

Submission ID #: 71691

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Title: Single-Cell Mass Spectrometry Imaging Using Desorption Electrospray Ionization Coupled to Orbitrap Mass Spectrometers

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? **No**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

Videographer: Please film the computer screen/ instrument display for all shots labelled as SCREEN

3. Filming location: Will the filming need to take place in multiple locations? **No**

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 28

Number of Shots: 50

Interviews

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

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Nathan Colwell**: Our research investigates how DESI-MSI can be adapted for accessible and reproducible molecular imaging at the single-cell level.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Nathan Colwell**: Current DESI-MSI methods struggle to reach single-cell resolution, so this protocol helps to make single-cell imaging more accessible for labs.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Nathan Colwell**: This protocol allows researchers to measure lipid and metabolic differences spatially across individual cells and cellular regions.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.4. **Dan Chen:** Following imaging, region-specific analyses, statistical comparisons, and molecular feature extraction can be performed on selected cells to generate additional biological insights.

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

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Dan Chen:** Future studies can extend this workflow to diverse biological systems and combine single-cell metabolomics with histological staining, transcriptomic, and genomic analyses to enable multi-omics characterization of cellular heterogeneity.

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

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

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

What problem in your field of research does your JoVE article address?

- 1.6. **Nathan Colwell, Ph.D. Candidate, University of Oklahoma**: (authors will present their testimonial statements live)
 - 1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

What value do you expect this video article to have for other researchers working in your field?

- 1.7. **Nathan Colwell, Ph.D. Candidate, University of Oklahoma**: (authors will present their testimonial statements live)
 - 1.7.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Preparation of Cultured Cells for Desorption Electrospray Ionization–Mass Spectrometry (DESI-MS)

Demonstrator: Nathan Colwell

2.1. To begin, place individual 28-millimeter gridded glass coverslips with a 50-micrometer grid spacing into the wells of a 6-well plate [1].

2.1.1. Talent placing individual 28 millimeter gridded glass coverslips into the wells of a 6-well plate. ***Videographer:** Please obtain CU shot as well for this shot.*

2.2. Add 2 milliliters of complete medium and 200 microliters of the prepared OVCAR-8 (OHV-car eight) cell suspension containing approximately 2×10^5 cells to each well [1]. Gently rock the 6-well plate in an X pattern to evenly distribute the cells across the coverslip surface [2].

2.2.1. Talent adding complete medium and the cell suspension to a well of the 6-well plate.

2.2.2. Talent gently rocking the 6-well plate in an X pattern.

2.3. Incubate the cells for approximately 15 to 20 hours to allow cell attachment [1].

2.3.1. Talent placing the 6-well plate into the incubator.

2.4. Prepare the isotonic ammonium formate wash buffer immediately before use by weighing 0.45 grams of ammonium formate [1] and dissolving it in 50 milliliters of LC/MS (L-C-M-S) grade water to obtain a final ammonium formate concentration of 0.14 molar [1]. Do not adjust the solution's pH [2].

2.4.1. Talent weighing 0.45 grams of ammonium formate

2.4.2. Talent dissolving ammonium formate in 50 milliliters of liquid chromatography/mass spectrometry-grade water.

2.4.3. Shot of the prepared ammonium formate solution.

2.5. In a new 6-well plate, add 2 milliliters of the prepared ammonium formate solution into 2 wells [1].

- 2.5.1. Talent adding 2 milliliters of ammonium formate solution into 2 wells.
- 2.6. Using sterile forceps, gently lift each coverslip containing adherent cells from the well while maintaining the cell-coated surface facing upward [1]. Briefly place the coverslip into the separate well pre-filled with 2 milliliters of cold 0.9% ammonium formate solution for 2 to 3 seconds [2].
 - 2.6.1. Talent lifting a coverslip with adherent cells using sterile forceps while keeping the cell-coated surface facing upward.
 - 2.6.2. Talent placing the coverslip into a separate well containing cold 0.9% ammonium formate solution.
- 2.7. Repeat the rinsing process for a total of two rinses using fresh ammonium formate solution for each rinse [1]. After the final rinse, remove the coverslip from the ammonium formate solution and allow it to air dry on a glass slide [1] at room temperature in a chemical fume hood for approximately 20 to 30 minutes before DESI (*DEH-see*) mass spectrometry imaging analysis [3].
 - 2.7.1. Talent transferring the coverslip into a fresh ammonium formate solution for an additional rinse.
 - 2.7.2. Talent removing the coverslip from the ammonium formate solution and keeping it on a glass slide.
 - 2.7.3. Talent placing the glass slide in a chemical fume hood for air drying.

3. Assembly of the DESI Sprayer and Orbitrap Interface

- 3.4. Install a custom-fabricated stainless-steel ion transfer tube designed to match the Orbitrap ion source inlet [1]. ~~Position the ion transfer tube so that it aligns directly with the Orbitrap inlet while extending sufficiently to allow unobstructed access to the DESI plume [2].~~ NOTE: This shot is moved here as per authors request. The VO for the deleted shot is removed
 - 3.4.1. Talent installing the custom-fabricated stainless-steel ion transfer tube onto the Orbitrap ion source inlet.
 - ~~3.4.2. Talent aligning the ion transfer tube with the Orbitrap inlet while maintaining unobstructed access for the desorption electrospray ionization plume. NOTE: Not filmed~~
- 3.1. Mount the DESI sprayer to the setup consisting of the customized FAIMS (*FAMES*) frame, motorized XYZ stage, gas regulator, an optical breadboard, and a CONEX-MFACC (*KOH-necks M-fack*) controller [1].
 - 3.1.1. Talent mounting the desorption electrospray ionization sprayer onto the

integrated setup.

- 3.2. Position the DESI sprayer at an angle of approximately 70 degrees relative to the sample surface [1]. Place the sample directly below the sprayer [2] and position the sprayer tip as close as possible to the Orbitrap inlet without physical contact [3].
 - 3.2.1. Talent adjusting the sprayer to an angle of approximately 70 degrees relative to the sample surface.
 - 3.2.2. Talent placing the sample directly below the sprayer.
 - 3.2.3. Talent positioning the sprayer tip close to the Orbitrap inlet without making contact.
- 3.3. Connect the DESI grounding metal to a ground source beneath the mass spectrometer
 - 3.3.1. Talent connecting the DESI grounding metal to the grounding source.
- 3.4. ~~Install a custom-fabricated stainless-steel ion transfer tube designed to match the Orbitrap ion source inlet [1]. Position the ion transfer tube so that it aligns directly with the Orbitrap inlet while extending sufficiently to allow unobstructed access to the DESI plume [2].~~ NOTE: This step is moved before 3.1
 - 3.4.1. ~~Talent installing the custom-fabricated stainless-steel ion transfer tube onto the Orbitrap ion source inlet.~~
 - 3.4.2. ~~Talent aligning the ion transfer tube with the Orbitrap inlet while maintaining unobstructed access for the desorption electrospray ionization plume.~~
- 3.5. ~~Use an ion transfer tube with a bent geometry to improve positioning of the DESI sprayer relative to the sample surface and Orbitrap inlet [1].~~
 - 3.5.1. ~~Talent positioning the bent ion transfer tube relative to the sample surface and Orbitrap inlet.~~ NOTE: 3.5 and 3.6 are not filmed
- 3.6. ~~Align the sprayer at an angle of approximately 70 degrees relative to the sample surface so that the DESI spray plume intersects the Orbitrap inlet region without contacting the sample surface [1].~~
 - 3.6.1. ~~Talent adjusting the sprayer angle to direct the spray plume toward the Orbitrap inlet region without contacting the sample surface.~~
- 3.7. ~~To define a stable spray plume, confirm the presence of stable mass spectra and consistent normalized level values during acquisition [1]. Adjust the sprayer position until a stable ion signal is observed [2].~~ NOTE: 3.7. and 3.8 are moved after 4.6
 - 3.7.1. ~~SCREEN: Stable mass spectra and consistent normalized level values being monitored during acquisition.~~

Authors: Please create screen capture videos of the shots labeled as SCREEN, create a screenshot summary, and upload the files to your project page as soon as possible: https://review.jove.com/files_upload.php?src=21865803

~~Videographer: Please film the computer screen/ instrument display for all shots labelled as SCREEN~~

~~3.7.2. Talent fine adjusting the sprayer position while observing the ion signal.~~

~~3.8. Set the sprayer to surface distance to approximately 1.0 to 1.5 millimeters as a starting condition [1]. Manually fine adjust the sprayer position while monitoring normalized level values to achieve the highest stable ion signal [2].~~

~~3.8.1. Talent positioning the sprayer approximately 1.0 to 1.5 millimeters above the sample surface.~~

~~3.8.2. Talent fine adjusting the sprayer position while monitoring normalized level values.~~

4. DESI-MSI Spray Optimization and Raster Imaging

4.1. Prepare a fresh spray solvent consisting of methanol, water, and formic acid before DESI-MSI analysis [1].

4.1.1. Talent preparing the methanol, water, and formic acid spray solvent mixture.

4.3. Install a C18 column in-line to provide sufficient backpressure for stable spray formation at low flow rates [1]. **NOTE: This is moved here as per authors request**

4.3.1. Talent installing the C18 column in-line with the solvent delivery system.

4.2. Deliver the solvent using an Ultimate 3000 nano-LC system operated as an isocratic solvent pump at 750 nanoliters per minute [1]. Connect the nano-LC outlet to the DESI sprayer using polyether ether ketone tubing [2].

4.2.1. Talent placing the solvent line into the Ultimate 3000 nano-liquid chromatography system.

4.2.2. Talent connecting the nano-liquid chromatography outlet to the desorption electrospray ionization sprayer using polyether ether ketone tubing.

~~4.3. Install a C18 column in-line to provide sufficient backpressure for stable spray formation at low flow rates [1]. **NOTE: 4.3 is moved before 4.2**~~

~~4.3.1. Talent installing the C18 column in-line with the solvent delivery system.~~

4.4. Set the mass spectrometer method to positive ion mode with Orbitrap detection, a spray voltage of 0.85 kilovolts, an Orbitrap resolution of 120,000 at mass-to-charge ratio 200, and a mass-to-charge ratio range of 150 to 1200 [1].

4.4.1. SCREEN: 71691_screenshot_2.mp4: 00:05-00:42

4.5. Set the normalized automatic gain control target to 100%, the maximum injection time to 100 milliseconds, the radio frequency lens to 45%, and microscans to 1 [1]. Disable quadrupole isolation and source fragmentation [2].

4.5.1. SCREEN: 71691_screenshot_3.mp4: 00:02-00:18

4.5.2. SCREEN: 71691_screenshot_4.mp4

4.6. Supply nitrogen sheath gas from a nitrogen generator or liquid nitrogen source at approximately 12 pounds per square inch to stabilize the DESI spray plume [1]. Use 12 pounds per square inch as the starting operating pressure and make minor manual adjustments only if needed to improve normalized level values and maintain a stable mass spectrum [2].

4.6.1. Talent connecting the nitrogen sheath gas supply to the desorption electrospray ionization system.

4.6.2. Talent making minor manual adjustments to the gas supply while monitoring normalized level values.

3.7 To define a stable spray plume, confirm the presence of stable mass spectra and consistent normalized level values during acquisition [1]. Adjust the sprayer position until a stable ion signal is observed [2]. **NOTE: 3.7 and 3.8 are moved here as per authors request**

3.7.1 SCREEN: 71691_screenshot_1.mp4

Videographer: Please film the computer screen/ instrument display for all shots labelled as SCREEN

3.7.2 Talent fine-adjusting the sprayer position while observing the ion signal.

3.8 Set the sprayer-to-surface distance to approximately 1.0 to 1.5 millimeters as a starting condition [1]. Manually fine-adjust the sprayer position while monitoring normalized level values to achieve the highest stable ion signal [2].

3.8.1 Talent positioning the sprayer approximately 1.0 to 1.5 millimeters above the sample surface.

3.8.2 Talent fine-adjusting the sprayer position while monitoring normalized level values.

~~5. Raster Imaging and Pixel Control~~ **NOTE: Merge this section with the previous section because of insufficient shots**

5.1. Identify a region of interest containing optically visible single cells using the gridded coverslips as a reference [1]. ~~Select isolated cells with clear visual separation from neighboring cells, intact adherent morphology, and no obvious overlap with debris or cell clusters [2].~~ **NOTE: The VO is edited for the deleted shot**

5.1.1. SCREEN: 71691_screenshot_5.mp4

~~5.1.2. SCREEN: An isolated cell with clear separation from neighboring cells and no visible overlap with debris or cell clusters is being selected.~~ **NOTE: Not filmed**

5.2. After defining the raster region, set the raster step size to 0.01 millimeters, the stage velocity to 0.01 millimeters per second, and the dwell time to 20 seconds [2]. **NOTE: The VO is edited for the deleted shot**

~~5.2.1. SCREEN: Fance Stage Controller software being opened. Raster region being defined. **NOTE: Not filmed**~~

5.2.2. SCREEN: 71691_screenshot_7.mp4

~~5.3. Use left to right raster scanning across each line, followed by movement to the next line in the y-direction [1]. Set the orthogonal step size between raster lines to 10 micrometers [2]. **NOTE: Not filmed**~~

~~5.3.1. SCREEN: Left to right raster scanning pattern being configured across each line. Movement to the next line in the y-direction being defined.~~

~~5.3.2. SCREEN: Orthogonal step size between raster lines is being set to 10 micrometers.~~

~~5.4. Adjust the stage velocity according to the Orbitrap acquisition rate to achieve the desired scan-direction pixel dimension [1]. Calculate the scan-direction pixel dimension using the presented equation [2].~~

~~5.4.1. SCREEN: Stage velocity being adjusted according to the Orbitrap acquisition rate. **NOTE: 5.4.1 is not filmed**~~

5.4.2. TEXT ON A PLAIN BACKGROUND.

$$\text{Pixel size } (\mu\text{m}) = \text{Stage velocity } \left(\frac{\mu\text{m}}{\text{s}} \right) \times \text{Acquisition time per scan (s)}$$

~~5.5. Using a stage velocity of 0.01 millimeters per second, acquire approximately 376 scans over 102 seconds to produce an acquisition time of 0.271 seconds per scan and a corresponding scan-direction pixel dimension of approximately 2.7 micrometers [1]. Synchronize stage movement and mass spectral acquisition by setting the Orbitrap acquisition timing slightly shorter than the combined stage movement and dwell timing parameters [2].~~

~~5.5.1. SCREEN: Stage velocity being set to 0.01 millimeters per second. Acquisition is being observed as ~376 scans over 102 seconds. Acquisition time per scan and scan-direction pixel dimension being displayed.~~

~~5.5.2. SCREEN: Orbitrap acquisition timing being adjusted to synchronize mass spectral acquisition with stage movement and dwell timing.~~

5.6. Acquire imaging data over the selected mass range until completion of the predefined raster region [1].

5.6.1. SCREEN: 71691_screenshot_13.mp4: 00:21-00:22, 01:31-01:37

5.7. ~~Generate RAW data files during acquisition [1]. Stop acquisition after completion of the specified x axis and y axis raster coordinates and the corresponding instrument method duration [2].~~ After the acquisition is complete, collect RAW data files, resulting in approximately 5 gigabytes of total data for 101 raster lines [1]. **NOTE: The VO is edited for the deleted shots**

5.7.1. ~~SCREEN: RAW data files being generated during acquisition.~~ **NOTE: 5.7.1 and 5.7.2 is not filmed**

5.7.2. ~~SCREEN: Acquisition stopping after completion of the specified raster coordinates and instrument method duration.~~

5.7.3. SCREEN: : 71691_screenshot_16.mp4

Results

6. Results

6.1. Brightfield microscopy images showed individual OVCAR-8 cells [1].

6.1.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the dark cell shapes in the panel labeled '(A)'.*

6.2. Representative ion images at different mass-to-charge ratios demonstrated heterogeneous molecular distributions across neighbouring cells [1].

6.2.1. LAB MEDIA: Figure 2B, C, and D

1. OVCAR-8
Pronunciation link: No confirmed link found
IPA: No verified IPA available
Phonetic Spelling: oh·vee·car·eight
2. Ammonium
Pronunciation link: <https://www.merriam-webster.com/dictionary/ammonium>
IPA: /ə'moʊ.ni.əm/
Phonetic Spelling: uh·moh·nee·uhm
3. Formate
Pronunciation link: <https://www.merriam-webster.com/dictionary/formate>
IPA: /'fɔr.meɪt/
Phonetic Spelling: for·mayt
4. Adherent
Pronunciation link: <https://www.merriam-webster.com/dictionary/adherent>
IPA: /əd'hɪr.ənt/
Phonetic Spelling: ad·heer·uhnt
5. Desorption
Pronunciation link: <https://www.merriam-webster.com/dictionary/desorption>
IPA: /di'zɔrp.ʃən/
Phonetic Spelling: dee·zorp·shuhn
6. Electrospray
Pronunciation link: <https://www.merriam-webster.com/dictionary/electrospray>
IPA: /ɪ'lek.troʊ.spreɪ/
Phonetic Spelling: ih·lek·troh·spray
7. Orbitrap
Pronunciation link: <https://www.howtopronounce.com/orbitrap>
IPA: /'ɔ:r.bi.træp/
Phonetic Spelling: or·bih·trap
8. FAIMS
Pronunciation link: No confirmed link found
IPA: No verified IPA available
Phonetic Spelling: faymz
9. CONEX-MFACC
Pronunciation link: No confirmed link found
IPA: No verified IPA available
Phonetic Spelling: koh·neks em·fak
10. Isocratic
Pronunciation link: <https://www.merriam-webster.com/dictionary/isocratic>
IPA: /,aɪ.sə'kræt.ɪk/
Phonetic Spelling: eye·suh·krat·ik
11. Polyether
Pronunciation link: <https://www.merriam-webster.com/dictionary/polyether>

- IPA: /ˌpɑː.liˈiː.θə/
Phonetic Spelling: pol·ee·ee·ther
12. Ketone
Pronunciation link: <https://www.merriam-webster.com/dictionary/ketone>
IPA: /ˈkiː.toon/
Phonetic Spelling: kee·tohn
13. Quadrupole
Pronunciation link: <https://www.merriam-webster.com/dictionary/quadrupole>
IPA: /ˈkwɑː.drəˌpoul/
Phonetic Spelling: kwah·druh·pohl
14. Microscans
Pronunciation link: No confirmed link found
IPA: No verified IPA available
Phonetic Spelling: my·kroh·skanz
15. Raster
Pronunciation link: <https://www.merriam-webster.com/dictionary/raster>
IPA: /ˈræs.tə/
Phonetic Spelling: ras·ter
16. Orthogonal
Pronunciation link: <https://www.merriam-webster.com/dictionary/orthogonal>
IPA: /ərˈθɑː.ɡə.nəl/
Phonetic Spelling: or·thog·uh·nuhl
17. Acquisition
Pronunciation link: <https://www.merriam-webster.com/dictionary/acquisition>
IPA: /ˌæk.wəˈzɪʃ.ən/
Phonetic Spelling: ak·wuh·zish·uhn
18. Microscopy
Pronunciation link: <https://www.merriam-webster.com/dictionary/microscopy>
IPA: /maɪˈkraɪ.skə.pi/
Phonetic Spelling: my·krah·skuh·pee
19. Heterogeneous
Pronunciation link: <https://www.merriam-webster.com/dictionary/heterogeneous>
IPA: /ˌhet̬.ə.əˈdʒiː.ni.əs/
Phonetic Spelling: het·uh·ruh·jee·nee·uhs
20. Chromatography
Pronunciation link: <https://www.merriam-webster.com/dictionary/chromatography>
IPA: /ˌkroʊ.məˈtɑː.grə.fi/
Phonetic Spelling: kroh·muh·tog·ruh·fee