



## Pre-Conference Workshops

**Sunday, June 22**

**12:00 p.m. – 2:00 p.m.**

### **W1: Part 1 of 2**

**Using web-based tools for integrative analysis of metabolomics and microbiome data for deep functional insights**

**\*\*Hands-On Session – Laptop Required**

#### **Presenters**

- TBD

#### **Description**

Metabolomics is increasingly used together with marker gene profiling and/or shotgun metagenomics to help researchers gain deep insights into community functions and host-microbiome interactions. However, proper data processing, statistical analysis, functional interpretation and multi-omics integration represent the major bottleneck. In this workshop, we will introduce a suite of user-friendly, freely available bioinformatics software tools that have been developed to comprehensively address this gap. The tool suite consists of four components:

1. Raw data processing based on OptiLCMS / Asari for metabolomics, and DADA2 / FunTaxSeq for microbiome data
2. MetaboAnalyst.ca for metabolomics data profiling
3. MicrobiomeAnalyst.ca for microbiome data profiling
4. MicrobiomeNet.com for functional interpretation and system biology.

The workshop will contain three components - lecture (~60 min), live demos & hands-on practices (~2.5 hours), and summary & discussion (~30 min). During the live demo, we will illustrate common workflows through three well-curated example datasets for three typical study designs. Users are expected to bring their laptops for hands-on practice following each demo. We will also create a comprehensive protocol (~80 pages) containing step-by-step instructions for common tasks.

#### **Workshop Objectives**

- Learn to process raw data generated in metabolomics & microbiomics
- How to perform statistical analysis
- How to perform multi-omics integration

#### **Learning Outcomes**

- Be able to comfortably perform common data analysis tasks in microbiome metabolomics studies.

**\*Part 2 of this workshop immediately follows at 2:15 p.m.**

*Updated April 15*

## W2: MetabolomeXchange: International Data Exchange and Data Representation Standards for Metabolomics

### Presenters

- TBD

### Description

Metabolomics data deposition to public open repositories such as MetaboLights, Metabolomics Workbench and GNPS/MassIVE continues to grow. These resources are key in enabling the FAIR principles and open data practices in the field. Historically repositories have acted independently to archive and share datasets – each with distinct practices, processes and policies. Now repositories have started to partner with efforts begun to standardise data and metadata worldwide (this is key in the context of Interoperability) and further encourage open data in the field.

This workshop will introduce the next generation MetabolomeXchange initiative, recently funded (Chan Zuckerberg Initiative (CZI)), akin to analogous efforts in other omics fields, to standardise open data practices in metabolomics globally. RefMet and ReDU as possible drivers for example. Data standardisation efforts across metabolomics (aimed at having common data standards across tools and repositories) also will be featured from the International Metabolomics Society / Metabolomics Standard Initiative (MSI) and Human Proteome Organization / Proteomics Standard Initiative (PSI). This workshop will conclude with discussions from the audience and panel to collate data standardisation efforts and initiate a roadmap with challenges and opportunities for the future."

### Workshop Objectives

- Communicate and collate the need for the generalisation of open data practices across metabolomics with example efforts
- Stimulate engagement and energise awareness of data standards
- Initiate a roadmap including the main challenges and opportunities for the future

### Learning Outcomes

- Describe the benefits of widespread open data practices and the role of the MetabolomeXchange project herein
- Understand the current landscape of data repositories and data standards across metabolomics
- Cite the existing Minimal Information guidelines in the field

**W1: Part 2 of 2****Using web-based tools for integrative analysis of metabolomics and microbiome data for deep functional insights****\*\*Hands-On Session – Laptop Required****Presenters**

- TBD

**Description**

Metabolomics is increasingly used together with marker gene profiling and/or shotgun metagenomics to help researchers gain deep insights into community functions and host-microbiome interactions. However, proper data processing, statistical analysis, functional interpretation and multi-omics integration represent the major bottleneck. In this workshop, we will introduce a suite of user-friendly, freely available bioinformatics software tools that have been developed to comprehensively address this gap. The tool suite consists of four components:

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**Learning Outcomes**

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**\*Part 1 of this workshop immediately prior at 12:00 p.m.**

## W3: Reverse Metabolomics: A hands-on workshop on big data discovery approach

**\*\*Hands-On Session – Laptop Optional**

### Presenters

- TBD

### Description

Reverse metabolomics is a data-driven strategy that transforms how we use untargeted metabolomics data. Unlike traditional LC-MS/MS-based untargeted metabolomics, where researchers start with specific samples to answer predefined questions and subsequently collect LC-MS/MS data, reverse metabolomics leverages over two billion MS/MS spectra and thousands of datasets publicly available with curated metadata to formulate their research question and hypothesis. By mining these resources, researchers can uncover phenotype-relevant insights, such as organ or biofluid distribution, disease states, intervention effects (e.g., pre- and post-intervention), organismal origins (e.g., mammals vs. non-mammals), geographic trends, and other biologically significant associations. This approach focuses on mining existing data sets to find meaningful associations, which directs the efforts for follow-up experiments. This hands-on workshop will introduce participants to the reverse metabolomics workflow demonstrating how one can add biological context to known or unknown MS/MS spectra.

### Workshop Objectives

- Introduction to reverse metabolomics workflow to mine public data.
- Provide guidance for validating the associations found using the reverse metabolomics workflow

### Learning Outcomes

- Getting an overview of reverse metabolomics by querying public datasets, filtering results based on relevant metadata, and interpreting findings to generate valuable biological insights using three small molecules (e.g., bile acid amides, N-acyl lipids).
- Getting equipped to use the reverse metabolomics workflow for their own research, empowering them to harness publicly available MS/MS data and deepen their understanding of metabolomics complexity.

## W4: Volatilomics - bringing small molecules in the multi-omics landscape for biomedical investigations

### Presenters

- TBD

### Description

The vast collection of metabolites presents in biological systems, i.e. the metabolome, is chemically diverse. In discovery-based, untargeted metabolomics where the goal is to detect and annotate as many metabolites as possible, the use of complementary analytical platforms is extremely important. No single analytical platform is capable of characterizing the degree of chemical diversity present in these types of samples on its own.

This workshop will focus on small volatile molecules fraction, i.e., volatilomics, and their high potential as molecular phenotype descriptors in biological systems. The main step of volatilomics will be covered in this workshop.

1. Understanding the specificity of volatilomics technology
2. the key role of QA/QC and the challenges of volatilomics methods
3. the conception of volatilomics workflow from sampling to processing
4. the high potential of multi-platform approaches
5. the high performance of multidimensional chromatography for non-targeted volatilomics, and the
6. the implementation of volatilomics in clinical studies.

### Workshop Objectives

- To illustrate the biological relevance of volatile molecules and their key relationships with phenotypes.
- To highlight the key steps of non-targeted metabolomics research and the specificity of volatilomics workflows.
- To increase awareness of the critical role modern GC-MS and direct MS instrumentation plays in complementary multi-platform metabolomics.
- To address the fundamental requirements of each step of volatilomics workflows, including QA/QC protocol design, technology/instrumental choice for confidently solving challenges in different assays, the high annotation capacity of GC-MS, and automated data processing and visualization.

### Learning Outcomes

Attendees will gain a broader understanding of the key attributes to consider in successfully non-targeted volatilomics research. Everything from experimental design and QA/QC, to sampling techniques, to routine and advanced sample analysis techniques, and finally data processing and statistical analysis strategies, will be covered.

## **W5: Untargeted metabolomics, multimodal MS data analysis, and interactive molecular networking in mzmine**

**\*\*Hands-On Session – Laptop Required**

### **Presenters**

- **Dr. Robin Schmid** (mzio GmbH)
- **Dr. Steffen Heuckeroth** (McGill University)
- **Corinna Brungs, Ph.D.** (University of Vienna)
- **Dr. Ansgar Korf** (mzio GmbH)

### **Description**

Untargeted high-resolution mass spectrometry (HRMS) of complex samples rapidly generates gigabytes of data in a single study - of which only a fraction can be annotated, leaving a vast unknown chemical space to explore. This creates a need for flexible and scalable software tools for data processing. The modular software mzmine provides a platform for users and developers with its vibrant open-source community. Recent contributions have expanded its capabilities to support various MS platforms, enabling multimodal MS data analysis of ion mobility spectrometry (IMS), LC-MS, GC-MS, direct infusion, as well as MS-Imaging. Those workflows enable large-scale metabolomics and lipidomics research by spectral preprocessing, feature detection, and various options for compound annotation for thousands of samples in parallel. The modern graphical user interface and interactive charts facilitate data exploration and validation of results from every processing step. In addition, the mzmine processing wizard (mzwizard) introduces easy-to-configure workflows that can be scaled to process large studies of thousands of samples.

This hands-on workshop will introduce the mzwizard, feature detection, compound annotation and interactive molecular networking in mzmine.

### **Workshop Objectives**

- Introducing untargeted metabolomics LC-MS/MS data processing in mzmine, focus on data processing optimization.
- Introducing the mzwizard to rapidly process data from various MS techniques (GC-MS, LC-IMS, MS-Imaging).
- Introducing Interactive Molecular Networking within mzmine.

### **Learning Outcomes**

- Getting an overview of MS data processing in mzmine by processing an example dataset.
- Optimize individual data processing steps for LC-MS, GC-MS, IMS and MALDI-Imaging data.
- Apply Interactive Molecular Networking to untargeted Metabolomics datasets.

## W6: Demystifying Stable Isotope Labelling Metabolomics

### Presenters

- TBD

### Description

Metabolite profiling and targeted metabolomics approaches are now routinely applied by researchers wanting to understand the physiology of their cell, tissue, organism, or biofluid of choice.

One of the largest challenges faced by metabolomics researchers in any study is relating changes in identified metabolites to their metabolic roles. Understanding this relationship is critical for understanding metabolic mechanisms in that biosystem.

Stable isotope labelling has rapidly emerged as a powerful tool in unravelling the precise structure and activity of metabolic pathways and how these influence/are caused by the physiological state of the biosystem. These techniques can reveal subtle alterations in metabolic activity that would not be possible with unlabeled approaches. This depth of information can provide valuable insight into the mechanisms that underlie various physiological conditions and aberrant processes, including infection and disease.

However, this technique is perhaps not used as widely as it might. This workshop will show how metabolic labelling studies are readily approachable and easily interpretable when performed correctly. We will show you how to design, run, and analyse stable isotope labelling experiments, and explain how these simple workflows are often sufficient for significant biological insight.

\*New for 2025! Simple in vivo labelling and imaging approaches\*

### Workshop Objectives

Attendees will leave the workshop:

- Knowing how to design a labelling project, including essentials to consider and potential pitfalls
- Knowing the basics of analysis of labelling data, including awareness of some of the available LC-MS and GC-MS software
- Understanding how to interpret data, including the importance of mass isotope distributions

### Learning Outcomes

Attendees will learn:

- That stable isotope labelling projects are easy to design, perform, and interpret
- That labelling experiments can bring intricate detail to many metabolomics projects, whether untargeted or targeted, or polar or lipid-focused
- Key principles for in vitro and in vivo labelling studies, combining labelling with mass spectrometry imaging, and multi-isotope approaches

## W7: From Research to Practice: Advancing Metabolomics for Clinical Implementation

### Presenters

- TBD

### Description

Metabolomics is rapidly evolving from a powerful research tool into a clinically relevant discipline. However, translating metabolomics findings into actionable clinical applications requires a robust framework that integrates harmonization, meta-analysis, and practical implementation within the clinical laboratory setting. This workshop delivered by the Advanced Metabolomics Team from GIOTEC-IISPV and the Metabolomics working group at International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), provides a comprehensive overview of the end-to-end workflow, beginning with data harmonization and meta-analysis techniques to enhance reproducibility, statistical power, and biomarker discovery. Participants will also explore the critical steps of integrating metabolomics into clinical diagnostics, including quality assurance, regulatory considerations, data interpretation, and clinician-laboratory communication. By bridging the gap between research and clinical practice, this session equips attendees with essential knowledge and strategies to ensure that metabolomics data is both scientifically rigorous and clinically meaningful.

### Workshop Objectives

- Understand the role of data harmonization and meta-analysis in improving the reproducibility and impact of metabolomics research.
- Identify the key components required to integrate metabolomics into clinical workflows, including study design, quality control, and standardization.
- Recognize regulatory and communication strategies essential for translating metabolomics findings into actionable clinical diagnostics.

### Learning Outcomes

- Explain how harmonization and meta-analysis improve biomarker discovery and enable robust, reproducible results.
- Design metabolomics workflows that meet clinical standards, incorporating quality assurance and regulatory requirements.
- Interpret and communicate metabolomics findings effectively within clinical teams to support diagnostic decision-making.

## W8: Next-Level Substructure Discovery in Mass Spectra using MS2LDA

**\*\*Hands-On Session – Laptop Required**

### Presenters

- The three workshop organizers are Rosina Torres Ortega, Jonas Dietrich, and Justin van der Hooft. We also plan to invite a guest speaker with MS2LDA experience to showcase its practical use in untargeted metabolomics studies.

### Description

Mass spectrometry data has significantly increased in availability and utility over recent years. This has triggered metabolite annotation tool development to derive structural and functional information from mass spectra. In 2016, MS2LDA was coined as a substructure discovery tool to identify patterns in mass spectral data known as 'Mass2Motifs,' which are associated with molecular substructures. Whilst MS2LDA has been widely used by the community, the tool has started to face challenges such as the limited scalability and the increasing time it takes for manual annotation and validation of Mass2Motifs discovered in large datasets.

In this workshop, we will do a conceptual as well as hands-on introduction of a new version of MS2LDA, addressing these limitations with enhanced scalability and by providing automated annotation guidance. We will also showcase integration to other python frameworks through the matchms and massql packages. The new version also comes with new visualizations that will be demonstrated. Participants will gain insight into the principles of substructure discovery, explore the key improvements in the updated tool, and apply these advancements through hands-on exercises using reference library and experimental data. Participants will need to bring a laptop with internet access to follow all workshop elements.

### Workshop Objectives

- Introduce participants to the motivation for and concept of substructure finding and introduce the new features of MS2LDA, highlighting its improvements in speed, guided annotation, and integration with other frameworks.
- Demonstrate the new MS2LDA workflow: Preprocessing MS/MS data using matchms. Running MS2LDA to discover Mass2Motifs. Annotating Mass2Motifs: Train participants on using Spec2Vec for Mass2Motif annotation guidance, providing hands-on examples of how these aid in improving data interpretation & Guide participants in structural analysis of mass spectra using fingerprints and SMILES, emphasizing practical use cases for chemical characterization. Downstream analysis and visualizing results: Showcase how to use massql for motifDB screening and integrating results into the analysis pipeline. MotifDB is a repository of MotifSets that contain validated annotated Mass2Motifs from various sources.
- Equip participants with the skills to incorporate MS2LDA into their research workflows by its integration with other Python-based frameworks.

### Learning Outcomes

- Understand motivation for and concept of MS2LDA substructure discovery.
- Apply MS2LDA to discover Mass2Motifs in MS/MS data.
- Perform guided annotation of Mass2Motifs with automated annotation.

## W9: Metabolomics Data Standardization and Harmonization for AI

### Presenters

- TBD

### Euretos Moderators/Discussants

- TBD

### Description

This workshop brings together leaders in large initiatives focused on integrating metabolomics and lipidomics data across studies, assays, laboratories, and repositories. Starting with updated recommendations from the Metabolomics Standards Initiative, the workshop will explore optimizing large-scale data use in open biorepositories, focus on standardizing lipid annotations and quantifications, and incorporate new approaches for data harmonization and FAIRification to coalesce different data types and existing knowledge. Finally, it will explore using digital twinning and AI to search across data resources. This workshop sets the stage for broader community discussions that include metabolomics and lipidomics societies, large initiatives, task groups, and interest groups to define the next steps in data reporting, integration, harmonization, data sharing, and visiting strategies.

### Workshop Objectives

- Metabolomics Lipidomics data standardization and harmonization complexities
- getting data ready for AI
- connecting biorepositories
- future data visitation

### Learning Outcomes

- better practices in metabolomics data and its reporting
- data harmonization approaches
- machine readable data for AI applications

## W10: EMN – MetaboMentors: Paving the way to successful mentorship

### Speakers (EMN Members):

- Aleš Kvasnička
- Ambrin Farizah Babu
- Diana Pinto
- Thomas Dussarrat

### Panelist speakers for the discussion:

- Álvaro Fernández Ochoa
- Biswapriya Misra
- Erin Baker
- Evelina Charidemou
- Lynn Vanhaecke
- Nicholas Ratray

### Description

Mentorship is well-recognized for shaping Early Career Researchers (ECRs) scientific careers and fostering leadership development. The workshop will cover the different facets of this mentor-mentee interaction through three sections that will help ECRs to forge and maintain fruitful mentor-mentee relationships. Section (1) “Why mentorship matters: evidence and strategies for success” will provide a comprehensive background on the benefits and key components of mentorship and shed light on the expectations encountered by mentors and mentees. Section (2) “Enhancing mentorship skills: from theory to practice” will seek to equip ECRs with practical directions and tips for effectively navigating the challenges of establishing and maintaining bidirectional relationships, while integrating mentorship strategies with diversity and inclusion initiatives. A list of recommended resources provided during the workshop will be available to participants. Section (3) will consist of an open discussion between the ECR audience, EMN members and invited panelists from academia and industry, including the perspective of mentees, their experiences, challenges and successes in mentoring relationships. This interactive discussion will offer the possibility of sharing experiences in mentorship with the panelists, EMN members and the rest of the audience.

### Workshop Objectives

- Section 1: Understanding Mentorship: Introduce the fundamentals of mentorship, including its benefits and the roles of both mentors and mentees. Impact of Mentorship: Present evidence on how mentorship positively affects career growth and the development of leadership qualities.
- Section 2: Building Relationships: Outline practical methods for creating and maintaining effective mentorship relationships, emphasizing goal setting and communication. Skill Development: Discuss tools and exercises that help bridge skill gaps through mentorship, preparing participants for advanced professional roles.
- Section 3: Global Perspectives: Connect participants with seasoned professionals who will share diverse mentorship experiences and insights from various fields, including a focus on the challenges and opportunities faced by mentees. Interactive Engagement: Facilitate dialogue and networking, encouraging participants to explore mentorship opportunities and apply workshop insights.

### Learning Outcomes

- Section 1: Understand mentorship models (traditional, peer, reverse, group) and benefits for mentees, mentors, and organizations. This includes understanding the role of mentorship in skill development, career advancement, and fostering inclusivity. Learn from other previous experiences and stories how a good mentorship experience can make a difference in someone's career development.
- Section 2: Learn how to build effective mentoring relationships: For mentors: learn strategies to create trust, how to provide constructive feedback, and empower mentees to achieve independence. For mentees: learn how to approach mentors, set goals, receive feedback and communicate effectively. Learn where to find tools and resources: templates for mentoring agreements, structuring frameworks, and progress tracking measurement. Participants will have access to a list of recommended resources, which can be used to further develop their mentoring skills and relationships.
- Section 3: Obtain actionable advice from experienced mentors and mentees about how to apply mentorship concepts in the context of academia and industry. Gain real-world insights and diverse perspectives from mentors and mentees across different regions, cultures, and professional backgrounds. Learn how to create a foundation for deeper engagement in the mentor-mentee relationships by discussing the main needs and challenges and how to overcome them.

## W11: Automated Compound Annotation with SIRIUS 6

**\*\*Hands-On Session – Laptop Required**

### Presenters

- **Fleming Kretschmer**, Friedrich Schiller University Jena
- **Markus Fleischauer**, Friedrich Schiller University Jena

### Description

In this workshop, you'll discover how SIRIUS (<https://bio.informatik.uni-jena.de/sirius/>) can transform your MS/MS data analysis by providing annotations from various sources unified in one application without the need for manual merging.

SIRIUS 6 integrates molecular formula, molecular structure, and compound class annotation from MS/MS data. It offers a user-friendly GUI and CLI that seamlessly incorporates web-services for specific computational tasks. From preprocessing complete LC-MS/MS datasets and spectral library search, to lipid annotation, structure database search via CSI:FingerID, de novo structure generation via MSNovelist and combinatorial fragmentation for the verification of your results — SIRIUS 6 is your all-in-one solution.

In this workshop, you'll learn to process entire LC-MS/MS datasets and annotate your MS/MS data with detailed structural information. You'll learn how to automatically sort and filter for high quality features and most confident hits, and to identify compounds of interest, such as transformation products, using custom databases.

Join us to experience how SIRIUS 6 can save you hours of manual work.

### Workshop Objectives

- Import/export data into and from SIRIUS. Generate your own custom databases.
- Perform analysis (molecular formula, molecular structure and compound class annotations)
- Interpret SIRIUS results

### Learning Outcomes

- Analyze whole datasets with multiple LC-MS/MS runs with SIRIUS
- Select suitable computational methods for your scientific question, define your own workflows, generate and store parameter presets for different applications and perform a meaningful analysis
- Interpret and evaluate computed results and select confident annotations

## W12: Beyond CID - Alternative fragmentation methods for metabolite/lipid identification

### Presenters

- TBD

### Description

Metabolite and lipid identification remains a major bottleneck in non-targeted analysis. Besides a small number of substances with reference spectra compared to the chemical diversity of metabolites and lipids, the information content is insufficient for detailed structural analysis. CID and HCD often fall short of indicative fragment peaks related to critical structural features such as the location of functional groups such as hydroxy groups, double bonds, etc. Novel fragmentation methods have recently evolved to resolve this issue. Examples include commercially available technologies Electron Induced Dissociation (EID), Ultraviolet Photodissociation (UVPD), Oxygen Attachment Dissociation (OAD), or experimental systems such as Ozone-induced dissociation (OzID). Each method shows distinct advantages and disadvantages for different metabolite/lipid classes. We will showcase several examples of selected methods from data generated in our library and how alternative fragmentation methods can aid metabolite and lipid identification.

### Workshop Objectives

- Explain alternative techniques and technical implementation
- Methods for lipid and FA analysis
- Methods for metabolomics
- Software workflows and libraries

### Learning Outcomes

Workshop participants will learn the basics of different alternative fragmentation methods beyond CID and their advantages and disadvantages. After attending the workshop, participants are able to select the most appropriate fragmentation method for their metabolite identification problem.

## W13: Advancing Best Practices with mQACC Living Guidance for LC-MS-Based Untargeted Metabolomics

### Presenters

- **Candice Z. Ulmer Holland**, U.S. Department of Agriculture
- **Julia Kuligowski**, Health Research Institute La Fe
- **Rick Dunn**, University of Liverpool
- **Dajana Vuckovic**, Concordia University

### Description

There is a critical and urgent need to standardize and implement quality assurance (QA) and quality control (QC) best practices in untargeted metabolomics to ensure high quality data generation and analysis. This workshop aims to disseminate the key findings collected by the metabolomics Quality Assurance and quality Control Consortium (mQACC) Best Practices Working Group to the broader metabolomics community and preview an open-access best practices living guidance document and accompanying website, as a go-to resource for QA/QC in LC-MS-based untargeted metabolomics. We will disseminate key practices identified through six years of community engagement across three relevant technical areas: system suitability testing, reference materials, and data quality review. Additionally, the workshop will act as a forum to encourage wide community adoption of best current practices and critically discuss the content of the mQACC living guidance including the revisions based on the feedback from 2024 workshops held on the topics of pooled QC samples, internal standards, and design of the analytical batch.

### Workshop Objectives

- To disseminate findings from the mQACC LC-MS Best Practices and Living Guidance Working Group's extensive community engagement efforts to establish best practices for LC-MS data collection in untargeted metabolomics.
- To solicit further feedback from the international metabolomics community on the open-access best practices living guidance document that will be freely accessible to researchers.

### Learning Outcomes

- Attendees will learn about minimum and best practices for untargeted LC-MS-based metabolomics as proposed in the mQACC living guidance. The guidance covered will include system suitability testing, use of reference materials, and data quality review.
- Attendees will learn how feedback from the 2024 workshops influenced revisions to minimum and best practices for pooled QC, internal standards, and analytical batch design. They will also learn how to participate in mQACC, including mechanisms to contribute to the best practices community engagement efforts and living guidance document.