QE MAP

NIGMS P41 QE-MAP Annual Symposium & Workshop August 16-18, 2022

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

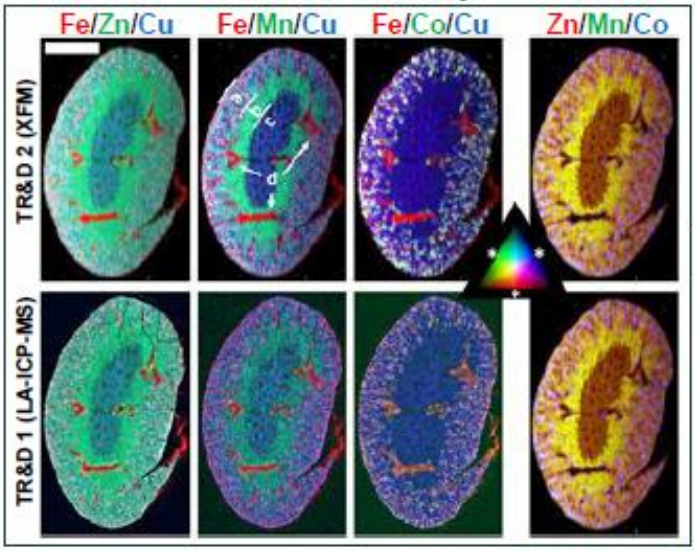
We're sending out this invitation for our first in person (at Michigan State University in East Lansing, MI) symposium and workshop for the P41 national research resource center for quantitative mapping in the life sciences (QE-Map).

Symposium/Workshop Format

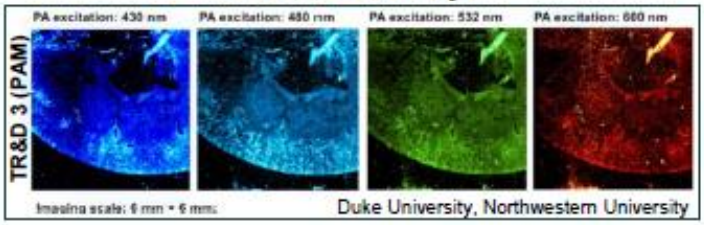
- Day 1: EAC meeting, half day of symposium talks
- Day 2: half day of symposium talks, half day of workshop
- Day 3: workshop

Please fill out the Microsoft Teams Poll:
https://forms.office.com/r/LjjZ27HSja

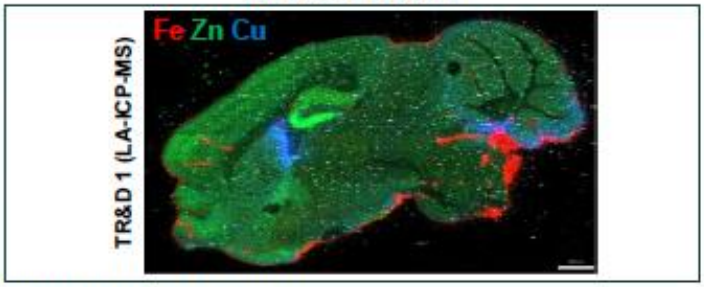
Mouse Kidney



Mouse Kidney



Mouse Brain



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Welcome

Dear Colleagues,

It is a great pleasure to welcome you to Michigan State University and the 1st annual NIGMS P41 QE-Map Symposium and Workshop in East Lansing, Michigan. While initially planned for 2021, we are very excited to organize this in-person event this year and hope you will enjoy it.

As we gather here, we are bringing together members of the NIGMS P41GM135018 grant as well as other members of the bio-element 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 setup 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*

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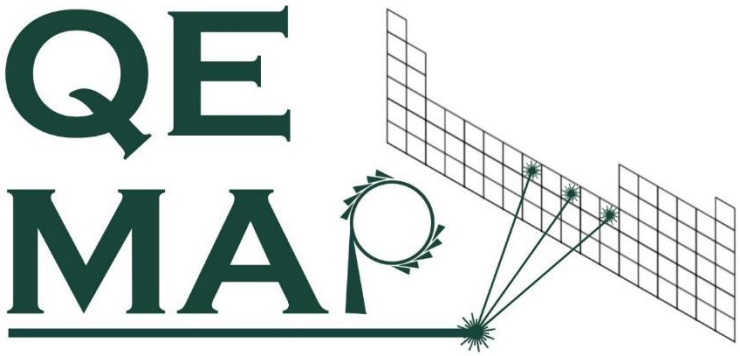

QE
MAP


College of Natural Science


College of Human Medicine
MICHIGAN STATE UNIVERSITY

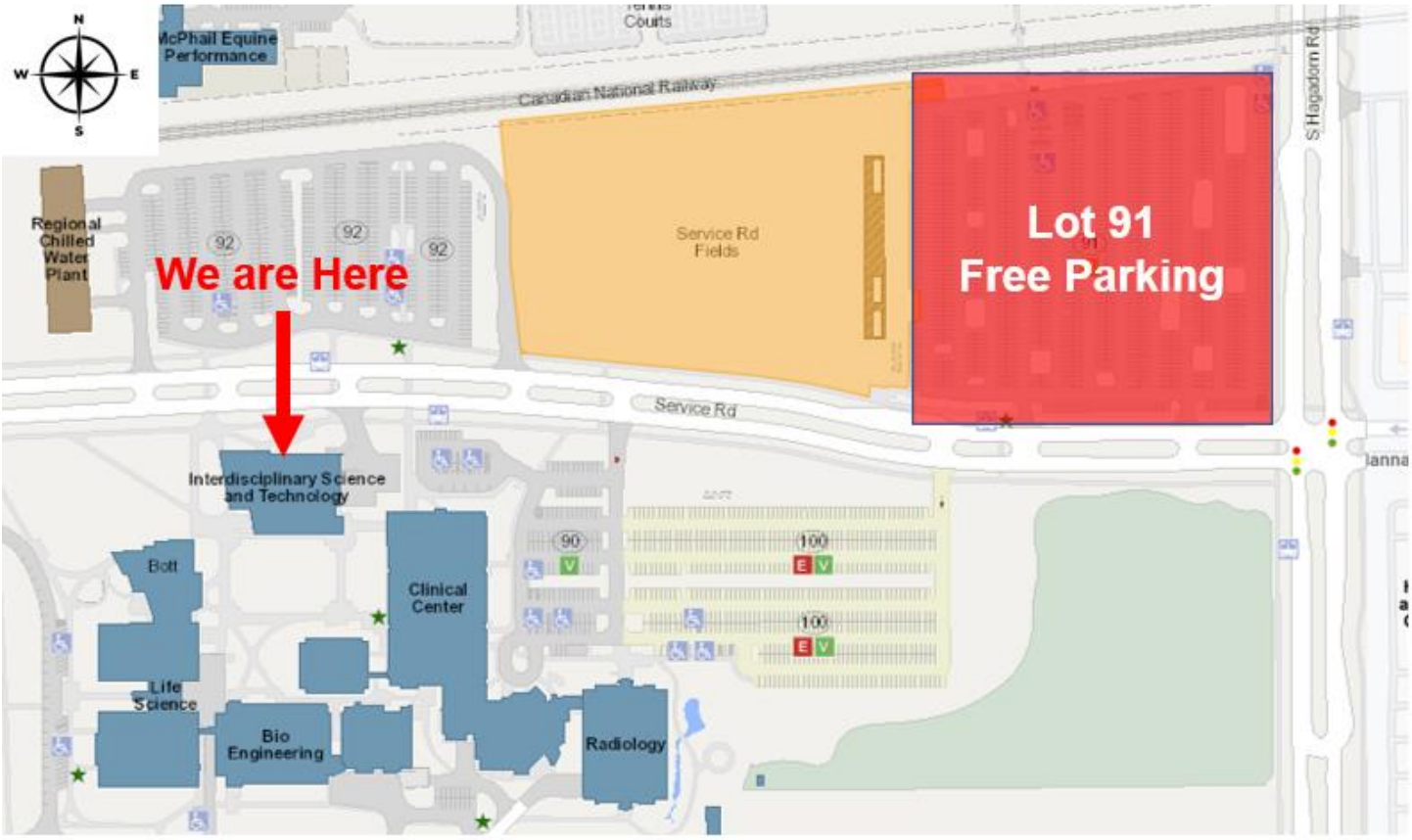
Conference Venue

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.

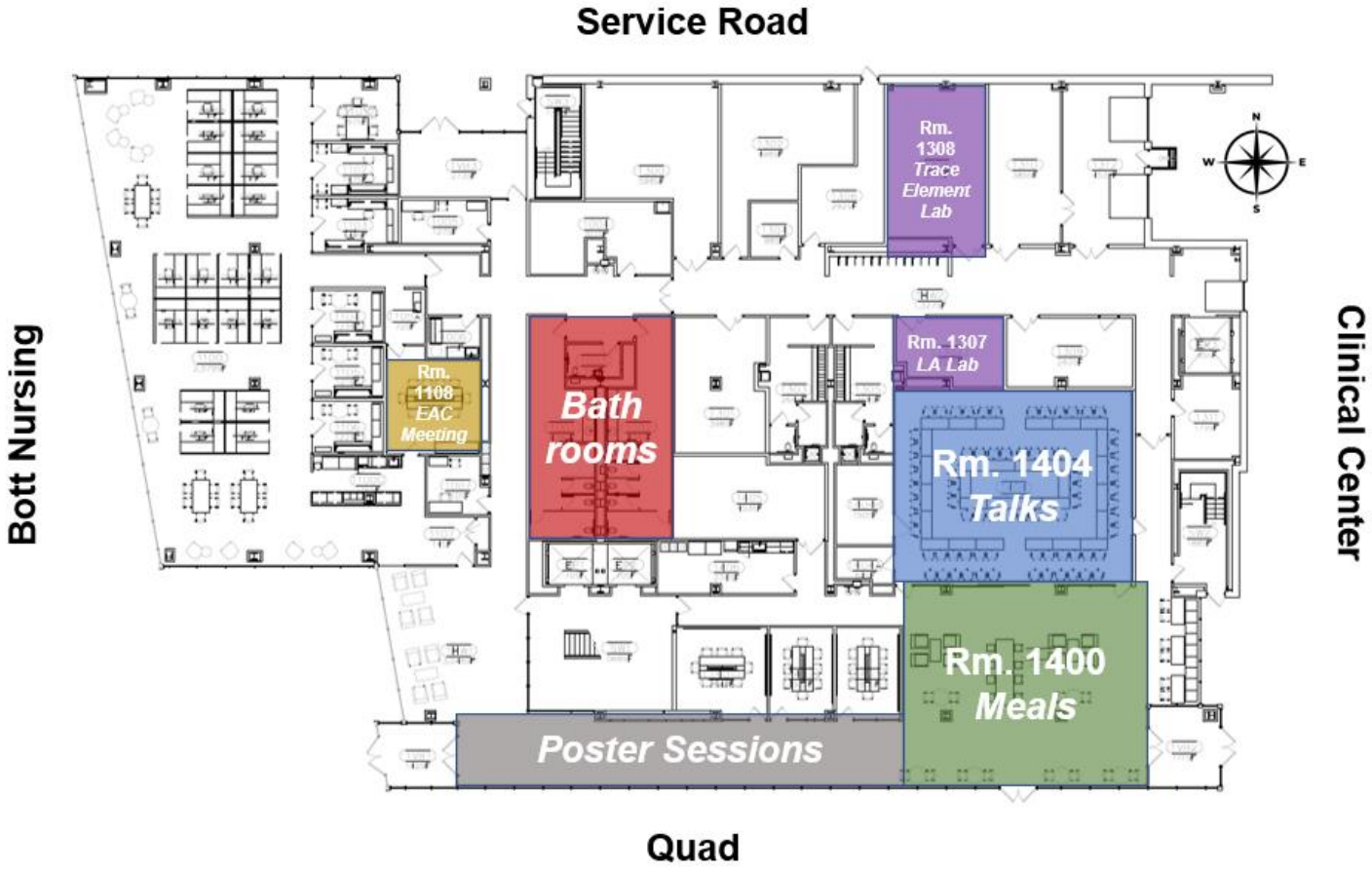
Venue Address: 766 Service Road, East Lansing, MI 48824

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 needing to 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 16th, 2022 (Morton's Fine Catering, ISTB Atrium Rm. 1400 and quad, weather permitting)

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

Break: 3:00-3:30 pm

Coffee with cookies from the MSU Bakery

Dinner: 6:00-8:00 pm

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

Wednesday August 17th, 2022 (Morton's Fine Catering, ISTB Atrium Rm. 1400 and quad, weather permitting)

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

Break: 2:30-3:00 pm

Ice Cream from MSU Dairy – ISTB Atrium and Quad

Dinner: Barbecue from Saddleback BBQ (Vegetarians Thai food from Taste of Thai)

Thursday August 18th, 2022 (Morton's Fine Catering, ISTB Atrium Rm. 1400 and quad, weather permitting)

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

Break: 2:30-3:00 pm

Assorted cookies from the MSU Bakery

Agenda Day 1 – Tuesday August 16, 2022

Start	End	Speaker	Affiliation	Session/Title	Page
8:00 AM	9:00 AM				
9:00 AM	9:30 AM				
9:30 AM	10:00 AM				
10:00 AM	10:30 AM				
10:30 AM	11:00 AM			Registration	
11:00 AM	11:30 AM				
11:30 AM	12:00 PM			Lunch/Registration: **ISTB 1st Floor Lobby Rm. 1400** EAC Meeting: **ISTB 1108**	
12:00 PM	1:00 PM				
Center Overview Session (All talks in ISTB Room 1404)					
1:00 PM	1:30 PM	Thomas O'Halloran	Michigan State University	*QE-Map Center Overview*	**17**
1:30 PM	2:00 PM	Keith MacRenaris	Michigan State University	***TR&D 1:*** *LA-ICP-MS and High Throughput Elemental Histology*	**18**
2:00 PM	2:30 PM	Chris Jacobsen	Northwestern University	***TR&D 2:*** *X-ray fluorescence: The Advanced Photon Source, beamline 8-BM, and correcting for self-absorption*	**19**
2:30 PM	3:00 PM	Cheng Sun	Northwestern University	***TR&D 3:*** *Photoacoustic Microsopy for In Vivo and Whole Tissue Imaging*	**20**
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	Eric Skaar	Vanderbilt University	*Metabolic changes associated with changes in cellular copper distribution*	**21**
4:00 PM	4:30 PM	Roger Guillory	Michigan Technological University	*Deciphering the tissue response of engineered bioresorbable metal materials for vascular implants using multimodal imaging*	**22**
4:30 PM	5:00 PM	Svetlana Lutsenko	Johns Hopkins University	*Metabolic changes associated with changes in cellular copper distribution*	**23**
5:00 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**	

Agenda Day 2 – Wednesday August 17, 2022

Start	End	Speaker	Affiliation	Session/Title	Page
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	9:30 AM	Sean Lawler	Brown University	*Improving drug delivery for the treatment of brain cancer*	**25**
9:30 AM	10:00 AM	Christoph Fahrni	Georgia Tech	*Redox-modulator or metal buffer? Elucidating the role of glutathione in cellular copper homeostasis*	**26**
10:00 AM	10:30 AM	Sara Michel	University of Maryland (Baltimore)	*How to find a needle in a haystack: Tracking iron nanomedicines in clinical samples*	
10:30 AM	11:00 AM	Coffee Break/Recap Location: **ISTB 1st Floor Lobby Rm. 1400**			
Student/Post Doctoral Session (All Talks in ISTB Rm. 1404)					
11:00 AM	11:20 AM	Sky Price (*Emily Que*)	University of Texas at Austin	*Fluorescent probes for monitoring metallo-b-lactamase metalation state*	**28**
11:20 AM	11:40 AM	Asia Wildeman (*Valeria Culotta*)	Johns Hopkins University	*The Fungal Pathogen Candida Ablicans Requires Mn for Morphogenesis, Cell Wall Assembly, and Virulence*	**29**
11:40 AM	12:00 PM	Amani Gillette (*Melissa Skala*)	University of Wisconsin Madison	*Autofluorescence imaging of endogenous fluorophores as a source of non-destructive contrast*	**30**
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	2:00 PM				
				Workshop Session #1	
2:00 PM	2:30 PM				
2:30 PM	3:00 PM	Break/Recap - Ice Cream from MSU Dairy Store Location: **ISTB 1st Floor Lobby Rm. 1400**			
3:00 PM	3:30 PM				
				Workshop Session #2	
3:30 PM	4:00 PM				
4:30 PM	5:30 PM	Poster Session Location: **ISTB 1st Floor Lobby**			
6:00 PM	9:00 PM	Dinner Location: **ISTB 1st Floor Lobby Rm. 1400**			

Agenda Day 3 – Thursday August 18, 2022

Start	End	Speaker	Affiliation	Session/Title	Page
8:00 AM	9:00 AM		Breakfast/Check In Location: **ISTB 1st Floor Lobby Rm. 1400**		
			Workshop Day 2		
9:00 AM	9:30 AM	Mirna Lerotic	2nd Look Consulting	*PyElements: a software platform for intrinsic element image visualization and analysis*	
9:30 AM	10:00 AM			***Workshop Session #3***	
10:00 AM	10:30 AM				
10:30 AM	11:00 AM		Coffee Break/Recap Location: **ISTB 1st Floor Lobby Rm. 1400**		
11:00 AM	11:30 AM			**Workshop Session #4**	
11:30 AM	12:00 PM				
12:00 PM	1:30 PM		Lunch/Partner Talks Location: **ISTB 1st Floor Rm. 1404**		
1:30 PM	2:00 PM	Andrew Crawford Keith MacRenaris	Michigan State University	***Data Analysis Workshop #1***	
2:00 PM	2:30 PM				
2:30 PM	3:00 PM		Break/Recap - Cupcakes from MSU Bakers Location: **ISTB 1st Floor Lobby Rm. 1400**		
3:00 PM	3:30 PM	Andrew Crawford Keith MacRenaris	Michigan State University	***Data Analysis Workshop #2***	
3:30 PM	4:00 PM				
4:00 PM	4:30 PM		End of QE-Map P41 Workshop Location: **ISTB 1st Floor Lobby**		

Lunch Seminars and Visits

Day 2 – Wednesday August 17th, 2022 (ISTB Rm. 1404)

12:00 – 12:15 - TofWerk

12:15 – 12:30 pm – Elemental Scientific Lasers

12:30 – 12:45 pm – Agilent

12:45 – 1:00 pm - Milestone

Day 3 – Thursday August 17th, 2022 (ISTB Rm. 1404)

12:00 – 12:30 – Tofwerk Discussion about peak fitting and data analysis using Tofware

12:30 – 1:00 – Elemental Scientific Lasers Discussion about data analysis using Iolite

Talk Abstracts

All presentations and abstracts will be posted on the QE-Map website at https://qemap.ehi.msu.edu/qe-map-workshop-2022 following the workshop.

Thomas V. O'Halloran (Center Overview) Michigan State University

Tuesday August 16, 2022 - 1:00 – 1:30 pm

Title: The intersection of nutrition and infection at the host-pathogen interface

Abstract

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 is integrating 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.

Keith MacRenaris (TR&D 1) Michigan State University

Tuesday August 16, 2022 - 1:30 – 2:00 pm

Title: LA-ICP-MS and High Throughput Elemental Histology

Abstract

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.

Chris Jacobsen (TR&D 2) Northwestern University

Tuesday August 16, 2022 - 2:00 – 2:30 pm

Title: X-ray fluorescence: the Advanced Photon Source, beamline 8-BM, and correcting for self-absorption

Abstract

X-ray fluorescence microscopy provides an excellent way to image the distribution of essential elements present at low concentrations. The Advanced Photon Source (APS) at Argonne provides several instruments that can be used for X-ray fluorescence microscopy; most are operated based on no-cost scientific proposals open to all, but QE-Map has special access for large sample studies at beamline 8-BM. Studies at sub-100 nanometer spatial resolution will be significantly improved by the upcoming APS Upgrade, but this will also entail an extended shutdown of access. For larger samples, self-absorption of the fluorescence signal can become significant and thus must be corrected for. I'll describe methods for correcting for this, and how they are being scaled up towards routine use as well as efforts for improved quantitation of elemental concentrations.

Cheng Sun (TR&D 3) Northwestern University

Tuesday August 16, 2022 - 2:30 – 3:00 pm

Title: Photoacoustic Microscope for In Vivo and Whole Tissue Imaging

Abstract

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.

Eric P. Skaar, Vanderbilt University

Tuesday August 16, 2022 - 3:30 – 4:00 pm

Title: The intersection of nutrition and infection at the host-pathogen interface

Abstract

Cells require nutrient metal to carry out essential biochemical processes. This requirement is something that the immune system has exploited to defend against infection by restricting microbial access to metal. This process of nutrient restriction during infection is called “nutritional immunity”. Bacterial pathogens evolved elaborate mechanisms to circumvent nutritional immunity and acquire metal during infection. This struggle for nutrient metal impacts microbial virulence as well as the immune response of the host, profoundly impacting the outcome of host-pathogen interactions. In this talk I will cover aspects of nutritional immunity and microbial countermeasures that are relevant to infectious diseases.

To study these interactions in more detail, we have developed a powerful imaging workflow that can be applied to murine models of infectious disease. All diseases, including infections, are characterized by distinct changes in tissue molecular distribution. Molecular analysis of intact tissues traditionally requires knowledge and reagents relevant to the targets of interest as well as destructive processing for downstream identification platforms. Tissue-based analyses therefore sacrifice discovery to gain spatial distribution of known targets or sacrifice tissue architecture for discovery of unknown targets. To overcome these obstacles, we developed a multi-modality, three-dimensional imaging platform for discovery-based molecular histology. We have applied this platform to the study of multiple murine models of infection, leading to the discovery of infection-associated alterations in the distribution and abundance of macromolecules and elements in tissue. These data provide a three-dimensional analysis of how disease impacts the molecular architecture of complex tissues in infected animals, enable diagnosis of infection through imaging-based detection of bacterial and host analytes, and reveal molecular heterogeneity at the host-pathogen interface.

Roger Guillory, Michigan Technological University

Tuesday August 16, 2022 - 4:00 – 4:30 pm

Title: Deciphering the tissue response of engineered bioresorbable metal materials for vascular implants using multimodal imaging

Abstract

Bioresorbable metal medical devices, such as arterial stents, are rapidly approaching translation to the clinic. These devices are engineered from alloys with a base composition consisting of either Mg, Fe, or Zn. While it has been widely accepted that supraphysiological concentrations of these metal ions in vitro and in vivo for vascular cell types are toxic, little is known about the in-situ tissue presence of implant derived metals within the vascular system. We have attempted to bridge this preclinical knowledge gap by implanting wires made from novel bioresorbable metal materials into the abdominal aorta of normal and transgenic mice. We have explored tissue cross sections using LA-ICP-TOFMS, SEM-EDX, and multiplexed immunofluorescent staining. Using this combinatorial approach, we can begin to decipher how implant derived metals exert a bioactive effect on the tissue response towards novel engineered materials.

Svetlana Lutsenko, Johns Hopkins University

Tuesday August 16, 2022 - 4:30 – 5:00 pm

Title: Metabolic changes associated with changes in cellular copper distribution

Abstract

Mammalian cells maintain tight copper homeostasis by regulating uptake, intracellular distribution, and export of copper. Disruptions of these mechanisms cause significant metabolic changes and may lead to cell death. Two copper transporters, ATP7A and ATP7B play the major role in regulating copper homeostasis in tissues. In this presentation, I will discuss how ATP7A and ATP7B work together to maintain copper homeostasis in the small intestine and in the choroid plexus and describe the metabolic consequences associated with ATP7B inactivation.

NOTES

Sean Lawler, Brown University

Wednesday August 17, 2022 – 9:00 – 9:30 am

Title: Improving drug delivery for the treatment of brain cancer

Abstract

Brain Cancers such as glioblastoma are extremely challenging to treat effectively, in part due to the inherent difficulties in drug access to the central nervous system (CNS). To address this question, we have developed a peptide-drug conjugate platform that can effectively deliver non-CNS penetrant drugs to tumors in the brain. Using cisplatin as a prototype we have shown that this strategy can be employed to deliver brain impenetrant drugs to tumors and improve survival in animals bearing intracranial tumors. We have also been performing biological studies to understand in more detail the molecular composition of the blood brain and blood tumor barriers to identify potential sensitivities. Using this approach, we have identified small molecules that improve drug uptake into brain tumors and ongoing studies are investigating the mechanisms involved.

Christoph Fahrni, Georgia Tech University

Wednesday August 17, 2022 – 9:30 – 10:00 am

Title: Redox-modulator or metal buffer? Elucidating the role of glutathione in cellular copper homeostasis

Abstract

The tripeptide glutathione (GSH) is ubiquitous in most organisms, where it plays a critical role in cellular redox homeostasis, detoxification pathways, and cell signaling. Present at millimolar concentrations, glutathione has also been implicated as a buffer ligand in cellular copper homeostasis. Despite the importance of the copper-glutathione equilibrium system, previous studies have not reached a consensus regarding the nature and stability of the complexes formed under physiological conditions, where glutathione is present in large excess over copper. To revisit the speciation and thermodynamics of the copper-glutathione system, we performed extensive spectrophotometric and potentiometric competition titrations using our suite of MCL-Cu(I) affinity standards. Corroborated by low-temperature phosphorescence studies, the titration data are consistent with the spontaneous assembly of a tetranuclear cluster as the predominant species at physiological pH. Based on the derived thermodynamic model, glutathione thus limits free aqua-Cu(I) to the sub-femtomolar concentration regime, three orders of magnitude lower than previously estimated. To explore whether cytosolic glutathione might be involved in cellular Cu(I) buffering, we developed an emission-ratiometric fluorescent probe, crisp-17, which offers a Cu(I)-dissociation constant slightly below the buffer window of glutathione. Employed in live mouse NIH 3T3 fibroblasts, the probe revealed a low fractional saturation, both under basal conditions and when cells were grown in copper-supplemented medium, thus indicating buffering at low attomolar levels, even under conditions of copper overload. Combined with the thermodynamic model of the glutathione-Cu(I) equilibrium system, the ratiometric imaging data thus indicate that glutathione does not serve as a Cu(I) ligand under regular physiological conditions. As glutathione-bound Cu(I) can catalyze the production of reactive oxygen species, low attomolar buffering might in fact be a necessity for normal cell physiology to avoid copper-induced oxidative stress.

Sarah Michel, University of Maryland (Baltimore)

Wednesday August 17, 2022 – 10:00 – 10:30 am

Title: How to find a needle in a haystack: Tracking iron nanomedicines in clinical samples

Abstract

Iron carbohydrate nanoparticles are used to treat iron deficiency anemia in patients with chronic kidney disease. These nanomedicines are administered intravenously, and in the U.S. eight iron-carbohydrate drugs have been FDA approved. One product, sodium ferric gluconate, is available as both a brand (Ferrlecit) and a generic form. Sodium ferric gluconate is a complex colloidal nanoparticle composed of an iron-hydroxide core and a carbohydrate shell. Concern has been raised by the EMA (FDA equivalent in Europe) that generic iron colloid products can be toxic. It has been hypothesized that toxicity occurs because iron is released differently from the brand versus generic. The biological target of iron released from the nanoparticles is the protein transferrin, which delivers iron to cytoplasmic proteins for use or storage. Iron overload leads to saturated transferrin, and the remaining iron, termed labile iron, is transported into the cell where it can participate in chemistry with oxygen species leading to toxicity. We conducted a two-way crossover pharmacokinetic study that involved administering each drug to healthy volunteers to determine if there were differences in iron release between the brand and generic iron gluconate drug products. We developed a highly sensitive bioanalytical approach to measure iron speciation in the blood plasma that involved coupling size exclusion chromatography to inductively coupled plasma mass spectrometry (LC-ICP-MS). This strategy allowed us to measure all the iron species in the plasma - total iron (TI), transferrin bond iron (TBI), drug bound iron (DBI) and labile iron (LI) simultaneously. This is first time that quantification of iron nanoparticles directly in patient samples has been achieved and resulted in the FDA issuing a new draft guidance for the approval of iron nanoparticle drugs utilizing this approach. Our clinical trial data, along with comparative physiochemical characterization of the two ferric gluconate drugs will be presented.

NOTES

Student/Post Doc Session

Sky Price, University of Texas at Austin *(PI: Emily Que)*

Wednesday August 17, 2022 – 11:00 – 11:20 am

Title: Fluorescent probes for monitoring metallo-b-lactamase metalation state

Abstract

Metallo-b-lactamases (MBLs) are enzymes that are capable of hydrolyzing most blactam antibiotics and all clinically relevant carbapenems. Limited zinc availability has been shown to adversely affect the antibiotic resistance conferred by some MBLs, but a number of clinical variants have emerged to overcome the selective pressure of zinc deprivation. In response to the difficulty of directly assessing enzyme metalation state, we developed a library of reversible fluorescent turn-on probes that are designed to directly bind to the dizinc active site of these enzymes. New Delhi Metallo-b-lactamase-1 (NDM-1) is shown to be susceptible to demetallation by intracellular and extracellular metal chelators in a live-cell model of zinc dyshomeostasis, whereas the NDM-15 metalation state is shown to be more resistant to zinc flux. Thus, we demonstrate the utility of these probes as a new sensor that can be used to study the dynamic metalation state of NDM in response to metal ion sequestration in host-pathogen interactions.

Asia Wildeman, Johns Hopkins University *(PI: Valeria Culotta)*

Wednesday August 17, 2022 – 11:20 – 11:40 am

Title: The Fungal Pathogen Candida Ablicans Requires Mn for Morphogenesis, Cell Wall Assembly, and Virulence

Abstract

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. *This work was supported by NIH grants:R35 GM136644 and R21 AI54726*

Amani Gillette, University of Wisconsin, Madison *(PI: Melissa Skala)*

Wednesday August 17, 2022 – 11:40 am – 12:00 pm

Title: Autofluorescence imaging of endogenous fluorophores as a source of non-destructive contrast

Abstract

Biological systems are rich in endogenous fluorophores that are used for autofluorescence molecular imaging in a convenient, label-free manner. Endogenous fluorophores are powerful biomarkers because their emission properties are often influenced by their microenvironment, as well as the morphology, metabolic state, and pathological conditions of the sample. Imaging endogenous fluorophores is advantageous because it avoids the administration of external fluorescent dyes, thus circumventing complications introduced by these contrast agents including nonspecific binding, toxicity, and interference with the biochemical and physiological functions of the sample. This presentation will focus on sources of endogenous contrast and applications that may be of interest for assessing samples prior to quantitative elemental mapping.

NOTES

Poster Abstracts

All presentations and abstracts will be posted on the QE-Map website at https://qemap.ehi.msu.edu/qe-map-workshop-2022 following the workshop.

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