into a PEEK union. Then, tighten the nut finger tight to swage on the ferrule.
4. Remove the needle from the tubing. Unscrew the nut and verify that the ferrule is swaged onto the tubing. Then, reconnect the syringe adapter to the PEEK union.

Connecting the Static NSI Probe to the Syringe Pump

The syringe pump provides backpressure to the static NSI probe.

❖ To connect the static NSI probe to the syringe pump

1. Prepare a syringe adapter as described in the previous section, "Creating a Syringe Adapter."
2. Connect the syringe adapter to a PEEK union.
3. Cut a 40 to 50 cm length of 0.030 in. ID Teflon tubing.
4. Use a fingertight nut and ferrule to connect one end of the 0.030 in. ID Teflon tubing to the PEEK union.
5. Use a flangeless nut and a flangeless ferrule to connect the other end of the 0.030 in. ID Teflon tubing (see Figure 66) to the gas inlet (see Figure 67)of the static NSI probe.

Figure 66. Syringe line

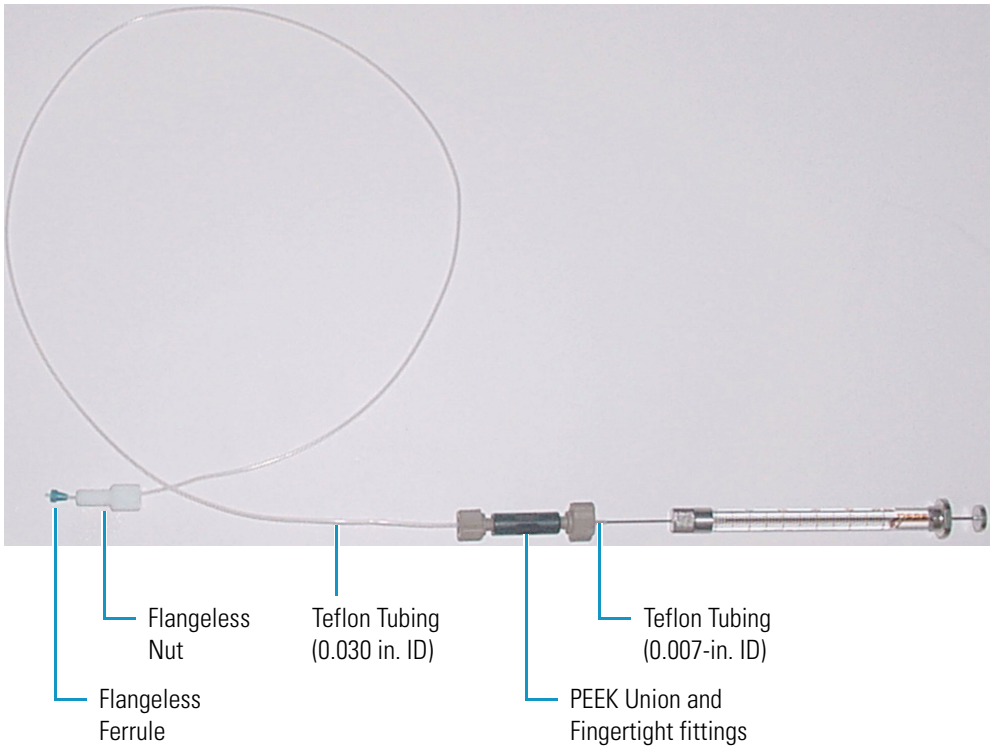
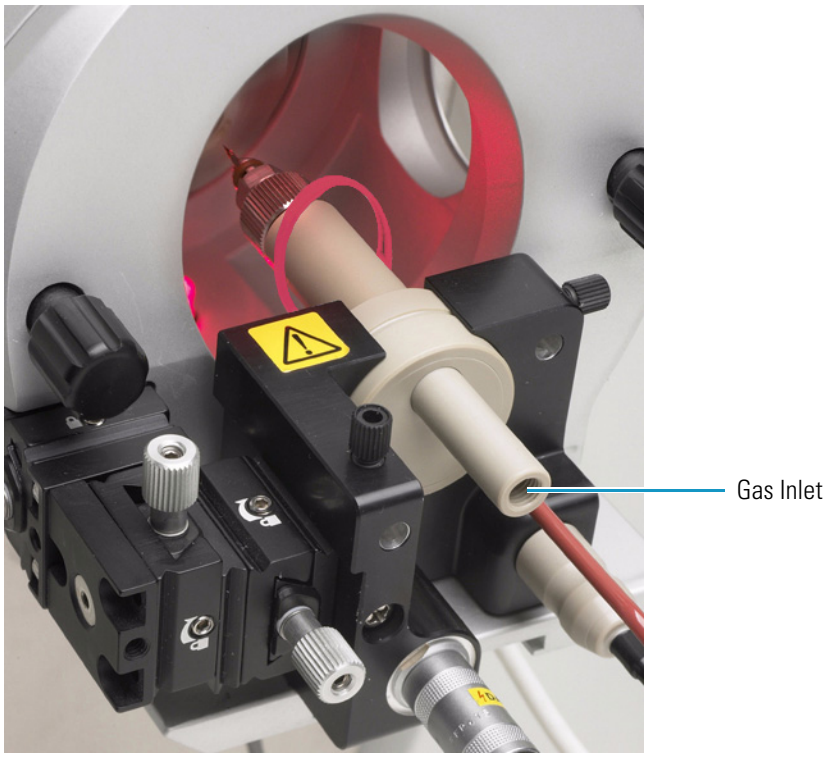


Figure 67. View of the static NSI probe, showing the gas inlet



6. Slip the syringe needle into the free end of the syringe adapter. Then, insert the empty syringe into the syringe pump on the front of your mass spectrometer as follows:
   - For an LCQ Deca, LCQ Duo, or LCQ Advantage mass spectrometer, insert the syringe into the syringe holder of the syringe pump. Press the black release button on the syringe pump handle. Then, move the handle forward until it just contacts the syringe plunger.
   - For an LCQ Deca XP Plus or LTQ Series mass spectrometer, insert the syringe into the syringe holder of the syringe pump. Squeeze the blue release levers on the pusher block. Then, slide the pusher block forward until it just contacts the syringe plunger.
   - For a TSQ Quantum Series mass spectrometer, insert the syringe into the syringe holder of the syringe pump. Press the black release button on the syringe pump handle. Then, lower the handle until it just contacts the syringe plunger.

Connecting the Dynamic NSI Probe to the Syringe Pump

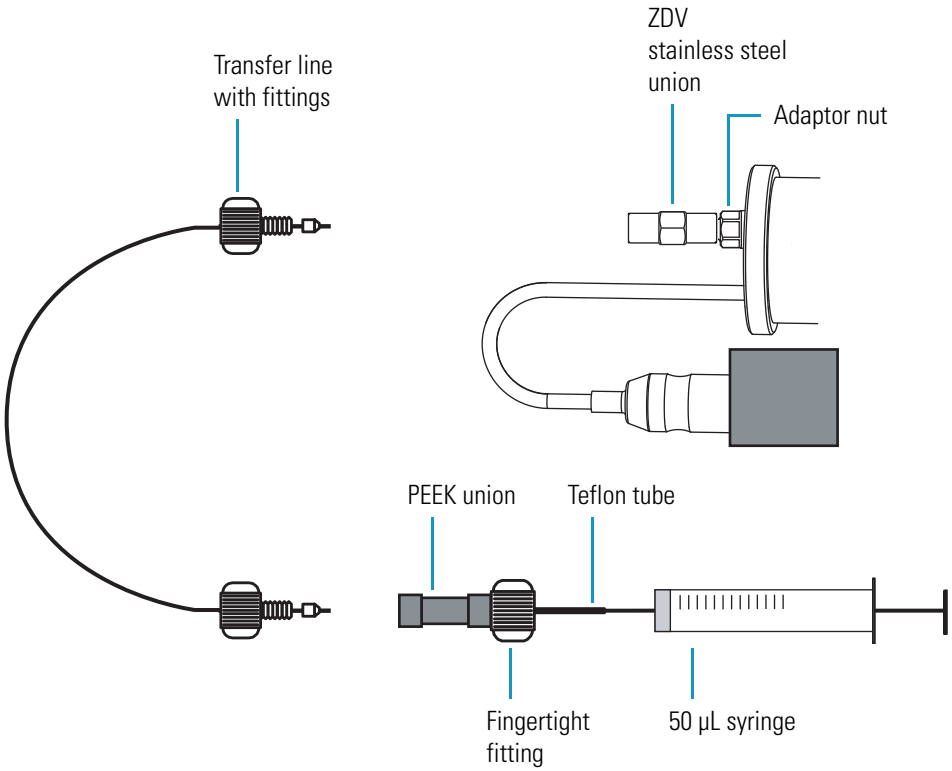
To tune your NSI/MS system using the dynamic NSI probe, you must first assemble the dynamic NSI probe with a tuning adapter instead of a column adapter, and then connect the tuning adapter to the mass spectrometer's syringe pump.

❖ To prepare an NSI/MS system with a dynamic NSI probe for tuning

1. Assemble the dynamic NSI probe with a tuning adapter as described in "Assembling the Dynamic NSI Probe" on page 49.
2. Insert the dynamic NSI probe into the base mount of the NSI source, and then reconnect the NSI source to the mass spectrometer.
3. Create a syringe adapter as described in "Creating a Syringe Adapter" on page 68.
4. Fill a 50 µL syringe with an appropriate tuning solution. For instructions on preparing an appropriate tuning solution, see Appendix C, "Tuning Solutions for the NSI Mode."
5. Slip the syringe needle into the free end of the syringe adapter, and then insert the empty syringe into the syringe pump on the front of your mass spectrometer as follows:
   - For an LCQ Deca, LCQ Duo, or LCQ Advantage mass spectrometer, insert the syringe into the syringe holder of the syringe pump. Press the black release button on the syringe pump handle. Then move the handle forward until it just contacts the syringe plunger.
   - For an LCQ Deca XP Plus or LTQ Series mass spectrometer, insert the syringe into the syringe holder of the syringe pump. Squeeze the blue release levers on the pusher block. Then slide the pusher block forward until it just contacts the syringe plunger.
   - For a TSQ Quantum Series mass spectrometer, insert the syringe into the syringe holder of the syringe pump. Press the black release button on the syringe pump handle. Then lower the handle until it just contacts the syringe plunger.

6. Using the appropriate fittings, connect a transfer line between the ZDV stainless steel union that is connected to back end of the dynamic NSI probe and the PEEK union that is connected to the syringe adapter. See Figure 68.

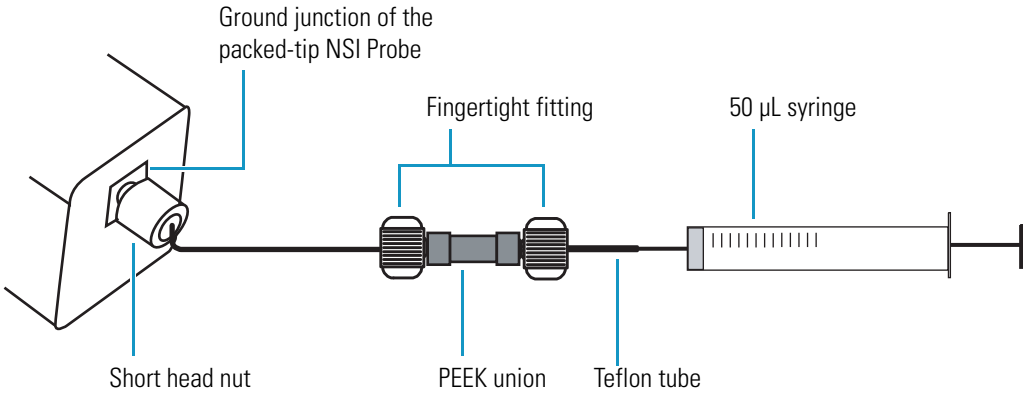
Figure 68. Connecting the dynamic NSI probe to the syringe pump



Connecting the Packed-Tip Probe to the Syringe Pump

To tune your NSI/MS system using the packed-tip NSI probe, you must replace the PicoFrit column with a TaperTip™ emitter. Then, using the appropriate fittings and tubing, connect the ground junction on the back of the probe to the syringe pump on the front of the mass spectrometer. See Figure 69.

Figure 69. Connection between the syringe pump and the packed-tip NSI probe



❖ To prepare an NSI/MS system with a dynamic NSI probe for tuning

1. Remove the cover from the body of the packed-tip NSI probe.
2. Pull the liquid junction out of the spring retainer.
3. Unscrew the packed-tip emitter from the Voltage Junction of the packed-tip NSI probe and replace it with a TaperTip™ emitter.

   Note You can order a TaperTip emitter from New Objective, Inc.

4. Insert the packed-tip NSI probe into the base mount of the NSI source. Then, reconnect the NSI source to the mass spectrometer.
5. Prepare a syringe adapter as described in "Creating a Syringe Adapter" on page 68.
6. Fill a 50 µL syringe with an appropriate tuning solution. For instructions on preparing an appropriate tuning solution, refer to Appendix C, "Tuning Solutions for the NSI Mode."
7. Slide the syringe needle into the free end of the syringe adapter. Then, insert the empty syringe into the syringe pump on the front of your mass spectrometer as follows:
   - For an LCQ Deca, LCQ Duo, or LCQ Advantage mass spectrometer, insert the syringe into the syringe holder of the syringe pump. Press the black release button on the syringe pump handle. Then, move the handle forward until it just contacts the syringe plunger.
   - For an LCQ Deca XP Plus or LTQ Series mass spectrometer, insert the syringe into the syringe holder of the syringe pump. Squeeze the blue release levers on the pusher block. Then, slide the pusher block forward until it just contacts the syringe plunger.

- For a TSQ Quantum Series mass spectrometer, insert the syringe into the syringe holder of the syringe pump. Press the black release button on the syringe pump handle. Then, lower the handle until it just contacts the syringe plunger.

8. Using the Short Head Nut and a ferrule, connect one end of a transfer line to the Ground Junction on the back of the packed-tip NSI probe. Using the appropriate fittings, connect the other end of the transfer line to the PEEK union that is connected to the syringe adapter.

Tuning an LCQ Series Mass Spectrometer

The Tune Plus application program is used to determine and optimize the instrument control parameters for an LCQ Series mass spectrometer.

The procedures in this chapter assume that you are familiar with your mass spectrometer and Xcalibur software. For additional information, refer to the data system Help or the Getting Connected Guide and Hardware Manual for your mass spectrometer.

Tip You can tune your system in either the static or dynamic nanospray mode prior to performing dynamic nanospray experiments. Tuning your NSI/MS system with the static NSI probe allows you to avoid contaminating the liquid junction of the dynamic NSI probe with the tuning solution.

Contents

Tuning an LCQ Series Mass Spectrometer in the Static NSI Mode

The procedures in this section apply to the static NSI mode and assume that you have already installed the static NSI probe and connected its gas inlet to the syringe pump of your LCQ Series mass spectrometer. For instructions on installing the static NSI source, refer to Chapter 6. For instructions on assembling the static NSI probe, refer to "Assembling the Static NSI Probe" on page 44.

To tune your LCQ Series mass spectrometer in the static NSI mode, follow these procedures:

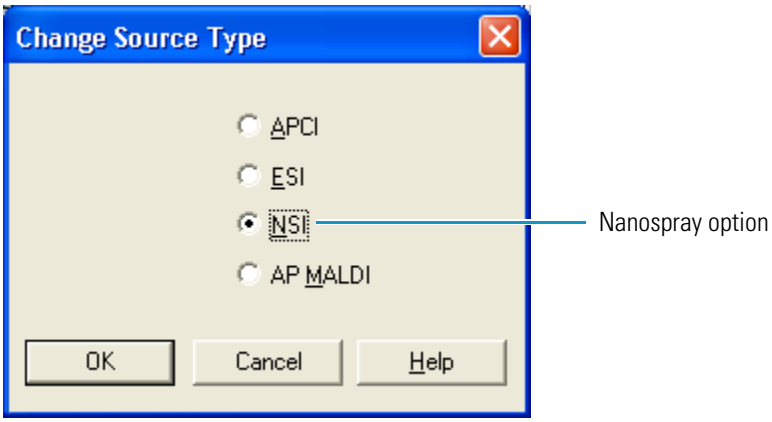
1. Setting Up the LCQ Series Mass Spectrometer for the Static NSI/MS Mode
2. Initiating Flow for Static Nanospray
3. Determining the Threshold and Operating Spray Voltage for the Static Nanospray Mode
4. Optimizing the Emitter Position for Static Nanospray
5. Automatically Optimizing the Voltages for Ion Detection in the Static NSI Mode

Setting Up the LCQ Series Mass Spectrometer for the Static NSI/MS Mode

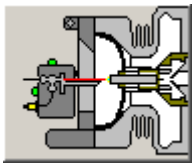
❖ **To set up your LCQ Series mass spectrometer for the static NSI/MS mode from the Tune Plus application program**

1. From the computer desktop, click the Tune icon to open the Tune Plus window.
2. On the Control / Scan Mode toolbar, which is the second toolbar from the top in the Tune Plus window, click the On/Standby button to turn on your LCQ Series mass spectrometer.
3. If it is not already selected, select NSI as the API source as follows:
   a. From the Tune Plus menu, choose **Setup > Change API Source Type** to open the Change Source Type dialog box shown in Figure 70.

   Figure 70. Change Source Type dialog box

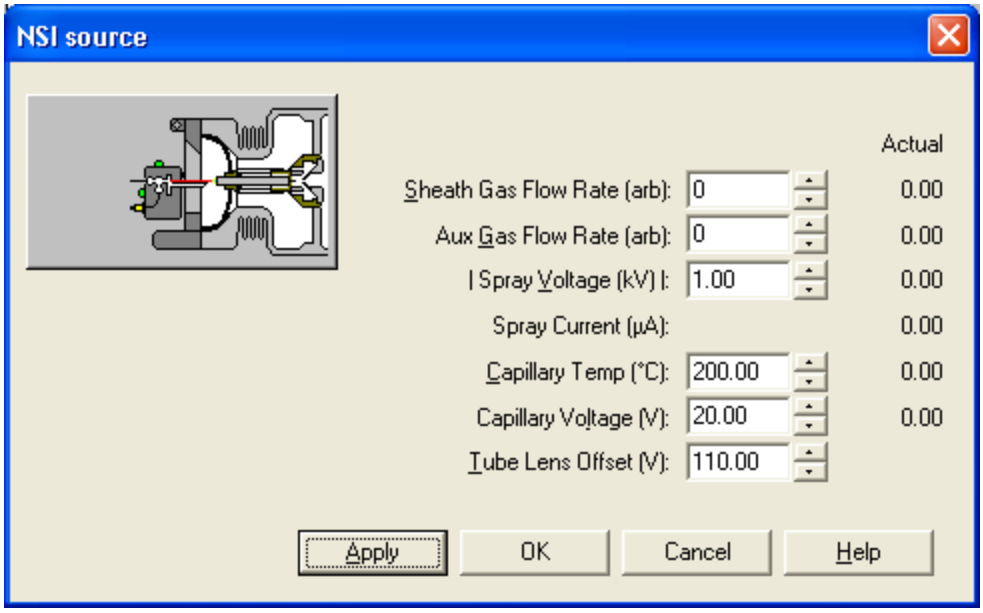


   b. Select the NSI option button.
   c. Click **OK** to close the Change Source Type dialog box.



4. From the Instrument Setup toolbar, click the API Source button to open the NSI Source dialog box. See Figure 71.

Figure 71. NSI Source dialog box



5. Ensure that the setting for the spray voltage is at (±)1.0 kV and that the setting for the sheath gas flow rate is at 0. Sheath gas is not used for a static NSI source.

IMPORTANT When switching to nanospray mode or when switching the ion polarity while operating in nanospray mode (for example, from positive ion mode to negative ion mode), an electrical arc might be created between the tip of the nanospray needle and the ion sweep cone if the initial spray voltage is set too high. To prevent arcing, increase the distance between the tip of the needle and the ion sweep cone or turn off the spray voltage and unplug the high voltage cable.

Initiating Flow for Static Nanospray

Thermo Fisher Scientific recommends these settings for initiating static nanospray flow are as follows:

- Distance from the spray tip to the ion transfer capillary: ~2 mm
- Spray voltage: (±)1.0 kV

❖ To initiate the flow for static nanospray

1. Using the imaging system or by visual inspection, ensure that the tip is approximately 2 mm away from the ion transfer tube.
2. If the NSI Source dialog box is not already open, open it.
3. Verify that the spray voltage is set to (±)1.0 kV.
4. Click **Apply** to download the parameters.

5. If you are using the syringe pump to provide backpressure, turn it on from the Syringe Pump dialog box. Allow the pump to run for approximately 5 seconds, then stop the pump and keep the syringe in place. For instructions on connecting a syringe line to the static NSI probe, refer to "Connecting the Static NSI Probe to the Syringe Pump" on page 68.

   Note Use the **minimum** required syringe pump setting to initiate static nanospray.

6. Set the scan parameters for tuning as follows:

   a. Click the Define Scan button to open the Define Scan dialog box.
   b. In the Scan Description group box, select the Full option button for the Scan Type.
   c. In the Scan Ranges Group box, set the scan range to begin at 400 m/z and end at 2000 *m/z*.
   d. In the Scan Time group box, make the following entries:
      i. In the Number of Microscans combo box, type **3** to set the total number of microscans to 3.
      ii. In the Maximum Inject Time combo box, type **200** to set the maximum injection time to 200 ms.
   e. Click **Apply** to download the parameters.
   f. Click **OK** to close the Define Scan dialog box.

7. Click the Injection Control toolbar button to open the Injection Control dialog box.
8. Verify the parameters are set to the following values. Change the values if needed:
   a. AGC is set to On.
   b. Full Scan MS Target is set to 1e+008.
   c. SIM Scan Mode Target is set to 2e+007.
   d. MSn Scan Mode Target must be set to 4e+007.
   e. Zoom Target must be set to 4e+007.
   f. Inject WaveForm is set to Off.
   g. Click **Apply** and **OK** to accept these values.

9. If necessary, click the Centroid/Profile button to toggle the data type to centroid. (The Centroid state of the button is shown on the left.)

10. Ensure that the polarity is set to the appropriate mode for your tuning compound. If necessary, click the Positive/Negative Polarity button to toggle the ion polarity mode.

Determining the Threshold and Operating Spray Voltage for the Static Nanospray Mode

❖ **To determine the threshold spray voltage and the operational spray voltage for static nanospray**

1. In the Tune Plus window, display the Spectrum view by clicking the Display Spectrum View button on the File/Display tool bar.
2. With the mass spectrometer On, observe the mass spectrum of the test solution in the Spectrum View of the Tune Plus window. If MRFA is used as the tuning solution, the peak of interest will be at *m/z* 524.30.
3. From the NSI Source dialog box, gradually increase (decrease if operating in negative polarity mode) the spray voltage in 50 V (0.05 kV) increments until the signal of the ion at *m/z* 524.30 is observed in full scan MS mode. Record this voltage as the threshold nanospray voltage. Typical values of the voltage for initiating spray vary from (±)1.0 to 2.0 kV.
4. Further increase the spray voltage setting to 100 to 200 V above the threshold voltage to ensure that stable nanospray is obtained. This nanospray voltage typically ranges from (±)1.2 to 2.2 kV.

IMPORTANT Spray voltages above (±)2.5 kV might cause arcing between the emitter tip and the ion transfer tube surface. This might damage the emitter coating.

Optimizing the Emitter Position for Static Nanospray

❖ **To set the emitter position while monitoring the signal intensity of the ion of interest**

1. Initiate the manual tuning process for the mass-to-charge ratio of the ion of interest as follows:
   a. On the Control/Scan Mode tool bar in the Tune window, click the Tune button to display the Tune dialog box.
   b. Click the Manual tab to display the Manual page.
   c. In the Mass spin box, type in the mass-to-charge ratio for MRFA (*m/z* 524.30) or the mass-to-charge ratio of interest for your tuning solution and select the adjacent check box.
   d. Click **Start**. A message box displays the following message: *Ensure that the syringe is full*. Click **OK** to close the message box and return to the Tune dialog box.

2. On the File/Display toolbar, click the Graph View button to monitor the intensity of the ion of interest using the Graph View.
3. Slightly turn the “X” and “Y” adjustment knobs on the XYZ stage to fractionally adjust the tip position for signal optimization.

4. Decrease or stop the syringe pump. The syringe pump speed should be set to the lowest possible value that can maintain a stable analyte signal. The backpressure required to *initiate* the spray is often higher than the one to *maintain* the spray. Decreasing the syringe pump speed typically results in a decreased flow rate and increased analysis time.

   Note For the maximum analysis time, turn off the syringe pump after the spray is initiated. The electrospray process itself will maintain a stable flow.

5. When you are satisfied with the signal intensity, click **Stop** to end the manual tune process.

Automatically Optimizing the Voltages for Ion Detection in the Static NSI Mode

❖ **To automatically optimize the voltages for ion detection in the static NSI mode**

1. On the Control/Scan Mode tool bar in the Tune window, click the Tune button to display the Tune dialog box.
2. Initiate the automatic tuning process for the ion of interest in your tuning solution:
   a. In the Tune dialog box, click the Automatic tab to display the Automatic page.
   b. In the Mass spin box, type **524.30** for MRFA to specify a tune value of *m/z* 524.30 or enter the mass-to-charge ratio for the ion of interest in your tuning solution.
   c. Click **Start**.
   Voltages for the ion transfer tube, the tube lens offset, ion lenses, and multipoles are automatically optimized.
3. Choose **File > Save As** to display the Save As dialog box and save the values of the optimized ion detection parameters in a Tune Method.

   Note The Tune Method created by following the procedures in this section is specific to the NSI source. This method will not be optimum for standard electrospray.

Your LCQ Series mass spectrometer is now successfully tuned in the static NSI/MS mode using a standard tuning solution. Recall the Tune Method and use it as a starting point for optimizing on a different analyte of interest or for acquiring data.

Tuning an LCQ Series Mass Spectrometer in the Dynamic NSI Mode

This section describes the operation of the Thermo Scientific NSI source in the dynamic NSI mode on a LCQ Series mass spectrometer using the Tune Plus application program.

You need to connect the syringe pump on the front of your mass spectrometer to your dynamic NSI probe or your packed-tip NSI probe to perform the tuning experiment.

Table 8 displays method parameters for operation of a LCQ Series mass spectrometer. As you progress to smaller ID columns (micro-capillary to nano-capillary), the suggested flow rate, emitter tip ID, and capillary temperature decrease.

Note The values listed in Table 8 can vary significantly from emitter to emitter. Refer to these values as guidelines, not specifications.

Table 8. LC/NSI/MS Parameters for a LCQ Series mass spectrometer

Column ID	Flow Rate	Tip ID*	Emitter Position†	Capillary Temperature
50 µm	<100 to 300 (nL/min)	5 to 15 µm	approx. 1 mm	100 to 180 °C
75 µm	150 to 800 (nL/min)	10 to 20 µm	1 to 2 mm	130 to 200 °C
180 µm	0.30 to 1.0 (µL/min)	20 to 30 µm	2 to 3 mm	150 to 250 °C
320 µm	1.0 to 5.0 (µL/min)	30 to 75 µm	3 to 5 mm	150 to 250 °C

* The size of the tip ID is chosen based on PicoTips information provided by New Objective, Inc., Cambridge, Massachusetts. For more product information on the various tip sizes and types, see New Objective's catalogs or web site (http://www.newobjective.com).

† Refers to the distance of the emitter tip from the surface of the ion transfer tube

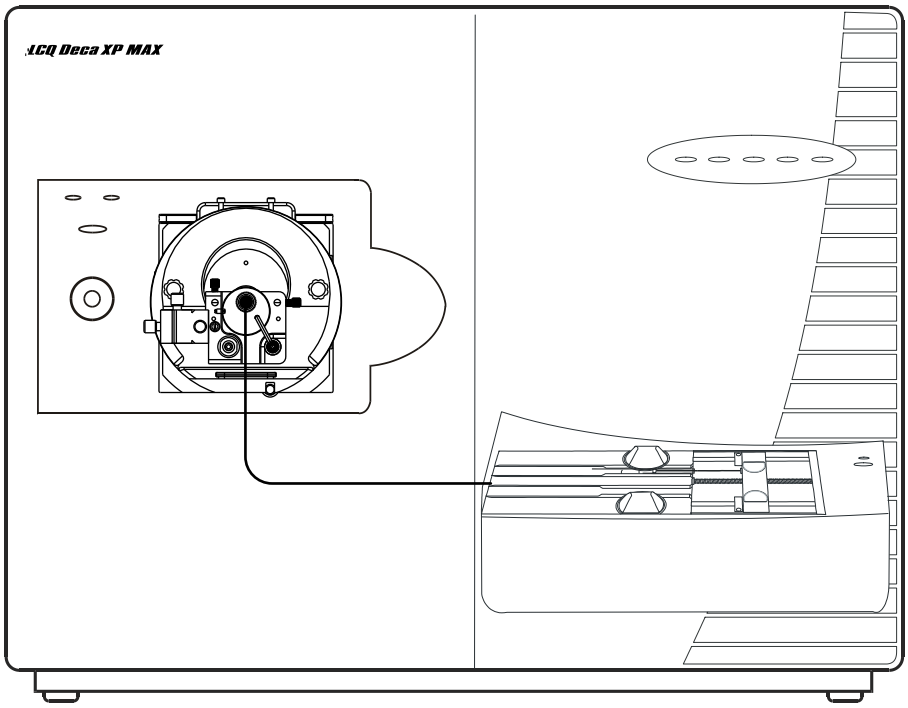
To tune your dynamic nanospray system, follow these procedures:

1. Preparing for Direct Infusion of the Tuning Solution
2. Setting Up the LCQ Series Mass Spectrometer for the Static NSI/MS Mode
3. Initiating Dynamic Nanospray by Using the Syringe Pump
4. Determining the Dynamic Nanospray Threshold and Operational Spray Voltages
5. Optimizing the Emitter Position for Dynamic Nanospray
6. Automatically Optimizing the Voltages for Ion Detection in the Dynamic NSI Mode

Preparing for Direct Infusion of the Tuning Solution

Use syringe pump on the front panel of your LCQ Series mass spectrometer as the tuning inlet. The syringe pump allows you to infuse a sample solution into the NSI source for extended periods of time. See Figure 72.

Figure 72. LCQ Series mass spectrometer set up for tuning with the dynamic NSI source



For instructions on connecting either the dynamic NSI probe or the packed-tip probe to the syringe pump, see Chapter 7.

See Table 3 on page 16 to find the appropriate flow rate for the selected emitter tip ID.

Tune the NSI/LCQ Series MS system with a test solution of either 1 to 5 pmol/µL MRFA in (50:50) 1% acetic acid in methanol / water or a tuning solution with a sample concentration of 1 to 5 pmol/µL. For instructions on preparing a tuning solution for your NSI/MS system, refer to "Tuning Solution for LCQ Series and LTQ Series Mass Spectrometers" on page 146 on page 146.

Setting up the Mass Spectrometer for Dynamic NSI/MS Operation

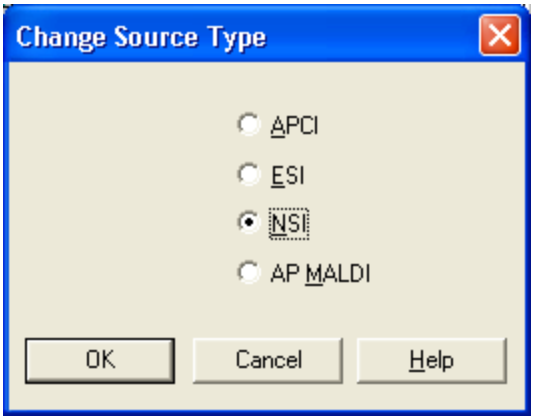
❖ **To set up the LCQ Series mass spectrometer for dynamic nanospray**

1. Open the Tune Plus window.
2. From the Control/Scan Mode toolbar, click the On/Standby button to turn on the mass spectrometer.

3. If it is not already selected, select NSI as the API source as follows:

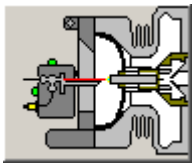
   a. From the Tune Plus menu, choose **Setup > Change API Source Type** to open the Change Source Type dialog box shown in Figure 73.

   Figure 73. Change Source Type dialog box



   b. Select the NSI option button.

   c. Click **OK** to close the Change Source Type dialog box.



4. If the Instrument Setup toolbar is not displayed, display it by choosing **View > Instrument Setup**. From the Instrument Setup toolbar, click the API Source button to open the NSI Source dialog box.

5. Settings in the NSI Source dialog box should approximate those in Figure 71 on page 77.

   Note Follow the guidelines provided in Table 8 for setting the capillary temperature.

6. Since neither are used with dynamic nanospray, set the auxiliary and sheath gas values to 0.

7. To set the scan parameters, do the following:

   a. Click the Define Scan button to open the Define Scan dialog box.

   b. Set the Scan Type to Full with a Scan Range of approximately 400 to 2000.

   c. Set the number of microscans to 3, for the LCQ Series mass spectrometer, and set the maximum ion inject time to 200 ms.

   d. Click **Apply** and close the Define Scan dialog box.

   e. If necessary, click the Centroid/Profile button to toggle the data type to centroid. (The correct state of the button is shown on the left.)

   f. Ensure that the polarity is set to the appropriate mode for your tuning compound. If necessary, click the Positive / Negative Polarity button to toggle the ion polarity mode.

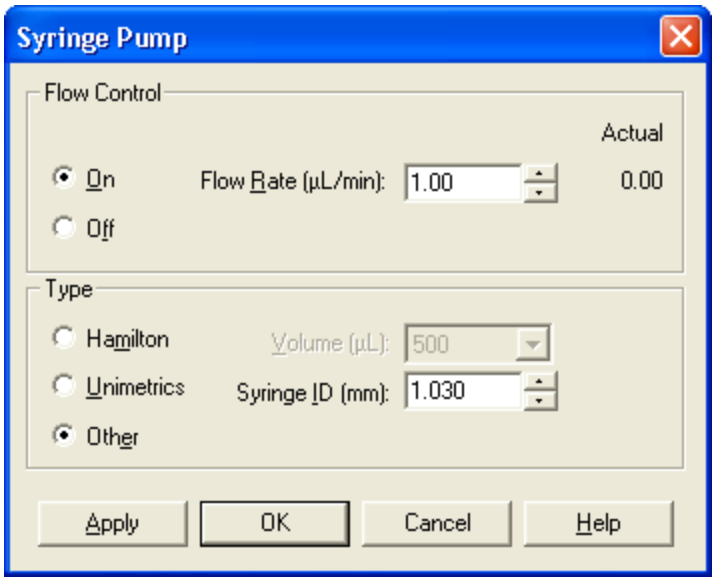
Initiating Dynamic Nanospray by Using the Syringe Pump

❖ **To initiate the dynamic nanospray by using the syringe pump**

1. In the Tune Plus window, click the Syringe Pump button to display the Syringe Pump dialog box.
2. In the (syringe) Type group box select the Other option button. This enables the Syringe ID spin box. Choose 1.030 mm for the Syringe ID to match the diameter of the 50 µL syringe (P/N 00301-19014) used in the infusion experiment.
3. In the Flow Control group box, type **1.00** in the Flow Rate spin box to set the flow rate to 1 µL/min. Select the On option button to start the pump. Click **OK** to apply the parameters and to close the dialog box.

   Figure 74 shows the appropriate settings for the syringe pump.

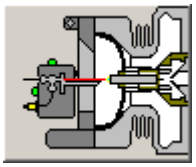
Figure 74. Syringe pump settings



4. Watch the emitter tip with the imaging system for the appearance of the spray. It will take several minutes for the test solution to fill the system.

5. If necessary, click the On / Standby button to turn on the mass spectrometer.
6. Check that the emitter tip is 2 to 5 mm from the ion transfer tube.

   You can view the emitter tip position from the imaging system or the window on the right-side of the Ion MAX adapter.



7. From the API Source dialog box, verify that the spray voltage is (±)1.0 kV.

Determining the Dynamic Nanospray Threshold and Operational Spray Voltages

❖ To determine the appropriate threshold and operational spray voltages

1. In the Tune Plus window, display the spectrum view by clicking the Display Spectrum View button on the File/Display toolbar.
2. Observe the test solution mass spectrum; if MRFA is used as the test solution, the peak of interest will be at *m/z* 524.30.
3. Open the NSI Source dialog box by clicking the API Source button. Then, increase the spray voltage gradually in 50 V (0.05 kV) increments. When the ion signal at *m/z* 524.30 (or the *m/z* for your tuning compound) shows in the full scan MS mode, record the voltage setting as the threshold nanospray voltage. The threshold voltage for initiating spray varies from (±)1.0 to 2.0 kV and depends, in part, on the syringe pump speed.

   Tip For dynamic LC-NSI-MS experiments, the threshold voltage depends on both the column flow rate and the mobile phase composition. The threshold spray voltage value increases as the surface tension of the mobile phase and the column flow rate increase. The surface tension of MeOH, ACN, and Water are 0.0226 N/m, 0.030 N/m, and 0.073 N/m, respectively.

4. Increase the spray voltage setting to 100 to 200 V above the threshold value to ensure stability of the nanospray. This nanospray value typically falls between (±)1.2 to 2.2 kV.

   IMPORTANT Voltage settings above (±)2.5 kV might cause arcing between the emitter tip and the ion transfer tube surface.

5. If no signal is noted and voltage has been raised beyond (±)2.2 kV, turn off the voltage and check the emitter tip for droplets. If none are present a clogged tip is likely. For more information on troubleshooting your dynamic NSI system, refer to "Troubleshooting Dynamic Nanospray" on page 118.

Optimizing the Emitter Position for Dynamic Nanospray

You can optimize the emitter position by monitoring the signal intensity of the ion of interest using the Spectrum view in the Tune Plus window.

❖ To optimize the emitter position

1. On the Control/Scan Mode tool bar, click the Tune button to display the Tune dialog box. Then click the Manual tab to display the Manual page.
2. In the Mass spin box, type in the mass-to-charge ratio for MRFA (*m/z* 524.30) or the mass-to-charge ratio of interest for the tuning solution and select the adjacent check box.
3. Click **Start**. A message box displays the following message: *Ensure* that the syringe is full. Click **OK** to close the message box and return to the Tune dialog box.

4. On the File/Display toolbar, click the Display Graph View button to monitor the intensity of the ion of interest using the Graph View.
5. Turn slightly the X and Y knobs of the XYZ stage to fractionally adjust the tip position for signal optimization, if necessary.
6. When the signal is satisfactory, click **Stop** to end the manual tune process.

Automatically Optimizing the Voltages for Ion Detection in the Dynamic NSI Mode

You can optimize the voltage settings for the ion transport elements while monitoring signal intensity of the ion of interest in the Spectrum View of the Tune Plus window.

❖ To automatically optimize the voltages for ion detection in the dynamic NSI mode

1. In the Tune dialog box, click the Automatic tab to display the Automatic page.
2. In the What to Optimize On group box, select the Mass option button and type in the spin box either **524.30** (the mass-to-charge ratio for MRFA) or the mass-to-charge ratio for the ion of interest in your tuning solution.
3. Click **Start**. A message box displays the following message: Ensure that the syringe is full. Click **OK** and return to the Tune dialog box.
4. Observe the Tune Plus window and the Tune dialog box during automatic tuning. The ion transfer tube, the tube lens offset, ion lenses and multipoles will have their voltage levels automatically optimized.
5. Choose **File > Save As** and save the values of the optimized parameters in a Tune method.

> **Note** The Tune Method created by following the procedures in this section is specific to dynamic nanospray with the particular operating conditions that were used. This method will not be optimum for static nanospray or standard electrospray.

Your LCQ Series mass spectrometer is now tuned in the NSI/MS mode. The Tune Method that you created by following the instructions in this section can be recalled and used as a starting point for optimization in the dynamic NSI/MS mode either for a different analyte or for loop injections.

Tuning an LTQ Series Mass Spectrometer

The Tune Plus application program is used to determine and optimize the instrument control parameters for an LTQ Series mass spectrometer.

The procedures in this chapter assume that you are familiar with your mass spectrometer and Xcalibur software. If you need additional information, refer to the Online Help, Getting Connected Guide, and/or MS detector Hardware Manual for your mass spectrometer.

If you have just installed an NSI source, ensure that the NSI mode is selected by following the instructions in the first section of this chapter. Then, proceed to the section that describes how to perform the tuning experiment for your nanospray application.

Tip You can tune your system in either the static or dynamic nanospray mode prior to performing dynamic nanospray experiments. Tuning your NSI/MS system with the static NSI probe allows you to avoid contaminating the liquid junction of the dynamic NSI probe with the tuning solution.

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Selecting NSI as the API Source

After you install an NSI source on your mass spectrometer, ensure that the NSI mode of operation is selected for the instrument configuration.

❖ To configure the LTQ Series mass spectrometer for the NSI mode

1. From the Windows taskbar, depending on the data system version, do one of the following:

   - For Xcalibur 2.0.7 or lower, choose **Start > Programs > Xcalibur > Instrument Configuration**.

     The Instrument Configuration application appears.

     Note The Instrument Configuration application has the same functionality as the Thermo Foundation Instrument Configuration dialog box.

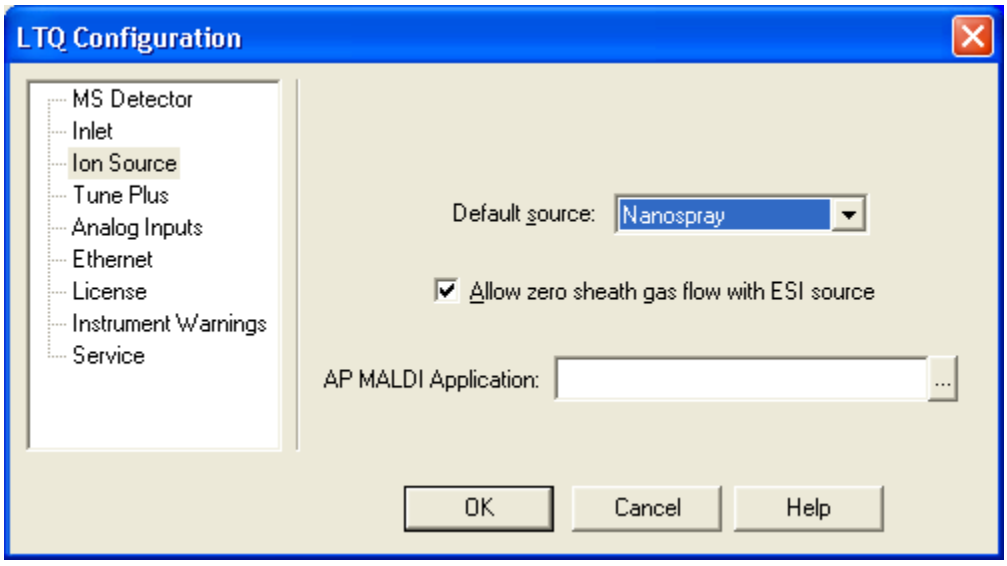
     –or–

   - For Xcalibur 2.1.*x*, choose **Start > Programs >Thermo Foundation 1.0 > Instrument Configuration**.

     The Thermo Foundation Instrument Configuration dialog box appears.

2. Click the LTQ MS icon in the Configured Devices area to open the LTQ Configuration dialog box.
3. Click the Ion Source listing in the directory tree.
4. From the Default Source list box, select *Nanospray*. See Figure 75.

   Figure 75. LTQ Configuration dialog box, showing Nanospray selected as the default source



5. Click the Service listing in the directory tree.
6. In the list of Service items, ensure that the Enable Ion Source Identification check box is selected.
7. Click **OK** to exit the LTQ Configuration dialog box.
8. Click **Done** to exit the Instrument Configuration or Foundation application.

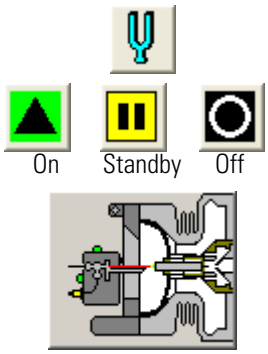
Tuning an LTQ Series Mass Spectrometer in the Static NSI/MS Mode

The procedures in this section apply to the static NSI mode and assume that you have already installed the static NSI probe and connected its gas inlet to the syringe pump of your LTQ Series mass spectrometer. For instructions on assembling the static NSI probe, refer to "Assembling the Static NSI Probe" on page 44. For instructions on installing a static NSI source, see to Chapter 6.

To tune and calibrate your LTQ Series mass spectrometer in the static NSI mode, follow these procedures.

1. Setting Up the Mass Spectrometer for Static NSI/MS Operation
2. Initiating Flow for Static Nanospray
3. Determining the Threshold and Operating Spray Voltage for the Static NSI Mode
4. Optimizing the Emitter Position for Static Nanospray
5. Automatically Optimizing the Voltages for Ion Detection in the Static NSI Mode

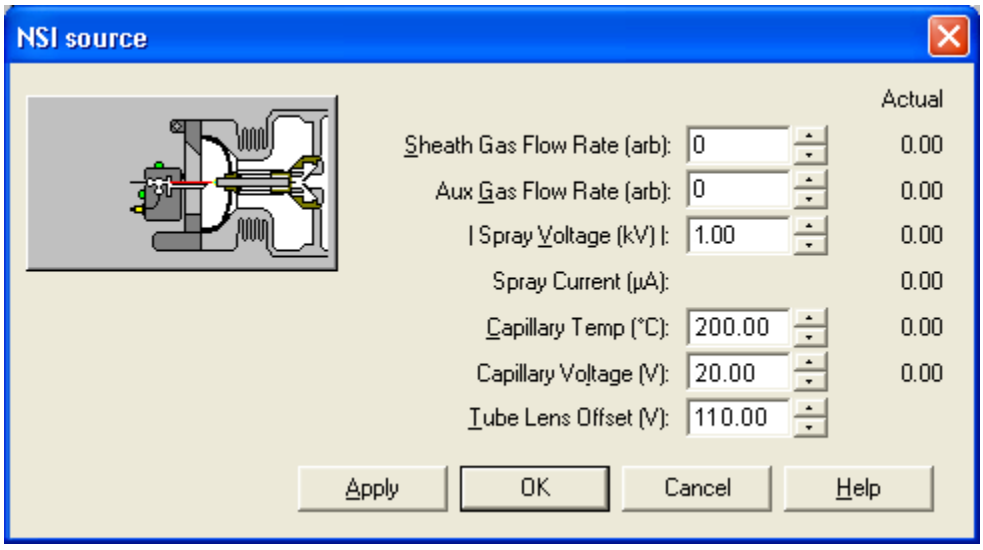
Setting Up the Mass Spectrometer for Static NSI/MS Operation



❖ **To set up your ion trap mass spectrometer for static NSI operation from the Tune Plus window**

1. From the Windows desktop, click the Tune icon to open the Tune Plus window.
2. On the Control/Scan Mode toolbar, which is the second toolbar from the top in the Tune Plus window, click the On/Standby button to turn on the LTQ Series mass spectrometer.
3. From the Instrument Setup toolbar, click the API Source icon to open the NSI Source dialog box. See Figure 76.

Figure 76. NSI Source dialog box



4. Ensure that the setting for the spray voltage is at (±)1.0 kV and that the setting for the sheath gas flow rate is at 0. Sheath gas is not used for a static NSI source.

IMPORTANT When switching to nanospray mode or when switching the ion polarity while operating in nanospray mode (for example, from positive ion mode to negative ion mode), an electrical arc might be created between the tip of the nanospray needle and the ion sweep cone if the initial spray voltage is set too high. To prevent arcing, increase the distance between the tip of the needle and the ion sweep cone or turn off the spray voltage and unplug the high voltage cable.

Initiating Flow for Static Nanospray

Thermo Fisher Scientific recommends these settings for initiating static nanospray flow:

- Distance from the spray tip to the ion transfer capillary: ~2 mm
- Spray voltage: (±)1.0 kV

❖ To initiate the flow for static nanospray

1. Using the imaging system or by visual inspection, ensure that the tip of the emitter is approximately 2 mm away from the ion transfer tube.
2. If you do not see the tip of the emitter on the screen of the imaging system, adjust its Z-axis position.
3. Open the NSI Source dialog box if it is not already open.
4. From the NSI Source dialog box, ensure that the spray voltage is set to (±)1.0 kV. Then, click **Apply**. See Figure 76 on page 89.

Note Use the **minimum** required syringe pump setting to initiate static nanospray.

5. If you are using a syringe to provide backpressure, turn on the syringe pump from the Syringe Pump dialog box. Allow the pump to run for approximately 5 seconds. Then, stop the pump and keep the syringe in place. For instructions on connecting a syringe line to the static NSI probe, refer to "Connecting the Static NSI Probe to the Syringe Pump" on page 68.
6. Set the scan parameters for tuning and optimization as follows:

   a. Click the Define Scan button to open the Define Scan dialog box.
   b. In the Scan Description group box, make the following selections:
      - Select *Normal* from the Mass Range list box.
      - Select *Normal* from the Scan Rate list box.
      - Select *Full* from the Scan Type list box.

c. In the Scan Ranges Group box, select *From/To* from the Input list box. Then, set the scan range to begin at 400 m/z and end at 2000 m/z.

d. In the Scan Time group box, make the following entries:

- Type **1** in the Microscans combo box to set the number of microscans to 1.
- Type **200** in the Max Inject Time combo box to set the maximum ion inject time to 200 ms.

e. In the Source Fragmentation group box, confirm that the Turn On check box in not selected to specify that the ion source fragmentation option is turned off.

f. Click **Apply** to download these settings to the mass spectrometer. Then, click **OK** to close the Define Scan dialog box.

7. If necessary, click the Centroid/Profile button to toggle the data type to centroid. (The Centroid state of the button is shown on the left.)

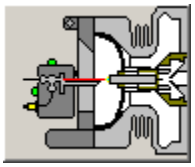
8. Ensure that the polarity is set to the appropriate mode for your tuning compound. If necessary, click the Positive/Negative Polarity button to toggle the ion polarity mode.

Determining the Threshold and Operating Spray Voltage for the Static NSI Mode

❖ **To determine the threshold spray voltage and the operational spray voltage for static nanospray**

1. In the Tune Plus window, display the Spectrum view by clicking the Display Spectrum View button on the File/Display tool bar.

2. With the mass spectrometer On, observe the mass spectrum of the test solution in the Spectrum View of the Tune Plus window. If MRFA is used as the test solution, the peak of interest will be at *m/z* 524.30.



3. Click the NSI Source icon to open the NSI Source dialog box. Then, gradually increase (decrease if operating in negative polarity mode) the spray voltage in 50 V (0.05 kV) increments until a signal for the ion at *m/z* 524.30 is observed in full scan MS mode. Record this voltage as the threshold nanospray voltage.

   Typical spray voltage values for initiating spray vary from (±)1.0 to 2.0 kV.

4. Further increase the spray voltage setting to 100 to 200 V above the threshold voltage to ensure that stable nanospray is obtained. This nanospray voltage typically ranges from (±)1.2 to 2.2 kV.

Note Spray voltages above (±)2.5 kV might cause arcing between the emitter tip and the ion transfer tube surface. This might damage the emitter coating.

Optimizing the Emitter Position for Static Nanospray

❖ **To set the emitter position, while monitoring the signal intensity of the ion of interest**

1. Initiate the manual tuning process for the mass-to-charge ratio of the ion of interest as follows:

   a. On the Control/Scan Mode tool bar in the Tune window, click the Tune button to display the Tune dialog box.

   b. Click the Manual tab to display the Manual page.

   c. In the Mass spin box, type in the mass-to-charge ratio for MRFA (*m/z* 524.30) or the mass-to-charge ratio of interest for your tuning solution and select the adjacent check box.

   d. Click **Start**. A message box displays the following message: *Ensure that the syringe is full*. Click **OK** to close the message box and return to the Tune dialog box.

2. On the File/Display toolbar, click the Graph View button to monitor the intensity of the ion of interest using the Graph View.

3. Slightly turn the "X" and "Y" adjustment knobs on the XYZ stage to fractionally adjust the emitter tip position for signal optimization.

4. Decrease or stop the syringe pump. The syringe pump speed should be set to the lowest possible value that will maintain stable analyte signal. The backpressure required to *initiate* the spray is often higher than the one to *maintain* the spray. Decreasing the syringe pump speed typically results in a decreased flow rate and increased analysis time.

   Tip For the maximum analysis time, turn off the syringe pump after the spray is initiated. The electrospray process itself will maintain a stable flow.

5. When you are satisfied with the signal intensity, click **Stop** to end the manual tune process.

Automatically Optimizing the Voltages for Ion Detection in the Static NSI Mode

❖ **To automatically optimize the voltages for ion detection in the static NSI mode**

1. On the Control/Scan Mode tool bar in the Tune window, click the Tune button to display the Tune dialog box.

2. Initiate the automatic tuning process for the ion of interest in your tuning solution:

   a. In the Tune dialog box, click the Automatic tab to display the Automatic page.

   b. In the Mass spin box, type **524.30** for MRFA to specify a tune value of *m/z* 524.30 or enter the mass-to-charge ratio for the ion of interest in your tuning solution.

   c. Click **Start**.

Examine the Tune plus window and the Tune dialog box while automatic tuning is in progress. Voltages for the ion transfer tube, the tube lens offset, ion lenses, and multipoles will be automatically optimized.

3. Choose **File > Save As** to display the Save As dialog box and save the values of the optimized ion detection parameters in a Tune Method.

Note The Tune Method that you have created by following the procedures in this chapter is specific to static nanospray. This method will not be optimum for dynamic nanospray or standard electrospray.

The mass spectrometer is now successfully tuned in NSI/MS mode using a standard tuning solution. Recall the Tune Method and use it as a starting point for optimizing the mass spectrometer using a different analyte of interest or for acquiring data.

Tuning an LTQ Series Mass Spectrometer in the Dynamic NSI Mode

The procedures in this section apply to the dynamic NSI mode and assume that you have already installed an NSI source containing either the dynamic NSI probe or the packed-tip NSI probe onto your mass spectrometer.

Note Thermo Fisher Scientific has discontinued the NSI-2 dynamic nanospray and packed-tip probes. For information about installing the NSI-1 dynamic nanospray probe, refer to the *Dynamic Nanospray Probe (NSI-1) Installation Guide*.

Table 9 and Table 10 display the NSI method parameters for operation on a LTQ Series mass spectrometer. As you progress to smaller ID columns (micro-capillary to nano-capillary), the suggested flow rate, emitter tip ID, and capillary temperature decrease.

IMPORTANT If you are transferring a nanospray method developed on an LTQ or LTQ XL mass spectrometer to an LTQ Velos mass spectrometer, raise the capillary temperature (for the ion transfer tube heater) by 50 °C. The minimum capillary temperature for the LTQ Velos mass spectrometer in the NSI mode is 250 °C.

IMPORTANT When you are developing a nanospray method for the LTQ Velos mass spectrometer, use a minimum capillary temperature of 250 °C. Setting the capillary temperature below 250 °C could result in contamination of the ion source interface and more frequent maintenance of the ion transfer tube.

Note The values listed in Table 9 and Table 10 can vary significantly from emitter to emitter. Refer to these values as guidelines, not specifications.

The tip ID sizes are based on PicoTips information provided by New Objective, Inc., Cambridge, Massachusetts. See New Objective's web site (http://www.newobjective.com) or catalogs for more product information on the various tip sizes and types.

The emitter position refers to the distance of the emitter tip from the surface of the ion transfer tube.

Table 9. LC/NSI/MS parameters for an LTQ or LTQ XL mass spectrometer

Column Size	Flow Rate	Tip ID	Emitter Position	Capillary Temperature
50 µm	<100 to 300 nL/min	5 to 15 µm	Approx. 1 mm	100 to 180 °C
75 µm	150 to 800 nL/min	10 to 20 µm	1 to 2 mm	130 to 200 °C
180 µm	0.3 to 1.0 µL/min	20 to 30 µm	2 to 3 mm	150 to 250 °C
320 µm	1 to 5 µL/min	30 to 75 µm	3 to 5 mm	150 to 250 °C

Table 10. LC/NSI/MS parameters for an LTQ Velos mass spectrometer

Column Size	Flow Rate	Tip ID	Emitter Position	Capillary Temperature
50 µm	<100 to 300 nL/min	5 to 15 µm	Approx. 1 mm	minimum 250 °C
75 µm	150 to 800 nL/min	10 to 20 µm	1 to 2 mm	minimum 250 °C
180 µm	0.3 to 1.0 µL/min	20 to 30 µm	2 to 3 mm	250 to 300 °C
320 µm	1 to 5 µL/min	30 to 75 µm	3 to 5 mm	250 to 300 °C

To tune and calibrate an LTQ Series mass spectrometer in the dynamic NSI mode, follow these procedures:

1. Preparing for Direct Infusion of the Tuning and Calibration Solution
2. Setting up an LTQ Series Mass Spectrometer for Dynamic NSI/MS Operation
3. Initiating Dynamic Nanospray by with the Syringe Pump
4. Determining the Dynamic Nanospray Threshold and Operational Spray Voltages
5. Optimizing the Emitter Position for Dynamic Nanospray
6. Automatically Optimizing the Voltages for Ion Detection in the Dynamic NSI Mode

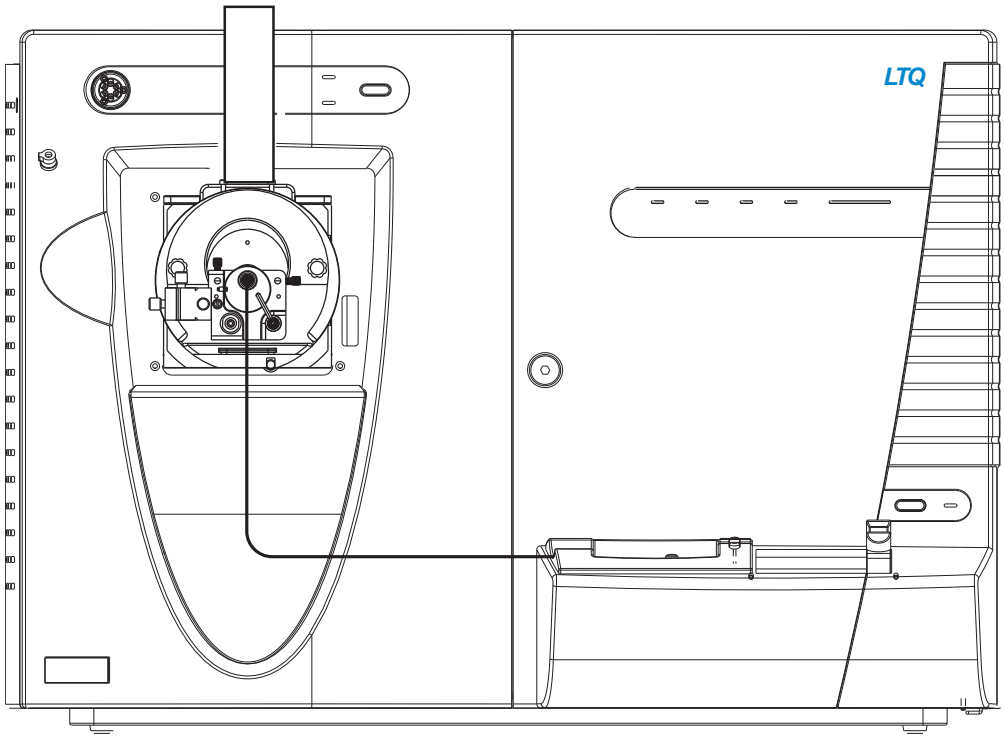
Preparing for Direct Infusion of the Tuning and Calibration Solution

Use the mass spectrometer's syringe pump as the inlet for tuning. The syringe pump allows you to infuse a sample solution into the NSI source for extended periods of time. Figure 77 shows the plumbing connections for NSI/MS sample introduction from the syringe pump. For detailed instructions on setting up your system for tuning, see Chapter 7.

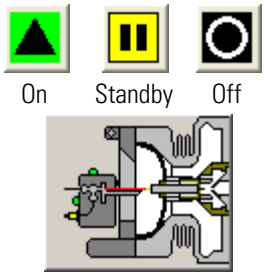
See Table 3 on page 16 to find the flow rate appropriate to the emitter tip ID selected.

Tune your NSI/LTQ Series mass spectrometer with a test solution of either 1 to 5 pmol/µL MRFA in (50:50) 1% acetic acid in methanol / water or a tuning solution with a sample concentration of 1 to 5 pmol/µL.

Figure 77. LTQ mass spectrometer, showing dynamic NSI probe connected to the syringe pump



Setting up an LTQ Series Mass Spectrometer for Dynamic NSI/MS Operation



❖ **To set up the LTQ Series mass spectrometer for dynamic nanospray**

1. Open the Tune Plus window. Click the On/Standby button to turn on the mass spectrometer.
2. From the Instrument Setup toolbar, click the API Source button to open the NSI Source dialog box.
3. Ensure that the settings in the NSI Source dialog box approximate those in Figure 76 on page 89.

Note Follow the guidelines provided in Table 9 for setting the capillary temperature.

4. Set auxiliary and sheath gas values to 0, since neither are used with dynamic nanospray.
5. Set the scan parameters as follows:

   a. Click the Define Scan button to open the Define Scan dialog box.
   b. Set the Scan Type to Full with a Scan Range of approximately 400 to 2000.
   c. Set the number of microscans to 1 for the LTQ Series mass spectrometer, and set the maximum ion inject time to 200 ms.
   d. Click **Apply** and close the Define Scan dialog box.

   e. If necessary, click the Centroid / Profile button to toggle the data type to centroid. (The correct state of the button is shown on the left.)

   f. Ensure that the polarity is set to the appropriate mode for your tuning compound. If necessary, click the Positive/Negative Polarity button to toggle the ion polarity mode.

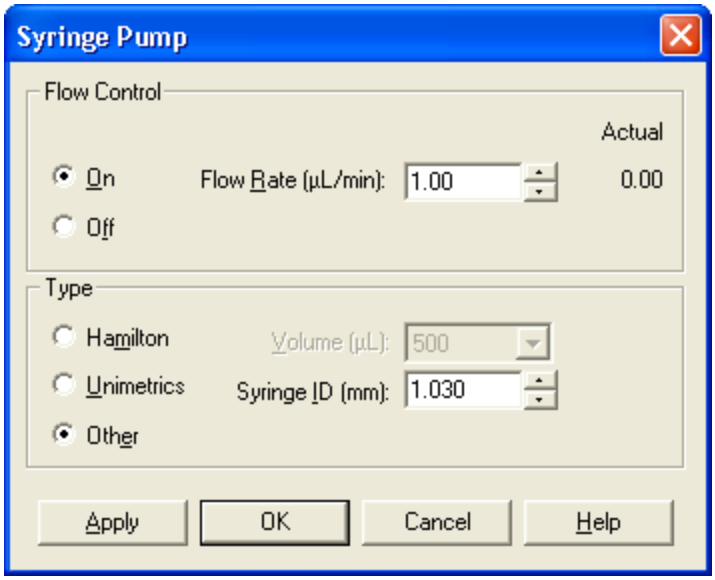
Initiating Dynamic Nanospray by with the Syringe Pump

❖ **To initiate the dynamic nanospray with the syringe pump**

1. In the Tune Plus window, click the Syringe Pump button to display the Syringe Pump dialog box. See Figure 78.
2. In the Type group box, select the Other option button. This enables the Syringe ID spin box. Choose *1.030* mm for the Syringe ID to match the diameter of the 50 µL syringe (P/N 00301-19014) used in the infusion experiment.
3. In the Flow Control group box, type **1.00** in the Flow Rate spin box to set the flow rate to 1 µL/min. Select the On option button to start the syringe pump. Click **OK** to apply the parameters and to close the dialog box.

   Figure 78 shows the appropriate settings in the Syringe Pump dialog box.

Figure 78. Syringe pump settings



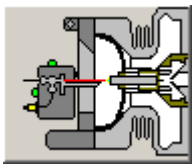
4. Click the On/Standby button to turn on the mass spectrometer if it is not already On.
5. From the imaging system, watch the emitter tip for the appearance of the spray. It will take several minutes for the test solution to fill the system.
6. Ensure that the emitter tip is 2 to 5 mm from the ion transfer tube.

   You can view the emitter tip position from the imaging system or from the window on the right-side of the Ion MAX adapter.
7. From the NSI Source dialog box, verify that the spray voltage is (±)1.0 kV.

Determining the Dynamic Nanospray Threshold and Operational Spray Voltages

❖ **To determine the appropriate threshold and operational spray voltages**

1. In the Tune Plus window, display the spectrum view by clicking the Display Spectrum View button on the File/Display toolbar.
2. Observe the test solution mass spectrum; if MRFA is used as the test solution, the peak of interest will be at *m/z* 524.30.



3. Open the NSI Source dialog box by clicking the NSI Source button. Then, increase the spray voltage gradually in 50 V (0.05 kV) increments. When the ion signal at *m/z* 524.30 (or the *m/z* for your tuning compound) shows in the full scan MS mode, record the spray voltage setting as the threshold nanospray voltage.

   The threshold spray voltage for initiating spray varies from (±)1.0 to 2.0 kV and depends, in part, on the speed of the syringe pump.

Tip For dynamic LC-NSI-MS experiments, the threshold voltage depends on both the column flow rate and the mobile phase composition. The threshold spray voltage value increases as the surface tension of the mobile phase and the column flow rate increase. The surface tension of MeOH, ACN, and Water are 0.0226 N/m, 0.030 N/m, and 0.073 N/m, respectively.

4. Increase the spray voltage setting to 100 to 200 V above the threshold value to ensure stability of the nanospray. This nanospray value typically falls between (±)1.2 to 2.2 kV.
5. If no signal is noted and voltage has been raised beyond (±)2.2 kV, turn off the voltage and check emitter tip for droplets. If none are present a clogged tip is likely. For more information, refer to "Troubleshooting Dynamic Nanospray" on page 118.

IMPORTANT Voltage settings above (±)2.5 kV might cause arcing between the emitter tip and the ion transfer tube surface.

Optimizing the Emitter Position for Dynamic Nanospray

You can optimize the emitter position for dynamic nanospray while monitoring the signal intensity of the ion of interest using the Spectrum view in the Tune Plus window.

❖ To optimize the emitter position

1. On the Control / Scan Mode tool bar, click the Tune button to display the Tune dialog box. Then, click the Manual tab to display the Manual page.
2. In the Mass spin box, type in the mass-to-charge ratio for MRFA (*m/z* 524.30) or the mass-to-charge ratio of interest for the tuning solution and select the adjacent check box.
3. Click **Start**. A message box displays the following message: *Ensure* that the syringe is full. Click **OK** to close the message box and return to the Tune dialog box.

4. On the File/Display toolbar, click the Display Graph View button to monitor the intensity of the ion of interest using the Graph View.
5. Turn slightly the "X" and "Y" knobs of the XYZ stage to fractionally adjust the tip position for signal optimization, if necessary.
6. When the signal is satisfactory, click **Stop** to end the manual tune process.

Automatically Optimizing the Voltages for Ion Detection in the Dynamic NSI Mode

You can optimize the voltage settings for the ion transport elements while monitoring signal intensity of the ion of interest in the Spectrum View of the Tune Plus window.

❖ To automatically optimize the voltages for ion detection in the dynamic NSI mode

1. In the Tune dialog box, click the Automatic tab to display the Automatic page.
2. In the What to Optimize On group box, select the Mass option button and type in the spin box either **524.30** (the mass-to-charge ratio for MRFA) or the mass-to-charge ratio for the ion of interest in your tuning solution.
3. Click **Start**. A message box displays the following message: Ensure that the syringe is full. Click **OK** and return to the Tune dialog box.
4. Observe the Tune Plus window and the Tune dialog box during automatic tuning. The ion transfer tube, the tube lens offset, ion lenses and multipoles will have their voltage levels automatically optimized
5. Choose **File > Save As** and save the values of the optimized parameters in a Tune method.

> **Note** The Tune Method created by following the procedures in this section is specific to dynamic nanospray with the particular operating conditions that were used. This method will not be optimum for static nanospray or standard electrospray.

The mass spectrometer is now tuned in the NSI/MS mode. The Tune Method that you created by following the instructions in this section can be recalled and used as a starting point for optimization in the dynamic NSI/MS mode either for a different analyte or for loop injections.

Tuning a TSQ Series Mass Spectrometer

This chapter describes how to tune your TSQ™ Series mass spectrometer using an NSI source. You tune your TSQ Series mass spectrometer and determine the optimum settings for a specific compound from the Tune Master application.

The procedures provided in this chapter assume that you are familiar with your mass spectrometer and Xcalibur software. If you need additional information, refer to the appropriate mass spectrometer Help, Getting Connected Guide, and/or MS detector Hardware Manual.

Tip You can tune your system in either the static or dynamic nanospray mode prior to performing dynamic nanospray experiments. Tuning your NSI/MS system with the static NSI probe allows you to avoid contaminating the liquid junction of the dynamic NSI probe with the tuning solution.

Contents

Tuning a TSQ Series Mass Spectrometer in the Static NSI Mode

To tune the TSQ Series mass spectrometer with a static NSI source, follow these procedures:

1. Setting up the Mass Spectrometer for Static NSI/MS Operation
2. Initiating Flow for Static Nanospray
3. Determining the Threshold and Operational Spray Voltages for Static Nanospray
4. Optimizing the Emitter Position for Static Nanospray
5. Optimizing Conditions for Ion Detection

Setting up the Mass Spectrometer for Static NSI/MS Operation

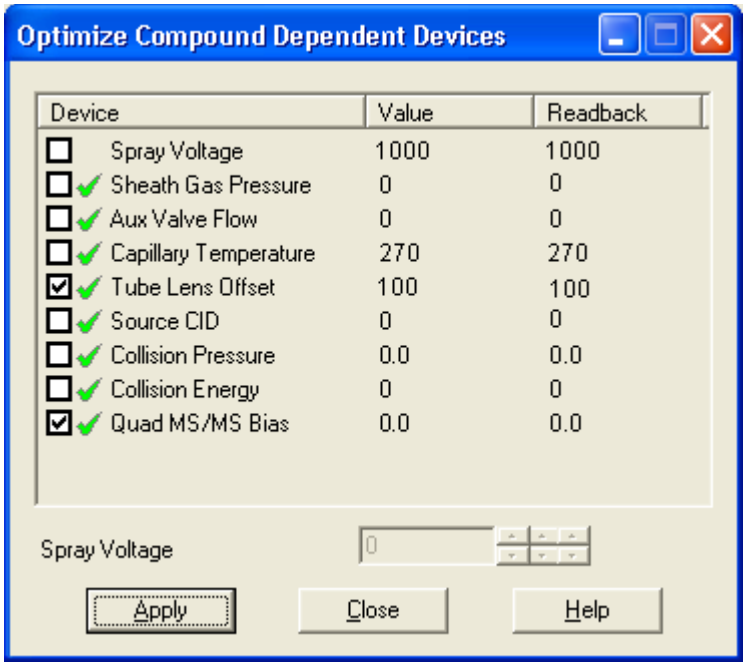
❖ To set up your TSQ Series mass spectrometer for static nanospray

1. Depending on the Xcalibur software version, open the tuning software by doing one of the following:
   - From the computer desktop, double-click the Quantum Tune icon. The Tune Master window appears.
   - From the taskbar, choose **Start > Programs > Xcalibur > Quantum Tune**. The Tune Master window appears.
   - From the taskbar, choose **Start > Programs > Thermo Instruments > TSQ > TSQ Tune**. The Thermo TSQ EZ Tune – EZ Workspace appears.
2. From the Tune Master (or EZ Tune) window, click the On / Standby button to turn on the mass spectrometer.

   When you turn on your mass spectrometer, you initiate the following events:
   - A high voltage is applied to the NSI probe.
   - The mass spectrometer begins scanning.
   - Tune Master (or EZ Tune) shows a real-time display in the Spectrum view.
3. From the menu, choose **Setup > Change Ion Source > NSI**.

4. On the Control/Scan Mode toolbar in the Tune Master window, click the Optimize Compound Dependent Devices button to open the Optimize Compound Dependent Devices view. Or, choose **Display > Compound Dependent Devices** from the menu. See Figure 79.

Figure 79. Optimize Compound Dependent Devices view



5. From the Compound Dependent Devices view, do the following:
   a. If the Spray Voltage is not already set to (±)1 kV, click Spray Voltage in the Device Display box. Then, enter a value of (±)1000 in the Device spin box.
   b. If the Sheath Gas Pressure is not already set to 0 units, click Sheath Gas Pressure in the Device Display box. Then, enter a value of 0 in the Device spin box.
   c. Click **Apply**.

Note Sheath gas is not used for a static NSI source.

Initiating Flow for Static Nanospray

Thermo Fisher Scientific recommends these parameters for initiating static nanospray flow:

- Distance from the spray tip to the ion transfer capillary: ~2 mm
- Nanospray voltage: (±)1.0 kV

❖ To initiate the flow for static nanospray

1. Ensure that your TSQ Series mass spectrometer is On.
2. Using the imaging system, or by visual inspection, ensure that the emitter tip is approximately 2 mm away from the ion transfer tube.
3. From the Compound Dependent Devices view, verify that the Spray Voltage is set to (±)1.0 kV.


OFF


ON

4. If you are using the syringe pump to provide backpressure, click the Syringe Pump button to turn on the syringe pump. Allow the pump to run for approximately 5 seconds. Then, stop the syringe pump and keep the syringe in place.

5. Set the scan parameters for optimizing on your compound as follows:

   a. Click the Define Scan button to display the Define Scan view.

   b. For Scan Type, click the Full Scan button to display the Scan Parameters group box.

   c. In the Scan Range group box, select the FM/LM option button.

   d. In the First Mass spin box, type **50** to set the low endpoint of the scan range to 50 u.

   e. In the Last Mass spin box, type **1500** to set the high endpoint of the scan range to 1500 u.

   f. Click **Apply**.

6. If necessary, click the Centroid/Profile button on the Control/Scan Mode toolbar to toggle the data type to centroid. (The Centroid state of the button is shown on the left.)

7. Ensure that the polarity is set to the appropriate mode for you tuning compound. If necessary, click the Positive/Negative Polarity button on the Control / Scan Mode toolbar to toggle the ion polarity mode.

Determining the Threshold and Operational Spray Voltages for Static Nanospray

❖ To determine the operational voltage for static nanospray

1. In the Tune Master window (or EZ Tune), ensure that the Spectrum view is displayed. If it is not already displayed, choose **View > Display Spectrum View** to display it.

2. With the mass spectrometer On, observe the mass spectrum of the test solution. If polytyrosine is used as the test solution, the peak of interest will be at *m/z* 508.208.

3. From the Compound Dependent Devices view, gradually increase (decrease if operating in negative polarity mode) the spray voltage in 50 V (0.05 kV) increments until you observe the signal of the ion at *m/z* 508.208 (or the mass-to-charge ratio of your tuning compound) in the full scan MS mode. Record this voltage as the threshold spray voltage. Typical values of the threshold voltage vary from (±)1.0 to 2.0 kV.

> **IMPORTANT** Voltage settings above (±)2.5 kV might cause arcing between the emitter tip and the ion transfer tube surface. This might damage the emitter coating.

4. Further increase the spray voltage setting to 100 to 200 V above the threshold voltage to ensure that stable nanospray is obtained. This operational nanospray voltage typically ranges from (±)1.2 to 2.2 kV.

> **Tip** For the maximum analysis time, turn off the syringe pump after the spray is initiated. The nanospray process itself will maintain a stable flow.

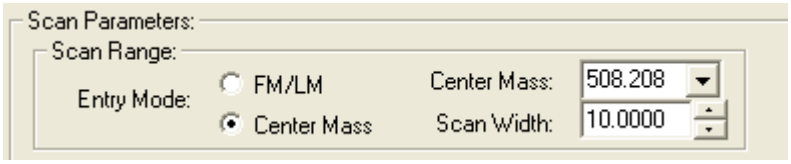
Optimizing the Emitter Position for Static Nanospray

You can optimize the emitter position for static nanospray while monitoring the signal intensity of the ion of interest using the Spectrum view in the Tune Master (or EZ Tune) window.

❖ To optimize conditions for static nanospray

1. On the Control/Scan Mode toolbar, click the Instrument Method Development Workspace button to display the Instrument Method Development workspace.
2. In the Scan Range group box, select the Center Mass option button for the Entry Mode. See Figure 80.

   Figure 80. Scan Range group box, showing settings for polytyrosine

3. In the Center Mass spin box, type **508.208** for polytyrosine to specify a tune value of *m/z* 508.208. Alternatively, enter the mass-to-charge value for the ion of interest in your tuning solution.
4. Click **Apply**.

5. On the File/Display toolbar, click the Display TIC button to monitor the intensity of the ion of interest using the TIC view.
6. If necessary, slowly turn the X and Y adjustment knobs on the XYZ stage to fractionally adjust the tip position for signal optimization.

Optimizing Conditions for Ion Detection

❖ To optimize conditions for ion detection

1. Open the Full Instrument Control workspace, if it is not already open by choosing **Workspace > Full Instrument Control**.

2. Open the Compound Optimization view by clicking the Compound Optimization button.
3. In the Compound Optimization view, select the Custom option button for the Optimization Options.

   The Optimize Compound Dependent Devices view appears.
4. In the Optimize Compound Dependent Devices view, select all of the check boxes that correspond to optimizable items for your application.

5. From the Compound Optimization view, do the following:
   a. In the Optimization Mass table, type **508.208** for polytyrosine to specify a tune value of *m/z* 508.208. Alternatively, enter the *m/z* value for the ion of interest in your tuning solution.
   b. Click **Apply**. Then, click **Start**.
6. Examine the Tune Master window while automatic tuning is in progress.

   Voltages for the ion transfer tube, the tube lens offset, ion lenses, and multipoles are automatically optimized.
7. Choose **File > Save As** to display the Save As dialog box, and save the values of the optimized parameters in a Tune Method.

   Note This Tune Method is specific to the NSI mode. It is not optimized for standard electrospray.

The Tune Method is now optimized in NSI/MS mode using a standard tuning solution. You can open the Tune Method and use it as a starting point for optimizing the mass spectrometer using a different analyte of interest or for acquiring data.

Tuning a TSQ Series Mass Spectrometer in the Dynamic NSI Mode

This section describes the operation of the Thermo Scientific NSI source in the dynamic NSI mode on a TSQ Series mass spectrometer using the Tune Master (or EZ Tune) application program.

Note Thermo Fisher Scientific has discontinued the NSI-2 dynamic nanospray and packed-tip probes. For information about installing the NSI-1 dynamic nanospray probe, refer to the *Dynamic Nanospray Probe (NSI-1) Installation Guide*.

To perform a tuning experiment, you must connect the syringe pump on the front of your mass spectrometer to your dynamic NSI probe or your packed-tip NSI probe.

Table 11 displays method parameters for operation on the TSQ Quantum family of mass spectrometers and Table 12 displays method parameters for operation on the TSQ Vantage mass spectrometer. As you progress to smaller ID columns (micro-capillary to nano-capillary), the suggested flow rate, emitter tip ID, and capillary temperature decrease.

IMPORTANT If you are transferring a nanospray method developed on a TSQ Quantum mass spectrometer to a TSQ Vantage mass spectrometer, raise the capillary temperature (for the ion transfer tube heater) by 50 °C. The minimum capillary temperature for the TSQ Vantage mass spectrometer in the NSI mode is 250 °C.

IMPORTANT When you are developing a nanospray method for the TSQ Vantage mass spectrometer, use a minimum capillary temperature of 250 °C. Setting the capillary temperature below 250 °C could result in contamination of the ion source interface and more frequent maintenance of the ion transfer tube.

Note The values listed in Table 11 and Table 12 can vary significantly from emitter to emitter. Refer to these values as guidelines, not specifications.

The tip ID sizes are based on PicoTips information provided by New Objective, Inc., Cambridge, Massachusetts. See New Objective's Web site (http://www.newobjective.com) or catalogs for more product information on the various tip sizes and types.

The emitter position refers to the distance of the emitter tip from the surface of the ion transfer tube.

Table 11. LC/NSI/MS parameters for the TSQ Quantum family of mass spectrometers

Column Size	Flow Rate	Tip ID	Emitter Position	Capillary Temperature
50 µm	<100 to 300 nL/min	5 to 15 µm	approx. 1 mm	225 to 275 °C
75 µm	150 to 800 nL/min	10 to 20 µm	1 to 2 mm	225 to 275 °C
180 µm	0.3 to 1.0 µL/min	20 to 30 µm	2 to 3 mm	225 to 275 °C
320 µm	1 to 5 µL/min	30 to 75 µm	3 to 5 mm	225 to 300 °C

Table 12. LC/NSI/MS Parameters for the TSQ Vantage mass spectrometer

Column Size	Flow Rate	Tip ID	Emitter Position	Capillary Temperature
50 µm	<100 to 300 nL/min	5 to 15 µm	approx. 1 mm	250 to 325 °C
75 µm	150 to 800 nL/min	10 to 20 µm	1 to 2 mm	250 to 325 °C
180 µm	0.3 to 1.0 µL/min	20 to 30 µm	2 to 3 mm	250 to 325 °C
320 µm	1 to 5 µL/min	30 to 75 µm	3 to 5 mm	250 to 350 °C

To tune your TSQ Series mass spectrometer in the dynamic NSI mode, follow these procedures:

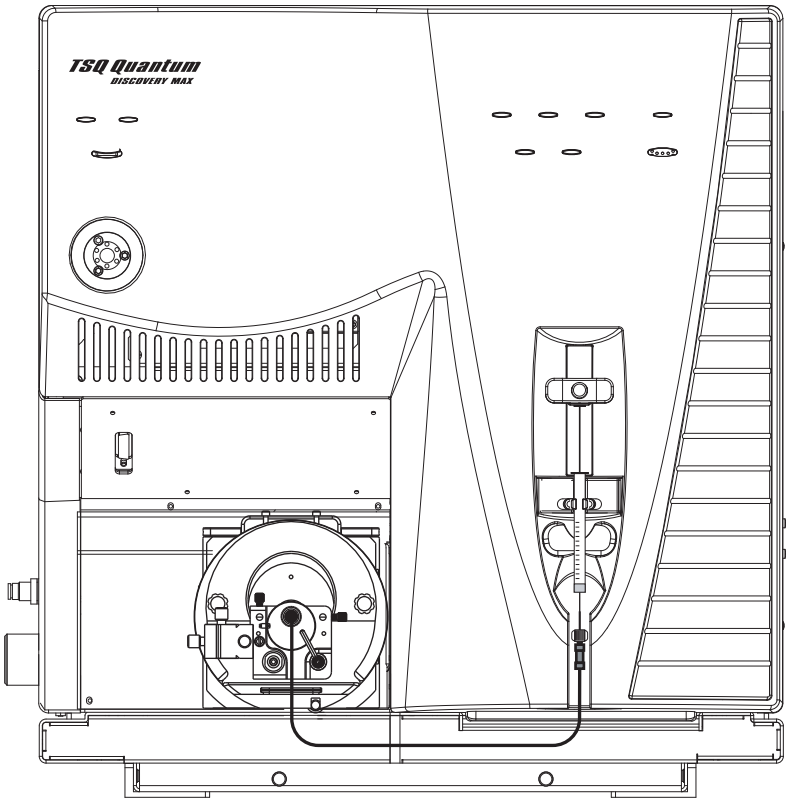
1. Connecting the Syringe Pump to the Dynamic or Packed-Tip NSI Probe
2. Setting Up the Mass Spectrometer for Dynamic NSI/MS Operation
3. Initiating Dynamic Nanospray
4. Determining the Threshold and Operational Spray Voltages
5. Optimizing the Emitter Position for Dynamic Nanospray
6. Optimizing the Ion Transport Voltage Settings for Dynamic Nanospray

Connecting the Syringe Pump to the Dynamic or Packed-Tip NSI Probe

The syringe pump on the front of the TSQ Series mass spectrometer is the sample introduction device that you use for the tuning procedure. The syringe pump allows you to infuse the tuning solution directly into the NSI source for extended periods.

The plumbing connections for NSI/MS sample introduction from the syringe pump are shown in Figure 81.

Figure 81. NSI/MS plumbing connections for direct infusion



Setting Up the Mass Spectrometer for Dynamic NSI/MS Operation

❖ **To set up the TSQ Series mass spectrometer for dynamic nanospray from the Tune window**

1. Open the Tune Master window or the Thermo TSQ EZ Tune – EZ Workspace (see step 1 on page 102.

2. From the Control/Scan Mode toolbar, click the On/Standby button to turn on the mass spectrometer.
3. Choose **Setup > Change Ion Source > NSI**.

4. From the Control/Scan Mode toolbar, click the Optimize Compound Dependent Devices button to open the Optimize Compound Dependent Devices view. Or, choose **Display > Compound Dependent Devices.** See Figure 79 on page 103.

5. In the Optimize Compound Dependent Devices view, do the following:

   a. Set auxiliary and sheath gas values to 0 units since neither are used with dynamic nanospray.

   b. Set the spray voltage to (±)1.0 kV.

   c. Enter an appropriate value for the capillary temperature.

      See Table 11 or Table 12 for the appropriate capillary temperature for your application.

> **IMPORTANT** If you are transferring a nanospray method developed on a TSQ Quantum mass spectrometer to a TSQ Vantage mass spectrometer, raise the capillary temperature (for the ion transfer tube heater) by 50 °C. The minimum capillary temperature for the TSQ Vantage mass spectrometer in the NSI mode is 250 °C.

6. On the Control/Scan Mode toolbar, click the Define Scan button to open the Define Scan view.

7. Set the Scan Type to Full Scan, with a mass range of about 150 to 1500.

8. Click **Apply**.

9. If necessary, click the Centroid/Profile button in the Control/Scan Mode toolbar to toggle the data type to centroid. (The correct state of the button is shown on the left.)

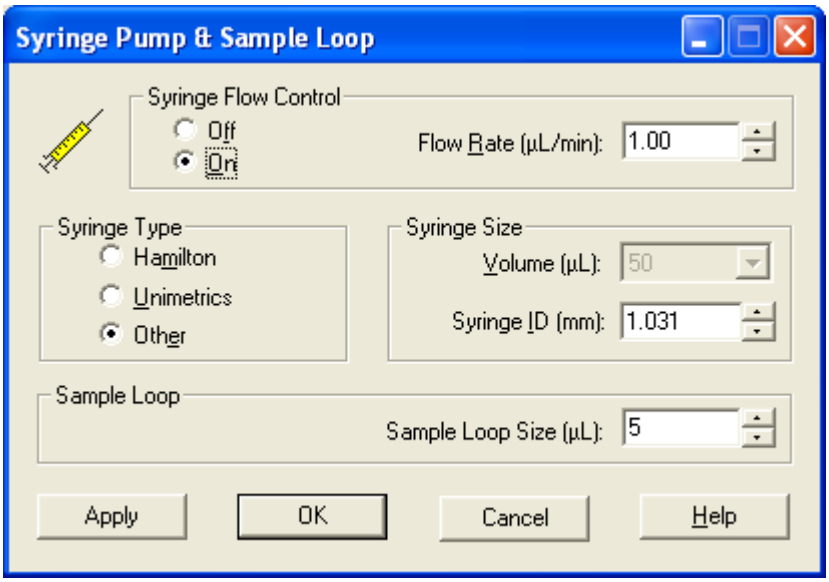

10. Ensure that the polarity is set to the appropriate mode for your tuning compound. If necessary, click the Positive/Negative Polarity button in the Control/Scan Mode toolbar to toggle the ion polarity mode.

Initiating Dynamic Nanospray

❖ **To initiate dynamic nanospray from the Tune Master (or EZ Tune) window**

1. In the Tune Master (or EZ Tune) window, choose **Setup > Syringe Pump and Sample Loop** to display the Syringe Pump dialog box. See Figure 82.

   Figure 82. Syringe Pump dialog box



2. In the Syringe Flow Control group box, select the On option button to enable the Flow Rate spin box. Then, set the syringe pump flow rate to 1 µL/min by typing **1** in the Flow Rate combo box.

3. In the Syringe Type group box, select the Other option button.

4. In the Syringe Size group box, select *1.031* mm for the syringe ID.

   This is the diameter of the 50 µL syringe (P/N 00301-19014) that is used in the infusion experiment.

5. Click **Apply** to apply the parameters and start the syringe pump.

6. From the imaging system, monitor the emitter tip for the appearance of the spray. It will take several minutes for the test solution to fill the system.

7. Ensure that the emitter tip is 2 to 5 mm from the ion transfer tube of the mass spectrometer. If the emitter tip is not displayed on the imaging screen, it is too far away.

8. Choose **Display > Instrument Status** to display the Instrument Status view. Then, click the Ion Source tab and verify that the spray voltage is (±)1.0 kV.

Determining the Threshold and Operational Spray Voltages

❖ **To determine the threshold spray voltage and the operational spray voltage for your dynamic NSI application**

1. In the Tune Master (or EZ Tune) window, display the Spectrum view by clicking the Spectrum view button on the File/Display toolbar.
2. Observe the test solution mass spectrum; if you are using polytyrosine as the test solution, the peak of interest will be at *m/z* 508.208.
3. From the Compound Dependent Devices view, increase the spray voltage gradually in 50 V (0.05 kV) increments. When you observe the ion signal at *m/z* 508.208 (or the *m/z* appropriate for your tuning compound) in the full scan MS mode, record the voltage setting as the threshold voltage. The threshold voltage setting for initiating spray varies from (±)1.0 to 2.0 kV and depends, in part, on the syringe pump speed.

IMPORTANT Voltages above (±)2.5 kV can cause arcing between the emitter tip and the ion transfer tube surface.

Tip For dynamic LC-NSI-MS experiments, the threshold voltage depends on both the column flow rate and the mobile phase composition. The threshold spray voltage value increases as the surface tension of the mobile phase and the column flow rate increase. The surface tension of MeOH, ACN, and Water are 0.0226 N/m, 0.030 N/m, and 0.073 N/m, respectively.

4. Increase the spray voltage setting to 100 to 200 V above the threshold value to ensure stability of the nanospray. This nanospray value typically falls between (±)1.2 to 2.2 kV.
5. If no signal is noted and the voltage has been raised beyond (±)2.2 kV, turn off the voltage and check emitter tip for droplets.

   If none are present, a clogged tip is likely. For more information, refer to "Troubleshooting Dynamic Nanospray" on page 118.

Optimizing the Emitter Position for Dynamic Nanospray

You can optimize the emitter position for dynamic nanospray while monitoring the signal intensity of the ion of interest using the Spectrum view in the Tune Master (or EZ Tune) window.

❖ **To set the emitter position**

1. On the Control/Scan Mode toolbar, click the Instrument Method Development Workspace button to display the Instrument Method Development workspace.
2. In the Scan Parameters group box, in the Scan Range group box, select the Entry Mode: Center Mass option button.

3. In the Center Mass combo box, type **508.208** for polytyrosine to specify a tune value of *m/z* 508.208. Alternatively, enter the *m/z* value for the ion of interest in your tuning solution.
4. Click **Apply**.
5.  On the File/Display toolbar, click the Display TIC button to monitor the intensity of the ion of interest using the TIC view.
6. Turn slightly the X and Y adjustment knobs on the XYZ stage to fractionally adjust the tip position for signal optimization, if necessary.

Optimizing the Ion Transport Voltage Settings for Dynamic Nanospray

You can optimize the voltage settings for the ion transport elements while monitoring the signal intensity of the ion of interest.

❖ **To optimize the voltage settings for the ion transport elements**

1.  On the Control/Scan Mode toolbar in the Tune window, click the Compound Optimization Workspace button to switch to the Compound Optimization workspace.
2. Select the Optimization Options: Custom options button.
3. In the Optimization Mass table, type **508.208** for polytyrosine to specify a tune value of *m/z* 508.208. Alternatively, enter the mass-to-charge ratio for the ion of interest in your tuning solution.
4. In the Optimize Compound Dependent Devices view, select all of the check boxes corresponding to optimizable items.
5. Click **Start**. Examine the Tune Master (or EZ Tune) window while automatic tuning is in progress. Voltages for the ion transfer tube, the tube lens offset, ion lenses, and multipoles will be automatically optimized.
6. Choose **File > Save As** and save the values of the optimized parameters in a Tune Method.

Note The Tune Method created by performing this procedure is specific to dynamic nanospray with the particular operating conditions that were used. It is not optimized for standard electrospray.

The Tune Method is now optimized in the NSI/MS mode. It can be recalled and used as a starting point for NSI/MS detector optimization either for a different analyte or for loop injections.

Maintaining and Troubleshooting Your NSI/MS System

This chapter contains information on maintaining and troubleshooting your LC/NSI/MS system.

Maintaining a scrupulously clean NSI/MS system is critical to your success at running the mass spectrometer in the NSI mode. After all calibrating and tuning procedures, you must scrupulously clean the ion source, transfer lines, and syringes. During operation of the NSI probe, you must avoid leaks inside the probe, which can lead to improper grounding and an unstable nanospray.

In addition to requiring scrupulous cleaning, all three NSI probes contain parts that require frequent replacement. It is a good practice to remove the NSI source from the mass spectrometer before you uninstall the NSI probe to replace the emitter or PicoFrit column. This practice helps you to avoid damaging the tip of the new emitter or PicoFrit column as you reinstall the NSI probe into the base mount of the NSI source.

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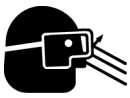
Cleaning the Components of the NSI/MS System

After all calibrating and tuning procedures, you must scrupulously clean the ion source, transfer lines, and syringes. Alternatively, you can dedicate the additional fittings provided (tubing and syringe) for tuning/calibration. Dedicating specific fittings, tubing, and syringes to tuning and calibration allows you to avoid extensive cleaning of the actual working apparatus.

Note For best results, set aside a dedicated set of labware (syringe, fittings, and tubing) for the sole purpose of calibrating the instrument. This will help you to avoid cross contamination.

Cleaning after calibration entails successive washings with acetone, isopropyl alcohol, and methanol in that order. These agents are pumped through all transfer lines and emitters with the syringe pump. You must also clean the spray shield and the exterior of the ion tube with successive solvent washes. After you have performed the above cleaning procedures, using a spray bottle or syringe/filter, successively spray the solvents listed above into the ion transfer tube and scour with the reaming bar after each solvent spray.



CAUTION Always wear protective gloves and safety glasses when you use solvents or corrosives. Also, contain waste streams and use proper ventilation. Refer to your supplier's Material Safety Data Sheet (MSDS) for proper handling of the particular solvent.

If you can still detect very small levels of residual calibration compound after following the cleaning steps described above, then proceed with a more thorough cleaning of the API stack components. Successively clean the stack with acetone, isopropyl alcohol, and methanol. For more information on how to clean API source components, refer to the appropriate hardware manual.

CAUTION AVOID BURNS. Some of the API stack components, such as the spray cone and ion transfer tube, can reach temperatures of 350 °C. Always allow the API components to cool down from operating temperature to room temperature before cleaning the components.

Uninstalling the NSI Source and the NSI Probe

It is a good practice to uninstall the NSI source before you attempt to replace an emitter or PicoFrit column.

❖ To uninstall the NSI source and dismount the NSI probe

1. Before you uninstall the NSI probe, ensure that you have a clean worksite where you can handle your micro fluidic connections.
2. Open the Tune Master (or EZ Tune) or Tune Plus window and choose **Control > Standby** to put the mass spectrometer in Standby mode. If you are running the LC pump, turn off its flow.

CAUTION AVOID ELECTRICAL SHOCK. Make certain that all electrical voltages are at ground potential before handling the NSI source

3. Remove the high voltage cable between the NSI base mount and the NSI extension assembly by turning the locking ring on the power cable counterclockwise. Then, gently pull the high voltage connector out of the receptacle on the NSI base mount.
4. Depending on your NSI probe configuration, disconnect the liquid transfer line, gas line, or syringe line from the rear of the NSI probe.

5. Loosen the flange retainer bolts and remove the NSI source from the NSI extension assembly.
6. Disconnect the high voltage lead of the NSI probe from the base mount. Then, slide the NSI probe backwards out of the NSI base mount.
7. Place the NSI probe on a lint free cloth in the area that you have reserved as your micro fluidic worksite.

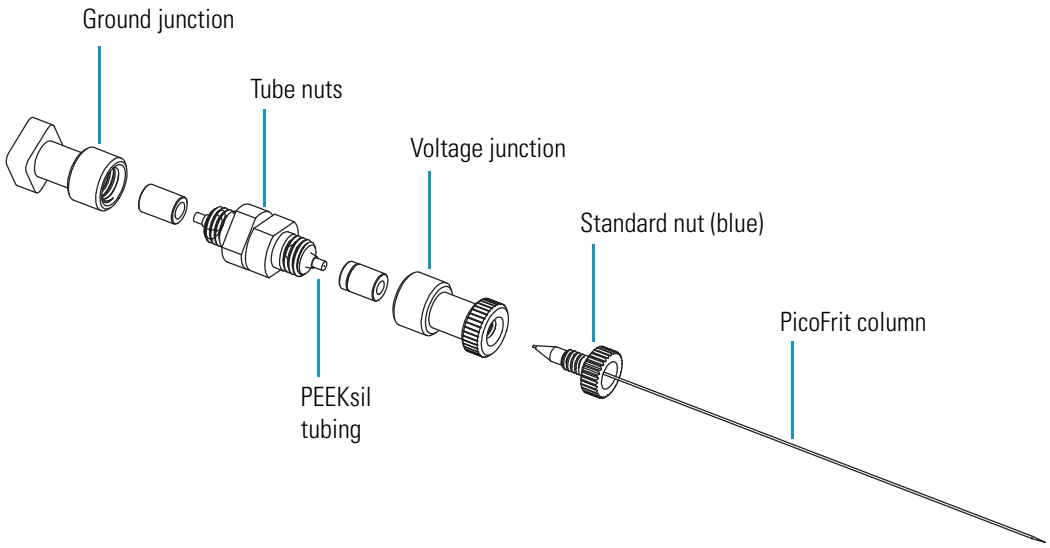
Replacing the PEEKsil Tubing of the Packed-Tip NSI Probe

Occasionally the PEEKsil tubing portion of the liquid junction for the packed-tip NSI probe becomes clogged.

❖ To replace the PEEKsil tubing

1. Put your LC/NSI/MS system in the Standby mode and ensure that the solvent flow from the LC pump is Off.
2. Detach the packed-tip NSI probe from the LC pump by unscrewing, and then removing the short head nut.
3. Detach the NSI source from the mass spectrometer.
4. Detach the packed-tip NSI probe from the NSI source.
5. Remove the clogged PEEKsil tubing as follows:
   a. Pull the probe cover off the probe body.
   b. Pull the liquid junction out of the spring retainer.
   c. Unscrew and remove the fingertight fitting.
   d. Using two 3/16 in. wrenches, unscrew the tube nuts from the ground junction and the voltage junction. See Figure 83.

Figure 83. Liquid junction of packed-tip nanospray probe



e. Discard the clogged PEEKsil tubing.

6. Reassemble the liquid junction as follows:
   a. Slide the tube nuts onto a new piece of PEEKsil tubing.
   b. Screw one tube nut into the ground junction and tighten the connection with a 3/16 in. wrench.
   c. Screw the other tube nut into the voltage junction and tighten the connection with a 3/16 in. wrench.
   d. Reattach the fingertight fitting and the PicoFrit column to the voltage junction.

   IMPORTANT It is critical that you avoid leaks surrounding the liquid junction of your NSI probe. If the exterior of the liquid junction becomes wetted, improper grounding occurs, leading to anomalously high and unstable spray currents.

7. Reinsert the liquid junction into the spring retainer.
8. Reattach the probe cover to the probe body.

Troubleshooting

This section provides troubleshooting tips for the problems you might encounter while running your NSI/MS system and contains the following topics:

- Troubleshooting Static Nanospray
- Troubleshooting Dynamic Nanospray
- Troubleshooting Microscale LC/MS

Troubleshooting Static Nanospray

Use the guidelines listed in Table 13 for troubleshooting static nanospray problems.

Table 13. Common static nanospray problems, their causes, and solutions

Problem	Possible Causes	Solution
No stable background ion signals are observed in full scan MS mode when the high voltage is set above (±)2 kV and the emitter is positioned approximately 2 mm from the ion transfer tube and is centered.	Most likely the electrical contact is discontinuous and the electrospray voltage is not being applied to the emitter.	Check: • HV connection at the high voltage leads beneath the probe cover • HV connection between NSI probe and mass spectrometer • the integrity of the emitter coating • contact between the coated tip and the contact clip to ensure electrical continuity
Analyte ion signal is observed only for a very short period of time, less than 2 to 5 minutes.	• No sample solution • air bubbles • emitter tip broken • backpressure is too high	Remove the emitter and check if any sample solution remains. A) If some sample solution remains in the capillary, the flow disruption is most likely due to air bubbles trapped inside the tip during sample loading. Reload the sample carefully following instructions provided in Chapter 4 B) If the entire sample is gone: • The emitter tip might be partially broken, resulting in a high flow rate. Change the emitter and reload the sample • The backpressure provided to initiate the flow is too high and the sample was ejected. Reload the sample into the tip and reduce the speed of, or turn off the syringe used for initiating the flow.
Analyte ion signal is not observed or is very weak. Some low mass background ions (for example *m/z* 371 and 445) are observed, but no high mass background ions are observed.	The spray conditions are not optimized, and either: • the flow rate is too low • the emitter is clogged	If increasing the spray voltage in 100 V increments does not solve the problem, increase the syringe pump speed, or turn the syringe pump on for 5 to 10 s to further push the liquid through the tip. If the symptom persists when the nanospray voltage is above (±)2 kV and the gas backpressure is above 50 units, remove the emitter and check the sample solution inside. If there is significant sample solution left in the capillary, the emitter tip is most likely clogged. Change the emitter and reload with clean sample.

Troubleshooting Dynamic Nanospray

Use the guidelines in the following table for troubleshooting dynamic nanospray problems.

Table 14. Common problems in dynamic nanospray, their possible cause, and solution(s)

Problem	Possible Cause	Solution
No stable background ion signals are observed in full scan MS mode when the high voltage is set above (±)2 kV and the emitter is positioned approximately 2 mm from the ion transfer tube and is centered	Most likely the electrical contact is discontinuous and the electrospray voltage is not being applied to the emitter.	Check: • high voltage connection • emitter coating to ensure electrical continuity • proper connection of high voltage pin leads, properly configured for liquid junction or tip • the emitter under a microscope and look for fractures. Replace the emitter if necessary
Analyte ion signal was observed intermittently	Most likely the spray is unstable due to either: • too low voltage • air bubbles in the line	Use the camera and monitor to observe the surface of the ion transfer tube. Use the X or Y adjustment knobs to move the emitter slightly off axis. If liquid does not continuously appear on the ion transfer tube, increase the spray voltage slowly. If a stable spray does not occur and the voltage is increased over the recommended value, turn off the voltage and check the emitter. If solvent appears to be flowing at the desired flow rate, the flow might have been disrupted due to air bubbles in the line. Degas the solvent to remove air and equilibrate your sample to room temperature to avoid outgassing of the solvent.
Analyte ion signal is not observed or is very weak. Some low mass background ions (for example *m/z* 371 and 445) are observed, but no high mass background ions.	The spray conditions are not optimized, and either: • the flow rate might be too low • the voltage might be too high	Turn off the voltage and check the emitter. If solvent does not appear to be flowing at the desired flow rate, the emitter might be clogged. Change the emitter, if necessary, and reload with clean sample that has been centrifuged. If the solvent flow rate appears to be too low, drop the voltage to (±) 0.9 kV and increase it in 50 V increments until spraying commences.
Anomalously high and unstable spray currents	If the exterior of the liquid junction becomes wetted, improper grounding occurs.	Avoid leaks surrounding the liquid junction of your NSI probe.

Troubleshooting Microscale LC/MS

Use the guidelines in Table 15 for troubleshooting problems with microscale LC/MS.

Table 15. Common problems in microscale LC/MS, their possible cause, and solution(s)

Problem	Possible Cause	Solution(s)
The flow rate seems to change over the course of the last several experiments.	It is likely that the column starts to foul and the split ratio is changed as a result of the increased back pressure of the column.	1. Adjust column flow rate. 2. Clean or replace column.
Flow rate is not low enough for optimum column performance, but it is impossible to lower the pump flow rate even more.	The split ratio of the LC-tee splitter might be incorrect.	Increase the split ratio by shortening the restrictor line.
The loop injection signal is very weak and spread out in time.	A large dead volume exists in the plumbing between the HPLC and the emitter.	Check all of the connections and ensure only ZDV unions are installed. Refer to Chapter 2 for guidelines on microfluidic plumbing.
A large droplet of solvent forms at the tip and requires spray voltage settings above the recommended range to break up.	Spray hysteresis caused by large surface tension of the solvent Spray instability caused by fracture to emitter tip	Carefully add a drop of organic solvent, for example, acetonitrile or methanol to the tip with a plastic pipette to overcome the large surface tension of the droplet. Replace the PicoTip or PicoFrit emitter/column.

Replaceable Parts

This chapter lists replaceable and consumable parts and part numbers for the Thermo Scientific NSI source. To ensure proper results in servicing the Thermo Scientific NSI source, order only the parts listed.

Note Thermo Fisher Scientific has discontinued the NSI-2dynamic nanospray and packed-tip probes. For information about ordering and installing the original dynamic nanospray probe, refer to the *Dynamic Nanospray Probe (NSI-1) Installation Guide*.

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Thermo Scientific Consumable Parts

You can order these parts from Thermo Fisher Scientific:

Fused silica, 50 µm ID, 360 µm OD, 5 meter lengths	00106-10512
HPLC column, 180 µm ID, 150 mm length, C8	00109-00504
HPLC column, 320 µm ID, 150 mm length, C18	00109-00505
Syringe, 50 µL, removable needle, gas tight	00301-19017
Teflon tubing, 15 cm, 1/16 in. 0.007 in. ID × 0.063 in. OD	00301-22803
Teflon tubing, 0.5 cm, 1/16 in. 0.003 in. ID × 0.063 in. OD	00301-22915
Polytyrosine – 1,3,6 Calibration Solution (liquid)	00301-22924
Polytyrosine – 1,3,6 Calibration Solution (solid)	00301-22925
Chemical; MS standards	97000-62042
Adapter ring, 1 in. for dynamic probes	97055-20415
Adapter ring, 2 in.	97144-60270
O-ring, 0.125 in. ID × 1/16 in OD, Viton	00107-02550
O-ring, 0.176 in. ID × 1/16 in OD, AS-008, Viton	00107-02575

Thermo Scientific Kits

You can order these kits from Thermo Fisher Scientific:

Nanospray Interface Ion MAX Kit
(for systems w/ an Ion MAX source OPTON-20050
Nanospray documentation CD 97055-64041
Ion MAX adapter . . . . . N/A
Assembly, adapter flange for Ion MAX, nanospray . . . . . N/A
Nanospray, flange, extension (1 in. adapter ring) . . . . . N/A
Nanospray, translation flange . . . . . N/A
Nanospray, base, Upchurch. . . . . N/A
NSI-2, source mount kit for LCQ Deca XP and LCQ Advantage XP . . . OPTON-20055
Nanospray documentation CD 97055-64041
Nanospray, translation flange . . . . . N/A
Nanospray, flange extension (1-in. deep adapter ring) . . . . . N/A
Adapter ring, 2 in. deep . . . . . N/A
Nanospray, base, Upchurch. . . . . N/A
Kit, video, nanospray, 230V, w/o display . . . . . N/A
NSI-2, source mount upgrade kit
(for LCQ Deca XP and LCQ Advantage XP OPTON-20054
Nanospray documentation CD 97055-64041
Nanospray, base, Upchurch. . . . . N/A
Nanospray, flange extension (1-in. adapter ring) . . . . . N/A
Static probe and accessory kit OPTON-20051
Dynamic probe, accessory kit, and column support . . . . . Discontinued
Packed-Tip probe and accessory kit . . . . . Discontinued
Kit, Dynamic Probe accessory 97055-98033
Adapter nut . . . . . N/A
Ferrule, high pressure, FingerTight II, 10 pack . . . . . N/A
Female nut . . . . . N/A
Front nut, conductive . . . . . N/A
MicroFerrule for 1/16 in. OD tubing . . . . . N/A
MicroFerrule, 0.025 in., black . . . . . N/A
MicroTight sleeve, green, PEEK . . . . . N/A
Tube, PEEKsil, 1/16-in. × 50 µm 8 cm, 2 each . . . . . N/A
Union, ZDV, stainless steel, 2 each . . . . . N/A
Kit, Static Probe accessory 97055-98039
Glass, PicoTip, 10 each. . . . . N/A
Contact, PicoTip . . . . . N/A
O-ring, 0.125 in. ID × 1/16 in OD, Viton . . . . . N/A
O-ring, 0.176 in. ID × 1/16 in OD, Viton . . . . . N/A
Kit, Packed-Tip Probe accessory 97055-98040
Union, conductive, sym, (2 each) . . . . . N/A
Union, conductive, inlet, (2 each) . . . . . N/A
Tube, connector, PEEKsil, (5 each) . . . . . N/A
Fitting, (2 each) . . . . . N/A

Ferrule, for 1/32 in. tubing, (5 each) . . . . . N/A
Ferrule, for 1/16 in. tubing, (5 each) . . . . . N/A
PicoFrit columns, (2 pack) . . . . . N/A
Fingertight fitting, (10 each) . . . . . N/A

Upchurch Scientific Parts

You can order the these parts from Upchurch Scientific:

Dynamic Probe Assembly S-2498-1
Kit, Dynamic Probe accessory S-2498-2
PEEKsil Tubing, 1/16 in. × 50 µm x 8 cm 6508-1
MicroFerrule, PEEK, Fingertight, 1/16-in. F-132
Ferrule, PEEK, Fingertight, 1/16-in.. . . . . F-142
MicroFerrule, 0.025 in., PEEK, black F-172
MicroTight Sleeve, green, PEEK, 0.015 in. ID × 0.025 in. OD F-185
Female nut P-416
Front nut, conductive S-2494-21
Adapter nut S-2494-16
ZDV union, 0.010 in. ID, stainless steel U-435-01

New Objective, Inc. Parts

You can order these parts from New Objective, Inc.:

Pre-cut, 5 cm length, 30 µm ID tip, PicoTip emitter . . . . . F360 - 50 - 30 - N - CT
Pre-cut, 5 cm length, 10 µm ID tip, PicoTip emitter . . . . . F360 - 25 - 10 - N - CT

Supplier Information

This appendix provides a partial list of lab equipment and chemical supply companies for your reference. To order nanoscale fittings and tubing, contact Upchurch Scientific. To order PicoTip emitters, SilicaTip emitters, and PicoFrit columns, contact New Objective, Inc.

Upchurch Scientific

619 Oak St.,
Oak Harbor, WA 98277

Phone: (800) 426-0191 or (360) 679-2528
Fax: (800) 359-3460 or (360) 679-3830
E-mail: upchurch@upchurch.com
Web address: http://www.upchurch.com

New Objective, Inc.

2 Constitution Way
Woburn, MA 01801-1023 USA

Phone: (888) 220-2998 or (781) 933-9560
Fax: (781) 933-9564
E-mail: sales@newobjective.com
Web address: http://www.newobjective.com

VWR International

300 Hadley Rd.
So. Plainfield, NJ 07080

Phone: (800) 932-5000 or 908-757-4045
Fax: (908) 757-0313
Web address: http://www.vwrsp.com

Fisher Scientific (US Headquarters)

2000 Park Lane Dr.
Pittsburgh, PA 15275-9943

Phone: (800) 766-7600 or 412-490-8300
Fax: (800) 926-1166
Web address: http://www.fishersci.com

Brinkman Instrument Inc.

One Cantiague Rd.
Westbury, NY 11590-0207

Phone: (800) 645-3050 or 516-334-7500
Fax: (516) 334-7506
E-mail: info@brinkmann.com
Web address: http://www.brinkmann.com

Installing the Osprey MultiMedia Capture Device, Software, and Drivers

This Appendix provides detailed, step-by-step procedures for installing the video capture card and the associated driver for the video capture system.

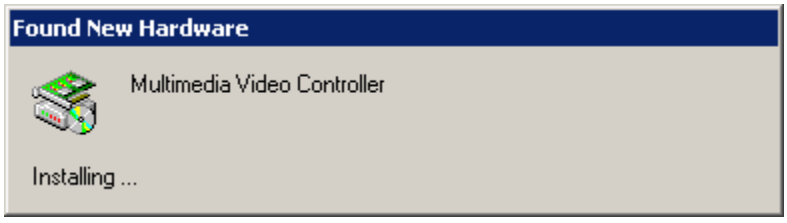
Contents

Installing the Video Capture Card

❖ **To install the video capture card**

1. Shut off the system computer.
2. Remove the cover from the computer.
3. Locate an available PCI slot to install the card, and remove the PCI slot dust cover.
4. Install the video capture card into the PCI slot.
5. Replace the cover on the computer.
6. Turn On the computer and log onto the operating system. Note, the computer detects the new hardware upon startup. See Figure 84.

Figure 84. Found New Hardware message



7. Cancel the Found New Hardware wizard, which starts automatically, by clicking **Cancel**. See Figure 85.

Figure 85. Found New Hardware Wizard – Welcome page

The drivers for the video capture card need to be installed before the video can be used. Go to the next section, "Installing the Device Driver."

Installing the Device Driver

❖ To install the Osprey device driver with the Osprey driver installation wizard

1. Insert the Osprey Software Installation CD into the CD drive. The Osprey Software Installation program starts automatically. See Figure 86.

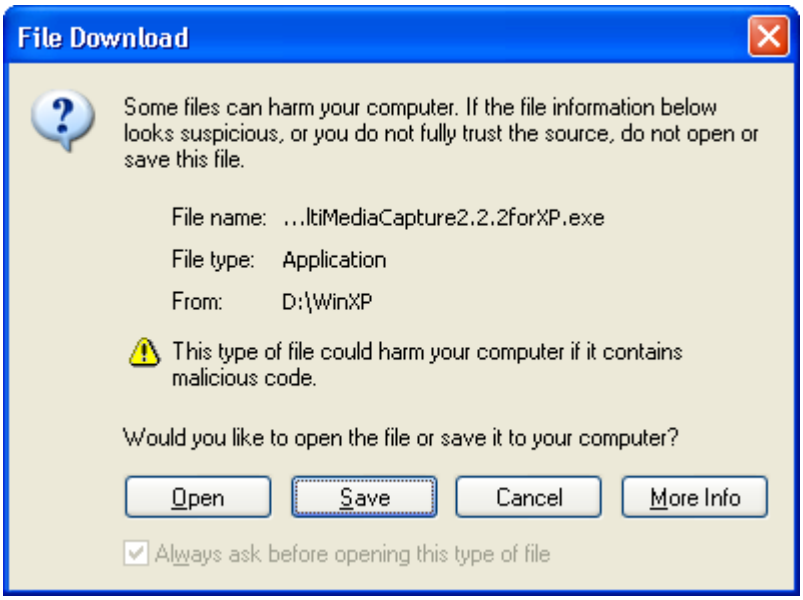
Figure 86. Osprey Software Installation page showing Welcome screen

2. In the navigation bar on the left, click Driver Installation to expand the list of Microsoft operating systems supported by the Osprey video capture system. See Figure 87.

Figure 87. Osprey Software Installation page with list of supported operating systems

3. From the Driver Installation list, select Windows XP to begin downloading the Osprey MultiMedia Capture 2.2.2 Windows XP driver installation application. See Figure 88.

Figure 88. File Download dialog box



4. In the File Download dialog box, click **Open** to begin downloading the Osprey MultiMedia Capture 2.2.2 Windows XP driver installation application to a temporary folder. See Figure 89.

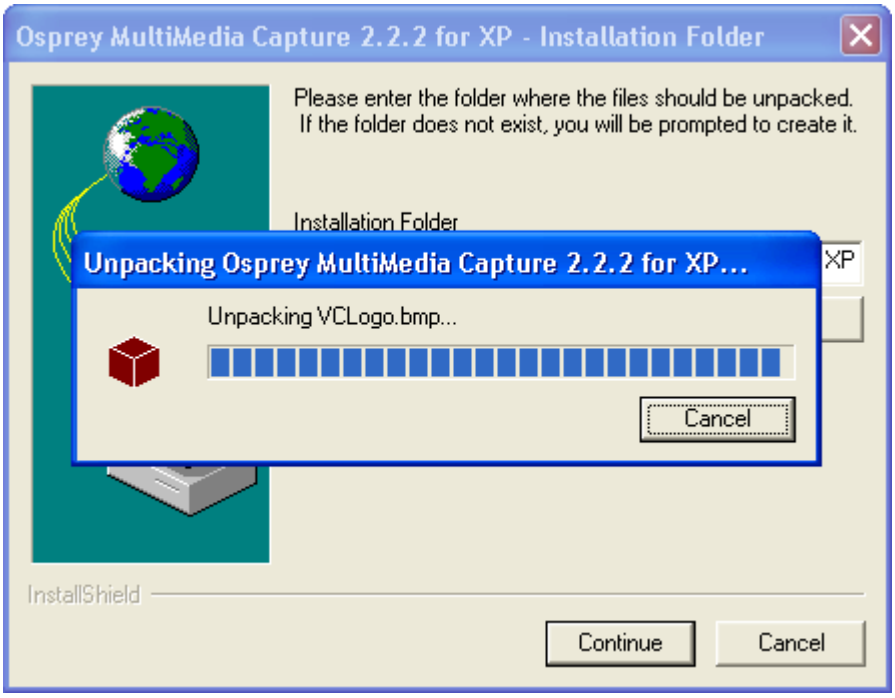
Figure 89. Installation Folder dialog box (for Windows XP)

5. From the Osprey MultiMedia Capture 2.2.2 Windows XP driver installation application – Installation Folder dialog box, click **Continue** to download the Osprey MultiMedia Capture 2.2.2 Windows XP driver installation application to a temporary folder. See Figure 90.

Figure 90. Folder Creation dialog box

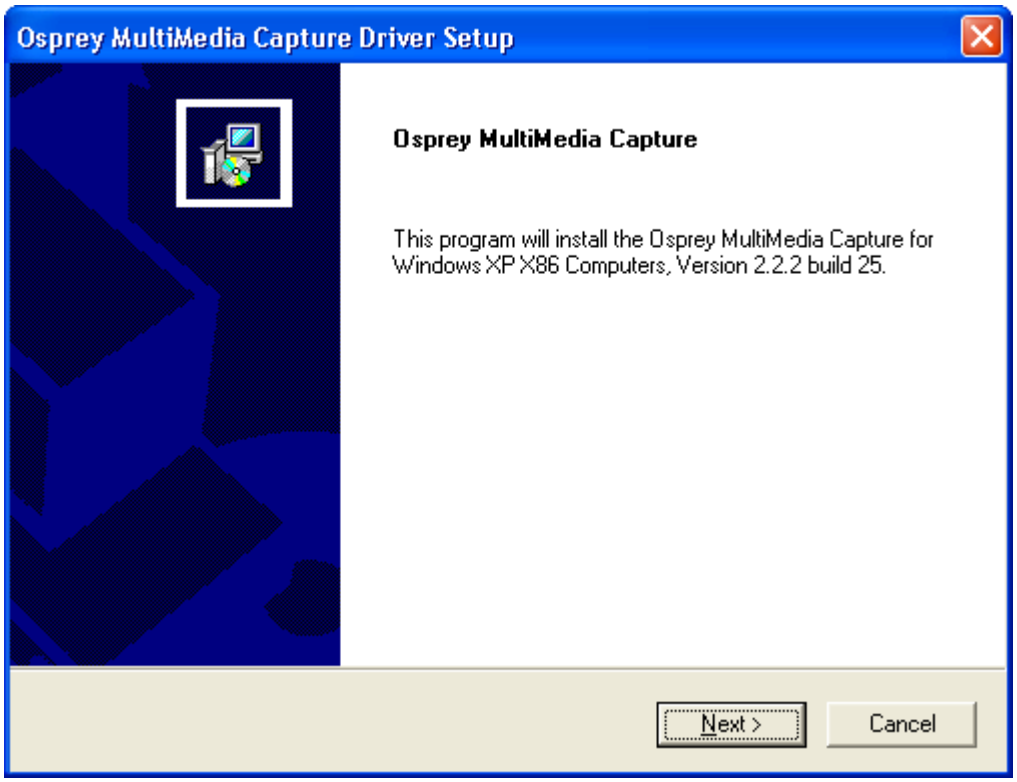
6. From the Osprey MultiMedia Capture 2.2.2 Windows XP driver installation application – Folder Creation dialog box, click **Yes** to create a temporary folder and to begin unpacking the application. See Figure 91.

Figure 91. Application Unpacking status box



7. After the Osprey MultiMedia Capture 2.2.2 Windows XP driver installation application is unpacked, the Osprey MultiMedia Capture Setup wizard is displayed. See Figure 92.

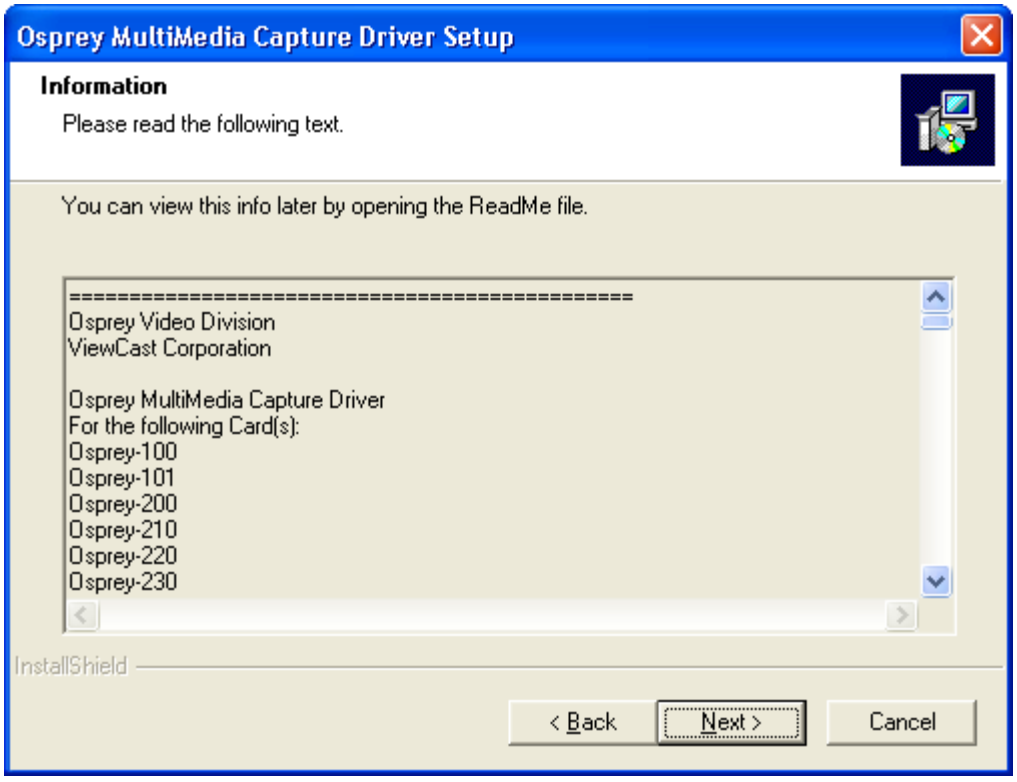
Figure 92. Osprey MultiMedia Capture Setup wizard – Introduction dialog box



8. In the Osprey MultiMedia Capture Setup wizard, click **Next** to display the Information dialog box. See Figure 93.

   The information dialog box indicates the types of cards and operating systems that the Osprey drivers are designed to operate with.

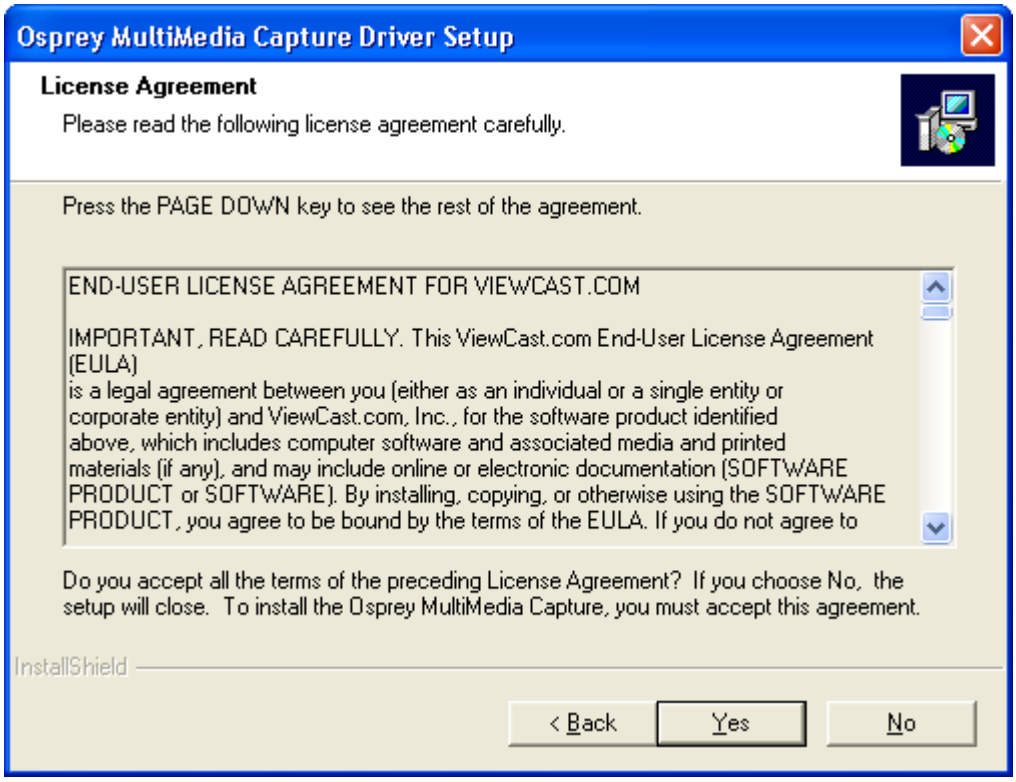
Figure 93. Osprey MultiMedia Capture Setup wizard – Information dialog box



9. Click **Next** to display the software license agreement. See Figure 94.

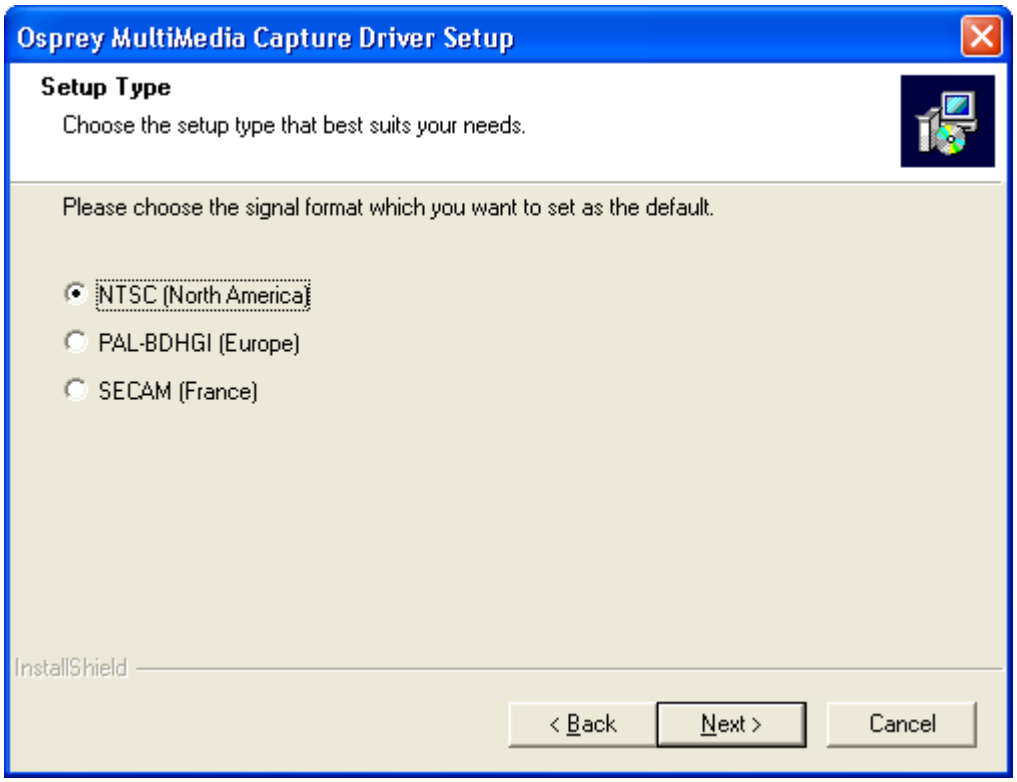
   Read the end user license agreement.

Figure 94. Osprey MultiMedia Capture Setup wizard – Software License Agreement dialog box



10. Click **Yes**. The Setup Type dialog box is displayed. See Figure 95.

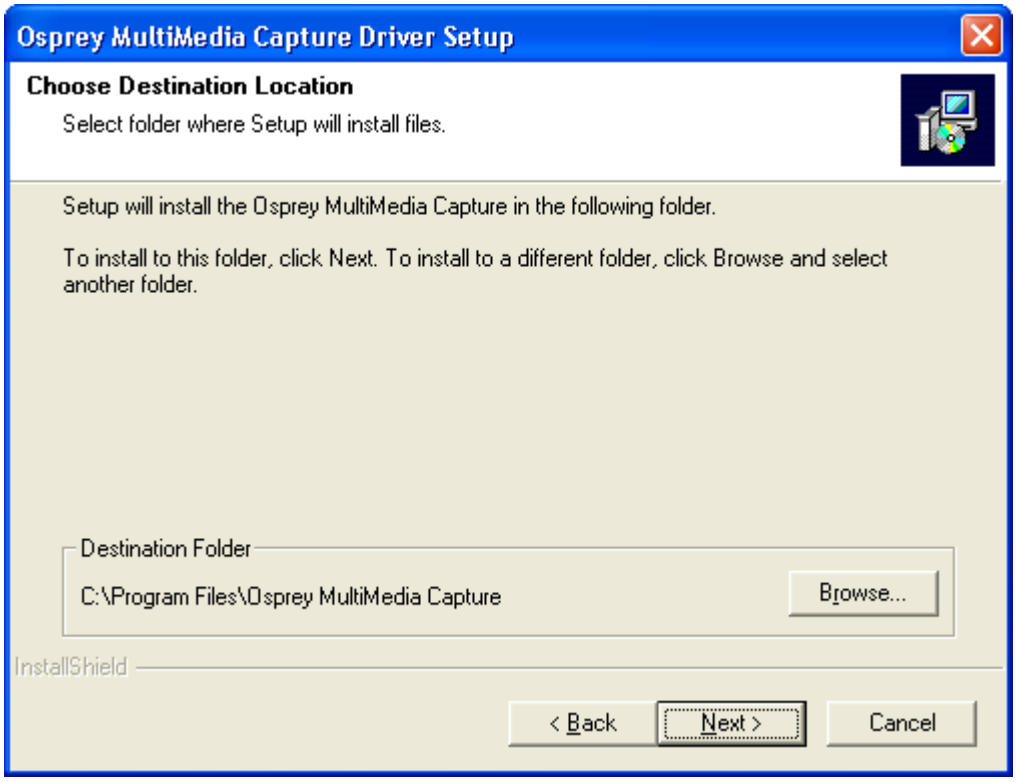
Figure 95. Osprey MultiMedia Capture Setup wizard – Setup Type dialog box



11. Ensure that the NTSC option button is selected. Click **Next** to display the Choose (driver) Destination Location dialog box. See Figure 96.

    The wizard shows the default location where the driver will be installed.

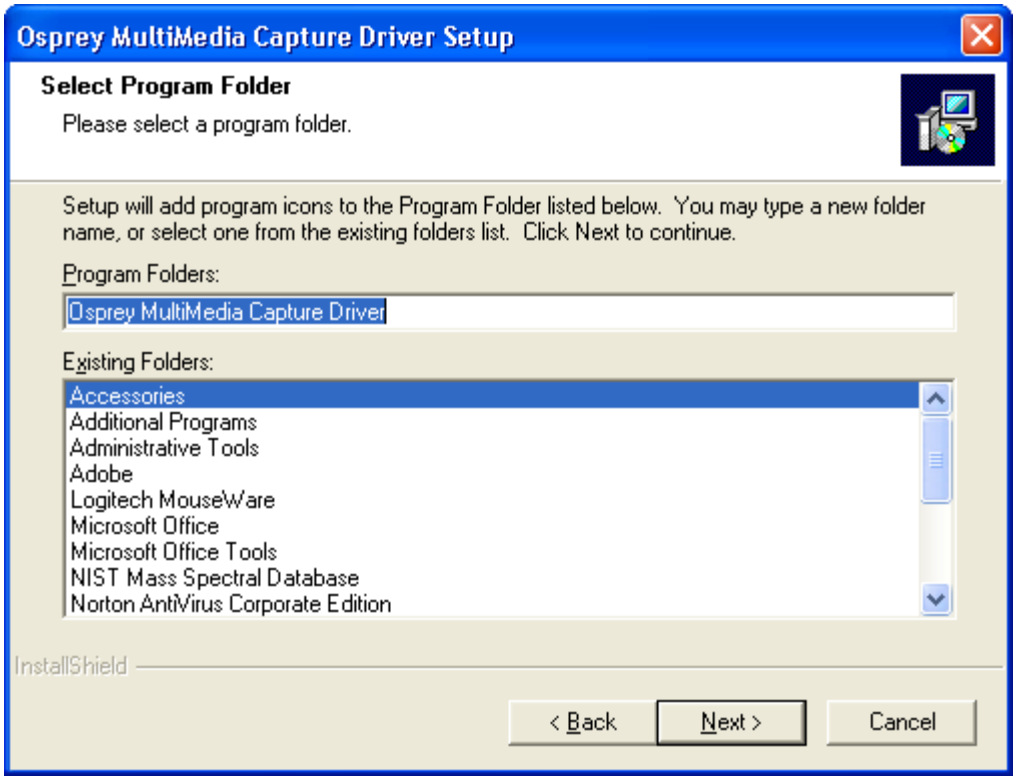
Figure 96. Osprey MultiMedia Capture Setup wizard – Choose Destination Location dialog box



12. Click **Next** to install the driver in the default location and to display the Select Program Folder dialog box. See Figure 97.

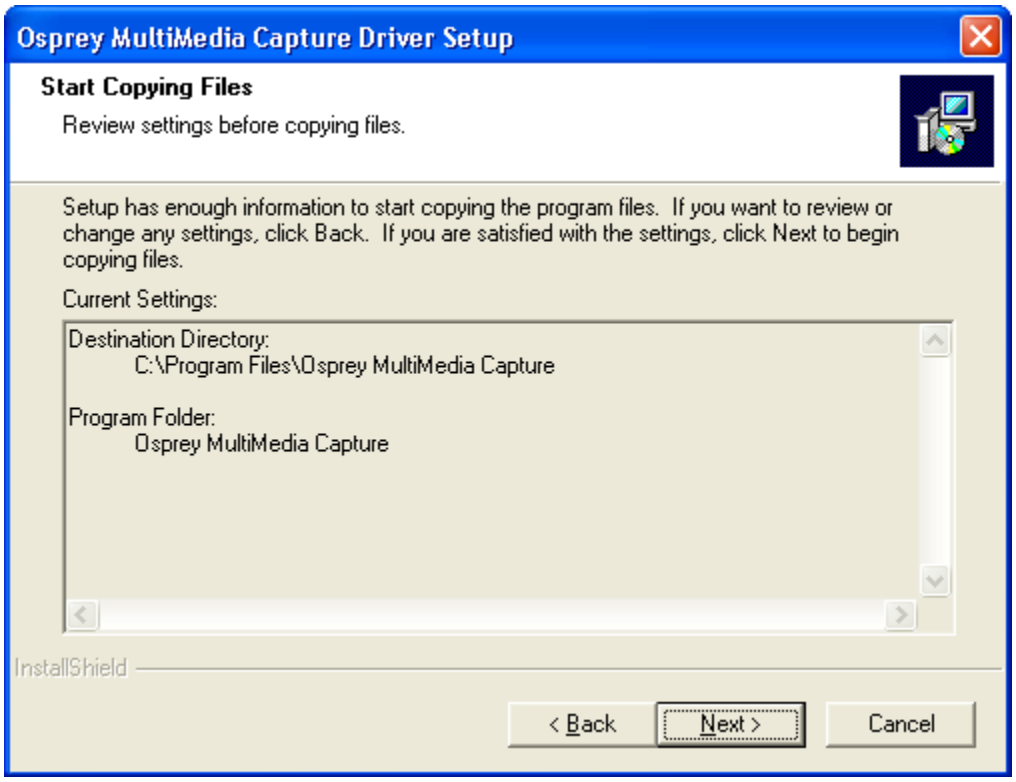
    The wizard indicates the program folder in which it will place the application shortcuts.

Figure 97. Osprey MultiMedia Capture Setup wizard – Select Program Folder dialog box



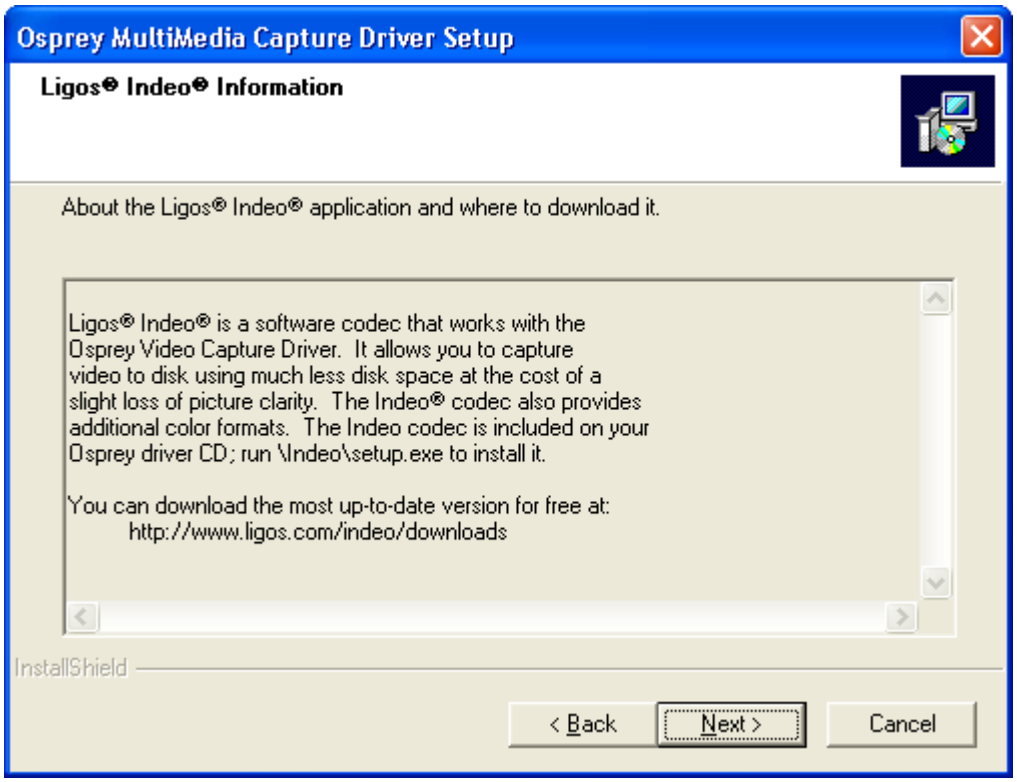
13. Click **Next** to continue. The Start Copying Files dialog box is displayed. See Figure 98.

Figure 98. Osprey MultiMedia Capture Setup wizard – Start Copying Files dialog box



14. Click **Next** to continue. An informational dialog box about codec (compression/decompression) software is displayed. See Figure 99.

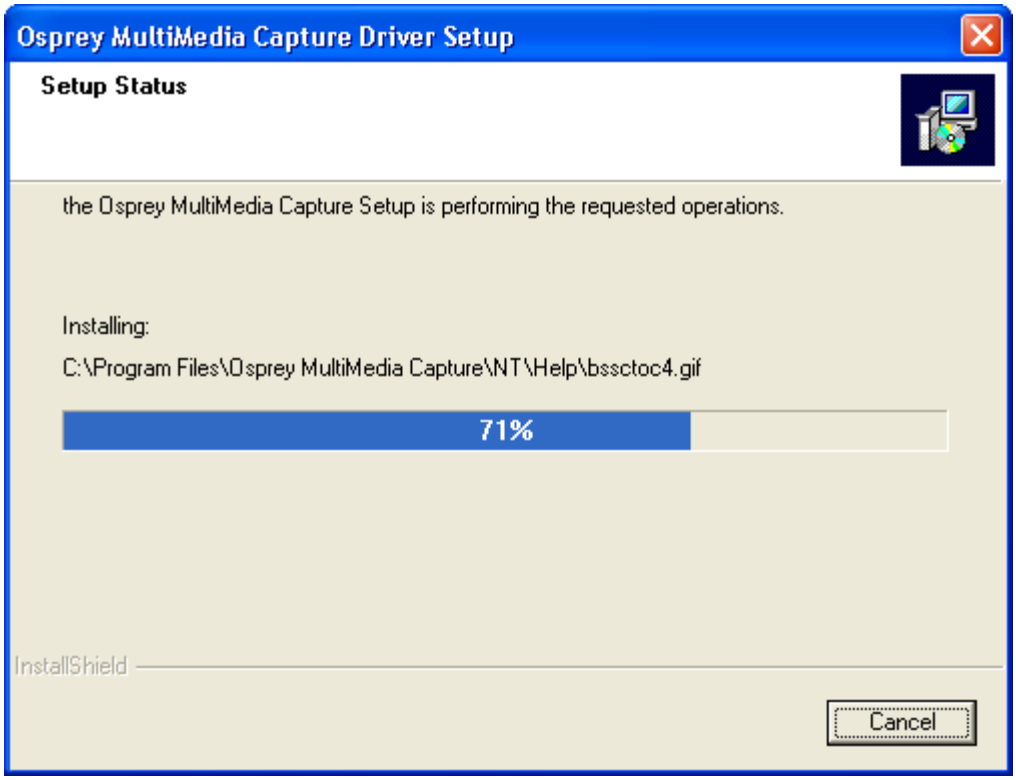
Figure 99. Osprey MultiMedia Capture Setup wizard – Information dialog box



15. Click **Next** to continue. The Setup Status dialog box is displayed. See Figure 100.

    The wizard copies the driver files onto the computer hard drive, which might take a couple of minutes.

Figure 100. Osprey MultiMedia Capture Setup wizard – Setup Status dialog box

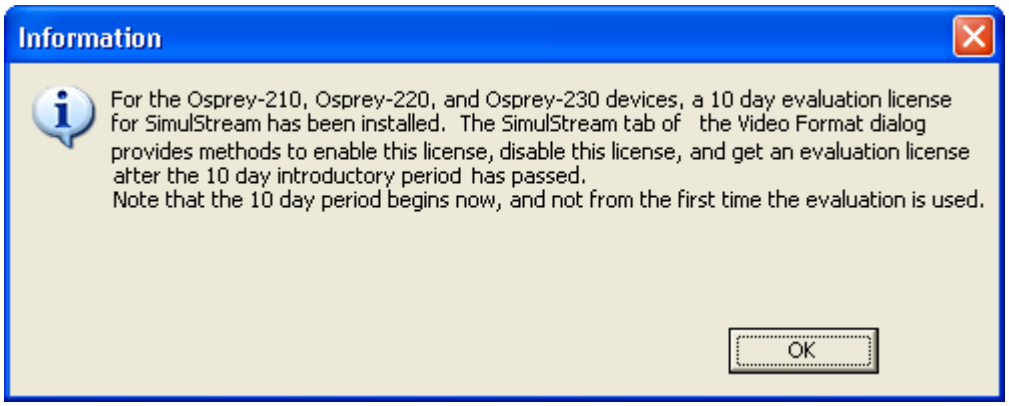


16. When the wizard finishes copying files to the hard drive, a dialog box is displayed that gives you the option to install an additional shortcut on the desktop. See Figure 101. This shortcut is not necessary; therefore, click **No**.

Figure 101. Osprey MultiMedia Capture Setup wizard – ViewCast Corporation / Osprey Video Division Special Offers Shortcut dialog box

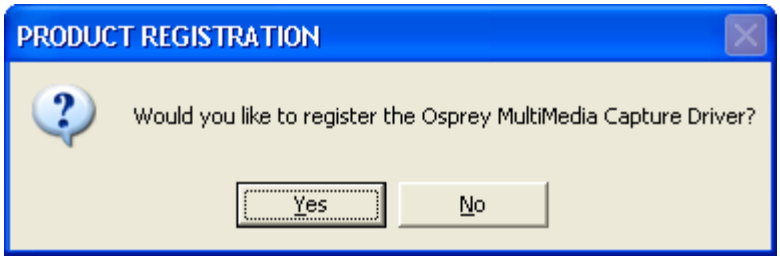
17. The wizard then displays another Information dialog box describing the terms of the SimulStream shareware that was installed on your computer. See Figure 102.

Figure 102. Osprey MultiMedia Capture Setup wizard – Information dialog box



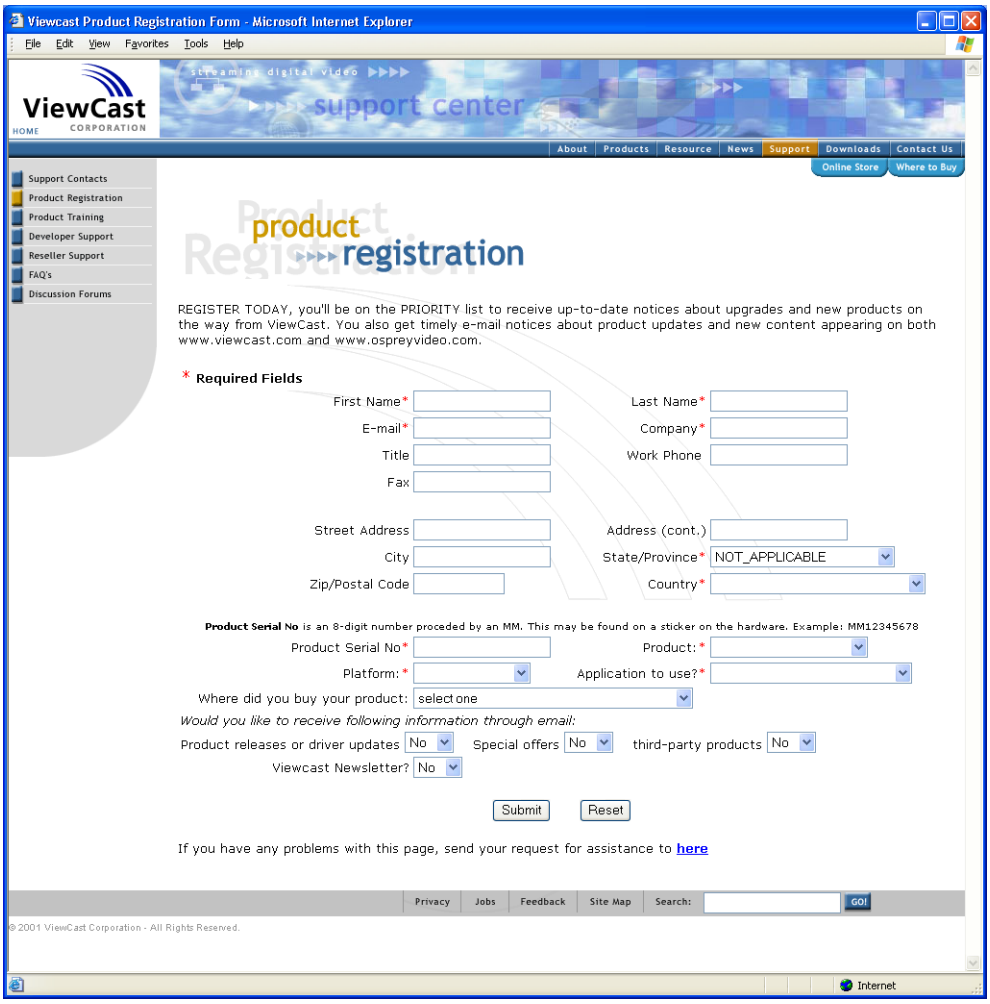
18. Acknowledge the Information dialog box by clicking **OK**. The Product Registration dialog box is displayed. See Figure 103.

Figure 103. Osprey MultiMedia Capture Setup wizard – Product Registration dialog box



19. In the Product Registration dialog box, choose **Yes** to launch the Product Registration HTML form. See Figure 104.

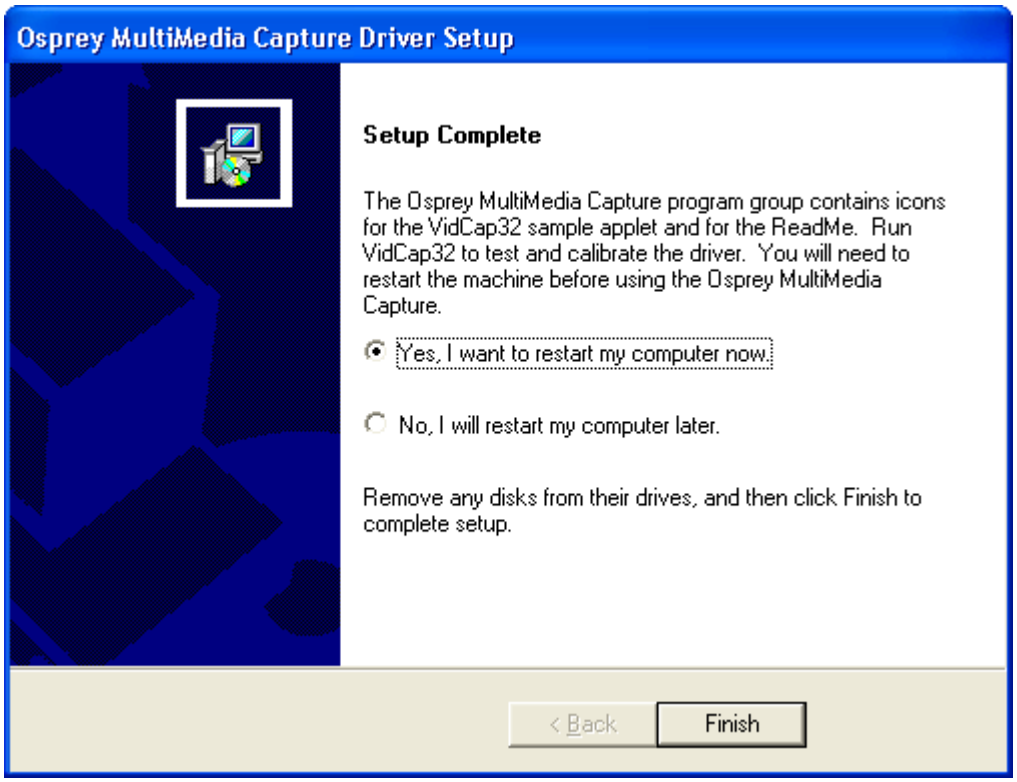
Figure 104. Product Registration HTML form



20. Complete the registration form and click **Submit** to register your software.

21. After you submit your registration, the Osprey MultiMedia Capture Setup wizard will display the Setup Complete dialog box (see Figure 105) remove any disk(s) from the computer disk drive(s) and click **Finish** to complete the setup and restart the computer.

Figure 105. Osprey MultiMedia Capture Setup wizard – Setup Complete dialog box



The Osprey MultiMedia Capture 2.2.2 Windows XP driver installation application has successfully installed the necessary software, drivers, and updates. Go to the next section, "Testing the Osprey MultiMedia Capture Application."

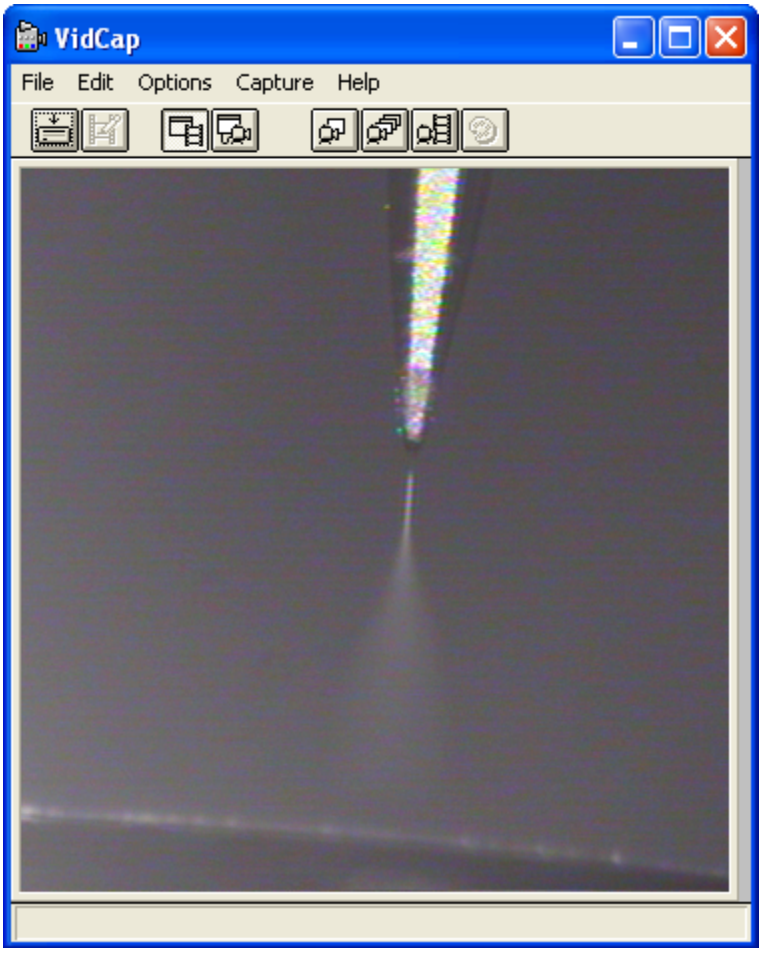
Testing the Osprey MultiMedia Capture Application

❖ **To restart the computer and check the video capture application**

1. Connect the video camera to the video capture card using a coaxial cable. The camera also needs to be connected to its power supply and turned on for the image to be viewed from the computer.

2. Start the Osprey MultiMedia Capture application to ensure proper operation of the video capture system by selecting **Start > Programs > Osprey MultiMedia Capture > SwiftCap**. See Figure 106.

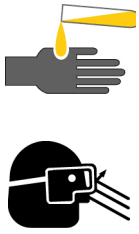
Figure 106. Osprey Video Capture application



The installation of the Osprey MultiMedia Capture system is completed successfully if you can view the nanospray tip from the Osprey MultiMedia Capture application.

Tuning Solutions for the NSI Mode

This appendix provides instructions for preparing the solution that is used to tune the mass spectrometer in the NSI mode.



CAUTION AVOID EXPOSURE TO POTENTIALLY HARMFUL MATERIALS. Always wear protective gloves and safety glasses when you use solvents or corrosives. Also, contain waste streams and use proper ventilation. Refer to your supplier's Material Safety Data Sheet (MSDS) for the proper handling of a particular solvent.

Always take safety precautions when you handle chemicals and unknown samples. **READ AND UNDERSTAND THE HAZARDS OF THE CHEMICALS USED IN THE FOLLOWING PREPARATIONS.** Dispose of all laboratory reagents by the appropriate method for a specific reagent or solvent.

Material Safety Data Sheets (MSDS) provide summarized information on the hazards and toxicity of specific chemical compounds. MSDSs also provide information on the proper handling of compounds, first aid for accidental exposure, and procedures for the remedy of spills or leaks. Producers and suppliers of chemical compounds are required by law to provide their customers with the most current health and safety information in the form of an MSDS. Read the MSDSs for each chemical you use.

Examples of potentially hazardous chemicals used in procedures throughout this manual are as follows:

- Acetic acid
- Formic acid
- Methanol

Contents

Tuning Solution for LCQ Series and LTQ Series Mass Spectrometers

The recommended tuning solution for a LCQ Series or LTQ Series mass spectrometer configured with an NSI source is either 1 to 5 pmol/µL MFRA in 1% acetic acid methanol /water 50:50 or a tuning solution with a sample concentration of 1 to 5 pmol/µL.

To prepare a solution of 5 pmol/µL MFRA in 1% acetic acid methanol /water, perform the procedures provided in this section in the order listed:

- Preparing the MRFA Stock Solution
- Preparing the MRFA Working Solution for NSI/MS

Preparing the MRFA Stock Solution

The concentration of the MRFA stock solution is 5.0 nmol/mL MRFA in 50:50 methanol/water.

❖ To prepare 1 mL of the MRFA stock solution

1. Obtain the vial of L-methionyl-arginyl-phenylalanyl-alanine acetate•H_2O (MRFA) in your API accessory kit. In this form, the MRFA sample has an average molecular weight of 607.7 u. Carefully weigh 2.6 mg of the MRFA sample.
2. Make up the MRFA solution to a total volume of 1.0 mL with a mixture of 50:50 methanol / water. Mix the solution thoroughly.
3. Label the vial *MRFA Stock Solution*.

Note Do not inject the MFRA stock solution into the mass spectrometer while it is configured with an NSI source.

Preparing the MRFA Working Solution for NSI/MS

The concentration of the MRFA working solution is 5.0 pmol/µL MRFA in 1% acetic acid methanol / water 50:50.

❖ To prepare 100 mL of MRFA working solution

1. Pipet 0.1 mL of the MRFA stock solution (5.0 nmol/µL) into a 100 mL volumetric flask.
2. Dilute to volume with 1% acetic acid in 50:50 methanol / water.
3. Label the flask *MRFA Tuning Solution for Nanospray*.

Tuning Solution for TSQ Series Mass Spectrometers

This section contains two procedures for preparing solutions of polytyrosine – 1, 3, 6 that are suitable for testing the performance of a TSQ Series mass spectrometer configured with an NSI source.

The first procedure describes how to reconstitute the performance test solution using the 20-mL vial that contains pre-weighed amounts of the polytyrosine components in dry powder form (P/N 00301-22925). The contents of the vial is so small that it has the appearance of a residue. This vial is supplied in the accessory kit.

The second procedure describes how to prepare the performance test solution from your stock of dry chemicals.

Your accessory kit also contains a 20-mL vial of polytyrosine – 1, 3, 6 in solution (P/N 00301-22924). You need to dilute this solution 100-fold before you infuse it into your NSI/MS system.

Table 16 provides a summary of the polytyrosine standards supplied in the accessory kit.

Table 16. Polytyrosine standards supplied in the accessory kit

Standard Description (on label)	Thermo Scientific Part Number	C S Bio Company Product Number
Polytyrosine Standard Liquid Form	00301-22924	CS0272L
Polytyrosine Standard Solid Form	00301-22925	CS0272S

Reconstituting the Polytyrosine – 1, 3, 6 Solution Using the Prepackaged Mixture

Polytyrosine is provides as a prepackaged mixture ((P/N 00301-22925).

❖ To reconstitute the polytyrosine – 1, 3, 6 solution

1. Obtain the vial of premixed polytyrosine chemicals from the accessory kit.
2. Prepare a concentrated stock solution of polytyrosine as follows:
   a. Add 20 mL of 0.1% formic acid in 50:50 methanol / water to the vial. Then, swirl the vial to dissolve the polytyrosine residue.

      This yields a solution of 4 ng/µL of Tyr, 12 ng/µL of $(Tyr)_3$, and 24 ng/µL of $(Tyr)_6$.

   b. Label the vial *Polytyrosine – 1, 3, 6 Nanospray Stock Solution*.

Note Do not inject the stock solution into your mass spectrometer while it is configured with an NSI source.

3. Prepare the 20 mL of working test solution as follows:
   a. Dilute the stock solution 100 fold with 0.1% formic acid in 50:50 methanol / water.
   b. Label the vial *Polytyrosine – 1, 3, 6 Nanospray Working Solution*.
4. Store the test solutions in a refrigerator until needed.

Preparing the Polytyrosine – 1, 3, 6 Tuning Solution Your Stock of Dry Chemicals

You can prepare the polytyrosine – 1, 3, 6 tuning solution from your stock of dry chemicals.

❖ **To prepare 250 mL of the polytyrosine – 1, 3, 6 tuning and calibration solution**

1. Weigh out and deliver 1 mg of L-tyrosine, 3 mg of $(Tyr)_3$, and 6 mg of $(Tyr)_6$ into a clean, dry, 250 mL volumetric flask.
2. Add approximately 50 mL of 0.1% formic acid in 50:50 methanol / water to the volumetric flask. Swirl the flask to dissolve the polytyrosine mixture. Then, fill the flask to volume. This yields a solution containing 4 ng/µL of L-tyrosine, 12 ng/µL of $(Tyr)_3$, and 24 ng/µL of $(Tyr)_6$.
3. Transfer the solution to a clean vial labeled *Polytyrosine – 1, 3, 6 Tuning and Calibration Solution*, and store it in a refrigerator until it is needed.

Table 17 summarizes the compounds used to prepare the tuning and calibration solution.

Table 17. Polytyrosine tuning and calibration stock standard summary

Compound	Formula	MW	Vendor	Vendor P/N
L-Tyrosine	$C_9H_{11}NO_3$	181.19	Sigma	T8566
Tyr-Tyr-Tyr	$C_{27}H_{29}N_3O_7$	507.54	Sigma	T2007
$(Tyr)_6$	$C_{54}H_{56}N_6O_{13}$	997.07	Sigma	T1780

Note You can order standard chemicals directly from Thermo Scientific, San Jose, or you can contact these chemical suppliers:

Sigma Chemical Company
P.O. Box 14508
St. Louis, MO, USA 63178-9916
(800) 325-3010 (in the USA or Canada)
[1] (314) 771-3750 (outside the USA or Canada)

C S Bio Company
1300 Industrial Road
San Carlos, CA, USA 94070
(800) 627-2461 (in the USA or Canada)
[1] (650) 802-0880 (outside the USA or Canada)

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