

Agilent G1992A Nanospray Ion Source

User Guide



Agilent Technologies

Notices

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

In This Guide...

This guide contains information to install and set up the Nanospray Ion Source.

1 Installation

In this chapter you install the source and prepare the system for analysis.

2 Mass Reference Solution

This chapter describes how to use the Mass Reference Solution to calibrate the Nanospray Ion Source.

3 Safety

This chapter contains safety information.

4 Reference

This chapter contains parts lists and other reference information.

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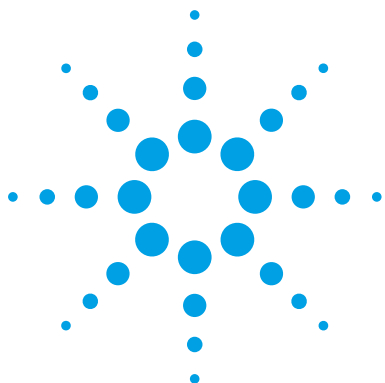
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Upgrade Preparation

The installation kit contains several upgrade kits for older instruments:

- gas distributor (p/n G1964-20303)
- spray shield kit (p/n G1988-60007)

If you have an installed instrument, inspect the instrument to determine which of these kits need to be installed. These parts are already installed on new instruments.

You also need to adjust the position of the [Counter Electrode](#). A 1.5 mm hex wrench, and a 7 mm spacing tool are provided for setting the distance of the [Counter Electrode](#) from the spray shield.

To remove the ESI or Agilent Jet Stream source

NOTE

Autotune is not supported with the Agilent G1992A Nanospray Ion Source. Before changing the source, do all tuning with the ESI or Agilent Jet Stream source. Calibration can still be done with the Nanospray Ion Source.

- 1 Put the instrument into standby mode and lower the source temperatures to start cooling down the source.
- 2 When the source is cool, turn off the nebulizer, drying gas, and sheath gas flow (if using the Jet Stream source).
- 3 Remove the existing source, install the protective cover and store in a safe place.
- 4 Remove the spray shield and store with the source.

To upgrade the gas distributor

Newer model gas distributors are required to support the improved resistive or dielectric capillary. If your gas distributor looks like the one in [Figure 1](#), replace it with the new one.

- 1 Remove the chamber and capillary high voltage cable leads from the T-nut connectors.

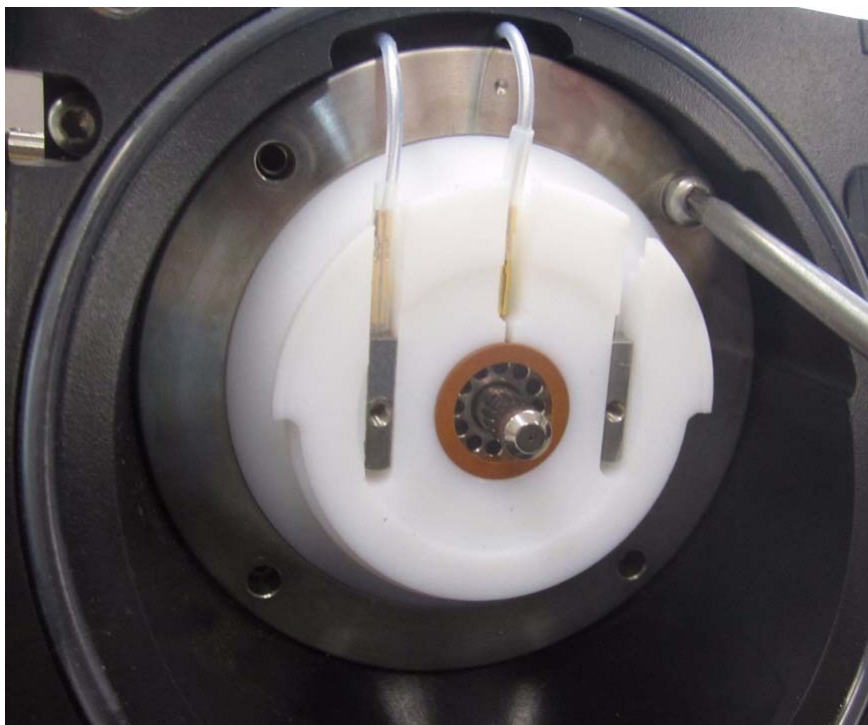


Figure 1 Remove the four T20 Torx screws from the metal ring.

- 2 Remove the four T-20 torx screws used to secure the metal ring that holds the PTFE spray shield support block in place.
- 3 Remove the PTFE spray shield support block to access the vespel gas distributor. Remove and discard the old gas distributor.

1 Installation

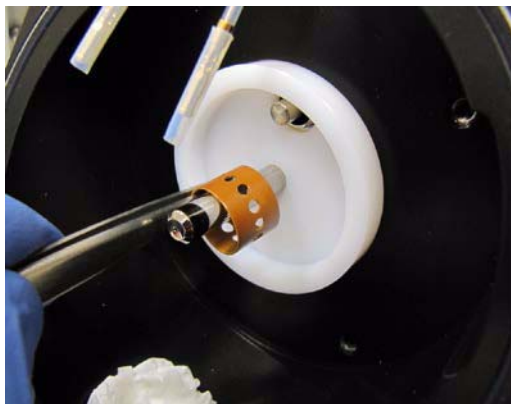


Figure 2 Remove the old gas distributor

- 4 Install the new [gas distributor \(p/n G1964-20303\)](#) with the solid side facing the PTFE wall. Position it so that it is flush with the PTFE plate.



Figure 3 G1964-20303 Gas Distributor

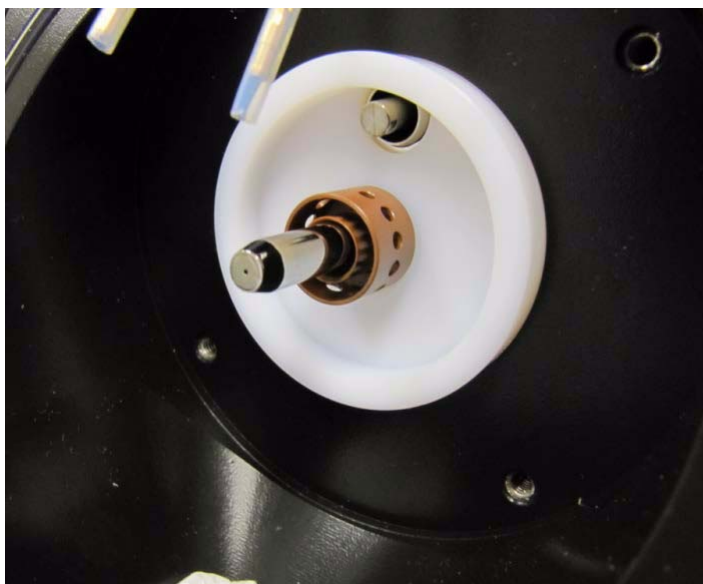


Figure 4 Install the new gas distributor

- 5** Reinstall the PTFE end plate support block with the stainless steel ring. Make sure that the new gas distributor is positioned properly before installing and tightening the screws to avoid damage to the new gas distributor.

To upgrade the stainless steel end plate

If the screws that hold the end plate in position are pan heads, install the [Nanospray Desolvation Upgrade Kit \(p/n G1988-60009\)](#). This upgrade reduces any arcing that can occur to the raised head screws.

Before you begin, make sure that you have the parts for [Nanospray Desolvation Upgrade Kit \(p/n G1988-60009\)](#) listed in [Table 7](#) on page 62.

- 1 Remove the end plate and the attached screws.
- 2 Remove the two T-nut high voltage connectors from the white PTFE block.
- 3 If the existing T-nut connectors are damaged, install the two new [T-nut connectors](#) into the PTFE block.
- 4 Install the new [stainless steel end plate](#). Use the two new [gold screws](#) to secure the end plate. Do not over-tighten these screws!

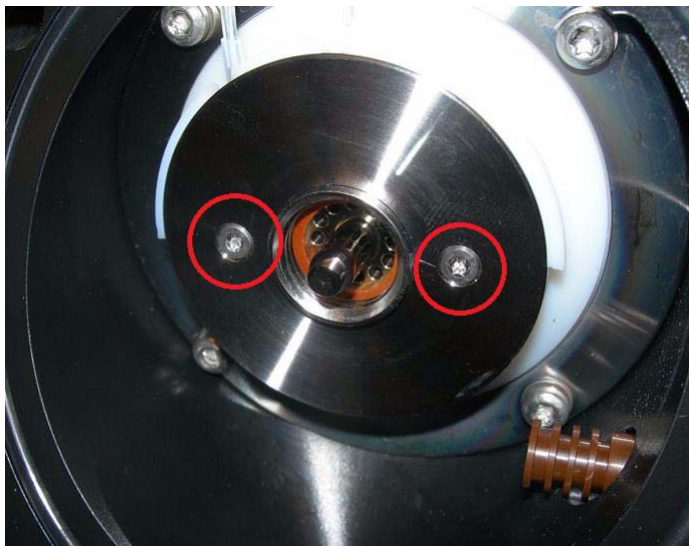


Figure 5 Flat head gold screws installed on new stainless steel end plate

To assemble and install the new spray shield and gas diverter assembly

Before you begin, make sure that you have the parts for the [Spray Shield Kit \(p/n G1988-60007\)](#) listed in [Table 8](#) on page 62.

- 1 Slide the [Wave washer](#) over the appropriate spray shield:
 - [Single bore spray shield](#) for single bore capillary in orthogonal orientation
 - [Single bore inline spray shield](#) for inline needle orientation
 - [Multibore vortex spray shield](#) for multibore capillary
- 2 Insert the [Radial gas diverter](#) insert into the spray shield. Make sure to align the pin in the [Radial gas diverter](#) with the notch on the End Cap.



Figure 6 Gas diverter pin aligns with the spray shield groove

- 3 Thread the vespel [Radial gas diverter](#) vespel into the [Winged spray shield nut](#). Make sure to thread the [Radial gas diverter](#) completely into the [Winged spray shield nut](#).

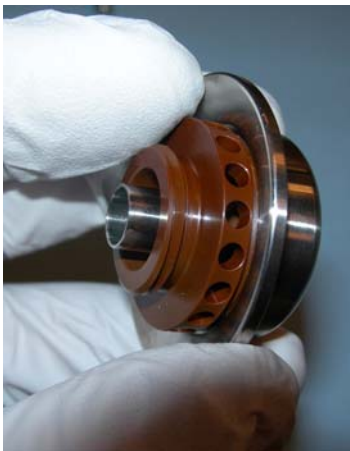


Figure 7 Radial gas diverter and winged nut

- 4 After all of the parts are assembled, rinse all of these parts with Isopropanol in a clean glass beaker at least three times.
- 5 After the parts are rinsed, remove the parts from the beaker and place them on a lint free cloth.
- 6 Insert the [Contact spring](#) into the cleaned spray shield assembly.



Figure 8 Contact spring installed onto spray shield assembly

- 7 Install the spray shield.



Figure 9 Installed spray shield assembly. (Multibore vortex spray shield shown)

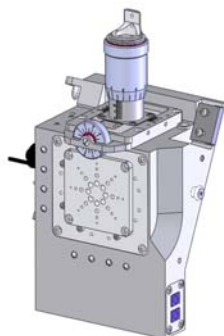
- 8 Verify that the desolvation chamber end plate screws have good clearance to the end plate.

WARNING

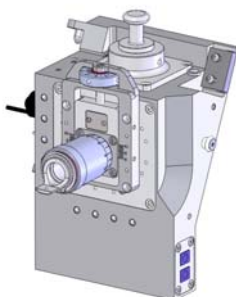
If the screw heads are too close to the winged spray shield nut, arcing can occur.

Installation

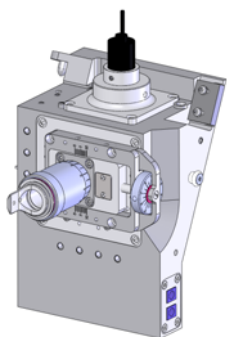
The source can be configured in three ways:



Orthogonal Orientation Sample enters from above. The needle is at right angles to the MS capillary. Adjustment is along Z and Y axes. Camera is to the left, viewing the spray orthogonally.

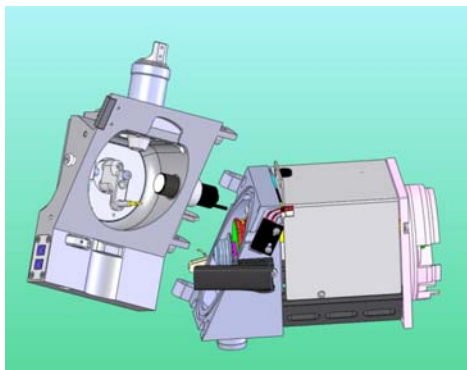


Inline Horizontal Orientation Sample enters from the front. The needle is parallel with the capillary. Needle movement is along Z and Y. The camera is mounted to the left, viewing the needle movement orthogonally. In this orientation, the camera plate is located over the top hole of the chamber. The camera plug covers the opening.

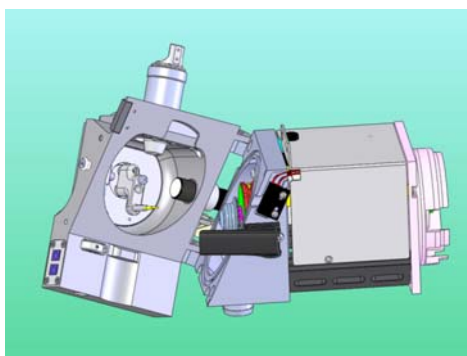


Inline Sideways Orientation Sample enters from the front. The needle is parallel to the capillary. Needle movement is forward and side-to-side (Z-X). The camera adapter plate is located over the top hole of the chamber, and the camera is mounted above, viewing the needle movement orthogonally. The camera plug is inserted into the hole vacated by the camera.

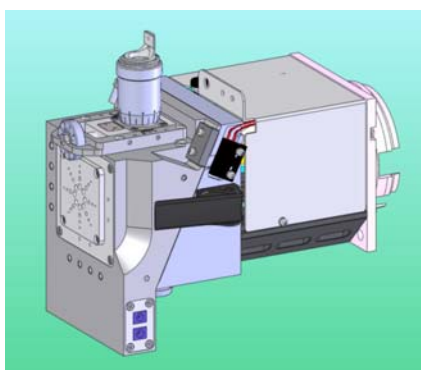
To install the source



- 1 Align the hinge pins with their respective holes on the desolvation assembly. The source must be at an angle of less than 90 degrees with respect to the desolvation chamber.



- 2 Slide the hinge pins down into their seated position, and rotate the source into contact with the desolvation chamber.



- 3 Latch the chambers together.

To set the position of the Counter Electrode

For best orthogonal Nanospray Ion Source operation, the [Counter Electrode](#) needs to be about 7 mm away from the end of the winged spray shield cap.

WARNING

The instrument must be in Standby mode or turned off to do this step. Otherwise, the spray chamber high voltage electronics will be energized and present a shock hazard.

Before you begin, make sure that you have:

- electrode spacing tool (p/n GT430-20358)
- 1.5 mm hex wrench

- 1 Loosen the four recessed Torx T10 screws which attach to the spray chamber body.
- 2 Remove the [Nanospray Slide Assembly](#).

Removal of the [Nanospray Slide Assembly](#) creates a larger hole for better access to the [Counter Electrode](#).

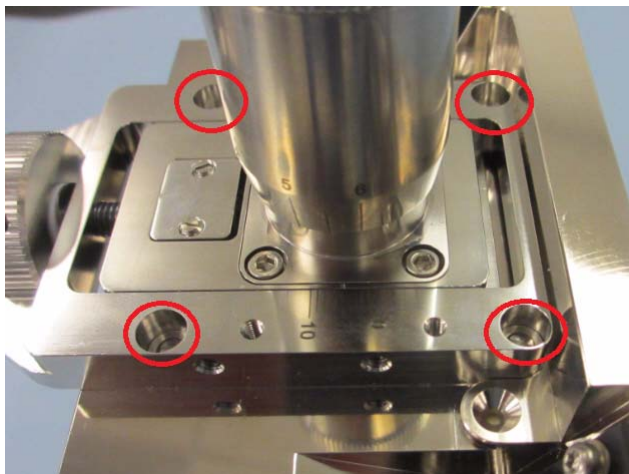


Figure 10 Loosen the 4 screws to remove the [Nanospray Slide Assembly](#).

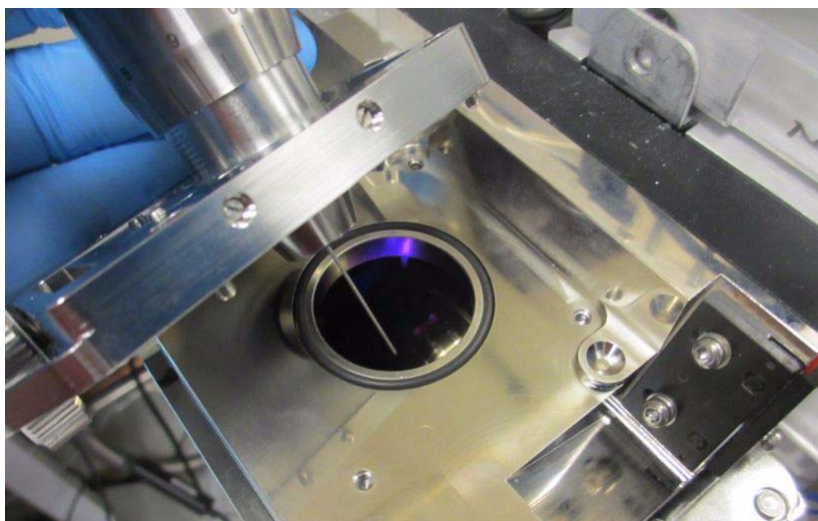


Figure 11 Nanospray Slide Assembly removed.

- 3 Loosen the set screw for the Counter Electrode using the 1.5 mm hex wrench

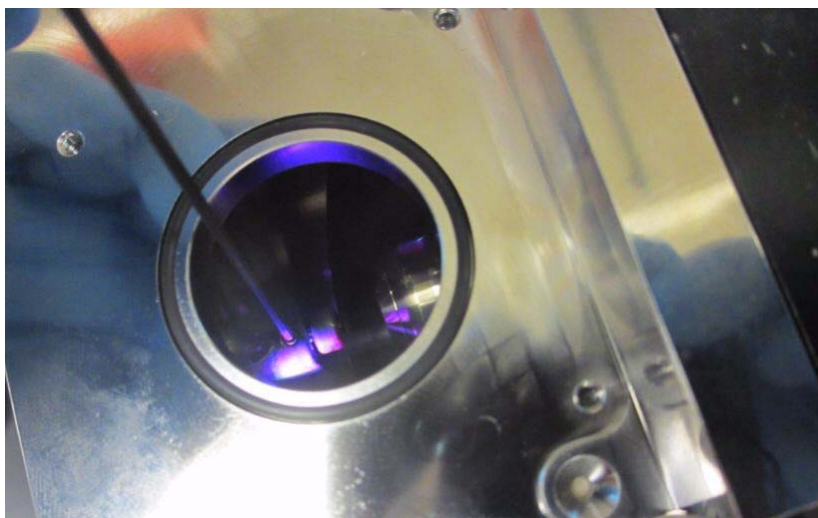


Figure 12 Removing the Nanospray Slide Assembly provides easier access to the set screw.

- 4 Insert the electrode spacing tool (p/n GT430-20358) between the Counter Electrode and the spray shield.

The tool must touch both the Counter Electrode and the spray shield for correct positioning. Lightly tighten the set screw to secure the position of the Counter Electrode.

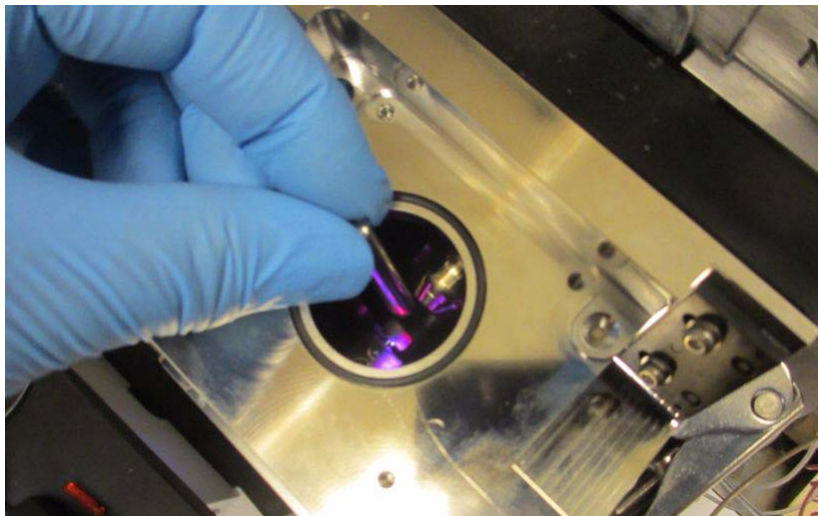


Figure 13 The electrode spacing tool touches both the spray shield and the Counter Electrode.

- 5 After tightening the set screw, make a final check with the electrode spacing tool.

The Counter Electrode must be properly positioned to generate proper spray.

- 6 Reinstall the Nanospray Slide Assembly.

To set up MassHunter program for the Nanospray Ion Source

- 1 In the MassHunter Acquisition program, make sure that the source type is set to **HPLC-Chip** in the Tune Panel.
- 2 Set the drying gas flow and temperature parameters. See [Table 1](#).
Operating the source with parameters above these set points can result in reduced performance, and can lead to frequent needle clogs.

Table 1 Maximum drying gas temperature and flow rate

| | Maximum Drying Gas Temperature | Maximum Flow Rate |
|---|--------------------------------|-------------------|
| I-Funnel instruments with Hexabore capillaries | 225°C | 15 L/minute |
| Non I-Funnel instruments with single bore capillaries | 365°C | 8 L/minute |

- 3 Set the **VCap** voltage to **900** volts.

To install the power cord and camera

- 1** Plug in the power source and camera power cords:
 - a** Locate the power contacts on the lower-right of the spray chamber.

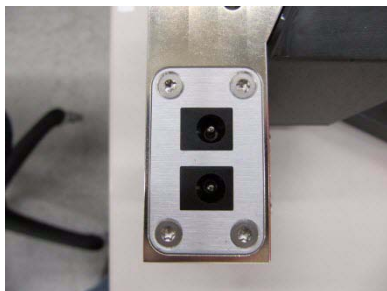


Figure 14 Power contacts

- b** Plug the camera power cord into either of the two power contacts.
 - c** Plug the 12V DC power supply for the source into the remaining power contact. Plug the other end to the wall outlet.

The internal LED light glows with a red light. The fan at the rear of the source turns on.
- 2** Install the video capture card into the computer and load the software. Follow the manufacturer's instructions.
- 3** Connect the camera to the video capture cards:
 - a** Connect the yellow male RCA plug from the video capture card to one female end of the BNC adapter (p/n 1250-3410).
 - b** Connect the yellow female BNC plug from the camera to the BNC adapter.

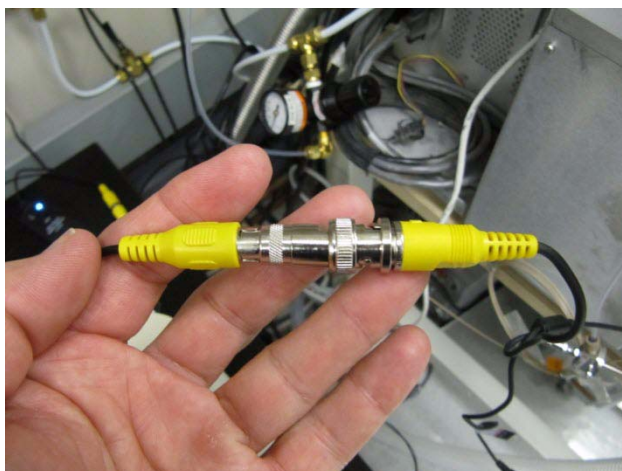


Figure 15 BNC adapter inserted between the camera plug (right) and the video capture card cable.

4 Ground the [Needle Sleeve Assembly](#):

- a** Attach one end of a push-clip ground line to the [Needle Sleeve Assembly](#).
- b** Attach the other end to a convenient ground location, such as a ground lug on the source.

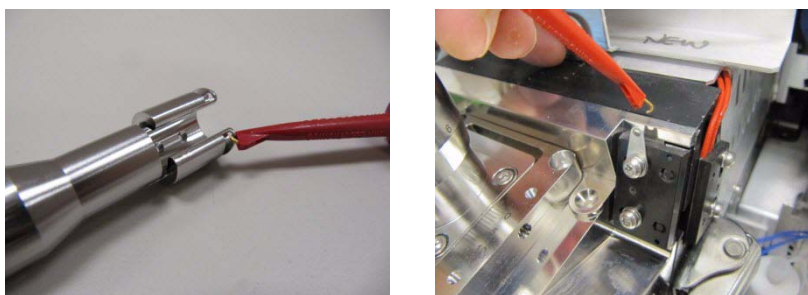


Figure 16 Push-clip ground line attached to the [Needle Sleeve Assembly](#) (left) and a ground lug on the source.

All of the external components of the Nanospray Ion Source are electrically grounded. However, additional grounding can improve performance.

5 Install the camera:

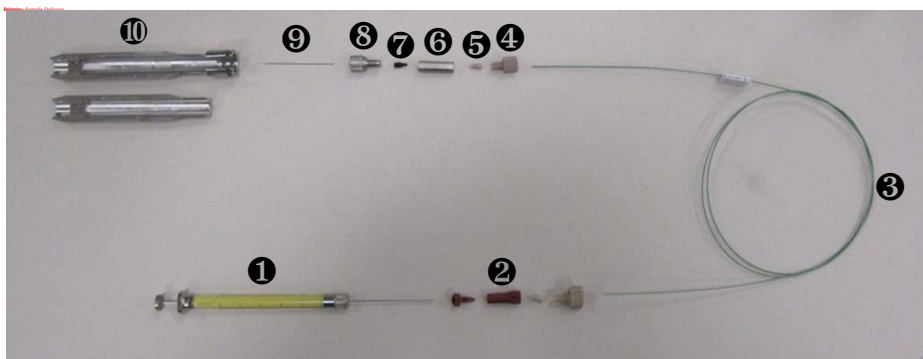
- a** Insert and rotate the Camera Ring (with the camera) until the detent on the ring snaps into alignment with the spring plunger on the source.
- b** Run the video capture software, until you get an image of the source interior.
- c** Loosen the setscrew on the Camera Ring using a 1.5mm hex wrench, and align the camera image so that the capillary is to the left.
- d** Focus the image by moving the camera in and out of the Camera Ring. You can temporarily install a [Needle Sleeve Assembly](#) and carefully focus on the needle.
- e** Lightly retighten the set screw on the Camera Ring. With the camera already focused in this way, the image stays aligned and in focus even when the camera is moved into its second location, in the Camera Plate on the top of the source, while doing inline experiments.

To assemble the Infusion Sample Delivery System

This task describes how to assemble and install an infusion sample delivery system. The assembly is used to introduce tuning mix for LC/MS calibration.

WARNING

Use extreme caution when setting up experiments with any type of needle, especially conductive ones. Touching a high voltage region inside the Nanospray Ion Source chamber with a conductive needle is extremely hazardous.



- 1 Connect the [LC-MS Connection Capillary](#) to the [Syringe Adapter](#) ②.
- 2 Install the other end of the [LC-MS Connection Capillary](#) ③ into the [Stainless Steel Zero Dead Volume Fitting](#) ⑥. Use the [Nut](#) ④ and [F113 Ferrule \(0.8mm ID\)](#) ⑤.
- 3 Install the [Needle](#) ⑨ into the [Conductive Needle Nut](#) ⑧ and [Conductive Ferrule](#) ⑦.
- 4 Loosely connect the [Needle](#) ⑨ into the [Stainless Steel Zero Dead Volume Fitting](#) ⑥.
- 5 Mount the [Syringe](#) ① to the syringe pump. Set the flow rate of the syringe pump to 18 $\mu\text{L}/\text{hour}$, and start the flow.
If you are not using a syringe pump, activate the syringe plunger by hand.
- 6 Push the plunger into the [Syringe](#) slowly and carefully until a small droplet of calibrant liquid begins to show at the needle tip.
- 7 Install the needle/union assembly into the [Needle Sleeve Assembly](#) ⑩.

To infuse solution through the Infusion Sample Delivery System

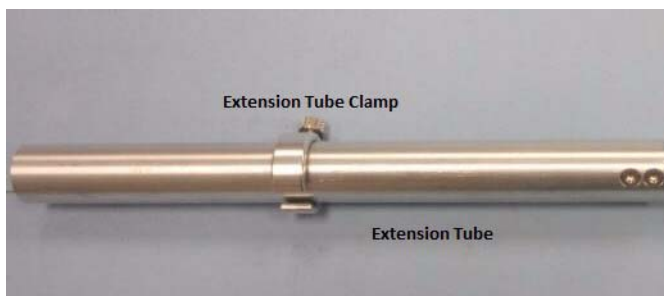
- 1** Draw calibrant solution into the [Syringe](#).
- 2** Connect the [Syringe](#) to the [Syringe Adapter](#) at the end of the assembled [Infusion Sample Delivery System](#).
- 3** Insert the [Needle Sleeve Assembly](#) into the [Nanospray Slide Assembly](#). See “To install the Needle Sleeve Assembly into the Nanospray Ion Source” on page 32.

To assemble the Extension Tube column support assembly for LC analysis

The LC transfer line is attached to the analytical column, which is attached to a needle assembly. The needle assembly is held in the [Needle Sleeve Assembly](#). The [Needle Sleeve Assembly](#) is inserted into the Nanospray Ion Source through the [Nanospray Slide Assembly](#). The extension tube adds support for the column.

Before you begin, make sure that you have:

- [Extension Tube](#)
- [Tube Clamp](#)
- T-8 Torx screws (p/n 0515-2975)



- 1 If the [Extension Tube](#) is not already attached to the [Needle Sleeve Assembly](#), use two T-8 Torx screws (p/n 0515-2975) to attach the [Extension Tube](#) onto the [Needle Sleeve Assembly](#).



1 Installation



Figure 17 Extension tube attached to the Needle Sleeve Assembly.

2 Attach the analytical column to the needle.



Figure 18 Analytical column attached to the needle, seated in the Needle Sleeve Assembly.

- 3 Attach the analytical column to the LC transfer line.

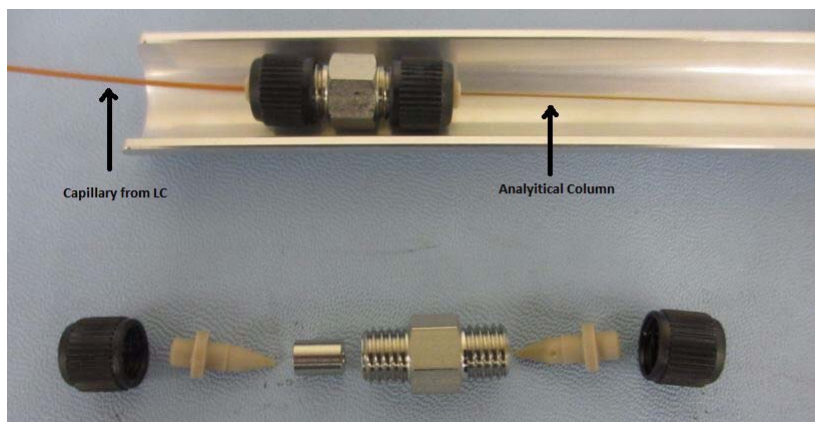


Figure 19 Analytical column attached to the LC transfer line.

- 4 Attach the **Tube Clamp** to the **Nut**. The **Tube Clamp** adds support to the column.



Figure 20 Tube Clamp attached to the Nut.

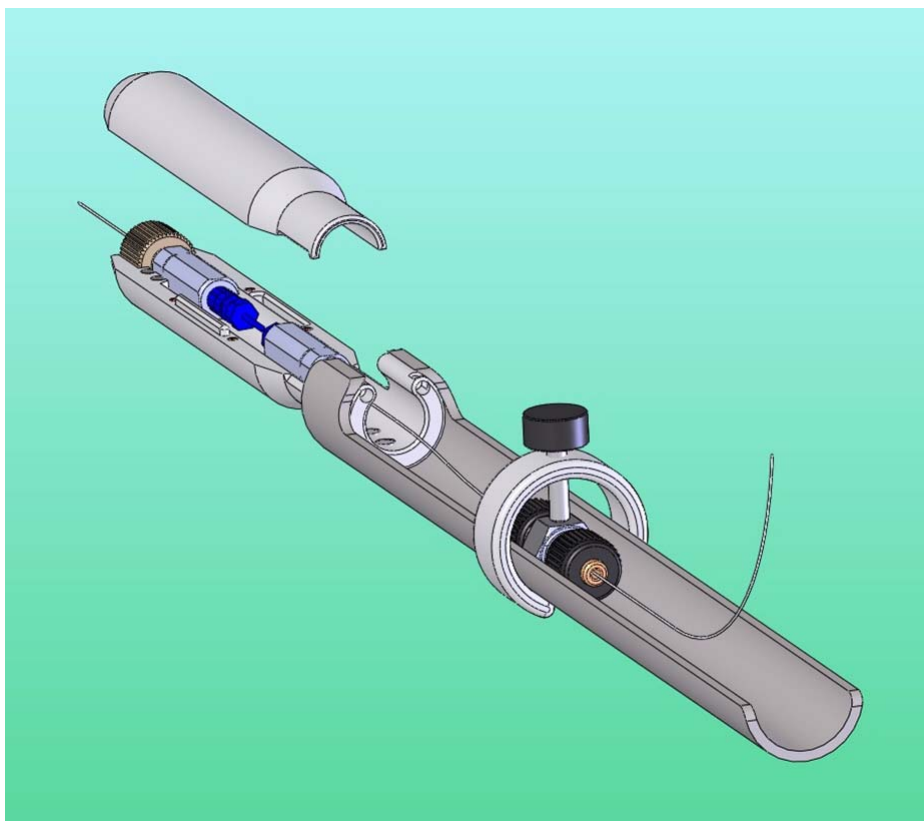


Figure 21 Completed [Extension Tube](#) column support assembly.

5 Install the [Nanospray Slide Assembly](#).

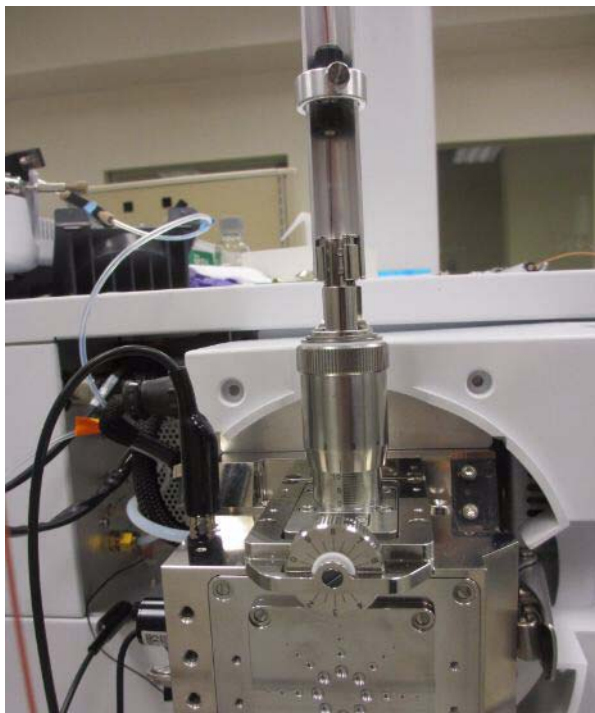


Figure 22 [Extension Tube](#) column support assembly installed in the orthogonal configuration.

If you need to change the orientation of the [Nanospray Slide Assembly](#) for inline analysis, see “[To install the Nanospray Slide Assembly for inline analysis](#)” on page 36

To install the Needle Sleeve Assembly into the Nanospray Ion Source

- 1 Start the video capture software so that the camera display is shown on the computer monitor.
- 2 Position the [Nanospray Slide Assembly](#) (on top of the Nanospray Ion Source) at the midway point of its travel length. You do this step to make sure that the [Needle Sleeve Assembly](#) does not hit the spray shield or the [Counter Electrode](#) when the needle is inserted into the source.

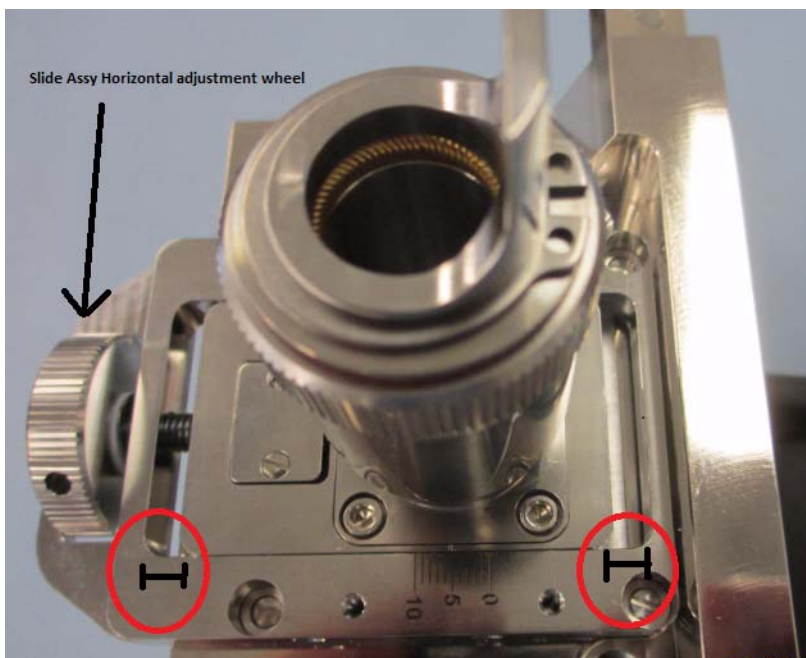


Figure 23 Before inserting that the [Needle Sleeve Assembly](#), position the [Nanospray Slide Assembly](#) mid-way between the spray shield and the [Counter Electrode](#). This action prevents damage to the needle when it is inserted into the source

- 3 Slowly insert the [Needle Sleeve Assembly](#) into the [Nanospray Slide Assembly](#) while you view the video capture screen. Make sure that the needle appears at the top of the screen roughly midway between the [Counter Electrode](#) and the spray shield.



Figure 24 The needle becomes visible at the top of the video capture screen.

- 4 After the needle is safely positioned in the spray chamber, fully insert the [Needle Sleeve Assembly](#) into the [Nanospray Slide Assembly](#). Make sure that the needle is roughly equidistant from the spray shield and the [Counter Electrode](#).



Figure 25 Needle safely inserted midway between the spray shield and [Counter Electrode](#).

- 5 Use the horizontal and vertical adjustment knobs to adjust the final position of the needle.

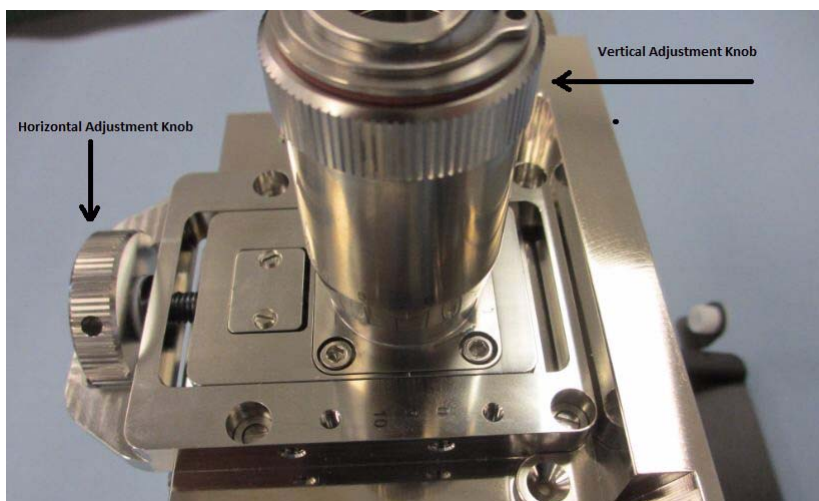


Figure 26 Adjustment knobs to position the needle.

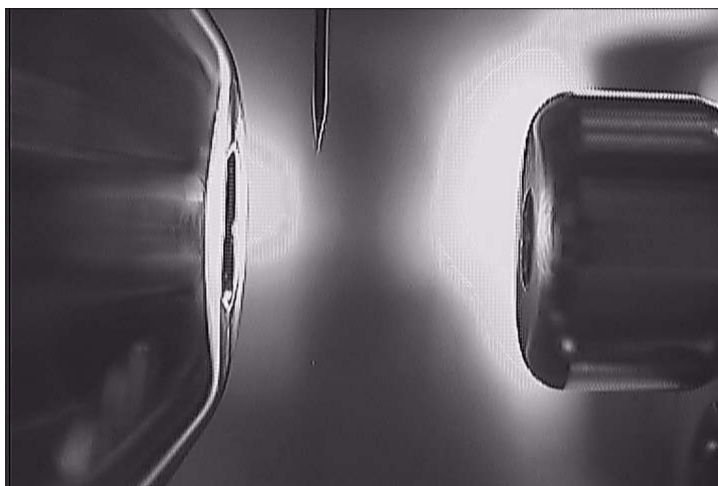


Figure 27 Correct needle position.

- 6 Infuse calibrant into the source at a flow rate of 18 $\mu\text{L}/\text{hour}$.
Look for a small droplet of calibrant solution to form at the needle tip.

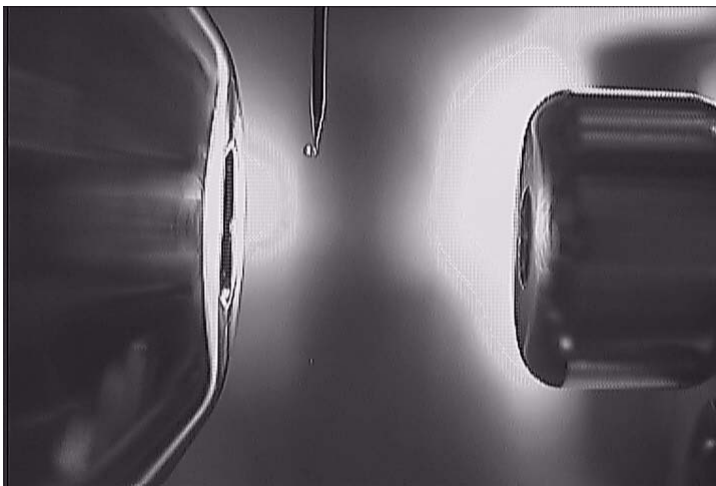
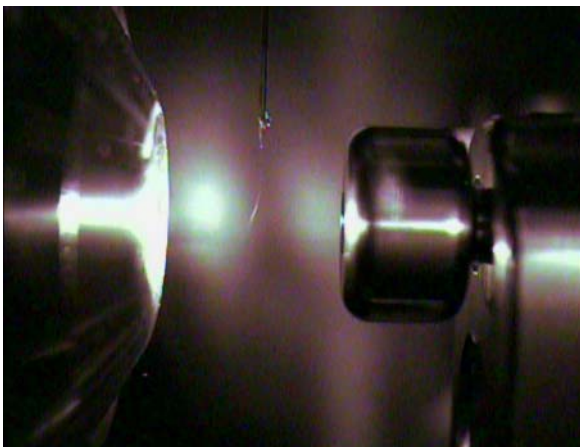


Figure 28 Small droplet forming at the needle tip. An increase in the capillary voltage causes the spray to form.

- 7 Slowly increase the V_{cap} voltage to start the spray formation.
Typical operating values for new needles are 1100 to 1200 volts. When the spray has started, calibrant ions appear in the spectra.



To install the Nanospray Slide Assembly for inline analysis

Do these steps to change the [Nanospray Slide Assembly](#) from the standard [Orthogonal Orientation](#) to either the [Inline Horizontal Orientation](#) or [Inline Sideways Orientation](#).

Before you begin, make sure that you have the [Camera Plate](#) and [Camera Plug](#).

WARNING

Do not change the [Nanospray Slide Assembly](#) configuration when the system is turned on. The high-voltage region inside of the source can cause bodily harm.

- 1 Make sure that the LC/MS instrument is in Standby mode or turned off.
- 2 Remove the [Needle Sleeve Assembly](#) from the [Nanospray Slide Assembly](#), if present.
- 3 Loosen the four captured Torx T10 screws that attach the [Nanospray Slide Assembly](#) to the top of the Nanospray Ion Source.

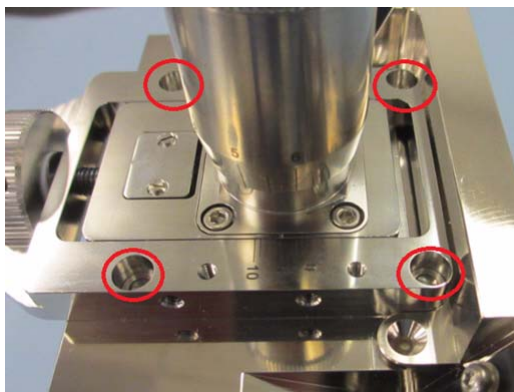


Figure 29 Four captured screws attach the [Nanospray Slide Assembly](#) to the Nanospray Ion Source.

- 4 Remove the [Nanospray Slide Assembly](#) from the spray chamber.



Figure 30 Removing the [Nanospray Slide Assembly](#) from the spray chamber.

- 5** Install the [Camera Plate](#) onto the top of the Nanospray Ion Source. Notice the orientation of the spring plunger in [Figure 31](#).

When the plate orientation is correct, the camera snaps into proper position. The camera image is in focus and the capillary appears on the left side.

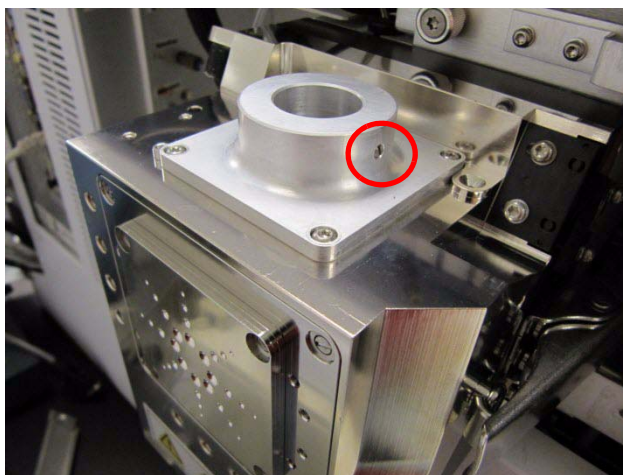


Figure 31 [Camera Plate](#) installed onto the [Nanospray Slide Assembly](#).

- 6 For Inline Horizontal Orientation, install the Camera Plug into the Camera Plate. The Camera Plug snaps into the Camera Plate.

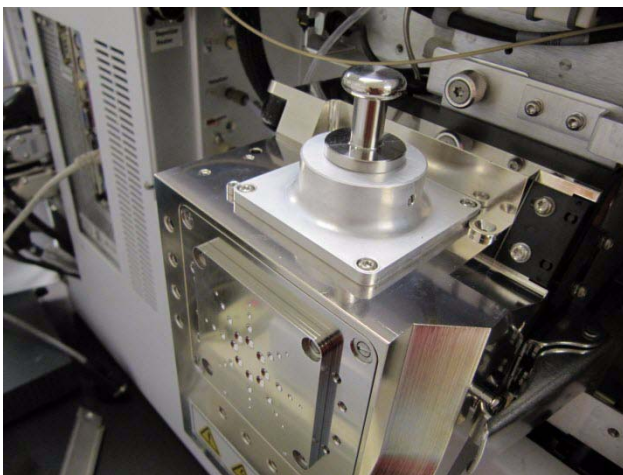


Figure 32 Camera Plug inserted into Camera Plate.

- 7 Remove the Nanospray Front Plate. Angle the Counter Electrode and pin to clear the opening in the spray chamber.

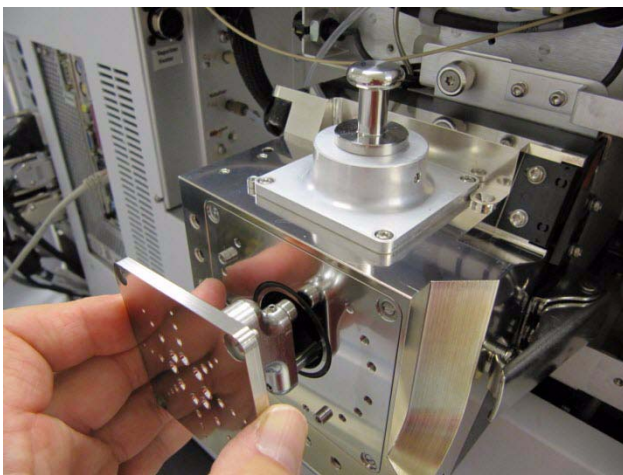


Figure 33 Removing the Nanospray Front Plate with Counter Electrode from the source.

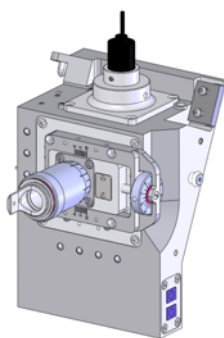
- 8** For **Inline Horizontal Orientation**, install the **Nanospray Slide Assembly**. Make sure that the slide movement is up and down.



Figure 34 Nanospray Slide Assembly installed for **Inline Horizontal Orientation**.

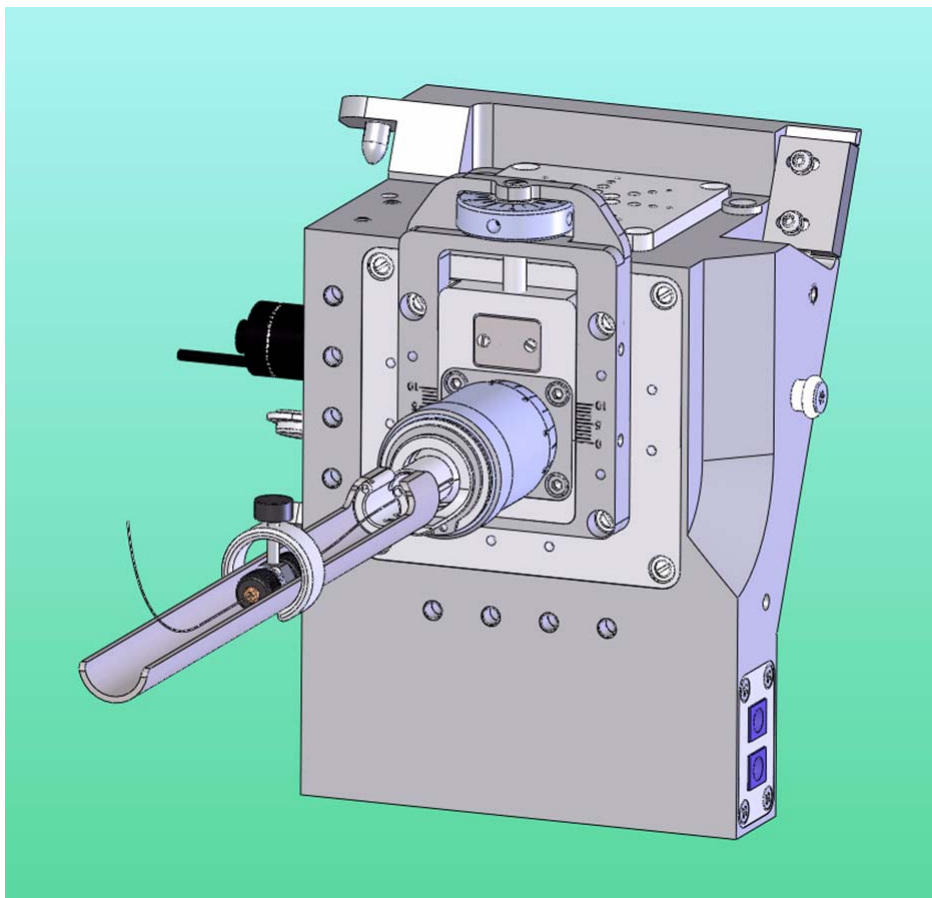
- 9** For **Inline Sideways Orientation**:

- a** Install the **Nanospray Slide Assembly** rotated clockwise as shown. Make sure the slide movement is left and right.



- b** Remove the **Camera Assembly** from the side of the Nanospray Ion Source and install it into the **Camera Plate** on top of the Nanospray Ion Source. Snap the **Camera Assembly** in place.

- 10** Install the extension tube column support assembly. See “To install the Needle Sleeve Assembly into the Nanospray Ion Source” on page 32.



To adjust the LED camera light

You can adjust the LED housing position to control glare and get the best spray image on the camera. The LED housing can be adjusted from outside the chamber, even while experiments are in progress.

- Turn the rotating knob as needed to rotate the LED housing.

You can rotate the LED housing more than 90 degrees. Do not over-rotate!

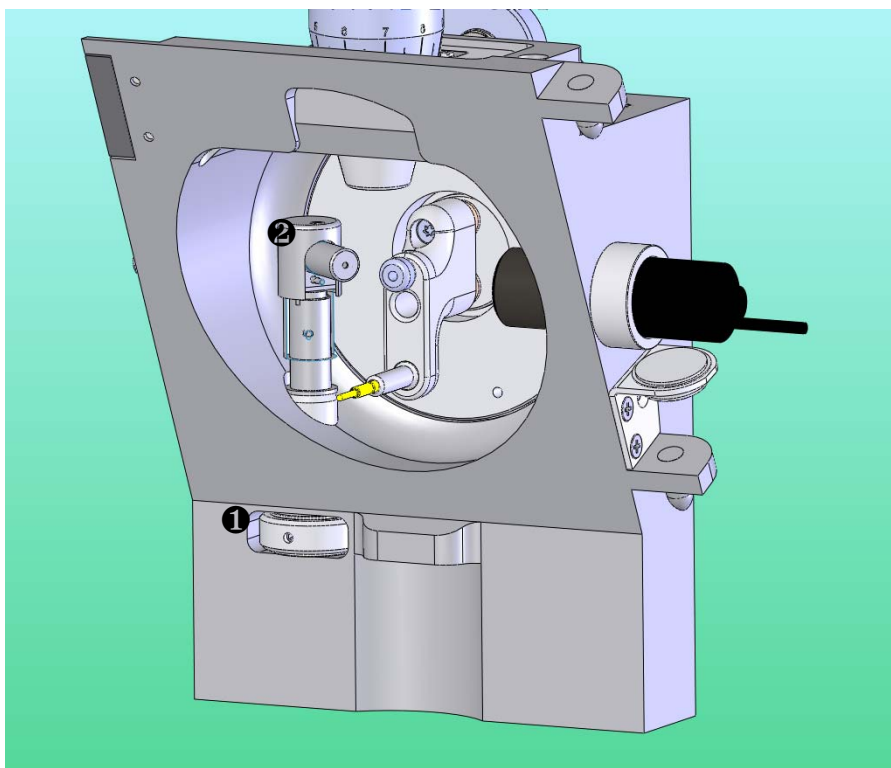
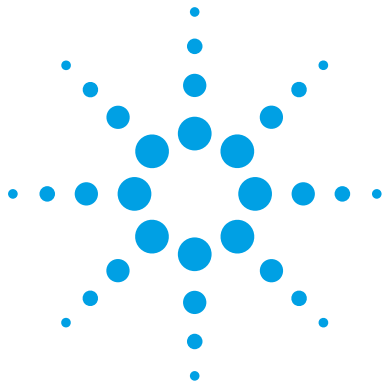


Figure 35 LED light components: ① rotating knob and ② LED housing.



2 Mass Reference Solution

- To prepare the Low Mass IRM stock solution 45
- To prepare the IRM working solution 46
- To apply the IRM solutions 47
- To enable reference mass correction 50
- To clean the wick and wick cover 52

Reference mass solutions are only used with the 6200 Series TOF LC/MS and the 6500 Series Q-TOF LC/MS. The 6400 Series Triple Quadrupole LC/MS does not use these solutions.

When you take accurate mass measurements on a TOF or Q-TOF instrument, the addition of compounds which ionize under the analysis conditions and provide known accurate mass values (“reference masses”) in real time allows you to correct the mass values in the acquired spectra for short-term variations.

This chapter describes how to use reference masses with TOF or Q-TOF instruments outfitted with a Nanospray Ion Source and operated in positive ion polarity. In summary, solutions are applied to an absorbent wick on the top inside of the source, and then the IRM compounds slowly evaporate and are ionized, providing signals for correction of the mass assignments.

The solution of internal reference mass standard(s) in acetonitrile (ACN) is applied to the wick and allowed to dry. Then the high mass solvent (FC-70) is applied. The use of FC-70 is optional. Signals from the IRM compounds are acquired without its use, but the response is more uniform and lasts longer when it is used.



2 Mass Reference Solution

NOTE

Do not mix acetonitrile and FC-70 together before you apply them to the wick. They are immiscible and will not form a solution.

They can be applied sequentially to the wick, however.

To prepare the Low Mass IRM stock solution

This step prepares 10 mL of 1000 µg/ml methyl stearate in acetonitrile.

Before you begin, make sure that you have:

- reagent-grade acetonitrile
- methyl stearate (p/n G1982-85003)

1 Into a suitable container, add the contents listed in [Table 2](#).

Table 2 Low Mass IRM stock solution

| Component | Amount |
|---|--------|
| methyl stearate (p/n G1982-85003) | 10 mg |
| reagent-grade acetonitrile | 10 mL |

2 Shake to dissolve.

Refrigerate this stock solution to keep it for more than a few days.

To prepare the IRM working solution

This step prepares 1 mL of 100 µg/mL methyl stearate and 2% (v/v) HP-1221 in acetonitrile.

Before you begin, make sure that you have:

- reagent-grade acetonitrile
- [Low Mass IRM stock solution](#)
- HP-1221 (p/n G1982-85001)

1 Into a vial, add the compounds in [Table 3](#).

Table 3 IRM working solution

| Component | Amount |
|---|--------|
| reagent-grade acetonitrile | 900 µL |
| Low Mass IRM stock solution | 100 µL |
| HP-1221 (p/n G1982-85001) | 20 µL |

2 Mix on a vortex mixer.

This working solution must be refrigerated between uses.

To apply the IRM solutions

You need to adjust the amount of solution to apply or its concentration to suit your needs.

These compounds are designed to persist in the ionization region and are difficult to remove completely. Therefore, for the first few applications, apply smaller amounts until the response of your system under your analysis conditions is known. Typically, 200 μL of the IRM working solution is applied and allowed to evaporate; then 200 μL of FC-70 is applied. The wick holds approximately 250 μL to 300 μL of solution before saturation occurs.

Before you begin, make sure that you have:

- reagent-grade acetonitrile
- [IRM working solution](#)
- FC-70 liquid (p/n G1982-85002)

- 1 In the MassHunter Acquisition program, set the instrument to **Standby** mode.
- 2 Briefly mix the [IRM working solution](#) on a vortex mixer to assure homogeneity.
- 3 Load a pipette with 200 μL of [IRM working solution](#). Adjust this volume as needed.
- 4 Carefully dispense the [IRM working solution](#) evenly between the two IRM filling ports on the top of the Nanospray Ion Source.

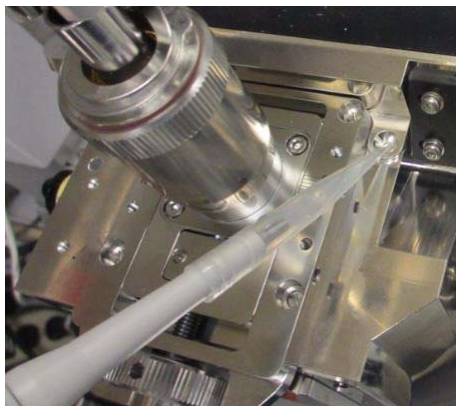


Figure 36 Dispensing solution into one of two IRM filling ports.

- 5 Wait about five minutes for the [reagent-grade acetonitrile](#) to evaporate at a typical standby source temperature of 300°C. The five minute pause minimizes the chances of arcing when the instrument is turned on.
- 6 Load a pipette with 200 µL of the [FC-70 liquid \(p/n G1982-85002\)](#). Add the [FC-70 liquid \(p/n G1982-85002\)](#) to the wick in the same way that you added the [IRM working solution](#). Dispense slowly (10 to 20 seconds) to avoid overfilling and to allow time for the wick to soak up the viscous solution.

Signals from the IRM compounds are displayed only when there is a spray present and the mobile phase contains electrolytes. The IRM signals are unstable during the startup and stabilization of the spray, which can take ten minutes or longer at a drying gas temperature of 350°C and flow of 4 L/minute.

The accurate mass values are:

Table 4

| Compound | <i>m/z</i> value for proton adduct (positive mode) |
|---|--|
| methyl stearate (p/n G1982-85003) | 299.294457 |
| HP-1221 (p/n G1982-85001) | 1221.990637 |

[Figure 37](#) shows an example spectrum of the IRM compounds taken on a 6510 Q-TOF LC/MS with these settings:

- 1 scan/second (9,816 transients/scan)
- 200 μL of **IRM working solution** allowed to evaporate, followed by 200 μL of **FC-70 liquid** (p/n G1982-85002)
- drying gas temperature: 325 $^{\circ}\text{C}$
- gas flow: 5 L/minute
- mobile phase: 300 nL/minute of 0.1% formic acid in 97/3 water/acetonitrile.

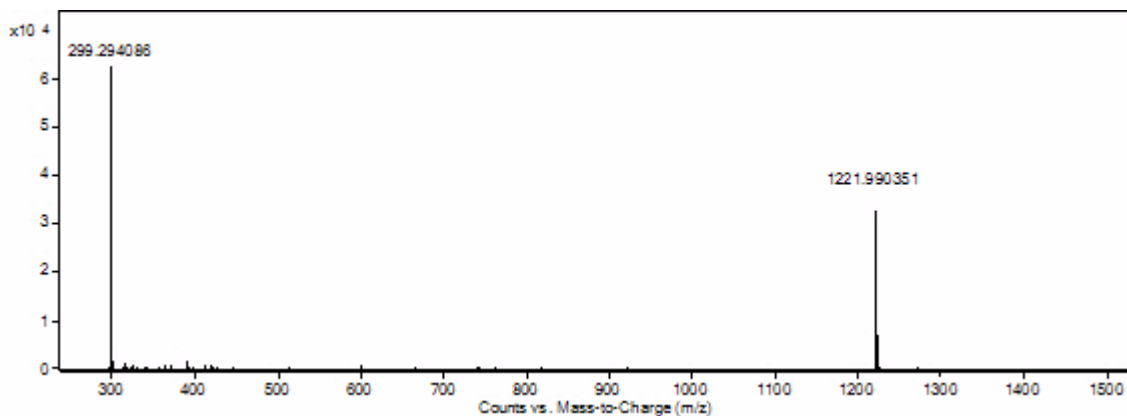


Figure 37 Example spectrum of the IRM compounds

To enable reference mass correction

- 1 In the MassHunter Acquisition program, in the **Reference Mass** tab, enter the m/z value. Mark the check the box in front of the entry.
- 2 Repeat for the other IRM compound if it is used.
- 3 Check the **Enable Reference Mass** box.
- 4 Set the acquisition mass range in MS mode to be wide enough to include the reference masses selected.
- 5 Save the method.

Shortly after loading the IRM solutions, the ion signals stabilize at around 20,000 to 150,000 counts abundance when the instrument is operated at approximately 5,000 to 10,000 transients per spectrum.

Actual values depend primarily on:

- the amount that was loaded
- cleanliness of the interface assembly
- operating parameters used for the MS
- mobile phase composition
- number of transients per scan

For best results:

- Keep the abundances above 5,000 counts for all scan speeds to obtain adequate ion statistics.
- Keep the abundances below the value of $90 \times (\text{number of transients per scan})$ to avoid saturation effects.
- For 2 scans/second (4,880 transients/scan), the desirable range is between 5,000 to 440,000 counts abundance.

The ion signals from the IRM compounds decay quickly at first but stabilize at a lower usable level for days. An increase to the drying gas temperature increases the abundances for the IRM compounds but also leads to a faster rate of decline of the IRM abundances. The IRM response can be reduced when using mobile phase compositions that contain high organic content, particularly high amounts of acetonitrile. (This is especially true for methyl stearate.)

When the IRM solutions are used repeatedly, a residual amount of the IRM compounds builds up in the interface assembly itself, requiring less frequent addition of the IRM solutions to maintain usable abundances.

The IRM compounds persist in the interface assembly and yield signals for an extended period of time. If you no longer require the presence of these IRM signals, clean the interface assembly with wipes and organic solvents (such as acetonitrile and isopropyl alcohol) to remove the IRM compounds.

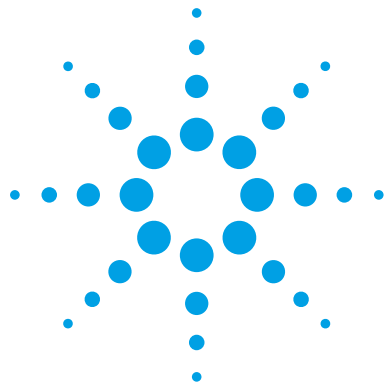
However, traces of the IRM compound remain and can be removed only by prolonged heating (350°C to 365°C and 10 to 12 L/minute drying gas flow for several days).

Over time, an increase in background signals can result from material being adsorbed on or deposited in the wick in the top plate. See [“To clean the wick and wick cover”](#) on page 52.

To clean the wick and wick cover

- 1** Use a T-6 driver to remove the four screws from the wick cover.
- 2** Use tweezers to remove the wick.
- 3** Soak the wick in a suitable organic solvent such as acetonitrile or LC flushing solvent to clean the wick.
- 4** Allow the wick to dry completely.
- 5** Clean the wick cavity with IPA or LC flushing solvent.
- 6** Use tweezers to reinsert the wick into the slot of the top plate, or press the wick firmly into place while wearing clean gloves.
- 7** Remove any stray fibers with tweezers, or blow them off with compressed air or nitrogen, and reinstall the wick cover.

A replacement wick is available if needed (part number G1988-20021).



3 Safety

| | |
|----------------|----|
| Safety Issues | 54 |
| Safety Symbols | 58 |

This chapter contains safety information.



Safety Issues

Agilent designed the Nanospray Ion Source to minimize safety hazards:

- ESI and nanospray needles are grounded.
- High voltage components are enclosed inside of chambers.

However, you still need to be aware of several other potential safety issues when you operate the Nanospray Ion Source.

Some of the procedures in this chapter require access to parts of the instrument and Nanospray Ion Source while it is in Shutdown state or shortly after it is turned off. If you do not perform these procedures correctly, you are exposed to dangerous temperatures, voltages, and chemical hazards. This topic describes the potential dangers.

Needle Hazard

The Nanospray Ion Source provides you with many options and configurations for a wide range of experiments. This flexibility includes the ability to use customer-drawn columns and needles. However, some combinations of needles can be dangerous when used with the Nanospray Ion Source.

WARNING

Use extreme caution when setting up experiments with any type of needle, especially conductive ones. Touching a high voltage region inside the Nanospray Ion Source chamber with a conductive needle is extremely hazardous.

WARNING

The needle in the needle holder is very sharp and can pierce your skin. Do not touch the tip, especially when you analyze toxic substances or when you use toxic solvents.

WARNING

The tip of the needle is very fragile. Do not touch the tip to any objects, such as the capillary cap or spray chamber. If you accidentally touch the needle tip, replace the needle.

WARNING

Use care when you adjust the needle. Do not damage the end of the needle.

WARNING

Do not use metal or otherwise conductive needles. If a conductive needle touches the spray shield, the source can short.

High temperatures

Some parts in the Nanospray Ion Source operate at or reach temperatures high enough to cause serious burns. These parts include, but are not limited to the capillary, capillary cap, and spray shield.

Do not touch these parts.

WARNING

Use extreme care when you work on an instrument that has recently been turned off. Certain parts remain hot for many minutes after the instrument is shut down or turned off. In particular the spray shield and the capillary cap could be very hot after working with APCI or ESI.

Hazardous voltages

Whenever the instrument is not in Standby, hazardous voltages are present on one or more interior parts. Parts that use hazardous voltages include, but are not limited to, the capillary cap and counter electrode.

These parts are usually covered or shielded. As long as the covers and shields are in place, you will not make contact with hazardous voltages.

WARNING

Never open the Nanospray Ion Source chamber while the instrument is in the Operate state or the HV voltage is turned on.

WARNING

Do not insert fingers or tools through the openings on the Nanospray Ion Source chamber. During operation the capillary and capillary cap are at high voltage up to 3 kV.

WARNING

If you connect the instrument to an ungrounded or improperly grounded power source, you create a shock hazard for the operator and can damage the instrument. Intentional interruption of instrument grounding is strictly prohibited.

WARNING

Do not wear dangling, conductive items such as necklaces, earrings, or other items that can enter the source while it is in operation. A shock can result.

Biohazardous residue

The Nanospray Ion Source interface does not ionize all of the sample and solvent. The vacuum pumps of the instrument removes the sample that is not ionized and solvent. The exhaust from these pumps can contain traces of sample and solvents. Vent all pump exhaust outside the building or into a fume hood. Comply with your local air quality regulations.

CAUTION

Do not vent exhausts into your laboratory. See the warning labels on the instrument. Comply with your local air quality regulations. The exhaust fumes from the vacuum system and spray chamber contain trace amounts of the chemicals you analyze. Health hazards include chemical toxicity of solvents, samples, buffers, and pump fluid vapor, as well as potentially biohazardous aerosols of biological samples. Vent all exhausts out of the building where they cannot be recirculated by environmental control systems.

- Fluid drained from the Nanospray Ion Source chamber is made of solvent and sample from your analyses. The fluid in the mechanical pump collects traces of samples and solvents. In addition, unnebulized solvent and sample collects at the bottom of the spray chamber. Connect the drain on the bottom of the spray chamber to a closed container.
- Handle and dispose of all fluids using precautions appropriate for their biohazardous and biological content. Comply with local environmental regulations.
- Handle all used pump fluid as hazardous waste. Dispose of used pump fluid as specified by your local regulations.

Power supply information

This equipment must be installed in an environment of Category II installation as defined in IEC 664. Check that the supply voltage does not fluctuate more than +10% or -10% of rated voltage.

Cleanliness

Cleanliness and the prevention of accidental contamination during maintenance are very important. Contamination of the interior of the vacuum system or the sample path can affect the results of your analyses.

- Always wear clean gloves when handling parts that come in contact with the sample path. Oil from your fingers is difficult to remove.
- When you set parts down, place them on clean, lint free cloths or clean aluminum foil, not directly on the laboratory bench.
- Keep parts covered so they do not get dirty.
- If possible, maintain a separate set of tools that have been thoroughly cleaned. Use these tools only when working on clean assemblies.
- With open ion sources, such as API, avoid dusty and fibrous environments. Dust particles can enter MS ion source and deposit on ion optics, causing sensitivity loss.

Safety Symbols



NOTE



These symbols are placed on the product where it is necessary for you to refer to the **manual** in order to understand a **hazard**.



WARNING

This symbol is placed on the product within the area where **hazardous voltage** is present or shock hazard can occur. Only trained service persons should perform work in this area.



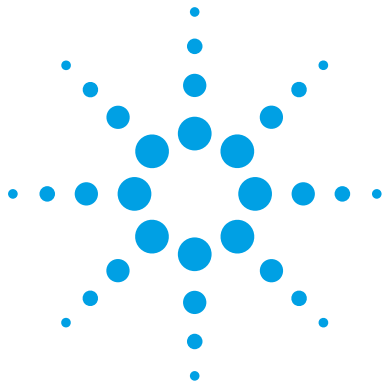
WARNING

This symbol is placed on the product within the area where **hot parts and surfaces** are present. Allow the product to cool before performing work in this area.



WARNING

This symbol is placed on the product within the area where **biohazards** are present. Handle these areas with the respective care.



4 Reference

| | |
|---------------------|----|
| Parts List | 60 |
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This chapter contains reference information.



Parts List

This section contains a description of all of the parts included with Nanospray Ion Source.

Nanospray Ion Source - Outside

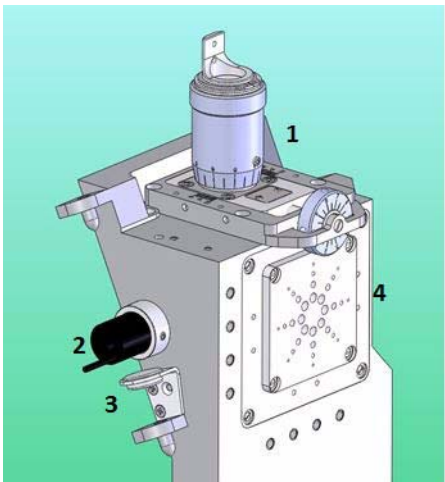


Table 5 Nanospray Ion Source - Outside

| Part | Description | Part Number |
|------|--------------------------|-------------|
| ❶ | Nanospray Slide Assembly | G1988-60002 |
| ❷ | Camera Assembly | G1982-60458 |
| ❸ | IButton Assembly | G1982-60450 |
| ❹ | Nanospray Front Plate | G1988-20001 |

Nanospray Ion Source - Inside

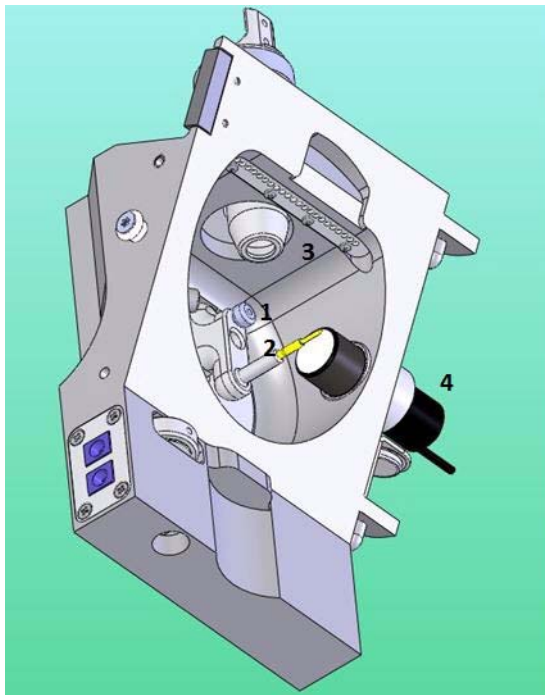



Table 6 Nanospray Ion Source - Inside

| Part | Description | Part Number |
|------|-----------------------------|-------------|
| ① | Counter Electrode | G1982-20008 |
| ② | Probe Receptacle 0.975 inch | 1400-3275 |
| ② | Probe Contact 1:32 inch | 1400-3276 |
| ③ | Nanospray Wick Cover | G1988-20006 |
| ③ | Nanospray IRM Wick | G1988-20021 |
| ④ | Camera Assembly | G1982-60458 |

Nanospray Desolvation Upgrade Kit (p/n G1988-60009)

Table 7 Nanospray Desolvation Upgrade Kit (p/n G1988-60009)

| | Part | Part Number |
|---|---------------------------|-------------|
|  | T-nut connectors | G1946-20111 |
| | stainless steel end plate | G1946-20156 |
| | gold screws | G1960-20148 |

Spray Shield Kit (p/n G1988-60007)

Table 8 Spray Shield Kit (p/n G1988-60007)



| | Part | Part number |
|---|---------------------|-------------|
|  | Contact spring | G1982-20005 |
|  | Radial gas diverter | G1982-20006 |

Table 8 Spray Shield Kit (p/n G1988-60007)

| | Part | Part number |
|---|---------------------------------|-------------|
|  | Winged spray shield nut | G1982-20011 |
|  | Wave washer | G1982-20012 |
|  | Single bore spray shield | G1982-20111 |
|  | Multibore vortex spray shield | G1988-20023 |
|  | Single bore inline spray shield | G1988-20038 |

Infusion Sample Delivery System

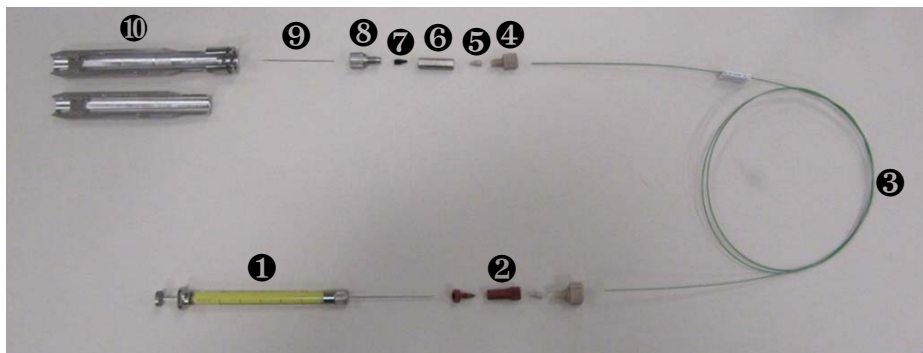


Table 9 Infusion Sample Delivery System

| Part | Description | Part Number |
|------|--|-------------|
| ① | Syringe • SST needle | 5190-1512 |
| ② | Syringe Adapter • F126S 1/32-inch nut • 1/32-inc to 1/16-inch reducing union • F113 ferrule (0.8mm ID) • F300 10-32 winged nut | 0100-2647 |
| ③ | LC-MS Connection Capillary | 5065-9906 |
| ④ | Nut | 0100-2437 |
| ⑤ | F113 Ferrule (0.8mm ID) | 0100-2436 |
| ⑥ | Stainless Steel Zero Dead Volume Fitting | 5022-2184 |
| ⑦ | Conductive Ferrule | 0100-2262 |
| ⑧ | Conductive Needle Nut | G1988-20030 |
| ⑨ | Needle | 9301-6424 |
| ⑩ | Needle Sleeve Assembly | G1988-60006 |

Sleeve Assembly and Extension Tube

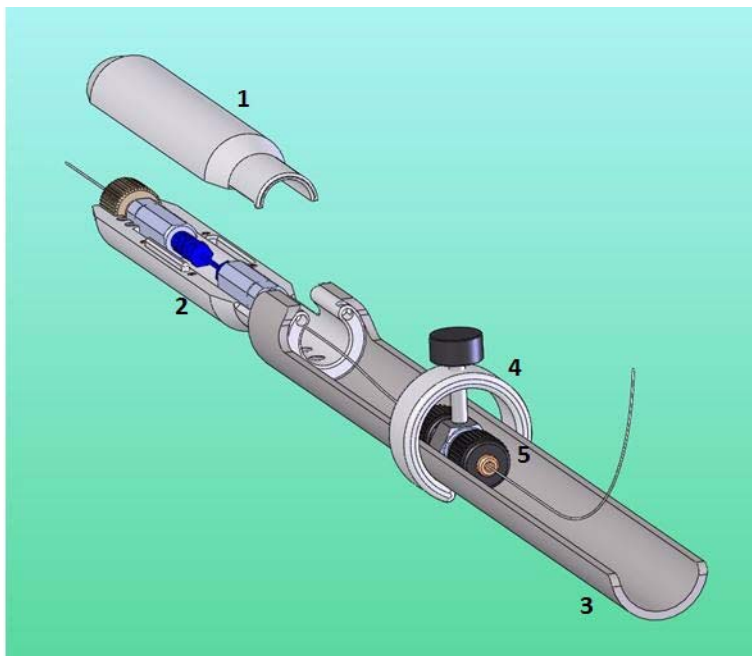


Table 10 Sleeve Assembly and Extension Tube

| Part | Description | Part Number |
|------|---|-------------|
| ❶ | Upper Sleeve Assembly • includes Conductive Gasket | G1988-60004 |
| ❷ | Lower Sleeve Assembly • includes Conductive Gasket | G1988-60005 |
| ❸ | Extension Tube | G1988-20033 |
| ❹ | Tube Clamp | G1988-20001 |
| ❺ | Idex M-572 Fitting | 0100-2652 |

Camera Plate and Plug

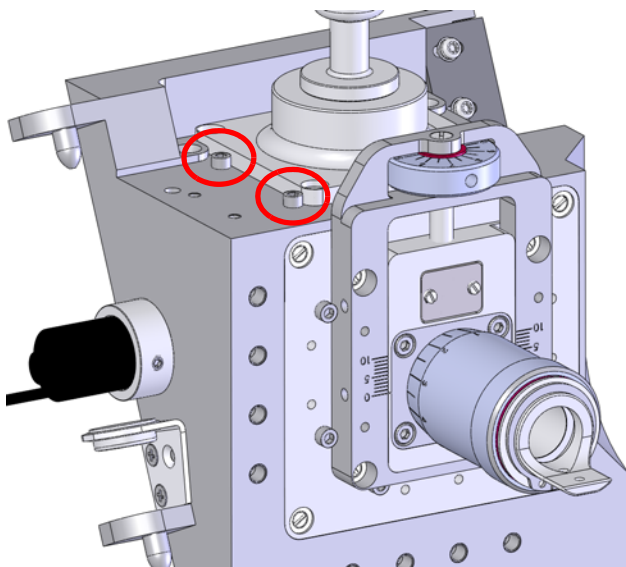


Table 11 Camera Plate and Plug

| Description | Part Number |
|--------------|-------------|
| Camera Plate | G1988-20002 |
| Camera Plug | G1988-20039 |

Component Alignment

When the [Nanospray Slide Assembly](#) is removed and reinstalled, small misalignments can occur. Stop Buttons are included to help realign the [Nanospray Slide Assembly](#) after removal. Slide the component into contact with the appropriate Stop Button, and tighten the component mounting screws. As long as the Stop Buttons remain in place, the [Nanospray Slide Assembly](#) is always positioned in a repeatable spot.



Hole Layout

Extra holes on the Nanospray Ion Source are to mount accessories.

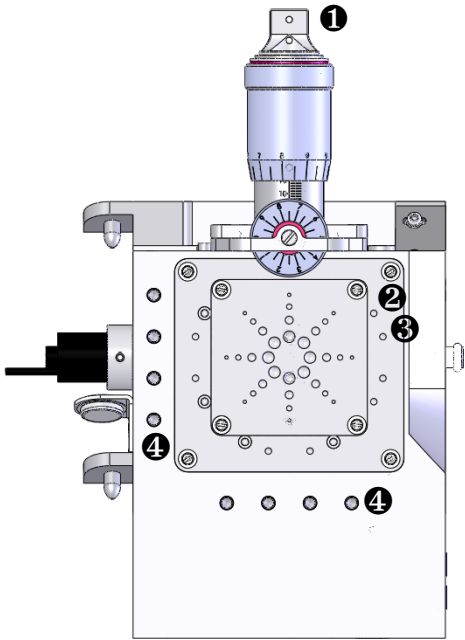
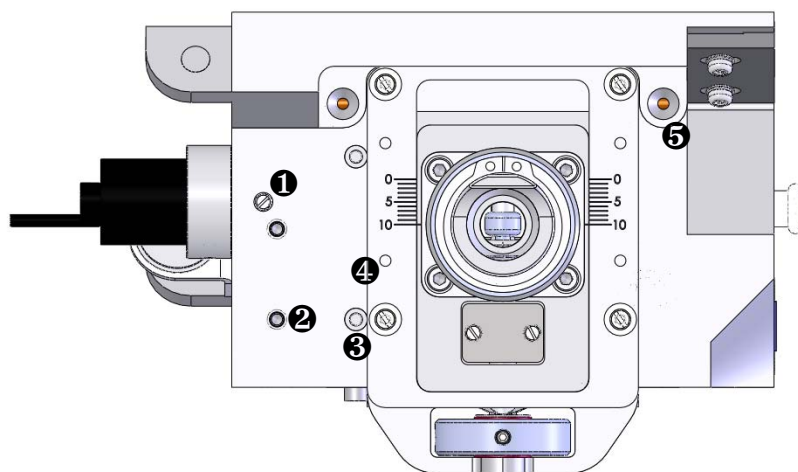


Table 12 Nanospray Ion Source front view

| Part | Description | Quantity |
|------|--|----------|
| ❶ | M3 holes to mount extra components | 2 |
| ❷ | M3 holes for Stop Buttons | 6 |
| ❸ | M3 holes to mount accessories | 6 |
| ❹ | M5 holes to mount accessories. The four holes on the left can be used to mount the Valve Mount Kit (G1988-64001) | 8 |

**Table 13** Nanospray Ion Source top view

| Part | Description | Quantity |
|------|--|----------|
| ① | M4 hole: Spring-loaded plunger for camera Ring | 1 |
| ② | M3 holes to mount extra components. | 2 |
| ③ | M3 holes for Stop Buttons. | 2 |
| ④ | M3 holes to mount extra components. | 4 |
| ⑤ | Holes to refill IRM compound. | 2 |

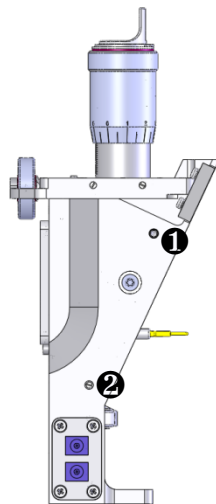
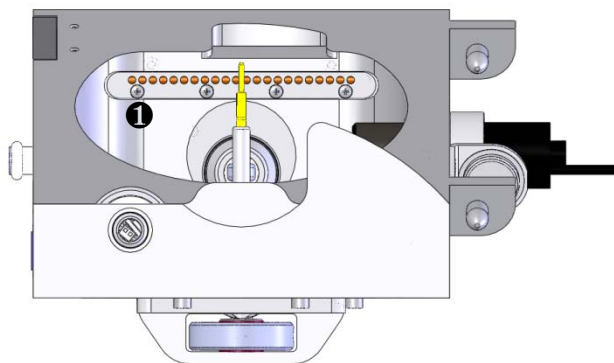


Table 14 Nanospray Ion Source side view

| Part | Description | Quantity |
|------|--|----------|
| ❶ | M4 hole to attach Protective Cover (G1988-60003) | 1 |
| ❷ | M4 hole for spring-loaded plunger; provides safety grounding for LED shaft | 1 |

**Table 15** Nanospray Ion Source inside view

| Part | Description | Quantity |
|------|---|----------|
| 1 | M2 holes to mount the Wick Cover. Be careful when you service the wick. Use light tightening force. | 4 |

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In This Book

This guide describes the step to upgrade, install, and use the Nanospray Ion Source.

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November, 2012



G1988-90000



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