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Home-built spinning apparatus for drying agarose-based imaging mass spectrometry samples

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Abstract

Matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) is a useful technique for mapping the spatial distribution of molecules across biological samples. Sample preparation is crucial for MALDI-IMS; samples must be flat, dry, and co-crystallized with a matrix prior to analysis. Agarose-based samples can be difficult to consistently prepare as they are susceptible to environmental changes, which can lead to inconsistent drying and wrinkling on the sample surface. Small height differences may cause low ionization of target analytes, or introduce artifacts in imaging data depending on the instrument used for analysis. To overcome the variations, a home-built robotic spinner was constructed and applied to agarose-based samples. This robotic-spinner is inexpensive, easy to assemble; and when applied to agarose-based samples, accelerated the drying process and reduced wrinkles, improving the overall quality of the resulting IMS data.

Keywords

agarose; imaging mass spectrometry; 3D cell culture; sample preparation; MALDI

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#Denotes equal contributions

Associated Content

Supporting Information – Experimental details and additional figures including:

- Optical images of manual and robo-spun plates
- Spectra resulting from the ionization of wrinkled and non-wrinkled locations
- Zoomed in ion images showing “dead spots” for better visualization
- Table listing the percent of “dead spots” in different replicates (N=5)
- A cost breakdown of materials used for spinner construction

Introduction

Matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) is a label free analytical technology for creating ion images of mass-to-charge ratios that can be overlaid with optical images to generate biological hypotheses. It has previously been applied to tissues, microbes on agar, and more recently to 3D mammalian cell cultures – with the goal of visualizing spatial chemical exchange in different biological conditions.^{1–3} In IMS, ionization occurs at specific sampling positions (x,y coordinates) across the surface of a sample, and a mass spectrum is obtained at each sampling position. These sampling positions become “pixels” which are combined to generate images, allowing researchers to visualize the spatial distribution of a selected mass to charge ratio (m/z) across the surface of a sample.

Ovarian cancer is the most lethal gynecologic malignancy and the fifth leading cause of cancer-related deaths among women.⁴ High grade serous ovarian cancer (HGSOC), the most common and lethal subtype, is characterized by a distinct and aggressive pattern of metastasis; originating in the fallopian tube with the transformation of fallopian tube epithelial cells (FTEs) and metastasizing first to the ovary, then commonly to the adipose-rich omentum.⁵ To probe chemical exchange at the interface of ovarian cancer cells and the tissues they frequently colonize during metastasis, our lab developed an IMS protocol for analyzing 3D co-cultures of tumorigenic FTEs and healthy murine tissues. With this agarose-based approach, metabolite exchange is easily visualized, as small-molecules secreted by interacting partners readily diffuse through the agarose.^{6,7} Using this model system, we determined that the co-culture of tumorigenic FTEs with healthy murine ovaries induced norepinephrine production from the ovary, while normal FTEs and other cell types did not.^{7,8}

Effective sample preparation procedures are crucial for reliable results using MALDI-IMS.^{9,10} Agar and agarose-based samples can be the most difficult to consistently prepare as they are susceptible to humidity and temperature fluctuations, which can lead to inconsistent drying and low ionization of target analytes, or artifacts induced in the resulting images.⁹ Ideally, samples should be flat, dry, and co-crystallized with a matrix prior to introduction of the sample into the mass spectrometer. Consistently obtaining dry, flat samples has been a challenge with our agarose-based 3D mammalian cell cultures, as they tend to dry unevenly and wrinkle toward the end of the drying process. Small height differences were not disruptive when using a Bruker Autoflex MALDI-TOF MS for analysis, but height differences are disruptive to analysis when using a Bruker timsTOF fleX, which is more sensitive to height differences on the sample surface. Analysis of 3D mammalian cell co-cultures using the timsTOF fleX resulted in “dead spots”, or areas where no spectra are detected on wrinkles – making replication of our previous results technically challenging. To alleviate wrinkling and “dead spots” on the sample, we constructed a home-built spinning apparatus to rotate samples as they dry. Herein, we describe the construction and application of this home-built robotic spinner to agarose-based IMS samples so that other research groups may be able to apply this technology toward the development of other IMS protocols.

Materials and Methods

Construction of spinner

Materials—All materials were commercially obtained except for pipette tip boxes which were already in house. A 5–6 RPM Turntable synchronous motor was purchased from Naive Blue. Rutland 500°RTV High Heat Silicone 2.7 Oz. Tube was purchased from Rutland Products. Silicone Coasters obtained from YQL. 13575 Adhesive Backed Aluminized Heat Barrier obtained from Thermo-Tec (Figure 1).

1x Pipette tip box (preferably 100–1000 μ L tips but any will work)

1x 5–6RPM Turntable synchronous motor set - motor and wiring (Naive Blue)

1x Rutland 500°RTV High Heat Silicone 2.7 Oz Tube (Rutland Products)

1x Adhesive Backed Aluminized Heat Barrier (Thermo-Tec)

1x Silicone Coaster (YQL)

1x Sigma Electric Round Steel Flat Box Cover For Wet Locations

Safety—When assembling this instrument, do so in fume hoods to avoid exposure to volatile chemicals. Let silicone glue dry in fume hood or open space, as it releases acetic acid while curing. During assembly ensure that the device is not able to access electricity.

Instrumentation Assembly—Begin assembly by placing the pipette box on a dry, clean surface (Figure 2A). If the pipette container size is not large enough to accommodate the turntable section of the motor, increase the size of one of the central holes with a screwdriver or similar tool, carving a 1–2 cm hole in the top of the box (Figure 2B). Using a 5–6 RPM Turntable Synchronous Motor set, assemble by placing the motor underneath the pipette tip holder through the hole previously carved. Utilizing the neighboring holes in both the motor and pipette tip holder, use two 1 inch tall screws and corresponding nuts to fasten the motor to the pipette tip holder. Then, using the hexagonal adapter, small black screws, and the small Allen wrench that came with the kit, attach the hexagonal adapter to the rotating part of the motor (Figure 2C). Once the motor is safely attached, feed the motor wiring (blue wire) through a hole in the pipette tip holder (Figure 2D) and connect the circuit to the switch plug cord (black wall outlet wiring), separating the black and red wires into the left and right segments of the connector terminal. (Figure 2E). Re-secure the pipette tip holder to the pipette tip box. Now, find the silicone coaster and the metal disc, using silicon glue, coat the metal disc with a thin layer of silicon glue and press the silicon coaster firmly on top of it (Figure 2F). Let it dry, but curing is not necessary. Next, use silicon glue to coat the top of the hexagonal adapter in a circular application, press down metal disc onto hexagonal adapter (Figure 2G). Let this dry and cure overnight. Before placing in the oven, use heat resistant aluminum, aluminum tape, or electrical tape to coat any exposed wiring, and use aluminum tape to cover the bottom of the pipette box if using high temperatures (Figure 2H). Youtube video detailing the construction process: https://www.youtube.com/watch?v=onnyQzvj_fc

Results and Discussion

Automated spinning of IMS samples while drying has several advantages; it minimizes the need for human intervention (manual turning) and reduces environmental variability which results from opening the oven door. This, in turn, helps samples dry more evenly and consistently across multiple replicates (N=5), and reduces wrinkling on the sample surface and “dead spots” in the resulting ion images (Figure 3, Figure S1–S3, Table S1). When applied to agarose-based IMS samples, robotic spinning also resulted in samples drying twice as fast at the same temperature, presumably due to increased air circulation. This more rapid robotic-facilitated desiccation enables tight control of the drying process; drying can be sped up by turning the spinner on or slowed by turning it off. Automated spinning can be useful for decreasing dry time at a specific temperature or optimizing drying protocols at lower temperatures to prevent heat-related degradation. Robotic spinning reduces labor by eliminating the need for manual intervention and accelerating the drying process, thereby reducing the overall “cost” associated with IMS experiments. The spinning apparatus is made from easily acquired and inexpensive materials, costing only \$13.55 for the construction of a single apparatus (Table S2), an expense which is easily offset by labor reduction and data quality improvement. Additionally, spinner construction is quick and simple, requiring less than one hour to complete.

For our purposes, the robotic spinner was used to optimize dry time to ~4 hours at a lower temperature, because lowering the temperature resulted in additional wrinkle reduction. Lowering the temperature without the robotic spinner was not possible due to the increase in dry time which required samples to be dried overnight; overnight drying increases the likelihood of analyte degradation and causes agarose-based samples to over-dry and flake – preventing IMS analysis. Prior to the introduction of the robotic spinner, agarose-based samples took four hours to dry at 37 °C, and over eight hours to dry at 30 °C. After the introduction of the robotic spinner, samples dried in four hours at 30 °C. IMS analysis of agarose-plugs spiked with 15 µM norepinephrine standard, or a water blank, on robo-spun and manual spun plates confirmed that robotic spinning and temperature reduction reduced wrinkles on the sample surface, and alleviated “dead spots” in the imaging data (Figure 3, Figures S1–S3). This “dead spot” reduction can be visualized in Figure 3, and Figure S3; a large “dead spot” was observed on the bottom right of the blank in manual spun samples but not in robo-spun samples (Figure 3, Figure S3). Sample homogeneity was also improved as evidenced by the spatial distribution of the α -cyano-4-hydroxycinnamic acid (CHCA) matrix signal m/z 190.02, which is more evenly distributed on robo-spun samples compared to manual spun (Figure 3). Five replicate experiments were performed to compare robo-spun and manual spun samples prepared side-by-side on the same day because environmental factors (i.e. humidity) may impact matrix co-crystallization and ionization efficiency. Analysis of five replicate experiments revealed the prevalence of “dead spots” in IMS data varied significantly between experiments conducted on different days with <1% “dead spots” on the low end and >20% on the high end (Table S1). This variation is likely due to the efficiency of matrix co-crystallization. While the prevalence of “dead spots” varied significantly between replicate experiments, within each replicate the percent “dead spots” in IMS data was consistently reduced in robo-spun samples compared to

manual spun with a 0.03% reduction on the low end and a 23.73% reduction on the high end (Table S1). The highest percent of “dead spots” observed on manual spun plates was 23.82%, while the highest percent observed on robo-spun plates was 3.51% (Table S1). This work demonstrates that automated spinning of IMS samples as they dry makes the process of using agarose-based samples more robust and less user error prone, improving the overall quality of IMS data.

Conclusion

The home-built robotic spinner described in this application note is inexpensive, easy to assemble, and has several advantages when applied to agarose-based IMS samples. Robotic spinning accelerates the drying process, while helping samples dry more evenly and consistently across multiple experiments by reducing human and environmental variability. It is anticipated that robo-spinning would also improve sample preparation consistency for agar based microbial imaging experiments as well. Overall, utilizing an autonomous system provides a robust preparation process that results in reduced artifacts and “dead spots” in IMS data.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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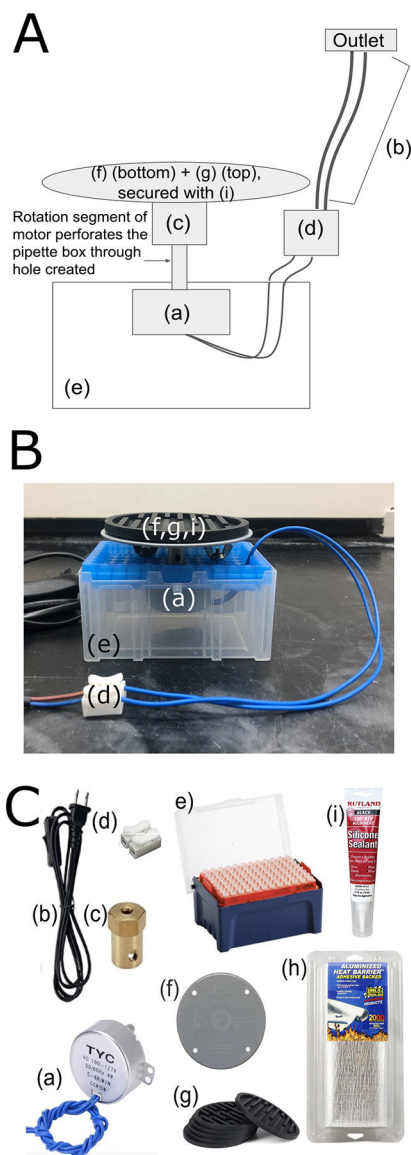


Figure 1:

(A) Schematic for assembly of the spinner (B) Picture of spinner (C) Materials needed for spinner assembly: (a-d) 5–6RPM Turntable synchronous motor set - motor and wiring (Naive Blue) [(a) motor, (b) switch plug cord (wiring and plug to outlet), (c) hexagonal adapter, (d) connection terminal], (e) Pipette-tip box, (f) Sigma Electric Round Steel Flat Box Cover For Wet Locations, (g) Silicone Coaster (YQL), (h) Adhesive Backed Aluminized Heat Barrier (Thermo-Tec), (i) Rutland 500°RTV High Heat Silicone 2.7 Oz Tube (Rutland Products)

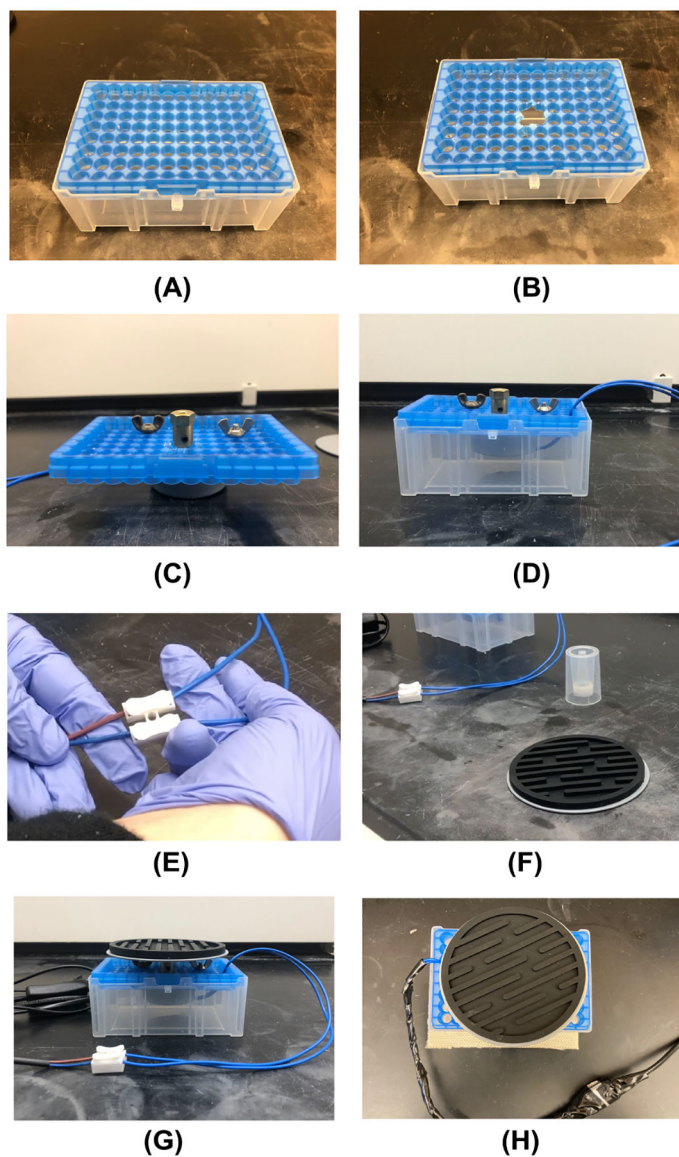


Figure 2:

(A) Picture of pipette tip box and pipette tip holder (B) Picture after ~2 cm hole carved into pipette tip holder (C) Picture after motor attached to pipette tip holder and hexagonal adapter attached to motor (D) Picture after wire is weaved through one of the preexisting pipette tip holder holes (E) Close-up picture of wiring attachment to connector terminal (F) Picture of silicone coaster attached to metal disc using silicone glue (G) Picture of silicone coaster attached to metal disc secured to hexagonal adapter using silicone glue (H) Picture of connected circuit wrapped in aluminum tape at the connector terminal and black electrical tape wrapped over the exposed wiring

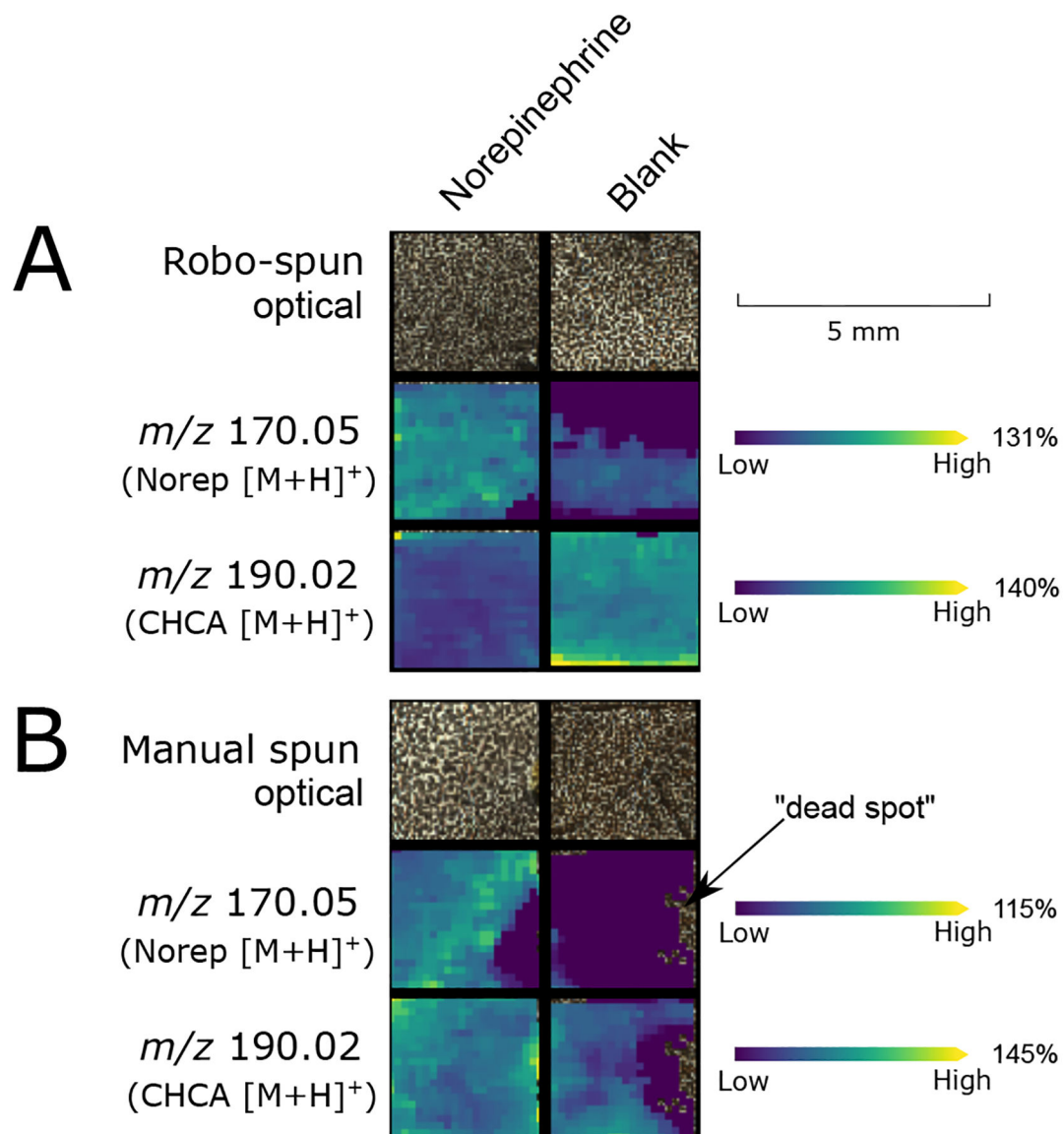


Figure 3:
Automated spinning reduces “dead spots” in IMS data. (A) Ion images of norepinephrine (m/z 170.05) and CHCA matrix (m/z 190.02) on robo-spun plate (B) Ion images of norepinephrine (m/z 170.05) and CHCA matrix (m/z 190.02) on manual spun plate.