



# Spectra Processing, Compound Annotation, Functional Insight and Causal Analysis using **MetaboAnalyst 6.0**

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# Schedule

## Part I: 2:15 pm – 4:15 pm

- **2:15 – 2:30:** General introduction
- **2:30 - 3:00:** Untargeted metabolomics
  - ✓ LC-MS & MS/MS spectral processing
  - ✓ From peaks to functions
- **3:05 – 3:25:** Live demo
- **3:25 – 4:15:** Hands on practice

## Part II: 4:30 p.m. – 6:30 p.m.

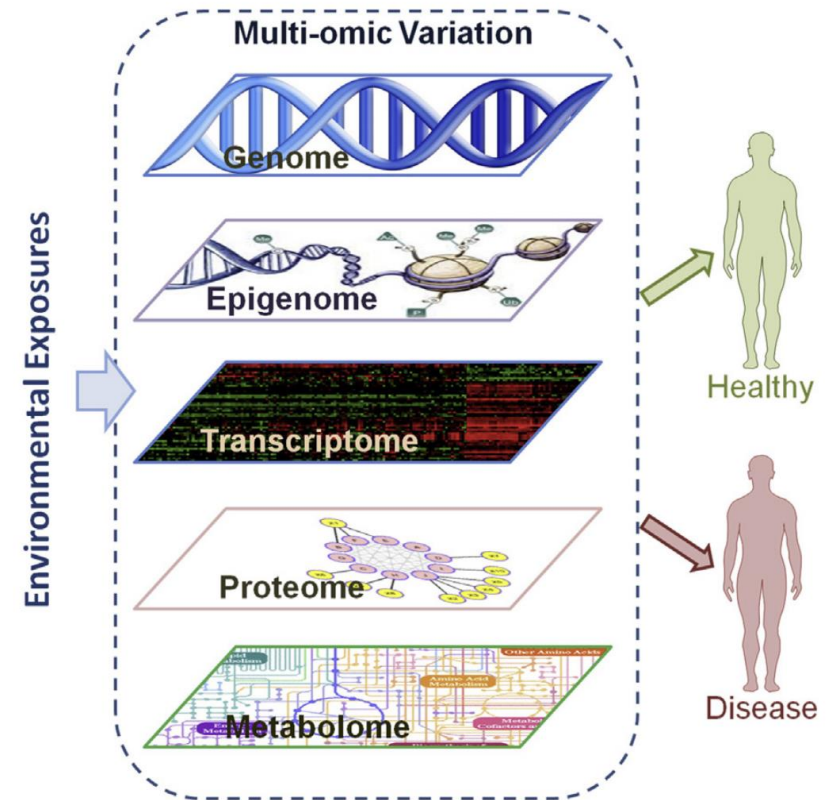
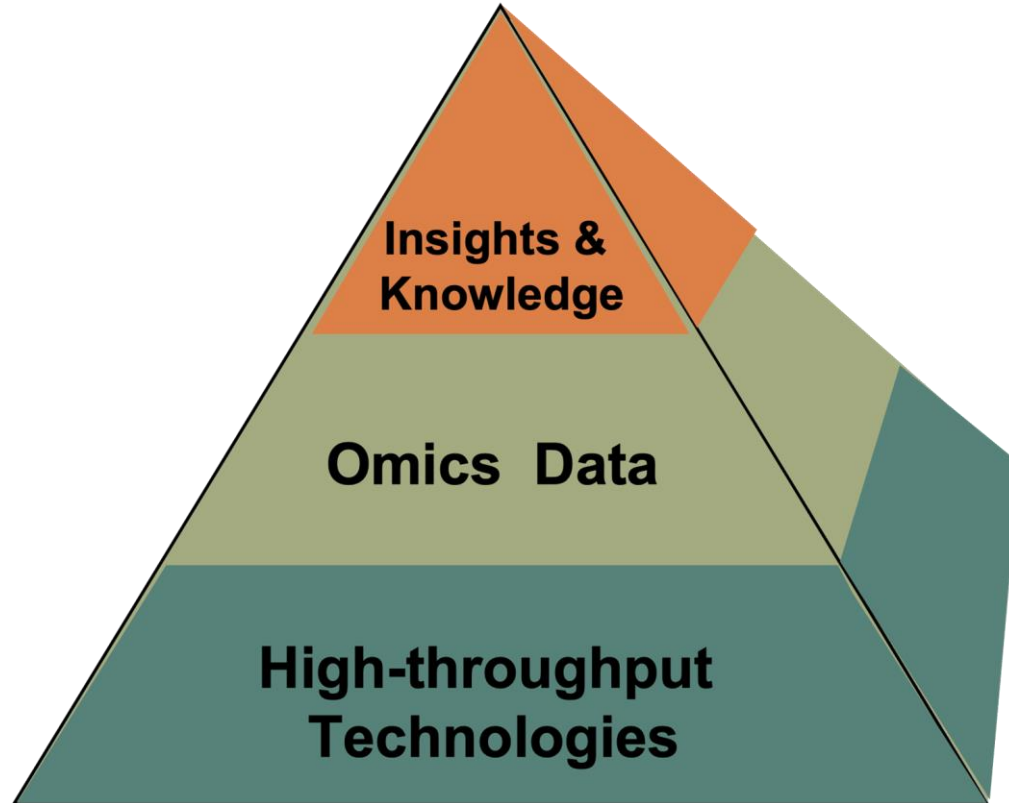
- **4:30 – 5:15:** Background
  - ✓ Statistical analysis
  - ✓ Causal analysis
- **5:15 – 5:35:** Live demo
- **5:40 – 6:20:** Hands on practice
- **6:20 – 6:30:** Summary & discussion

# Github Repository

- [https://github.com/xia-lab/Metabolomics\\_2024](https://github.com/xia-lab/Metabolomics_2024)
- Slides (in PDF format);
- Example data;
- Reference literatures;
- Contact information.

# General Intro to Omics Data Science

# Omics & multi-omics era



# Two distinct challenges

## Size challenge (raw data)

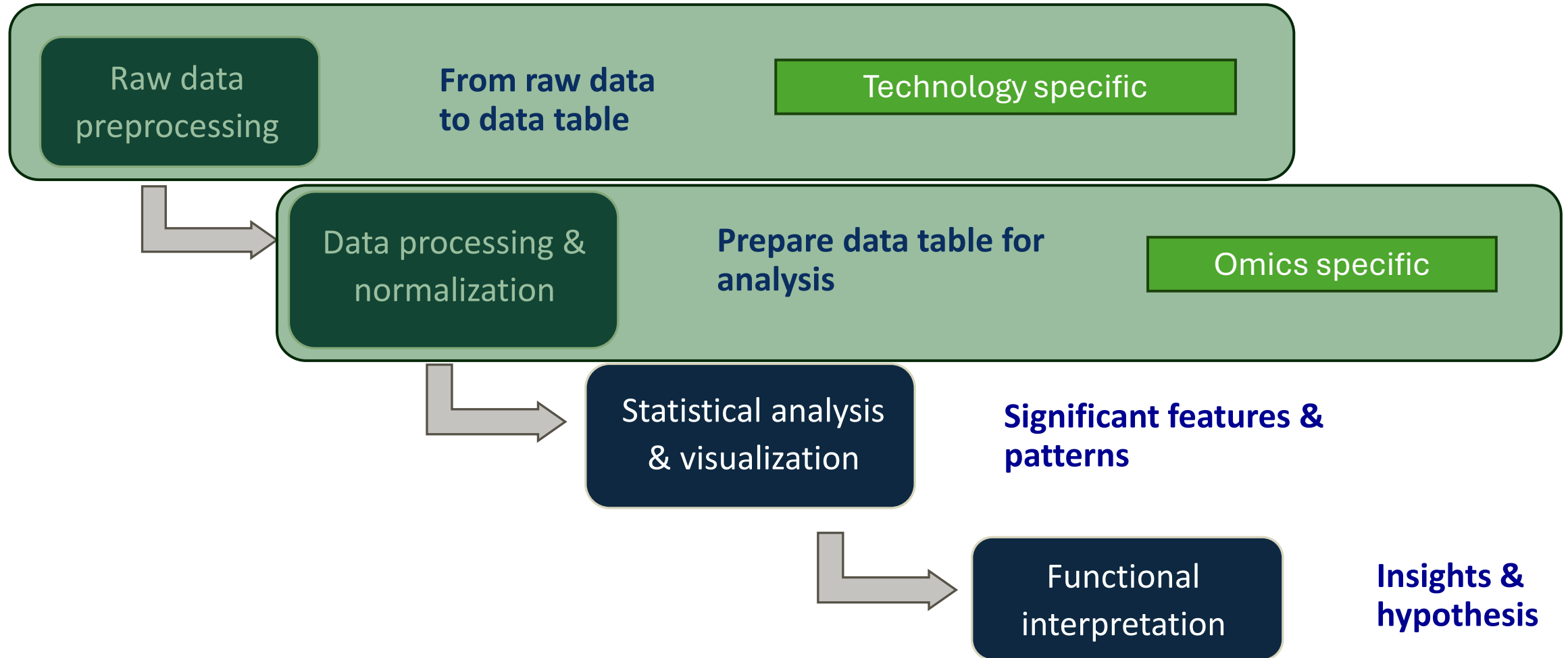
- Raw reads, spectra, images
- Large (GB ~TB)
- Large storage and computing resources




## Complexity challenge (feature table)

- Feature table (abundance, intensities)
- Small (100s KB ~ MB)
- High-dimensional, missing values
- Data analysis starts here


# General workflow for single omics



# Unified frameworks for single omics analysis



MicrobiomeAnalyst -- comprehensive statistical, functional and integrative analysis of microbiome data

Home | Formats | Forum | Updates | Resources

Please choose a module based on your data

- Marker Data Profiling
- Shotgun Data Profiling
- Taxon Set Analysis

Analyze marker gene counts data | Analyze shotgun metagenomics data | Discover enriched microbial signatures

Co-a

## MicrobiomeAnalyst 2.0: comprehensive statistical, functional and integrative analysis of microbiome data

Yao Lu<sup>1</sup>, Guangyan Zhou<sup>2</sup>, Jessica Ewald<sup>3,2</sup>, Zhiqiang Pang<sup>2</sup>, Tanisha Shiri<sup>2</sup> and Jianguo Xia<sup>1,2,3,\*</sup>

Nucleic Acids Research, 2023, 1  
<https://doi.org/10.1093/nar/gkad407>


2023

nature communications

Article

<https://doi.org/10.1038/s41467-023-38785-y>

## ExpressAnalyst: A unified platform for RNA-sequencing analysis in non-model species



MetaboAnalyst 6.0 - from raw spectra to biomarkers, patterns, functions and systems biology

Home | Data Formats | Tutorials | User Forum | MetaboAnalystR | Publications | Update History | Databases | APIs | User Stats | About | Contact

NSERC CRISNG

Canada Research Chairs

News & Updates

- Pre-registration is now open for our [Omics Data Science Training Course](#) - 1 week Bootcamp (Aug. 5-9) or 12 weeks (Sep - Nov);
- Check out our latest publication on [MetaboAnalyst 6.0](#): towards a unified platform for metabolomics data processing, analysis and interpretation
- Please help complete a brief [user survey](#) on behalf of The Metabolomics Innovation Centre (TMIC)
- Check out our latest Nature Protocol on [web-based multi-omics integration](#)
- Enhanced support for customizing colors and shapes in 2D score plots in Statistical Analysis modules (04/16/2024);
- Fix the issue on name mapping for fumarate based on user feedback (04/10/2024);
- Updated the tutorials for all new and updated modules in MetaboAnalyst version 6.0 (03/12/2024);
- Added support for MGF (mass generic format) files for MS2 spectra upload (03/11/2024);
- Enhanced compound databases and name mapping algorithm based on user feedback (02/16/2024);
- The Spectra Processing module now supports MS2 spectra upload (01/10/2024);

Click here to start

Overview

MetaboAnalyst is a web-based platform dedicated for comprehensive metabolomics data analysis, interpretation and integration with other omics data. Over the past decade, MetaboAnalyst has evolved from statistical and functional analysis for targeted metabolomics data, towards more streamlined analysis for both quantitative and untargeted metabolomics data. In addition to many feature enhancements, the version 6.0 contains three new modules - tandem MS spectral processing and **compound annotation**, dose response analysis for **chemical risk assessment**, and leveraging metabolite-genome wide association analysis and Mendelian randomization for **causal analysis**.

Nucleic Acids Research, 2024, 1-9  
<https://doi.org/10.1093/nar/gkae253>  
Web Server Issue



## MetaboAnalyst 6.0: towards a unified platform for metabolomics data processing, analysis and interpretation

Zhiqiang Pang<sup>1</sup>, Yao Lu<sup>2</sup>, Guangyan Zhou<sup>1</sup>, Fiona Hui<sup>1</sup>, Lei Xu<sup>1</sup>, Charles Viau<sup>1</sup>, Aliya F. Spigelman<sup>3</sup>, Patrick E. MacDonald<sup>3</sup>, David S. Wishart<sup>4</sup>, Shuzhao Li<sup>5,6</sup> and Jianguo Xia<sup>1,2,\*</sup>

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<sup>2</sup>Department of Microbiology and Immunology, McGill University, Montreal, Quebec, Canada

<sup>3</sup>Department of Pharmacology and Alberta Diabetes Institute, University of Alberta, Edmonton, Alberta, Canada

<sup>4</sup>Departments of Biological Sciences and Computing Science, University of Alberta, Edmonton, Alberta, Canada

<sup>5</sup>The Jackson Laboratory for Genomic Medicine, Farmington, CT, USA

<sup>6</sup>University of Connecticut School of Medicine, Farmington, CT, USA

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### Abstract






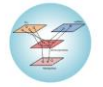

We introduce MetaboAnalyst version 6.0 as a unified platform for processing, analyzing, and interpreting data from targeted as well as untargeted metabolomics studies using liquid chromatography - mass spectrometry (LC-MS). The two main objectives in developing version 6.0 are to support tandem MS (MS2) data processing and annotation, as well as to support the analysis of data from exposomics studies and related experiments. Key features of MetaboAnalyst 6.0 include: (i) a significantly enhanced Spectra Processing module with support for MS2 data and the asari algorithm; (ii) a MS2 Peak Annotation module based on comprehensive MS2 reference databases with fragment-level annotation; (iii) a

2024



# Omics Data Science Training Course

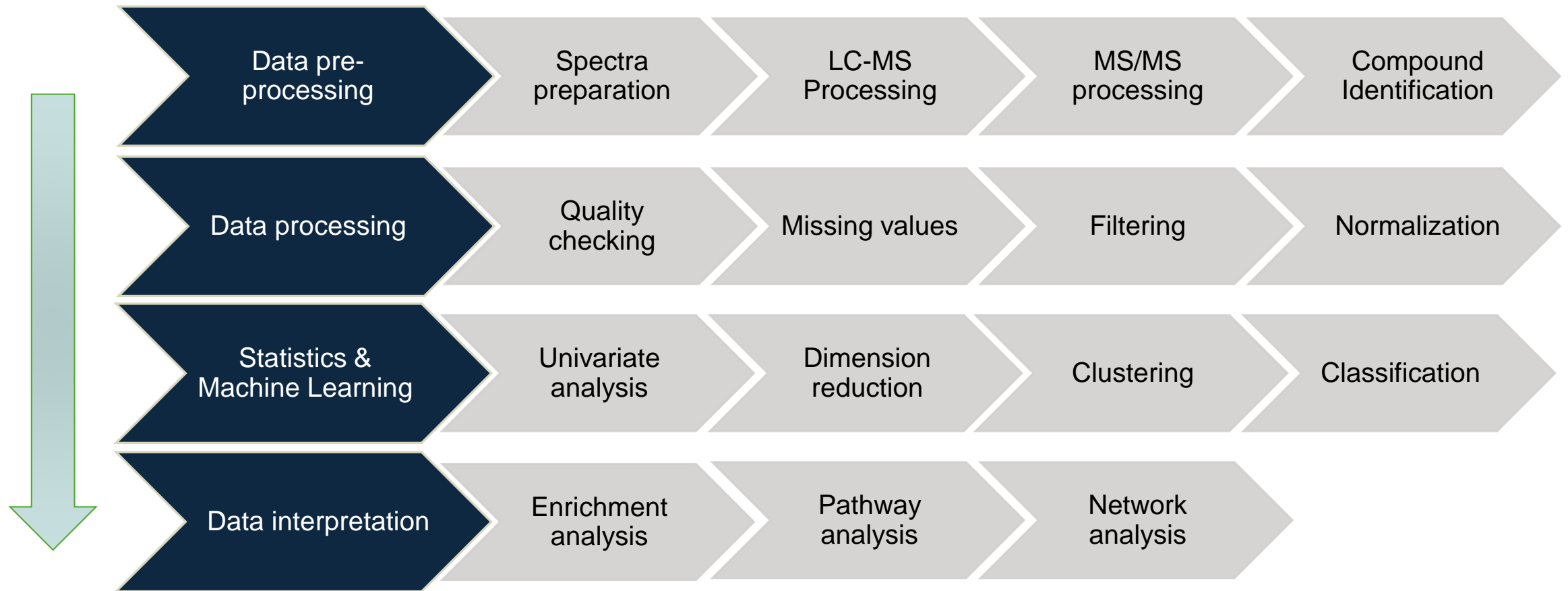
- Summer Bootcamp
  - Aug. 5 - 9, 9:30 - 16:30
- Regular Session
  - Saturday morning 9:30 - 12:00, Sept. - Nov.

Topics	Tools	Key Publications
Transcriptomics	 ExpressAnalyst	1. Nature Communications (2023) 2. Current Protocol (2023)
Biological Networks	  NetworkAnalyst miRNet	3. Methods in Molecular Biology (2022) 4. Nucleic Acids Research (2020) 5. Nucleic Acids Research (2019)
Metabolomics	 MetaboAnalyst	6. Nucleic Acids Research (2024) 7. Nature Protocols (2022) 8. Current Protocols in Bioinformatics (2019)
Microbiomics	 MicrobiomeAnalyst	9. Nucleic Acids Research (2023) 10. Nature Protocols (2020)
Multi-omics	  OmicsNet OmicsAnalyst	11. Nature Protocol (2024) 12. Nucleic Acids Research(2022) 13. Nucleic Acids Research (2021)
Special Topics	Biomarker analysis & meta-analysis	14. Metabolomics (2013) 15. Nature Protocols (2015)
	RNAseq processing for non-model species	16. Genome Research (2021)
	LC-MS and MS/MS spectra processing	17. Nature Communications (2024)



## Key Publications

- Liu, P., Ewald J., Legrand E., Jeon, Y.S., Sangiovanni, J., Hacariz, O., Pang, Z., Zhou, G., Head, J., Basu, N., and Xia, J. @ (2023) "A unified platform for RNA-seq analysis in non-model species", Nature Communications 14, 2995.
- Ewald J., Zhou, G., Lu, Y., and Xia, J.@ (2023) "Using ExpressAnalyst for Comprehensive Gene Expression Analysis in Model and Non-Model Organisms" Current Protocols 3 (11), e922
- Chang, L., Xia, J.@ (2022) "MicroRNA Regulatory Network Analysis Using miRNet 2.0" Chapter Transcription Factor Regulatory Networks, 185-204, Methods in Molecular Biology, Humana Press, New York, NY.
- Chang, L., Zhou, G., Soufan, O. and Xia, J.@ (2020) "miRNet 2.0: network-based visual analytics for miRNA functional analysis and systems biology" Nucleic Acids Research (doi: 10.1093/nar/gkaa467)
- Zhou, G., Soufan, O., Ewald, J., Hancock, REW, Basu, N. and Xia, J.@. (2019) "NetworkAnalyst 3.0: a visual analytics platform for comprehensive gene expression profiling and meta-analysis" Nucleic Acids Research 47 W234-241
- Pang, Z., Lu, Y., Zhou, G., Hui, F., Xu, L., Viau, C., Spigelman, A., MacDonald, P., Wishart, D., Li, S., and Xia, J. @ (2024) "MetaboAnalyst 6.0: towards a unified platform for metabolomics data processing, analysis and interpretation" Nucleic Acids Research (doi: 10.1093/nar/gkac253)
- Pang, Z., Zhou, G., Ewald, J., Chang, L., Hacariz, O., Basu, N., Xia, J.@ (2022) "Using MetaboAnalyst 5.0 for LC-HRMS spectra processing, multi-omics integration and covariate adjustment of global metabolomics data" Nature Protocols (doi: 10.1038/s41596-022-00710-w).
- Chong, J., Wishart, D.S. and Xia, J.@ (2019) "Using MetaboAnalyst 4.0 for Comprehensive and Integrative Metabolomics Data Analysis". Current Protocols in Bioinformatics 68, e86 (doi: 10.1002/cpbi.86)
- Lu, Y., Zhou, G., Ewald, J., Pang, Z., Shiri, T., and Xia, J.@ (2023) "MicrobiomeAnalyst 2.0: comprehensive statistical, functional and integrative analysis of microbiome data" Nucleic Acids Research (doi: 10.1093/nar/gkad407).
- Chong, J., Liu, P. and Xia, J.@. (2020) "Using MicrobiomeAnalyst for comprehensive statistical, functional and meta-analysis of microbiome data" Nature Protocols (doi: 10.1038/s41596-019-0264-1)
- Ewald J., Zhou, G., Lu, Y., Kolic, J., Ellis, C., Johnson, J., Macdonald P., and Xia, J. @ (2024) "Web-based Multi-omics Integration Using the Analyst Software Suite", Nature Protocols (doi: 10.1038/s41596-023-00950-4
- Zhou, G., Pang Z., Lu, Y., Ewald, J., Xia, J.@ (2022) "OmicsNet 2.0: a web-based platform for multi-omics integration and network visual analytics" Nucleic Acids Research (doi:10.1093/nar/gkac376).
- Zhou, G., Ewald, J., and Xia, J.@ (2021) "OmicsAnalyst: a comprehensive web-based platform for visual analytics of multi-omics data" Nucleic Acids Research (10.1093/nar/gkab394)
- Xia, J., Broadhurst, D., Wilson, M. and Wishart, D. (2013) "Translational biomarker discovery in clinical metabolomics: an introductory tutorial". Metabolomics (doi: 10.1007/s11306-012-0482-9)
- Xia, J@., Gill, E., and Hancock, R.E.W@. (2015) "NetworkAnalyst for Statistical, Visual and Network-based Approaches for Meta-analysis of Expression Data" Nature Protocols 10 (6), 823-844
- Liu, P., Ewald, J., Galvez, J., Head, J., Crump, D., Basu, N. and Xia, J.@ (2021) "Ultrafast functional profiling of RNA-seq data for nonmodel organisms" Genome Research (doi: 10.1101/gr.269894.120)
- Pang, Z., Xu, L., Viau, C., Lu Y., Salavati, R., Basu, N., and Xia, J.@ (2024) "MetaboAnalystR 4.0: a unified LC-MS workflow for global metabolomics" Nature Communications (doi:10.1038/s41467-024-48009-6)

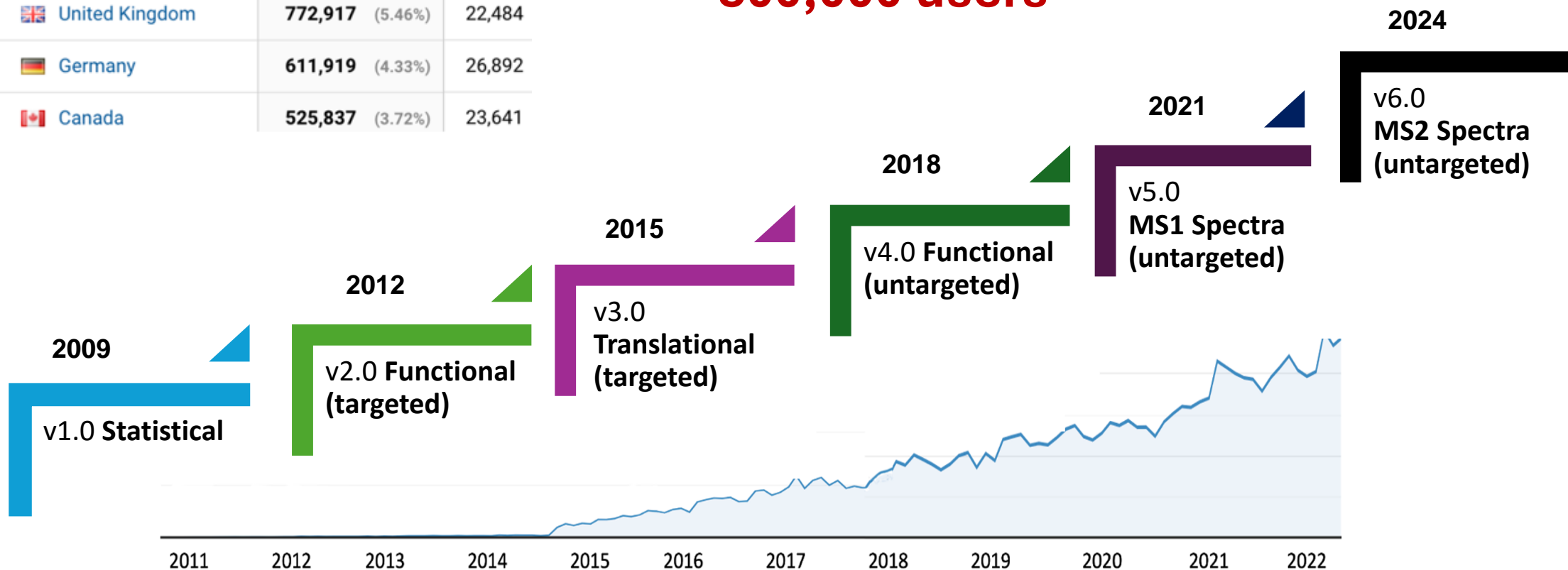
# Common Tasks for Metabolomics



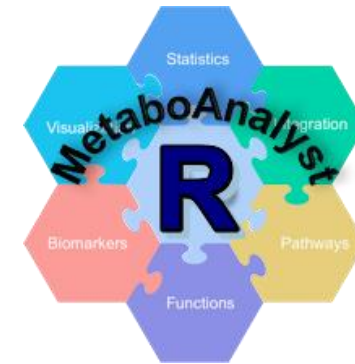
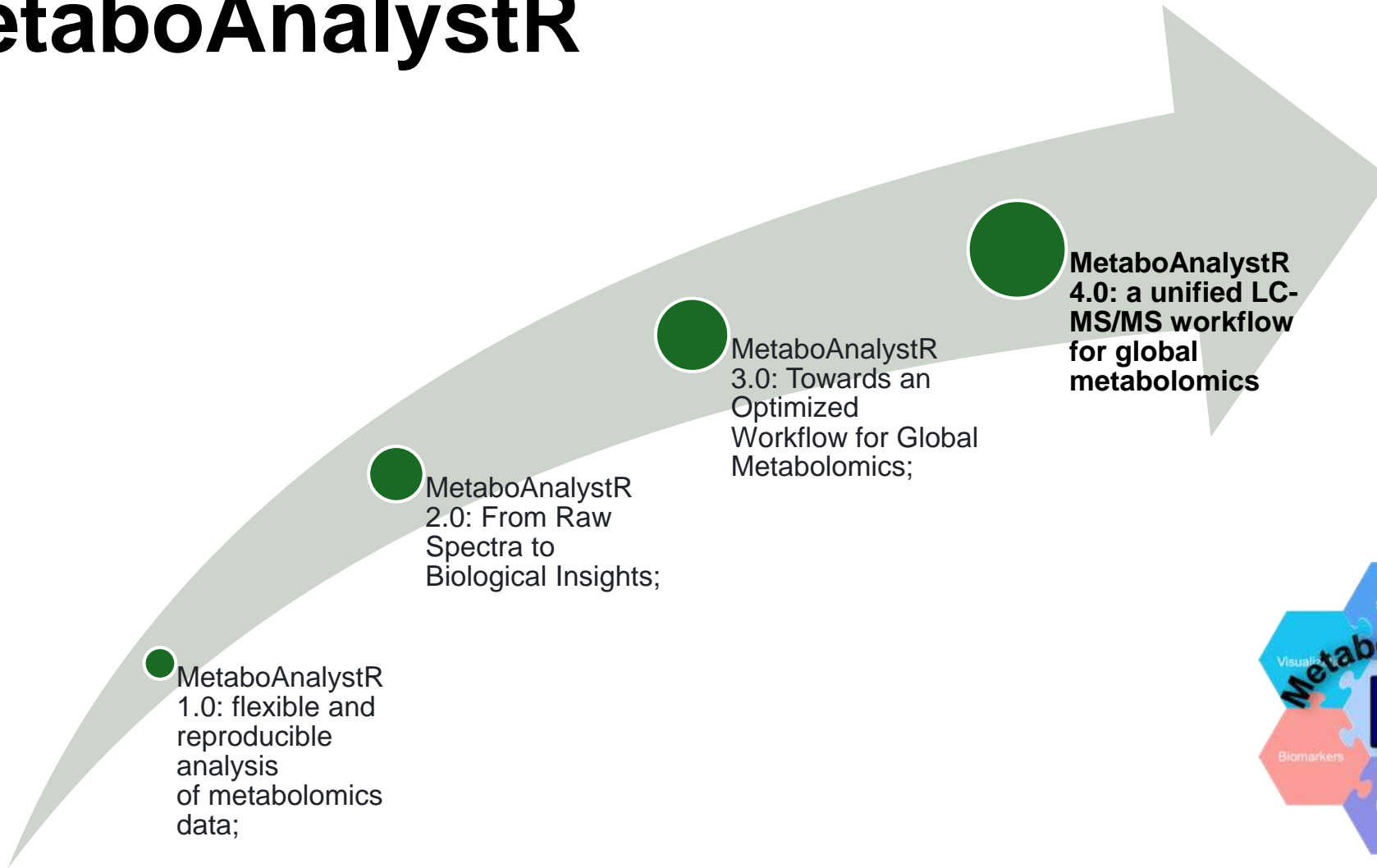
# MetaboAnalyst roadmap

Countries	Jobs	Users
1.  United States	3,695,032 (26.12%)	120,929
2.  China	3,145,812 (22.24%)	179,934
3.  United Kingdom	772,917 (5.46%)	22,484
4.  Germany	611,919 (4.33%)	26,892
5.  Canada	525,837 (3.72%)	23,641

**~ 500,000 users**



# MetaboAnalystR



# MetaboAnalyst 6.0 Modules

Input Data Type	Available Modules (click on a module to proceed, or scroll down to explore a total of 18 modules including <a href="#">utilities</a> )				
LC-MS Spectra (mzML, mzXML or mzData)			Spectra Processing [LC-MS w/wo MS2]		
MS Peaks (peak list or intensity table)		Peak Annotation [MS2-DDA/DIA]	Functional Analysis [LC-MS]	Functional Meta-analysis [LC-MS]	
Generic Format (.csv or .txt table files)	Statistical Analysis [one factor]	Statistical Analysis [metadata table]	Biomarker Analysis	Statistical Meta-analysis	Dose Response Analysis
Annotated Features (metabolite list or table)		Enrichment Analysis	Pathway Analysis	Network Analysis	
Link to Genomics & Phenotypes (metabolite list)			Causal Analysis [Mendelian randomization]		

# Resources for this workshop

Slides & materials:

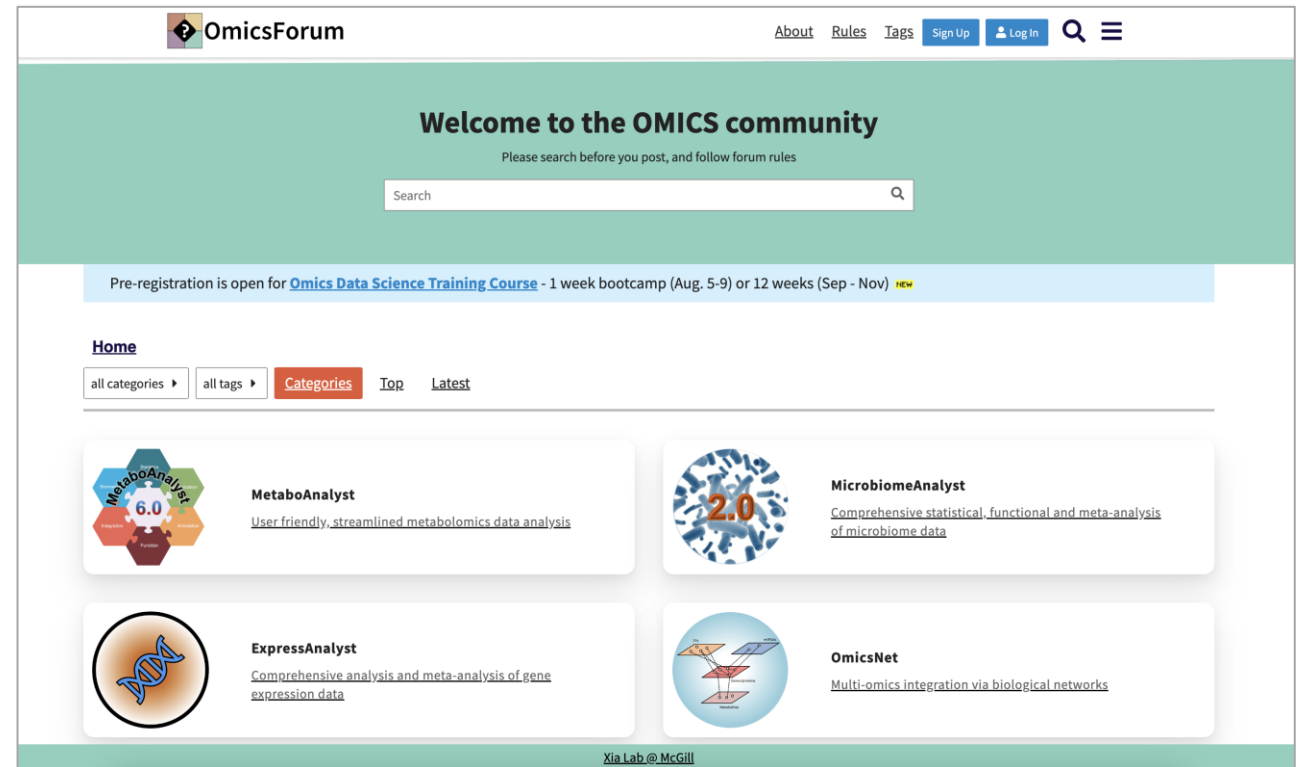
- [https://github.com/xia-lab/Metabolomics\\_2024](https://github.com/xia-lab/Metabolomics_2024)

MetaboAnalyst servers:

- <https://www.metaboanalyst.ca>
- <https://new.metaboanalyst.ca>
- <https://dev.metaboanalyst.ca>

User Forum:

- <https://omicsforum.ca>



# Cautions & recommendations

- **Do not open multiple tabs!**
  - Results will overwrite each other!
- Form a group and share computers
  - Make new friends
  - Help each other & reduce stress
- Page display may be **slow** due to bandwidth limitation
  - Be patient
  - Reduce bandwidth consumption by forming group