

## Introduction

Matrix-assisted laser desorption/ionization-mass spectrometry imaging (MALDI-MSI) is vital to in situ metabolomics, offering spatial and molecular insights from biological samples. METASPACE<sup>1</sup>, a key open-source tool, visualizes metabolites and exports pixel-to-pixel intensities from multiple databases for untargeted analysis, but overlapping annotations create duplicate values. Manual workflows using tools like Excel to average intensities and remove overlaps are slow and error-prone. In addition, integrating MSI data across samples manually is inefficient, driving the need for automation. Therefore, we developed MetaSync to streamline METASPACE MSI data trimming and aggregation for disease profiling and biomarker discovery.

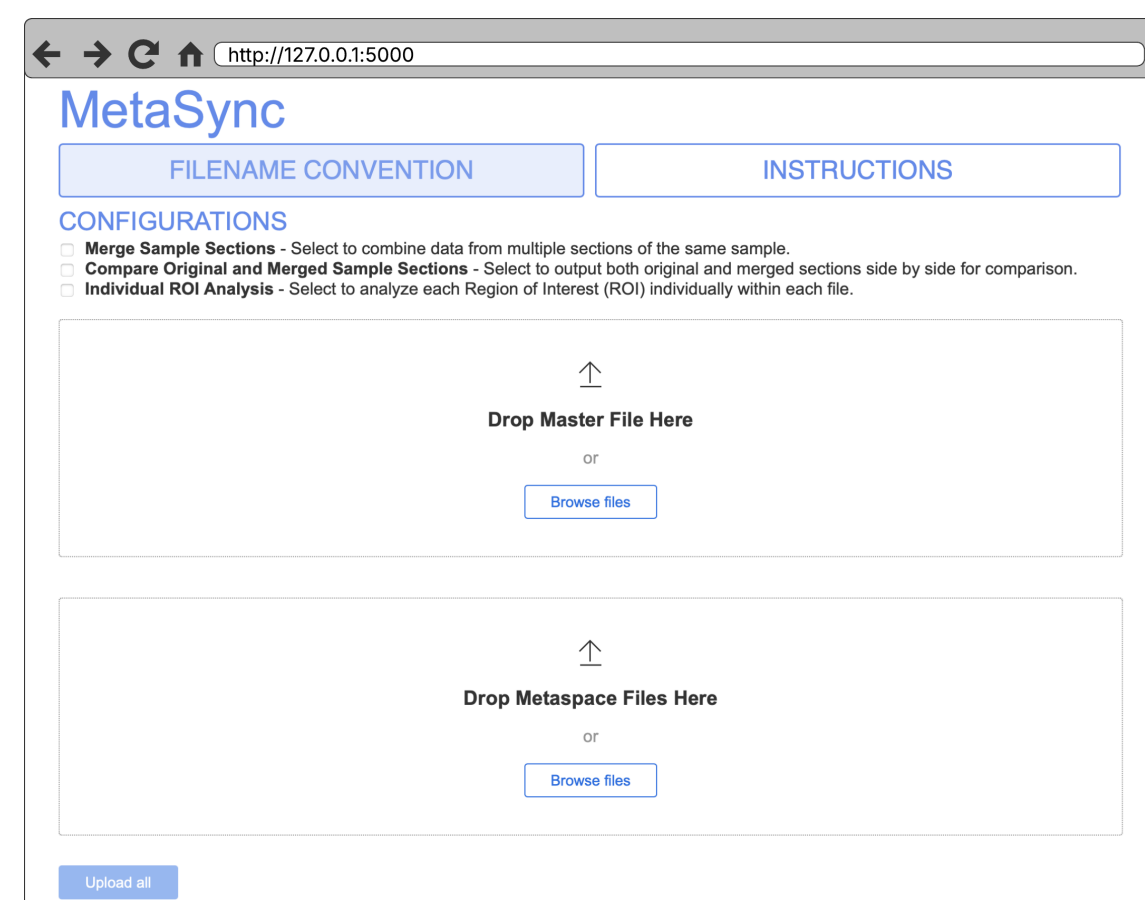


Fig. 1 User Interface(UI)

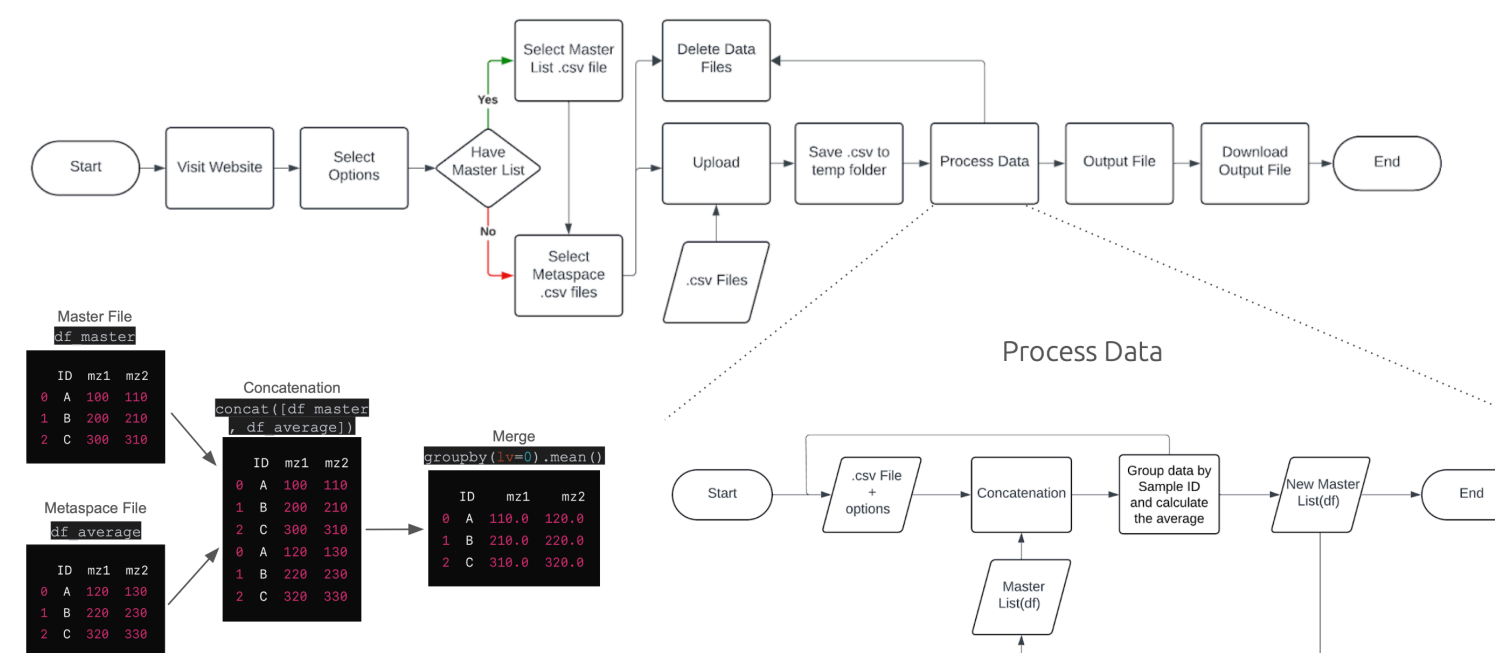


Fig. 2 MetaSync Logic Diagram

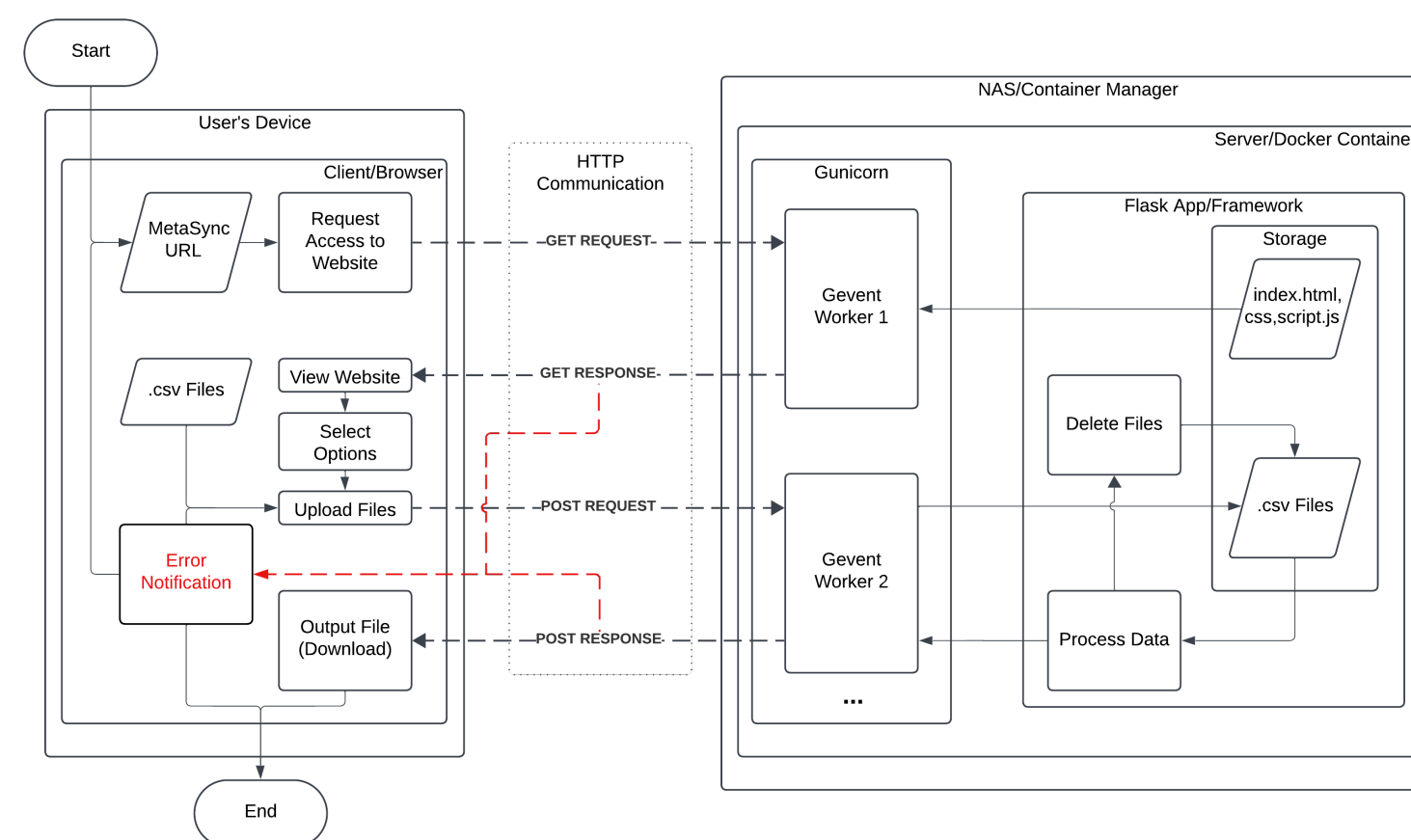


Fig. 3 MetaSync System Architecture

## Methods

- Male C57BL6 wild-type (WT) mice (8-10 weeks age, n=8 per group) received either water alone (Veh) or 5 mM adenine by drinking water (Ad) for 1 week
- Harvested kidney cortex via slow-freezing with isopentane.
- Selected 3 representative cortex samples per group for MALDI-MSI analysis.
- Export METASPACE ROI pixel intensity CSV files.
- Chose one MetaSync analysis option.
- Uploaded METASPACE CSV files.
- Processed uploaded files in MetaSync to aggregate and analyze MSI data.
- Downloaded processed data as a single CSV file.
- Analyzed it in MetaboAnalyst 6<sup>2</sup> for statistical insights.

## Results

### MetaSync Performance Results:

- Uploaded and processed 5.84GB of data (equivalent to 122 METASPACE files) in 5 minutes, versus more than 3 hours manually.
- Demonstrates O(n·m) time complexity (n = files, m = intensities) for efficient large-scale MSI data handling.
- Ran efficiently on a lab server, scalable to enhanced hardware or self-hosted setups tailored to operational needs.

### Untargeted Metabolomic Profile in Adenine-Treated Mouse Kidney:

- Processed 6 wild-type(WT) mice kidney MSI files—each with over 1200 metabolites, including METASPACE<sup>1</sup> overlaps.
- Identified 76 significantly downregulated and 85 significantly upregulated features (p < 0.05) from WT files—each with over 1200 unique metabolites—using MetaSync's output in MetaboAnalyst.

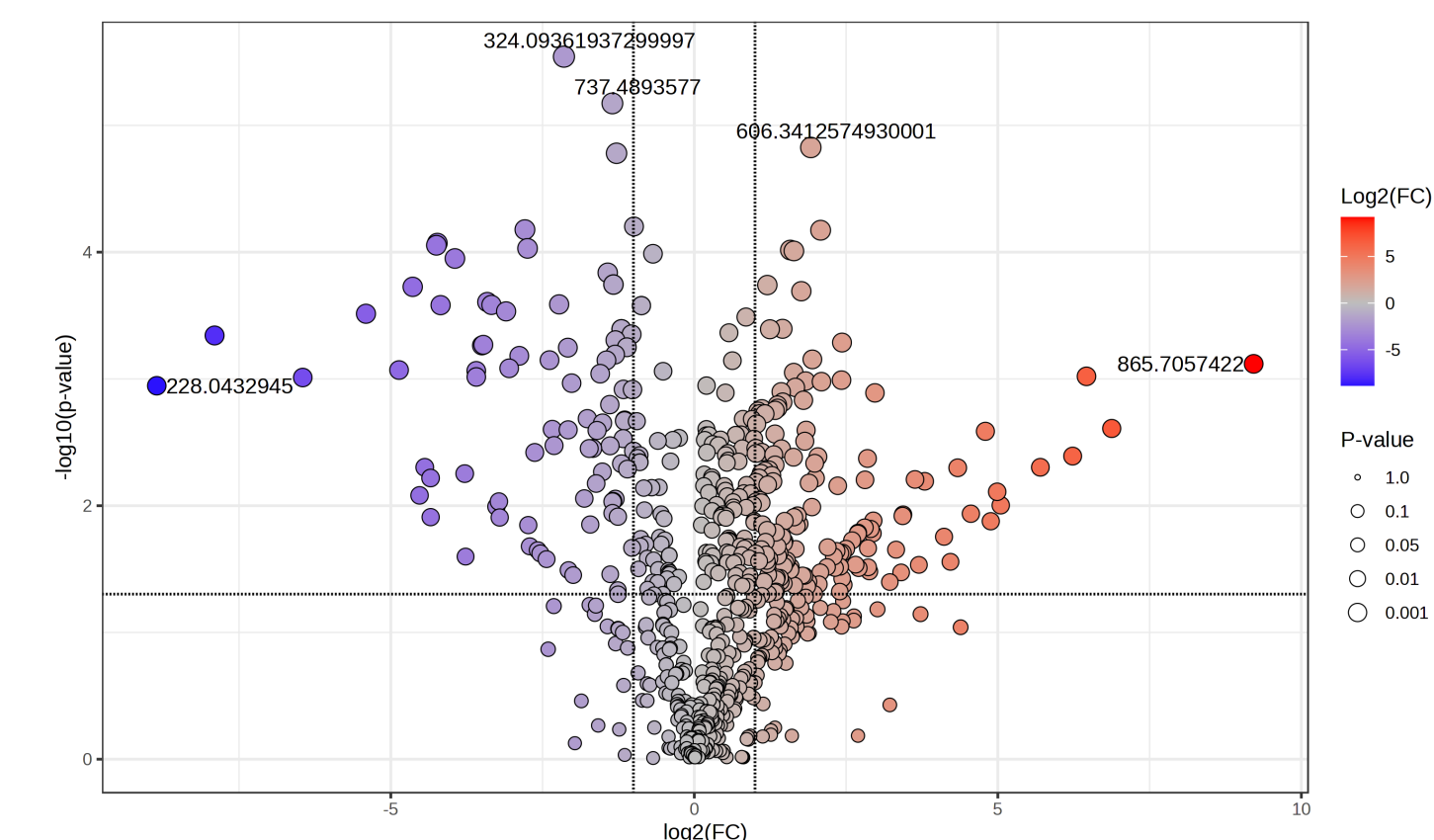


Fig. 4 WT-Veh vs WT-Ad Volcano Plot

## Conclusion

MetaSync streamlines MSI analysis by reducing processing time and ensuring data integrity, enhancing its utility in spatial metabolomics. Its performance mice kidney model, managing thousands of metabolites, shows it can process high-resolution datasets. Future scalability and analytics enhancements may expand its role translational research.

## Funding

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## References

- Palmer A, et al. Nat Methods. 2017;14:57-60.
- Xia J, et al. Nucleic Acids Res. 2024;52:W398-W406.

## Approach

- Developed in Python, utilizing Pandas, Flask, Unicorn, and Docker, featuring an intuitive drag-and-drop interface for ease of use.
- Enables uploading an optional master CSV file along with multiple MATASPACE<sup>1</sup> CSV files.
- Aligns data by mass-to-charge (m/z) ratios and averages metabolite intensities across samples, guided by a structured filename convention (e.g., sampleID\_sectionNumber\_slideNumber.csv).
- Offers three customizable output options:
  - Merging multiple sections within a sample for a unified dataset.
  - Producing side-by-side comparisons of original and merged data for validation.
  - Analyzing individual regions of interest (ROIs) separately to preserve spatial granularity.
- Delivers processed results as a single CSV file, facilitating seamless downstream analysis.