



ASMS Fall Workshop DIA and Getting to More Biology

November 7 – 8, 2022

Catamaran Resort Hotel
San Diego, CA

Organizers

Michael MacCoss, *University of Washington*
& Jennifer Van Eyk, *Cedars-Sinai Medical Center*

Name: _____

MONDAY, NOVEMBER 7

7:30 – 9:00 **Enhanced Continental Breakfast & Badge pick-up**, *Kon Tiki Ballroom*

9:00 - 9:05 **Welcome**, Michael MacCoss, *University of Washington*
and Jennifer Van Eyk, *Cedars-Sinai Medical Center*

DIA-MS INTRODUCTION

9:05 – 9:35 **01 Introduction to DIA Concepts, Drive for Reproducible Large Scale Datasets and Comparison of Different Data Acquisition Methods for Q-Orbitrap, Q-TOF, IMS-TOF, etc.**
Brian Searle, *Ohio State University*

9:35 – 10:05 **02 Peptide-Centric Approaches for Data Analysis (e.g. Open-SWATH, Skyline, EncyclopeDIA, Spectronaut)**
Lilian Heil, *University of Washington*

10:05 – 10:30 **Coffee Break**

10:30 - 11:00 **03 Spectrum-Centric Approaches for Data Analysis (e.g. Pular-X, DIA-Umpire)**
Alexey Nesvizhskii, *University of Michigan*

11:00 - 11:30 **04 Skyline Tools and Applications Including Quantification**
Brendan MacLean, *University of Washington*

11:30 – 12:00 **05 Small Group Discussion: Current Challenges and Issues in DIA-MS, Specifically around Robustness and Scalability**
Michael MacCoss, *University of Washington*, Hannes Röst, *University of Toronto*
and Niveda Sundararaman, *Cedars-Sinai Medical Center*

12:00 - 1:00 **Group Photo & Lunch hosted by ASMS**, *Beach North (outside)*

DIGGING INTO THE NITTY GRITTY OF SOFTWARE AND ANALYTICS AROUND DIA-MS

1:00 – 1:30 **06 Hands-On Skyline Tutorial**
Brendan MacLean, *University of Washington*

Hands-On Tutorials Using Thermo Fisher Orbitrap and Bruker timsTOF Instruments on the Same Real Data

1:30 – 2:15 **07 Part 1: Effect of Missingness and Linearity on Quantitation: Orbitrap MS (Exploris 480) Data Analysis by DIA-NN and EncyclopeDIA**
Michael MacCoss, Deanna Plubell and Aaron Maurais, *University of Washington*

2:15 – 3:00 **08 Part 2: Effect on Library Size on Protein Identification and Quantification timsTOF (TimsTOF Pro) by TIMS-DIA-NN**
Qin Fu and Niveda Sundararaman, *Cedars-Sinai Medical Center*

3:00 – 3:30 **Coffee Break**

MONDAY, NOVEMBER 7

NEW AND EMERGING APPROACHES

In this session, each new approach will outline the novelty and the pros and cons compared to peptide- and spectrum- centric approaches based on showing the data generated on the same model data set as above.

3:30 – 3:50 **09 New Approach: DeepSearch and Orbitrap Data**

Gautam Saxena, *DeepDIA*

3:50 – 4:10 **10 New Approach: AI and TimsTOF Data**

Robin Park, *Bruker*

4:10 – 4:30 **11 Update on New Approaches with a Focus on Unmet Needs of DIA-MS**

Hannes Röst, *University of Toronto*

4:30 – 5:00 **12 Panel Discussion: Current Challenges in Data Acquisition**

and Data Handling. The focus is on establishing the issues around misinterpreting spectra and establishing what should be the gold standard.

Brian Searle, Michael MacCoss, Niveda Sundararaman

5:00 – 6:00 **RECEPTION**

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TUESDAY, NOVEMBER 8

7:30 – 9:00 **Enhanced Continental Breakfast & Badge pick-up**, *Kon Tiki Ballroom*

ESTABLISHING REPRODUCIBLE DIA-MS RESEARCH

The goal of this session is to provide real-life examples focusing on quality control, reproducibility of data and highlighting lessons learned sets.

9:00 – 9:20 **13 Sample Preparation and Automation**

Qin Fu, *Cedars-Sinai Medical Center*

9:20 – 9:40 **14 Setting Up Systems Suitability, Process Control and QC for Sample Preparation**

Deanna Plubell, *University of Washington*

9:40 – 10:00 **15 Full System Automation with Tracking and Automated Data Reports**

Sarah Parker, *Cedars-Sinai Medical Center*

10:00 – 10:30 **16 What Is Wrong with This Data?**

Michael MacCoss, *University of Washington*

10:30 – 10:50 Coffee Break

10:50 – 11:30 **Small Group Discussion**

Discuss the challenges and potential solutions based on a series of targeted questions. Questions will include:

- **17** What are criteria for quantification if based on a single peptide, and then extract those criteria to if you have to quantify 20,000 peptides?
- **18** Is identification equal to quantification? Is quantification equal to precision on different instruments?
- **19** What is the strategy for using DIA-MS on PTMs that can be isolated in different DIA-windows (e.g., mono-, di- versus tri-methylation)? What is the strategy for using DIA-MS on PTMs that cannot be isolated in different DIA-MS windows (e.g., citrullination, deamidation, etc.)?

11:30 – 12:00 **20 Full Group Discussion and Brainstorming**

Alek Binek, *Cedars-Sinai Medical Center* and Michael MacCoss, *University of Washington*

12:00 – 1:00 **LUNCH**, Beach North (outside)

PUSHING DIA-MS INTO LARGE SCALE REPRODUCIBLE SCIENCE

The goal of this session is to provide real-life examples of the lectures, focusing on real questions and reproducibility of data and highlight the lessons learned.

1:00 – 1:05 **21 Introduction of Case Studies**

Jennifer Van Eyk, *Cedars-Sinai Medical Center*

1:05 – 1:45 **22 Case Study 1: 350 CSF Samples from Different Disease Categories.**

Challenge: Sample Numbers and Individual Variation

Michael MacCoss, *University of Washington*

1:45 – 2:25 **23 Case Study 2: 1,000 IPSC Derived Motor Neurons for Answer ALS.**

Challenge to overcome are samples arriving over time. DIA-MS carried out on 6600 triple tof

Jennifer Van Eyk and Niveda Sundararaman, *Cedars-Sinai Medical Center*

TUESDAY, NOVEMBER 8

2:25 – 2:50 Coffee Break

2:50 – 3:30 **24 Case Study 3: Single Cell (Aorta).** Challenge: Scaling down the input and scaling up data acquisition and analysis
Sarah Parker, *Cedars-Sinai Medical Center*

3:30 – 4:30 **25 Panel Discussion**
Will summarize needs and next steps in DIA-MS applications.
Moderated by Qin Fu, *Cedars-Sinai Medical Center*
and Lilian Heil, *University of Washington*

4:30 – 5:00 **Summary and Conclusion**
Michael MacCoss, *University of Washington*
Jennifer Van Eyk, *Cedars-Sinai Medical Center*