QE MAP

3rd Annual NIGMS P41 QE-MAP Symposium & Workshop August 22-24, 2023

MICHIGAN STATE UNIVERSITY
Interdisciplinary Sciences and Technology Building (ISTB) 1ST Floor
766 Service Road, East Lansing MI, 48824

We're sending out this invitation for our 2nd in person (at Michigan State University in East Lansing, MI) symposium and workshop for the P41 national research resource center for quantitative mapping in the life sciences (QE-Map).

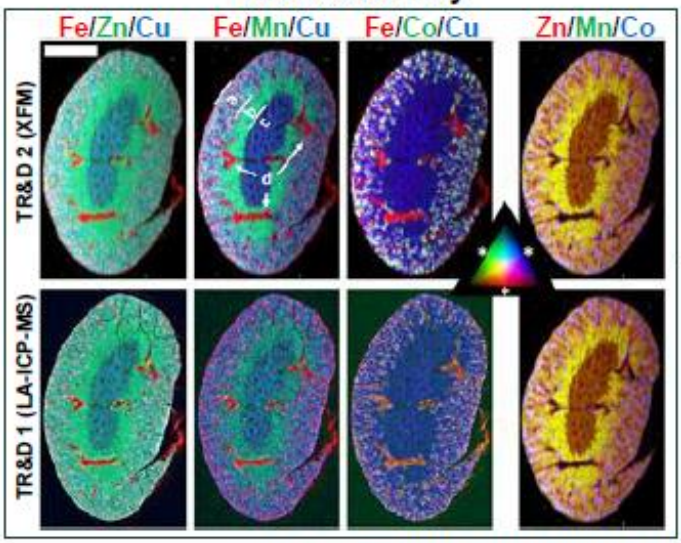
Symposium/Workshop Format

- Day 1: EAC meeting, half day of symposium talks
- Day 2: half day of symposium talks, half day of workshop
- Day 3: full day of workshop

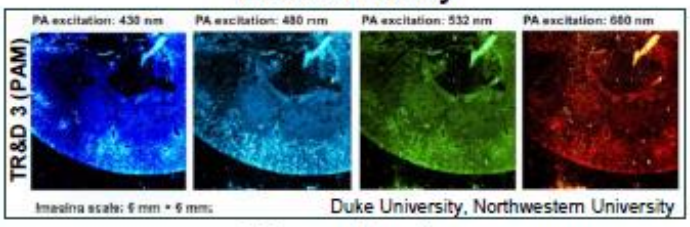
Please fill out the Microsoft Teams Poll:

https://forms.office.com/r/Mz3XJvuX9D

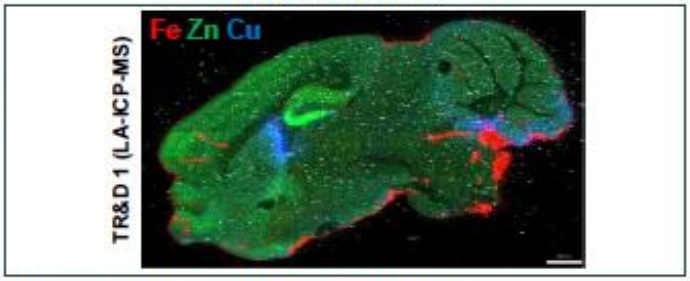
Mouse Kidney



Mouse Kidney



Mouse Brain



SPONSORED BY NIH/NIGMS P41GM135018 | ehi.qemap.msu.edu

Table of Contents

Welcome

Dear Colleagues,

It is a great pleasure to welcome you to Michigan State University and the 3rd annual (2nd in-person) NIGMS P41 QE-Map Symposium and Workshop in East Lansing, Michigan.

As we gather here, we are bringing together members of the NIGMS P41GM135018 grant as well as other members of the bioelement imaging and analysis community. Over the next few days, we will discuss the National Research Resource Center for Quantitative Mapping in the Life Sciences (QE-Map) and how technology research and development (TR&D) projects facilitate research among the driving biological projects (DBP) and beyond. We will hear about progress in laser ablation inductively coupled plasma time-of-flight mass spectrometry (LA-ICP-TOF-MS, TR&D 1), synchrotron-based X-ray fluorescence microscopy (XFM, TR&D 2), and photoacoustic microscopy (PAM, TR&D 3) and how we can answer fundamental biological questions using the aforementioned technologies.

We will also have a workshop on days 2 and 3 to expose students, postdoctoral researchers, and faculty to the cutting-edge instrumentation that we are developing in this resource. We encourage open dialogue and discussions throughout the event and hope you will all actively participate.

We are highly grateful for the support and generosity of our sponsors and have set up lunchtime partner talks where we will here directly from the instrument manufacturers about development of new technologies and implementation of their current ones.

On behalf of the NIGMS P41 QE-Map Executive Advisory Committee, we wish you a warm welcome to East Lansing and Michigan State University and look forward to an engaging symposium and workshop.

Thomas V. O'Halloran
Principal Investigator P41 National Research Resource (QE-Map)

P41 Members
Technology Research and Development (TR&D) Leads

TR&D 1, PI: **Thomas V. O'Halloran**, *Michigan State University*

TR&D 2: **Chris Jacobsen**, *Northwestern University/Argonne National Laboratory*

TR&D 3: **Cheng Sun**, *Northwestern University*

Driving Biological Projects (DBP)

Yevgenia Kozorovitskiy, *Northwestern University*

Svetlana Lutsenko, *Johns Hopkins University*

Somshuvra Mukhopadhyay, *University of Texas-Austin*

Valeria Culotta, *Johns Hopkins University*

Eric Skaar, *Vanderbilt University*

Christoph Fahrni, *Georgia Institute of Technology*

Carole LaBonne, *Northwestern University*

Hossein Ardehali, *Northwestern university*

Malek El Muayed, *Northwestern University*

Donald McClain, *Wake Forest University*

Executive Advisory Committee

Christine Austin, *Mount Sinai Hospital*

Graham George, *University of Saskatchewan*

Robert Hausinger, *Michigan State University*

Eric Hegg, *Michigan State University*

Michael Marleta, *University of California-Berkeley*

Sarah Michel, *University of Maryland-Baltimore*

Elizabeth Nolan, *Massachusetts Institute of Technology*

James Penner-Hahn, *University of Michigan-Ann Arbor*

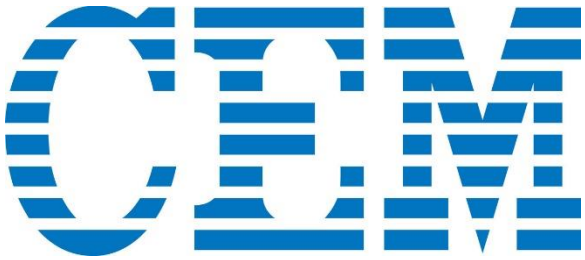
JoAnne Stubbe, *Massachusetts Institute of Technology*

Emily Que, *University of Texas-Austin*

Junjie Yao, *Duke University*

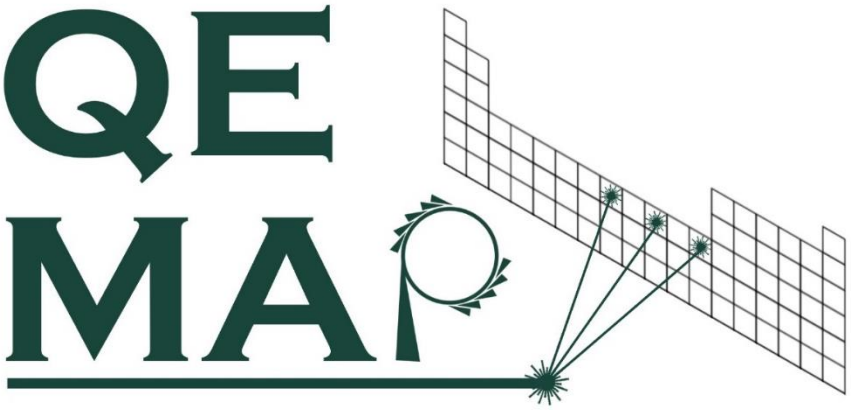
Partners

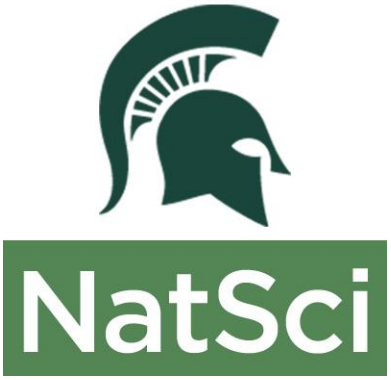
Platinum Sponsors



Sponsors


National Institute of
General Medical Sciences


QE
MAP


NatSci

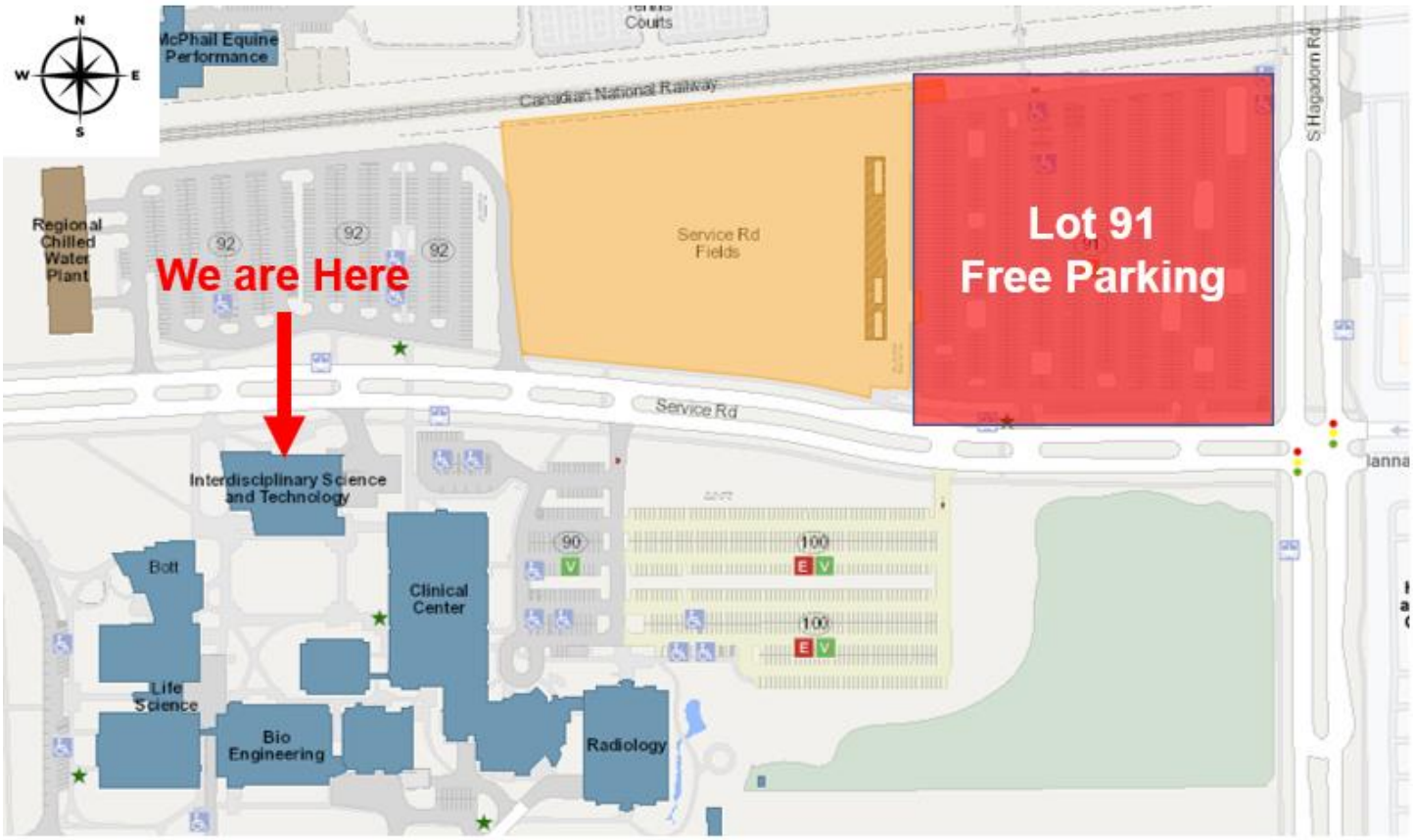
Conference Venue

The venue for the 2022 QE-Map Symposium/Workshop is Michigan State University and the Interdisciplinary Science and Technology Building (ISTB). ISTB was built in 2019 to provide state-of-the-art laboratories for diverse research groups including engineering, biology, computer science, and chemistry.

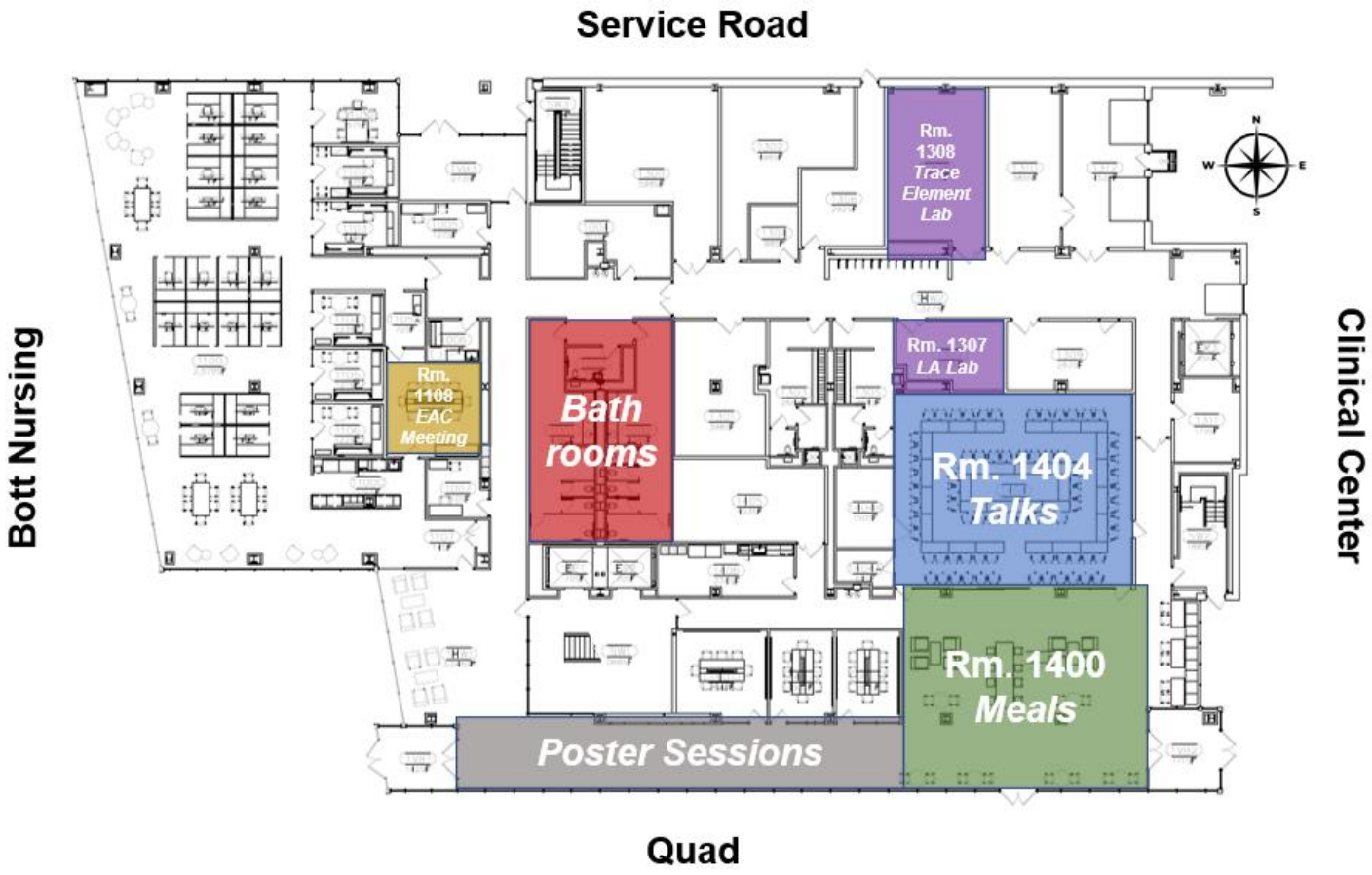
Venue Address: 766 Service Road, East Lansing, MI 48824

Parking

Parking is free in Lot 91 for the summer! It's on the corner of Service Road and South Hagadorn Road and is a short 2-3 block walk from Lot 91 to the ISTB building.



Floor Plan



Internet Access

MSU guests and visitors can connect to MSUNet Guest Wireless without needing an MSU NetID or register their devices.

Guest users without an MSU NetID can join the Wi-Fi network (SSID) MSUnet Guest or MSUnet Guest 3.0 under your device's wireless connection options. You'll also need to agree to abide by the MSU Acceptable Use Policy for Information Technology Resources.

Social Program (Food)

Tuesday August 22nd, 2023 (Morton's Fine Catering, ISTB Atrium Rm. 1400)

Lunch: 11:30 am-1:00 pm

Meat option: Rosemary chicken thighs w/ roasted garlic, lemon, and rosemary

Vegetarian option: Caprese mostaccioli w/ fresh mozzarella baked in penne pasta with marinara, torn basil leaves

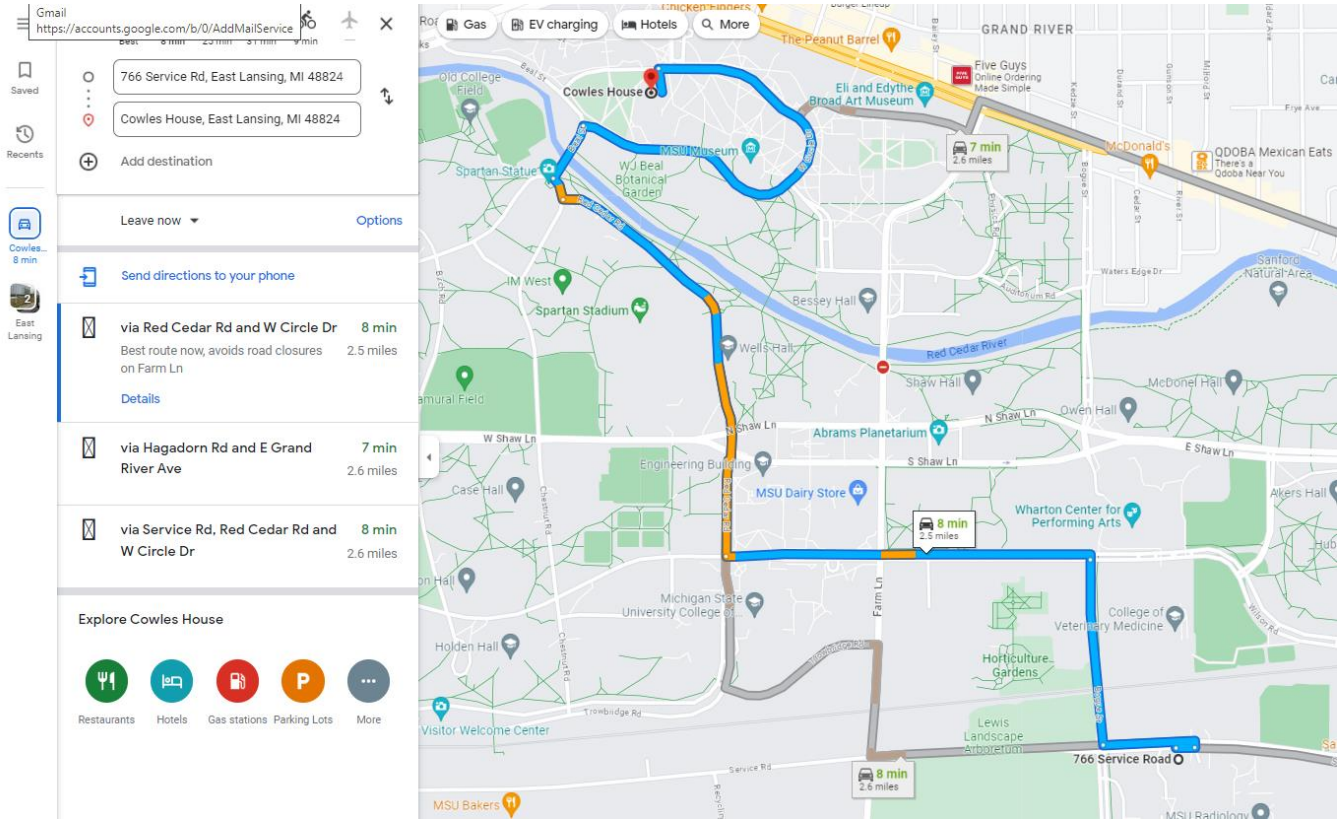
Sides: Roasted red potatoes, seasonal vegetable medley, artisan breadbasket

Dessert: Brownie bites

Coffee: 3:00-3:30 pm

Coffee and Tea with cookies from the MSU Bakery

Dinner: 6:00-9:00 pm (Backyard Barbecue Dinner at Cowle's House, 1 Abbot Rd.)



Wednesday August 23rd, 2023 (Morton's Fine Catering, ISTB Atrium Rm. 1400)

Breakfast: 8:00-9:00 am

Breakfast Sandwiches: Fresh Egg, Ham, Swiss, Sausage, Colby-Jack, fresh fruit display

Coffee: 10:30-11:00 am

Coffee, tea, and snacks

Lunch: 12:00-1:00 pm

Roasted vegetable lasagna with pepper, eggplant, tomatoes, mushrooms, zucchini with mozzarella and ricotta, house marinara

Sides: Caesar salad, green beans almandine, artisan breadbasket

Dessert: Brownie bites

Coffee: 2:30-3:00 pm

Coffee and Tea with cookies from the MSU Bakery

Dinner: *Meat option:* Wild mushroom pork loin with mushroom demi-glace

Vegetarian option: Ratatouille with French Provençal stewed vegetables such as eggplant, zucchini, peppers, squash with white beans in herbed tomato sauce

Sides: Wild rice pilaf, garden salad, artisan breadbasket

Dessert: Lemon bars

Thursday August 24th, 2023 (Morton's Fine Catering, ISTB Atrium Rm. 1400)

Breakfast: 8:00-9:00 am

Breakfast Burritos: Scrambled eggs, and choice of sausage, bacon, and black beans in a flour tortilla w/ salsa, hot sauce, and sour cream on the side. Fresh fruit display

Coffee: 10:30-11:00 am

Coffee, tea, and snacks

Lunch: 12:00-1:00 pm

Meat Option: Lemon caper chicken breast w/ lemon caper wine sauce, fresh thyme, and lemon zest

Vegetarian Option: Zucchini and chickpea tagine w/ Moroccan spices

Sides: Basmati rice pilaf, artisan breadbasket

Dessert: lemon bars

Coffee: 2:30-3:00 pm

Coffee and Tea with assorted cookies from the MSU Bakery

AGENDA

Day 1 - August 22, 2023				
Start	**End**	**Speaker**	**Affiliation**	**Session/Title**
8:00 AM	9:00 AM			
9:00 AM	9:30 AM			
9:30 AM	10:00 AM			
10:00 AM	11:30 AM	EAC MEETING: ISTB 1108		
11:30 AM	1:00 PM	Lunch/Registration: **ISTB 1st Floor Lobby Rm. 1400**		
Center Overview Session (All talks in ISTB Room 1404)				
1:00 PM	1:30 PM	Thomas O'Halloran	Michigan State University	***P41 QE-Map Overview:*** *Advancements in Elemental Imaging and Analysis*
1:30 PM	2:00 PM	Keith MacRenaris	Michigan State University	***TR&D 1:*** *LA-ICP-MS and High Throughput Elemental Histology*
2:00 PM	2:30 PM	Chris Jacobsen	Northwestern University	***TR&D 2:*** *X-ray fluorescence at Argonne: Before and After the Upgrade of the Advanced Photon Source*
2:30 PM	3:00 PM	Cheng Sun	Northwestern University	***TR&D 3:*** *Photoacoustic Microsopy for In Vivo and Whole Tissue Imaging*
3:00 PM	3:30 PM	Break/Recap - Cookes from MSU Bakers Location: **ISTB 1st Floor Lobby Rm. 1400**		
Symposium Session 1 (All Talks in ISTB Rm. 1404)				
3:30 PM	4:00 PM	Svetlana Lutsenko	Johns Hopkins University	*Intracellular Copper Redistribution During Cell Differentiation*
4:00 PM	4:30 PM	Christoph Fahrni	Georgia Technological University	*Zinc-Selective Fluorescent Probes for Two-Photon Excitation Microscopy*
4:30 PM	5:00 PM	Young-Ah Seo	University of Michigan	*Manganese: Unsung Hero for Physiology?*
5:00 PM	6:00 PM	Poster Session Location: **ISTB 1st Floor Lobby**		
6:00 PM	9:00 PM	Dinner Location: **Cowle's House, 1 Abbot Rd.**		

Day 2 - August 23, 2023

Start	End	Speaker	Affiliation	Session/Title
8:00 AM	9:00 AM	Breakfast/Check In Location: **ISTB 1st Floor Lobby Rm. 1400**		
Symposium Session 2 (All Talks in ISTB Rm. 1404)				
9:00 AM	9:25 AM	Weilue He	Michigan Technological University	*Mapping of Bioresorbable Stent Derived Rare Earth Metals in Mouse Aorta*
9:25 AM	9:50 AM	Graham George	University of Saskatchewan	*Examining the Interplay Between Toxic Elements using Synchrotron Radiation*
9:50 AM	10:15 AM	Bong Hong	Michigan State University	*Effect of Iron Deficient Diet on Metal Homeostasis of Mouse Tissues, and Inorganic Signatures of Heart Failure Mouse Models*
10:15 AM	10:40 AM	Soo Hyun Ahn	Michigan State University	*Iron Deposition in Epididymis Linked to Fibrosis and Infertility in Autoimmune Regulator Deficient Male Mice*
10:45 AM	11:00 AM	Coffee Break Location: **ISTB 1st Floor Lobby Rm. 1400**		
Student/Post Doctoral Session (All Talks in ISTB Rm. 1404)				
11:00 AM	11:20 AM	Aidan Reynolds (*Tian Qiu*)	Michigan State University	*Optimization of LA-ICP-TOF and MALDI-TOF Imaging Mass Spectrometry for Caenorhabditis elegans Research*
11:20 AM	11:40 AM	Jacquelyn Spathies (*Eric Skaar*)	Vanderbilt University	*Multimodal Imaging of the Gastrointestinal Tract of Zebrafish*
11:40 AM	12:00 PM	Adelita Mendoza (Kornfeld/Diwan)	Washington University	*Lysosomes Contain an Expansion Compartment that Mediates Zinc Transporter Delivery to Promote Zinc Homeostasis in C. elegans*
12:00 PM	1:00 PM	Lunch/Partner Talks Location: **ISTB 1st Floor Rm. 1404**		
Workshop Day 1				
1:00 PM	1:30 PM	Keith MacRenaris	Michigan State University	***Workshop Overview***
1:30 PM	2:30 PM	***Workshop Session #1***		
2:30 PM	3:00 PM	Coffee Break Location: **ISTB 1st Floor Lobby Rm. 1400**		
3:00 PM	4:30 PM	***Workshop Session #2***		
4:30 PM	6:00 PM	Poster Session Location: **ISTB 1st Floor Lobby**		
6:00 PM	8:00 PM	Dinner Location: **ISTB 1st Floor Lobby Rm. 1400**		

Day 3 - August 24, 2023

Start	End	Speaker	Affiliation	Session/Title
8:00 AM	9:00 AM		Breakfast/Check In Location: **ISTB 1st Floor Lobby Rm. 1400**	
			Workshop Day 2	
9:00 AM	10:30 AM			***Workshop Session #3***
10:30 AM	11:00 AM		Coffee Break Location: **ISTB 1st Floor Lobby Rm. 1400**	
11:00 AM	12:00 PM			**Workshop Session #4**
12:00 PM	1:00 PM		Lunch Location: **ISTB 1st Floor Rm. 1404**	
1:00 PM	2:30 PM	Andrew Crawford Keith MacRenaris	Michigan State University	***Data Analysis Workshop #1***
2:30 PM	3:00 PM		Coffee Break Location: **ISTB 1st Floor Lobby Rm. 1400**	
3:00 PM	4:00 PM	Andrew Crawford Keith MacRenaris	Michigan State University	***Data Analysis Workshop #2***
4:00 PM	4:30 PM		End of QE-Map P41 Workshop Location: **ISTB 1st Floor Lobby**	

Lunch Seminars and Visits

Day 1 – Tuesday August 22nd, 2022 (ISTB Rm. 1404)

12:00 – 12:30 – Elemental Scientific Lasers discussion about bioimaging

12:30 – 12:45 pm – TofWerk discussion about LA/single cell ICP-TOF-MS

Day 2 – Wednesday August 23rd, 2022 (ISTB Rm. 1404)

12:00 – 12:30 – Agilent discussion about ICP-QQQ-MS and laser ablation/single cell applications

12:30 – 1:00 – CEM discussion about sample preparation and microwave digestion of biological samples

Workshops at-a-glance

Workshop #1 – Gelatin standards preparation, cryosectioning, slide mounting, and storage

Location – ISTB 3202 Histology Laboratory

Workshop Leader – Niharika Sinha, PhD

Workshop #2 – Microwave digestion (CEM MARS6), ICP-QQQ-MS (Agilent 8900), and ICP-OES (Agilent 5800) of various mouse tissues

Location – ISTB 1308 Trace Element Analysis Laboratory

Workshop Leader – Aaron Sue, PhD

Workshop #3 – Slide scanning (Zeiss Axioscan 7) and LA-ICP-TOF-MS (Tofwerk S2 ICP-TOF-MS w/ an ESL Bioimage 266 laser ablation system) of gelatin standards and mouse tissues

Location – ISTB 1307 Bioelement Mapping Laboratory

Workshop Leader – Keith MacRenaris, PhD

Workshop #4 – Virtual Synchrotron-Based X-ray Fluorescence Microscopy Workshop

Location – ISTB 1404 Seminar Room

Workshop Leader – Andrew Crawford, PhD

Data Analysis Workshop #1 – X-ray fluorescence data analysis and fitting for quantitative elemental mapping at the beamline

Location – ISTB 1404 Seminar Room

Workshop Leaders – Andrew Crawford, PhD and Ben Roter PhD

Data Analysis Workshop #2 – LA-ICP-TOF-MS data analysis including new software tools for peak fitting (AutoSpec and InSpec) and image analysis and standardization using Iolite software.

Location – ISTB 1404 Seminar Room

Workshop Leaders – Andrew Crawford, PhD and Keith MacRenaris, PhD

Talk Abstracts

All presentations and abstracts will be posted on the QE-Map website at https://qemap.ehi.msu.edu/qe-map-workshop-2023 following the workshop.

P41 QE-Map Overview: Advancements in Elemental Imaging and Analysis

Thomas V. O'Halloran[1,2,3] (ohallor8@msu.edu)

[1]Department of Microbiology and Molecular Genetics and Chemistry, Michigan State University, East Lansing, MI, USA.
[2]Elemental Health Institute, Michigan State University, East Lansing, MI, USA
[3]Quantitative Bio Element Analysis and Mapping (QBEAM) Center, Michigan State University, East Lansing, MI, USA

The National Resource for Quantitative Elemental Mapping for the Life Sciences (QE-Map) is developing novel analytical and imaging technologies that enable biomedical research teams to image changes in metal localization in a quantitative manner across length scales from the cellular level to tissue, and whole animal. QE-Map integrates multiple technologies to create transformative approaches to answer compelling biological questions about the functions of metals and other essential elements in health and disease.

The BTRR is composed of three Technology Research and Development Projects (TRDs), and 4 thematic areas for Driving Biomedical Research Projects (DBPs). The three TRD projects were identified based on complementary potentials to (a) enable accurate analysis of inorganic elements across length scales; (b) achieve sensitivity sufficient to allow single cell analysis; (c) accurately determine metal localization; and (d) measure dynamics of intracellular and extracellular metals in response to fluctuations in response to physiologic signals. We are addressing current limitations of laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) and scanning x-ray fluorescence microscopy (SXFM) in a wide variety of tissue samples as well as developing photoacoustic methods (PAM) and probes to enable studies at the tissue level and, eventually, in living mammalian models. We are developing workflows and software that allow co-registration of images and standardization of quantitative data that will maximize the impact of these technologies and accelerate their application to a broad range of biomedical research questions.

TR&D 1: LA-ICP-MS and High Throughput Elemental Histology

Keith MacRenaris[1,2,3] (macrenar@msu.edu)

[1]Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI, USA.
[2]Quantitative Bio Element Analysis and Mapping (QBEAM) Center, Michigan State University, East Lansing, MI, USA
[3]Elemental Health Institute, Michigan State University, East Lansing, MI, USA

Cells must accumulate several metals, such as zinc and iron, to millimolar levels to survive. Fluctuations in the metal content can control processes as varied as the mammalian cell cycle, pathogen infection, and neurological function. The critical regulatory role of metals is emphasized by the observation that one-third of all protein-encoding genes in the human genome encode metal-dependent proteins. To gain a complete picture of the role metals play in systems biology, multiple imaging modalities are being deployed including, laser ablation inductively coupled plasma time-of-flight mass spectrometry (LA-ICP-TOF-MS; *TR&D 1*), synchrotron-based X-ray fluorescence microscopy (XFM; *TR&D 2*), and photo-acoustic imaging (*TR&D 3*). To unleash the full potential of elemental imaging using multi-modal approaches we must be able to image the same sample using different platforms. This will allow us to corroborate findings between the different imaging modalities while offering true pixel-by-pixel overlays which will provide fully quantitative elemental maps in a multitude of tissues and cell types. In this talk I will discuss, in detail, substrate selection and their effects on sample preparation and analysis across all TR&D imaging modalities.

TR&D 2: X-ray fluorescence at Argonne: Before and After the Upgrade of the Advanced Photon Source

Chris Jacobsen[1,2,3] (c-jacobsen@northwestern.edu)

[1]Department of Physics and Astronomy, Northwestern University, Evanston, IL, USA
[2]Advanced Photon Source, Argonne National Laboratory, Lemont, IL, USA
[3]Chemistry of Life Processes Institute, Northwestern University, Evanston, IL, USA

Scanning fluorescence x-ray microscopy is available at several beamlines at the Advanced Photon Source at Argonne. Some experimental setups have used zone plate optics for 50-200 nm spatial resolution (including one that can work with frozen hydrated specimens), while others have used mirror optics to obtain 3-30 micrometer resolution. The endstation 8-BM has been used heavily by our resource for scanning larger tissue samples, with upgrades in detectors and optics tested before the April 17 shutdown for upgrading the APS. A brief summary of recent results will be provided, along with a discussion of capabilities one might expect after the APS Upgrade is completed in 2024.

TR&D 3: Photoacoustic Microscope for In Vivo and Whole Tissue Imaging

Cheng Sun[1] (c-sun@northwestern.edu)

[1]*Department of Mechanical Engineering, Northwestern University, Evanston, IL, USA*

Functional photoacoustic microscopy (PAM) has been studied extensively for its unique capability in noninvasive label-free imaging of biological samples in 3D. PAM Photoacoustic generation employs a ns-pulse laser to illuminate light-absorbing materials. The transient thermo-expansion and the following rapid thermal relaxation by the light-absorbing material upon the absorption of the laser energy led to a temporally confined photoacoustic wave, which is proportional to the tissue absorption. Thanks to reduced acoustic attenuation in tissue, PAM nearly doubles the penetration depth of confocal microscopy using the same wavelength. However, the commonly used sizeable and opaque piezoelectric ultrasonic detectors featuring limited ultrasound detection bandwidth often impose a serious constraint. To this end, optical-based ultrasonic detection techniques may offer a more desirable solution. Because light oscillates more than five orders of magnitude faster than ultrasonic waves, optical-based detection methods can potentially allow more sensitive ultrasonic detection over a much wider frequency band. We have thus developed a coverslip-style optically transparent ultrasound detector based on a polymeric optical micro-ring resonator (MRR). We have demonstrated an optically transparent ultrasound detector with the total thickness of 250 um. It enables highly sensitive ultrasound detection over a wide receiving angle with a bandwidth from DC to 140 MHz, which corresponds to a photoacoustic saturation limit of 287 cm−1, at an estimated noise-equivalent pressure (NEP) of 6.8 Pa. We also established a theoretical framework to provide general design guideline for optical-based ultrasound detectors. The optimal design was further validated experimentally for its key sensing characteristics including sensitivity, bandwidth, angular dependence, and functional imaging capabilities including lateral/axial resolution and saturation limit. We have further demonstrated the functional integration of PAM with the optical microscope and endoscope, by making use of the transparent MRR detectors. In a recent study, we have successfully integrated the MRR to the inner surface of cranial window, which enables the experimental demonstration of long-term in vivo intravital cortical photoacoustic microscopy of live rodents over a 28-day period.

NOTES

DBP A: Intracellular Copper Redistribution during Cell Differentiation

Svetlana Lutsenko[1] (lutsenko@jhmi.edu)

[1]*Department of Physiology and Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA*

Emerging evidence suggests that copper plays an important role in cells proliferation, differentiation, morphology, and motility. Not only the total levels of copper have to be maintained within the homeostatic range when cells transition from their proliferative to differentiated state, but the intracellular distribution of copper has to be tightly regulated. This presentation will describe examples of intracellular copper redistribution during cell differentiation and consequences of copper mis-localization.

DBP C: Zinc-Selective Fluorescent Probes for Two-Photon Excitation Microscopy

Christoph J. Fahrni[1,2] (fahrni@chemistry.gatech.edu)

[1]*School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA, USA*
[2]*Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA, USA*

The spatiotemporal detection of exchangeable Zn(II) ions within the complex chemical environment of live cells requires sensitive analytical tools and reagents. Developed more than 30 years ago for the visualization of calcium in neurobiology, ratiometric fluorescence imaging stands out as it allows for quantitative dynamic visualization of metal ion fluxes in live cells. To harness also the benefits of two-photon excitation microscopy (TPEM), which include increased depth penetration, reduced phototoxicity, and intrinsic 3D imaging capabilities, we developed a family of Zn(II)-selective fluorescent probes that offer a significant two-photon absorption cross-section and respond with an emission shift upon Zn(II)-binding suitable for ratiometric image analysis. In addition to achieving a balanced two-photon absorption cross section between the free and Zn(II)-saturated probes, significant efforts were devoted towards minimizing undesired non-radiative deactivation pathways, including suppression of a twisted intramolecular charge transfer state and an excited state proton transfer process. With Zn(II) dissociation constants in the low nanomolar range, the probes are well suited for visualizing physiologically relevant changes of mobile zinc levels in cells and tissues.

This research was supported by the National Institutes of Health through grant GM136404

Manganese: Unsung Hero for Physiology?

Young-Ah Seo[1] (youngseo@umich.edu)

[1]*Department of Nutritional Sciences, University of Michigan School of Public Health, Ann Arbor, MI, USA*

Manganese (Mn) is an essential yet underappreciated nutrient required for proper growth and physiological processes, such as bone formation, immune response, and carbohydrate metabolism. The essentiality of Mn stems from its role as a cofactor for various enzymes, including galactosyl transferase, Mn superoxide dismutase, arginase, and glutamine synthetase, and pyruvate decarboxylase. Genetic, dietary, or environmental factors can cause imbalances in this essential metal, resulting in either deficiency or excess. Sequelae of such imbalances not only pose scientific challenges but also offer opportunities to unravel the underlying molecular underpinnings, thus yielding profound implications for public health and clinical practice. Despite its vital importance, little is known regarding how Mn is regulated in the body and how its dysregulation contributes to various human diseases. The overarching goal of my research program is to decipher fundamental roles of Mn and its transporters in physiology and related disorders. Currently, my research program focuses on new functions of Mn and its transporters, which undergo mutations linked to a variety of traits and diseases, including intestinal and neurological disorders. We employs novel combinatorial approaches, comprising reverse genetics, murine phenotyping, primary organoid cultures, and cutting-edge functional genomics utilizing next-generation sequencing. I will introduce one of our lab's projects regarding the physiological roles of Mn in the intestines and its involvement in the development of inflammatory bowel disease (IBD). Our research holds substantial translational potential to develop new diagnostic and therapeutic strategies to treat disorders of metal imbalance.

NOTES

Elemental Mapping of Mg-Based Bioabsorbable Vascular Implant Derived Metals

Weilue He[1] (weilueh@mtu.edu), Amani Gillette[2], Keith MacRenaris[3,5,6], Thomas O'Hallaran[3,5,6], Roger J. Guillory II[4*] (rguillory@mcw.edu)

[1]Department of Biomedical Engineering, Michigan Technological University, Houghton MI, USA
[2]Morgridge Institute for Research, Madison, WI, USA
[3]Quantitative Bio Element Analysis and Mapping (QBEAM) Center, Michigan State University, USA
[4]Department of Biomedical Engineering, Medical College of Wisconsin, Milwaukee, WI, USA
[5]Departments of Microbiology and Molecular Genetics and Chemistry, Michigan State University, East Lansing, MI, USA.
[6]Elemental Health Institute, Michigan State University, East Lansing, MI, USA

To minimize post-surgical complications, newly engineered stents must support healthy reendothelialization, prevent or avoid excessive inflammation, and avoid smooth muscle cell hyperplasia responses during the lifetime of the stent. The new-generation stents to address these limitations are bioresorbable metallic stents. Magnesium (Mg) is the most prominent candidate, which has shown preclinical safety, biocompatibility, and encouraging corrosion profile and mechanical strength *in vitro* and *in vivo*. To enhance the overall mechanical properties of Mg alloys, solid solution and particle dispersion strengthening via adding rare earth elements (REEs) has been the dominant mechanism, including REEs such as yttrium (Y) and neodymium (Nd). As common formulation of the Mg-Y-REEs alloys ("WE" series) dominates the transportation industry, concerns of the biocompatibility of exotic REEs grow. The major challenge in understanding REE biocompatibility is the lack of information regarding *in situ* local implant derived metals, and their subsequent cellular/tissue fate and concentrations. In this study, we used the REE bearing Mg alloy WE22 wire implanted within the abdominal aorta of transgenic hypercholesterolemia mice (APOE-/-) to simulate a bioresorbable vascular prosthesis in a diseased artery for up to 30 days. We demonstrate that *in situ* laser ablation inductively coupled plasma time of flight mass spectrometry (LA-ICP-TOFMS) is a critically important elemental mapping tool to aid in biocompatibility analysis of bioabsorbable metal alloys, both qualitatively and quantitatively.

Examining the Interplay between Toxic Elements using Synchrotron Radiation

Graham George[1] (g.george@usask.ca)

[1]*Department of Geological Sciences and Chemistry, University of Saskatchewan, Saskatoon, Saskatchewan, Canada*

Three elements that pose significant problems for human populations are arsenic, mercury and selenium. Arsenic compounds are notorious poisons, and the compounds of mercury can be more deadly than those of any other non-radioactive heavy element. Arsenic in drinking water gives rise to health problems for more than 100 million people worldwide, while mercury pollution has been dramatically increasing in recent years and is now at an all-time high. The compounds of selenium are also toxic at approximately the same level as arsenic, with the important difference that at levels lower than those that are toxic, selenium is essential for health, playing vital roles in redox homeostasis and the endocrine system. The difference in dose between the toxic levels and those required for good health is narrower than for any other element. Both mercury and arsenic have a complex relationship with selenium, which can both cancel and exacerbate the toxicities of the compounds of both arsenic and mercury. X-ray fluorescence imaging, in combination with X-ray absorption spectroscopy, gives insights into the complex interplay between selenium and the other two toxic elements. Recent results will be discussed, with an emphasis on risks posed to human health.

DPB D: Effect of Iron Deficient Diet on Metal Homeostasis of Mouse Tissues, and Inorganic Signatures of Heart Failure Mouse Models

Bongjin Hong[1,4] (hongbong@msu.edu), Yuki Tatekoshi[5], Jason S. Shapiro[5], Qiaoling Jin[6,7], Keith MacRenaris[1,3,4], Hossein Ardehali[5], and Thomas V. O'Halloran[1,2,3,4]

[1]Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI, USA
[2]Department of Chemistry, Michigan State University, East Lansing, MI, USA
[3]Quantitative Bio-Element Analysis and Mapping (QBEAM) Center, Michigan State University, East Lansing, MI, USA
[4]Elemental Health Institute, Michigan State University, East Lansing, MI, USA
[5]Feinberg Cardiovascular Research Institute, Northwestern University, Evanston, IL, USA
[6]Department of Physics and Astronomy, Northwestern University, Evanston, IL, USA
[7]Advanced Photon Source, Argonne National Laboratory, Lemont, IL, USA

Iron is a critical element for cellular function, but because disorders of iron homeostasis are common and often result in serious diseases, an iron level in a body is tightly regulated. Iron deficiency (ID) is the most prevalent nutritional disorder in the world, affecting over a quarter of the world's population. Usually, ID is diagnosed by examining blood iron and protein contents. To understand how the ID disturbs metal homeostasis in a body which leads to relevant diseases, however, its effect should be scrutinized at a tissue and cell level. Previous animal studies reported that ID could change the homeostasis of various metals in tissues and its effect vary depending on tissue types, but these results are often conflicting each other, probably due to limitation in detection accuracy and sensitivity of their measurement methods. In addition to the animal studies, recent clinical studies also showed a big controversy in iron therapy in in patients with cardiac diseases. While intravenous (IV) iron therapy provides symptomatic benefit to patients with heart failure, chelation therapy also improves outcome in patients with cardiovascular disease. Thus, given the significant controversy in the field and widespread use of IV iron in patients with heart failure, it is critical to perform a comprehensive analysis of the cardiovascular system in ID conditions. With the aid of an inductively coupled plasma mass spectrometry (ICP-MS), X-ray fluorescence microscopy (XFM), and laser ablation-inductively coupled plasma-time-of-flight mass spectrometry (LA-ICP-TOF-MS), I have been studying the effect of ID diet on metal homeostasis of various mouse tissues and determining the levels of iron and other essential metals in normal heart and in heart failure at baseline and with systemic ID. In this presentation, the relevant research results will be demonstrated.

Iron Deposition in Epididymis Linked to Fibrosis and Infertility in Autoimmune Regulator Deficient Male Mice

Soo Hyun Ahn[1] (ahnsooh1@cvm.msu.edu), Geoffrey Grzesiak[1], Katrina Halgren, Margaret G. Petroff[1,2,3], and Thomas V. O'Halloran[4,5,6,7]

[1]Department of Pathobiology Diagnostic Investigation, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA.
[2]Institute for Integrative Toxicology, Michigan State University, East Lansing, MI, USA.
[3]Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI, USA
[4]Department of Chemistry, Michigan State University, East Lansing, MI, USA
[5]Elemental Health Institute, Michigan State University, East Lansing, MI, USA
[6]Quantitative Bio-Element Analysis and Mapping Center (QBEAM), Michigan State University, East Lansing, MI, USA

The epididymis is an elongated tubule where testicular sperm mature and gain fertilization competence. Inflammation of the epididymis - epididymitis - is a contributing factor to male infertility, which can arise from an underlying autoimmune disorder such as Autoimmune Polyglandular Syndrome Type 1 (APS-1). APS-1 is caused by a monogenic mutation in Autoimmune regulator (Aire) which is a transcription factor that is expressed in the thymus. By regulating the expression of peripheral tissue-specific antigens, Aire ensures generation of a T cell receptor repertoire that is tolerant to self-antigens but averse to foreign antigens. Among many symptoms, men with APS-1 can suffer from testicular insufficiency and infertility. The objective of this research is to determine the lesions in the male reproductive tract that cause male infertility in APS-1. Mice with targeted mutations in Aire were generated by CRISPR/Cas9 recombination, resulting in multiple base-pair deletion in Exon 2 of Aire (Aire-/-), as well as Aire-/-;Rag1 -/-, which lack both Aire and Recombinase Activating Gene 1 (Rag1), and therefore are T- and B cell deficient. Wild type mice served as controls. Immunohistochemistry and Masson's trichrome were used to characterize the immune cells and fibrosis in the epididymis, respectively. Laser assisted Inductively coupled plasma time of flight mass spectrometry (LA-ICP-TOF-MS) was used to spatially map and quantify iron concentration in the epididymis. ICP-QQQ-MS was used to quantify concentration of trace elements in the epididymis. Aire-/- males had severely reduced fertility compared to WT and Aire-/-;Rag1-/- males. All Aire-/- males (n=10) had epididymitis, infiltration of CD8+ T and CD19+ B cells and fibrosis. LA-ICP-TOF-MS revealed increased iron deposition in the interstitial space of the epididymis, particularly in the corpus and cauda epididymis, but absent in the caput, and were observed in male mice as young as 9 weeks of age. No immune cell infiltration, fibrosis, or iron deposition were observed in Aire-/-;Rag1-/- mice, suggesting that T and B cells target the epididymis and contribute to fibrosis. These results reveal the importance of Aire-mediated immune tolerance to the male reproductive tract and suggest that in the absence of Aire, T and B lymphocytes target the epididymis and contribute to fibrosis and iron deposition. Further studies will reveal the specific role of lymphocytes in causing these changes, and the significance of iron deposition.

NOTES

Student/Post Doc Session

Optimization of LA-ICP-TOF and MALDI-TOF Imaging Mass Spectrometry for *Caenorhabditis elegans* Research

Aidan Reynolds[1] (reyno569@msu.edu), Aaron Sue[2,3], Ryutaro (Einar) Jacobson[1], Elizabeth Smith[1,3], Keith MacRenaris[2,3,4], Thomas O'Halloran[1,2,3,4], Tian (Autumn) Qiu[1]

[1]Department of Chemistry, Michigan State University, East Lansing, MI, USA
[2]Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI, USA
[3]Quantitative Bio-Element Analysis and Mapping (QBEAM) Center, Michigan State University, East Lansing, MI, USA
[4]Elemental Health Institute, Michigan State University, East Lansing, MI, USA

Elucidating the spatial distribution and co-localization of endogenous metabolites, proteins, and xenobiotics enables a deeper understanding of biological processes. Caenorhabditis elegans (C. elegans) is an established model organism for in vivo research due to its tractable genetics, ease of culturing, and rapid growth rate. While many optical and genetic tools were developed to understand the spatial biology of C. elegans, their small body size has posed limitations to profile elements and chemicals with spatial resolution through imaging mass spectrometry (IMS) techniques. Additionally, C. elegans has a collagenous cuticle which prevents the detection of analytes inside the body via "soft ionization" IMS techniques including the commonly used matrix-assisted laser/desorption ionization (MALDI). Herein we present advancements made in C. elegans sample preparation, method development and optimization to facilitate IMS using laser ablation inductively coupled plasma time-of-flight (LA-ICP-TOF) and MALDI-TOF to perform in situ analysis of C. elegans and map their elemental and chemical distributions. We optimized sample preparation and instrumental analysis of LA-ICP-TOF IMS and successfully showed the distribution of endogenous and toxic heavy metals with a pseudo-2µm spatial resolution achieved by oversampling, while preserving major anatomical regions of L4 and gravid adult C. elegans. Embedding and cryo-sectioning methods was developed for MALDI-TOF IMS and we successfully showed the spatial distribution of lipids and metabolites with 10-µm spatial resolution. Ultimately, we aim to develop multi-modal IMS methods to provide a toolkit to study multiple classes of analytes within C. elegans and link spatial -omics profiles to biology.

Multimodal Imaging of the Gastrointestinal Tract of Zebrafish

Jacquelyn Spathies[1,2] (jacquelyn.spathies@vanderbilt.edu), Caitlin C. Murdoch[2,3], Eric P. Skaar[2,3], Jeffrey M. Spraggins[1-4]

[1]*Mass Spectrometry Research Center, Vanderbilt University, Nashville, TN, USA*
[2]*Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN, USA*
[3]*Vanderbilt Institute for Infection, Immunology and Inflammation, Vanderbilt University School of Medicine, Nashville, TN, USA*
[4]*Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN, USA*

Pathogens who inhabit mammalian hosts must attain metals as essential trace nutrients in the face of host nutritional immunity. The role of manganese in bacterial virulence is well established; however, the role of Mn in eukaryotic pathogenesis is by comparison, poorly understood. *Candida albicans* is an opportunistic and polymorphic fungal pathogen that can cause systemic infection in immunocompromised individuals. During a murine model of systemic candidiasis, total Mn levels in infected tissues such as the kidney decline, indicative of host limitation of manganese, but impacts of low manganese on fungal growth and pathogenesis has not been investigated. We aim to develop a deeper understanding of how *C. albicans* accesses the trace nutrient Mn, and to define the role of Mn in fungal virulence. *C.albicans* has a gene family of four NRAMP transporters, a gene class of divalent metal transporters that was originally identified in human macrophages for withholding metal nutrients from microbial pathogens. Three out of the four *C. albicans* NRAMP transporters are uncharacterized. We generated CRISPR null mutations in each of the uncharacterized NRAMP transporters and found that two members of this family, Smf12 and Smf13, are Mn transporters that have nonredundant roles in Mn acquisition and Mn- dependent enzyme activity. The single mutants *smf12Δ* and *smf13Δ* have a tenfold reduction in cellular Mn, but no change in total cellular Fe or Cu, and the effect of a double *smf12 smf13* mutants is additive. Decreased cellular manganese in these mutants impacts Mn- dependent enzymes. The mutants have defective SOD activity for both the mitochondrial SOD2 and the novel cytosolic Mn SOD3 of this organism, as well as loss of Mn-dependent mannosyl transferase (MNT) activity. Defects in MNT activity result in decreased protein mannosylation of vacuolar and cell wall proteins. Moreover, as mannose residues comprise the major outer layer of the fungal cell wall, the *smf12* and *smf13* mutants both exhibit deficiencies in the protective phospho-mannan layer of the cell wall. Furthermore, both mutants have a clear defect in hyphal morphology, showing a deficiency in the transition from yeast-form to invasive hyphal filament morphology. Using the murine disseminated model of candidiasis where kidney is the major target organ, we find that both *smf12Δ* and *smf13Δ* mutants have virulence defects. The roles of Mn in both fungal growth and host recognition of the fungi in this reduced virulence will be discussed. Altogether, this work provides the first linkage between the nutritional requirement of Mn and virulence in a fungal pathogen.

Lysosomes Contain an Expansion Compartment that Mediates Zinc Transporter Delivery to Promote Zinc Homeostasis in *C. elegans*

Adelita D. Mendoza[1] (adelitadmendoza@wustl.edu), Kerry Kornfeld[1]

[1]*Department of Developmental Biology, Washington University School of Medicine, St. Louis, MO, USA*

Zinc is a transition metal that is essential for all life. The Kornfeld lab uncovered conserved, homeostatic mechanisms respond to zinc excess and zinc deficiency in *C. elegans*.

Lysosomes are a site of zinc storage and release. Zinc mobilization from the lysosome is mediated by zinc transporters CDF-2 and ZIPT-2.3 that are reciprocally regulated at the mRNA and protein levels. Super resolution microscopy experiments showed that in addition to housing CDF-2 and ZIPT-2.3, lysosomes alter their morphology in response to cytosolic zinc and form acidified and the expansion compartments. ZIPT-2.3 and CDF-2 surround the acidified compartment, while CDF-2 surrounds the expansion compartment. The acidified compartment is comprised of LysoTracker and zinc regions. This configuration is significant because it allows the lysosome to participate in canonical degradation activities while conducting zinc metabolic activities.

The expansion compartment is highly dynamic and deviates greatly in size. Our model proposes that lysosome restructuring facilitates the rapid turnover of transporters needed to store or release zinc. We therefore predicted that the expansion compartment grows and shrinks to accommodate zinc. Time course super resolution microscopy with worms expressing CDF-2::GFP and ZIPT-2.3 mCherry in excess zinc shows a time-dependent increase of the expansion compartment, suggesting the expansion compartment continually stores zinc. Preliminary genetic analysis with hlh-30 and hizr-1 lof mutants shows that these genes are necessary for the assembly of compartments.

Labile zinc occupies the acidified compartment. However, CDF-2 presence on the expansion compartment membrane suggests total zinc is present in the lumen. Preliminary X-Ray Fluorescence Microscopy (XFM) on isolated lysosomes shows elevated total zinc levels in lysosomes from worms grown in zinc excess conditions, and presence of total zinc in the expansion compartment, suggesting that CDF-2 is mobilizing zinc into the lysosome.

Our work with *C. elegans* shows that lysosomes are a critical site of zinc trafficking and metabolism by utilizing zinc transporters and by altering morphology to compensate for perturbations in zinc levels. Future studies will involve elucidating how the structure facilitates zinc homeostasis in *C. elegans* and human model systems.

NOTES

Poster Abstracts

All presentations and abstracts will be posted on the QE-Map website at https://qemap.ehi.msu.edu/qe-map-workshop-2023 following the workshop

Quantitative Bio Element Analysis and Mapping (QBEAM) Center

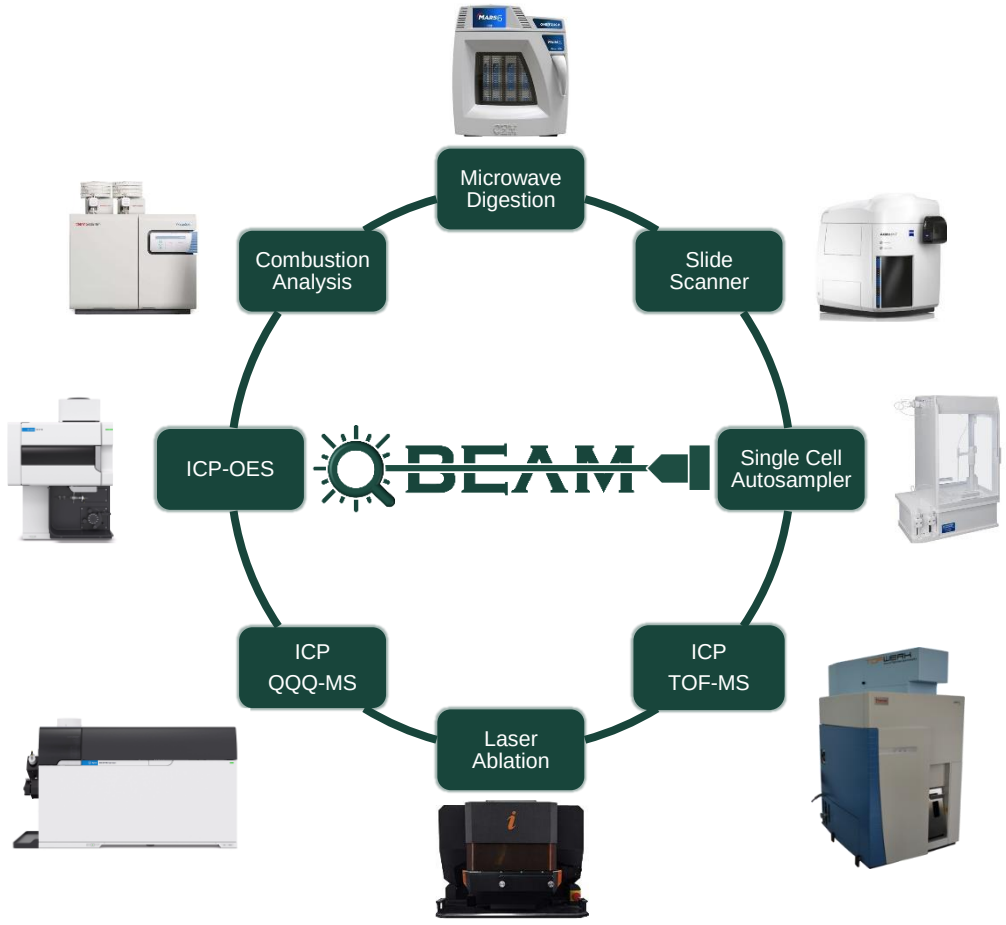
Keith MacRenaris[1,2,3] (macrenar@msu.edu), Aaron Sue[1,2,3], and Thomas V. O'Halloran[1,2,3]

[1]Departments of Microbiology and Molecular Genetics and Chemistry, Michigan State University, East Lansing, MI, USA
[2]Elemental Health Institute, Michigan State University, East Lansing, MI, USA
[3]Quantitative Bio-Element Analysis and Mapping Center (QBEAM), Michigan State University, East Lansing, MI, USA

The Quantitative Bio Element Analysis and Mapping (QBEAM) center was founded by Professor Thomas V. O'Halloran at MSU in January of 2021 as an interdisciplinary center within the elemental Health Institute (EHI) focused on elemental mapping and analysis. QBEAM's mission is examining elemental quotas in biology from single cells to whole organisms and how the interplay between metals and systems biology can be harnessed to develop therapeutics, elucidate the mechanisms of disease, and further our understanding of the ecological and environmental consequences of metal exposure.

The center has developed a suite of high-resolution instruments capable of quantitatively imaging biologically essential elements in individual cells. QBEAM's instrumentation has enabled teams of physical, life, and material scientists to begin analyzing metal quotas at scales ranging from the subcellular level to entire ecosystems shaping global biogeochemical cycles. This work is expected to yield a fundamental understanding of the co-evolution of microbial and eukaryotic life within a broad range of challenging chemical environments.

Complementing QBIC's research mission is its role as a shared resource facility serving investigators within the Northwestern scientific community and beyond. In this capacity, the Center provides researchers with access to state-of-the-art imaging and quantification instrumentation while supporting its use with an expert technical staff that offers a range of services, including instrument training, sample preparation and analysis, experiment design, and grant proposal assistance.



Functional Studies on Human ZIP Transporter using Non-Radioactive Metal Substrates

Yuhan Jiang[1,2] (jiangy60@chemistry.msu.edu), Jian Hu[1,2]

[1]Department of Chemistry, Michigan State University, East Lansing, MI, USA
[2]Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, USA

The Zrt-/Irt-like protein (ZIP, SLC39) family is one of the two Zn transporter families in human and it also plays crucial roles in homeostasis for other divalent d-block metals such as Fe and Mn. Despite the increasing structural knowledge in the past few years, more biochemistry data for this transporter family are required. Here, we present a non-radioactive measurement by combining stable isotopes and inductively coupled plasma-mass spectrometry (ICP-MS). The culture media was firstly treated with chelex-100 resin to remove over 90% of the Zn, and enriched-70Zn was added. The resulting 70Zn-enriched media was then used to incubate cells and facilitate quantification of 70Zn by ICP-MS. Our new method demonstrated same pattern in time-dependent and dose-dependent assays for human ZIP4 as it showed in radioactive assays. Taking advantage of this platform we calculated Kcat for human ZIP4 and two mutants, and evaluated the metal efflux during metal uptake assays. We also explored laser ablation-ICP-MS (LA-ICP-MS), a potential high-throughput method for future biochemical studies.

Using Small Molecules to Identify Critical Host-Cellular Pathways for *Brucella* Macrophage Infection

Thomas Kim[1] (kimthom6@msu.edu), Erika Lisabeth[2], Richard Neubig[2], Bill Hong[3], Thomas O' Halloran[3], Aretha Fiebig[1], Sean Crosson[1]

[1]*Department of Microbiology & Molecular Genetics, Michigan State University, East Lansing, MI, USA*
[2]*Department of Pharmacology & Toxicology, Michigan State University, East Lansing, MI, USA*
[3]*Department of Chemistry, Michigan State University, East Lansing, MI, USA*

Brucellosis is a highly contagious zoonosis caused by exposure to animal products contaminated with bacteria of the genus *Brucella.* In addition to the burden it inflicts on human health, brucellosis causes devasting losses to the livestock industry and small-scale livestock holders in many areas of the world. Current understanding of *Brucella* infection and macrophage trafficking have been characterized through the identification of bacterial virulence factors, however, the host-factors required for infection remain elusive. The identification of host-cellular processes involved in *Brucella* infection is critical for the development of novel host-targeted therapeutic interventions. Host-directed therapy is a treatment strategy that aims to target essential host-cellular processes that are required for pathogen survival. By targeting the host rather than the bacteria itself, antimicrobial resistance is less likely to develop, mitigating the development of multi-drug resistance. We have used a pharmacologic approach to identify possible host-cellular processes that are involved in *Brucella* infection. We conducted a small molecule screen utilizing the non-human pathogen *Brucella ovis*, a close relative of zoonotic causing *Brucella.* We screened 8000 small molecules in THP-1 macrophage-like cells infected with a constitutively expressing luminescent strain of *Brucella ovis*. Our screening efforts resulted in 147 primary hits and multiple hits consisted of FDA approved small molecules that target Ca^{2+} homeostasis, many of which are clinically indicated for cardiovascular diseases. Our follow up studies have confirmed that the Ca^{2+} channel targeting small molecules kill *Brucella ovis* and *Brucella abortus* intracellularly in our THP-1 infection model. These preliminary data show that FDA approved Ca^{2+} modulators are an effective host-directed small molecule in vitro and more importantly show that Ca^{2+} homeostasis may play an essential role in *Brucella* pathogenesis in the macrophage.

Mapping of Elemental Content in Semisoft Biological Samples

Aaron Sue[1,5,6] (sueaaron@msu.edu), Kyleen Hall[1], Aidan Reynolds[2], Keith MacRenaris[1,5,6], Brandon Reagan[3], Federica Brandizzi[3,4], Tian (Autumn) Qiu[2], Thomas O'Halloran[1,2,5,6]

[1]*Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI, USA*
[2]*Department of Chemistry, Michigan State University, East Lansing, MI, USA*
[3]*Department of Plant Biology, Michigan State University, East Lansing, MI, USA*
[4]*MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI, USA*
[5]*Elemental Health Institute, Michigan State University, East Lansing, MI, USA*
[6]*Quantitative Bio-Element Analysis and Mapping Center (QBEAM), Michigan State University, East Lansing, MI, USA*

Cells have several strategies for mobilizing elements in response to intrinsic and extrinsic stimuli, which may alter the elemental distribution within a cell, tissues, organs, and even whole organisms. Identifying these fluxes in elements can indicate possible impacts on biological processes, or how disturbances in biological processes can affect how an organism meets elemental quota. Significant advances in technology and methodology have given an unprecedented view of elements in both spatial resolution and sensitivity for a variety of human and mice tissues. As biological studies using elemental mapping techniques become more prevalent in understanding the interface between metals and biology, new challenges will arise as a result of employing other model systems besides mammals. Although nonhuman mammalian model systems are generally considered the most representative of human biology, understanding the metal content of evolutionarily distant organisms is useful for uncovering elemental biology that is applicable beyond the scope of just that species. Samples that have cell walls or chitinous or collagenous exoskeletons may alter not only sample preparation, but also microscopic or spectroscopic behaviors. This necessitates developing methodologies for mapping elements in biological samples that may have variable physical properties.

We utilized two model organisms, *Caenorhabditis elegans* and *Arabidopsis thaliana*, to investigate preparation and mapping techniques that will allow for optimal structure preservation and highest resolution mapping. Both systems are fast growing, isogenic, genetically tractable, have a low cost of maintenance, and have shared biology with distantly related species. *C. elegans* are small enough to be mapped in their entirety, providing a view of the elemental content of an intact system, while we investigated *A. thaliana* pistils due to the large size of the whole *Arabidopsis* flower. Both samples were mapped using a laser ablation inductively coupled plasma time-of-flight mass spectrometer (LA-ICP-TOF-MS). We found that flash freezing nematodes in a uniform layer of optimal cutting temperature (OCT) compound was sufficient to preserve nematode structure and allowed for complete ablation. *Arabidopsis* provided a greater challenge due to the impermeability of the cell wall to OCT as well as pistil thickness. Despite suboptimal sectioning conditions, we were still able to visualize accumulation of specific elements in specific structures of the pistil. Although sample preparation optimization is not complete, we uncovered interesting elemental signatures in these model systems, particularly the manganese distribution in both nematodes and thale cress pistils. Given the sparse data on manganese biology in nematodes and manganese reproductive biology in thale cress, these data demonstrate the value of examining elemental distributions, and how samples can require specific preparation techniques.

MALDI Mass Spectrometry Imaging of *Caenorhabditis elegans*

Ryutaro E. Jacobson[1](jacob433@msu.edu), Elizabeth Smith[1], Tian (Autumn) Qiu[1]

[1]*Department of Chemistry, Michigan State University, East Lansing, MI, USA*

The chemical and biological heterogeneity of cells and tissues is crucial for their biological functions and responses in the spatial context. The nematode *Caenorhabditis elegans* is a classical model organism for biomedical sciences. With its transparent body and tractable genetics, many optical tools have been developed to understand the biological mechanisms at specific locations. However, the spatial chemical profiles of *C. elegans* have not been resolved in situ due to the challenges from its small body size (~1 mm long and ~50 µm width for an adult hermaphrodite) and their thick collagenous cuticle structures. To address this gap, we developed a sample preparation workflow to enable matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) of *C. elegans* with high spatial resolution. Synchronized adult *C. elegans* were mixed with embedding medium, followed by freezing. A series of embedding media compositions and different freezing methods were tested to optimize the embedding process for the subsequent cryosectioning. To aid the identification of cryo-sectioned worm sections, we utilized a *C. elegans* transgenic strain with pan-neuronal fluorescent reporter (otIs356 [rab-3p(prom1)::2xNLS::TagRFP] V). Cryo-sectioned thin slices of 5-20 µm thickness were thaw-mounted onto conductive slides and examined under bright field and fluorescence microscope. Chemical matrix was then spray-coated onto the cryo-sectioned slices for MALDI-MSI. We found that the fluorescence transgenic markers are preserved in embedded, cryo-sectioned *C. elegans* to guide the identification of *C. elegans* anatomy with brightfield images. MS imaging with 10 µm spatial resolution was achieved, and differential distribution of m/z features for metabolites and lipids was observed in worm cross-sections at different locations. Future work will optimize the embedding protocol for minimal structural deformation, the matrix spraying methods for better analyte extraction, and mass spectrometry methods for better sensitivity and higher resolution.

Statistical Heterospectroscopy of MALDI Imaging and NMR Spectroscopy Data for Evaluation of Breast Tumor Models

Dalton Brown[1], Caitlin M. Tressler[1, 2], Cole Johnson[1], Sophia Nakuchima[1], Ethan Yang[1, 2], Kristine Glunde[1, 2] (kglunde1@jhmi.edu)

[1]Johns Hopkins AIMS Core, Baltimore, MD; [2]Johns Hopkins School of Medicine, Baltimore, MD

Introduction

Matrix Assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) Mass Spectrometry Imaging (MSI) generates extensive peak lists with spatial encoding yet identifying analytes by tandem mass spectrometry (MS) is time-consuming. Proton High Resolution Nuclear Magnetic Resonance (^{1}H-HR-NMR) spectroscopy conversely provides analyte identification and quantitative information, but no spatial information. Combining these two complementary approaches will significantly enhance our knowledge gained. Here we have developed Statistical Heterospectroscopy (SHY) of ^{1}H-HR-NMR and MALDI-TOF MSI, supplementing the lack of spatial resolution of H^1-HR-NMR with spatially resolved MALDI imaging data while adding ^{1}H-HR-NMR based analyte identification.

Methods

Standards as detailed below were prepared in 50% ACN with 0.1% TFA and spotted on an MTP 384 target plate. Spectra were collected on a Bruker RapifleX MALDI TOF/TOF instrument in reflectron positive ion mode with 300 shots per pixel and a 20 µm laser for m/z 40-1000 Da. Similar solutions were prepared in deuterated water for NMR experiments. Proton spectra were collected with a 5-mm TXI probe on a Bruker Avance-III 750 MHz NMR spectrometer with 8 scans. Spectra were binned from either Topspin for NMR data or mMass for MALDI imaging data and exported as a .txt file. These .txt files were imported into MATLAB where necessary transformations and correlation computations were performed.

Preliminary Data

SHY has previously been reported for UHPLC data correlated with NMR data, however, no code has been published to allow others to use this method. We have built a MATLAB-based software which allows for the correlation of the spectral domain of MALDI imaging data with NMR spectroscopy data. To test our new software, we have acquired NMR, MALDI target plate, and MALDI imaging data from a set of standards, including glutamine, glutamate, phenylalanine, taurine, and a 1:1:1:1 mixture of all four components. These standards were measured by both ^{1}H-HR-NMR and on a MALDI target plate. Spectra were binned to the same size using 7923 data points and imported into MATLAB for analysis. We are currently testing the homebuilt software with these data sets. We have also harvested SUM159 and MDA-MB-231 tumor xenografts from athymic nude mice. We have chosen these two tumor models as they are well studied models of triple negative breast cancer. Currently, triple negative breast cancer has no targeted treatment options and has the highest rate of mortality in breast cancer patients. These tumors have been cryosectioned for MALDI imaging with interleafed samples taken for dual phase extraction for NMR spectrometry. We have begun measuring these tumors using both modalities. For MALDI imaging experiments, we are measuring tumor sections using negative ion mode from m/z 0-500 Da to detect metabolites including lactate, taurine, and amino acids, among others. We are using positive ion mode from m/z 400-1000 Da to measure phospholipids. For NMR spectroscopy, we are measuring both the aqueous phase containing water-soluble metabolites and the organic phase containing phospholipids. We will bin and correlate these data in our new software to investigate the similarities and differences in both small molecules and lipids in our two triple negative breast cancer tumor models.

Innovative Software Solutions for Measuring Analyte Delocalization in MALDI Imaging

Cole C Johnson[1]; Dalton R Brown[1]; Caitlin M. Tressler[1]; Nathan Riemann[1]; Jason Fan[1]; Kristine Glunde[1] (kglunde1@jhmi.edu)

[1]*Johns Hopkins School of Medicine, Baltimore, MD*

Introduction

Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) can be used to detect the spatial distribution of a wide range of analytes in tissue sections. As the spatial information provided by MALDI MSI is its primary advantage over non-imaging mass spectrometry approaches, preserving the localization of analytes throughout the MALDI MSI pipeline is of paramount importance. We have previously shown that delocalization of analyte signals in MALDI MSI depends on the method of matrix deposition. Our previous work evaluated the effects of matrix spraying parameters on delocalization, however, established methods of manually measuring delocalization were found to be imprecise. Here we developed a new software tool that measures delocalization and applied it to MALDI MSI experiments to minimize delocalization.

Methods

Fresh frozen heart, kidney, and brain tissues harvested from athymic nude mice were cryo-sectioned at 10 µm thickness onto indium tin oxide (ITO) slides using a Leica cryostat. Samples were sprayed with 10 mg/mL 1,5-diaminonapthalene (DAN) matrix in 50%, 70%, or 90% acetonitrile (ACN) with 0.2% trifluoroacetic acid (TFA) using an HTX M5 Sprayer. Sections were then imaged using a Bruker MALDI TOF/TOF rapifleX instrument running in reflectron positive mode with 100-micron raster, 100-micron m5 imaging laser, and mass range of *m/z* 0-3200. Delocalization was measured using a home-built software written in R that subsets MALDI imaging data into on-tissue and off-tissue sections, then measures the shortest distance from each point on the delocalization front to the tissue boundary.

Preliminary Data

To test our software, we re-analyzed data sets published in Tressler et al (JASMS 2021) which demonstrated the use of factorial design of experiments (DOE) to optimize spraying methods for matrix deposition in MALDI imaging. These data sets were re-analyzed using our home-built R-based software and compared with the published manual delocalization measurements. Our software measures delocalization along the entire tissue boundary rather than only at four representative averaged points as in the manual approach. For several of the tested matrix spraying conditions, we found a significant difference in the amount of delocalization when comparing manual with automated delocalization measurements. We then re-ran the DOE based on the automated delocalization measurements to determine new optimized spraying conditions. These re-analyzed DOE data show that tray temperature, flow rate, nozzle speed, and the combination of flow rate and nozzle speed were all key factors that affected the extent of delocalization. Previously, when basing the DOE optimization on manual delocalization measurements, only the first three factors were significant. Using our automated delocalization measurement software, we calculated a new optimized set of spraying parameters, which we tested experimentally, and which resulted in less delocalization than previously reported for DOE optimization based on manual delocalization measurements. One predominant theory suggests that higher water content in the solvent for matrix spraying leads to more analyte delocalization. To test this theory, we are currently working with data sets in which we are using our new software to measure the delocalization under different solvent conditions for matrix spraying. Our data indicate that not all analytes delocalize under the same solvent conditions for matrix spraying. We have selected four different peaks across the m/z range measured in four different organs for analysis with our delocalization software to understand the role of organ type and solvent composition on analyte delocalization.

MALDI Imaging of Live Patient Brochoalveolar Lavage Samples from COVID-19 Patients

Caitlin M. Tressler[1], Alvaro Ordonez[2], Kristine Glunde[1,3] (kglunde1@jhmi.edu)

[1]The Russell H. Morgan Department of Radiology and Radiological Science and [2]Department of Pediatrics, [3]The Sidney Kimmel Comprehensive Cancer Center, The Johns Hopkins University School of Medicine, Baltimore, MD, United States

Introduction

The SARS-CoV-2 is a respiratory virus responsible for the COVID-19 pandemic results in a wide range of severity from mild cold or flu-like symptoms to hospitalization and death. In cases of hospitalization, many patients have to be intubated due to difficulty breathing and excessive fluid in their lungs. Patients who ultimately recover from COVID-19 are often left with prolonged pulmonary, cardiac, and neurological side effects. Both immediate, severe disease and long-term side effects are believed to be a result of a dysregulated immune response. Mechanistic information of the molecular events leading to severe COVID-19 disease and recovery are key in understanding the long-term effects and potential novel treatment strategies.

Methods

Bronchoalveolar lavage samples were taken from consenting patients admitted to the Johns Hopkins Hospital during 2020 with severe COVID-19 disease requiring intubation. Samples were deposited on poly-L-lysine coated indium tin oxide slides using a cytospin centrifuge and formalin fixed to ensure decontamination of the SARS-CoV-2 virus. Samples were stored at -20 °C until imaging. Samples were sprayed on an HTX M5 sprayer with 40 mg/mL 2,5-dihydroxybenzoic acid in 50% acetonitrile with 0.2% trifluoracetic acid. Samples were imaged at 20-micron spatial resolution in positive ion mode with 200 laser shots per pixel from m/z 0 to 2000. Data was analyzed in FlexImaging and SCiLs Lab software.

Preliminary Data

BAL samples from patients are primarily composed of mucus and immune cells including T-cells, macrophages, neutrophils, among others. The distribution and type of cells vary widely from patient to patient with some patients having one primary cell type and other having a mixture of cell types. No evidence of live virus was found in the samples obtained. The most notable result from these experiments shows an increase of m/z 400.3 which was identified as palmitoylcarnitine (PalC) using tandem MS in patients who survived. In nearly all cases, attempts to isolate or replicate viruses from BAL samples were unsuccessful demonstrating that these patients were past the infectious stage of COVID-19 disease. The two surviving patients showed a significant increase in PalC when compared to the thirteen in the study who did not survive. This lipid is not usually observed in the lungs and is known to activate apoptosis through a caspase cascade in T cells. Our preliminary data demonstrates that this lipid is increased in patients who ultimately survive, indicating a potential mechanism of recovery and biomarker for recovery. We are currently further stratifying the samples based on patient data including flow cytometry data of the makeup of immune cells in each patient. We are also currently imaging hACE2 mouse lungs utilizing the same sample preparation in order to determine overlapping metabolite and lipid in mouse models as compared to patients. These mice express the human ACE2 receptor in their airway epithelia which is the point of entry of the SARS-CoV-2 virus. These data will allow us to better understand the mechanisms of death and recovery in COVID-19 patients and the appropriateness of the hACE2 mice as a model system.

Applied Imaging Mass Spectrometry (AIMS) Core / Service Center

Kristine Glunde[1]

[1]Applied Imaging Mass Spectrometry (AIMS) Core/Service Center, The Johns Hopkins University School of Medicine, Baltimore, MD, United States

The Johns Hopkins Applied Imaging Mass Spectrometry (AIMS) Core / Service Center offers rapid matrix-assisted laser desorption/ionization (MALDI) imaging at high spatial resolution, which includes sample preparation, on-tissue digests and derivatizations, and data analysis. Spatially resolved MALDI imaging measurements are directly taken from a frozen or formalin-fixed paraffin-embedded (FFPE) tissue section without destroying it. MALDI imaging combines mass spectrometric analyses of biomolecules with simultaneous histological evaluation to analyze intact proteins, tryptic peptides (on-tissue tryptic digest), N-glycans (on-tissue PNGase digest), peptides, lipids, metabolites, drug molecules, and drug metabolites in a spatially resolved manner.

In 2022, we have added a second MALDI imaging instrument to the AIMS core through an NIH high-end instrumentation grant (S10 OD030500). This instrument is a timsTOF flex MALDI-2 instrument, which brings three new technologies to the AIMS core: (1) MALDI-2 ionization for significantly enhanced imaging sensitivity, (2) simultaneous trapped ion mobility spectrometry (tims) separation, (3) nano-LC-MS/MS capabilities.

In 2023, we will be adding a laser ablation (LA) inductively coupled plasma (ICP) time of flight (TOF) mass spectrometry imaging instrument to the AIMS Core through an NIH high-end instrumentation grant (S10 OD034239). This instrument will bring multiplexed elemental and metallomic tissue and cell imaging capabilities to the AIMS Core.

The Johns Hopkins AIMS Core is located in the Cancer Research Building 2 (CRBII) in the lower basement in rooms LB03E and LB08.

Machine Learning to Guide Selection of Single E. coli Cells in X-ray Fluorescence Microscopy

Kiwon Ok[1] (okkiwon@msu.edu), M. Arshad Zahangir Chowdury[2], Yanqi Grace Luo[3,4], Si Chen[3,4], Aniket Takewade[2], Thomas V. O'Halloran[1,4,5]

[1]Departments of Microbiology and Molecular Genetics and Chemistry, Michigan State University, East Lansing, MI, USA
[2]Data Science and Learning Division, [3]X-ray Science Division, [4]Advanced Photon Source, Argonne National Laboratory, Lemont, IL, USA
[3]MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI, USA
[4]Elemental Health Institute, Michigan State University, East Lansing, MI, USA
[5]Quantitative Bio-Element Analysis and Mapping Center (QBEAM), Michigan State University, East Lansing, MI, USA

The microscopy research at the Bionanoprobe (beamline 9-ID of Advanced Photon Source) of Argonne National Laboratory focuses on applying synchrotron X-ray fluorescence (XRF) techniques to obtain trace elemental mappings of cryogenic biological samples to gain insights about their role in critical biological activities. The elemental mappings and the morphological aspects of the biological samples, in this instance, the bacterium Escherichia coli (E. coli), also serve as label-free biological fingerprints to identify live and dead cells. The key limitations of achieving good identification performance are the extraction of cells from raw XRF measurement via binary conversion, definition of features, noise floor and proportion of differently treated cells in the measurement. Automating the cell extraction from raw XRF measurements across different types of chemical treatment and the implementation of machine learning models to distinguish cells from the background and their differing treatments will be described. Principal components are calculated from domain knowledge specific features and clustered to distinguish healthy and poisoned E. coli cells from the background without manual annotation.

Effect of Iron Deficient Diet on Metal Homeostasis of Mouse Tissues, and Inorganic Signatures of Heart Failure Mouse Models

Bongjin Hong[1,4] (hongbong@msu.edu), Yuki Tatekoshi[5], Jason S. Shapiro[5], Qiaoling Jin[6,7],Keith MacRenaris[1,3,4], Hossein Ardehali[5], and Thomas V. O'Halloran[1,2,3,4]

[1]Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI, USA
[2]Department of Chemistry, Michigan State University, East Lansing, MI, USA
[3]Quantitative Bio-Element Analysis and Mapping (QBEAM) Center, Michigan State University, East Lansing, MI, USA
[4]Elemental Health Institute, Michigan State University, East Lansing, MI, USA
[5]Feinberg Cardiovascular Research Institute, Northwestern University, Evanston, IL, USA
[6]Department of Physics and Astronomy, Northwestern University, Evanston, IL, USA
[7]Advanced Photon Source, Argonne National Laboratory, Lemont, IL, USA

Iron is a critical element for cellular function, but because disorders of iron homeostasis are common and often result in serious diseases, an iron level in a body is tightly regulated. Iron deficiency (ID) is the most prevalent nutritional disorder in the world, affecting over a quarter of the world's population. Usually, ID is diagnosed by examining blood iron and protein contents. To understand how the ID disturbs metal homeostasis in a body which leads to relevant diseases, however, its effect should be scrutinized at a tissue and cell level. Previous animal studies reported that ID could change the homeostasis of various metals in tissues and its effect vary depending on tissue types, but these results are often conflicting each other, probably due to limitation in detection accuracy and sensitivity of their measurement methods. In addition to the animal studies, recent clinical studies also showed a big controversy in iron therapy in in patients with cardiac diseases. While intravenous (IV) iron therapy provides symptomatic benefit to patients with heart failure, chelation therapy also improves outcome in patients with cardiovascular disease. Thus, given the significant controversy in the field and widespread use of IV iron in patients with heart failure, it is critical to perform a comprehensive analysis of the cardiovascular system in ID conditions. With the aid of an inductively coupled plasma mass spectrometry (ICP-MS), X-ray fluorescence microscopy (XFM), and laser ablation-inductively coupled plasma-time-of-flight mass spectrometry (LA-ICP-TOF-MS), I have been studying the effect of ID diet on metal homeostasis of various mouse tissues and determining the levels of iron and other essential metals in normal heart and in heart failure at baseline and with systemic ID. In this presentation, the relevant research results will be demonstrated.

CONTACT LIST

TR&D Leads				
Last Name	**First Name**	**Title**	**University**	**Email**
O'Halloran	Thomas	Professor	Michigan State University	ohallor8@msu.edu
Jacobson	Chris	Professor	Northwestern University	c-jacobsen@northwestern.edu
Cheng	Sun	Associate Professor	Northwestern University	c-sun@northwestern.edu
Initial DBPs				
Last Name	**First Name**	**Title**	**University**	**Email**
Kozorovitskiy	Yevgenia	Associate Professor	Northwestern University	yevgenia.kozorovitskiy@northwestern.edu
Lutsenko	Svetlana	Professor	John Hopkins University	svlinbalto1@gmail.com
Mukhopadhyay	Somshuvra	Associate Professor	University of Texas, Austin	som@austin.utexas.edu
Culotta	Valeria	Professor	John Hopkins University	vculott1@jhu.edu
Skaar	Eric Patrick	Ernest W. Goodpasture Professor	Vanderbilt University	eric.skaar@vumc.org
Fahrni	Christoph J.	Professor and Chair	Georgia Institute of Technology	fahrni@chemistry.gatech.edu
LaBonne	Carole	Erastus O. Haven Professor	Northwestern University	clabonne@northwestern.edu
Woodruff	Teresa K	President, Professor	Northwestern University	tkw@msu.edu
Ardehali	Hossein	Thomas D. Spies Professor	Northwestern University	h-ardehali@northwestern.edu
El Muayed	Malek	Assistant Professor	Northwestern University	m-muayed@northwestern.edu
McClain	Donald A	Professor	Wake Forest University	dmcclain@wakehealth.edu
Newer DBPs				
Last Name	**First Name**	**Title**	**University**	**Email**
Petroff	Margaret	Associate Professor	Michigan State University	petrof10@cvm.msu.edu
Chen	Chen	Associate Professor	Michigan State University	chen2@msu.edu
Schrenk	Matt	Associate Professor	Michigan State University	schrenkm@msu.edu
Guillory II	Roger	Assistant Professor	Michigan Technological University	rguillory@mcw.edu
Tewari-Singh	Neera	Assistant Professor	Michigan State University	tewarisi@msu.edu
Lawler	Sean	Associate Professor	Brown University	sean_lawler@brown.edu
Wale	Nina	Assistant Professor	Michigan State University	walenina@msu.edu
Duncan	Francesca	Assistant Professor	Northwestern University	f-duncan@northwestern.edu
Qiu	Tian (Autumn)	Assistant Professor	Michigan State University	qiutian8@msu.edu
Lee	Kin Sing	Assistant Professor	Michigan State University	sing@msu.edu
Crosson	Sean	Assistant Professor	Michigan State University	crosson4@msu.edu
Administration				
Last Name	**First Name**	**Title**	**University**	**Email**
MacRenaris	Keith	Research Associate Professor	Michigan State University	macrenar@msu.edu
Garwin	Seth	Research Associate	Michigan State University	garwinse@msu.edu
Crawford	Andrew	Senior Research Associate	Michigan State University	andrew.crawford@northwestern.edu
Connerty	Cristin	Administraitve Assistant	Michigan State University	cristin.connerty@northwestern.edu
Maxey	Evan	Beamline Scientist	Argonne National Laboratory	emaxey@anl.gov
Jin	Qiaoling	Postdoctoral Fellow	Northwestern University, Argonne National Laboratory	qiaoling.jin@northwestern.edu
Lai	Barry	Physicist, Group Leader	Argonne National Laboratory	blai@anl.gov
Vogt	Stefan	Associate Division Director	Argonne National Laboratory	svogt@anl.gov
Hong	Bong	Research Assistant Professor	Michigan State University	hongbong@msu.edu
Ok	Kiwon	Postdoctoral Fellow	Michigan State University	okkiwon@msu.edu
EAC				
Last Name	**First Name**	**Title**	**University**	**Email**
Austin	Christine	Assistant Professor	Icahn School of Medicine, Mount Sinai Hospital	christine.austin@mssm.edu
George	Graham	Professor	University of Saskatchewan	g.george@usask.ca
Hausinger	Robert	Professor	Michigan State University	hausinge@msu.edu
Hegg	Eric	Professor	Michigan State University	erichegg@msu.edu
Marleta	Michael	Professor	UC-Berkeley	marletta@berkeley.edu
Michel	Sarah	Professor	University of Maryland-Baltimore	smichel@rx.umaryland.edu
Nolan	Elizabeth	Professor	MIT	lnolan@mit.edu
Penner-Hahn	James	Professor	University of Michigan - Ann Arbor	jeph@umich.edu
Agar	Nathalie	Professor	Haravard	nagar@rics.bwh.harvard.edu
Que	Emily	Associate Professor	University of Texas - Austin	emilyque@cm.utexas.edu
Yao	Junjie	Assistant Professor	Duke University	junjie.yao@duke.edu
Presenters				
Last Name	**First Name**	**Title**	**University**	**Email**
Seo	Young-Ah	Assistant Professor	University Michigan	youngseo@umich.edu
He	Weilue	Postdoctoral Reseach Associate	Michigan Technological University	weilueh@mtu.edu
Ahn	Soo Hyun	Assistant Reseach Professor	Michigan State University	ahnsooh1@cvm.msu.edu
Reynolds	Aidan	Graduate Student	Michigan State University	reyno569@msu.edu
Spathies	Jacquelyn	Graduate Student	Vanderbilt University	jacquelyn.spathies@vanderbilt.edu
Mendoza	Adelita	Postodtoral Research Associate	Washington University in St. Louis	adelitamendoza@wustl.edu
Jiang	Yuhan	Graduate Student	Michigan State University	jiangy60@chemistry.msu.edu
Kim	Thomas	Graduate Student	Michigan State University	kimthom6@msu.edu
Sue	Aaron	Postdoctoral Reseach Associate	Michigan State University	sueaaron@msu.edu
Jacobson	Ryutaro	Graduate Student	Michigan State University	jacob433@msu.edu
Glunde	Kristine	Professor	Jons Hopkins University	kglunde1@jhmi.edu