Hardware and software solutions for implementing nanospray desorption electrospray ionization (nano-DESI) sources on commercial mass spectrometers

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Abstract:

Nanospray desorption electrospray ionization (nano-DESI) is an ambient ionization mass spectrometry imaging (MSI) approach that has been used for imaging of biological and environmental samples with high spatial resolution and throughput. Because nano-DESI has not yet been commercialized, researchers develop their own sources and interface them with different commercial mass spectrometers. Previously, several protocols focusing on the fabrication of nano-DESI probes have been reported. In this tutorial, we discuss different hardware requirements for coupling the nano-DESI source to commercial mass spectrometers, such as the safety interlock, inlet extension, and contact closure. In addition, we describe the structure of our custom software for controlling the nano-DESI MSI platform and provide detailed instructions for its usage. With this tutorial, interested researchers should be able to implement nano-DESI experiments in their own labs.

I. Introduction

Prototyping plays an important role in designing new mass spectrometry instrumentation and coupling of new ionization sources to commercial instruments.[1] Our research is focused on the development of nanospray desorption electrospray ionization (nano-DESI), an ambient ionization technique that has been extensively used for mass spectrometry imaging (MSI) applications.[2] Nano-DESI employs a liquid extraction-based probe that forms a dynamic liquid bridge on a sample surface. Analytes are desorbed from the sample into the liquid bridge and subsequently transferred to a mass spectrometer where they are ionized using electrospray (ESI)-like ionization. Since its initial demonstration as an MSI technique in 2012,[3] nano-DESI has undergone significant advancements in terms of its sensitivity, quantitative capabilities, spatial resolution, and throughput. The spatial resolution of 6-10 μm has been demonstrated in several studies.[4–7] The throughput of the technique has increased by at least an order of magnitude.[8–11] The range of analytes that can be detected in nano-DESI MSI experiments has been expanded from metabolites,[12–15] lipids,[16–18] and drugs[19] to glycans,[20] peptides, and proteins.[21–23] The molecular coverage has been expanded using ion mobility separation,[24–27] online derivatization,[17] ionization enhancement,[28] tandem mass spectrometry (MS/MS),[29,30] selected ion monitoring (SIM) acquisition modes,[31] and single ion detection.[32] Nano-DESI MSI can be implemented on any mass spectrometer equipped with an ESI source. For example, our group has coupled nano-DESI sources with six commercial mass spectrometers.

The implementation of nano-DESI MSI on commercial instruments requires some modifications to the hardware and synchronization of the source with a mass spectrometer acquisition software. So far, several groups have successfully coupled nano-DESI MSI with mass spectrometers in their labs.[23,33–40] To facilitate broader access to nano-DESI MSI, our group has published two protocols detailing how to fabricate nano-DESI probes.[4,16] This tutorial is focused on other aspects of the instrumentation development and coupling of nano-DESI MSI to commercial instruments. We briefly describe the advances in the design of nano-DESI probes, discuss the requirements for coupling of nano-DESI to different mass spectrometers, and describe the LabVIEW software for controlling nano-DESI MSI platforms developed in our laboratory.

II. Fabrication of nano-DESI probes

The conventional nano-DESI probe is comprised of two glass capillaries assembled at a ~90-degree angle, as shown in **Figure 1a**. The primary capillary delivers the extraction solvent to the sample while the secondary capillary transfers the extracted analytes to a mass spectrometer. A detailed protocol for setting up this nano-DESI probe can be found in Ref.16. This probe is relatively easy to assemble and align at the instrument inlet. It provides a spatial resolution of 100-150 µm and is typically used at a scan rate of 20-100 µm/s.

To enhance the spatial resolution of nano-DESI MSI, both glass capillaries are finely pulled, as shown in **Figure 1b**. The probe assembled using pulled capillaries generates a substantially smaller liquid bridge on the sample surface resulting in a spatial resolution of better than 10 µm. This probe is typically operated using a scan rate of 10-40 µm/s. The fabrication and alignment of the high-resolution nano-DESI probe is more challenging in comparison with the probe shown in **Figure 1a**. A protocol for achieving high spatial resolution in nano-DESI MSI has been published.[4]

In both types of capillary-based nano-DESI probes, the solvent flow through the secondary capillary is assisted by the vacuum suction from the MS inlet. Although a self-aspirating probe was used in the initial implementation of nano-DESI, its stability is affected by air bubbles in the nanospray capillary.[2] Meanwhile, stable performance of the probes has been achieved using vacuum-assisted flow.[3] The solvent flow through the probe is adjusted by varying the distance between the probe and MS inlet. Despite the success of these types of probes, their performance is dependent on the vacuum at the MS inlet. To address this challenge, Duncan et al. have developed a pneumatically assisted nano-DESI probe,[41] in which a nebulizer gas is used to assist solvent flow through the secondary capillary as shown in **Figure 1c**. The nebulizer gas is introduced through a PEEK tee. In this probe, the secondary capillary can be much longer (~5 cm) as compared to the conventional probe, in which the length of the secondary capillary is 1-2 cm. The solvent flow rate through the secondary capillary can be adjusted by varying the nebulizer gas pressure. This pneumatically assisted nano-DESI probe shows enhanced sensitivity for metabolite species. The improved desolvation of the microdroplets generated by this probe is beneficial to using pure water as the extraction solvent. A protocol for setting up the pneumatically assisted nano-DESI probe has been reported.[42]

Microfluidic nano-DESI probes (MFP) have been developed to improve the robustness of the technique.[43] As shown in **Figure 1d**, the MFP uses two microfluidic channels to replace the primary and secondary capillaries. After the channels are fabricated, the probe is designed by carefully polishing both the sampling port and nanospray emitter. The MFP eliminates the positioning step required for the conventional nano-DESI probe. Because the relative position of primary and secondary channels remains fixed, the MFP is robust and can be operated at higher scan rates of up to 400 µm/s.[9] Both intermediate (~30 µm) and high (~10 µm) spatial resolution has been achieved using different designs of the MFP.[6,43] Recently, we have fabricated microfluidic fused-silica probes using femtosecond selective laser-assisted etching (SLE) technology, which substantially simplifies the fabrication of the probe and reduces (or completely eliminates) the laborious manual polishing and grinding steps.[10] This approach will make nano-DESI MSI more accessible to the scientific community.

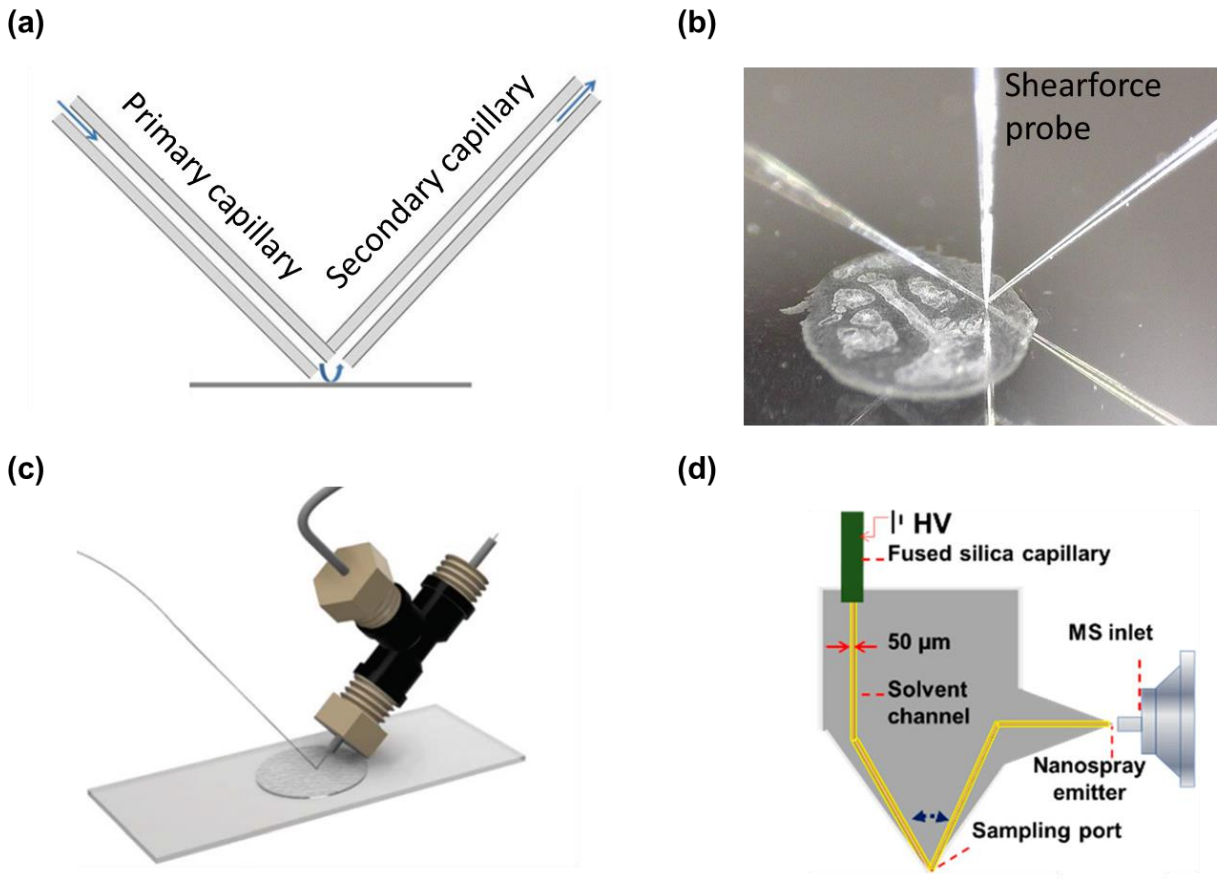

Figure 1. Different types of nano-DESI probes: (a) The moderate-resolution probe composed of two glass capillaries, adapted from Ref.[16] with permission from Springer Nature; (b) The high-

resolution probe composed of pulled glass capillaries and a shear force probe, adapted from Ref.[4] with permission from Springer Nature; (c) A pneumatically assisted probe, adapted from Ref.[41] with permission from RSC publication; (d) An integrated microfluidic probe, adapted from Ref.[43] with permission from John Wiley and Sons.

III. Coupling of a nano-DESI source to a mass spectrometer

1. Safety interlock

To integrate a custom-designed nano-DESI source with a mass spectrometer, it is necessary to bypass the safety interlock mechanism of the commercial ESI source. This process varies depending on the specific instrument and may requires either an electronic or physical device. For example, on Thermo Scientific instruments, a 15-Position D-Sub Plug Connector shown in **Figure 2a** is used to establish a shortcut between pins 9 and 10 and install a 10 kΩ resistor between pins 7 and 8. Alternatively, on Agilent instruments, disabling the interlock can be achieved by affixing a magnetic plate to the side of the ESI source, as depicted in **Figure 2b**. On Bruker timsTOF instruments, the interlock is bypassed by inserting a pin into a designated hole to activate the button, as shown in **Figure 2c**.

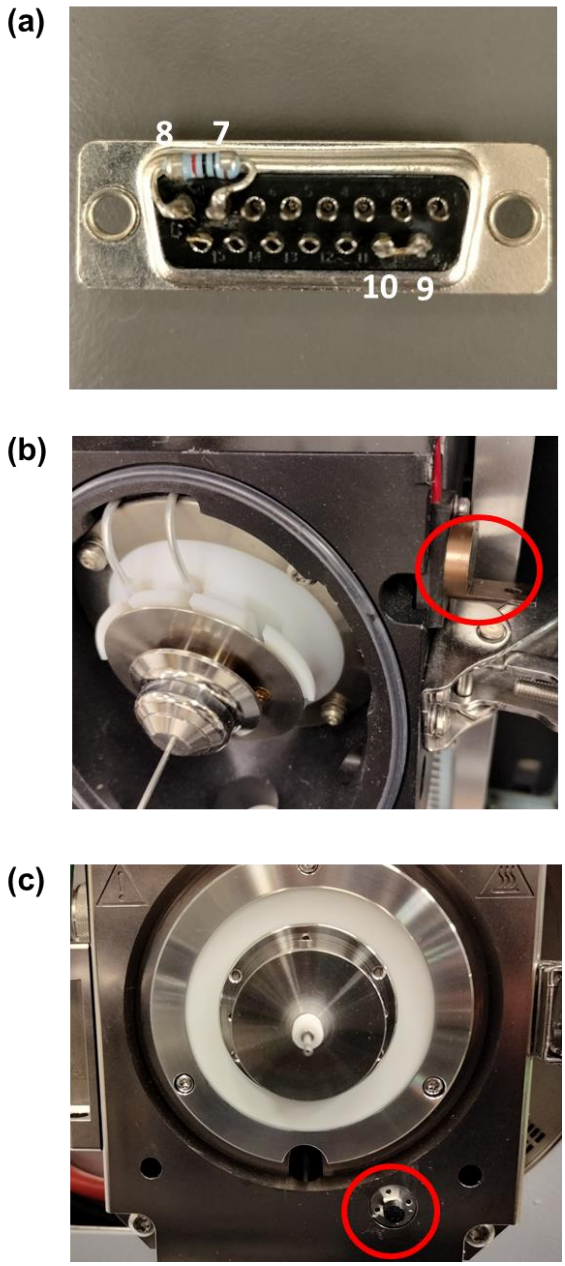

Figure 2. Methods for disabling safety interlocks on commercial mass spectrometers from: (a) Thermo Scientific, (b) Agilent, and (c) Bruker.

2. Mass spectrometer inlet extension

For most of the mass spectrometers equipped with a commercial ESI source, the nano-DESI probe can be installed directly in front of the inlet. However, this limits the accessible area of the sample surface due to the constrained space between the nano-DESI probe and instrument inlet. To address this limitation, a source inlet extension is utilized to expand the accessible area. These source inlet

extensions can be either designed and fabricated or obtained commercially. **Figure 3a** shows a photograph of an ion transfer tube with extension tubing for Thermo Scientific instruments, such as LTQ and Q-Exactive HF-X Orbitrap. This part can be used to replace the regular ion transfer tube and is commercially available through Scientific Instrument Services (Palmer, MA, USA). For Agilent and Bruker instruments utilizing a glass capillary as an ion transfer tube, an inlet extension design is depicted in **Figure 3b**. This design incorporates a cap adapter connecting the glass capillary to a stainless steel tube. An O-ring is used to seal the extension against the glass capillary to ensure proper vacuum suction from the instrument that both minimizes ion losses in the extension and facilitates solvent flow through the nano-DESI probe.

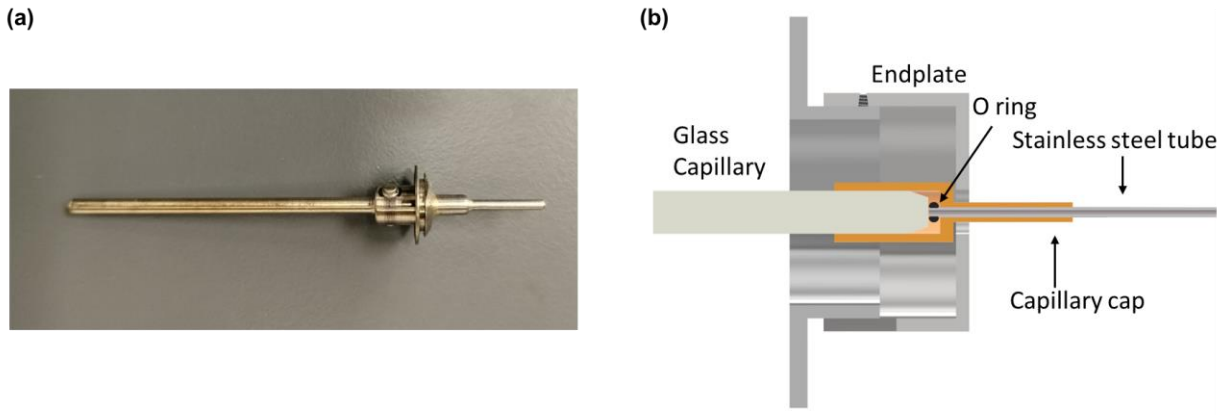

Figure 3. Mass spectrometer source inlet extension examples: (a) Thermo Scientific instruments and (b) Agilent and Bruker instruments, adapted from Ref. [24] with permission from American Chemical Society.

3. Contact closure

As for any other non-commercial ionization source, synchronization between the nano-DESI source and a mass spectrometer is achieved using an external trigger commonly referred to as contact closure.[44] In nano-DESI MSI, data acquisition occurs in discrete lines. Each line scan is initiated using contact closure. Most commercial mass spectrometers support external instrument control via an interface triggered by a pulsed DC voltage. The specifications of this interface are typically described in the instrument manual. To generate the required pulsed signal, we use a multifunctional I/O device (model no. USB-6009) from National Instruments (Austin, TX, USA)

shown in **Figure 4a**, which is interfaced it with a LabVIEW program controlling the nano-DESI source. The initialization process for the device is depicted in **Figure 4b**, where the digital port P0.0 is turned on. **Figure 4c** shows the code that generates the trigger signal. It deactivates the same port for 1000 ms before resetting it, thereby producing a pulsed signal shown in **Figure 4d**. It should be noted that the data acqusition starts at the time the P.0.0 is deactivated because the mass spectrometer is triggered by a negative pulse.

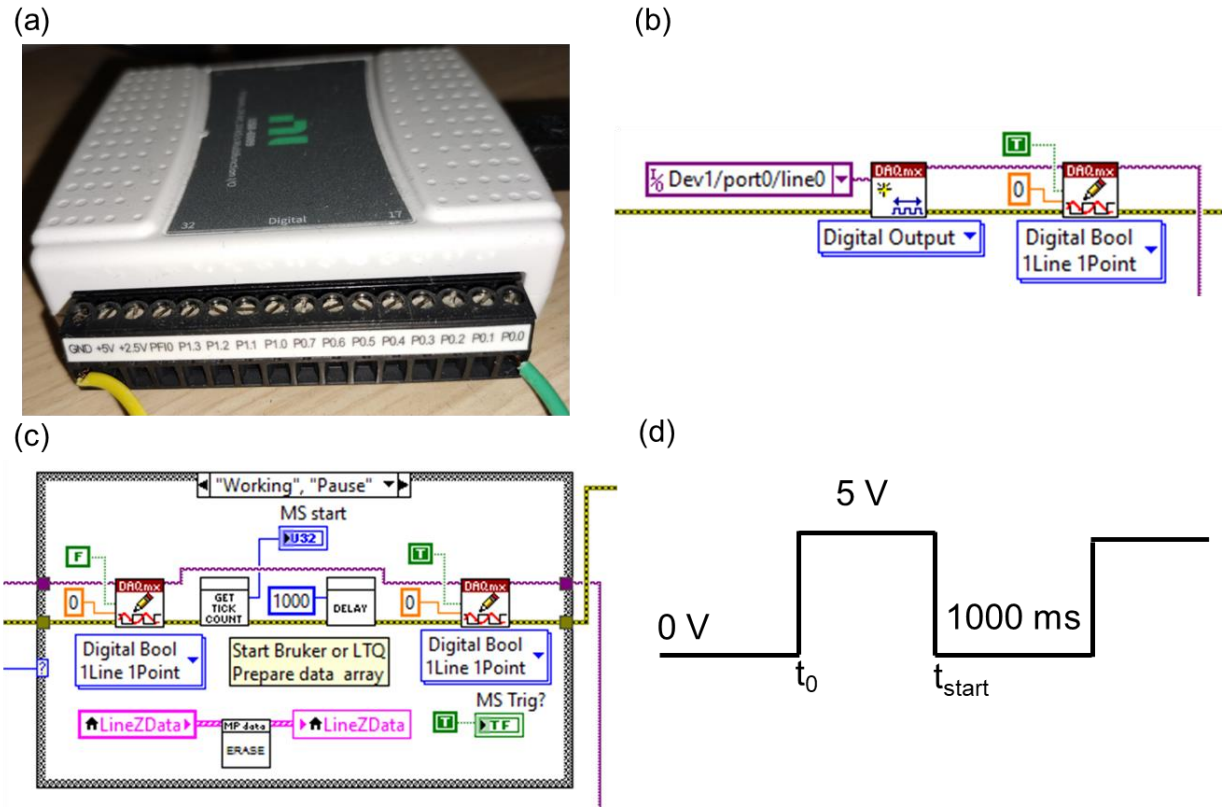

Figure 4. (a) A multifunctional I/O device that generates a pulsed signal. The green wire connects to the trigger signal while the yellow wire is grounded. The LabVIEW code for controlling the device is illustrated in (b) and (c). The resulting output signal is depicted in (d).

IV. LabVIEW stage control software

In nano-DESI MSI experiments, mass spectra are acquired as the sample is scanned under the probe in lines. To ensure consistent contact between the liquid bridge and the sample surface throughout the sampling process, the distance between the sample and the probe is precisely controlled. This is achieved by mounting the sample onto a computer-controlled motorized XYZ stage. Different implementations of the nano-DESI source utilized either the MFA-CC linear

stages from Newport (Newport Corporation, Irvine, CA, USA) or custom-designed XYZ stages from Zaber (Zaber Technologies Inc., Vancouver, Canada). The XYZ stages are controlled using a custom-designed modular LabVIEW code, which also interfaces the nano-DESI source with a mass spectrometer to facilitate data acquisition. In the following section, we describe the architecture and usage of the LabVIEW stage control program for nano-DESI MSI experiments. Interested parties can request access to the software from the corresponding author.

1. Overview

The programming is based on a producer/consumer architecture, functioning as an ordered list that dictates the workflow. The program comprises two major loops: the producer loop with an Event structure and the consumer loop with a Case structure, as shown in **Figure S1** (supporting information). The Event structure loop manages the user interface, reading user inputs and placing corresponding actions in the queue. Meanwhile, the Case structure loop executes the actions from the queue sequentially. The advantage of the two-loop architecture is that the responsiveness of the user interface can be maintained even while processor-intensive actions are being performed in the main loop. When the program starts to run, default actions are added to the queue to initialize the devices. Subsequently, user-triggered actions are appended to the end of the queue to avoid disrupting the ongoing processes. Certain critical actions, such as an Emergency Stop, are prioritized at the front of the queue. The Event structure loop is designed for readability and customization. Each action within the loop can be individually selected in the Case structure menu and modified without impacting other actions. For instance, "Z Init" governs the line scan starting method, "Line Motion" specifies the line scanning technique, and "MS Trig" manages the contact closure signal. These actions can be easily adjusted to serve specific purposes, allowing for flexibility and tailored functionality.

The interface of the software is shown in **Figure 5**. It encompasses the setup of the XYZ stage, nano-DESI MSI parameters, distance control methods, and the plot displaying results. For the first time of use, the XYZ stage device ports need to be configured. In this software, the X and Y stages share the same port because they are serially connected. The Z stage is separated for a faster response time and the baud rate is set to be 115200 bits per second. Those settings can be retained for future use. A separate window will be popped up to adjust the XYZ stage in real-time by a joystick or a keyboard, as shown in **Figure S2**. Line scan parameters must be specified for each

experiment, with "main motion" denoting line scanning direction and "strip motion" indicating line spacing direction. The start and end positions define the corresponding sampling range, while the velocity of line scanning and distance between the lines (strip lines) are determined by the desired spatial resolution. Line scan time is automatically calculated, which may be useful for data acquisition. The Z start position marks where the stage starts at the beginning of the line scan, while the Z out position signifies its position when the stage moves from the end of one line to the start of the next line. The Z out position is typically several hundreds of μm lower than the Z start position.

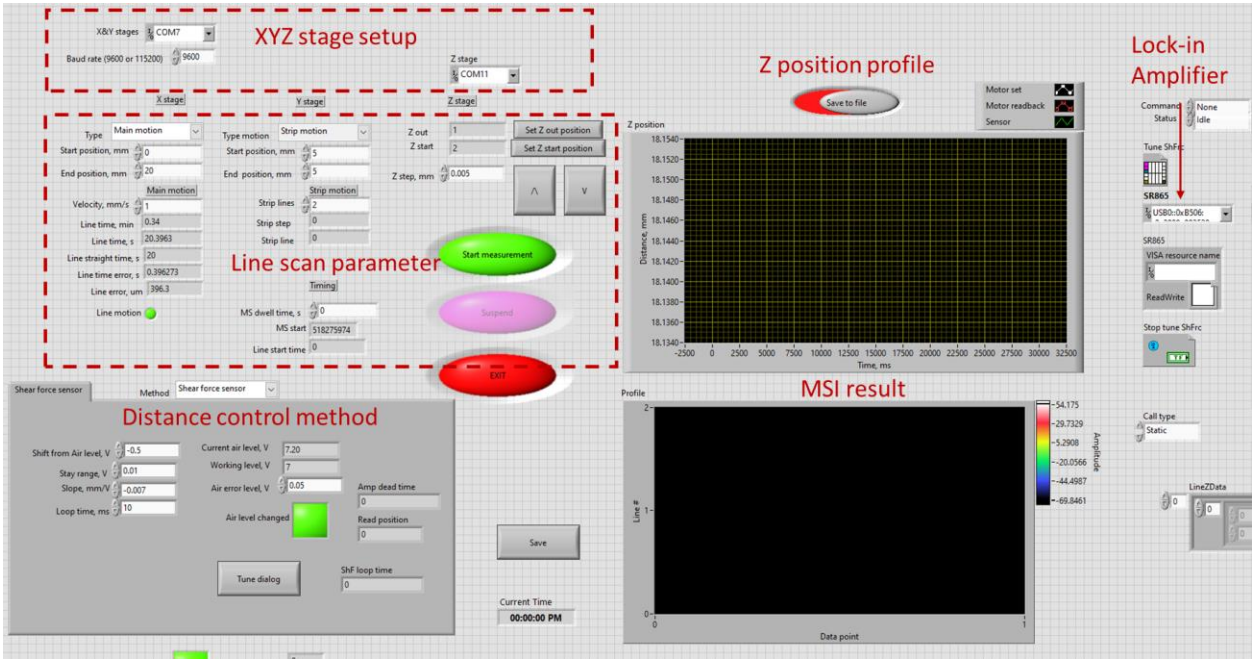

Figure 5. The interface of the LabVIEW stage control software for nano-DESI MSI.

2. Distance control methods

A constant distance control between the nano-DESI probe and sample surface is critical for achieving high spatial resolution experiments or large samples. This LabVIEW program offers support for various distance control methods, such as 3-point method, shear force sensor, laser distance sensor. Among them, the 3-point method is the simplest approach to maintain a stable liquid bridge between the nano-DESI probe and the sample surface. The shear force microscope is a more precise method to control the distance between the nano-DESI probe and the sample surface, which has been extensively used by our group. We have also tested and integrated laser-based sensors into the program. Although in our experience, distance control between the nano-

DESI probe and thin tissue sections using laser-based sensors is challenging, a recent nano-DESI MSI experiment using a laser distance sensor has been reported. [35]

2.1 Three-point plane calibration

As shown in **Figure 6a**, the three-point plane method is implemented by determining the tilt of the glass slide and dynamically adjusting the Z stage position during nano-DESI imaging based on the tilt.[45] The reference coordinates are the start positions of X, Y, and Z. In practice, three locations around the sample region are selected, and at each location, the Z stage is adjusted until a liquid bridge is formed between the nano-DESI probe and the glass surface. Clicking the set point button records these positions. After configuring the three points, it is crucial to check the Z step time highlighted in **Figure 6b** to ensure that it is not excessively large. The Z step time is dependent on the length of each step and the tilt of the glass slide. This parameter determines how often the Z stage moves during the line scan. It should be noted that the actual line scan time is the integer multiple of the Z step time. Reducing the Z step value results in a smaller Z step time. In the three-point plane method, the Z start position determines the position of the Z stage when the line scan starts. The Z out position is where the Z stage stays after the line scan. The three-point plane

method is effective for thin tissue sections with a flat surface and is compatible with low-resolution glass capillary-based nano-DESI probes and MFP probes.

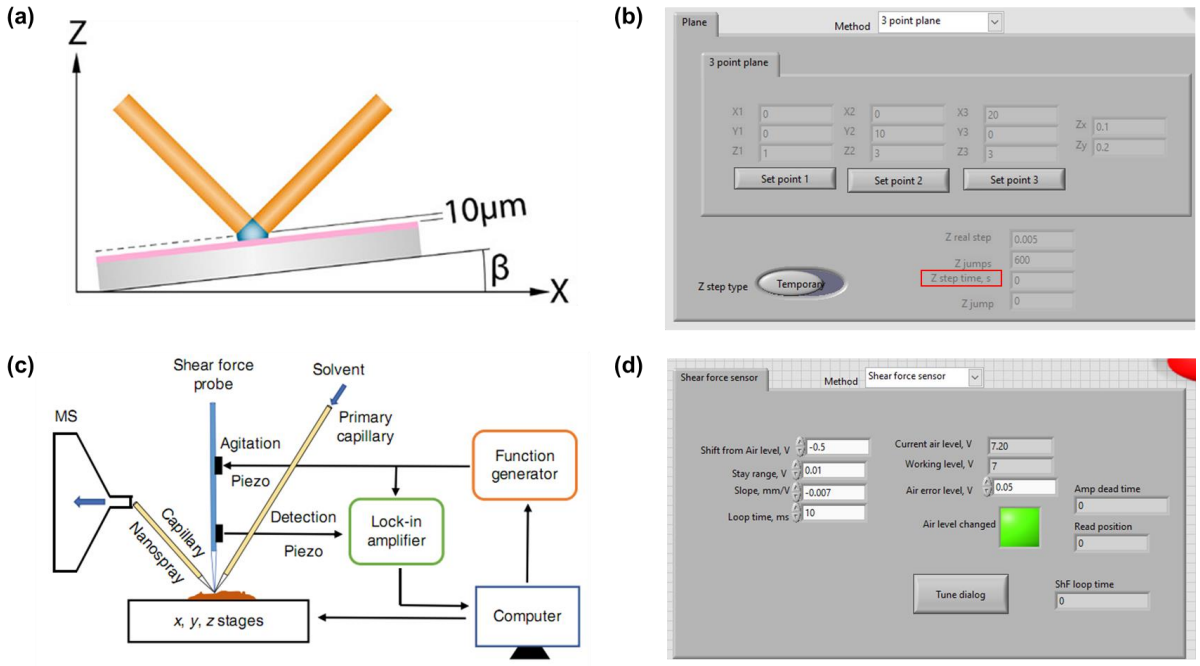

Figure 6. Distance control methods used in nano-DESI MSI. (a) An illustration of the three-point plane method, adapted from Ref. [45] with permission from American Chemical Society; (b) The interface for the three-point plane method in the LabVIEW stage control software; (c) An illustration of the shear force sensor method with a pulled nano-DESI probe, adapted from Ref. [46] with permission from American Chemical Society; (d) The interface for the shear force sensor method in the LabVIEW stage control software.

2.2 Shear force probe

As shown in **Figure 6c**, in the shear force sensor method, a glass capillary probe with two piezo plates is placed near the nano-DESI probe.[46] An AC signal from a function generator is applied to agitate one piezo plate, while a lock-in amplifier is employed to detect the electrical signal generated by the other piezo plate. Some of the natural oscillations of the shear force probe are affected by the surface. To find the optimal working frequency, a frequency scan program is designed. By comparing the frequency response of the shear force probe in the air and on the surface, the frequency that is most sensitive to the sample surface is selected.

In practice, a lock-in amplifier (model SR865) from SRS (Stanford Research Systems, Sunnyvale, CA, USA) is connected to the LabVIEW software. The USB port may need to be refreshed before each run in the "SR865 window" in **Figure 5**. Reconnecting the lock-in amplifier may solve most of the errors. In the method window of **Figure 5**, choose the shear force sensor and a window will appear as illustrated in **Figure 6d**. To configure the parameters for the shear force probe, it is necessary to initiate the frequency scan program. By clicking the Tune dialog button, the frequency scan window is activated as shown in **Figure 7**. The agitation AC signal voltage and frequency are adjusted in the RS856 parameters window. The frequency start, stop, and step values are specified in the scanning parameters window. The frequency scan is typically acquired twice both in the air and on the surface to determine the frequencies that are most affected by the sample. The best values of the frequencies are suggested in a list by the LabVIEW program as shown in **Figure 7**. Next, an approach curve is acquired by selecting one of the suggested frequencies and recording the amplitude of the vibration at this frequency as a function of the distance between the shear force probe and the sample surface. This is achieved by setting the Z start position in which the shear force probe is in the air and the Z end position in which the shear force probe contacts the surface. The approach curve is displayed on the screen as shown in **Figure 7**. The slope of the curve and the amplitude difference between air and surface are used for setting up the shear force probe parameters. By exiting this window, the user returns to the main window.

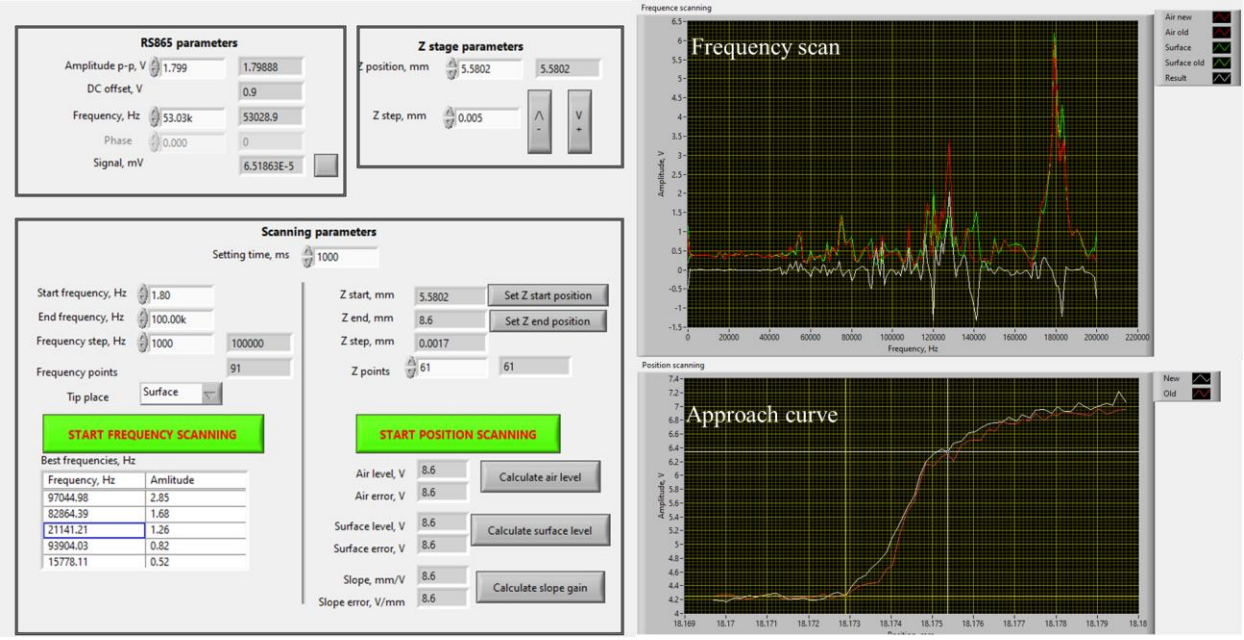

Figure 7. The interface of the frequency scan program that finds the best working frequency for the shear force probe.

For the shear force sensor method, three important parameters need to be adjusted: “Shift from air level”, “Stay range”, and “Slope”, as shown in **Figure 6d**. The shift from the air level should be approximately 20% of the difference between the air and surface levels, and it determines the working distance of the shear force probe. The sign of the shift from the air level is determined by the Z stage installation direction. If increasing the Z value causes the nano-DESI probe to approach the sample surface, the sign of the shift from the air level should be opposite to the slope gain. The stay range signifies the range in which the Z stage does not move when the shear force output signal is within the range of (air level − shift from air level) ± stay range. Typically, the stay range is set to be 20% of the shift from air level. The slope value is determined by the frequency scan program. For smooth and flat samples, an absolute value between 0.001 and 0.0005 is recommended. This setting helps prevent over-adjustment of the distance between the shear force probe and the surface. The loop time defines how quickly the LabVIEW reads the lock-in amplifier signal. It is worth noting that the meaning of Z start in the shear force sensor method is different from that in the 3-point method. Z start is the starting point where the shear force microscope begins working, and it should be 20-50 μm from the sample surface to avoid damaging the shear force probe.

V. Conclusions and Outlook

Advancements in the spatial resolution, throughput, selectivity, molecular coverage, and quantitative capabilities of nano-DESI MSI have prompted several research groups to implement this imaging capability in their laboratories. To promote further growth of the nano-DESI MSI community, we provide an overview of the current nano-DESI probe designs, describe hardware modifications necessary for coupling of the nano-DESI source to various mass spectrometry platforms, and describe the LabVIEW software that controls the position of the sample during imaging experiments and interfaces the custom-designed source with a mass spectrometer. We also provide step-by-step instructions for setting up the constant distance mode nano-DESI MSI experiment with different distance control methods. We believe that this tutorial will be of interest to researchers interested in implementing nano-DESI experiments in their labs.

Supporting Information

Additional figures showing the LabVIEW software for nano-DESI (PDF).

Acknowledgements

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