Enhancing Metabolite Coverage for Matrix-Assisted Laser Desorption/Ionization-Mass Spectrometry Imaging Through Multiple On-Tissue Chemical Derivatizations

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Abstract

The ability to study and visualize metabolites on a cellular and sub-cellular level is important for gaining insights into biological pathways and metabolism of multicellular organisms. Matrix-assisted laser desorption/ionization (MALDI)- mass spectrometry imaging (MSI) is a powerful analytical tool for metabolomics experiments due to its high sensitivity and small sampling size. The spatial resolution in MALDI-MSI is mainly limited by the number of molecules available in a small sampling size. When the sampling size is low enough to achieve cellular or sub-cellular spatial resolution, signal intensity is sacrificed making poorly ionized metabolites difficult to detect. To overcome this limitation, on-tissue chemical derivatization reactions have been used to enhance the desorption/ionization efficiency of selected classes of compounds by adding a functional group with a permanent positive charge or one that can be easily ionized. By utilizing several chemical derivatizations in parallel, metabolite coverage can be drastically improved. This chapter outlines methodology for sample preparation and data analysis for on-tissue chemical derivatization using various derivatization reagents.

Keywords

metabolomics, on-tissue derivatization, mass spectrometry imaging, matrix-assister laser desorption/ionization, metabolite coverage

Running Title: On-Tissue Chemical Derivatization for MALDI-MSI

1. Introduction

Mass spectrometry imaging (MSI) has become a widely used analytical tool for mapping metabolites and has led to a deeper biological understanding of various multicellular organisms. MSI has led to innovations in drug discovery and development[1,2], disease research[3-5], biomarker discovery[6], plant biology[7], and forensic science[8]. Each of these fields involves highly complex organisms and biological systems; thus, metabolomics experiments should be able to determine differences on cellular and sub-cellular levels in order to get the most accurate information. Matrix-assisted laser desorption/ionization (MALDI)-MSI can achieve small sampling size, which allows for imaging at the cellular and sub-cellular level. Along with high chemical versatility and sensitivity, these traits have allowed MALDI-MSI to become increasingly popular for spatial mapping of metabolites across different biological systems.

Laser spot size is most critical in limiting the spatial resolution in MALDI-MSI. It has been reduced down to 5-10 μm by simply modifying optical systems in commercial instruments.[9,10] Additionally, transmission mode geometry MALDI (t-MALDI) and t-

MALDI-2 technology has recently been implemented to reduce the laser spot size below 1 μm.[11,12] Our group has established a MALDI-MSI platform with 5-10 μm spatial resolution, and this has been applied to visualize metabolites in maize roots[13,14], leaves[9,15,14], and seeds[16,17]. Moreover, this platform has shown that metabolite distribution is sometimes heterogeneous even between the same type of cells[9,18] and the sub-cellular localizations can have important biological implications[19].

Although MALDI-MSI provides high-spatial resolution information that is unprecedented in traditional metabolomics, there are some issues such as a reduction in analyte signal owing to small sampling size, a limited number of analytes due to the selectivity of the matrix, and lack of chromatographic separation. Chemical derivatization strategies can be used to overcome these limitations to enhance the ionization efficiency of metabolites. In a recent report by Dueñas et al., a combination of chemical derivatization reactions was adopted to selectively enhance metabolite signals of specific functional groups in maize. For instance, 4-hydroxy-3-methoxycinnamaldehyde (coniferyl aldehyde; CA) was used for the derivatization of primary amines[20,21], Girard's reagent T (GT) for carbonyl groups[22-24], and 2-picolylamine (2-PA) for carboxylic acids.[25] The general reactions used for these modifications are shown in **Scheme 1**. Implementing chemical derivatization reactions such as these on serial tissue sections dramatically expands the metabolite coverage by enhancing the signal of certain classes of compounds at a time. This strategy has been demonstrated by our group and proven to be beneficial for greater metabolite coverage on maize roots and leaves.[14]

In this chapter, we describe a sample preparation method for on-tissue chemical derivatization using CA, GT, and/or 2-PA as derivatization reagents. This strategy is applicable to any tissue; however, this chapter uses plant tissue as an example. **Figure 1** shows a general

workflow using maize roots as the example tissue. Briefly, maize roots are grown hydroponically in wetted paper towels. Once the desired length is reached, the roots are harvested, embedded in gelatin, and flash frozen in liquid nitrogen. Then the roots are cryo-sectioned to 10 μm thickness and vacuum dried. Next, the derivatization agent and matrix are applied to the tissue sections prior to the MSI experiment. The end result is a dramatic increase in ionization efficiency for many analytes and visualization for compounds that was not previously possible.

2. Materials

2.1 Cryo-sectioning

1. Cryo-microtome: set at -20 °C.
2. Glass microscope slides and a microscope slide box (see Note 1).
3. Scotch tape.
4. Plant tissue (for example, maize root tissue as shown in **Figure 1**).
5. 300 bloom gelatin.
6. High-purity water
7. Cryomold
8. CryoJane® adhesive tape (Leica Biosystems, Buffalo Grove, IL, USA; see Note 2).
9. Liquid nitrogen.
10. Disposable pipettes.
11. 70% (v/v) ethanol solution.
12. Optimal Cutting Temperature (OCT) compound.
13. Styrofoam container with dry ice.

14. Roller tool (see Note 3).

2.2 Tissue Drying

1. Vacuum chamber with a vacuum pump capable of reaching pressures in the mTorr range (see Note 4).
2. Heat sink: Aluminum block precooled to -80 °C.

2.3 Derivatization and matrix application via TM Sprayer

1. TM Sprayer (HTX Technologies, LLC. Chapel Hill, NC, USA) (see Note 5).
2. Syringe pump.
3. CA reagent: 20 mg/mL coniferyl aldehyde in methanol.
4. 2-PA reagent: 6 mM 2-picolylamine, 30 mM 2,2-dipyridyldisulfide (DPDS), 30 mM triphenylphosphine (TPP) in acetonitrile.
4. Matrix solution: 40 mg/mL 2,5- dihydroxybenzoic acid (DHB) in 70% methanol.
5. 1 mL luer lock glass syringes with fill needles.
6. Tank of compressed nitrogen.

2.4 Derivatization Application via ESI deposition

1. Power supply (up to 5 or 10 kV)
2. Syringe pump
3. Fused silica capillary: 363 μm O.D, 150 μm I.D., 40 mm long

4. Capillary sleeve: 0.025” OD, 0.0155” ID
5. PEEK tubing: 1/16” OD, 0.010" ID, 3 – 5’ long
6. Finger tight PEEK tubing fitting (x2): 1/16” OD.
7. Luer lock syringe
8. Luer lock adaptor for 1/16” OD PEEK tubing.
9. Zero volume stainless steel connector for 1/16" PEEK tubing
10. Ring stand (or anything that can hold the ESI capillary sprayer).
11. GT reagent: 10 mg/mL Girard’s Reagent T, 2% trifluoroacetic acid (TFA) in methanol.
12. 1 mL glass syringe with needles.

2.5 Matrix Application via Sputter Coater

1. Sputter Coater.
2. Methanol.
3. Tank of compressed argon (minimum 99.9% purity).
4. Vacuum pump.
5. Gold sputter targets.

3. Methods

3.1 Tissue Sectioning

This sub-section aims to explain how to prepare tissue samples prior to derivatization, matrix application, and MSI.

1. Prepare a 10% w/v solution of gelatin in high-purity water and heat at ~40 °C until the gelatin has completely dissolved and the solution is clear.
2. Using a disposable pipet, fill a cryo-mold with the gelatin solution. Place the tissue of interest in the cryo-mold, and immediately flash freeze the sample, by placing the cryo-mold on the liquid nitrogen.
3. When the gelatin is ~80% opaque (see Note 6), place the mold in a -20 °C freezer and allow the sample to thermally equilibrate (wait at least 30 minutes, but 24 hours is ideal for best results).
4. Transport the mold to the cryo-stat in a Styrofoam cooler filled with dry ice to prevent melting. Make sure the cryostat has been pre-chilled to -20 °C.
5. Remove the sample from the mold and affix it to the aluminum cryo-stat chuck using OCT compound (see Note 7). Wait until the OCT compound turns white before cryo-sectioning the tissue.
6. Place the cryo-stat chuck onto the microtome sample stage, and orient the sample in the desired position
7. Clean the blade with a 70% ethanol solution before sectioning and cryo-section the tissue at the desired thickness (see Notes 8-10).
8. Collect the tissue on CryoJane adhesive tape by first removing the backing from the CryoJane tape and then placing the tape over the tissue sample. Use a roller tool to press the tape against the gelatin to ensure adhesion. Cryo-section the tissue at 10-15 μm thickness.

a. Place the CryoJane tape with section attached face-up on a pre-chilled glass slide. Attach the CryoJane tape to the glass slide by taping both ends to the slide with Scotch tape. (see Note 11)
b. To thaw mount, place the CryoJane tape with section attached face-down on a pre-chilled glass slide, use a roller tool to ensure the sample is flat, and then hold your finger firmly on the bottom of the glass slide under the sample. Once the sample melts, place the glass slide on a cold metal block. When the sample refreezes (gelatin will turn opaque) slowly peel the CryoJane tape from the glass slide. The sample and gelatin should remain on the glass slide.

9. Repeat step 8, until the desired number of tissue sections have been collected.
10. Store all collected tissue sections at a -80 °C freezer until analysis. Once again, transport sections in a Styrofoam cooler with dry ice.

3.2 Tissue Drying

This sub-section describes how to dry tissue samples and bring them up to room temperature while minimizing compound delocalization.

1. Connect vacuum chamber to a vacuum pump.
2. Remove tissue samples from the -80 °C freezer, and place the glass slides with the tissue sections onto the heat sink (see Note 12).
3. Quickly place the heat sink in the vacuum chamber and turn on vacuum pump to evacuate chamber.

4. Once the samples have dried and the heat sink has warmed to room temperature, vent the chamber and remove the tissue samples.

3.3 On-Tissue Derivatization with TM Sprayer

This sub-section explains how to derivatize tissue sections using a TM Sprayer. This technique is optimal for CA and 2-PA derivatization. **Figure 2** includes screenshots of the TM Sprayer software with numbered labels for features mentioned. To further improve ionization through cation adduct formation, cation solutions (e.g., potassium acetate) can be separately applied to the tissues using TM sprayer.

1. Fill a syringe with 50:50 methanol:water and place it in the syringe pump.
2. Turn on the syringe pump. Make sure the diameter is correctly set to match the diameter of the syringe you are using. Set the flow rate between 0.05 -0.2 mL/min, start the flow, and turn on the nitrogen gas. Allow the solution to run for several minutes until the system is flushed with solvent.
3. Meanwhile, create a method using the TM Sprayer software using the methods tab (**1**) located in the upper right hand corner of the software interface. The following parameters are used to coat the tissue section for both derivatization reagents: 30 °C nozzle spray temperature, 0.03 mL/min flow rate, 1200 mm/min linear velocity, and 8 passes in a crisscross pattern (CC) and 3 mm track spacing. The gas pressure was set to 10 psi and the nozzle height to 40 mm (see Note 13).
4. Using the TM Sprayer software, under the sample tab (**2**) located in the upper left hand corner of the software interface, choose the area over which to spray. This can be done

either from the “plate selection” drop down menu (**3**) or by inputting values to the “X Range” and “Y Range” boxes (**4**) that correspond to that area of the grid on the sample stage.

5. Once the system has been completely flushed with solvent, fill a second syringe with derivatization regent solution (CA or 2-PA reagent) and switch out the syringes on the syringe pump (see Note 14). If the syringe is a different size, make sure to change the diameter on the syringe pump. Adjust the flow rate to 0.03 mL/min to match the method.
6. Place the slide onto the sample stage and orient it in the desired orientation. Tape the slide down to keep it from moving while being sprayed.
7. Move to the “cycle” tab (**5**) on the TM Sprayer software and press start (**6**). If using a syringe pump, there is no reason to move between “load” and “spray” (see Note 15). So, after pressing start, when it prompts you to move to “spray” mode, you can immediately press continue.
8. Monitor the syringe to ensure the reagent solution does not run out. Make use of the “estimated cycle usage” (**7**) provided by the software.
9. When the method has completed, remove the sample, and switch the derivatization reagent syringe out for the solvent syringe once again, and flush the system before exiting the system (see Note 16).
10. Turn off the nitrogen gas and wait for the low pressure light to come on before exiting the software. Confirm that a drop of solvent forms on the spray tip to ensure there are no clogs. Exit the software before turning off the TM Sprayer.

3.4 Derivatization Application via ESI deposition

This section will explain how to apply derivatization agent via Electrospray Ionization (ESI) deposition. ESI deposition is another method of on-tissue derivatization[25] where the high voltage (see Note 17) applied to the spray facilitates the formation of microdroplets. ESI deposition works very well for charged derivatization agent (e.g., GT)[14]. First step is to construct the ESI deposition apparatus as shown in **Figure 3**.

1. Insert the fused silica capillary (see Note 18) into the capillary sleeve and then into a PEEK tubing fitting.
2. Connect a PEEK tubing to a syringe luer adaptor on one side and another Peek tubing fitting on the other side.
3. Insert both PEEK tubing fittings into a zero volume stainless steel connector. This metal connection will provide electrical contact to the liquid flow by connecting to the power supply.
4. Connect the ground connection to the base so there is an electric field between the sprayed surface (see Note 19) and the sprayer. Mount the PEEK tubing setup to a ring stand but keep the metal connection shielded from the ring stand to avoid grounding out the electrical connection. The fused silica capillary should be 3 cm above the surface that will be sprayed.
5. Fill the 1 mL syringe with a derivatization solution (e.g., GT reagent) and connect to the PEEK tubing. Place the syringe into the syringe pump and set the flow rate to 0.3 mL/hr. Make sure the diameter is correctly set to match the diameter of the syringe you are using.
6. Allow the solution to completely fill the tubing and begin to form droplets at the fused silica capillary tip. Once droplets begin to form, turn on the 5kV power supply.

7. Carefully place the sample (see Note 20) to be sprayed under the capillary and spray around 200 μL of derivatization agent (will need to adjust on a per sample basis).
8. After finishing ESI deposition, stop the flow, turn off the voltage and remove the sample. Make sure to flush the tubing with methanol to remove any leftover reagent.

3.5 Matrix Application via Sputter Coater

This section will explain how to use a sputter coater for matrix application. Sputter coating involves the physical deposition of a thin metal layer onto the sample surface through argon plasma generated by an electrical current. Sputter coating is a convenient method for application of nanometer-thick metal matrices because it is very quick, highly reproducible, and homogenous. Inert metals such as gold and silver are commonly used matrices for various applications. CA and 2-PA derivatization both use gold as a matrix in this example. **Figure 4** is a labelled schematic of the Sputter Coater. More information about sputter coating is available in the work by Hansen et al.[26]

1. Place sample slide onto the Sputter Coater sample table (see Note 21). The distance between the metal target and the sample is about 4 cm.
2. Clean the target using methanol and place the target into the holder located on the inside of the chamber top plate. This is done by removing the dark space shield, unscrewing the threaded clamp ring, placing the target inside, and replacing the threaded clamp ring.
3. Turn on the sputter coater, then the vacuum pump, and finally open the argon gas tank to 5 psi.

4. Set the sputter time by holding down the pause button while pressing the up or down arrows until the timer display reads the desired time. The optimal time for our setup is 20 seconds.
5. Set the current to 40 mA by holding down the “set mA” button while pressing the up or down arrows until the display reads 40 mA.
6. Wait until the pressure in the chamber is between 0.05 and 0.1 mbar, and then hit the cycle button, which will automatically move through the steps for sputtering. It will begin by flushing out the chamber twice with argon gas (pressure ~8 psi) before bringing it back down to vacuum pressure. Then the plasma will form and the timer will begin to count down (see Note 22).
7. Once the sputter coating has been finished, turn off the sputter coater, vacuum pump, and close the argon gas tank.
8. Allow the chamber to vent and remove samples (see Note 23).

3.6 Matrix Application via TM Sprayer

This section discusses matrix application using a TM Sprayer. It is important to note that there are other methods of matrix application for organic matrices, such as sublimation or other nebulized sprayer alternatives[27]. Charged analytes, such as those derivatized with GT, work the best with DHB matrix according to our experience.

1. Follow the same steps in section 3.3 except for the spray parameters.
2. Choose a method from the existing list or create a new one.

3. Our method for matrix application included the following parameters: 75 °C nozzle spray temperature, 0.1 mL/min flow rate, 1200 mm/min linear velocity, and 8 passes in a crisscross pattern (CC) and 3 mm track spacing. The gas pressure was set to 10 psi and the nozzle height to 40 mm.

3.7 Data Acquisition

This section will describe how to perform a mass spectrometry imaging experiment on a Thermo MALDI Linear Ion Trap (LIT)- Orbitrap Instrument (MALDI-LTQ-Orbitrap Discovery; Thermo Finnigan, San Jose, CA, USA). Similar data analysis can be performed for other types of imaging mass spectrometers following the vendor-specific user manual.

1. Scan an image of the tissue to load into the TunePlus instrument software.
2. Optimize instrument parameters such as laser energy and number of laser shots in the TunePlus software. Save a tune file containing this information.
3. Open the MALDI window of TunePlus and select the desired area to image using the scanned optical image. Select the raster step size and save this information as a position file.
4. Set up a new instrument method using Xcaliber software. The method should include the desired mass range, resolution, polarity, and the saved tune file from step 2.
5. Set up a new sequence using Xcaliber software. Include a name for the data file, a file destination folder, the saved instrument method, and saved position file. Repeat this with all the tissue sections you wish to run.
6. Select all of the samples to be analyzed and select “run sequence.”

3.8 Analysis of Derivatized Data Sets

This section will explain how to analyze derivatized data sets, identify derivatized peaks, and address some challenges. The exact masses added due to derivatization (Δm_{der}) are 160.052 u, 114.103 u, and 90.058 u for CA, GT, and 2-PA derivatization, respectively. Subtracting these masses from the mass values of the peaks only present in derivatized samples may help identify the derivatized metabolites. GT contains a permanently positive charge, so derivatized compounds are detected as Δm_{der} adducts. Products from CA and 2-PA derivatization commonly form potassium adducts in plant tissues and are therefore detected as Δm_{der} + K adducts.

1. Export the mass list to Excel from Xcaliber (Thermo).
2. Unique features not present in the underivatized data sets can be identified in the derivatized data sets using several filtering steps. Filtering can be done manually in Excel as described in steps 3-5 or using a python script on the text file.
3. Remove *m/z* values with a signal intensity of less than 500 (or signal-to-noise-ratio of 20) from the peak list (see Note 24).
4. Remove all ^{13}C isotope peaks, alkaline ion adducts, and known contamination peaks.
5. Generate MS images for the remaining *m/z* values using MSiReader[28] to ensure they are localized to the tissue; remove any peaks localized off-tissue.
6. Identify unique features using database search with accurate masses of experimental data and a mass tolerance of 5 ppm. Make sure to use the underivatized mass for database search. The databases utilized for maize application were METLIN (https://metlin.scripps.edu) and Maize Genetics and Genomics Database (https://corncyc-b73-v4.maizegdb.org).

7. Filter mass values based on the functional groups present. For example, a match for a feature from a CA dataset should contain a primary amine group.
8. If applicable, perform MS/MS experiments on tissue sections to confirm derivatization. For example, a fragment at *m/z* 161 indicates derivatization with CA.
9. Use imaging software to create the images for unique features and label with identifications as seen in **Figure 5,** which shows the MS images of several amino acids in maize root tissue with and without derivatization with CA. Without derivatization, only three amino acids were detected, while after derivatization, twelve were detected with good intensity.

4. Notes

1. For MALDI-Linear Ion Trap (LIT)-Orbitrap instruments it is not necessary to use only conductive materials because of the relatively low voltage needed to extract ions. Therefore, normal scotch tape and glass microscope slides are acceptable. If using another system, such as a MALDI-TOF, conductive materials such as indium tin oxide (ITO) glass slides will be needed.
2. CryoJane® adhesive tape is removable from tissue sections after flash UV curing. In this method, we use CryoJane tape without UV curing, but CryoJane Tape-Transfer System can be used to produce high-quality frozen sections of difficult tissues.
3. The roller tool can be anything small and cylindrical. A small paintbrush handle, pen, or pencil would all work.
4. A lyophilizer with a vacuum system can be used instead.
5. Other similar devices that can homogeneously deposit organic matrix might also work.

6. When embedding samples, leaving the gelatin completely frozen in liquid nitrogen can cause the gelatin to crack. Therefore, we suggest to remove the mold once the gelatin is about 80% frozen (opaque). Alternatively, the sample can be embedded under milder conditions by flash freezing the sample in dry ice chilled isopropanol[29].
7. As OCT can cause background signals in the mass spectrometer, it cannot be used as an embedding material but a minimum amount of OCT can be used to fix the gelatin to the cryo-stat chuck.
8. Before collecting your tissue, use the cryo-stat to section several sections of gelatin until the surface is flat and the tissue sample is visible. Depending on the tissue, it may be helpful to look at an optical image of the sectioned tissue before beginning to collect sections. If there is a specific morphology or cell type you are interested in, make sure it is present in the portion of the tissue sectioned. To ensure the region of interest is collected, we typically observe one or two sections under a bright field microscope before beginning collection. Since the tissue goes from -20 °C to room temperature when visualizing under the microscope, the tissue will break and the cells will burst. Discard this section as it cannot be used for MSI.
9. If you choose to look at tissue sections under a bright field microscope before collecting sections, it helps to put an additional chilled glass slide under the one that has the tissue attached. This slows the amount of time it takes for the gelatin to melt completely. In order to visualize an intact sample, complete this step within 10 seconds after removing from the cryo-stat.

10. When sectioning, it can be helpful to use a forceps to collect the section. Holding the bottom edge of the tape with a forceps while using the microtome is more efficient than using your hands.
11. In this method, we attach the tape with the tissue on a MALDI glass slide, which may not work for MALDI-MS with high extraction field. High extraction field instruments require a coherent field on the sample, so samples directly mounted to ITO slides by thaw mounting or thermal adhesion are necessary. Samples on non-conductive tape attached to the slides would not work.
12. The heat sink could be a variety of different materials; however, we use an aluminum block that is 18 cm x 6 cm x 0.65 cm. With this heat sink, the vacuum drying and thermalization process for plant tissues takes 75 to 90 minutes.
13. While we suggest conditions that worked best for our system, some additional optimization may be necessary depending on the apparatus condition and sample.
14. If using the syringe pump with the TM Sprayer (as opposed to a HPLC pump), switch between syringes as quickly as possible to limit the amount of time there is nothing running through the lines.
15. Since the sample is in the syringe and constantly being pumped, the sample loop does not need to be filled. Hence, it does not matter whether the sample loop is in the “Load” or “Spray” position. This will not be true if a HPLC pump is used instead of a syringe pump.
16. It is necessary to flush the TM sprayer with several milliliters of solvent. If any derivatization agent remains in the tubing or nozzle, the nozzle may become clogged.

17. As a note of caution, the ESI setup requires a high voltage power supply and can be dangerous to operate. Be careful not to touch the spray or any metal connected to the voltage with bare skin.
18. The end tip of capillary should be cut clean to achieve a good spray. Alternatively, commercially available precut capillary can be used.
19. Due to the necessity of a stable electric field, it may be helpful to mount the whole setup onto a square aluminum, or other conductive metal, slab. This allows you to easily connect the ground as well as a more consistent field.
20. The ESI setup requires a strong electric field between the spray surface so the sample slide must also be conductive, stainless steel slides or ITO glass slides are good options.
21. When sputtering samples, make sure slide is placed as close to the center of the sample stage as possible.
22. In some instances, the plasma does not form immediately when using the sputter coater. If this happens, wipe down the target and metal holder once again with methanol as metal residue will sometimes form and hinder the plasma formation.
23. It is useful to wipe down the inside of the sputter coater chamber immediately after sputtering with methanol to keep everything clean. If metal residue accumulates over time in the chamber, it is much harder to remove.
24. This threshold can be changed depending on the specific application.

Acknowledgements

This work is supported by National Science Foundation (NSF) (Award# 1905335).

Figure Caption

SCHEME 1. Three on-tissue chemical derivatization reactions used in this method: (a) coniferyl aldehyde (CA) for a primary amine, (b) 2-picolylamine (2-PA) for a carboxylic acid, and (c) Girard's reagent T (GT) for a carbonyl group. TPP: triphenylphosphine. DPDS: 2,2-dipyridyldisulfide.

FIG1. Workflow for MSI experiment using maize roots as an example tissue sample.

FIG2. Screenshots from TM Sprayer software with reference numbers corresponding to the text.

FIG3. Image of a home-made ESI deposition setup.

FIG4. Photo of sputter coater with labels for the major components.

FIG 5. MS images for the 12 amino acids detected in Mo17 maize roots. The first 2 columns are control roots without CA derivatization and the last column is with CA derivatization. 'M+K' indicates the non-derivatized amino acids detected as potassium ion adduct. 'M+CA+K' indicates the derivatized amino acids detected as potassium ion adduct. The numbers below each amino acid label correspond to the maximum intensity scale used to produce the false color image. The scale bar is 500 μm.

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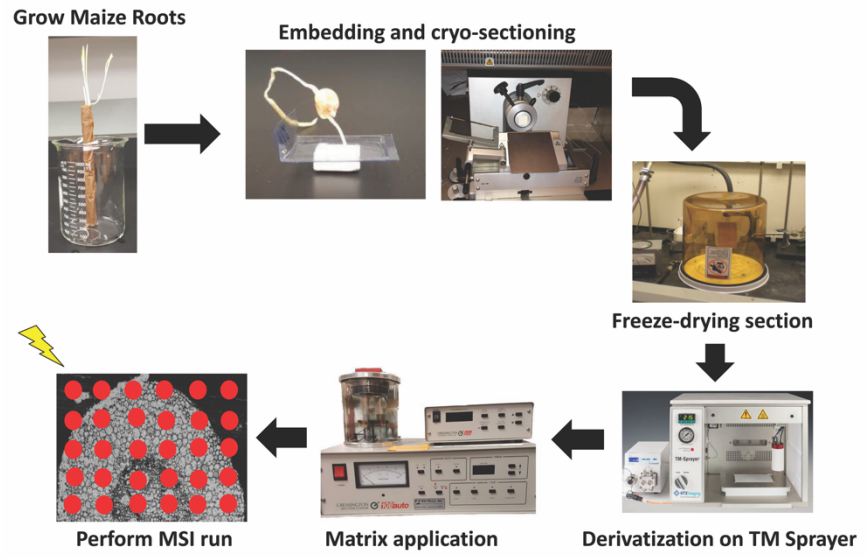
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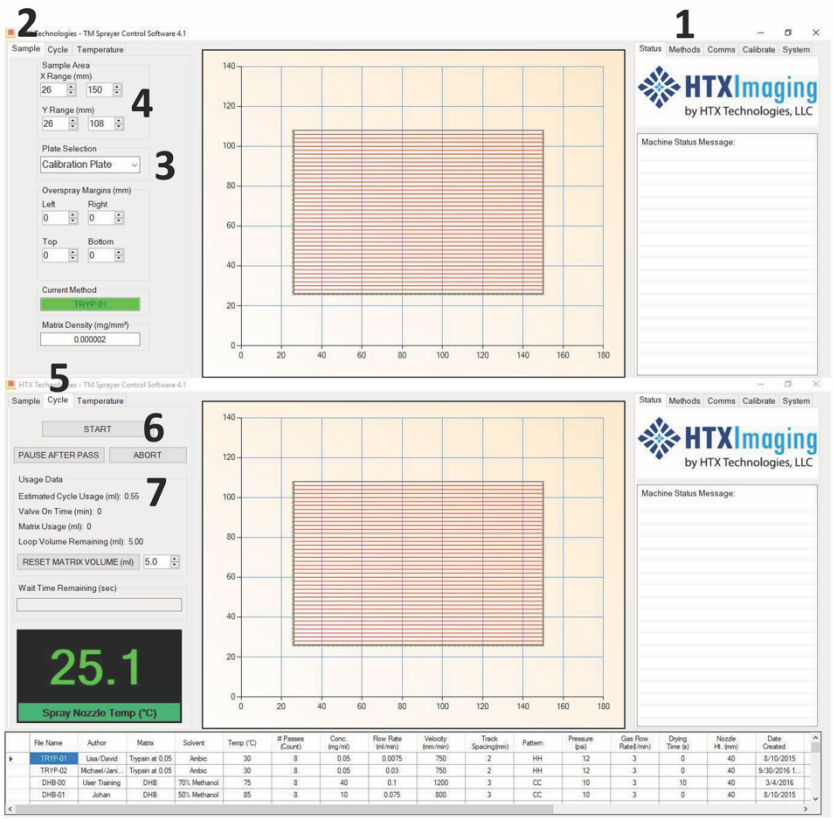
Embedding and cryo-sectioning
Freeze-drying section
Derivatization on TM Sprayer
Matrix application
Perform MSI run

Fig. 1


1
2
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HTXImaging
by HTX Technologies, LLC
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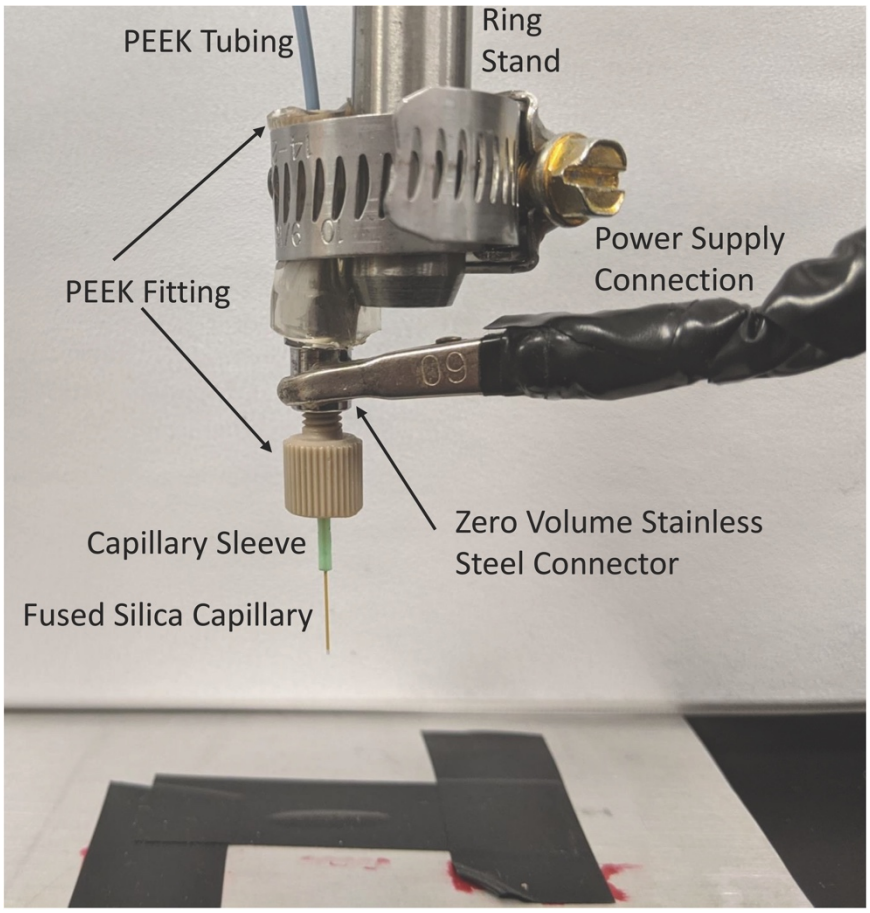
ABORT
25.1
Spray Nozzle Temp (°C)

Fig. 2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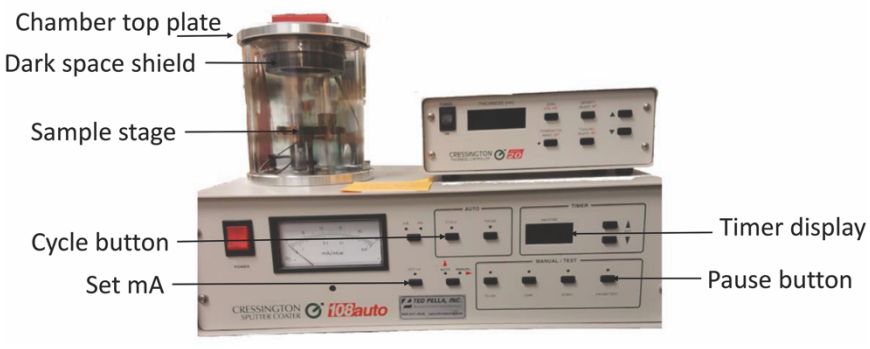
Power Supply Connection
PEEK Fitting
Zero Volume Stainless Steel Connector
Capillary Sleeve
Fused Silica Capillary

Fig. 3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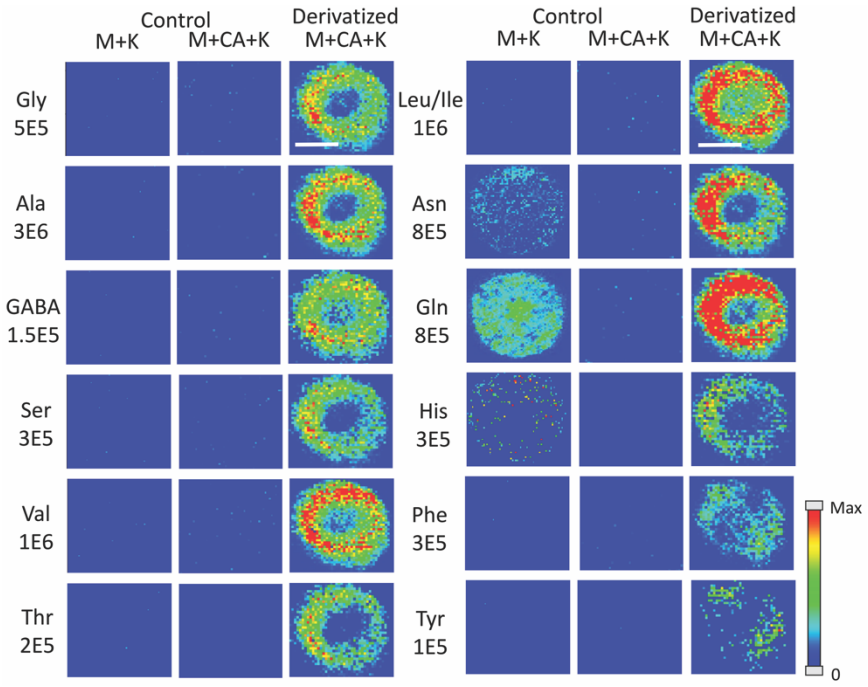
Dark space shield
Sample stage
Cycle button
Set mA
Timer display
Pause button
CRESSINGTON
108auto

Fig. 4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1E6
Ala
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Asn
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GABA
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Gln
8E5
Ser
3E5
His
3E5
Val
1E6
Phe
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Thr
2E5
Tyr
1E5
Max
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Fig. 5