FINAL SCRIPT: APPROVED FOR FILMING

**Submission ID #: 68376**
**Scriptwriter Name: Debopriya Sadhukhan**
**Project Page Link: https://review.jove.com/account/file-uploader?src=20854728**

# Title: Fluorescence-Guided Matrix-Assisted Laser Desorption/Ionization With Laser-Induced Postionization Mass Spectrometry of Individual Rat Neural Cells

## Authors and Affiliations:

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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 something similar? **Yes**

   Can you record movies/images using your own microscope camera?

   **Yes**

   **Zeiss Axio Imager M2**

   If a dissection or stereo microscope is required for your protocol, please list all shots from the script that will be visualized using the microscope (shots are indicated with the 3-digit numbers, like 2.1.1, 2.1.2, etc.).

   **2.1.1, 2.1.2., 2.2.1, 2.2.2**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

3. **Filming location:** Will the filming need to take place in multiple locations? **Yes**

   If **Yes**, how far apart are the locations? **100 feet**

**Current Protocol Length**
Number of Steps: 24
Number of Shots: 46

# Introduction

***Videographer: Obtain headshots for all authors available at the filming location.***

**REQUIRED:**

1.1. **Jonathan Sweedler:** This research focuses on developing high-throughput, image-guided single-cell MALDI workflows to reveal cellular heterogeneity and link molecular profiles to cell identity, function, and response in complex samples.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.2.1, 2.2.2.*

What technologies are currently used to advance research in your field?

1.2. **Siheun Lee:** Advanced instrumentation, such as MALDI-2 and high-spatial-resolution mass spectrometers, enables targeted, spatially resolved analysis of single cells, enhancing sensitivity and expanding the scope of molecular profiling in complex tissues.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 3A.*

What are the current experimental challenges?

1.3. **Siheun Lee:** Current challenges include limited sensitivity, reproducibility across samples, and complex data analysis. MALDI data are often sparse, and large datasets with thousands of cells and hundreds of molecules demand robust computational tools.

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

What research gap are you addressing with your protocol?

1.4. **Seth Croslow:** This protocol addresses the gap in high-throughput methods for analyzing individual cells and even organelles, enabling detailed studies of heterogeneity and molecular insights into cellular biology and function.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 3D.*

What advantage does your protocol offer compared to other techniques?

1.5. **Timothy Trinklein:** This protocol allows rapid, targeted analysis of cells scattered across a slide, eliminating the need for cell manipulation or full-area scanning, and significantly increasing throughput.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1.*

***Videographer: Obtain headshots for all authors available at the filming location.***

**Testimonial Questions:**

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.6. **Jonathan Sweedler:** Publishing with JoVE will enhance our visibility by providing a visual, step-by-step demonstration of our protocol and the software microMS, making it easier for other labs to adopt and apply in their own research.

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

**Ethics Title Card**

This research has been approved by the Illinois Institutional Animal Care and Use Committee

# Protocol

## 2. Microscopy Slide Preparation and Image Acquisition for MALDI-2 Analysis

**Demonstrator:** Seth Croslow

2.1. To begin, rinse each slide with 2 to 3 milliliters of 150 millimolar ammonium acetate to remove glycerol and salt crystals that can interfere with microscopy and MALDI *(Mal-dee)* matrix application **[1]**. Then, dry the slides under a gentle stream of nitrogen or allow them to air dry completely **[2]**.

2.1.1. WIDE: Talent rinsing the slide with ammonium acetate solution.

2.1.2. Talent drying the slides under a stream of nitrogen or air drying the slides on a benchtop. NOTE: The videographer filmed this shot.

2.2. Load the dried slide into the microscope stage **[1]**. Focus the microscope using at least 10 support points distributed evenly across the entire slide **[2]**.

2.2.1. Talent placing the slide into the microscope stage.

2.2.2. SCREEN: 2.2.2_r.mkv 00:09-00:16.

***Videographer: Please record the screen for all SCREEN shots as a backup.***

2.3. Using filters suitable for DAPI *(Dapi)* and brightfield imaging, acquire a tiled fluorescence image of the entire slide at 5x *(five-ex)* to 10x *(ten-ex)* magnification, ensuring that the fiducial markers on the microscopy slides are clearly captured in the image **[1-TXT]**.

2.3.1. SCREEN: 2.3.1.1_r.mkv 00:01-00:21, 00:26-00:33, 00:48-00:53, 03:53-03:58, 09:23-09:34 **TXT: DAPI:; Excitation: 335-383 nm; Emission: 420-470 nm** *Video Editor: Speed up the video as required.*

2.4. Stitch the tiled images using microscopy software such as ZEN (Zeiss) *(Zen Zice)* **[1]**. Verify that the vertically adjacent tiles are not offset **[2-TXT]**.

2.4.1. SCREEN: 2.4.1_r.mkv 00:05-00:25, 01:48-01:52. *Video Editor: Speed up the video as required.*

2.4.2. SCREEN: 2.4.2_r.mkv 00:02-00:10. **TXT: Consider acquiring tiled images with a large tile overlap (20% - 30%)**

2.5. Process and export each stitched image as a BigTIFF *(Big-T-I-F)* file using the microscopy

software, or as a standard TIFF *(T-I-F-F)* if the final image size is less than 2 gigabytes **[1]**.

2.5.1. SCREEN: 2.5.1_r.mkv 00:03-00:40. *Video Editor: Speed up the video as required.*

## 3. Matrix Application via Sublimation for Slide Preparation

**Demonstrator:** Timothy Trinklein

3.1. Dissolve 20 milligrams of 2,5-dihydroxyacetophenone *(two-five-di-hydroxy-aceto-phe-none)* in 1.5 milliliters of acetone **[1]**. Place the slides into the holder of the sublimation chamber **[2]**, then place the holder into the sublimation apparatus **[3]**.

3.1.1. Talent dissolving 2,5-dihydroxyacetophenone in 1.5 milliliters of acetone.

3.1.2. Talent placing slides into the holder.

3.1.3. Talent placing the holder into the sublimation apparatus.

3.2. Pipette the dissolved matrix solution onto the ceramic wafer and allow the acetone to evaporate completely **[1]**. Then, close the sublimation chamber to seal it **[2]**.

3.2.1. Talent dispensing the dissolved matrix solution onto the ceramic wafer.

3.2.2. Talent closing the sublimation chamber.

3.3. Fill the coolant chamber with an ice-water slush **[1]** and place it securely on top of the sublimation chamber **[2]**.

3.3.1. Talent filling the coolant chamber with an ice-water slush.

3.3.2. Talent placing the filled coolant chamber on top of the sublimation unit.

3.4. Turn on the vacuum pump and allow the system to equilibrate for 5 minutes **[1-TXT]**.

3.4.1. Talent switching on the vacuum pump. **TXT: The pressure in the system should be <40 mbar**

3.5. Begin the sublimation by heating the chamber to 200 degrees Celsius for 5 minutes **[1]**.

3.5.1. Talent heating the chamber.

3.6. After 5 minutes, remove the ice-water bath from the chamber **[1]**. Turn the temperature to 25 degrees Celsius **[2]** and place a heatsink on top of the chamber **[3-TXT]**.

3.6.1. Talent removing the ice-water bath from the chamber.

3.6.2. Control interface adjusting the temperature setting to 25 degrees Celsius.

3.6.3. Talent placing a heatsink on top of the chamber. **TXT: Allow the system to warm up to RT for 5 min to avoid condensation**

3.7. Slowly vent the sublimation chamber to release pressure **[1]**. Open the chamber and carefully remove the slides **[2]**.

3.7.1. Talent rotating the valve to slowly vent the system.

3.7.2. Talent opening the chamber and removing the slides.

## 4. Procedure for Single-Cell MALDI MS

**Demonstrator:** Siheun Lee

4.1. Open microMS *(Micro-M-S)* and decimate the bigTIFF microscopy images using the **Image Group** option to accommodate both brightfield and fluorescence channels **[1]**.

4.1.1. SCREEN: 4.1_r.mkv 00:01-00:16, 01:28-end.

4.2. Navigate to the **Blob Options** tool and adjust the maximum and minimum blob size to define the acceptable blob size range **[1]**. Set the threshold of the fluorescence channel for blob detection, specify the circularity value to define how circular the identified cells need to be for consideration, and choose the color **[2]**. NOTE: The VO has been edited.

4.2.1. SCREEN: 4.2_r.mkv 00:00-00:09.

4.2.2. SCREEN: 4.2_r.mkv 00:10-end. NOTE: The timestamps for 4.2.2 and 4.2.3 are merged.

~~4.2.3. SCREEN: To be provided by authors: Setting the circularity value.~~

4.3. Use the Blob find option to detect blobs **[1]**. When prompted, save the blob list under a desired name **[2]**.

4.3.1. SCREEN: 4.3_r.mkv 00:06-00:19.

4.3.2. SCREEN: 4.3_r.mkv 00:20-end.

4.4. Use the Distance filter tool to set the minimum distance between each cell **[1]**. Test the error via test points to accurately determine the offset error **[2]**.

4.4.1. SCREEN: 4.4.1_r.mkv 00:01-end.

4.4.2. SCREEN: 4.4.2_r.mkv 00:04-end.

4.5. Load the slides into the instrument using the MTP Slide Adapter II *(two)* **[1]**. After returning to the computer with microMS, access the mass spectrometer computer via a remote desktop application **[2]**.

4.5.1. Talent loading the slides into the instrument using the MTP Slide Adapter II.

4.5.2. SCREEN: 4.5.2_r.mkv 00:25-end.

4.6. If using the instrument for the first time, verify its position by navigating to **Tools,** followed by **Instrument Settings [1]**. In the popup window, view the set of coordinates with their X and Y positions, and select each of these specific points on the Slide Adapter II geometry **[2]**. Update the X and Y positions in the microMS window **[3]**.

4.6.1. SCREEN: 4.6_r.mkv 00:05-00:08.

4.6.2. SCREEN: 4.6_r.mkv 00:08-00:33,

4.6.3. SCREEN: 4.6_r.mkv 00:34-00:52.

4.7. Using the mass spectrometer's camera and stage controls, navigate to an easily identifiable location on the slide and copy the instrument coordinates **[1]**. In microMS, locate the same position in the microscope image, right-click, and input the coordinates into the popup window. Round the coordinates to the nearest integers and separate them by a space **[2-TXT]**.

4.7.1. SCREEN: 4.7_r.mkv 00:00-00:12.

4.7.2. SCREEN: 4.7_r.mkv 00:13-end. **TXT: Repeat this step to register at least 12 fiducial markers across the entire slide**

4.8. After three registration points have been added, one of the circles will turn red, indicating that it is the most off position from the registration **[1]**. Delete the registration point using **Ctrl** + right-click *(Control plus right click)* and try again **[2]**.

4.8.1. SCREEN: 4.8_r.mkv 00:13-00:33.

4.8.2. SCREEN: 4.8_r.mkv 00:37-end.

4.9. Under **File**, go to **Save** and then **Registration** to save the registration file **[1]**.

4.9.1. SCREEN: 4.9_r.mkv 00:00-end.

4.10. With the blobs visible on the slide, go to **File**, **Save**, and then **Instrument Positions** to save the instrument position file **[1]**. Then, using remote desktop software, transfer this file to the instrument computer **[2]**.

4.10.1. SCREEN: 4.10.1_r.mkv 00:00-end.

4.10.2. SCREEN: 4.10.2_r.mkv 00:00-end.

4.11. Open the custom xeo *(Custom X-E-O)* file and copy its contents into the **MTP Slide Adapter II .xeo** *(M-T-P-Slide-Adapter-two-dot-X-E-O)* file on the instrument computer **[1]**. Save the file to update this geometry file with the cells' locations **[2]**. NOTE: The sentence numbers of the narration have been adjusted to accommodate the added shot.

4.11.1. SCREEN: 4.11_r.mkv 00:02-00:30.

Added shot: 4.11_r.mkv 00:30-end.

4.12. Click on the **Automation** tab and select **New…** *(New)* to create a new automatic run **[1]**. Drag across the displayed sample region to select the cells and right-click to add them to the analysis list **[2]**. Save the automatic run and click **Start Automatic Run** to begin acquisition **[3]**.

4.12.1. SCREEN: 4.12.rc.mkv 00:00-00:18.

4.12.2. SCREEN: 4.12.rc.mkv 00:18-00:23.

4.12.3. SCREEN: 4.12.rc.mkv 00:25-00:45, 01:04-01:09. *Video Editor: Speed up the video as required.*

# Results

**5. Results**

5.1. This figure illustrates how single-cell MALDI-2 *(Mal-dee-two)* mass spectrometry profiling reveals lipid-based cellular heterogeneity across and within distinct brain regions **[1]**. Uniform manifold approximation and projection or UMAP *(U-M-A-P)* analysis separated cells by brain region, forming distinct clusters for striatum, hippocampus, and cortex **[2]**.

5.1.1. LAB MEDIA: Figure 3A.

5.1.2. LAB MEDIA: Figure 3A. *Video Editor: Highlight green dots when the VO says "striatum", yellow dots when the VO says "hippocampus", and blue dots when the VO says "cortex".*

5.2. Leiden clustering revealed four lipid-based cell subpopulations **[1]**.

5.2.1. LAB MEDIA: Figure 3B.

5.3. Additionally, cluster-specific lipid signatures were observed, with distinct intensity profiles across annotated lipids **[1]**. Mass spectra from six cortical cells also showed consistent lipid detection across slides **[2]**. Furthermore, Cortical cells from different slides showed overlapping UMAP distributions, indicating minimal batch effects **[3]**.

5.3.1. LAB MEDIA: Figure 3C.

5.3.2. LAB MEDIA: Figure 3D.

5.3.3. LAB MEDIA: Figure 3E.

## Pronunciation Guides:

**1. Ammonium acetate**

**Pronunciation link:**
https://www.merriam-webster.com/dictionary/ammonium
https://www.merriam-webster.com/dictionary/acetate
**IPA:**

- Ammonium: /əˈmoʊniəm/
- Acetate: /ˈæsəˌteɪt/
  **Phonetic Spelling:** uh-moh-nee-um ass-uh-tayt

**2. Microscopy**

**Pronunciation link:**
https://www.merriam-webster.com/dictionary/microscopy
**IPA:** /maɪˈkrɑːskəpi/
**Phonetic Spelling:** my-krah-skuh-pee

**3. MALDI (Matrix-Assisted Laser Desorption/Ionization)**

**Pronunciation link:**
https://www.howtopronounce.com/maldi
**IPA:** /ˈmæl.di/
**Phonetic Spelling:** mal-dee

**4. DAPI (a fluorescent stain)**

**Pronunciation link:**
https://www.howtopronounce.com/dapi
**IPA:** /ˈdæpi/
**Phonetic Spelling:** da-pee

**5. 2,5-dihydroxyacetophenone**

**Pronunciation link:**
https://www.howtopronounce.com/2-5-dihydroxyacetophenone
**IPA:** /ˌtuː.faɪv.daɪˌhaɪˌdrɒksi.əˌsiːtoʊˈfiːnoʊn/
**Phonetic Spelling:** two-five-dye-hock-see-uh-see-toh-fee-nohn

**6. Sublimation**

**Pronunciation link:**
https://www.merriam-webster.com/dictionary/sublimation
**IPA:** /ˌsʌbləˈmeɪʃən/
**Phonetic Spelling:** sub-luh-may-shun

**7. microMS**

**Pronunciation link:**
No confirmed link found
**IPA:** /ˈmaɪkroʊ ɛm ɛs/
**Phonetic Spelling:** my-kroh M-S

**8. MTP Slide Adapter II**

**Pronunciation link:**
No confirmed link found
**IPA:** /ɛm ti pi slaɪd əˈdæptər tuː/
**Phonetic Spelling:** M-T-P slide uh-dap-ter two

**9. Leiden**

**Pronunciation link:**
https://www.howtopronounce.com/leiden

**IPA:** /ˈlaɪdən/
**Phonetic Spelling:** lie-dun