Metabolite Explorer

A SOFTWARE TOOL FOR TARGETED ANALYSIS OF MASS SPECTROMETRY IMAGING DATA

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Intro

The increasing throughput of mass spectrometry imaging (MSI) experiments mandates efficient, streamlined processes to analyze the resulting data [1]. Moreover, MSI datasets typically feature a **variety of concomitant meta-information**, such as characteristics of the analyzed tissue. Such meta-information can be leveraged for structured analysis of multiple MSI experiments, e.g., for stratification or statistical comparisons between groups.

We introduce Metabolite Explorer, a software tool that facilitates high-throughput, targeted data analysis, given data from multiple MSI experiments. Its design emphasizes **human interpretability** via intuitive, user-defined screening criteria, facilitating in particular applications like drug delivery, biomarker discovery and diagnostics. In this work, we perform a drug delivery study using Metabolite Explorer, where the goal is to determine which potential drug metabolites warrant further investigation. **A typical workflow is depicted in Figure 1**.

Pre-filtering

A list of potential drug metabolites is generated using Mass-Metasite (Molecular Discovery Ltd.) resulting in ~300 ions of interest. All data related to these metabolites is extracted from each dataset and pooled into **a single imzML file** using SCiLS Lab 2020Pro (Bruker Daltonik GmbH).

Import MSI data into Metabolite Explorer

Metabolite Explorer combines the pooled imzML file with relevant meta-information that can be related to both tissues (e.g., animal ID, dosage level) and candidate metabolites (e.g., SMILES formulas, related biotransformations). 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**.

Visualize and explore

List of imported candidate metabolites, together with provided meta-data, such as the related biotransformation or SMILES formula.

The panel above shows the **intensities for the drug compound** in this study. We import two dose groups, "Control" and "High Dose", which are laid out in a grid based on the tags provided by the meta data (rows represent technical replicates, columns represent different animals). We observe a clear difference in ion expression between control and dosed tissues (first and subsequent columns, respectively).

The tool provides a wide variety of options to visualize ion images, while **minimizing perceptual bias**, including several perceptually uniform color maps [2,3] and winsorizing intensities to reduce the impact of outlier pixels.

Moreover, the tool 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 compared to the substrate). Finally, an interactive intensity histogram is displayed below each ion image to compare intensity distributions across tissues, or to identify e.g. bi-modal intensity distributions.

Data acquisition

We demonstrate the tool using kidney tissues from a **nonclinical drug safety study in rats**, involving a total of 12 tissues, originating from 1 control and 3 treated animals. Treated animals were dosed once daily with 150 mg/kg of a Factor Xa antagonist for 14 consecutive days. Cryosections (15 µm) were spray-coated with DHB matrix, and MSI data was acquired on a solariX 7T FT-ICR instrument (Bruker Daltonik GmbH), at a 100 µm raster width, focusing on the 100 to 800 m/z range.

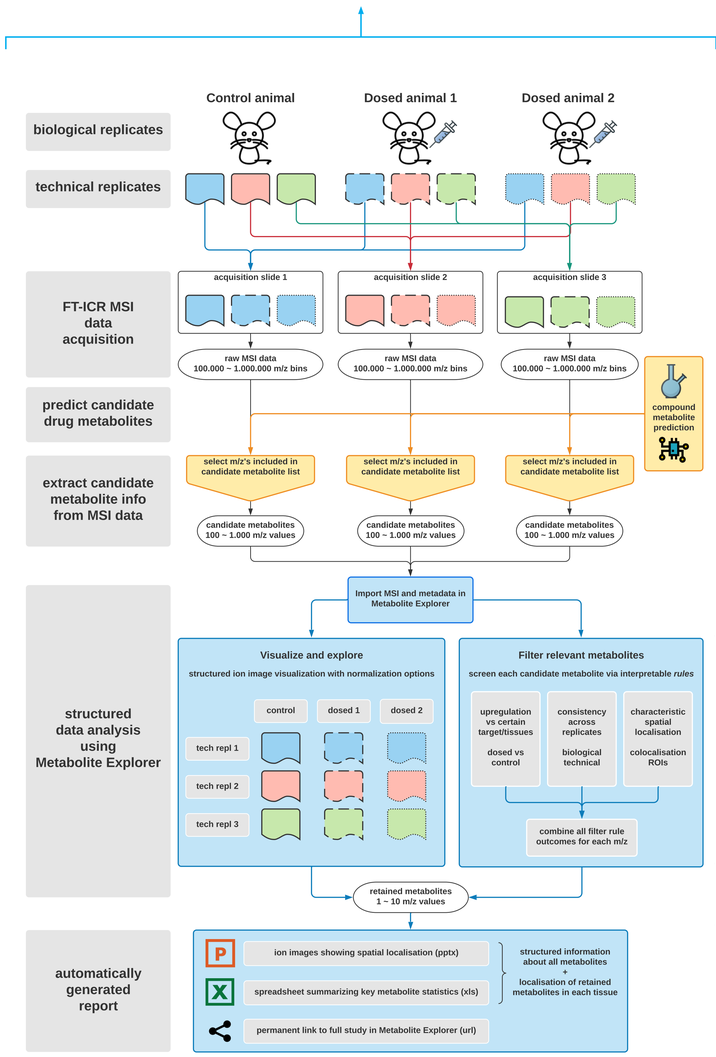
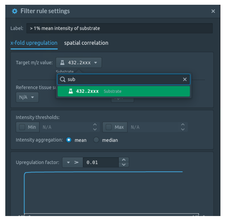


Figure 1. Workflow of a drug discovery study using Metabolite Explorer.

Filter relevant metabolites



Define filter rules to retrieve metabolites of interest.

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

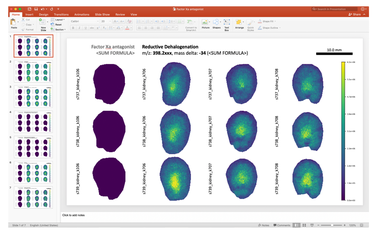
Metabolite Explorer allows the user to define a screening workflow by combining **configurable, human-interpretable filter** rules, which assess specific properties of each candidate metabolite, given the experimental data and meta-information.

In this example, we construct a screening pipeline that consists of two filter rules, namely (i) minimal candidate metabolite abundance compared to the substrate ion within dosed tissues and (ii) at least a 10-fold upregulation of the candidate metabolite in the dosed tissue compared to the control tissue. Furthermore, filters can be added to screen for spatial correlation to a target ion, or a relevant biomarker.

In this example, the aforementioned filter pipeline greatly reduces the number of potential drug metabolites that require in-depth investigation from ~300 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 a Powerpoint file, which captures visualizations as shown in the tool, and an Excel file, containing candidate metabolite statistics.



Importantly, once a report is exported, the analysis gets locked. 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.

Conclusion

Having a dedicated tool that provides a streamlined workflow is **invaluable for high throughput** applications, such as the drug delivery studies performed at Boehringer Ingelheim. By imposing a structured yet flexible workflow, Metabolite Explorer **encourages standardization and reproducibility** between team members as well as across studies. Finally, the tool's automated reporting capabilities eliminate tedious, repetitive tasks in manually constructing reports, thus saving time while simultaneously reducing the risk of human error.

Want to see Metabolite Explorer in action?

Join our upcoming webinar! Details available at
www.aspect-analytics.com/asms2020/#webinar
or contact us at info@aspect-analytics.com

References

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