



Setting up a nanospray emitter into the MS source head

Nanoelectrospray ionization

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Introduction

Nano electrospray ionization (nano ESI), known as nanoelectrospray ionization (nanoESI or NSI), is a form of ESI that uses smaller diameter emitters (10 to 100 μm I.D.) and flow rates of 10 to 1000 nL/min. Compared with conventional ESI, nanoESI produces smaller droplets resulting in more efficient ionization and significant increases in sensitivity. Besides, it also tolerates a wider range of liquid compositions including pure water and requires lower sample volume. NanoESI generally does not require a drying gas or thermal heating.

Step 1: Prepare a borosilicate emitter

Borosilicate nanoESI emitters can be either prepared in-house using a tip puller or purchased directly from the Sutter website. A protocol of tip pulling is available at: <https://nativems.osu.edu/protocols>

Once a suitable emitter is obtained, the emitter length is shortened using a glass cutter to fit on the platinum wire of nanoESI source and minimize the contact between the sample and the glass walls. A typical length of emitter ranges from 1.5-3.0 cm. Examine the emitter under a microscope before loading samples because small glass particles inside the tip may cause emitter to clog.

Step 2: Load sample onto the borosilicate emitter

The sample solution is introduced directly into the emitter using a 10 μL Hamilton syringe or a gel-loading tip. The syringe/gel-loading tip is often inserted as far as possible into the capillary and the solution is deposited slowly to prevent the formation of air bubbles. The presence of bubbles may cause unstable spray. To eliminate bubbles, the capillary can be shaken down by flicking your wrist or using a table centrifuge. The emitter is then examined under a microscope to ensure the tip orifice doesn't break and no bubbles are present.

- To avoid contaminating the emitter with contaminants on skin, always wear powder-free, sterile gloves when handling the emitter.
- To avoid damaging the emitter, always grasp the emitter by its larger outer body and do not grasp or hold it by its proximal (spray) end.

Step 3: Insert the borosilicate emitter

CAUTION!

Before installing or removing the emitter, always make sure that high voltage is off or the instrument is in stand-by to prevent personal injury.

Note: Your source setup may differ from the figures on the next pages. Check the corresponding manual before following the protocol.

continued



Adding a platinum wire to a Thermo nESI source

a) use a piece of foil to wrap the end of the platinum wire to avoid wagging; b) thread the platinum wire on the source head; tighten the platinum wire with the front screw; c) attach the backing pressure line to the back of the source head by tightening the back screw and positioning the source head on the stage.



Inserting the borosilicate emitter into a Thermo NanoSpray Flex Series Ion Source

- I. Pull the source bottom away from the MS along sliding rails to fully expose the source head from the observation cylinder;
- II. Thread the sample-loaded emitter into the platinum wire and push emitter through the wire to insert the distal end of the emitter into the nebulizer sheath.

If a fitting is equipped on the source head, the emitter can be secured by finger-tightening the fitting to provide an airtight fit. To provide backing pressure, connect the red syringe cap, which is attached to the tubing, to the syringe tip. To keep pressure on the syringe, place it in the static air pressure device with the backing plate. To avoid unintentionally breaking the proximal end of emitter, pull the source head away from MS using X-manipulator arm before pushing the source bottom back to the observation cylinder.

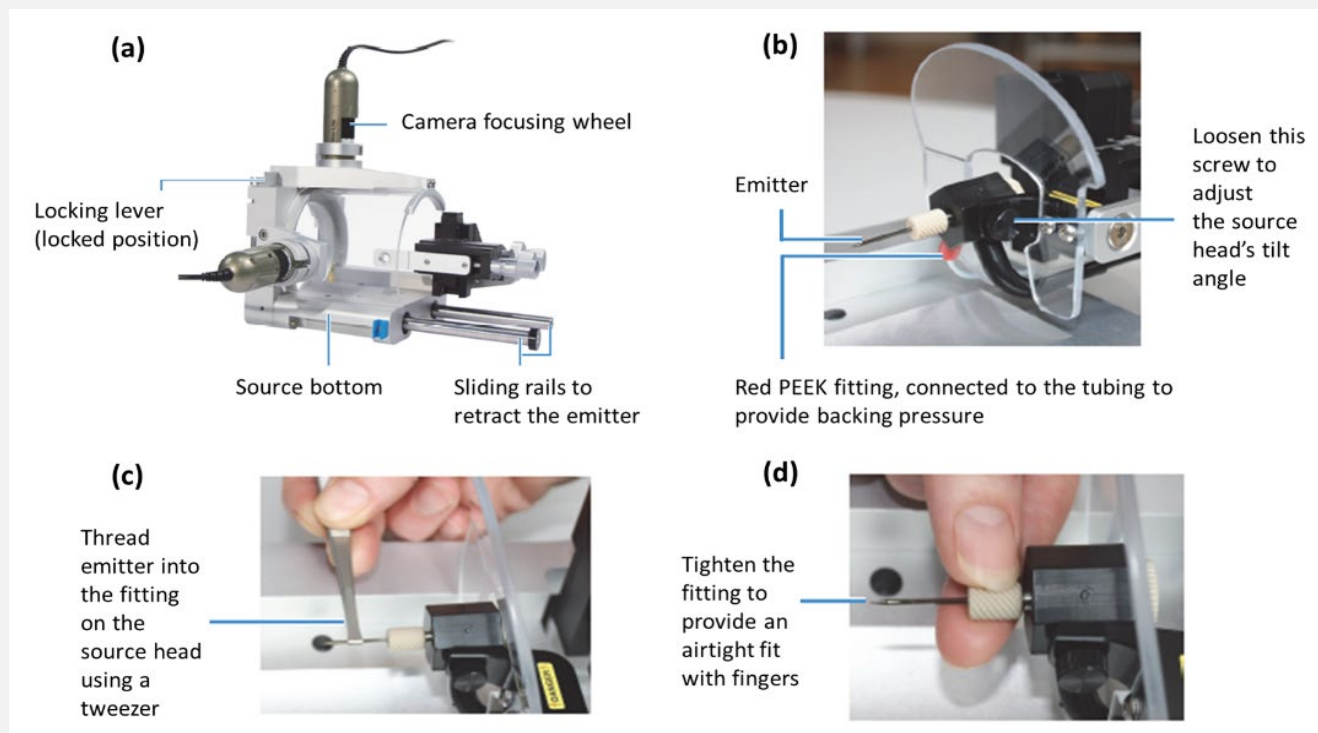


Figure 1. (a) Thermo Nanospray Flex ion source (ES072) ES071) with the USB cameras (ES218). (b) Source head with a borosilicate emitter. (c) and (d) Installing the borosilicate emitter into the source head.¹

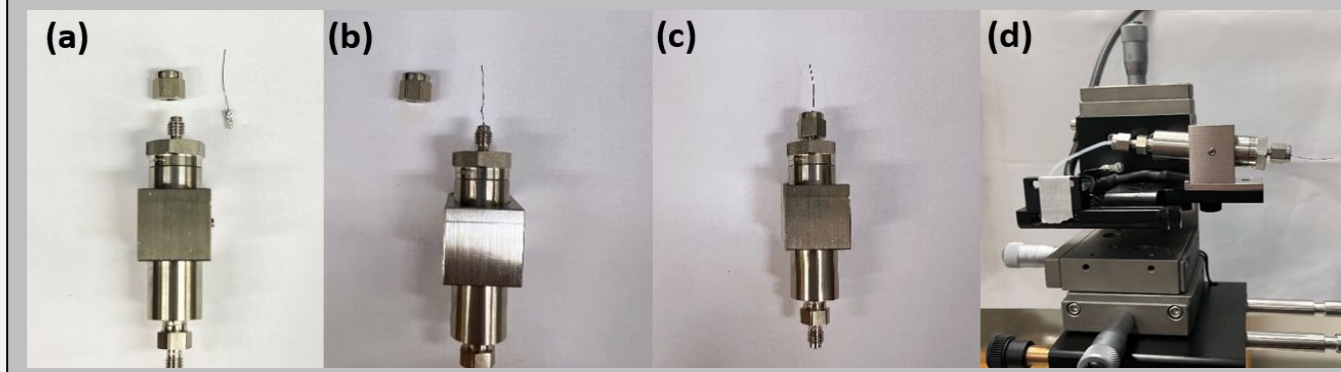
Adding a platinum wire to a Waters Cyclic nESI source

(a) use a piece of foil to wrap the end of the platinum wire to avoid wagging

(b) thread the platinum wire on the source head

(c) tighten the platinum wire with the front screw

(d) attach the backing pressure line to the back of the source head by tightening the back screw and positioning the source head on the stage.



Inserting the borosilicate emitter into a Waters NanoFlow Sprayer

- I. Loosen the stop screw and slide the source stage back from the source region.
- II. Thread the distal end of emitter through the nebulizer sheath carefully.

To ensure enough space to load emitter, the sprayer head can be removed from the stage by loosening the captive thumbscrew, located underneath the stage platform.

To place the sprayer back on the stage platform, align the slot in the base of the sprayer with the tab on the platform. Thread the thumbscrew into the hole in the sprayer base and tighten it to secure the sprayer on the stage platform.

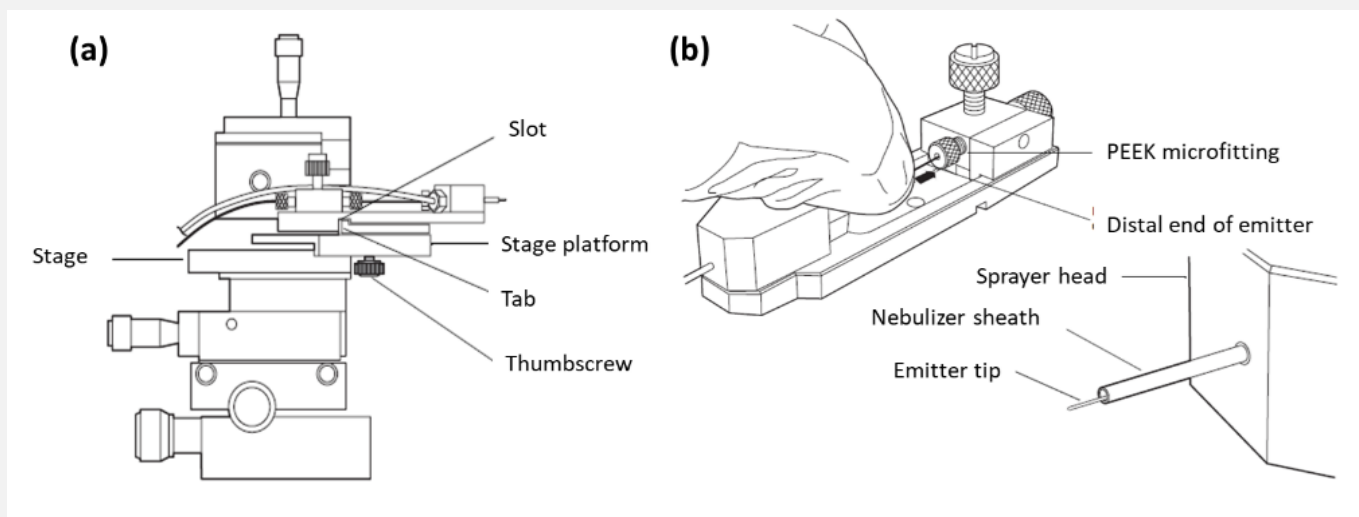


Figure 2. (a) Attaching the sprayer to the stage platform. (b) Guiding the end of the emitter into the PEEK microfitting.²

4. Position the borosilicate emitter

After loading the emitter into the platinum wire, use the XYZ-manipulator knobs to position the emitter tip almost on-axis with the ion transfer tube. The distance between emitter and the outlet of the spray cone is usually 1–3 mm and fine adjustment can be made and checked on the monitor of cameras.

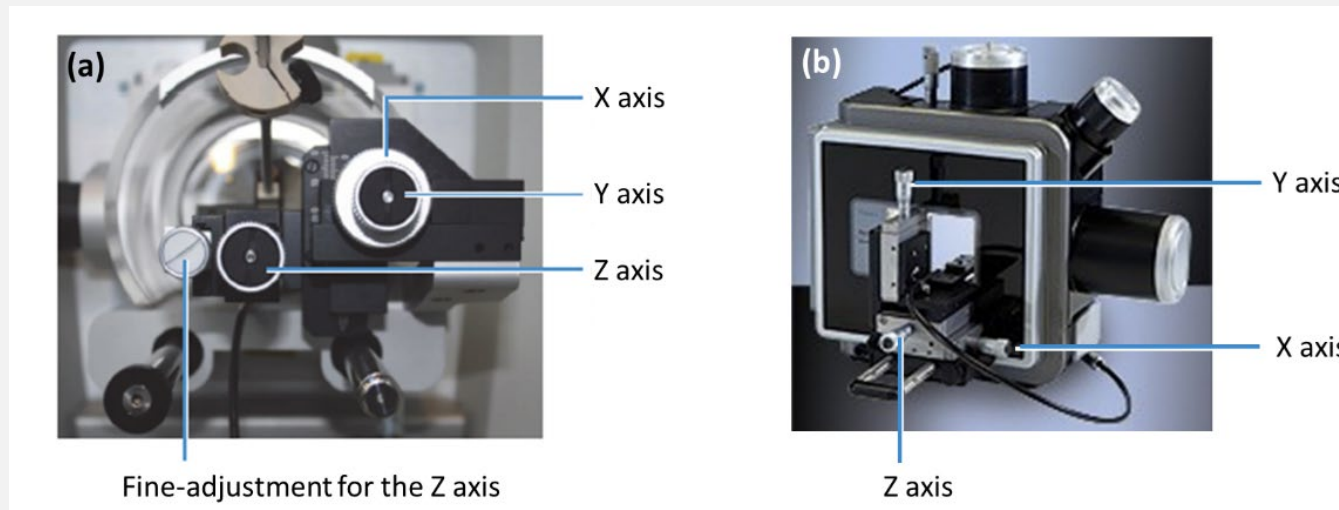


Figure 3. Adjustment knobs on the XYZ manipulator of Thermo Nanospray Flex Series Ion Source¹ (a) and Water NanoFlow Sprayer² (b). X axis: horizontal emitter adjustment; Y axis: vertical emitter adjustment; Z axis: forward-backward adjustment (toward or away from the MS ion transfer tube)

5. Obtain a stable, low flow spray

Once the emitter is correctly positioned, voltage can be turned on and applied gradually. If there is no signal or the spray stops, a higher voltage (1.2-1.5V) can be applied briefly to initiate the spray and then lowered to get a stable spray. The voltage for nanoESI is usually 0.6-1.5V and the optimal voltage varies according to emitter size and sample solution. Typically, a stable spray can last at least 30 minutes with 1 μ L of sample solution.

If the spray stops before depleting the sample, the most likely reason is that emitter is blocked. The emitter can be taken off from the nanoESI source head and the orifice can be opened by the tweezers manually under microscope. Practice this before using important samples because the emitter may break if you bend the emitter too far.



Figure 4. Open the orifice of the emitter using a tweezers under microscope

References:

1. Thermo Fisher Scientific Nanospray Flex Series Ion Source User Guide, Revision B, June 2018
2. Waters Universal NanoFlow Sprayer Installation and Maintenance Guide, Revision C, 2007