Integrated chemical imaging of biogeochemical processes and interfaces: molecular to ecosystem scale

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Environmental Molecular Sciences Laboratory

Organizers: Arunima Bhattacharjee, Tamas Varga, and Sam Webb

Day 1

8:30 a.m. Welcome, opening remarks

Chemical Imaging Modalities

8:35 a.m. | Challenges and Vision for Integrated Chemical Imaging | Tamas Varga, EMSL

Abstract: This talk will provide a brief overview of the chemical imaging capabilities available at EMSL as well as some of the opportunities to use them in a multimodal or integrated fashion to address BER science questions.
I will discuss some of the needs for and the challenges in integrating different imaging modalities. Finally, to provide preliminary guidance to the discussions in this workshop, I will outline specific points with respect to some of the scientific grand challenges, suitable chemical imaging approaches and data integration/correlation/fusion needs as informed by recent chemical imaging vision discussions at EMSL as well as a recent breakout session held on this topic at the ESS PI Meeting.

9 a.m. | Investigating the Molecular Processes Controlling Potassium Translocation by Fungi | Jocelyn Richardson, SLAC, Stanford University

Abstract: Potassium (K) is a critical nutrient for plants and is known to play a role in mitigating the effect of drought in a number of plant and microbial species; a trait that is increasingly important due to climate change. However, many environments are K limited due to (1) the amount of K removed during plant harvesting or deforestation exceeds K input, even in cases where fertilizers are used, and (2) up to 98% of soil K is structurally bound in minerals such as K-feldspar and micas, which is considered non-bioavailable. A bio-sustainable pathway for increasing K bioavailability without the need for fertilizer is potassium-solubilizing microbes (KSM). However, there is a lack of understanding of the molecular processes governing K translocation from mineral to microbes and to plants. One reason for this is the lack of techniques that can visualize and spatially characterize the form of K in complex, heterogeneous biological and environmental samples. Although previously unknown, K XAS of biological compounds are feature-rich within 15 eV of the absorption edge, meaning that chemical imaging of different forms of K is possible with a combination of μ-XRF imaging and XAS. In this research, we used a combination of TerraForms, multi-omics and mass spectrometry imaging (MSI) at EMSL, with

synchrotron X-ray Fluorescence (μ-XRF) imaging and X-ray Absorption Spectroscopy (XAS) at SSRL to probe the molecular mechanisms controlling K uptake, storage and transport by the saprotrophic fungus *Fusarium sp. DS 682* and the model crop *Brachypodium distachyon*. Preliminary synchrotron IR imaging (at the ALS) of *B. distachyon* grown in TerraForms helps to bridge MALDI-MSI data from EMSL, with K XRF imaging at SSRL. This multi-modal, multi-institutional approach lays the groundwork for K bioimaging in complex soil-microbe-plant systems.

9:30 a.m. | Synchrotron Hard X-ray Techniques for Mul8scale Imaging of Biological and Environmental Systems | Zou Finfrock, APS, Argonne National Laboratory

Abstract: Synchrotron-based techniques offer invaluable insights for characterizing biological and environmental systems. However, these resources remain largely underutilized by many research communities. The Advanced Photon Source (APS) facility has recently undergone a major upgrade, featuring a new light source design, advanced instrumentation, and innovative applications. The upgraded APS now stands as one of the world's brightest light sources, delivering x-rays up to 500 times brighter than before. The new instruments are designed to fully exploit the increased brightness and coherence of the x-ray source. High-energy x-rays will enable the study of unaltered large bulk samples with significant scientific relevance. The enhanced brightness allows researchers to achieve macroscopic fields of view with nanometer resolution, while improved coherence provides the highest spatial resolution even in highly heterogeneous environments. These advancements position the APS as a leading x-ray light source for imaging applications across numerous scientific disciplines.

10 a.m.| Coffee break

Combining Imaging Modalities

10:30 a.m. | Angstroms to acres: scaling insights from nanoscale chemical imaging to soil ecosystem processes | Angela Possinger, Virginia Tech

Abstract: Identifying the biogeochemical controls on soil carbon storage and turnover is critical for accurately predicting soil carbon response to environmental change. The high spatial and chemical complexity of the soil environment can make it challenging to identify the mechanisms controlling biogeochemical processes at the scale at which they occur (e.g., the interfaces between soil organic and mineral phases). Chemical imaging of soil architecture and organo-mineral and organo-organic associations can help "see" processes that are not visible using bulk measurements alone and can provide new ideas and hypotheses to be tested at larger spatial scales. In this presentation, I will highlight examples of how direct observations of organo-mineral and organo-organic associations using techniques such as analytical electron microscopy and secondary ion mass spectrometry imaging align (or do not align) with patterns in bulk soil measurements, and how these new insights are being used to inform ecosystem and up to continental-scale experiments.

11 a.m. | Multimodal 2D and 3D Geochemical and Physical Characterization of Soil Structures | Odeta Qafoku, EMSL

Abstract: Soil structure is a fundamental soil property that refers to the spatial arrangement of individual soil particles and aggregates, along with the pore network within these aggregates. This property has an essential role in soil functions and processes, which includes water flow dynamics, oxygen and gas exchange, redox conditions, and carbon and nutrient cycling. Soil aggregates are 3- dimensional (3D) structures that form when soil mineral particles are bound to organic material from plants and microorganisms through physicochemical, geo and biological processes. Soil aggregates are the building blocks of soil structure and vary in size ranging from nanosized organomineral associations to microaggregates (< 250 μm) and macroaggregates (> 250 μm). They coexist in a hierarchical organization, creating a network of connected pores and dead voids surrounded by minerals and organic matter. We present here a multimodal approach that integrates spatial and chemical imaging instrumentations for characterizing soil aggregates 2D to 3D. Physical characterization of soil structures is collected using X-ray computed tomography (XCT), a non-destructive imaging technique that provides 3D characterization of intact aggregate structure and enables assessment of aggregate porosity and pore connectivity. We couple the physical characterization of soil aggregates with 2D biogeochemical characterization of cross sections of these structures, using Scanning Electron Microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX). Following embedding and thin sectioning, SEM/EDX chemical characterization provides insights into spatial distribution of major elements and nutrients composing soil aggregates. This effort aims to elucidate soil functionality, carbon cycling, and overall soil health through the application of an integrated multimodal approach on soil samples collected from different ecosystems.

11:20 a.m. | Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) imaging for spatial metabolomics in environmental research | Dušan Veličković, EMSL

Abstract: MALDI-MSI is one of the most utilized EMSL capabilities for unveiling cell-type specific molecular signatures and for untargeted molecular imaging. Herein, I will cover the basics of MALDI MSI, its advantages and limitations, and highlight its value in analyses of environmental samples, including rhizosphere, plant roots, and microbial communities. Traditional MALDI-MSI workflows are not sensitive enough to map many physiologically important molecules due to their low abundance, poor ionization, tissue suppression, and instrument mass cut-offs. As such, the specific focus will be on our recently developed capability to overcome these challenges and map elusive plant and microbial natural products. The capability is based on on-target chemical derivatization (OTCD) using 4-APEBA (4-(2-((4-bromophenethyl)dimethylammonium)ethoxy)benzenaminium dibromide) that boosts the sensitivity of molecules with carbonyl groups. It enables the

detection of more than 500 phyto, rhizo, and microbial compounds revealing their localization and gradients across the environmental samples, such as plant roots, interacting soil microbes, and rhizosphere microfluidic models. The last part of the workshop will cover our recently developed capability, called RhizoMAP, a nondestructive MALDI-MSI-based approach for revealing hot spots of metabolic activity, biological origin of molecules, and microscale molecular gradients from root base to root tips in the rhizosphere.

11:40 a.m. | Multimodal chemical imaging of atmospheric particles | Swarup China, EMSL

Abstract: Atmospheric aerosol affects the climate directly by scattering and absorbing sunlight and indirectly by participating in warm and cold cloud formation. Particle size distribution, chemical composition, and their mixing state influence the optical properties of aerosol and their ability to form warm and cold cloud formation. Detailed measurements of single particle aerosol composition using multi-modal chemical imaging techniques are limited but needed. A single technique not able to provide simultaneous measurements of particle size, morphology, internal structure, surface chemistry, mixing state and molecular composition, a multi-modal approach is essential to better understand complex atmospheric particles. To improve the understanding of impacts of the vertical distribution of aerosol chemical composition, we perform a detailed physicochemical characterization of aerosol particles using a multi-modal approach. We utilized the high-resolution microscopy and X-ray spectroscopy techniques to probe their physicochemical properties. I will highlight results from recent field studies.

Noon | Lunch break

Multimodal Data Processing, Integration and Automation

1:30 p.m. | Combining synchrotron X-ray fluorescence imaging with conventional microscopies to obtain multi-modal dataset for the Earth Sciences | Sam Webb, SLAC

Abstract: X-ray fluorescence (XRF) imaging from synchrotron sources has become a "go-to" technique across the Earth Sciences for the spatial determination of elemental distributions on scales from the nano-scale to the macro-scale. Synchrotrons provide both high brightness and tunability of the incident x-ray energy to provide unique sensitivities and to perform spectroscopy for chemical species information. With any technique, the ability to expand informational content with other techniques is often highly desired and important for data interpretation. Imaging techniques across different modalities can be difficult to treat in more than a qualitative manner due to the difficulty in the registration of images from different techniques. This is often apparent when the different imaging modalities produce vastly different distributions of data, that provide little in terms of common reference points.

This presentation will show how a multi-modal data collection and analysis approach is being implemented at the Stanford Synchrotron Radiation Lightsource, using imaging

data from optical/fluorescence light microscopy, FTIR/Raman microscopy, Mass Spec imaging, and XRF imaging. Data is processed in a data pipeline as a coherent multi-modal set of data, where each pixel of the data image stack contains information from each of the data modalities. Applications of this type of data collection approach to the Earth Sciences will be discussed.

2 p.m. | Covered Affairs: Multimodal Predictions with Confidence | Peter Zwart, LBNL

Abstract: When performing multimodal imaging and experiments, the ability to produce reliable joint estimates across different modalities is crucial. Machine learning stands out as a powerful tool for this task, enabling us to make integrated predictions that account for various data sources. However, beyond generating these estimates, it is essential to quantify their uncertainty to ensure robustness and confidence in our results.

This talk will delve into the application of quantile regression and conformal prediction for providing comprehensive uncertainty estimates in multimodal predictions. By leveraging these techniques, we can not only deliver accurate predictions but also offer a measure of the reliability of these estimates. We will present initial findings from our experimental data and model systems, showcasing the effectiveness of our approach in real-world scenarios and its potential for future advancements in multimodal analysis.

2:20 p.m. | SoilTwin: AI-enabled model-data-experiment workflow for upscaling soil datasets for PFLOTRAN | Maruti Mudunuru, PNNL

Abstract: Soils store more carbon than all the vegetation on the Earth's surface. Soil structure influences water retention and movement, root penetration, carbon storage, and degradation. We refer to soil structure as the arrangement of solids and pore spaces within the soil. Pore structure and its connectivity give us insight into the microbial world of carbon cycling. The size and continuity of soil pores surrounding the aggregates are important for air, water, and nutrient transport. Soil porosity refers to the fraction of the total soil volume taken up by the pore space. Mainly, pore spaces facilitate the availability and movement of air or water within the soil environment. Soil microbial communities can directly affect soil structure and functionality by cycling soil nutrients and storing carbon. The pore size distribution and connectivity affect aeration, soil organic carbon, water holding capacity, and soil drainage capacity. However, there are still many unanswered questions about which processes favor soil accumulation. How can soil pore structure and 3D mineral distribution be accurately characterized? How can soil carbon accumulation and protection be better simulated? The proposed SoilTwin capability will address these two timely fundamental scientific challenges that must be overcome to successfully automate microstructure analysis and accelerate the model-data-experiment loop for the MONet initiative: (1) Efficiently and accurately integrate multiscale and multi-modal datasets to describe the 3D chemical composition of soil samples. (2) Transfer this informative data to multi-physics process models to better understand soil organic carbon cycling, stability, and mobility.

2:40 p.m. | Integrating multimodal imaging to enable chemically informed segmentation of x-ray computed tomography data | Devin Rippner, USDA-ARS

Abstract: X-ray computed tomography (CT) and micro-computed tomography (microCT) are powerful tools for investigating objects that are opaque to visible light in 3D. This capability has led to the ubiquitous usage of CT instrumentation in medical, environmental, and materials sciences. A limitation to the technique is its inability to collect exact chemical information from samples. One solution to this challenge is to cut up the object of interest for 2D chemical analysis using another technique (scanning electron microscopy-energy dispersive x-ray spectroscopy [SEM-EDX], x-ray fluorescence microscopy, etc.). The drawback to this approach is the inability to extend 2D chemical data back into the 3D object space. To solve this dilemma, 2D chemical data can be co-registered with 2D CT data and then used to train neural networks to predict chemical information throughout the entire 3D CT scan. A case study will be presented showing how staining (I2 or Os) paired with dual energy microCT or SEM-EDX can be used to enable the prediction of carbon containing particles and other elements in microCT scans of soil aggregates using a Pytorch implementation of a fully convolutional network (FCN).

3 p.m. | Coffee break

3:30 p.m. | Round table discussion of challenges and future directions

Day 2

8:30 a.m. | Welcome, opening remarks

8:40 a.m. | Tutorial on multimodal data |Hands-on demonstration and tutorial for data analysis of multi-modal datasets with the MicroAnalysis Toolkit | Sam Webb, SLAC

Description of tutorial: This session will go through the basic data loading and integration of data sets from multiple modes of experimentation. Demo will cover advanced tools that can be used to visualize, explore, and analyze data. For early downloading, see www.sams-xrays.com for the MicroAnalysis Toolkit (SMAK) installation instructions.

10:30 a.m. | Break

Visioning: Future of Integration of Chemical Imaging Modalities

10:45 a.m. | Deciphering plant-soil interactions driving biological invasion through multiplatform metabolomics | Malak Tfaily, University of Arizona

Abstract: Understanding the mechanisms underlying the success of invasive plants is crucial for effective management strategies. While traditional plant invasion ecology has focused on functional traits and interactions between plants, the role of the soil environment and plant-soil-microbe interactions remains underexplored. We employed a multi-platform metabolomics approach including Nuclear Magnetic Resonance (NMR),

Liquid chromatography mass spectrometry (LC-MS), Fourier transform ion cyclotron resonance (FT-ICR), and MALDI-MS to compare root- and rhizosphere metabolomes of the invasive Lehmann lovegrass (Eragrostis lehmanniana) and native grass Arizona cottontop (Digitaria californica). We also investigated metabolomes under mesquite canopies and in exposed conditions, where the latter acts as a proxy for drought stress and low soil fertility.
By integrating information across platforms, we reveal contrasting nitrogen use strategies, defensive chemical profiles, root exudate patterns, and responses to environmental stress between the two species. Our findings provide metabolomic evidence supporting multiple mechanisms, including nutrient acquisition, enemy release, and climate adaptation, contributing to the successful invasion of Lehmann lovegrass. This study highlights the power of metabolomics in unraveling the complex chemical interactions underlying plant invasions, offering novel insights for the development of targeted management approaches.

11:15 a.m. | Future of Data Integration at EMSL | Satish Karra and Kelly Stratton, EMSL

Abstract: EMSL Integrated Research Platform (IRP) leaders in the Computing, Analytics, and Modeling Science Area will discuss how their IRPs can help EMSL users with data integration and modeling of imaging data. Topics will include how imaging and bulk data have been combined in previous projects and how systems modeling can leverage imaging data. Existing tools and models, as well as areas for potential development will be highlighted.

11:35 a.m. | A Visual Omics platform for measuring biomolecular processes across scales | Chris Anderton, EMSL

Abstract: Heterogeneity is a natural phenomenon that exist across all scales of biology, and the heterogeneity that occurs across the different domains of scale can regulate and influence processes in adjacent size domains. For example, heterogeneous interactions of proteins and metabolites, as well as proteins and proteins, can regulate localized transcriptional changes and metabolic pathways within a cell, where these cells can then influence microbial neighborhoods, which can influence large scale processes of organisms, which in turn can influence what happens across landscapes. This influence across scales is bidirectional, where environmental perturbations can affect soil microbiome composition and metabolic cycling related to carbon sequestration, for example. Here, I will highlight recent successes we've had in creating new methodologies and integrated approaches for visualizing biomolecular processes across ecological scales, using an array of DOE resources that includes advanced light sources, spatially resolved mass spectrometry assays, and electron-based microscopies. I will also review the major challenges identified by attendees at the breakout session on Chemical and Physical Imaging at the 2024 DOE BER-ESS Annual Meeting. Lastly, I will discuss the future directions that EMSL is pursuing to generate a Visual Omics platform to address some of these issues and enable EMSL Users to capture biomolecular processes that occur across scales.

Noon | Concluding remarks