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Title: Sample Preparation for Metabolic Profiling Using MALDI Mass Spectrometry Imaging

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **N**

2. **Software:** Does the part of your protocol being filmed demonstrate software usage? **Y**

   If **Yes**, we will need you to record using screen recording software to capture the steps. If you use a Mac, QuickTime X also has the ability to record the steps. Please upload all screen captured video files to your project page as soon as reasonably possible.

Videographer: Please film the screen for all SCREEN shots as a backup

3. **Interview statements:** Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until the videographer steps away (≥6 ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **51**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Rinat Abzalimov**: MALDI-MSI is a unique advancement in the field of metabolomics that helps us to identify and visualize relative metabolite abundance and distribution, which are indicative of an organism's physiological and pathological conditions **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Ye He**: The main advantage of MALDI imaging is its ability to detect metabolites in situ without the need of labeling. **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Introduction of Demonstrator on Camera

1.3. **Ye He**: Demonstrating the procedure will be Sami Sauma, a graduate student from Dr. Patrizia Casaccia's lab, and Yuki Chen and Kelly Veerasammy, student researchers from my laboratory **[1][2]**.

1.3.1. INTERVIEW: Author saying the above
1.3.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera

Protocol

2. Tissue Preparation

2.1. After harvesting, immediately place the tissue into a liquid nitrogen-cooled aluminum foil boat in a polystyrene box **[1-TXT]** and close the lid of the polystyrene box to freeze the tissue for 2-10 minutes according to the size of the tissue **[2-TXT]**.

2.1.1. Added shot: Folding the aluminum boat (Extra 2.1.1)

2.1.1. WIDE: Talent placing tissue into boat *Videographer: Important step* **TEXT: Do not perfuse animal with PBS after euthanasia**

2.1.2. Talent closing box *Videographer: Important step* **TEXT: e.g., P1 mouse brain: 2 min; P21 mouse brain: 5 min; P60 mouse brain: 7 min; P60 rat brain: 10 min**

2.2. When the tissue is sufficiently frozen, use forceps to remove the boat **[1-TXT]**, and transport the tissue, secured within the foil, on dry ice to the cryostat **[2-TXT]**.

2.2.1. Talent removing boat **TEXT: Caution: Do not overfreeze**

2.2.2. Talent transporting sample on dry ice **TEXT: Optional: Store at -80 °C ≤24 mo until sectioning**

3. Cryosection Acquisition

3.1. Before sectioning the tissue, taking care not to breathe on the slides, use gloves and a voltmeter set to resistance to test the conductivity of the appropriate number of MALDI (mall-dee)-compatible indium tinoxide coated glass slides for the analysis **[1-TXT]**.

3.1.1. WIDE: Talent testing slide(s) on clean paper towel **TEXT: MALDI: matrix-assisted laser desorption/ionization**

3.2. After labeling, place the slides on a clean paper towel in a 70% ethanol-cleaned cryostat set to minus 20 degrees Celsius **[1]**.

3.2.1. Talent placing slide(s) into cryostat *Videographer: Important step*

3.3. Place the tissue sample into the cryostat **[1]** and set the temperature of the cryostat chamber and specimen head according to the type of the tissue **[2-TXT]**.

3.3.1. Talent placing sample into cryostat

3.3.2. Talent setting temperature **TEXT: e.g., -14 °C for liver; -20 °C for muscle; -25 °C for skin**

3.4. After allowing the tissue to equilibrate for 30 minutes, use cryo tissue embedding compound to mount the tissue to the chuck **[1]** and place and lock a clean blade into the stage **[2]**.

3.4.1. Tissue being mounted

3.4.2. Talent placing and/or locking blade

3.5. Adjust the position of the stage and the angle of the specimen to achieve the desired cutting angle **[1]** and section the tissue until the region of interest is revealed **[2]**.

3.5.1. Stage and/or specimen angle(s) being adjusted

3.5.2. Tissue being sectioned

3.6. When the desired region has been reached, obtain 10-12-micron-thick sections **[1-TXT]**, using a pre-cooled brush to carefully but quickly collect each section onto the labeled side of each slide as they are acquired **[2]**.

3.6.1. Section being acquired *Videographer: Important/difficult step* **TEXT: Adjust temperature to optimize tissue acquisition as necessary**

3.6.2. Section being collected into slide *Videographer: Important step*

3.7. Place a finger under the slides to warm the sections to ensure secure mounting to the slide. The sections will become transparent in 5-10 seconds **[1]** and turn opaque after about 30-60 seconds **[2]**.

3.7.1. Finger being placed under tissue/tissue turning transparent *Videographer: Important/difficult step*

3.7.2. Tissue turning opaque *Videographer: Important step*

3.8. When all of the sections have been collected, place the slides in slide holder and carry on dry ice to a desiccator. Alternatively, slides can be carried in a vacuum box **[1-TXT]**. Place the slides in a desiccator with desiccant **[2]**, and vacuum-dry the slides for 45-60 minutes **[3]**.

3.8.1. Talent placing slide into slide transporter, then placing transporter into dry ice. **TEXT: Hold slides in cryostat until all sections collected.**

3.8.2. Talent placing slide into desiccator

3.8.3. Talent vacuum drying slides

3.9. After drying, if not using immediately, place the slides into the slide transporter **[1]** and fill the transporter with nitrogen **[2]**.

3.9.1. Talent placing slides into slide transporter

3.9.2. Talent filling transporter with nitrogen Videographer NOTE: 3.9.2 was not filmed

3.10. Seal the holder with parafilm **[1]** and place the holder into a zip bag **[2]**.

3.10.1. Talent sealing holder

3.10.2. Talent placing transporter into bag

3.11. Then place the first zip bag into a second, labeled, zip bag containing desiccant for minus 80-degree Celsius storage for up to 6 months **[1]**.

3.11.1. Talent placing bag into bag

4. Matrix Preparation

4.1. After dehydration, use a bold point silver marker to place “X” marks on the blank spaces of the slides outside of the tissue sections **[1]** and use a black, fine point marker to place a second, black “X” on top of each silver “X” **[2]**.

4.1.1. WIDE: Talent drawing silver X *Videographer: Important step*

4.1.2. Talent drawing black X *Videographer: Important step*

4.2. Load one slide into the MALDI slide metal target **[1]** and place a plastic cover over the slide **[2]**.

4.2.1. Talent loading slide

4.2.2. Talent placing cover onto slide

4.3. Outline the sample location on the plastic cover **[1]** and place the slide and MALDI target onto the surface of a flatbed scanner **[2-TXT]**.

4.3.1. Sample being outlined

4.3.2. Talent placing slice onto scanner. **TEXT: Target surface screws will prevent sample damage or contamination by scanner**

4.4. Then preview the slide and select the desired area **[1]**. Scan the slide in 16-bit grayscale and 2400 dots per inch and save the image for later use **[2]**.

4.4.1. Talent at scanner. SCREEN: 62008_screenshot_1.mp4. 0:55-1:10. The slide preview and the desired slide area being selected.

4.4.2. SCREEN: 62008_screenshot_1.mp. 41:22-1:28 and 1:45-2:23 Slide being scanned. *Video Editor: Please speed up the footage. Videographer: Please film the screen*

5. Matrix Deposition

5.1. To apply matrix to the slides, turn on an automatic matrix sprayer unit, making sure that the valve is positioned at **LOAD [1]**, and launch the sprayer software **[2]**.

5.1.1. WIDE: Talent turning on unit/confirming valve position

5.1.2. Talent launching software, with monitor visible in frame

5.2. Check that the exhaust fan is operating properly **[1-TXT]** and confirm under the **Comms** tab that the system is communicating correctly **[2]**.

5.2.1. Talent checking exhaust fan **TEXT: Do not start solvent pump if active venting not functioning properly**

5.2.2. SCREEN: 62008_screenshot_2.mp4. 01:00-01:08. Comms tab being opened/shot of communicating systems *Video Editor: Highlight the 'Comms' tab being pressed at 01:01. Videographer: Please film the screen*

5.3. Start the solvent pump at 100 microliters/minute with a backpressure of approximately 500 pounds per square inch **[1]** and set the nitrogen tank to 30 pounds per square inch to start compressed air flow to the matrix sprayer **[2]**.

5.3.1. SCREEN: 62008_screenshot_2.mp4. 01:22-01:28. Pump being started *Video Editor: Highlight the 'Pump' tab being pressed at 01:23. Videographer: Please film the screen*

5.3.2. Talent setting tank to 30 psi

5.4. Adjust the pressure regulator on the front of the sprayer to 10 pounds per square inch **[1]** and set the sprayer nozzle temperature as desired **[2]**.

5.4.1. Talent adjusting regulator

5.4.2. Talent setting temperature

5.5. With the valve in the **LOAD** position, use a syringe to flush the loop with 7 milliliters of 70% methanol **[1]** before filling the loop with 6 milliliters of matrix **[2-TXT]**.

5.5.1. Talent flushing loop

5.5.2. Talent filling loop with matrix **TEXT: See text for matrix preparation details**

5.6. Place a blank glass slide into the holder in the sprayer, taping down both ends to prevent movement **[1]**, and check that the flow rate and temperature are stable **[2]**.

5.6.1. Talent placing/taping slide into holder *Videographer/Video Editor: shot will be used again*

5.6.2. Talent checking flow rate and temp and selecting method

5.7. Press **Start** to set the nozzle temperature and to adjust the pump flow rate to match the selected method **[1]**, switch the valve to from **Load** to **Spray**, and click **Continue** **[2]**.

5.7.1. Talent pressing Start

5.7.2. SCREEN: 62008_screenshot_2.mp4. 25:10-25:25. Valve being switched from load to spray, then continue being clicked *Video Editor: Emphasize 'yellow highlights being popped on the right side of screen at 25:13. Emphasize 'Continue' tab being clicked at 25:21. Videographer: Please film the screen*

5.8. Allow the system to run to completion **[1]**. When the deposition is finished, switch the valve from **Spray** to **Load** and click **Continue** **[2]**.

5.8.1. Slide being coated/System depositing matrix

5.8.2. SCREEN: 62008_screenshot_2.mp4: 47:31-47:36. Valve being switched from Spray to Load, then Continue being clicked *Videographer: Please film the screen*

5.9. Examine the pattern of matrix coating under a microscope **[1]**. If an even layer of fine matrix crystal is observed, deposit the matrix on the appropriate sample slides as just demonstrated **[2]**.

5.9.1. Talent at microscope, viewing slide *Videographer: Please film the screen*

5.9.2. Use 5.6.1. Talent taping slide into place

5.10. When all of the slides have been treated, clean the system according to the manufacturer's instructions to prevent clogging of the sprayer nozzle **[1-TXT]**.

5.10.1. Talent cleaning system **TEXT: Immediately after deposition analyze slides by MALDI**

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see? Please list 4 to 6 individual steps.
2.1., 3.2., 3.6., 3.7., 4.1.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success? Please list 1 or 2 individual steps from the script above.
3.6., 3.7. Be gentle when touching the tissue section with the tip of brush and act fast to transfer the tissue section to the ITO glass slide.

Results

6. Results: Representative Output with Selected m/z Spectra from Mass Spectrometry Acquired at 50-micron Lateral Resolution

6.1. Here output images from MALDI MS (M-S) imaging data analysis of mass to charge spectra selected from every 100-Dalton interval, clearly depicting the utility for the identification of spectra from small molecule metabolites to high molecular weight lipids, are shown **[1]**.

6.1.1. LAB MEDIA: Figure 2A **TEXT: MSI: mass spectrometry imaging**

6.2. Each row depicts respective ion heat maps containing both spatial and spectral information of a specific metabolite species across three tissues collected at postnatal days 2, 21, and 60 **[1]**.

6.2.1. LAB MEDIA: Figure 2A *Video Editor: please sequentially emphasize P1, P21, and P60 image rows*

6.3. A strength of the MALDI MSI methodology is the ability to discern the specificity of certain species identified by the mass to charge ratio to developmental milestones or specific anatomical structures **[1]**.

6.3.1. LAB MEDIA: Figure 2A *Video Editor: please emphasize m/z numbers at the top of each set of images*

6.4. In this analysis, some metabolites were observed to be enriched in postnatal day 1 neonates **[1]** or postnatal day 60 adults **[2]** or to be uniformly distributed across the tested ages **[3]**.

6.4.1. LAB MEDIA: Figure 2A *Video Editor: please emphasize P1 m/z 320.1 image*
6.4.2. LAB MEDIA: Figure 2A *Video Editor: please emphasize P60 m/z 864.5 image*
6.4.3. LAB MEDIA: Figure 3A *Video Editor: please emphasize m/z 480.3 images*

6.5. Other molecular species were observed to be specifically enriched in gray matter **[1]**, white matter **[2]**, or cerebral spinal fluid and ventricles **[3]**.

6.5.1. LAB MEDIA: Figure 2A *Video Editor: please emphasize 117.1, 524.3, and 765.1 images*
6.5.2. LAB MEDIA: Figure 2A *Video Editor: please emphasize 673.4 and 906.6 images*
6.5.3. LAB MEDIA: Figure 2A *Video Editor: please emphasize 239.0 image*

6.6. The spatial distribution of representative metabolites **[1]**, including hypoxanthine **[2]**, glutamic acid **[3]**, N-acetyl-L-aspartic acid **[4]**, arachidonic acid **[5]**, and several lipids were also analyzed **[6]**.

6.6.1. LAB MEDIA: Figure 2B
6.6.2. LAB MEDIA: Figure 2B *Video Editor: please emphasize m/z 135.0 images*
6.6.3. LAB MEDIA: Figure 2B *Video Editor: please emphasize m/z 146.1 images*
6.6.4. LAB MEDIA: Figure 2B *Video Editor: please emphasize m/z 174.0 images*
6.6.5. LAB MEDIA: Figure 2B *Video Editor: please emphasize 303.2 images*
6.6.6. LAB MEDIA: Figure 2B *Video Editor: please emphasize m/z 478.3, 500.3, 524.3, 838.5, and 885.6 images*

Conclusion

7. Conclusion Interview Statements

7.1. **Yuki Chen**: To maintain the fidelity of the metabolomics data, appropriate sample dissection, storage, and crysosectioning are crucial to preventing artifactual metabolic changes **[1]**.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (2.1., 2.2., 3.5.)

7.2. **Kelly Veerasammy**: After performing your MALDI experiment, you may want to verify the identities of the metabolites you've found. This can be achieved by microextraction and liquid chromatography tandem MS **[1]**.

7.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

7.3. **Sami Sauma**: MALDI MS imaging facilitates metabolomics-based inquiry with a spatial resolution and affords investigators the opportunity to ascertain metabolic data in a quantitative and visual medium **[1]**.

7.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

7.4. **Patrizia Casaccia:** Closing statements on the impact and future of MALDI (depending on which version was chosen for the final video).