AP-MALDI MSI

Coupling AP-SMALDI MS Imaging technology with a modified Orbitrap Hybrid mass spectrometer

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Abstract

Purpose: Present AP-SMALDI coupled to the new Thermo Scientific™ Orbitrap™ Excedion™ mass spectrometer

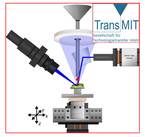
Methods: AP-SMALDI[5]AF ion source coupled to Thermo Scientific Orbitrap Excedion mass spectrometer for MS Imaging application using the full bandwidth of capabilities to create highly-resolved images w/o oversampling.

Results: **MS1 imaging** and localization of Purkinje cells in cerebellum with 5 um spatial resolution,
MS2 imaging of imatinib-dosed worm *Fasciola hepatica* confirming the MS1 suggested location of imatinib,
MS1 imaging of mating river fluke *Schistosoma Mansoni* with clear localization of the drug zotatifin in the female worm

Introduction

Atmospheric-pressure scanning microprobe matrix-assisted laser desorption/ionization mass spectrometry imaging (AP-SMALDI MSI) combined with Thermo Scientific™ Orbitrap™ technology provides unmatched performance regarding mass resolution, spatial resolution and sensitivity. Studies demonstrated the spatially resolved analysis for numerous classes of biomolecules using Thermo Scientific™ Orbitrap Exploris™ MS platform.

Materials and methods



MS Imaging – Samples and sample preparation:
tissue samples used
(1) healthy mouse brain tissue (refer to **Figure 1**),
(2) Imatinib-dosed worm *Fasciola Hepatica* en.wikipedia.org/wiki/Fasciola_hepatica
(refer to **Figure** 2) were sectioned at –20° C using a cryotome and thaw-mounted on regular glass slides. Tissue section of **(3)** *Schistosoma mansoni* (blood fluke) (refer to **Figure 3, and below**) en.wikipedia.org/wiki/Schistosoma_mansoni dosed with drug Zotatifin, was embedded in gelatine for sectioning.

MALDI matrix DHB was applied using the SMALDIPrep device (TransMIT GmbH, Giessen, Germany; www.smaldi.de/en). The SMALDIPrep device pneumatically sprayed the matrix solution onto the tissue applying matrix- and tissue specific (user-modifiable) protocols.
Measurements were performed using an **ion source:** AP-SMALDI[5] AF, TransMIT GmbH, Giessen, Germany; www.smaldi.de/en coupled to a **mass spectrometer, here** the new Thermo Scientific™ Orbitrap™ Excedion™ mass spectrometer (Bremen, Germany).
Regular MALDI - Samples and sample preparation (refer to **Figure 4**): CHCA and DHB matrices were used according to established protocols and prepared in dried droplet-manner on a stainless-steel plate together with a (non-complex)solution of compounds of amino acids (**Phe** and **Ile**) and others (data not shown)). Intensity ratios of respective precursor and fragment ions were investigated for their outcome when varying the RF-funnel set value.

Ion image generation (Figures 1, 2, 3) was performed using TransMIT's MIRION software (www.smaldi.de/en)

Data for **(3)** *Schistosoma Mansoni* (blood fluke):
MS1 and MS2 spectra were collected from adjacent pixels with 10 um distance each resulting a the provided 20 um pixel size image - data acquisition is demonstrated by the following scheme: 20 µm pixel size, 20 µm pixel size equals 2x 10x20 µm, w/ MS1 and tMS2.

Results 1

AP-SMALDI[5] AF ion source and Thermo Scientific Orbitrap Excedion mass spectrometer – MS Imaging technique applied to Healthy Mouse Brain Tissue – here imaging Purkinje cells in *cerebellum*.

Figure 1.
Healthy mouse brain tissue – cerebellum, 619x620 pixels with pixel size of 5 µm between MS[1] scans.

To the left: RGB image of AP-SMALDI MSI experiment depicting the distribution of three m/z signals in a horizontal mouse brain section with 5 µm lateral resolution. RF-level 50 units, positive-ion mode from m/z 250 – 1000, resolution setting 240k at *m/z* 200.
Experiment was carried out by coupling an AP-SMALDI[5] AF ion source to an Orbitrap Excedion mass spectrometer.
To the right: Optical image of the mouse brain section after MSI analysis and after haematoxylin and eosin staining. Matrix was rinsed off the sample before staining. The analyzed area is visible through lightly stained histological features, while tissue structures are well preserved. This facilitates the comparison with the MSI Images.
Anatomical features of the cerebellum (green and blue channel) and even the single layer of Purkinje cells (red channel) can be seen between the outer molecular layer and the inner granule cell layer of the cerebellum.

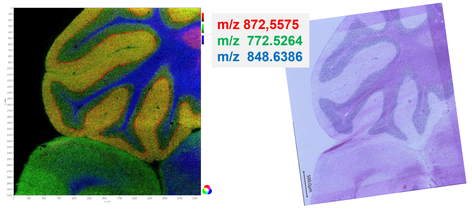
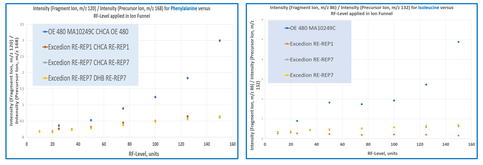


Figure 4. Plot: Intensity ratios of fragment ion and its respective precursor ion for two amino acids, known for their likely MS[1]-fragmentation.
To the left: **Phenylalanine in its transition from *m/z* 168 to *m/z* 120.**
To the right: **Isoleucine in its transition from *m/z* 132 → m/z 86**
As in ESI experiments (data not shown here), the Excedion ion funnel interface proves – also in MALDI application – its pronounced softness expressed by a significantly lower rate of MS[1] fragmentation compared to the ion funnel used with Orbitrap Exploris 480 mass spectrometer.



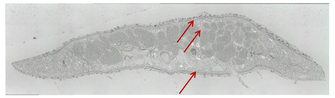
It is interesting to note that the two quite different matrices result in quite similar outcomes of the respective intensity ratios – the matrix choice due to collisional cooling under AP-conditions is obviously not predominately determining MS[1] fragmentation.

Results 2

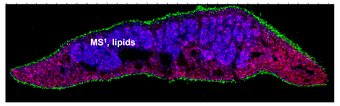
AP-SMALDI[5] AF ion source and Thermo Scientific Orbitrap Excedion mass spectrometer – MS Imaging technique applied to drug-dosed tissue – here imaging and confirming the drug imatinib and per its fragment ions in worm *Fasciola Hepatica*.

Figure 2.
Drug-dosed worm *Fasciola Hepatica*, 119x334 pixels with pixel size of 20 um between MS1 scans (and with MS2 scans between adjacent MS1 scans) – all data from one raw file acquisition.

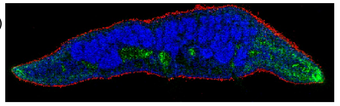
a) Optical image of cross-section of drug-dosed worm



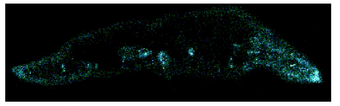
b) MS[1] image - three lipids extracted to display the worm's shape and inner organs.
***m/z* 734.5698**
***m/z* 828.5965**
***m/z* 713.4521**



c) MS[1] image with imatinib in green (precursor ion imatinib) at *m/z* 494.2663 along with two lipids observed:
***m/z* 828.5965**
***m/z* 713.4521**

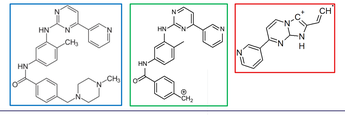


d) MS[2] image of three signals in context of imatinib fragmentation with the remaining precursor at *m/z* 494.2663 (blue), and two indicative, imatinib-specific fragment ions at *m/z* 217 (red) and *m/z* 393 (green).



Overlay - **MS[2]** scans, **blue** (remaining precursor ion of Imatinib at *m/z* 494 and fragment ions in **red** at *m/z* 217 and in **green** at *m/z* 394.

Chemical structures of precursor and fragment ions are shown to the right.



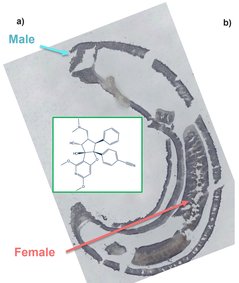
Results 3

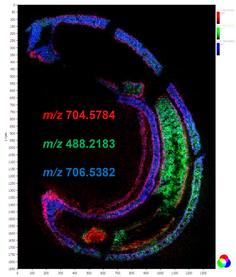
AP-SMALDI[5] AF ion source and Thermo Scientific Orbitrap Excedion mass spectrometer – MS Imaging technique applied to drug-dosed tissue – here imaging of drug zotatifin in *Schistosoma mansoni* (blood fluke).

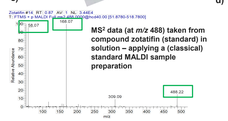
Figure 3.
Cryosection of drug-dosed worms *Schistosoma mansoni*, 277x371 pixels with pixel size of 5 um between MS[1] scans – all data from one raw file acquisition.
Here illustrated for the adult mated couple:
- **Outer, mainly blue part is the male**
- **Inner, mainly green and red part is the female.**
- **upon mating the female lives inside the male worm – with:**

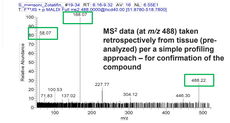
a) microscopic image before matrix application and measurement
b) MS image of lipids (*m/z* 704.5784 and *m/z* 706.5382) and Zotatifin (*m/z* 488.2183; $C_{28}H_{30}N_3O_5$).

c) Reference MS[2] spectrum of Zotatifin with characteristic ions (among others) at *m/z* 168 and *m/z* 58. – standard MALDI sample prep (DRUID project, refer to the Acknowledgements) – unique spectrum, no data available in data bases.
d) MS[2] data taken in retrospective manner from tissue - two months after first data acquisition for image shown in Figure 3b – sample with DHB matrix still preserved and sufficient for the profiling approach applied here.









Conclusions

- Thermo Scientific Excedion MS platform and AP-SMALDI technique is the perfect choice when it comes to MS Imaging application in combination with Orbitrap mass spectrometry.
- The data showcases MS Imaging applicability with MS[1] images as well as with combinations of MS[1] and MS[2] images – all within one single raw data file.
- All data presented were acquired with the new Thermo Scientific Orbitrap Excedion mass spectrometer. Our data prove that Orbitrap Excedion Platform (Orbitrap Excedion Pro mass spectrometer and Orbitrap Excedion MS) are MALDI-ready).
- Our MALDI data reveal and prove – like ESI- data – that the ion funnel interface with the Excedion platform is preventing MS[1] fragmentation significantly – avoiding false positives and respective faulty interpretation.
- The funnel interface of Excedion Platform is significantly softer than the funnel interface of Orbitrap Exploris 480 mass spectrometer: Significantly less MS[1] fragments are produced with the softer interface of the Orbitrap Excedion platform – these results obtained by AP-SMALDI resemble the results of the same compounds obtained under electrospray ionization (data not shown here).
- The results present the extension of AP-SMALDI to the next generation platform of Orbitrap Hybrid instrumentation.
  The data strongly suggest significant improvements in ion collection (softer funnel) and image sharpness (less MS[1] fragmentation).

Acknowledgements

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Stability data in Figure 4 ('MS[1] fragmentation' in different ion funnel interfaces) were partly acquired with MassTech's AP/MALDI UHR ion source in addition to TransMIT's AP-SMALDI[5] AF ion source to confirm the softness of the funnel interface of Orbitrap Excedion mass spectrometer independent of the ion source model. K.S. gratefully acknowledges the opportunity to borrow the ion source from MassTech Inc. (Columbia, MD, USA) for some weeks in early 2026.
S.B., C.D., K.S. are employees of Thermo Fisher Scientific, a manufacturer of scientific instruments. J.W., K.C.S., S.G., C.M.M., M.A.M. are employees of TransMIT GmbH, a manufacturer of scientific instruments. B.S. is consultant of TransMIT GmbH and professor at University of Gießen.

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