Metabolite Explorer

A software tool for targeted analysis of mass spectrometry imaging data

High throughput mass spectrometry imaging (MSI) experiments require efficient, streamlined processes to analyze the resulting data[1]. MSI datasets increasingly feature a variety of meta-information that reflect characteristics of the analyzed tissue, which can be leveraged for structured data analysis e.g. for group stratification or statistical comparisons. **Metabolite Explorer** facilitates high-throughput, targeted data analysis for multiple MSI experiments. We demonstrate the use of **Metabolite Explorer** in a drug delivery study with the aim of determining which potential drug metabolites warrant further investigation.

Approach

Import MSI data

Metabolite Explorer combines the pooled imzML file with relevant meta-information related to both tissues (e.g., animal ID, dosage level) and candidate metabolites (e.g., SMILES formulas, related bio-transformations). During import, the tool partitions the imzML file into its constituent tissues, and associates each tissue with the meta-information to facilitate group-based comparisons of tissues within filter rules. The complete approach is outlined in figure 1.

Data Acquisition

Rat kidney tissues from a nonclinical drug safety study were collected from 1 control and 3 treated animals, for a total of 12 tissues. Treated animals were dosed with 150 mg/kg of a Factor Xa antagonist daily for 14 consecutive days. Cryosections (15 µm) were spray-coated with DHB matrix, and MSI data acquired on a solariX 7T FT-ICR instrument (Bruker Daltonik GmbH), at a 100 µm raster width, over mass range m/z 100-800.

Pre-filtering

All data from each measurement were extracted and pooled into a single imzML file using SCiLS Lab 2020Pro (Bruker Daltonik GmbH). Separately, a list of potential metabolites for Factor Xa was generated using Mass-Metasite (Molecular Discovery Ltd.) containing ~300 ions of interest.

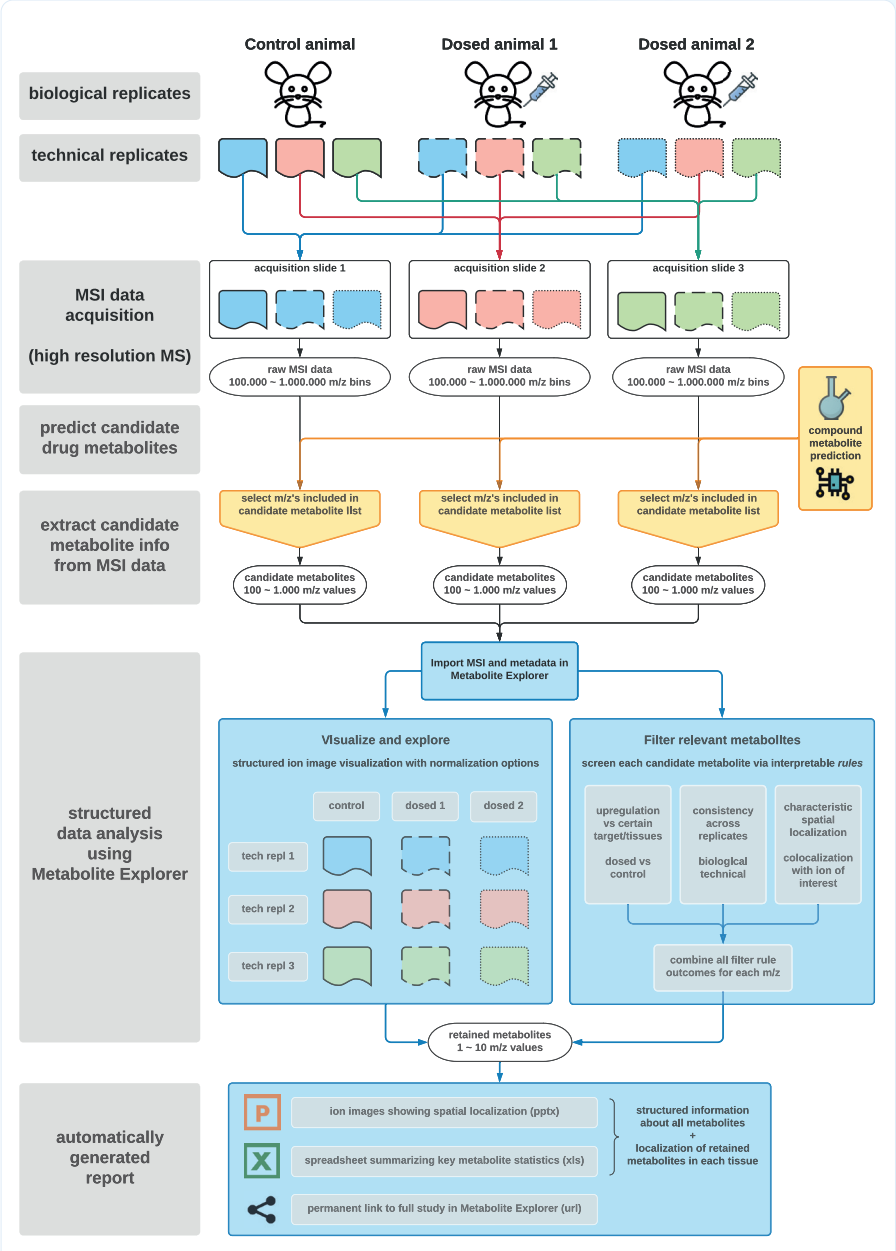
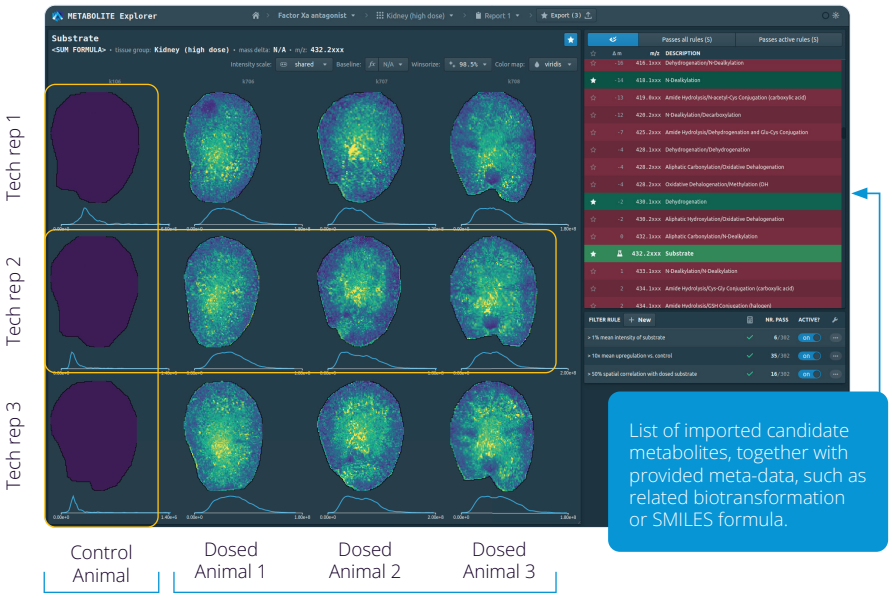


Fig. 1: Example drug discovery study workflow using Metabolite Explorer for structured data analysis

Application



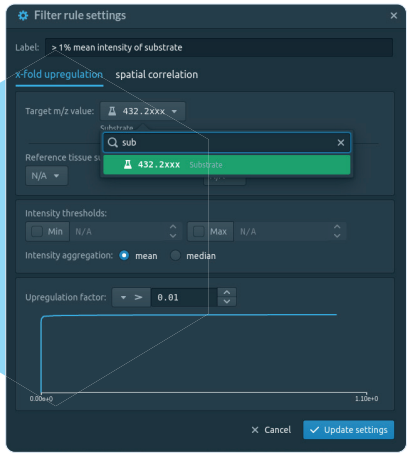
This panel shows intensities for the drug compound Factor Xa antagonist. Two dose groups were imported: "Control" and "High Dose". The groups are laid out in a grid based on the metadata tags, with technical replicates in rows and different animals in columns.

Visualize and explore

The software provides a variety of visualisation options while minimizing perceptual bias, e.g. several perceptually uniform color maps[2,3] and winsorizing intensities to reduce the impact of outlier pixels.

The tool also offers the ability to share the ion intensity scale across all tissues, or to scale intensities relative to a chosen reference ion (e.g., to assess metabolite intensity versus the substrate).

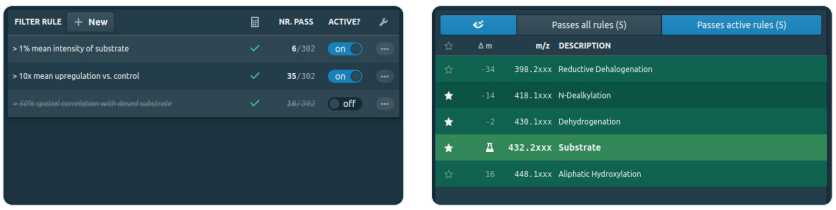
An interactive intensity histogram is displayed below each ion image e.g. for comparison of intensities across tissues, or identify bi-modal intensity distributions.



Define filter rules to retrieve metabolites of interest.

Filter relevant metabolites

Metabolite Explorer allows the user to define their own screening workflows by combining configurable, human-interpretable filter rules, which then allows assessment of specific properties of each candidate metabolite, given the experimental data and meta-information.



Combine multiple rules into a screening pipeline. Metabolites passing all rules are listed for further investigation.

For this specific study, the screening pipeline was constructed using two specific filter rules:

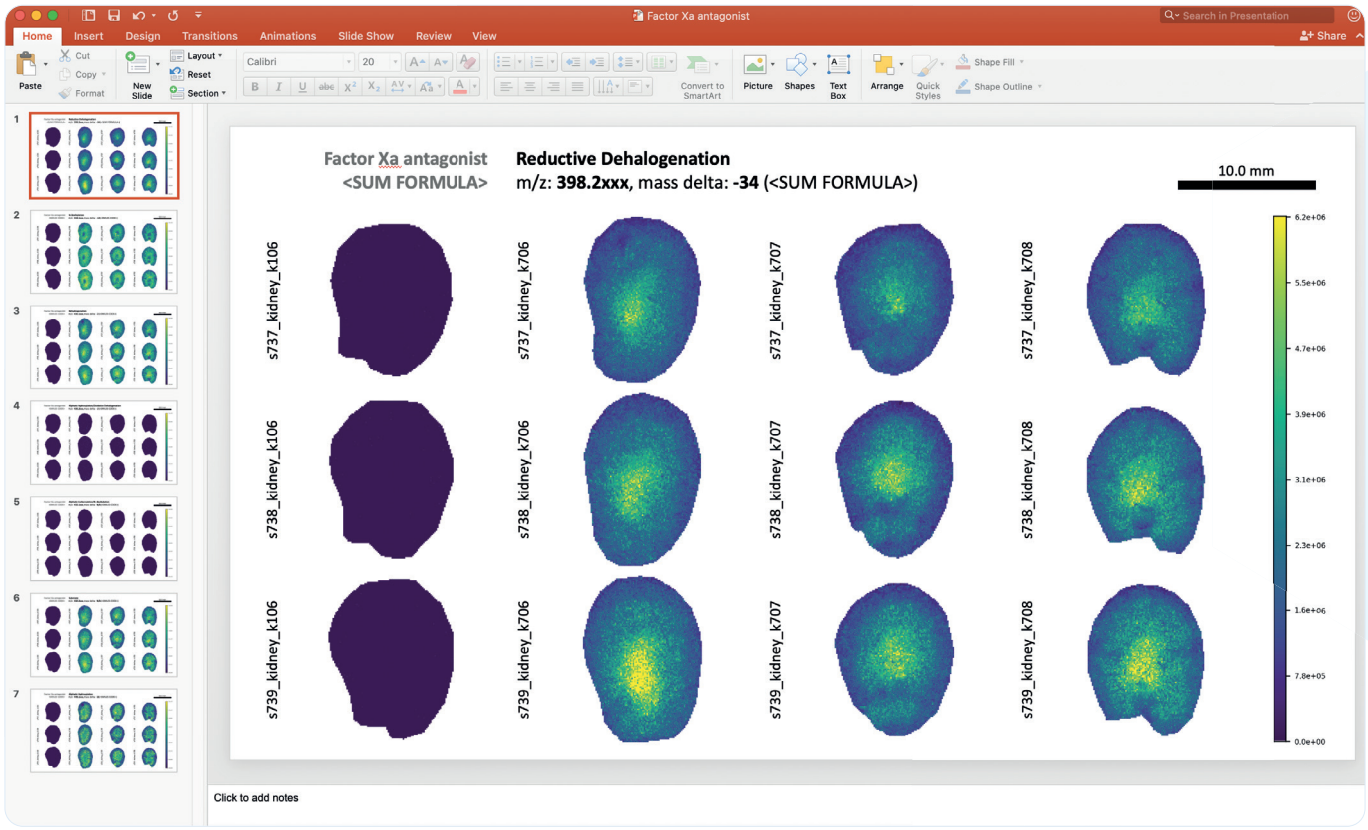
i. A minimal candidate metabolite abundance compared to the substrate ion within dosed tissues, and
ii. The candidate metabolite must have at least a 10-fold up-regulation in the dosed tissue compared to the control tissue.

Additional filters can be added, for example to screen for spatial correlation to a target ion, or a relevant biomarker.

Using these two rules, the pipeline greatly reduced the number of potential drug metabolites that required in-depth investigation from an initial ~300 peaks to a limited list of 5 molecular ions.

Reporting

Once the analysis is finalized, Metabolite Explorer generates a structured report that summarizes key conclusions. This process is fully automated, thus significantly reducing human effort.



Each report comprises of:

- a Powerpoint file, which captures visualizations as shown in the tool,
- an Excel file, containing candidate metabolite statistics, and
- a permalink within Metabolite Explorer that interactively shows the full report details.

Importantly, the analysis is locked once a report is exported. In this way, a report can always be traced back to a certain state of the analysis. Due to the web-based nature of the tool, a link pointing to the analysis results can be shared with collaborators and stored for permanent future reference. New analyses can be created based on an existing locked analysis in case users want to modify certain settings to create a new report.

Summary

- **Metabolite Explorer** is a dedicated tool that provides a streamlined workflow and is invaluable for high-throughput applications.
- The software design emphasizes human interpretability via intuitive, user-defined screening criteria, to facilitate applications like drug delivery, metabolism and biomarker discovery.
- Features include organization of study groups based on metadata and automated reporting capabilities which eliminate tedious, repetitive tasks, thus saving time while simultaneously reducing the risk of human error.
- By imposing a structured yet flexible workflow, **Metabolite Explorer** encourages standardization and reproducibility between team members as well as across studies.

References

[1] Schulz S, Becker M, Groseclose R, Schadt S, Hopf C (2019), Current Opinion in Biotechnology (55)

[2] Nuñez JR, Anderton CR, Renslow RS (2018) PLoS ONE 13(7)

[3] Race AM, Bunch J, (2015) Anal Bioanal Chem. 407(8)

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Additional links

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