thermoscientific

From Spectra to Molecules in Spatial Metabolomics: Data processing & metabolite annotation of AP-SMALDI-Orbitrap data using METASPACE

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ABSTRACT / OBJECTIVES

- **To demonstrate the complete MS Imaging workflow** from sample to compound annotation for spatial metabolomics, when approached by TransMIT's AP-SMALDI[5] AF ion source coupled with Thermo Scientific™ Orbitrap Exploris™ mass spectrometers. The **MS Imaging workflow** starts with optimized matrix application of NEDC matrix and ends with data processing including compound assigment of the MS Imaging data.
- **To apply the METASPACE platform** (https://metaspace2020.eu) as the processing tool for compound annotations of the here presented MS Imaging data sets.

For this purpose, the poster

- **Illustrates specific, useful features** supporting spatial metabolomics data interpretation in the METASPACE platform such as
  - Confirmation of interpretation by displaying co-localization of
    - isotopes in a pattern, in parallel
    - related quasi-molecular ions, in parallel
  - Displaying the spatial organization of metabolic activities at a given location

INTRODUCTION

Atmospheric-**p**ressure **s**canning microprobe **m**atrix-**a**ssisted **l**aser **d**esorption/**i**onization **m**ass **s**pectrometry **i**maging (**AP-SMALDI MSI**) combined with Thermo Scientific Orbitrap technology provides unmatched performance regarding spatial resolution, mass resolution, mass accuracy and sensitivity.

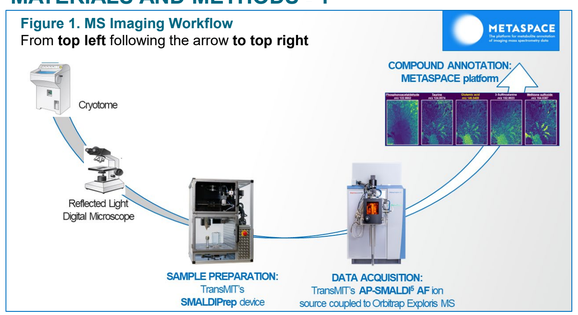
This positions **AP-SMALDI-Orbitrap** among the most prominent techniques for the emerging field of **spatial metabolomics** with applications in biology, medicine, and pharmacology. Metabolite identification is essential for applications but remains a key challenge in MSI.

Here, we describe a spatial metabolomics pipeline integrating data acquisition using AP-SMALDI-Orbitrap with processing and metabolite identification in the **METASPACE platform**, a public knowledge base and FDR-controlled metabolite annotation (*) platform. This pipeline converts large MSI data generated by AP-SMALDI MSI into a list of metabolites, supplied with molecular images in an untargeted, rapid, and automatic fashion.

* : in this poster presentation, compound annotations which have isomers or isobars are indicated with an asterisk * at the end of the name, see e.g., Figure 5 with glucose*, as there are other hexoses besides glucose. Potential isomers and isobars are provided by METASPACE for further structural validation.

MATERIALS AND METHODS - 1

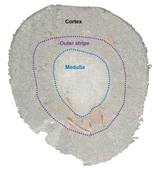
Figure 1. MS Imaging Workflow
From **top left** following the arrow **to top right**



- **Tissue slicing** in a cryotome
- **Visual inspection** of the sample during various stages of the analysis by a digital microscope
- **Matrix deposition** in TransMIT's SMALDIPrep device
- **MS Imaging analysis** using TransMIT's AP- SMALDI[5] AF ion source / Orbitrap Exploris MS
- **Compound annotation** using METASPACE, https://metaspace2020.eu/

MATERIALS AND METHODS - 2

Figure 2. Optical image of the explored model system mouse kidney tissue.



Sample system:
- Mouse kidney tissue sections (16 µm thickness)
- High metabolic activities including continuous nutrient uptake, removing waste products & balancing body fluids.
- Mouse kidney illustrates a complex tissue architecture consisting of the medulla, outer stripe and cortex region.

Matrix application:
- 10 mg/mL of NEDC (see reference 1), i.e., N-naphthylethylene-diamine dihydrochloride, in MeOH/H_2O (7:3)
- Ultrafine pneumatic spraying of 200 µL matrix solution via SMALDIPrep using a flowrate of 5 µL/min

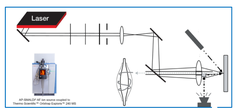
MS Imaging instrumentation:
- TransMIT AP-SMALDI[5] AF ion source + Thermo Scientific Orbitrap Exploris 480 mass spectrometer

Experimental parameters:
- Mass resolution: 240,000 at *m/z* 200, Mass range: *m/z* 120 to 440, negative ion mode
- Internal lock mass correction by EASY-IC (scan-to-scan mode) enabled for high mass accuracy
- Ion source was operated in the 2D pixel mode, pixel size was 10 µm and 15 µm, respectively
- Measurement time was about 12 h for each experiment (~ 72k pixels)

Figure 3. Workflow from *.raw to high-confidence compound annotation

1. **High-level workflow from sample to image w/ AP-SMALDI[5] AF source – Orbitrap MS Imaging, see Figure 1.**

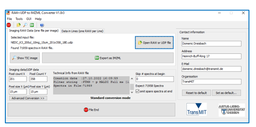
2. **AP-SMALDI[5] AF ion source coupled with Orbitrap Exploris for MS Imaging**



Schematics of **TransMIT's AP-SMALDI[5] AF ion source** / Thermo Scientific Orbitrap Exploris coupling.

AP-SMALDI[5] AF ion source with its
- co-axial source design
- 5 micrometer laser spot size
- pixelwise-autofocusing capability

3. **imzML converter - raw data file + position information – here for *.raw and *.udp – are converted to imzML format** (see reference 2)

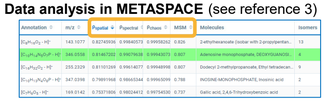


Various imzML converters are available, here the most recent one from TransMIT GmbH is shown.
By conversion to imzML format, initial profile data are reduced to centroid data.
An intuitive user interface guides the user to select respective *.raw (MS data) files and *.udp (position) files of the MS imaging project. The dimensions (number of pixels in *x* and *y*) and pixel size are extracted automatically.

4. **Upload imzML file to METASPACE platform**

The *.imzML file is uploaded to the METASPACE platform. The user enters respective metadata (such as ion source, detector, matrix, mass resolution, pixel size) – see to the right.
From a respective menu, the user can select up to 4 out of 14 databases for the search. For the metabolomics questions presented herein, for example, **CoreMetabolome** or **ChEBI databases** were chosen.

5. **Data analysis in METASPACE** (see reference 3)

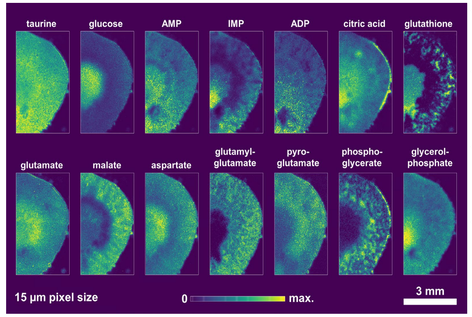


The estimated **Metabolite-Signal Match (MSM)** score ranks the **molecular formulas** by their likelihood (see reference 3). An MSM score of 1 indicates the maximal likelihood of the signal corresponding to the ion.

The MSM score is computed by multiplication of the following measures (see orange rectangle in table above):
- The measure of **spatial chaos** quantifies spatial informativeness within the image of the principal peak.
- The **spectral** isotope measure quantifies the spectral similarity between a theoretical isotopic pattern and relative sampled isotopic relative intensities.
- The **spatial** isotope measure quantifies spatial colocalization between isotopic ion images.

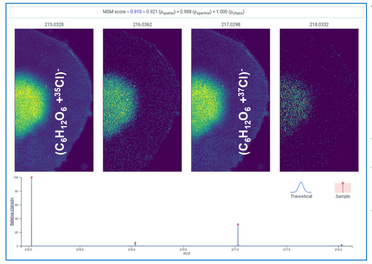
RESULTS - 1

Figure 4. Overview of spatial metabolomes for mouse kidney, 15 micrometer



- Here, data of mouse kidney (Material & Methods) with 15 micrometer pixel size are shown
- METASPACE platform enables for convenient browsing of metabolite annotations and their corresponding ion images
- Here, **up to 466 metabolite annotations** for the mass range *m/z* 100 to 400 (CoreMetabolome database) were found
- Metabolite annotations can be structured by various filter settings, including co-localization scoring (see below).
- The **QR code** directs to the METASPACE project.

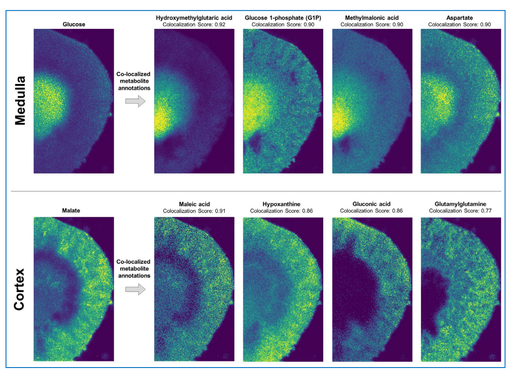
Figure 5. Demonstrating Metabolite-Signal Match scoring with the example of glucose



The ion image of metabolite **glucose*** $C_6H_{12}O_6$ is shown, displayed with its respective isotopes and displayed as $[glucose+Cl]^-$ ion in which the Cl^--adduct is deriving from the NEDC matrix. The respective $[glucose-H]^-$ ion signal (not shown here) is available in the data set with minor intensity, confirming the above interpretation.
MSM score is calculated as 0.91:
- High **spatial similarity** between four different isotopes is found w/ $rho_{spatial}$ = 0.921.
- High **spectral similarity** relative to the theoretical isotope pattern is found w/ $rho_{spectral}$ = 0.988.
- The measure of spatial chaos quantifies spatial informativeness within the image of the principal peak; the (high) value of rho_{chaos} = 1 indicates that all signals of this compound are found inside of the sample.

- Here, glucose* nicely illustrates details on the isotopic pattern and isotopologue images, in particular, because of the characteristic chlorine isotopic pattern.

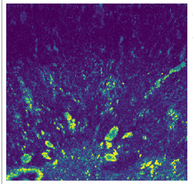
Figure 6. Automated co-localization scoring to reveal region-specific metabolic activities



- Ion images of various co-localized metabolites for the specific tissue regions are shown:
- First, two metabolites showing charactersitic distributions for the
  - **medulla** (glucose*) and
  - **cortex** (malate*)
  regions were selected. (see left)
- Next, METASPACE automatically displayed other metabolite annotations that have similar spatial distributions, based on the calculated **co-localization score**.

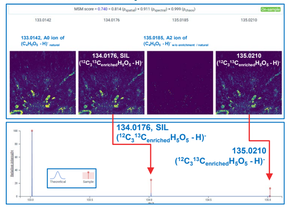
RESULTS - 2

Figure 7. METASPACE annotates spatially-resolved stable-isotope-labeled metabolites



Also taken from kidney tissue, here with **stable isotope labeled (SIL) metabolite** of malate* $C_4H_6O_5$.

Ion images displayed with its respective isotopic ions, $[M-H]^-$ ions.



Left: Monoisotopic ion image of $^{12}C_4H_6O_5$ enlarged.
Right: Ion images of respective isotopologues are shown w/ respective mass spectral information & ion abundances. That spatially-resolved isotope labeling can reveal tissue metabolic activities, has been published in 2022 by Wang et al. (see reference 4).
Herein, to our knowledge, we present the first interrogation of MSI data from SIL-experiments in METASPACE. Despite the higher intensity of the SIL malate, malate is correctly annotated. Its intensities are higher than the theoretically expected intensities for naturally occuring malate (thus rho_spectral decreases). Due to the increased intensity resulting from the ^{13}C enrichment, their ion images are more pronounced. It can be assumed that the higher intensities made the spatial correlation even higher compared to what would be seen for unlabelled malate in a similar tissue where no SIL was applied.

CONCLUSIONS

- **TransMIT's AP-SMALDI[5] AF ion source** coupled with Thermo Scientific Orbitrap Exploris mass spectrometer is perfectly suited to generate mass spectral raw data and imaging position information for an upload to the METASPACE platform.
- The **METASPACE platform** is a freely available, open-source, community-populated knowledge base for spatial metabolomes. It can be used for annotating small molecules, metabolites, lipids, drugs and allied.
- The METASPACE platform provides highly confident annotation of compounds resulting from High Resolution Accurate Mass (HRAM) MS imaging data.
- **Orbitrap technology** allows for ultra-high resolving power and **mass accuracy** of ≤ 1.5 ppm across the full scan range, i.e., of importance for **MS1-based annotations**.
- Databases enable access to efficient large scale analysis of MS Imaging data sets
- **Co-localization of isotopologues** and/or other corresponding quasi-molecular ions of a given metabolite, contributes to confidence of data interpretations (Fig. 5)
- **Spatial co-localization** analysis reveals **metabolites** with spatial localization as defined by a marker (Fig. 6)."
- **First-time use** of METASPACE for spatial ^{13}C-labelled isotope tracing (Fig. 7) is shown.

REFERENCES

1. Rui Chen et al. 2012, NEDC (N-(1-naphthyl) ethylenediamine dihydrochloride https://doi.org/10.1021/ac202438a | Anal. Chem. 2012, 84, 465–469
2. The imzML input format is an open standard supported by all major mass spectrometer vendors, and all code is open-source. For more details refer to https://metaspace2020.eu/help and How to export imaging mass spectrometry data into the imzML format
3. References to METASPACE: http://metaspace2020.eu, Palmer et al, 2017; see https://www.nature.com/articles/nmeth.4072, https://speakerdeck.com/metaspace2020/metaspace-theory and Palmer et al, 2017; see https://www.nature.com/articles/nmeth.4072
4. Lin Wang et al. 2022; Spatially resolved isotope tracing reveals tissue metab. activity, https://doi.org/10.1038/s41592-021-01378-y

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TRADEMARKS/LICENSING

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