The International Mass Spectrometry Imaging Society

Americas

Advances in Multimodal Imaging

Summer Workshop
at
Vanderbilt University

July 26th – 28th
2026

Welcome to the IMSIS-Americas Summer Workshop 2026!

We're absolutely delighted to have you join us here in Nashville, TN, home of the Country Music Hall of Fame and the first MALDI-MS imaging experiments! I think many of us workshop attendees would feel the latter is more important.

Perhaps it is with great symmetry that I write this last letter as IMSIS-Americas President for a workshop being help at the place I got my first hands-on experience with MALDI! I first visited Vanderbilt in 2013 as part of their 2nd annual AIMS course (the first year they had coffee). I learned about this new automated MALDI matrix sprayer by a small startup company called *HTX Technologies*. It was only 1 of like 4 sprayers in the world at the time. (Thank you for your sponsorship, HTX!) I also learned about this new MS imaging data software company called *SCiLS Lab*, which had yet to be purchased by one of our Gold Sponsors (thank you, Bruker).

What I remember most about the AIMS course is the people I met. Peggi Angel, Erin Seeley, Boone Prentice, **Jeff Spraggins (thanks for hosting!)** … and more. Folks that I have continued to work and network with over the last 13 years. Folks that have grown into powerhouse scientists of their own. I hope you have the opportunity to connect with your peers, share the great science you're doing, and learn about the amazing science they are doing at this workshop. And perhaps in 13 years, you too can reminisce about this meeting and see how far the field has come and how far folk's careers have grown.

This year's workshop theme is "**Advances in Multimodal Imaging**", and Vanderbilt University is an excellent venue for such theme. The team here have moved beyond the early MALDI-MS imaging days to really drive innovative approaches to connect MS imaging data with other spatial modalities to advance our understanding of biology.

Looking ahead, **I am excited about IMSIS 4 in Kyoto Japan this November,** as well as future IMSIS-Americas workshops in the future. I hope to see all of you there.

A heartfelt thank you goes out to the volunteers whose tireless efforts made this workshop possible. Thanks to the organizing team that helped in planning, obtaining sponsors, and dealing with all the little things that it takes to host a workshop like this. Finally, **a special shoutout to Maddie Colley (host)** whose herculean efforts made this event happen.

Here's to a **fantastic and inspiring workshop** for all. Let's make it memorable!

Warm Regards,
Chris Anderton
IMSIS-Americas President

And thank you to all our sponsors!!!

Schedule at a Glance

Sunday 7/26

8:30 am – Registration Opens	4:00 pm – Registration & Vendor Visits
9:00 am – Tutorial 1: Antibodies and transcriptomics, sample prep to acquisition	5:00 pm – Opening Address by Professor Jeffrey Spraggins and Plenary by Professor Ken Lau
11:30 am – Independent lunch in Midtown Nashville & Registration	6:30 pm – Setup posters and explore Nashville
1:30 pm – Tutorial 2: Leveraging generative AI for data analysis	

Monday 7/27

8:00 am: Registration & Coffee	1:30 pm: Oral Session 2: Advances in Multimodal Imaging (part 2)
8:30 am: Oral Session 1: Microscopy Enhanced MSI	3:30 PM: Poster Session Sponsored by SynCell
10:30 am: Coffee break	3:45 PM: Poster Session & Happy Hour
11:00 am: Oral Session 2: Advances in Multimodal Imaging Workflows (part 1)	6:00 pm – Vendor Demonstrations
12:00 pm: Lunch Sponsored by HTX Imaging	

Tuesday 7/28

8:30 am: Oral Session 3: Multimodal Imaging of Complex Microenvironments (part 1)	2:50 pm: Coffee Break
10:30 am: Coffee break	3:00 pm: IMSIS Business Meeting & Awards
11:00 am: Multimodal Imaging of Complex Microenvironments (part 2)	3:30 pm: Closing Plenary – The State of the Field by Professor Peggi Angel
12:00 pm: Lunch Sponsored by Agilent Technologies	4:30 pm: Farewells & Removing Posters
1:30 pm: Oral Session 4: Spatial Multi-Omics from Single Tissue Sections	5:00 pm: Unofficial Happy Hour in Nashville

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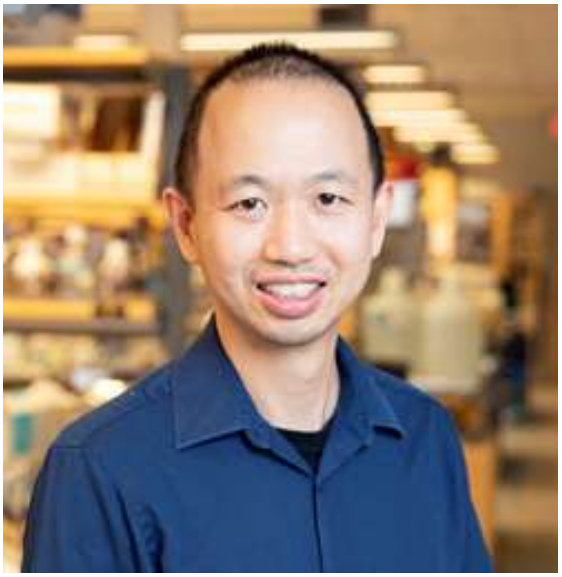
Opening Plenary

Professor Ken S. Lau, Ph.D.

Professor of Cell and Developmental Biology, Professor of Surgery, Director for the Center for Computational Systems Biology at Vanderbilt University School of Medicine



Dr. Lau is internationally recognized in field of single-cell systems biology. His research focuses on gut biology, specifically the molecular events that occur at the intersection of mucosal damage, inflammation, and tumor initiation. His laboratory applies systems biology approaches to investigating interactions between cells and their microenvironment in modulating cellular identity, development, and the origins of cancer. Serving as co-chair of the NCI Human Tumor Atlas Network, he has led efforts to map human cancers at single-cell resolution, setting new standards for integrative cancer research. He is currently a full professor in the Department of Cell and Developmental Biology at Vanderbilt University, and the director of the Center for Computational Systems Biology.

Closing Plenary

Professor Peggi M. Angel, Ph.D.

Professor, Department of Pharmacology & Immunology, Co-Director, MS Imaging & MUSC Proteomics, Bruker Center of Excellence for Clinical Glycomics at the Medical University of South Carolina

Peggi Angel is tenured Professor in the Department of Pharmacology & Immunology at the Medical University of South Carolina and Co-Director of Mass Spectrometry Imaging. Dr. Angel's work focuses on the contribution of spatial chemical biology to the external, endogenous environmental and mechanical forces in disease risk, progression, and therapeutic resistance. She has developed multiple mass spectrometry imaging approaches to spatial biology designed for use on clinically archived human specimens of tissues, cells and fluids. Notably, she pioneered a spatial method targeting the collagen proteome and other extracellular matrix glycoproteins from formalin-fixed, paraffin-embedded tissues, integrative with other spatial modalities. She has further developed this approach as a way to target the extracellular matrix in cell culture and in biofluids. Dr. Angel has over 14 years cumulative experience in 5 biotech startups including Glycopath, Inc., a company that leveraged glycosylation patterns as a prognostic or diagnostic tool; she currently serves on the board of N-Zyme Scientifics, a company that produces enzymes for targeted mass spectrometry imaging. Dr. Angel is committed to creating a collaborative mass spectrometry imaging community and serves as Past President for the Americas Region of the International Mass Spectrometry Imaging Society, as Scientific Advisor for the US Human Proteome Organization, and as scientific advisor for Mass Spectrometry & Advances in the Clinical Lab. Dr. Angel is devoted to coaching and mentoring serving on multiple committees to advise and mentor young scientists in entrepreneurship within multidisciplinary teams.

Dr. Angel's Closing Plenary will discuss the "State-of-the-Field Mass Spectrometry Imaging.

Full Schedule

Sunday 7/26

8:30 AM: Coffee and Registration

9:00 AM: Tutorial 1: Antibodies and transcriptomics, sample prep to acquisition taught by Caleb Marlin (The Oklahoma Medical Research Fund) and Thai Pham (Vanderbilt University)

11:30 AM: Off-campus, independent lunch

1:30 PM: Tutorial 2: Leveraging generative AI for data analysis taught by Nico Verbeek and (Aspect Analytics) and Sadi Quinones-Al-Muhtaseb (University of Florida)

4:00 PM: Registration & Vendor Visits

5:00 PM: Opening Address by Professor Jeffrey Spraggins and Plenary by Professor Ken Lau

6:00 PM: Conclusion of Daily Program

Monday 7/27

8:00 AM: Coffee and Registration

8:15 AM: Welcome Address by Madeline E. Colley

Oral Session 1: Microscopy-Enhanced MSI

8:30 AM: Alyssa Moore, Purdue University: “Understanding Neuronal Excitability using Mass Spectrometry Imaging of SCN2A-deficient Organoids “

8:50 AM: Siva Swapna Kasarla, Johns Hopkins Medicine Baltimore, MD United States: “A novel MALDI-MSI workflow for imaging polar metabolites and its application to organophosphate neurotoxicity studies”

9:10 AM: Tommy Zhang, Purdue University: “Enhancing on-tissue intact proteoform analysis using multi-segmented capillary electrophoresis”

9:30 AM**:** Fiorella Yantani, Johns Hopkins University: “OPNA Poisoning and treatment to explore the changes in Metabolism and Lipid Regulation in a Humanized Mouse Model”

9:50 AM: Matthew Bonet, The University of Maryland Baltimore: “Exploring the lipid droplet-associated lipid landscape in mouse hippocampus in neurodegenerative disease”

10:10 AM: Waters Gold Sponsor Talk

10:30 AM: Coffee Break

Oral Session 2: Advances in Multimodal Imaging Workflows (part 1)

11:00 AM: Shanika Weerasinghe, Iowa state university: “Mapping Double-Bond Positional Isomers of Phosphatidylcholines in Zebrafish Using OzMALDI Mass Spectrometry Imaging”

11:20 AM: Maddison Hibbard, University of Illinois - Chicago: “iGAMSI: An immunohistochemistry-compatible framework for single-cell spatial lipidomics in intact neural tissues”

11:40 AM: Tae-Hun Hahm, Johns Hopkins: “FluoMALDI Microscopy: A Multimodal Strategy for Spatial Molecular Discovery Integrating Fluorescence Labeling Strategies with MALDI Imaging”

12:00 PM: HTX Sponsored Lunch

Oral Session 2: Advances in Multimodal Imaging Workflows (part 2)

1:30 PM: Lauren Hill, Medical University of South Carolina: “Multi-Omic MALDI MSI to Evaluate Drug-Induced Metabolic Reprogramming of Peripheral Blood Mononuclear Cells at Single Cell Resolution”

1:50 PM: Jade Macdonald, Vanderbilt University: “Extracellular Matrix, N-Glycan and Immune Cell Remodeling Define Dysplastic Microenvironments in Early-Onset Colorectal Precancer”

2:10 PM: Conor Convery, Johns Hopkins: “An Analysis of Standard Matrix Deposition Methodology for Three Negative Ion Mode Matrices”

2:30 PM: Mithunjha Anandakumar, University of Illinois Urbana Champaign: “Learning When to Stop: Adaptive Temporal Acquisition for FT-ICR Mass Spectrometry Imaging”

2:50 PM: Erin Seeley, UT MD Anderson Cancer Center: “So Much Data, So Little Tissue: New Strategies for Multi-Omics Data Integration “

3:10 PM: Timothy Trinklein, University of Illinois Urbana-Champaign: “A whole-brain molecular atlas by integrating accelerated MALDI MSI and in vivo MRI”

3:30 PM: SynCell Poster Session Welcome Seminar

3:45 PM: Poster Session & Happy Hour

6:00 PM: Vendor Demos

6:30 PM: Conclusion of Daily Program

Tuesday 7/28

8:00 AM: Coffee and Registration

Oral Session 3: Multimodal Imaging of Complex Microenvironments (part 1)

8:30 AM: Angela Kruse, The Ohio State University: “A multimodal spatial atlas of lipid metabolism in the human intestine”

8:50 AM: Tialfi Bergamin de Castro, University of Maryland Baltimore: “Lung multiomics reveals dynamic molecular remodeling and immune responses in SARS-CoV-2 infected mouse lungs”

9:10 AM: Lauren Emmerson, Vanderbilt University: “Decoding the Molecular Architecture of S. aureus Osteomyelitis via Multimodal Imaging Mass Spectrometry”

9:30 AM: Kyle Duncan, Vancouver Island University: “Spatially resolved metabolomics of optimal cutting temperature compound (OCT)-embedded tumors”

9:50 AM: Marija Velickovic, Pacific Northwest National Lab: “Metabolome-Informed Proteome Imaging of Complex Heterogeneous Biological Systems”

10:10 AM: Robert Ernst, Department of Microbial Pathogenesis - University of Maryland Baltimore: “Imaging-Guided Analysis of BECC-Adjuvanted Influenza Microneedle Vaccines and Skin Phospholipid Remodeling”

10:30 AM: Coffee Break

Oral Session 3: Multimodal Imaging of Complex Microenvironments (part 2)

11:00 AM: Kristine Glunde, Johns Hopkins Applied Imaging Mass Spectrometry (AIMS) Core, Radiology Department, Johns Hopkins School of Medicine: “FluoMALDI, RaMALDI, and QMALDI expand MALDI imaging by enabling precise spatial targeting, multimodal integration, and quantitative analysis, providing powerful tools for understanding complex tissue microenvironments”

11:20 AM: Stephanie Cologna, Department of Chemistry, University of Illinois Chicago: “Comprehensive investigation of spatiotemporal lipid alterations in the whole brain of NPC1 mouse models using mass spectrometry imaging”

11:40 AM: Sizun Jiang, Beth Israel Deaconess Medical Center/Harvard Medical School: “Fantastic Cells and Where to Find Them”

12:00 PM: Agilent Sponsored Lunch

Oral Session 4: Spatial Multi-Omics from Single Tissue Sections

1:30 PM: Megan Ward, Vanderbilt University: “Segment-Resolved Lipidomic Exploration of the Human Proximal Tubule using Integrated MALDI IMS and Multiplexed Immunofluorescence”

1:50 PM: Gargey Yagnik, Ambergen, Inc.: “Multiplexed, Multiomic and Multimodal DESI-immunohistochemical Imaging of Biomarkers Including Drugs in Tissues using Novel Photocleavable Mass-Tag Antibody Probes”

2:10 PM: Bruker Gold Sponsor Talk

2:30 PM: Christopher Anderton, Pacific Northwest National Lab: “MOSAICS: An End-to-End Spatial Multiomics Approach for Analyzing a Single Tissue Section”

2:50 PM: Coffee Break

3:00 PM: IMSIS Business Meeting & Awards

3:30 PM: Closing Plenary: The State of the Field by Professor Peggi M. Angel

4:30 PM: Farewells & Unofficial Off Campus Happy Hour

5:00 PM: Conclusion of Program

Poster Session

Sponsored by SynCell

Poster #	Author Name	Author Title	Author Affiliation
1	Alexis Pope	Mapping Calprotectin-Mediated Molecular Adaptation in Staphylococcus aureus Biofilms	Vanderbilt University
2	Qingjun Wang	Mapping Retinal Lipid Alterations in CLN3 Disease Using Multimodal and Multi-Resolution Mass Spectrometry Imaging: Exploring the Data Analysis Workflow	University of Kentucky
3	Jacqueline Van Ardenne	Defining the Molecular Trajectory of Staphylococcus aureus Abscesses by Spatial Multiomics	Vanderbilt University
4	Bindesh Shrestha	MALDI and DESI Imaging with Cyclic IMS Using Wideband Enhancement Workflows for Improved Molecular Sensitivity and Specificity	Waters
5	Lyndsay Young	High-Throughput Single-Cell Array Profiling of N-Glycan and Lipid Signatures in Healthy and Pancreatic Cancer Circulating Immune Cell Populations	Medical University of South Carolina
6	Kameron Molloy	Multimodal Characterization of Human Alzheimer's Disease Brain Tissue Using Salt-Enhanced MALDI-2 IMS	Vanderbilt University
7	Shanaliz Natta	Defining Immunolipidomic Dysregulation During Pseudomonas aeruginosa Infection in a Cystic Fibrosis Model	University of Maryland Baltimore
8	Zhen Wang	Spatial lipidomic analysis reveals age- and cataract-related lipid modifications in human lenses	Vanderbilt University
9	Yinyue Zhu	TIMSImaging: An open and interoperable workflow for trapped ion mobility mass spectrometry imaging data processing and visualization	Northeastern University

10	Madeline Colley	Resolving Biology with High Spatial and Spectral Resolution Imaging through timsMRMS	Vanderbilt University
11	Yasaman Abbasi	Spatial Lipidomic Analysis of Gingiva in Mice with Periodontitis	University of Maryland Baltimore
12	Ryan Coyle	A High-Throughput, Reproducible Workflow Integrating MALDI Imaging and Automated LC–MS/MS for Lipid and Metabolite Annotation	Vanderbilt University
13	Abigail Weatherford	N-glycan mass spectrometry imaging characterization of intraductal papillary mucinous neoplasms and pancreatic cancer tissues	Medical University of South Carolina
14	Bindesh Shrestha	Multimodal DESI Imaging of Photocleavable Mass-Tagged Antibodies Using Targeted and Untargeted Mass Spectrometry Workflows	Waters
15	Timothy Hendrigsman	Incorporating a standardized instrument QC framework into large-scale MALDI IMS studies for reproducibility	Vanderbilt University
16	Silas Porter Crenna	Using modified sample preparation procedures to preserve heterogeneity within multi-layered biological systems	University of Victoria
17	David Anderson	Imaging Human Ocular Globes and Optic Nerve: 2 and 3-D Molecular Mapping of Anatomical Features Necessary for Vision	Vanderbilt University
18	Sumankalai Ramachandran	Assessment of Matrix Sublimation for MALDI HiPLEX-IHC Mass Spectrometry Imaging of Metabolic Heterogeneity in Lung Cancer Cell Lines	Bruker
19	Xiaowei Lu	DESI Imaging of Fucosylated N-Glycan with Histology-Compatible Tissue Preservation	Stanford University Mass Spectrometry Core Facility
20	Bindesh Shrestha	High-Speed DESI Metabolite Imaging at 200 Hz Using a Multi-Reflecting Time-of-Flight Mass Spectrometer	Waters

21	Jacquelyn spathies	Revealing the Spatial Organization of Polyunsaturated Lipids and Bile Salts Across the Zebrafish Body Axis with MALDI Imaging	Vanderbilt University

Tutorial Session

Antibodies and Transcriptomics, Sample Prep to Acquisition

Tutorial Leads

M. Caleb Marlin, Ph.D.
Senior Staff Scientist and Imaging Project Manager, Arthritis and Clinical Immunology Research Program at the Oklahoma Medical Research Foundation

Thai Pham, Ph.D.
Postdoctoral Researcher at Vanderbilt University
Mass Spectrometry Research Center & the Lab of Jeffrey M. Spraggins

Drs Marlin and Pham will introduce the concept of multi-modal imaging on the same sample and introduce important concepts pertaining to antibody- and probe-based imaging techniques, as they relate to Imaging Mass Spectrometry. Participants will see real world examples of workflows that involve antibody- and probe-based imaging and how these modalities can enrich Imaging Mass Spectrometry datasets and elucidate underlying biology.

Tutorial Session

Leveraging Generative AI for Data Analysis

Tutorial Leads

Nico Verbeeck, Ph.D.
Director of Mass Spectrometry Imaging
Aspect Analytics NV

Sadi Quinones-Al-Muhtaseb, Ph.D.
Postdoctoral Research Fellow at the University of Florida
The Lab of Ramon C. Sun

Drs Quinones and Verbeeck will introduce how generative AI and large language models (LLMs) can support mass spectrometry imaging data analysis. The tutorial will cover an overview of the current tool landscape, the fundamentals of effective prompting, and common pitfalls to keep in mind. Through live examples with imzML datasets, the presenters will demonstrate how to work productively with LLMs to explore and analyze data, giving participants a foundation to start running their own analyses. The tutorial will close with a practical demonstration of a generative AI-based analysis in the Weave multi-omics platform.

Abstracts

Oral Session

Microscopy-Enhanced MSI

Chairs

Kyle Duncan, Ph.D.
Assistant Professor at Vancouver Island University

Timothy J. Trinklein, Ph.D.
Postdoctoral Researcher at University of Illinois Urbana-Champaign
The Lab of Jonathan V. Sweedler

Understanding Neuronal Excitability using Mass Spectrometry Imaging of SCN2A-deficient Organoids

Alyssa Moore
Department of Chemistry, College of Science, Purdue University, West Lafayette, IN

Introduction: Human induced pluripotent stem cell (hiPSC)-derived organoids are powerful models for studying disease states without the use of animal models and can be grown to mimic different biological systems. In this study, we use nano-DESI MSI to understand alterations in molecular signaling in SCN2A-deficient iPSC-derived organoids developed as models of neuronal excitability in neurodevelopmental disorders. We used nano-DESI MSI to examine molecular differences between wild type (WT) and SCN2A+\/- (heterozygous knockout; HET) iPSC-derived cortical organoids.

Methods: We examined both fresh frozen (FF) and formalin fixed paraffin embedded (FFPE) cortical organoids. Imaging experiments were performed on a Q-Exactive HF-X Orbitrap mass spectrometer in both positive and negative ionization modes. Lipid alterations were determined through statistical analysis. For FFPE organoid and tissue sections, we developed a sample preparation protocol to remove paraffin while preserving lipid signals on tissue.

Results: Through comparison between WT and HET cortical organoids we identified multiple lipids that were altered in the SCN2A-deficient systems. In the HET organoids, multiple polyunsaturated phosphatidylserine (PS) and phosphatidylethanolamine (PE) lipids were observed to be upregulated when compared to WT organoids. These trends mirror prior observations in mouse brain models and are consistent with LPCAT3-associated remodeling. We also tested methods for analyzing FF organoid sections, which enhanced the sensitivity of nano-DESI MSI experiments by generating higher lipid signals than FFPE sections. In the fresh frozen HET cortical organoids multiple PS lipids and gangliosides were downregulated when compared to WT organoids. These trends may be due to hindered neuronal development in the HET organoids caused by SCN2A deficiency. This will be confirmed with immunofluorescence imaging of the different neuronal layers. Distinct spatial localizations of lipids were also observed within the organoids which indicate location-dependent cell differentiation. Our results highlight the capabilities of nano-DESI for imaging iPSC-derived organoids of different disease states, providing insights into cell differentiation within organoids.

Significance: First quantitative nano-DESI MSI study revealing lipid alterations associated with neuronal excitability in SCN2A-deficient human iPSC-derived organoids.

Additional Authors: Xiaoling Chen, Yang Yang, Julia Laskin

Additional Author Departments: Department of Medicinal Chemistry and Molecular Pharmacology, College of Science, Purdue University, West Lafayette, IN
Department of Medicinal Chemistry and Molecular Pharmacology, College of Science, Purdue University, West Lafayette, IN, Department of Chemistry, College of Science, Purdue University, West Lafayette, IN

A novel MALDI-MSI workflow for imaging polar metabolites and its application to organophosphate neurotoxicity studies

Siva Swapna Kasarla
Johns Hopkins Medicine Baltimore, MD United States

Introduction: Organophosphate nerve agents (OPNA) cause irreversible inhibition of acetylcholinesterase (AChE) which surplus severe cholinergic toxicity, disruption of neuronal energy homeostasis and long-term neurological dysfunction. Although atropine and pralidoxime (2-PAM) therapy improved survival against OPNA exposure, the associated long-term metabolic consequences remain poorly studied. Spatial metabolomic characterization of these alterations using MALDI-MSI remains challenging due to their poor ionization, low abundance and ion suppression. We developed a MALDI-MSI workflow by combining tissue washing, novel dual-matrix system, and subsequent MALDI-2 post-ionization to enhance spatial metabolomic analysis which is further employed to characterize persistent metabolic alterations upon OPNA exposure in KIKO mouse model.

Methods: The male and female KIKO mice were exposed to sarin (OPNA) along with atropine and 2-PAM therapy to enable survivable intoxication and long-term evaluation. Brain tissues were then collected at 30 min, 24 hours, 6 and 12 weeks (n=5) to capture acute, early, and chronic metabolic responses associated with organophosphate neurotoxicity. The tissues were thaw mounted at 10 µm thickness on indium tin oxide (ITO) glass slides. Prior to MALDI-MSI analysis, the tissue slides underwent basic hexane wash followed by the deposition of the novel dual-matrix system using HTX M5 sprayer. The MSI measurement was performed using timsTOF fleX MALDI2 (Bruker Daltonics) in the mass range of 60–650 m/z. Further data analysis and visualization was performed using SCiLS Lab (Bruker Daltonics). The statistical analysis was performed using Metaboanalyst (v6.0).

Results: Improving sensitivity for small polar metabolites was initially tested on control mouse kidney, brain and heart tissues. In brief, tissue sections underwent a basic hexane wash prior to analysis and were then coated using a novel matrix mixture consisting of N-DAN. Preliminary findings demonstrate the combination of matrices has shown improved intensity fold change (> 2) for most metabolites from glycolysis, TCA cycle and amino acid metabolic pathways compared to traditional single matrix application methodology among all the studied tissues. Further, MALDI-2 post-ionization parameters were fine tuned for small molecules and were incorporated to enhance ionization and improve metabolite coverage. Our findings demonstrated that MALDI-2 showed significant improvement in signal intensity and coverage with basic hexane washed tissues compared to unwashed tissues, which is

likely due to reduced endogenous lipid background and improved detection of low-abundant small molecules.

Later, the optimized protocol was subsequently applied to characterize metabolic changes in the mouse brain tissues upon OPNA exposure along with atropine and 2-PAM therapy. Distinct temporal metabolic signatures were observed across acute and chronic time points, suggesting metabolic remodeling on sarin exposure. Preliminary analyses additionally revealed the region-specific differential metabolite patterns in brain reflecting disruption of central carbon metabolism and mitochondrial function.

Overall, this work presents a refined analytical workflow for spatial metabolomic interrogation of challenging polar metabolites from various metabolic pathways. Also, provided new insights into the persistent metabolic consequences following sarin exposure along with standard antidotal intervention which is critical for identifying biomarkers of neurotoxicity and therapeutic intervention strategies.

Significance: We introduce the use of a dual polarity matrix for broad coverage of metabolites and apply this methodology to a novel mouse model of sarin exposure.

Additional Authors: Fiorella Yantani Coto, Conor Convery, Benjamin Wadsworth, C. Linn Cadiuex, Caitlin M. Tressler

Additional Author Departments: Johns Hopkins Medicine Baltimore MD United States, Johns Hopkins Medicine Baltimore MD United States, United States Army Medical Research Institute of Chemical Defense MD USA, United States Army Medical Research Institute of Chemical Defense MD USA, Johns Hopkins Medicine Baltimore MD United States

Enhancing on-tissue intact proteoform analysis using multi-segmented capillary electrophoresis

Tommy Zhang
Purdue University, West Lafayette, IN

Introduction: Spatial mapping of individual proteoforms using nanospray desorption electrospray ionization (nano-DESI) provides insights into the functional states of individual cells within heterogeneous tissues. Expanding the depth of coverage in proteoform imaging is critical to capturing the full diversity of protein isoforms and posttranslational modifications. Here, we integrate high-throughput capillary electrophoretic separation of proteoforms with direct sampling from tissues using nano-DESI. We employ a novel spray-capillary, sheathless capillary electrophoresis (CE) ionization interface to achieve high-throughput separation using multi-segmented sample injections. The multi-segmented CE is performed by landing the nano-DESI probe onto a tissue section followed by injection of the spacer solution without interrupting sampling. This hybrid approach enhances signal-to-noise and protein coverage, enabling spatial profiling of proteins that were previously undetectable in tissue sections using conventional mass spectrometry imaging platforms.

Methods: Proteins were extracted from mouse brain tissue sections using nano-DESI coupled to a spray capillary, separated by capillary electrophoresis, and analyzed using a ThermoFisher Orbitrap Exploris 480. Solvent containing background electrolyte is delivered through the nano-DESI primary capillary, which extracts analytes from the tissue section. The analyte is aspirated into the spray-capillary at an ultra-low flow rate, driven by ionization and electroosmostic flow. The electrophoretic separation voltage is applied to the syringe end of the nano-DESI capillary, and the electrospray voltage is applied to sheathless interface of the spray-capillary. Multi-segmented sample injection into the spray-capillary is performed by landing the nano-DESI probe onto tissue for 10 seconds followed by periodic blank injections for 60 seconds to space out different segments. Proteoform identification was performed by utilizing UniDec and mouse brain proteoform atlas.

Results: We have developed a hybrid platform for spatial profiling and identification of individual proteoforms in biological tissues by integrating nano-DESI sampling and high-throughput CE separation. Conventional nano-DESI detects high-abundance proteins directly from tissue sections, but lower-abundance proteins can be more challenging to detect and identify due to spectral complexity. By integrating a separation step prior to mass analysis, we enhance the depth of proteoform coverage and the accuracy of proteoform identification. Integration of separation approaches with mass spectrometry imaging modalities is challenging due to the low

throughput and low sensitivity of separation as well as difficulties of extracting and injecting samples into the separation stage. Unlike typical chromatographic separations, CE is more readily compatible with nano-DESI sampling from tissues. When implemented in multi-segmented injection mode, the time required for CE separation is dramatically reduced, making this hybrid approach practically useful for spatial profiling of proteoforms.

The newly-developed proteoform imaging platform integrates automated surface sampling using a nano-DESI probe that delivers an extraction solvent serving also as the supporting electrolyte, multi-segmented injection, and continuous data acquisition to enable high-throughput analysis directly from tissue sections. The platform was tested by profiling proteoforms in mouse brain tissue sections and the results were compared with proteoform maps obtained using conventional nano-DESI imaging. Both methods detect high-abundance proteoforms, including polyubiquitin-B (P0CG490), myelin basic protein (P04370-8), hemoglobin subunits alpha and beta (P01942\/ P02088), and cytochrome c oxidase (COX) subunit 7c mitochondrial (P17665). CE separation enables the detection of less abundant species including COX 6A1 mitochondrial (P43024), 6C (Q9CPQ1), and FA4 (Q62425). This novel platform enables discrete, multi-segmented surface sampling for spatial profiling with improved throughput, establishing a practical route for integrating CE separation into mass spectrometry imaging workflows.

Significance: Spray-capillary-based nano-DESI enables electrophoretic separation of proteins, enhancing depth of coverage of proteoforms directly sampled from tissue sections.

Additional Authors: Zhitao Zhao, Si Wu, Julia Laskin

Additional Author Departments: University of Alabama, University of Alabama, Purdue University

OPNA Poisoning and treatment to explore the changes in Metabolism and Lipid Regulation in a Humanized Mouse Model

Fiorella Yantani
Johns Hopkins University, Baltimore, MD United States

Introduction: Organophosphate nerve agent (OPNA) poisoning could lead to seizures, cholinergic crisis, and death, and in the long run, OPNA exposure is associated with neurodegenerative diseases and brain cancers. This is due to the inhibition of acetylcholinesterase (AchE), causing a buildup of acetylcholine. AchE is primarily found in the brain, however, the heart also contains the second most abundant population of AchE. We are studying the impacts of OPNA poisoning on the heart to determine potential short- and long-term side effects of OPNA poisoning.

Methods: Each animal received a first injection consisting of a median lethal dose of OPNA. The second and third injections consisted of 2-PAM and Atropine. The blood, brain, heart, and spinal cord tissues were immediately collected and cryopreserved. Hearts were cryosectioned on to indium tin oxide slides at 10 micron thickness. CHCA, THAP, or 1,5-DAN matrix was applied using an HTX M5 sprayer. MALDI imaging was performed on a Bruker timsToF Flex MALDI-2 or a Bruker RapifleX ToF\/ToF at 50 micron spatial resolution. Data was imported into SCiLS lab for analysis.

Results: In order to evaluate potential lipid deregulation associated with OPNA exposure, we imaged the hearts in three different matrices. CHCA and THAP were imaged in positive ion mode at 50-micron spatial resolution. 1,5-DAN was imaged in negative ion mode at 50-micron spatial resolution. An n of five males and five females was used for each group. Receiver operating characteristic curve analysis was performed on all three matrix conditions to determine peaks which were significantly different between unexposed and exposed groups. THAP matrix showed a striking difference in both males and females, especially at 24 hours. However, differences were observed out to 12 weeks. These analytes were observed over the entire m\/z range including small molecule metabolites and lipids. These data, taken together, indicate substantial alterations to both metabolomic and lipidomic profiles in the heart. The sections are also being hematoxylin and eosin stained for coregistration to MALDI imaging data sets.

Significance: This is the first MALDI imaging study to examine the metabolism and lipidome of OPNA exposed hearts in a novel mouse model.

Additional Authors: Conor Convery, Ian Spears, Siva Swapna Karsala, Benjamin Wadsworth, C. Linn Cadieux, and Caitlin M. Tressler

Additional Author Departments: Johns Hopkins University School of Medicine Department of Radiology and US Army Medical Research Institute for Chemical Defense

Exploring the lipid droplet-associated lipid landscape in mouse hippocampus in neurodegenerative disease

Matthew Bonett
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Introduction: Lipid droplets (LDs) are implicated in Alzheimer's Disease (AD) and other age-related neurodegenerative disorders, yet they remain understudied. The most common proteins that form lipid droplets are the perilipin family (PLIN1-5), with PLIN2 and PLIN3 being widely reported in the brain. The relationship between these LD brain locations and compositions remains poorly understood, especially relating to non-canonical PLIN coated LDs. Most LD isolation techniques are tailored for liver extraction, leaving a gap in knowledge around isolated LD populations from other tissues. Our goal is to establish the lipid profile of LDs in the hippocampus under normal aging and neurodegenerative conditions.

Methods: Brain tissue was collected from several AD mouse models. Brain tissue was sectioned, coated with matrix, and the lipids mapped by MALDI-MSI at both 5 and 50µm resolution using a Bruker timsTOF Flex. Sections were analyzed by immunohistochemistry with markers for PLINs, neurons (NeuN), microglia (Iba1), astrocytes (GFAP), and amyloid plaques (Methoxy X04). The resulting subregion lipid profiles were then compared. Liver, brain, and adipose tissue were collected from C57BL⁄6 wildtype (WT), 5xFAD early onset AD (EOAD), and E4FAD mice. Lipid droplets were isolated from the hippocampus, liver, and adipose tissue. Protein from the lipid droplets was then separated by PAGE, transferred, and then probed for PLINs to evaluate LD populations.

Results: Differential lipid signatures were detected in the PLIN positive lipid droplets in tissue depending on PLIN type. This suggests a region-specific lipid fingerprint corresponds with different PLINs during aging and development of neurodegenerative diseases. A differential distribution of LDs in the hippocampus in 5xFAD mice compared to WT mice was detected. To further characterize the LD populations, we isolated lipid droplets from brain, liver, and adipose tissue. We observed different migration patterns on a sucrose gradient between brain and liver lipid droplets, suggesting different compositions. We detected PLINs in the murine brain, liver, and adipose, but protein size discrepancies suggest specific proteoform expression was observed in each tissue. This preliminary work suggests that there are tissue-dependent modifications that drive specific LD types and states.

Significance: We identified a relationship between LD types and the regional lipid fingerprint in the surrounding brain tissue.

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Oral Session

Advances in Multimodal Imaging Workflows

Chairs

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Mapping Double-Bond Positional Isomers of Phosphatidylcholines in Zebrafish Using OzMALDI Mass Spectrometry Imaging

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Introduction: Lipids are essential biomolecules with various structures. Precise structural characterization, especially of their double-bond positions, is critical for understanding their metabolic functions. We have recently developed a novel technique for matrix-assisted laser desorption\/ionization (MALDI) mass spectrometry imaging (MSI) to determine and visualize double-bond positions of unsaturated lipids by inducing ozonation inside the MALDI source, dubbed OzMALDI. In this study, OzMALDI-MSI is combined with MALDI-MS\/MS of lithium adducts to observe organ-specific localizations of double-bond positions in zebrafish phosphatidylcholine (PC) lipid isomers. This study demonstrates its potential for application to disease models to discover alterations in lipid synthesis, revealing novel biological information.

Methods: Adult zebrafish were selected and euthanized by immersion in an ice-water bath, followed by flash-freezing in liquid nitrogen. The zebrafish were transected from the anal fin to the dorsal fin and immediately stored at -80°C. Samples were equilibrated at -20 °C, cryosectioned to 25 µm, and thaw-mounted onto platinum sputtered slides. They were vacuum-dried for 75 min and washed three times with a chilled (4 °C) 150 mM ammonium acetate solution. 2,5-Dihydroxyacetophenone (DHAP) was applied as a matrix using a TM Sprayer. The data were acquired on a Q-Exactive HF Orbitrap MS (Thermo) with a MALDI source (Spectroglyph). For MALDI-MS\/MS of lithium adducts, lithium acetate was also sprayed onto the samples. Ozone gas was introduced directly into the MALDI source, enabling an in-source ozonolysis reaction that simultaneously targets all unsaturated lipids. The formed ozonides are then fragmented in the HCD cell to determine the double-bond positions.

Results: MALDI-MSI was used to identify and spatially visualize PCs in adult zebrafish tissues, providing an overview of their relative abundance and anatomical distributions. Following MALDI-MSI data acquisition, the same tissue section was stained with H&E to facilitate annotation of major organs. The dominant lipid species detected were monounsaturated and polyunsaturated PCs, represented by PC 34:1 and PC 38:6, respectively. In addition to unsaturated PCs, saturated PCs, such as PC 32:0 and PC 34:0, were also detected. PCs were broadly distributed across the liver, pancreas, ovary, heart, and dorsal musculature, largely independent of fatty acyl composition. For example, OzMALDI-MSI of the PC 34:1 ozonide showed losses of C7H14O2 (FA 7:0) and C9H18O2 (FA 9:0), indicating two isomers with n-7 and n-9 double-bond positions. Combining with MALDI-MS\/MS of the Li adduct data, this

suggests two PC 34:1 isomers are present in zebrafish, PC 16:0_18:1 (n-7) and PC 16:0_18:1 (n-9).OzMALDI-MSI of the PC 38:6 showed the losses of FA 3:0, FA 6:1, FA 9:2, FA 12:3, FA 15:4, and FA 18:5, indicating double-bond positions of n-3, 6, 9, 12, 15, and 18, respectively, in FA 22:6. Overall, we characterized 27 double-bond positional isomers across 15 PC species and mapped their spatial distributions within tissues. Monounsaturated PCs consistently exhibited two distinct db-positional isomers, n-7 and n-9, which showed markedly different spatial localizations across zebrafish organs. In contrast, di- and polyunsaturated PC isomers did not exhibit pronounced differences in spatial distribution, except for PC 18:0_18:3(n-6,9,12), which showed specific enrichment in the intestine.

Significance: A novel OzMALDI-MSI technique is applied to study lipid metabolism in zebrafish and its implications in its disease models.

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iGAMSI: An immunohistochemistry-compatible framework for single-cell spatial lipidomics in intact neural tissues

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Introduction: The recently developed Gel-Assisted Mass Spectrometry Imaging (GAMSI) method overcomes the inherent spatial resolution constraints of conventional mass spectrometers by physically magnifying the biological specimen, thereby achieving sub-micrometer spatial lipidomics across intact cells and tissues. GAMSI-processed specimens, however, remain incompatible with commonly used cell markers as antigenic epitopes and cellular ultrastructure are often lost during the GAMSI procedure. To overcome this limitation, we developed immunohistochemistry-compatible GAMSI (iGAMSI) to enable cell-type-specific spatial lipidomics at the single-cell level. Using iGAMSI, we demonstrate concurrent mapping of proteomic and lipidomic profiles of individual cells in intact brain slices by correlating MALDI-TOF mass spectrometry (MS) and fluorescence microscopy images.

Methods: Mice were anesthetized and transcardially perfused with 4% paraformaldehyde (PFA) in 1xPBS. Following dissection, the brains were post-fixed and sectioned to ~25 μm. The brain slices were immunostained with fluorescently labeled antibodies and pre-expansion fluorescence images were acquired. The tissue slices were subject to a typical expansion microscopy workflow; chemically anchored and polymerized to form a superabsorbent sample-hydrogel composite, homogenized and subsequently expanded. Afterwards, the sample-hydrogel composite was immobilized onto a glass slide and analyzed via matrix assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI). Following MALDI-MSI analysis, the sample was fluorescently imaged post-MSI. A custom registration pipeline was developed utilizing the immunofluorescence (IF) and mass spectrometry (MS) images to extract single-cell mass spectra with a precision of ~3.75 μm.

Results: To demonstrate the capacity for cell-type-specific lipidomic mapping, we applied the iGAMSI pipeline to extract and compare the lipidomic profiles of cerebellar Purkinje cells (PCs) in wild-type mice and those in a model of Niemann-Pick disease, Type C1 (NPC1), a fatal neurodegenerative disorder characterized by lysosomal accumulation of lipids. As a result, we observed significant elevation of a number of key glycosphingolipids [GM3 (d36:1), GM2 (d36:1), and GM1(d36:1)] in the Purkinje cells (PCs) of the mutant animals within the flocculonodular lobule X of the cerebellum. To assess the spatial lipidomic variances across the cerebellar lobules, we mapped lipidomic profiles of cells from both anterior (e.g., IV⁄V) and flocculonodular (e.g., X) lobules. Notably, we found that a sphingolipid subclass of sulfatides, SHexCer, showed significant variations across the lobules in the NPC1 mouse cerebella. These findings

underscore the potential of the iGAMSI pipeline to perform mass-spectrometry imaging (MSI)-based single-cell spatial lipidomics studies with both cell-type and functional specificity.

Significance: iGAMSI circumvents current technical barriers to provide an integrated modality for cell-type-specific and functionally annotated single-cell spatial lipidomics.

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FluoMALDI Microscopy: A Multimodal Strategy for Spatial Molecular Discovery Integrating Fluorescence Labeling Strategies with MALDI Imaging

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Introduction: FluoMALDI microscopy inverts the conventional workflow of combining fluorescence microscopy with MALDI imaging on the same sample by depositing MALDI matrix before fluorescence acquisition. This approach revealed that aromatic MALDI matrices form co-crystals with fluorophores through J-type π–π stacking, substantially enhancing fluorescence brightness rather than diminishing it. Because both modalities interrogate the identical cells and tissue sections, FluoMALDI eliminates post-hoc registration of serial sections. We further developed this approach across three labeling paradigms including organic dyes, immunofluorescence, and genetically encoded HaloTag, enabling fluorescence-guided segmentation with multiplexed metabolite and lipid detection in the same tissue section.

Methods: FluoMALDI microscopy involves fluorescence labeling, MALDI matrix deposition, slide-scanning fluorescence microscopy (SSFM), and subsequent MALDI imaging. Immuno-FluoMALDI employed indirect CD31 immunofluorescence staining of blood vessels in human hippocampal brain sections with Alexa-647 and nuclear staining, followed by α-cyano-4-hydroxycinnamic acid (CHCA) or norharmane (nH) matrix spraying. HaloTag-FluoMALDI utilized genetic labeling of neural crest cells with tetramethylrhodamine (TMR) ligands bound to the HaloTag protein in chick embryos, followed by CHCA sublimation. For dye-FluoMALDI, human induced pluripotent stem cell (hiPSC)-derived astrocytes were labeled with actin, Golgi and nuclear fluorescent dyes, followed by CHCA or nH matrix sublimation. We carried out matrix depositions on HTX M3+ Sprayer or SubliMATE, SSFM on Olympus SlideView VS200, and MALDI imaging on Bruker timsTOF fleX. Data were co-registered and analyzed in ImageJ and SCiLS Lab.

Results: Depositing MALDI matrix amplified the fluorescence signal across all labeling strategies. Single-crystal X-ray diffraction revealed the structural basis. CHCA–rhodamine B co-crystals showed J-type staggered π–π stacking at 3.79Å interplanar separation, and nH–Alexa Fluor 488 co-crystals showed stacking below 3.49Å, both satisfying Kasha excitonic coupling criteria that preserve radiative transitions. Single-component matrix crystals lacked these aromatic contacts, confirming that enhancement arises specifically from co-crystallization.

In human hippocampal vasculature, immuno-FluoMALDI enhanced CD31 immunofluorescence 1.7-fold with nH and 18-fold with CHCA (p; 0.0001), and was capable of resolving lipid compartmentalization at single-vessel scale: PA(18:1/18:0) was enriched in arterial walls, PI(16:0/22:4) colocalized with CD31-positive endothelium, and SHexCer(18:1;O2/24:1) localized exclusively to perivascular regions, all confirmed by iprm-PASEF .

In the neural crest of chick embryos, HaloTag-FluoMALDI enhanced TMR-HaloTag fluorescence 2.3-fold following CHCA sublimation, and enabled us to show that overexpression of the sphingomyelinase 2 (nSMase2) SMPD3 altered 257 lipid features (FDR < 0.05), with ceramide species comprising approximately 55% of enriched lipids and C24:1 ceramide elevated alongside reciprocal sphingomyelin depletion, consistent with nSMase2 enzymatic activity.

In human iPSC-derived astrocytes, dye-FluoMALDI amplified SYTOX Green nuclear fluorescence 5.7-fold, Golgi (Alexa–568) fluorescence 1.7-fold, and actin (phalloidin-Alexa–647) fluorescence 1.8-fold with CHCA, allowing us to resolve subcellular sulfatide ST(18:3;O4;S) enrichments in actin-delineated membrane domains over Golgi compartments (p; 0.05). SYTOX Green was also detected as $[M+K]^+$ at m/z 318.1, creating a built-in cross-modal registration landmark.

In conclusion, exogenous fluorescence labeling strategies with dyes, immunofluorescence, and genetically encoded HaloTags combine fluorescence-guided segmentation with MALDI imaging.

Significance: FluoMALDI leverages co-crystallization of MALDI matrix with fluorophores as signal-amplifying strategy, enabling fluorescence-guided spatial omics within a single specimen across a range of biological systems.

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Multi-Omic MALDI MSI to Evaluate Drug-Induced Metabolic Reprogramming of Peripheral Blood Mononuclear Cells at Single Cell Resolution

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Introduction: Pharmacological agents frequently reprogram cellular metabolism, biosynthetic pathways, and signaling networks, directly influencing cellular fitness, lineage commitment, and drug resistance. Defining how therapeutics reshape metabolic states in immune cell populations is therefore essential when evaluating efficacy, identifying metabolic biomarkers, and optimizing treatment strategies. Here, we focus on a panel of therapeutic agents known to disrupt nucleotide biosynthesis and different glycan processing pathways.. We applied our new multi-omic, label-free matrix-assisted laser desorption\/ionization mass spectrometry imaging (MALDI MSI) single cell analysis workflow to evaluate drug uptake and downstream changes in metabolites, lipids, and N-glycans in heterogenous lymphoid and myeloid cell populations.

Methods: Peripheral blood mononuclear cells (PBMCs) were thawed and rested overnight. Cells were plated at 100,000 cells per ml and treated with different concentrations of Brequinar, NGI-1, and Eligustat for 24 to 48 hours. Following treatments, cells were washed and seeded at 20,000-30,000 cells in 1 ul spots on amine-reactive slides for bulk cell analysis. For single cell capture, patterned grids using antibodies to immune cell sub-types were created using PDMS stamps at 60um and 100um spacing for approximately 15,000 single cells acquired per slide. SoloCell software was used to identify and localize single cells on the slide. MALDI-MSI workflows were performed utilizing CHCA matrix in positive ion mode for lipid and N-glycan detection and NEDC matrix in negative ion mode for metabolites. Data was acquired using a Bruker timsTOF fleX and analyzed in SCILS software.

Results: Our established single-cell MALDI-MSI platform was used to profile drug uptake and metabolic remodeling across PBMC populations. Single-cell capture was achieved using PDMS stamps coated with antibodies to immune cell marker proteins (e.g., CD4), enabling unbiased selection while preserving intrinsic cellular heterogeneity. Therapeutic compounds were selected for their conjugated structural ring systems and membrane-localizing properties. Brequinar inhibits de novo pyrimidine synthesis and indirectly suppresses glycolysis through depletion of nucleotide-sugar intermediates such as UDP-glucose. NGI-1 is an inhibitor of N-glycosylation, and Eliglustat is a glucosylceramide synthase inhibitor of glycosphingolipid biosynthesis. These drugs were readily ionizable by MALDI, with their specific m/z values detected. In untreated cell populations, over 30 metabolites,

200 lipid species and 40 N-glycans are detected. Results reveal coordinated metabolic remodeling following treatment, with each drug eliciting distinct drug-induced changes in glycans, lipids, and metabolites such as ATP. Notably, there were distinct metabolic alterations that were detected within each immune cell class (CD4, CD8, CD19, CD14, and CD16) following treatments. Within each single cell population for each immune cell class, drug uptake was also highly variable, which could be assessed across 1,000 to 2,000 single cells. Ongoing efforts aim to delineate how these specific alterations reflect cellular viability and function, as well as link the analytes that are changing to quantitative mass spectrometry determinations. Overall, our single-cell MALDI-MSI workflow is a robust platform for interrogating drug uptake and metabolic response in immune cell populations, that we expect can be applied to many ongoing cellular and immunotherapy clinical trials.

Significance: This workflow can identify drug induced molecular changes to immune cells for eventual translational application to cellular immunotherapies.

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Extracellular Matrix, N-Glycan and Immune Cell Remodeling Define Dysplastic Microenvironments in Early-Onset Colorectal Precancer

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Introduction: The colorectal precancerous microenvironment is complex, comprising dysplastic epithelial cells, collagen-rich stroma, glycoproteins and diverse immune cell populations. Microenvironmental remodeling facilitates cancer initiation and shapes downstream tumor architecture and immune infiltration. However, studies integrating matrix-assisted laser\/desorption ionization (MALDI) imaging of N-glycans and ECM peptides with orthogonal microscopy modalities to provide complementary cell phenotyping, cellular neighborhoods, and ECM structure are limited. Here, we present a same-tissue multimodal spatial imaging strategy that combines second harmonic generation imaging, multiplexed immunofluorescence, histological staining, and MALDI imaging platforms to spatially resolve crosstalk between the ECM, N-glycans and immune cells in early-onset colorectal tissues.

Methods: This study sequentially integrates: 1) Second harmonic generation (SHG, 840nM excitation wavelength, AX\/AXR Multiphoton Confocal Microscope); 2) Multiplexed immunofluorescence (CODEX) with a 33-marker antibody panel focused on extracellular matrix proteins and immune cells (Akoya Biosciences); 3) Peptide N-Glycosidase F-directed N-glycan MALDI imaging mass spectrometry (IMS) (timsTOF Flex, Bruker); 4) hematoxylin and eosin (H&E) stain; 5) Collagenase type 3-directed ECM peptide MALDI IMS (timsTOF Flex and MALDI FTICR, Bruker); and 6) Targeted ECM LC-MS\/MS-based proteomics for peptide library generation (timsTOF Pro, Bruker). All modalities were sequentially performed on the same formalin-fixed paraffin-embedded (FFPE) tissue. Data were co-registered using sequential autofluorescence images with image2image. Neighborhood analyses were performed in Python and QuPath. IMS preprocessing and analysis was performed using in-house developed AutoIMS.

Results: Using an FFPE colorectal cancer tissue, we optimized a multimodal spatial workflow integrating SHG multiphoton imaging, CODEX, N-glycan and ECM peptide MALDI IMS, picrosirius red stain microscopy and H&E stain microscopy on the same tissue. MALDI imaging spatially profiled 239 N-linked glycans and 288 putative ECM peptides. Performing CODEX prior to MALDI IMS resulted in significantly increased signal intensity for both N-glycan (intensity = 435±50; p = 3.2*10-7) and ECM peptide imaging (722±83; p = 3.8*10-38) compared to IMS-only controls (295±35 and 278±32, respectively). Biantennary (m/z = 1501.529, 1663.581), fucosylated (m/z = 1757.585, 2122.717), and sialylated (m/z = 1976.659, 2100.735) N-linked glycans localized to regions of ECM-remodeling (TGFBI+, Fibronectin+), CD16+ natural killer cells, and

hypoxia (HIF1+). Putative ECM peptides from IMS experiments co-localized with stromal regions (H&E), markers of collagen type I and fibroblast activating protein (CODEX) as well as collagen fibers (SHG), demonstrating compatibility of high-resolution imaging of collagen fibers and immunofluorescence with downstream IMS analyses. These results establish a robust foundation for integrating immune phenotyping with ECM-targeted spatial mass spectrometry.

Ongoing studies apply this workflow to a cohort of precancerous colorectal adenomas with early (n = 39, age 59) disease to evaluate N-glycan, ECM, and immune cell spatial interactions associated with disease onset. Future work integrates G4X spatial transcriptomics and picrosirius red staining. Together, this approach is expected to profile spatial crosstalk within the precancerous colorectal microenvironment, providing insights into mechanisms of early-onset disease initiation and identifying potential therapeutic targets.

Significance: N-glycan and extracellular matrix imaging IMS is integrated with same-tissue multiplexed immune cell immunofluorescence and multiphoton imaging in colorectal adenomas.

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An Analysis of Standard Matrix Deposition Methodology for Three Negative Ion Mode Matrices

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Introduction: Matrix assisted laser desorption ionization (MALDI) imaging is an incredible tool capable of measuring large numbers of analytes label free which opens doors for incredibly insightful observations about cellular mechanics. The field however lacks standardized protocols when it comes to sample preparation. Matrix application is a critical step in this process which can have major repercussions for the final imaging experiment. The two most common methods of matrix application or spraying and sublimation. In this work, we explore the differences between these methods for negative ion mode matrices.

Methods: 1,5-Diaminonaphthalene(DAN), 9-aminoacridine(9AA) and norharman(nH) were used to analyze the matrix deposition methods. 10-micron thick sections of kidney, heart, and brain were taken from wild type C57Bl\/6 mice and placed on indium tin oxide coated slides. The slides are weighed before and after matrix deposition to determine the amount of matrix deposited on each slide. To spray the slides an HTX m5 sprayer was used and to sublimate the HTX SubliMATE was used. The slides were then imaged on a microscope scanner at 40X magnification with 95o polarized light and a 10micron zstack. The slides are then scanned in a RapifleX MALDI ToF/ToF instrument in negative ion mode with a 50-micron raster size. Then ROC analysis was done between matrix deposition methods and tissues to determine the statistical significance of notable peaks. MS-2 was conducted on notable peaks to confirm their authenticity of the peaks

Results: A two tailed paired t-test was performed on the matrix weight differences, and it found that the matrix weight differences were not significant enough to cause the differences in imaging data that was observed. The crystal sizes were found to be smaller in sublimated samples compared to sprayed samples for DAN and nH but for 9AA the sublimated samples had massive off tissue crystals around nucleation sites. On tissue areas of the 9AA sublimated slides contained smaller crystals then their sprayed counter parts much lie the other matrices. nH deposited slides had crystals that were to hard to definitively resolve on at 40x magnification but based on basic visual observation it was seen that the crystals were smaller in sublimate slides compared to the sprayed slides. All imaging and MS-2 were collected on a Bruker RapifleX ToF/ToF at 50 micron resolution. The imaging spectra that was collected showed matrix deposition-dependent peaks. In 9AA more peaks were observed in higher intensity in the 800-950 m/z range and the 150-200 m/z range. For DAN there was a more tissue specific response with the brain showing more

intensity with sprayed matrix while the heart had higher intensity spectra with the sublimated method. nH had consistent higher intensity with sublimation over spraying.

Significance: This work is the first example of directly comparable spray and sublimation methods for negative ion mode imaging.

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Learning When to Stop: Adaptive Temporal Acquisition for FT-ICR Mass Spectrometry Imaging

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Introduction: Fourier transform mass spectrometry imaging provides detailed spatial-chemical characterization of biological samples with exceptional mass resolution and rich chemical coverage. However, the long transient acquisition time limits effective throughput and wide adoption. Do we need to acquire transients with the same duration at different pixels across a heterogeneous tissue section? Some lipid-rich regions may require longer sampling to accurately resolve ions, while others may contain a limited useful signal. A fixed transient time for every pixel may be inefficient. We propose a deep learning-based adaptive acquisition framework that predicts when to stop transient acquisition at each pixel.

Methods: We observed that different pixels across a tissue section require different transient durations to achieve comparable reconstruction quality. We developed a deep learning-based adaptive acquisition framework to dynamically predict the transient duration needed at each pixel to preserve molecular fidelity while reducing unnecessary acquisition time. The core of our approach is a MAMBA-based model, which extrapolates partially acquired transient signals and estimates the expected marginal reduction in reconstruction error from acquiring the next transient segment as a function of acquisition length.

During inference, acquisition at each pixel is terminated once the predicted marginal reduction in reconstruction error falls below a user-specified tolerance, indicating that additional transient acquisition is unlikely to substantially improve the reconstructed spectrum. Conversely, pixels for which additional segments are predicted to provide meaningful error reduction are acquired for longer durations to preserve spectral and molecular fidelity.

Results: The proposed method was evaluated using data acquired on a Bruker 7T SolariX FT-ICR system. Fully sampled data were collected from sagittal rat brain sections (12 µm thickness) at 50 µm lateral resolution (negative ion mode), covering an m\/z range of 200–2400 with 1M timepoints/pixel (65035 transients in total). As a proof-of-concept, we focus on m/z 1100–2400, emphasizing molecular content primarily associated with gangliosides.

In a retrospectively simulated dynamic sampling, an initial segment of 64k-points was used to predict the expected marginal reduction in reconstruction error of all the subsequent 64k-point segments, and acquisition was terminated once the predicted reduction in error dropped below a threshold, chosen empirically from the training dataset. This adaptive sampling strategy achieved approximately 40% acceleration (mean stopping point: 9.24 ± 1.22 segments). The variable-length transients were then reconstructed using the MAMBA-based model. With standard Fourier reconstruction, molecular information was well preserved, achieving 76 ± 4% spectral similarity and 86 ± 5% peak correlation relative to the full-length transient. These results demonstrate the potential of the proposed method to substantially accelerate acquisition while preserving both spectral integrity and spatial information. The method can be optimized for either global spectral quality or a targeted m⁄z range.

Significance: Finally, we outline future work for task-specific adaptive sampling, integrating our method with dynamic spatial sampling and validations on diverse tissue types.
We introduce a novel computational framework for adaptive temporal acquisition and reconstruction of FTMS data for information-efficient, accelerated MSI.

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So Much Data, So Little Tissue: New Strategies for Multi-Omics Data Integration

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Introduction: Spatial-omics data rarely consists of just one dataset to be analyzed in isolation. Analysis of clinical samples often involves the collection of multiple spatial datasets to paint a more complete picture of the disease being studied. Data may be collected from the same tissue section (e.g. sequential MSI of different molecular classes) or from serial sections (e.g. spatial transcriptomics, immunofluorescence, histological staining). Regardless, the goal of these multi-omics studies is the integration of these datasets to enable a deeper understanding of molecular processes at play in disease progression. This presentation will explore new strategies for data integration across spatial modalities.

Methods: Sequential mass spectrometry imaging (metabolites, lipids, glycans, peptides) was acquired from a single tissue section. Serial sections were analyzed by spatial transcriptomics and\/or sequential immunofluorescence. Two different approaches were explored for data integration. MSI data were converted to OME.tiff files for registration to other spatial data in Visiopharm or segmentation data from Visiopharm in the form of multilayer data (MLD) files were converted to SCiLS region (SEF) files using custom tools. SEF regions were imported into SCiLS and ROIs generated for downstream analysis. The SCiLS Ion Image Mapper was used to co-register sequential MSI files to each other, allowing the imported annotations to be used in all MSI datasets.

Results: Traditionally, integration of multi-omics data has occurred via brute force. An annotated serial section was merged with an optical image of the section for MSI of a single analyte class and then ROIs were manually created by retracing the annotations. This process was quite tedious and error prone, especially when dealing with multiple serial sections for different analyte classes for MSI. More recently, the collection of multiple mass spectrometry images from the same tissue enabling more facile comparisons between analyte classes for the MSI data. However, these are still generally visualized one class at time in separate SCiLS files. The SCiLS Ion Image Mapper allows datasets collected from the same section to be mapped to each other using a common optical image so that the datasets may be integrated for visualization.

Visiopharm is a commonly used tool for the analysis and segmentation of spatial transcriptomics and immunofluorescence data. It is desirable to apply this segmentation data to MSI data to perform analyses on specific histological regions

or cell types. A custom tool has been developed, SCiLS MLD Converter, which converts MLD files from Visiopharm into SCiLS region files that can be imported into the dataset. The tool allows for disparate areas of the same histology to be combined into a single region per tissue in SCiLS allowing for statistical analysis of matched histological regions between samples. When combined with co-registered MSI files, the same annotations can be passed between all layers of MSI data, enabling analysis of combined molecular classes.

Significance: A new analysis strategy for the integration of spatial transcriptomics and immunofluorescence with MSI multi-omics data.

A whole-brain molecular atlas by integrating accelerated MALDI MSI and in vivo MRI

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Introduction: Mass spectrometry imaging is typically performed on 2D tissue sections, limiting its ability to capture disease pathology distributed across whole organs. Scaling measurements to 3D is challenging due to the extensive time needed for imaging many sections and the difficulty in placing imaged serial sections accurately within their native anatomy. To address these challenges, we present an approach for whole-brain 3D MSI leveraging in vivo MRI, sparse sampling, and a blockface registration "intermediate". We then apply our method to map metabolites and lipids across the entire brain in Alzheimer's disease (AD) model animals.

Methods: Each animal was first imaged in vivo by T2-weighted MRI to provide a subject-specific anatomical reference. Animals were then sacrificed and the brains were serially cryosectioned in the coronal plane, cutting at a section thickness of 16 μm. Every slice was retained, with sections alternately mounted on ITO-coated slides for MALDI or standard microscopy slide for fluorescence imaging. This yielded approximately 800 sections in total, with ~400 sections for MSI or microscopy, respectively. Throughout the sectioning process, an image of the exposed tissue block before cutting, i.e., the "blockface", was obtained with a digital camera. These images were coregistered together to generate a 3D volume to use as a registration intermediate. Sections were coated with the MALDI matrix NEDC using an HTX Sprayer (HTX Technologies). Sections were imaged on a MALDI timsTOF flex (Bruker) with a raster step of 30 μm in both X and Y. Given that alternate sections cut at 16 μm were retained, this yielded an isotropic (3D) resolution of approximately 30 μm. To facilitate imaging hundreds of sections per animal, we employed line-wise sparse sampling, randomly skipping 60% of lines in each acquisition. Every 10th section was fully sampled.

Results: We first reconstruct the MSI data using a self-supervised learning via sparse sampling, combined with deep unrolling, a physics-guided learning approach (Yaman et al., Magn. Reson. Med.. 2020). Next, we developed a pipeline to register each MSI slice within the MRI volume. Registering individual slices directly, one-by-one to MRI poses several challenges. First, the cutting angle rarely matches the MRI acquisition plane. Second, the brain deforms after removal from the skull and freezing breaking correspondence with the in vivo geometry. Third, each slice must be accurately placed within its correct z-position in the 3D volume. To address this, we developed a registration pipeline that uses the blockface as an intermediate for "registration transfer." (Sinha et al., Nat Methods, 2008). We first constructed a blockface volume

by serially registering each blockface image. This blockface volume was then registered to the MRI volume by affine registration initialized with manually selected landmarks, then refined with deformable B-spline registration. Because each MSI section is intrinsically paired with its blockface image, registering each MSI section to its corresponding blockface image is straightforward and accomplished by affine registration. Next, we applied the blockface-to-MRI transform to each MSI section to bring it into the MRI volume. We then extracted region-specific lipid and metabolite profiles by registering the Allen Reference Atlas Labels to the MSI volume. This pipeline enables lipid and metabolite distributions to be interpreted within their subject-specific, in vivo MRI anatomy.

Significance: This work reports a platform for anatomically-accurate, whole-brain 3D MSI, demonstrated with volumetric mapping of lipid and metabolite alterations in Alzheimer's disease.

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Oral Session

Multimodal Imaging of Complex Microenvironments

Chairs

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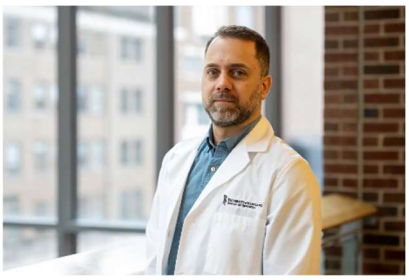

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A multimodal spatial atlas of lipid metabolism in the human intestine

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Introduction: The intestine is a highly heterogeneous organ that undergoes changes in cellular composition and metabolic activity along functionally distinct regions of the small and large intestine. The intestine is responsible for digestion and reuptake of lipids; however, how lipid metabolism varies across intestinal regions and individual cell types remains poorly understood. This gap results from a lack of integrative data linking metabolism across molecular, cellular, and tissue scales. To address this, we combined four complementary technologies—multiplexed spatial protein imaging, spatial lipidomics, bulk lipidomics, and single-cell RNA sequencing—to generate a comprehensive, spatially resolved metabolic atlas of the healthy human intestine, providing a molecular framework for understanding metabolic dysregulation across disease states.

Methods: We analyzed tissue samples from eight functionally distinct intestinal regions: the duodenum, proximal jejunum, mid-jejunum, and ileum in the small intestine, along with the ascending, transverse, descending, and sigmoid regions in the colon, from four human donors. Autofluorescence microscopy was used to establish tissue morphology and guide image co-registration. CODEX multiplexed immunofluorescence microscopy was performed using a 54-antibody panel targeting diverse epithelial, immune, and stromal cell types. Matrix-assisted laser desorption\/ionization imaging mass spectrometry (MALDI-IMS) lipidomic analysis was performed at 10 µm spatial resolution using a timsTOF fleX mass spectrometer. MALDI-IMS lipid annotations were validated by LC-MS\/MS analysis of serial tissue sections. Multimodal co-registration was achieved using Image2Image and custom algorithms, enabling cross-modality mining of cellular and lipidomic features.

Results: Here, we integrated multiple technologies to define the spatial and molecular heterogeneity of the human intestine. CODEX microscopy enabled us to capture the expression of cell-surface and nuclear markers at subcellular resolution, thereby facilitating cell type annotation. Clustering algorithms were then used to identify cellular neighborhoods, communities, and tissue units with distinct cellular organization profiles at different spatial scales. These multi-hierarchical cellular neighborhoods identified using CODEX microscopy were used to cross-mine spatial lipidomic profiles, linking lipid species to functionally distinct regions. We developed a shape-based alignment strategy to map CODEX-derived organizational units to MALDI-IMS data from serial sections, enabling correlation of lipid distributions with cell-type composition and neighborhood structure. We began by comparing tissue

regions broadly between the small intestine and colon. We identified sterols, such as ST 27:1;O;S, that are exclusive to the small intestinal mucosa. We then examined variation between the eight sampled intestinal regions. Interestingly, the transverse colon displayed a unique lipidomic profile enriched in specific sulfatide, phosphatidylcholine, and phosphatidylinositol lipids. We further compared lipidomic profiles of cellular neighborhoods, such as the transit-amplifying and secretory zones, which were enriched in phosphatidylinositol, phosphatidylethanolamine, and phosphatidylserine lipids. Analyses at the most granular level revealed spatial relationships, such as the exclusive presence of glycerophospholipid PMeOH 18:1_18:1 within the colonic muscularis mucosa. This lipid was negatively correlated with CD36+ endothelial cells, which play a role in fatty acid absorption. We are further expanding this atlas by identifying lipid pathways such as fatty acid metabolism that are consistently associated with functional spatial neighborhoods. In summary, the integration of these multimodal data links lipidomic profiles to specific multi-hierarchical cell neighborhoods at unprecedented spatial resolution and chemical specificity.

Significance: We integrated spatial proteomics, spatial and bulk lipidomics, and single-cell transcriptomics to generate a multiscale human intestinal metabolic atlas.

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Lung multiomics reveals dynamic molecular remodeling and immune responses in SARS-CoV-2 infected mouse lungs

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Introduction: The COVID-19 pandemic highlighted the need to understand spatial molecular responses driving lung injury and immune dysregulation. Severe infection with SARS-CoV-2 induces profound remodeling of pulmonary architecture, including inflammation, mucus accumulation, and epithelial damage. Lipid metabolism, particularly phospholipid homeostasis, is tightly linked to viral replication and host immune signaling. However, lipid alterations occur alongside broader metabolic and glycosylation changes that remain poorly characterized in situ. Mass spectrometry imaging (MSI) enables label-free spatial mapping of biomolecules within tissues. Here, we apply a multiomics MSI strategy to characterize phospholipids, cholesterol, metabolites, and N-glycans in infected mouse lungs.

Methods: All infection and analytical procedures were conducted under BSL3 conditions. Mice were intranasally inoculated with SARS-CoV-2, while control animals received PBS only. Lungs were collected at 2- and 4-days post-infection, inflated with gelatin, snap-frozen, and cryosectioned (10 µm). Serial sections were used for multimodal MSI and histological analyses. Phospholipids were detected using MALDI-MSI with norharmane matrix in both polarities. Metabolites were analyzed using an optimized 9-aminoacridine (9-AA) matrix. Cholesterol was detected using silver nitrate (AgNO3) LDI and N-glycans were released on-tissue via PNGase F digestion followed by CHCA matrix application and MSI acquisition. Data were acquired on a timsTOF Flex at 20 µm spatial resolution. Complementary immunofluorescence staining was performed to visualize immune cell infiltration and mucin accumulation. Co-registration of MSI and fluorescence images enabled spatial correlation between molecular alterations and cellular features.

Results: SARS-CoV-2 infection induced pronounced spatial remodeling across all molecular classes analyzed. Phospholipid distributions showed a marked shift from homogeneous patterns in control lungs to highly localized, high-intensity regions in infected tissues, consistent with focal inflammatory responses. Several phosphatidylinositols and phosphatidylglycerols displayed increased intensity in regions associated with immune infiltration, while other lipid species were depleted globally. Cholesterol exhibited regional accumulation, particularly in areas of tissue consolidation, suggesting membrane reorganization and viral replication niches. Metabolite mapping revealed distinct metabolic microenvironments, with altered distributions of energy-related and inflammation-associated metabolites co-

localizing with infected regions. These changes indicate metabolic rewiring linked to viral burden and immune activation. N-glycan imaging demonstrated significant alterations in glycosylation patterns, including increased abundance of complex glycans in inflamed regions, potentially reflecting host-pathogen interactions and immune modulation. Integration with immunofluorescence revealed strong spatial correlations between molecular alterations and biological features. Regions enriched in specific lipids and metabolites overlapped with dense immune cell infiltration, while areas with altered glycan profiles corresponded to epithelial disruption. Notably, mucin accumulation co-localized with distinct lipid and glycan signatures, suggesting coordinated regulation of barrier function and inflammatory responses. Temporal analysis indicated progressive molecular reorganization, with early infection characterized by localized changes in lipids and metabolites, followed by widespread remodeling and N-glycan alterations at later stages. These findings demonstrate that SARS-CoV-2 infection drives coordinated, spatially resolved multiomic changes in lung tissue.

Significance: This study reveals spatial multiomic signatures of infection, linking lipid, metabolic, and glycosylation remodeling to immune responses and lung pathology in a COVID-19 disease model.

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Decoding the Molecular Architecture of S. aureus Osteomyelitis via Multimodal Imaging Mass Spectrometry

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Introduction: Infiltration of Staphylococcus aureus into the bone is the most frequent cause of osteomyelitis, a debilitating disease characterized by inflammation of the bone and bone marrow. The resulting inflammatory response drives staphylococcal abscess formation in the bone microenvironment, reducing antibiotic penetration and compromising treatment efficacy. Utilizing a multimodal imaging approach integrating traditional microscopy, matrix-assisted laser desorption\/ionization (MALDI) imaging mass spectrometry (IMS), and spatial transcriptomics (ST) has revealed distinct spatiomolecular profiles across a broad range of molecular classes (e.g. lipids, metabolites, N-glycans, and RNA transcripts). Collectively, these data yield a more comprehensive understanding of osteomyelitis pathogenesis and establish a foundation for future therapeutic advances.

Methods: Intra-femoral injection of PBS (mock-infection) or fluorescent S. aureus was delivered to C57BL6\/J mice. At 14 days post-infection, femurs were harvested and either flash frozen in CMC\/gelatin for lipid and metabolite MALDI IMS workflows or formalin-fixed, EDTA-decalcified, and paraffin-embedded for N-glycan and spatial transcriptomic analyses. For MALDI IMS workflows, femurs were sectioned and mounted onto ITO slides followed by matrix application of DHA (lipids), NEDC (metabolites), or CHCA (N-glycans). IMS was performed using a Bruker timsTOF instrument with pixel sizes ranging from 10–20 µm, and acquisition parameters were optimized for each molecular class across an m/z range of 100–4000. 10X Visium HD was used to collect ST data to visualize RNA transcript localizations, and complementary fluorescence microscopy and histological stains aided in characterizing unique tissue regions. Data analysis was performed using in-house developed software, SCiLS (Bruker), and Space Ranger (10X Genomics).

Results: Previous studies utilizing fluorescence microscopy, histological staining, and MALDI IMS of lipids have uncovered unique molecular profiles at the host-pathogen interface. These data revealed the presence of macrophages with an altered lipid phenotype, as shown by the accumulation of triglycerides and cholesterol esters at the abscess border, prompting further investigation of the molecular profiles at this interface. Metabolite MALDI IMS workflows for fresh-frozen bone were developed, elucidating altered host and bacterial metabolic profiles surrounding and within the staphylococcal abscess. An increase in 1-glycerylphosphorylethanolamine (m/z 214.048) and citrate/isocitrate (m/z 191.019) surrounding the abscess border support

the presence of macrophages in this tissue region. Additionally, staphyloferrin A (m/z 479.118), a S. aureus-specific metabolite to scavenge iron from these host cells is seen within the abscess, further emphasizing the complex interactions between bacteria and host in the bone marrow microenvironment. This multimodal approach has been expanded to detect and visualize N-glycans, with preliminary N-glycan IMS experiments resulting in the tentative identification of numerous N-glycans with distinct spatial localizations, such as the central staphylococcal abscess community (m/z 1971.687 and m/z 2284.768), inflamed bone marrow (m/z 1905.634, [Hex9HexNAc2 + Na]), and cortical bone (m/z 2470.787, [Hex5dHex2HexNAc5NeuAc1 + 2Na]). Lastly, the 10X Visium HD ST workflow has been applied to mock- and S. aureus-infected femurs, uncovering the immune cell states and signaling pathways at play at the host-pathogen interface. Altogether, these data underscore the extensive molecular reprogramming within discrete cellular niches in and surrounding abscess lesions.

Significance: Multimodal molecular imaging reveals altered molecular landscapes resulting from S. aureus infiltration into the bone.

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Spatially resolved metabolomics of optimal cutting temperature compound (OCT)-embedded tumors

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Introduction: The majority of retrospectively-collected fresh-frozen tumor tissues are embedded in optimal cutting temperature compound (OCT) to preserve tissue integrity. However, mass spectrometry imaging (MSI) researchers have been resistant to analyzing OCT-embedded tissues due to abundant polymers (PVA, PEG) in OCT. Here, we demonstrate that spatial metabolomics data can be directly collected from OCT-embedded tissues using nanospray desorption electrospray ionization (nano-DESI). Proof-of-principle data was collected on contrived tissue homogenates and rat brain tissue sections with known histology. Following, our spatial metabolomics workflow was applied to image OCT-embedded murine tumors and biobanked human ovarian tumor tissue.

Methods: Nano-DESI was performed on an Orbitrap Exploris 120 using a custom interface and control software. Fused-silica capillaries (150 μm OD; 50 μm ID) were used to generate a liquid bridge (9:1 MeOH:H2O). Rat brain homogenates were embedded in OCT or the MSI compatible embedding material carboxymethylcellulose (CMC). Lymphomas were grown in mice fed a complete (control) or methionine-restricted (0.06%) diet. Upon collection, tumors were halved and embedded in OCT or CMC. MSI data was converted to imzML and processed using Cardinal, Python, and MetaboAnalyst. As a final proof-of-principle, our spatial metabolomics workflow was applied to a series of biobanked human ovarian tumors embedded in OCT. Biobanked samples were also anlazyed by MALDI using a Bruker timsTOF Flex2. HiPlEX on the same tissue section was carried out using commercial AmberGEN probes.

Results: Tissue homogenates embedded in OCT and CMC exhibited strikingly similar metabolite coverage, with negligible interfering OCT peaks below m/z 500. For lipids (>m/z 480), abundant OCT may drive reduced S/N and introduce isobaric interference. Tissue localization to expected anatomical features was preserved in OCT-embedded rat brains, however, minor metabolite diffusion into the embedding media was observed for both OCT- and CMC-embedded tumors (< 1mm). This systematic characterization of OCT- and CMC-embedding demonstrates spatial metabolomics is not compromised for OCT-embedded tissues.

To demonstrate MSI of OCT-embedded tumors, a non-targeted comparison of methionine-restricted vs. control lymphoma tumors was carried out. We show that

metabolic perturbances across CMC- and OCT-embedded tumors are conserved, including reduced methionine and increased S-adenosylmethionine with dietary methionine restriction. These data demonstrate that comparable spatial metabolomic profiles can be obtained from OCT- and CMC-embedded tumor tissues. We then applied our nano-DESI spatial metabolomics workflow to image biobanked human ovarian tumors. Pulsed polarity switching enabled high metabolomic coverage (> 300 structurally diverse metabolites in each polarity) and non-targeted investigation of tumor metabolism. MALDI-MSI of lipids was performed on a serial section for image segmentation, followed by MALDI-HiPLEX IHC for detection of targeted proteins. OPAL IHC on a serial section was completed for identification of targeted cell types. All three MSI datasets were co-registered with the OPAL IH data, which enabled regions of interest (ROI) analysis of metabolic microenvironments. Overall, these samples reveal unique metabolic fingerprints to specific cell types within these highly heterogeneous tumors.

Significance: We demonstrate OCT-embedded tissues are compatible with ambient MSI spatial metabolomics technologies, enabling integrated multi-omics characterization of metabolically heterogenous and cellular diverse biobanked ovarian tumors.

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Metabolome-Informed Proteome Imaging of Complex Heterogeneous Biological Systems

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Introduction: Metabolome-Informed Proteome Imaging (MIPI) is a spatial multi-omics approach that integrates high-resolution molecular imaging with ultrasensitive microscale proteomics to link metabolite distributions to their corresponding enzymes, enabling spatial reconstruction of biochemical pathways in biological systems. Its initial implementation combined metabolite imaging and proteomic characterization resolving lignin-degradation pathways in microbial consortium. An advanced MIPI workflow incorporated lipidomic imaging and expanded metabolomic coverage through on-tissue chemical derivatization for guiding proteomics analysis, enabling reconstruction of active pathways and highlighting functional differences among placental villous compartments. Building on these advances, the latest MIPI development enables integrated multi-omics profiling from a single poplar root section.

Methods: For traditional MIPI workflow, sample cryosections were collected on the conductive slide for Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI-MSI) and polyethylene naphthalate (PEN) slides for microPOTS (Processing in One pot for Trace Samples) proteomics. For newly developed MIPI-STS single-tissue-section workflow PEN slides were used only. For MALDI-MSI, sections were derivatized using 4-(2-((4-bromophenethyl)dimethylammonio)ethoxy) benzenaminium dibromide (4-APEBA) and then covered with 2,5-dihydroxybenzoic acid (DHB) using a TM Sprayer and analyzed with 25 µm step size by a 12T solariX Fourier-Transform Ion Cyclotron Resonance (FTICR)-MS in positive mode. Underivatized adjacent sections were sprayed with 2,5-dihydroxyacetophenone (DHA) and analyzed in both polarity modes with 15 µm step size using MALDI-UHMR-HF Orbitrap MS. Guided by MALDI-MSI results, replicates of distinct microscopic regions were microdissected and collected in into microwell chips using a PALM MicroBeam laser capture microdissection (LCM) system. Sample preparation was carried out on-chip, following peptide mass analysis using an Orbitrap Exploris MS.

Results: The initial implementation of MIPI enabled molecular characterization of microhabitats involved in lignocellulose deconstruction within a heterogeneous, multi-member microbial community. Spatial metabolomics by MALDI-FTICR was employed to map metabolite signatures across embedded sections and identify lignin-degradation hot spots, which were then subsequently analyzed by microPOTS metaproteomic to uncover taxon-specific enzymatic roles and reconstruct lignin degradation pathways.

An advanced MIPI approach broadens molecular coverage and improves sensitivity for difficult-to-ionize compound classes in biomedical tissue. Untargeted MALDI imaging of human placenta tissue sections revealed spatially distinct molecular patterns that identified regional differences between placenta villi subregion: syncytiotrophoblast (STB) and core. Using the METASPACE annotation platform we tentatively annotated 680 metabolites and 180 lipid signatures across placenta sections. Guided by these spatially defined metabolomic and lipidomic features, we microdissected replicates villous subregions from sections adjacent to MALDI-imaged tissue. Deep proteome profiling using microPOTS identified 2,821 proteins, whereas over 20% of them were differentially expressed between the villous subregions, highlighting their distinct molecular compositions and suggesting subregion-specific metabolic pathways. Spatial multi-omics integration reconstructed active biological pathways within morphologically distinct villous subregions, including ketone body oxidation in the core and pathways related to estrogen synthesis, fatty acid transport, and energy production in the STB. The latest MIPI-STS development enabled multi-omics MS profiling from a poplar root section on a PEN slide while retaining sensitivity across all MS modalities. These advances position MIPI as a robust, versatile workflow for obtaining spatially resolved pathway-level insights that support mechanistic hypotheses about microbial community function and disease-related processes.

Significance: MIPI demonstrates the power of integrative spatial multi-omics analyses to provide detailed, pathway-level insights not obtainable with bulk measurements or single imaging approaches.

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Imaging-Guided Analysis of BECC-Adjuvanted Influenza Microneedle Vaccines and Skin Phospholipid Remodeling

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Introduction: Dissolvable microneedle patches (MNPs) represent a promising strategy for influenza vaccine delivery by targeting the skin's immune-rich microenvironment while enabling minimally invasive administration. In this study, we investigated the incorporation and imaging-based evaluation of influenza hemagglutinin H1 (HA) combined with TLR4-based BECC lipid A adjuvants in dissolvable MNPs. BECC-derived lipid A mimetics have demonstrated potent adjuvant activity, including enhanced humoral and cellular immune responses and antigen dose sparing in murine influenza models. Here, imaging-guided analysis was applied to monitor MNP application, dissolution, and delivery performance while assessing local phospholipid alterations in mouse skin following lipid A administration.

Methods: C57BL/6 mouse dorsal skin was clipped prior to application of dissolvable microneedle patches containing 5 µg of influenza hemagglutinin H1 (HA-H1) and 50 µg of the synthetic TLR4-based BECC470s lipid A adjuvant. Control animals received empty microneedle patches. Patches were applied to the skin for up to 5 minutes to allow insertion and dissolution. The treated skin regions were subsequently dissected, snap frozen, and cryosectioned at 10 µm thickness. Tissue sections were mounted onto SuperFrost Gold glass slides for multimodal imaging analyses. Norharmane matrix (7.5 mg\/mL) was applied, and mass spectrometry imaging was acquired using a timsTOF Flex at 10µm spatial resolution. Lipid A-related ions and phospholipid species were analyzed in both positive and negative ion modes on the same tissue section using an offset acquisition strategy in a mass range between m/z 400-2000. Immunofluorescence staining was additionally performed to visualize influenza protein localization and lipid A distribution within the skin tissue.

Results: Imaging analyses demonstrated successful incorporation of BECC-adjuvanted H1 HA formulations into dissolvable microneedle patches with preserved structural integrity during fabrication and application. Microscopy-based monitoring confirmed uniform microneedle morphology and consistent dissolution behavior after insertion into mouse skin, supporting efficient cargo release within the targeted tissue microenvironment. Spatial imaging further enabled visualization of antigen and adjuvant distribution throughout the patch matrix and during skin delivery, providing critical insight into release kinetics and formulation stability. In parallel, lipidomic analysis of mouse skin following lipid A injection revealed localized phospholipid remodeling associated with innate immune activation. Distinct alterations were observed in phospholipid distributions within regions exposed to the BECC lipid A

adjuvant, including changes in phosphatidylcholines (PCs), phosphatidylethanolamines (PEs), and lysophospholipid species. Increased lysophospholipid abundance suggested active membrane remodeling and inflammatory signaling responses triggered by TLR4 stimulation. Spatially resolved analyses demonstrated that these lipid alterations were concentrated near microneedle insertion sites, indicating localized immunometabolic activation within the skin microenvironment. The combination of imaging-guided formulation assessment and phospholipid profiling established a practical framework for optimizing dissolvable MNP vaccine platforms. These findings support the use of BECC lipid A adjuvants in skin-targeted influenza vaccination strategies and demonstrate how multimodal imaging approaches can inform patch performance, delivery efficiency, and local biological responses.

Significance: This work advances the development of scalable dissolvable microneedle vaccines with enhanced immunogenic potential and improved translational applicability for influenza immunization.

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Deciphering Complex Tissue Microenvironments with Multimodal MALDI Imaging: FluoMALDI, RaMALDI, and QMALDI

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Introduction: To further advance matrix-assisted laser desorption\/ionization (MALDI) imaging, we have developed three innovative, application-driven technologies that expand its analytical capabilities across spatial biology, multimodal molecular discovery, and quantitative analysis. These approaches (1) integrate MALDI imaging with fluorescence microscopy to enable precise spatial targeting of defined tissue regions and cellular compartments, (2) combine MALDI imaging with Raman microscopy to facilitate complementary biomolecular discovery and improved molecular identification, and (3) establish quantitative MALDI imaging methodologies for the detection and spatial mapping of drug metabolites and imaging contrast agents. We show our latest data on deciphering complex tissue microenvironments by leveraging these multimodal workflows.

Methods: FluoMALDI microscopy integrates fluorescence microscopy and MALDI imaging on the same tissue section. Co crystallization of fluorophores with MALDI matrices enhances fluorescence intensity and enables improved spatial co-registration. Fluorescence-guided targeting can be applied using endogenous signals, genetic reporters, and immunofluorescence. RaMALDI microscopy is a multimodal workflow combining Raman spectroscopic imaging (RSI) and MALDI mass spectrometry imaging (MSI) on a single tissue section using a unified preparation protocol. Sequential acquisition and computational data fusion enables integration of complementary chemical information. Quantitative MALDI (QMALDI) imaging measures spatial distributions of drug metabolites and imaging agents. Aspirin and its metabolite salicylic acid can be mapped within the complex tumor microenvironment in murine breast cancer xenograft models following intravenous administration. Quantification is achieved through calibration approaches and normalization strategies to enable comparison across tissue regions.

Results: FluoMALDI microscopy enables robust integration of fluorescence microscopy and MALDI imaging on the same biological sample. Co-crystallization of fluorophores with specific MALDI matrices results in markedly increased fluorescence brightness, facilitating improved sensitivity and precise spatial alignment between modalities. Across applications in neuroscience, developmental biology, and cell biology, this approach enables fluorescence-guided targeting of specific tissue regions and cellular populations. As a result, FluoMALDI provides comprehensive molecular profiling of defined microenvironments within a single tissue section, combining high spatial resolution with multiplex molecular detection. RaMALDI imaging successfully integrates Raman and MALDI modalities into a unified workflow. By combining the label-free chemical specificity of Raman spectroscopy with the high sensitivity and molecular coverage of MALDI-MSI, RaMALDI generates complementary biomolecular maps with improved interpretability. This approach enables consistent co-registration of data across modalities and reveals spatially resolved molecular features across diverse tissue types with complex microenvironments. Integration of Raman and MALDI datasets enhances biomolecular identification and expands analytical depth, supporting applications in pathology, cell biology, and translational research. QMALDI imaging enables quantitative spatial analysis of drug metabolism within complex tissue microenvironments. Mapping of aspirin metabolites demonstrates pronounced accumulation of salicylic acid in the kidney medulla and within tumor regions, particularly in vascularized areas of the tumor rim. These findings highlight the spatial heterogeneity in drug distribution and metabolism. Together, these results demonstrate that FluoMALDI, RaMALDI, and QMALDI substantially enhance the scope, sensitivity, and quantitative capabilities of multimodal MALDI imaging for spatially resolved biological and biomedical investigations in complex tissue microenvironments.

Significance: FluoMALDI, RaMALDI, and QMALDI expand MALDI imaging by enabling precise spatial targeting, multimodal integration, and quantitative analysis, providing powerful tools for understanding complex tissue microenvironments.

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Comprehensive investigation of spatiotemporal lipid alterations in the whole brain of NPC1 mouse models using mass spectrometry imaging

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Introduction: Niemann-Pick disease type C1 (NPC1) is a rare, hereditary lysosomal storage disorder characterized by disrupted intracellular cholesterol transport, leading to the accumulation of cholesterol and glycosphingolipids in the late endosomal/lysosomal system. Clinical manifestations include cerebellar ataxia, dystonia, vertical gaze palsy, and cognitive impairment. Dysregulated lipid trafficking results in abnormal lipid distribution in the brain and other visceral organs.

Methods: The brain is a lipid-rich organ in which lipids are vital structural components and critically influence neuronal homeostasis; therefore, disruption can contribute to many pathological states. Dysregulated lipid metabolism and transport are linked to many neurodegenerative diseases, including NPC1, in which the threat arises mainly from dysregulated homeostasis of cholesterol, fatty acids, and sphingolipids, leading to neuropathological cascades. Several studies have expanded knowledge of lipid dysregulation in NPC1; however, the exact mechanisms linking dynamic changes in lipid metabolism to disease progression remain poorly understood, particularly across different brain regions, including the correlation with patterned degeneration of cerebellar lobules. In addition to capturing multiplexed spatiotemporal data on different molecules in biological samples across disease progression, lipidomics via high-sensitivity mass spectrometry imaging (MSI) provides spatially heterogeneous lipid distributions critical to cellular functions in complex diseases such as NPC1.

Results: Prior work from our lab using MSI has revealed a correlation between ceramide lipid accumulation and the neurodegenerative pattern in the cerebellum of Npc1-/- mice, as well as defects in phosphoinositide lipids. In addition, our recent preliminary data suggest that a few other non-ceramide lipids also correlate with the neurodegeneration pattern. In this study, we present a comprehensive lipid distribution across brain substructures in Npc1-/- and Npc11061T/11061T mice during disease progression (ranging from presymptomatic to advanced stages) using high-resolution timsTOF fleX (Bruker Daltonics, Germany) for MSI analysis.

Significance: The findings of this study offer spatiotemporal perspectives on lipid alterations, highlighting lipid metabolism imbalances in both Npc1-/- and Npc11061T/11061T mouse brains.

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Fantastic Cells and Where to Find Them

Sizun Jiang
Beth Israel Deaconess Medical Center/Harvard Medical School

Unraveling the complex choreography of host–disease interactions is essential to illuminate how immune responses are shaped; whether through the immunomodulation of viral infections, cancer progression, or the dynamic remodeling of epithelial barriers during disease. Today, cutting-edge spatial-omics technologies developed by our laboratory and others have opened an unprecedented window into these processes, enabling the in situ dissection of cell states, viral features, and spatially resolved mechanisms that underlie pathology and immune control. I will discuss how our lab is leveraging AI and spatial multi-omics to decode the systems-level immunological responses (or lack-there-of) towards pathways of inflammation and new therapeutic targets. I will highlight our spatially resolved analyses across various tumor ecosystems, uncovering how spatial architecture and immune checkpoint pathways together shape the tumor microenvironment. Besides our advacements in experimental and computational spatial biology, I will discuss our recent and ongoing efforts into building AI Foundation Models, and applications of AI agents towards meaningful applications in spatial-omics and systems immunology.

Oral Session

Spatial Multi-Omics from Single Tissue Sections

Chairs

Erin Seeley, Ph.D.
Director, Mass Spectrometry Imaging Core at MD Anderson Cancer Center

Jade MacDonald, Ph.D.
Postdoctoral Researcher at Vanderbilt University
Mass Spectrometry Research Center & the Lab of Jeffrey M. Spraggins

Segment-Resolved Lipidomic Exploration of the Human Proximal Tubule using Integrated MALDI IMS and Multiplexed Immunofluorescence

Megan Ward
Mass Spectrometry Research Center, Vanderbilt University, Nashville, TN, USA & Chemical Physical Biology Program, Vanderbilt University School of Medicine, Nashville, TN

Introduction: The proximal tubule plays a key role in fluid, electrolyte, and nutrient balance within the kidney and subdivides into convoluted and straight tubules (PCT and PST). Specialized epithelial cells undergo structural changes within the PCT and PST, underpinning the different functional roles for the two subdivisions. A spatial approach is required to examine the molecular fingerprints associated with the structural and functional alterations along the nephron axis. Herein, we integrate matrix-assisted laser desorption\/ionization imaging mass spectrometry (MALDI IMS) and multiplexed immunofluorescence to generate segment-resolved lipidomic mapping of the PCTs and PSTs and observe how the molecular landscape shifts in disease.

Methods: Human kidney tissues were cryosectioned at 6 µm thickness and mounted onto indium tin oxide-coated slides. Samples were washed with chilled 150 mM ammonium formate, and autofluorescence microscopy was collected with a Zeiss Axioscan.Z1 slide scanner. An aminated cinnamic acid analog matrix was sublimed onto the tissue with an HTX SubliMATE. Positive (m/z 350-1250) and negative (m/z 410-1600) polarity data were collected using a Bruker timsTOF fleX mass spectrometer at 10 µm pitch. Lipids were identified through exact mass matching to a custom LC-MS/MS library. Multiplexed immunofluorescence was performed after MALDI IMS using a custom panel of 37 antibodies, including key proximal tubule markers to differentiate between the PCT and PST. Segmentation masks for the two subdivisions were generated from the multiplexed immunofluorescence microscopy data and applied to the MALDI IMS data to extract segment-resolved mass spectra. All modalities were co-registered and analyzed using in-house developed tools, including image2image and autoIMS.

Results: Previously, our group has defined lipidomic biomarker candidates for the key functional tissue units (FTUs) in the kidney: glomeruli, proximal tubules, thick ascending limbs, distal tubules, and collecting ducts. These tissue features can exhibit functional changes based on position within the tissue, such as medulla or cortex, and along the nephron axis, underscoring the need for increased granularity to examine the molecular heterogeneity within a singular FTU. Particularly, the proximal tubule possesses molecular, structural, and functional differences between the convoluted

and straight portions. A multiplexed immunofluorescence panel consisting of 37 antibody markers helped identify major kidney cell types, vasculature, nerves, and immune cells. This distinguished the proximal tubule from other FTUs, and key proximal tubule markers, such as megalin, sodium-glucose cotransporter 2, and αB crystallin, separated the PCT from the PST. Modifications to the MALDI IMS acquisition parameters allowed subsequent multiplexed immunofluorescence to be performed on the same tissue section, permitting direct lipid mapping to the PCT and PST. Molecular heterogeneity can be observed at the lipid class and species level. For example, polyunsaturated phosphatidylethanolamines (PEs) display higher signal intensity in the PCT. Notable lipids include PE 38:4 (m/z 766.54) which displays higher signal intensity within the PCT and sulfated hexosylceramide 42:1;O3 (m/z 906.64) which shows higher signal intensity in the PST. The combination of MALDI IMS and multiplexed immunofluorescence elucidates the molecular landscape of the PCT and PST, and current efforts are underway to explore alterations within these molecular landscapes in the context of hypertension.

Significance: Enables the association of molecular changes to structural and functional changes along the nephron axis

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Multiplexed, Multiomic and Multimodal DESI-immunohistochemical Imaging of Biomarkers Including Drugs in Tissues using Novel Photocleavable Mass-Tag Antibody Probes

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Introduction: Desorption Electrospray Ionization Mass Spectrometry Imaging (DESI-MSI) is the most widely used ambient mass spectrometry technique. Unlike MALDI-MSI, which requires matrix-assisted laser desorption, DESI-MSI utilizes a charged solvent spray under atmospheric conditions. Importantly, DESI is well suited for detection of low molecular weight molecules such as metabolites, lipids and drugs in tissue. We have developed novel DESI-photocleavable mass tags (DESI-PCMTs) conjugated to antibodies for multiplexed, multiomic and multimodal DESI-immunohistochemistry (DESI-IHC). We report DESI-IHC workflows for imaging of targeted proteins along with unlabeled lipids, small molecules and exogenous drugs for both mouse brain and human tonsil tissues.

Methods: DESI-IHC was performed on fresh frozen mouse brain tissue from APP-SAA Alzheimer's mice dosed with clozapine or memantine, and on FFPE human tonsil tissues. DESI-MSI was performed on a Waters Xevo™ TQ Absolute mass spectrometer equipped with a DESI™ XS source in MRM mode with CID at 25 and 50 µm spatial resolution. Novel DESI PCMTs were synthesized and used to perform DESI-IHC analogous to protocols described for MALDI-IHC [Yagnik, Liu et al. (2021) J. Am Soc Mass Spectrom 32: 977-988] except not requiring matrix assisted laser desorption. The PCMTs incorporated an internal photocleavable linker, thiol or amine reactive moieties, and fluorophores as N-terminal or internal modifications. Following near UV photocleavage, the PCMTs released a doubly charged reporter ion containing the fluorophore. PCMTs were conjugated to different antibodies and used for multiplexed DESI-IHC imaging.

Results: Over 10-plex DESI-IHC imaging of both mouse brain and human tonsil tissue was achieved using a novel set of PCMTs designed for use with DESI-MSI. DESI-IHC sample preparation and staining with DESI-PCMT antibody probes followed protocols previously reported for MALDI-IHC, but without matrix deposition and using MRM based DESI-MSI. To assess background signal, species and isotype-matched immunoglobulins conjugated to the same DESI-PCMTs as the antibodies and imaged on adjacent tissue sections. Probe performance was validated by comparison with fluorescence microscopy images acquired from the same tissue sections using the endogenous fluorophores incorporated within the DESI-PCMTs. In the case of human tonsil, the evaluated probes targeted proteins useful in immuno-oncology studies, along with tissue architecture markers, including collagen, CD8, histone, beta-actin,

vimentin, HLA-DR, PanCK, ECAD, Caveolin-1, CD20 and CD3. Low abundant proteins and\/or relatively rare cell populations in the tonsil were all detected demonstrating the sensitivity of the approach. For mouse brain tissue, APP-SAA Alzheimer's mice were dosed intraperitoneally with either clozapine or memantine and harvested fresh frozen brain tissue sections were mounted on IHC slides. DESI-MSI was first performed in the positive ion mode followed by staining with a 10-plex neurological panel of DESI-PCMT antibodies. The slide was then imaged using fluorescence microscopy followed by DESI-MSI. This workflow enabled integrated imaging of lipids, exogenous drugs, and targeted proteins from a single tissue section using DESI-MSI.

Significance: Novel photocleavable mass-tag probes designed for multiplexed, multiomic and multimodal DESI-IHC imaging workflows are demonstrated including imaging of drug dosed APP-SAA Alzheimer's mouse brain tissue.

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MOSAICS: An End-to-End Spatial Multiomics Approach for Analyzing a Single Tissue Section

Christopher Anderton
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Introduction: Unlocking the underlying mechanisms regulating human health and disease requires tools that can broadly characterize diverse biomolecular classes, from the same cells across functionally distinct tissue regions. The rapidly evolving field of spatial omics has enabled visualization of location-specific gene expression and metabolic activity. However, obtaining spatial multiomics measurements from the same cell or functional regions of a sample remain challenging. Here, we introduce an approach and computational framework for data integration called MOSAICS (Multi-Omic Spatial Analysis In Cycles on a Section) for obtaining spatial transcriptomic, proteomic, and metabolic data directly from a single tissue section.

Methods: Formalin-fixed, paraffin-embedded (FFPE) human lung tissue blocks and tissue microarrays (TMAs) were sectioned and mounted on Xenium and indium tin oxide (ITO)-coated slides. Samples mounted on ITO slides were used as controls. Xenium In Situ Gene Expression assay (480 genes) was performed according to the manufacturer's protocol. Tissue samples were then imaged using the Ultrafast Focused Light-based Imaging & Photonics platform (U-FLIP), which combines two-photon fluorescence (TPF), second harmonic generation (SHG), and stimulated Raman scattering (SRS) into a single microscope. N-glycans and extracellular matrix proteins were imaged sequentially with enzyme-assisted matrix-assisted laser desorption\/ionization (MALDI)-MS (timsTOF fleX, Bruker Daltonics). Highly multiplexed immunofluorescence (MxIF) was then performed using a custom 53 antibody panel (Akoya Biosciences). Lastly, the tissues were histochemically stained and imaged. A custom workflow was developed for a multi-level data integration approach that enabled cross-referencing across multi-omics data to provide spatially explicit biomolecular and mechanistic insights.

Results: Given the differences in tissue handling and preservation methods used for each spatial omics assay, we identified potential pitfalls and implemented modifications to support multimodal imaging and ensure high-quality data from each assay. For data integration, we registered all data to the Xenium images to permit identification of cell types and structural segmentations, which is then used to guide molecular profiling across all data types. Using established computational pipelines, spatial transcriptomics data was used to create cell type annotations with a variety of epithelial, immune, endocrine, and other cell type markers. U-FLIP was able to identify changes in lipid abundance and saturation shifts within regions of the tissue,

as well as provide an overview of metabolic activity and fibril collagen-rich regions. Additionally, this data provided high-resolution lipid-subtyping, enabling the identification of cell-specific metabolic signals. Finally, we applied MOSAICS to multi-donor TMAs. This approach permitted direct observation of molecular and cellular trends within the tissues. For example, we noted increases in the abundance of COL3 and COL6 with high-mannose N-glycans in the alveolar parenchyma—both of which are indicative of the alveoli's role in gas exchange. Type 3 and 6 collagens provide a flexible ECM for cell adhesion, while high-mannose N-glycans are often associated with high energy expenditure. We envision that each technique could be applied individually to identify molecularly significant subregions in the tissue but combining them will allow us to classify the abundance of multiple molecular classes within cell types or functional regions of a tissue section.

Significance: MOSAICS captures spatial multiomics data directly from a single tissue section, facilitating co-registration, integration, and correlative analysis of diverse molecular distributions.

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