FINAL SCRIPT: APPROVED FOR FILMING

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Title: Spatial Molecular Imaging of the Glycome Using Mass Spectrometry

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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? **Yes, all done**

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. **No**

Current Protocol Length
Number of Steps: 25
Number of Shots: 57 (8 SC)

Introduction

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

INTRODUCTION:

Alison Ryan: We are using exciting new mass spectrometry-based technologies to map biomolecules in biological samples for spatial biology.

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

1.2. **Alison Ryan:** Key current challenges include rigor and reproducibility coming from sample preparation and limitations in sensitivity and resolution.

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

CONCLUSION:

1.3. **Isabella Caffee:** We utilize spatial molecular imaging to uncover the molecular, metabolic, and cellular underpinnings of cellular biology, physiology, and disease pathology.

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

1.4. **Reece Larson:** These findings will allow molecular imaging of the glycome in diverse biological sources and disease states.

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

1.5. **Craig Vander Kooi:** Future research will allow detection of different glycome classes, and increase the sensitivity and resolution to a single cell level.

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.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Florida.

Protocol

2. Slide Preparation for MSI of Glycomics

Demonstrators: Charles Soto and Roberto Ribas

2.1. To begin, prepare one or more dialysis cups by placing them in high-performance liquid chromatography or HPLC water for 15 minutes **[1]**. Dialyze 200 microliters of stock isoamylase using a 500-microliter dialysis cup in 14.3 milliliters of HPLC water for 2 hours at 4 degrees Celsius without stirring **[2-TXT]**.

2.1.1. WIDE: Talent placing dialysis cups into a conical tube filled with HPLC water.

2.1.2. Talent pipetting 200 microliters of stock isoamylase into a dialysis cup filled with 14.3 milliliters of HPLC water. **TXT: Replace the water after 16 h and then after 8 h**

2.2. Using a pipette, gently remove the isoamylase from the dialysis cup and place it into a 1.5-milliliter microcentrifuge tube **[1]**. Measure the total volume of the isoamylase solution after dialysis **[2]**. Based on the stock activity of 200 units per milliliter and the final volume, calculate the total volume required for 3 units of isoamylase **[3]**.

2.2.1. Talent using a pipette to collect isoamylase from the dialysis cup into a labeled 1.5 milliliter microcentrifuge tube.

2.2.2. Talent measuring the final volume of isoamylase using a pipette or microbalance.

2.2.3. Talent writing in a notebook.

2.3. Pipette aliquots containing 3 units of isoamylase into thin-walled PCR tubes **[1]**. Snap-freeze the tubes in liquid nitrogen **[2]** and store them at minus 80 degrees Celsius **[3]**.

2.3.1. Talent pipetting 3-unit aliquots of isoamylase into labeled PCR tubes.

2.3.2. Talent submerging the tubes in liquid nitrogen for snap freezing.

2.3.3. Talent placing the frozen PCR tubes into a minus 80 degrees Celsius freezer for storage.

2.4. When tissues are processed and ready, prepare the humidity chamber by placing a paper towel soaked with deionized water below the metal rack **[1]**. Place the chamber

in a 37 degrees Celsius incubator for equilibration **[2]**.

2.4.1. Talent placing a soaked paper towel under the rack in the humidity chamber.

2.4.2. Talent positioning the humidity chamber inside a 37 degrees Celsius incubator.

2.5. Then, fill the food steamer reservoir with tap water to the top **[1]** and set the timer to 60 minutes to turn the unit on **[2]**. Fill each slide mailer with the freshly prepared citraconic buffer **[3]**. Place the slide with the tissue facing inward to avoid contact with the sides and to ensure effective antigen retrieval **[4]**.

2.5.1. Talent pouring tap water into the food steamer reservoir until full.

2.5.2. Talent setting the timer to 60 minutes and switching on the steamer.

2.5.3. Talent carefully inserting tissue slides into the mailers with tissue facing inward.

2.5.4. Talent pouring citraconic buffer into multiple slide mailers, clipping one side.

2.6. Verify that steam is being emitted from the food steamer **[1]**. Place the mailers in the steamer and incubate them for 30 minutes **[2]**. Then, transfer the mailers to a deionized water bath for 5 minutes **[3]**. Replace half of the citraconic buffer in each mailer with deionized water and allow it to stand for another 5 minutes **[4]**.

2.6.1. Shot of the Steam visibly rising from the food steamer outlet.

2.6.2. Talent placing slide mailers into the steamer for incubation.

2.6.3. Talent moving the mailers from the steamer into a deionized water bath.

2.6.4. Talent pouring out half of the buffer and replacing it with deionized water.

2.7. Remove all solvent and wash the slides by adding 100 percent deionized water to each mailer and then removing it **[1]**.

2.7.1. Talent adding deionized water to the slide mailers, swirling gently.

2.8. On the back of each slide, draw a circle and a triangle to mark internal standard locations **[1]**. Apply 1 microliter of 1 milligram per milliliter horseradish peroxidase at the circle mark **[2]**, and 1 microliter of 10 milligrams per milliliter rabbit liver glycogen at the triangle mark **[3-TXT]**.

2.8.1. Close-Up of talent wiping the back of the slide and then marking a circle and triangle on the back of the slide.

2.8.2. Talent applying 1 microliter of horseradish peroxidase solution at the circle mark.

2.8.3. Talent applying 1 microliter of rabbit liver glycogen at the triangle mark. **TXT: Place the slides in a desiccator for 15 min**

2.9. Thaw one tube of 100 micrograms of lyophilized peptide N-glycosidase F and one tube containing 3 units of isoamylase for 10 minutes **[1]**. Centrifuge the tubes at approximately 2000 *g* for 5 seconds **[3]**.

2.9.1. Talent placing both tubes on the bench to thaw for 10 minutes.

2.9.2. Talent loading the tubes into a microcentrifuge.

2.10. Pipette 50 microliters of deionized water into each lyophilized PNGase F tube **[1]**. After vortexing gently, centrifuge the tube at approximately 2000 *g* for 5 seconds **[2]**. Pipette the resulting 50 microliters of PNGase F solution directly into the isoamylase tube **[3]** and add HPLC water to bring the total volume to 1 milliliter **[4-TXT]**.

2.10.1. Talent adding 50 microliters of deionized water into the PNGase F tube using a pipette.

2.10.2. Talent briefly spinning the tube in a microcentrifuge.

2.10.3. Talent pipetting the PNGase F solution into the isoamylase tube.

2.10.4. Talent adding HPLC water to reach a final volume of 1 milliliter. **TXT: Vortex the mixture and centrifuge (2000 x *g*, 5 s)**

3. Enzyme Spraying and Digestion

Demonstrator: Franca Bucco Paolasso

3.1. Turn on the HTX M5 sprayer and open the HTX M5 software on the connected computer **[1]**.

3.1.1. Talent pressing the power button on the HTX M5 sprayer.

3.2. Turn off the Knauer pump by pressing the **Stop** button on the front right side **[1]** and power on the external syringe pump using the switch on the back panel **[2]**.

3.2.1. Talent pressing the **Stop** button on the Knauer pump.

3.2.2. Talent reaching behind the syringe pump and switching the power on.

3.3. In the HTX software, under the **Methods** tab, select the appropriate method for dual enzyme spraying **[1]**. Navigate to the **Temp** tab and set the spray nozzle temperature to 45 degrees Celsius **[2]**.

3.3.1. SCREEN: SCREEN_3.3.1.mp4.

3.3.2. SCREEN: SCREEN_3.3.2.mp4.

3.4. Open the ultra-high purity nitrogen gas valve and ensure the sprayer pressure is set to 10 pounds per square inch **[1-TXT]**.

3.4.1. Talent turning the nitrogen gas valve and checking the pressure gauge to confirm 10 psi. **TXT: Syringe pump: 95 µL/min**

3.5. Using a fresh syringe and needle, fill it with 4 milliliters of HPLC-grade water **[1]**. After removing the bubbles, run 2 milliliters of water through approximately a 6-inch section of the sprayer line into a waste beaker **[2]**.

3.5.1. Talent drawing 4 milliliters of HPLC-grade water into a new syringe.

3.5.2. Talent adding 2 milliliters of water into the sprayer line.

3.6. Attach the syringe to the sprayer line **[1]** and run water through the syringe pump by starting the system at a flow rate of 95 microliters per minute for 5 minutes **[2]**.

3.6.1. Talent connecting the syringe to the sprayer line.

3.6.2. Show the syringe pump display as the water is pumped through.

3.7. While the syringe pump is flushing, tape the prepared slides to the bottom-left corner of the heated tray using a metal alignment guide **[1-TXT]**. Under the **Sample** tab, define the X and Y parameters for the spray region **[2]**.

3.7.1. WIDE: Talent securing slides onto the heated tray and then using the metal alignment guide to define coordinates. **TXT: Ensure that the tissue lies completely within the spray area**

3.7.2. SCREEN: SCREEN_3.7.2.mp4. 00:05-00:22

3.8. After 5 minutes, pause the syringe pump and disconnect the syringe **[1].** Draw air into it and push the air through the sprayer line to purge any residual water **[2]**.

3.8.1. Talent disconnecting the syringe from the sprayer line.

3.8.2. Talent drawing air into the syringe barrel and pushing air through the line.

3.9. Then, load the prepared dual enzyme solution into a new syringe **[1]** and connect it securely to the syringe pump **[2]**.

3.9.1. Talent filling a clean syringe with the mixed dual enzyme solution.

3.9.2. Talent connecting the loaded syringe to the syringe pump.

3.10. Attach the loaded syringe containing the dual enzyme solution to the sprayer line **[1]**. On the syringe pump, adjust the flow rate to 25 microliters per minute **[2]**.

3.10.1. CLOSE-UP: Talent connecting the enzyme-loaded syringe to the sprayer line.

3.10.2. SCREEN: SCREEN_3.10.2.mp4. 00:07-00:15

3.11. Once the spray nozzle temperature reaches 45 degrees Celsius **[1]**, press **Start** under the **Cycle** tab **[2]**. When prompted to turn on the Knauer pump, click **No [3]**. Press **Start** on the syringe pump to begin enzyme spraying **[4]**.

3.11.1. SCREEN: SCREEN_3.11.1.mp4. 01:00-01:07

3.11.2. SCREEN: SCREEN_3.11.2_3.11.3_take2.mp4. 00:04-00:12

3.11.3. SCREEN: SCREEN_3.11.2_3.11.3_take2.mp4. 00:14-00:20

3.11.4. Talent pressing the **Start** button on the syringe pump to initiate spraying.

3.12. Place a blank slide under the spray nozzle to confirm visible enzyme deposition **[1]**. Once the enzyme spray is visibly even, click **Continue** on the software **[2]**.

3.12.1. Talent positioning a blank slide directly under the spray nozzle.

3.12.2. SCREEN: SCREEN_3.12.2.mp4. 00:00-00:10

3.13. Next, place the glass cover on the sprayer and monitor for even application, ensuring uniform wetting across the slide surface **[1]**.

3.13.1. Talent placing the transparent glass cover over the sprayer.

3.14. When the spray cycle is complete, press **Stop** on the syringe pump **[1]**. Turn off the nitrogen gas valve and click **Valve load confirm** in the software **[2]**.

3.14.1. Talent pressing the **Stop** button on the syringe pump control panel.

3.14.2. Talent turning off the nitrogen gas valve by rotating it clockwise.

3.15. Incubate the sprayed slides in the HTX humidity chamber at 37 degrees Celsius for 2 hours, ensuring the slides are placed facing upward **[1]**. Once incubation is complete, transfer the slides to a desiccator and dry for 15 minutes **[2-TXT]**.

3.15.1. Talent placing slides face-up on the metal rack inside the 37 degrees Celsius humidity chamber.

3.15.2. Talent moving the slides from the humidity chamber into a desiccator and sealing it for drying. **TXT: Perform MALDI-MSI; Visualize ion intensity maps of the glycome**

Results

4. Results

4.1. Glycogen-derived oligosaccharides and N-linked glycans were detected across a wide mass-to-charge range in the liver using matrix-assisted laser desorption/ionization mass spectrometry imaging **[1]**.

4.1.1. LAB MEDIA: Figure 1B. *Video editor: Highlight the black peaks*.

4.2. Glycogen was broadly localized throughout liver hepatocytes, while it was absent from vessels and connective tissue in the portal tracts **[1]**.

4.2.1. LAB MEDIA: Figure 1C. *Video editor: Highlight the Regions around the labeled "Large portal tracts"*.

4.3. The spatial distribution of glycogen and its chain-length distribution were both measurable using MALDI-MSI **[1]**.

4.3.1. LAB MEDIA: Figure 1D. *Video editor: Emphasize the curve in the graph.*

4.4. One N-linked glycan species was widely distributed throughout liver hepatocytes **[1]**.

4.4.1. LAB MEDIA: Figure 1E.

4.5. Another N-linked glycan species displayed a more regional distribution pattern consistent with liver sinusoidal structure **[1]**.

4.5.1. LAB MEDIA: Figure 1F. *Video editor: Emphasize the patchy distribution of green-yellow signals scattered throughout the liver section*.

4.6. A distinct N-linked glycan localized specifically to portal tract elements **[1]**.

4.6.1. LAB MEDIA: Figure 1G. *Video editor: Highlight the bright yellow-green clusters near the edges of the tissue section*.

4.7. Histological staining confirmed the anatomical structures of the portal tract, including portal vein, bile duct, and connective tissue **[1]**.

4.7.1. LAB MEDIA: Figure 1H. *Video editor: Highlight the labels "Portal vein", "Bile duct with ductules", and "Connective tissue" in the stained tissue section.*

- Isoamylase

Pronunciation link: https://www.howtopronounce.com/isoamylase How To Pronounce+2definitions.net+2
IPA: /ˌaɪsoʊˈæmɪleɪz/
Phonetic Spelling: eye-soh-AM-ih-layz

- Citraconic (as in citraconic buffer)

Pronunciation link: https://howjsay.com/how-to-pronounce-citraconic howjsay.com+2oed.com+2
IPA: /ˌsɪtrəˈkɒnɪk/ or /ˌsɪtrəˈkɑnɪk/ (US)
Phonetic Spelling: sith-ruh-KON-ik

- Lyophilized

Pronunciation link: https://www.merriam-webster.com/dictionary/lyophilize merriam-webster.com+1
IPA: /ˌlaɪəˈfɪlaɪzd/
Phonetic Spelling: lie-uh-FIL-ized

- Microcentrifuge

Pronunciation link: https://www.howtopronounce.com/microcentrifuge How To Pronounce+2howjsay.com+2
IPA: /ˌmaɪkroʊˈsɛntrəˌfjuːdʒ/
Phonetic Spelling: MY-kroh-SEN-truh-fyo͞oj

- Serological

Pronunciation link: https://www.howtopronounce.com/serological How To Pronounce+1
IPA: /ˌsɛrəˈlɒdʒɪkəl/ or in US transfer: /ˌsɛrəˈlɑdʒɪkəl/
Phonetic Spelling: ser-uh-LOJ-ih-kul

- MALDI

Pronunciation link: https://www.howtopronounce.com/maldi How To Pronounce+1
IPA: /ˈmældi/
Phonetic Spelling: MAL-dee

- Antigen

Pronunciation link: https://www.merriam-webster.com/dictionary/antigen
IPA: /ˈæn.tɪˌdʒɛn/
Phonetic Spelling: AN-tih-jen

- Centrifuge (used for higher-speed device)

Pronunciation link: https://www.merriam-webster.com/dictionary/centrifuge
IPA: /ˈsɛn.trə.fjuːdʒ/
Phonetic Spelling: SEN-truh-fyo͞oj

- Dialysis (as in dialysis cups)

Pronunciation link: https://www.merriam-webster.com/dictionary/dialysis
IPA: /daɪˈæləsɪs/
Phonetic Spelling: die-AL-uhsis

- Lyophilized

Pronunciation link: https://www.merriam-webster.com/dictionary/lyophilize
IPA: /ˌlaɪəˈfɪlaɪzd/
Phonetic Spelling: lie-uh-FIL-ized

- Protamine

Pronunciation link: https://www.howtopronounce.com/protamine
IPA: /ˈproʊtəˌmiːn/
Phonetic Spelling: PROH-tuh-meen