High resolution atmospheric-pressure mass spectrometry imaging of biological samples using a matrix-free ionization-assisting DIUTHAME foil

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Introduction

- A key characteristic of mass spectrometry imaging (MSI) is the achievable lateral resolution
  - ➢ For matrix assisted laser desorption/ionization (MALDI) MSI, subcellular resolution was shown[1]
- Sample preparation is crucial for high resolution MALDI MSI
  - ➢ Matrix imperfections or inhomogeneities have a higher negative influence on image quality
- Nanostructured surfaces can also assist desorption/ionization (SALDI), without being ionized[2]

Experimental

- DIUTHAME (Desorption Ionization using through hole alumina membrane, Hamamatsu Photonics, Japan)[3]
  - ➢ Premanufactured 5 µm thin alumina membrane
  - ➢ Nanostructured with ⌀200 nm through-holes
- AP-SMALDI5 AF (TransMIT GmbH, Giessen, Germany) or home-built ion source
  - ➢ Coupled to Q Exactive HF or Q Exactive (Thermo Fisher Scientific, Bremen, Germany), respectively
- Imaging data analysis was carried out with Mirion[4]

Sample preparation

- DIUTHAME can be placed self-adhesively on glass slides (Figure 1)
  - ➢ No pressure, force or solvent is applied
- Tissue sections must be frozen and > 50 µm thick (Figure 2)
  - ➢ Microscopic images of the sample must be taken with the membrane attached
- No histological staining possible, since DIUTHAME cannot be removed

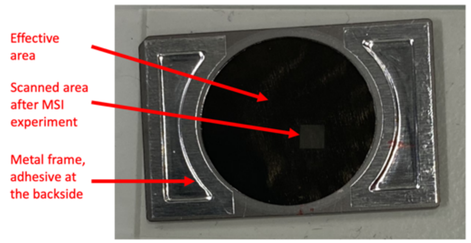

Figure 1: Photo of a DIUTHAME substrate attached to a glass slide. The tissue is not visible through the membrane.

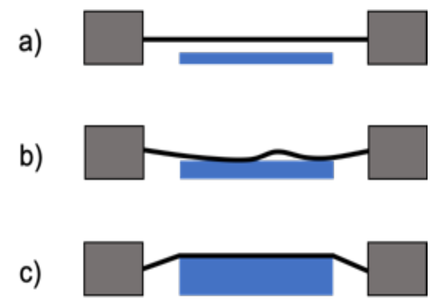

Figure 2: Scheme of a possible DIUTHAME (black) attachment on a tissue sample (blue). a,b) Tissue section is too thin, no or incomplete attachment. c) Tissue section > 50 µm, complete attachment of DIUTHAME.

Mass spectra from tissue

- In our MSI setup, DIUTHAME worked in positive ion mode
  - ➢ No signal from tissue in negative ion mode, independent of organism investigated
- Negligible background signal for DIUTHAME (NL ≈ 5)
- One order of magnitude lower signal intensity for DIUTHAME than for MALDI on mouse brain tissue (Figure 3)
  - ➢ Independent of tissue type and origin
  - ➢ Phospholipids annotated to DIUTHAME signals by LIPIDMAPS[5] are mostly a subset of those found by MALDI (Figure 4)
- Phospholipid signal composition (classes, adducts, ratios) comparable between MALDI and DIUTHAME

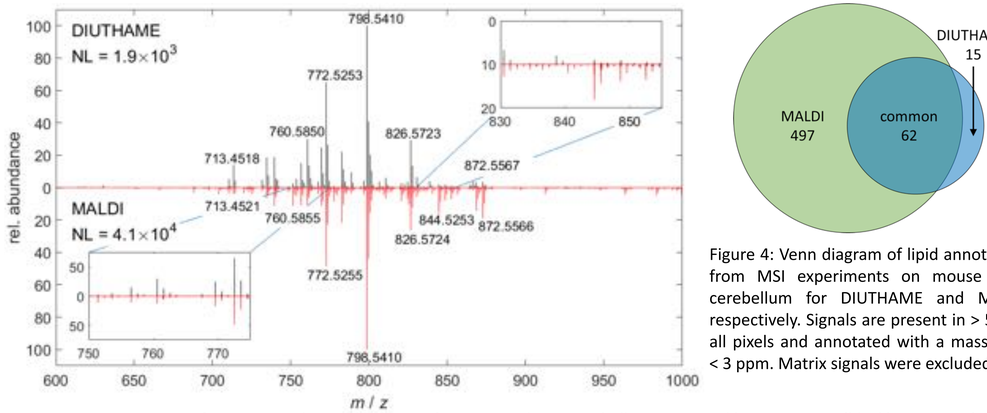

Figure 3: Comparison of 100 summed up mass spectra from mouse brain cerebellum measured by DIUTHAME (black) or MALDI (red).

Figure 4: Venn diagram of lipid annotations from MSI experiments on mouse brain cerebellum for DIUTHAME and MALDI, respectively. Signals are present in > 5 % of all pixels and annotated with a mass error < 3 ppm. Matrix signals were excluded.

MSI results

- Comparable results between DIUTHAME and MALDI (Figure 5)
  - ➢ Up to 5 µm lateral resolution achievable with DIUTHAME
- Higher contrast and homogeneity for DIUTHAME
- Higher number of images available for MALDI
- Applicable on many tissue types from different biological origin (Figure 6)

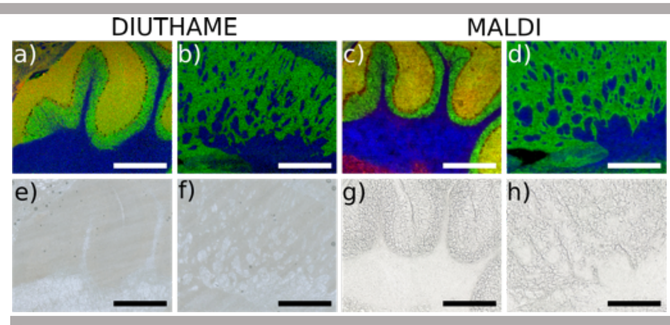

Figure 5: MS images of mouse brain cerebellum (a,c) or striatum ventral region (b,d) measured by DIUTHAME (a,b) or MALDI (c,d), respectively, for 300x250 pixels with a lateral resolution of 5 µm. e-h) Corresponding optical microscopy images of a-d). Scale bars: 500 µm.

- *m/z* 769.5620 [SM d36:1 + K]$^+$
- *m/z* 772.5253 [PC 32:0 + K]$^+$
- *m/z* 838.6086 [PC O-38:2 + K]$^+$

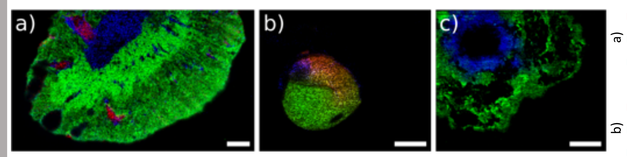

Figure 6: DIUTHAME MS images of a) mouse kidney (276x161 pixels, 30 µm pixel size), b) germinating rapeseed section (297x245 pixels, 20 µm pixel size), c) *Spodoptera littoralis* (caterpillar) section (300x250 pixels, 20 µm pixel size). Scale bars: 1 mm.

a)
- m/z 616.1769 [heme]$^+$
- m/z 849.5787 [PA 44:3 + K]$^+$
- m/z 687.5478 [CE 18:2 + K]$^+$

b)
- m/z 909.6985 [LacCer d36:0 + NH_4]$^+$
- m/z 923.7504 [TG 54:3 + K]$^+$
- m/ 6761.5719 [PG O-36:2 + H]$^+$

c)
- m/z 741.5174 [PC 32:5 + NH_4]$^+$
- m/z 915.4613 [PI 38:9(OH) + Na]+.

Conclusion & Outlook

- DIUTHAME is a useful supplement to MALDI in MSI
  - ➢ High lateral resolution up to 5 µm possible
  - ➢ Easy to use, even for untrained users
  - ➢ Lower ionization efficiency compared to MALDI
- DIUTHAME might be further developed to be applicable on thinner tissue sections
- Ionization efficiency of DIUTHAME should be improved

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Literature

(1) Kompauer, M.; Heiles, S.; Spengler, B., *Nature methods* **2017**, *14*, 90–96.
(2) K. P. Law; James R. Larkin. *Anal Bioanal Chem* **2011**, *399*, 2597–2622.
(3) Naito, Y. *et al.*, *Rapid communications in mass spectrometry,* **2018**, *32*, 1851–1858.
(4) Paschke, C. *et al.*, *Journal of the American Society for Mass Spectrometry* **2013**, *24*, 1296–1306.
(5) Sud, M. *et al., Nucleic Acids Research* **2006**, *35*, D527-32