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Applications of 1,5-diaminonaphthalene for MALDI imaging of lipids in neurodegenerative disorders

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Introduction

The selection of a suitable matrix and deposition technique is a critical step for a successful matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI) measurement. Recently, 1,5-diaminonaphthalene (DAN) has been used for lipid analysis, because of the rich lipid signals in both positive and negative mode. DAN is usually sublimed to achieve high spatial resolution [1-3].

Alzheimer's disease (AD) is the most common neurodegenerative disorder worldwide. AD is pathologically characterized by the accumulation of hyperphosphorylated tau neurofibrillary tangles and β-amyloid plaques in the brain [4], and recent evidence has shown that lipids are also involved in AD pathology [3-4]. However, the exact underlying mechanism of AD is still not fully understood.

Aim

In this study, we compared three techniques of matrix deposition with DAN as matrix, i.e., sublimation and spraying with ImagePrep and iMatrixSpray. The methods were targeted for the analysis of lipid distribution in the brains of mouse models of AD, namely, APP/PS1 mice, a widely used transgenic model of AD-like Aβ pathology.

Materials and methods

- APP/PS1 male mice and controls C57BL male mice were sacrificed with pentobarbital inhalation. Tissue samples (brain) were frozen in dry-ice-chilled isopentane.
- Samples were cut in sections of 12 μm thickness with a cryotome (CM1950, Leica, Germany) at - 18 °C. The sections were thaw-mounted on ITO glass slides (Bruker, Germany).

Sublimation of 1,5 DAN was performed in sublimation apparatus (constructed in IOCB AS CR) at constant vacuum 0.4 mbar. Sublimation time was 5 min with temperature 140 °C in oil bath.

Spraying

- Optimized solution for both sprayers was 10 mg/ml 1,5 DAN in ACN/H_2O, 7/3 (v/v).
- ImagePrep (Bruker, Germany) with sensor control assuring homogenous coverage over entire tissue surface
- iMatrixSpray (Tardo Gmbh, Switzerland) with typical spray parameters for dry spray [5]. Spray height 60 mm, speed 180 mm/s, line distance 1 mm, density 2 μm/cm^2, break 60 s in 5 cycles.

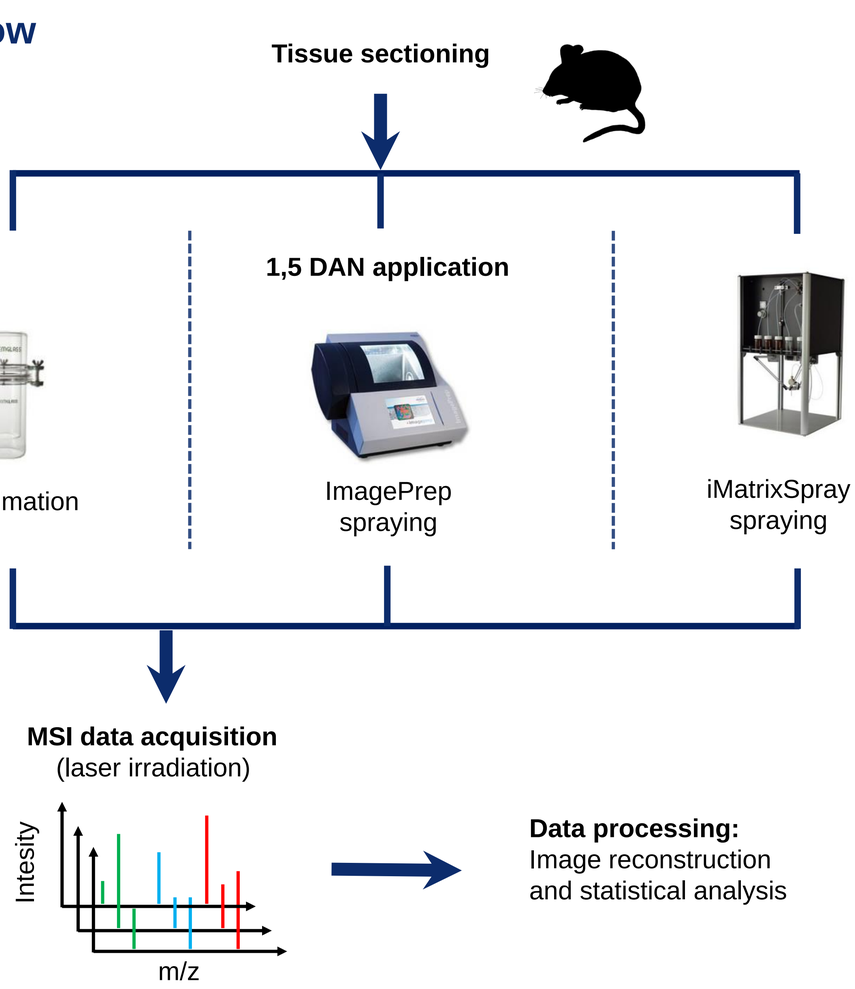
MALDI-MSI

- MALDI experiments were performed using UltrafleXtreme MALDI TOF instrument equipped with a 1 kHz laser (Bruker, Germany) in positive and negative mode with mass range 400 – 2 000 m/z.

Fluorescent amyloid staining

- After MALDI MSI analysis, the samples were rinsed in EtOH for 120 s, fixed in 95% EtOH/5% AcOH at -20 °C (8 min), 70% EtOH at -20 °C (30 s), and 70% EtOH (30 s).
- Samples were blocked in 5% goat serum (1 h, RT).
- Incubation with the APP/β-amyloid mouse primary antibody (NAB228, 1/100) was performed overnight at 4 °C and with mouse secondary antibody (Alexa Fluor 488, 1/400) for 1 h at RT.
- TBS/0.2% Triton X-100 was used for dilution of serum and antibodies.
- Imaging was performed using a Andor xD revolution spinning disc confocal microscope (Andor) on Olympus platform (software iQ3, Andor).

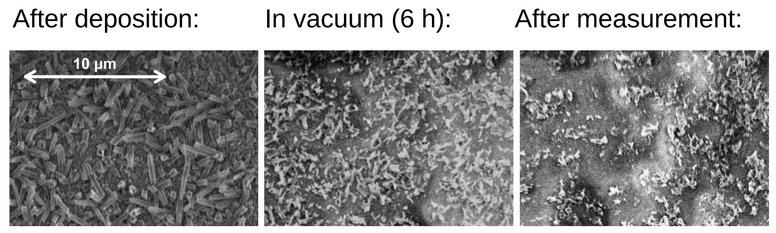
Workflow



Results

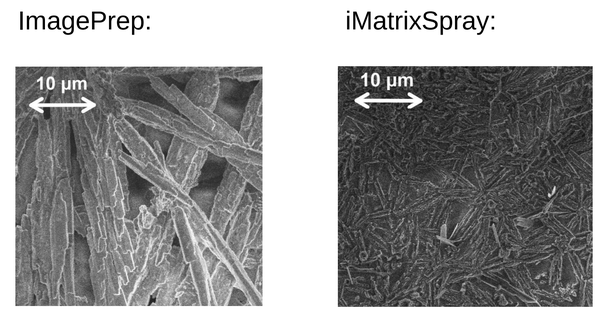
Optimalization of 1,5 DAN matrix deposition

Sublimation of 1,5 DAN was found highly irreproducible for measurement because of the matrix sublimation in the high vacuum during measurement.



ImagePrep and iMatrixSpray instruments provided homogenous coating of the sample. Applied matrix was stable during the measurement and provided reproducible datasets. The optimized solution for spraying was 10 mg/ml DAN in 70% acetonitrile for both sprayers. Both sprayer methods yielded datasets with about the same number of detected compounds and at a similar signal intensity.

However, iMatrixSpray has several technical advantages. Specifically, a faster matrix deposition and a formation of smaller matrix crystals.

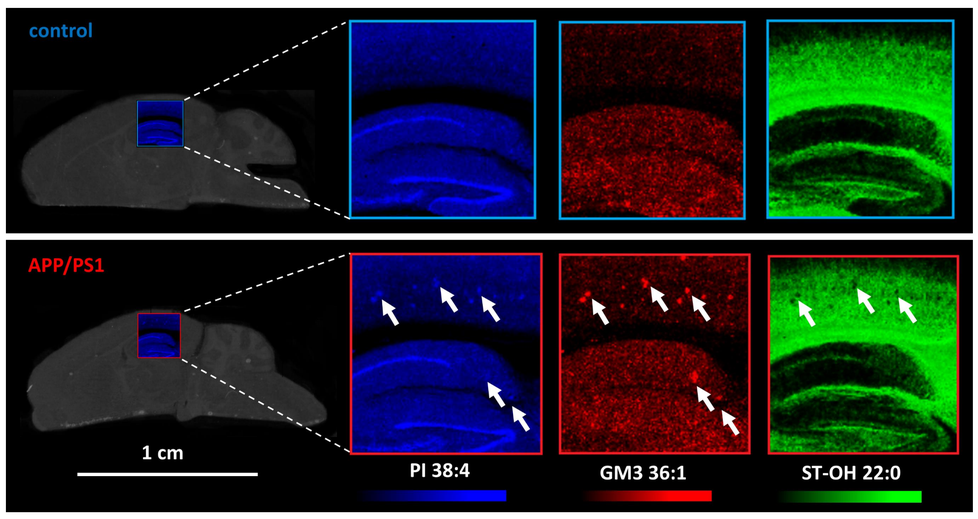


Application: comparison of lipid composition in the APP/PS1 mouse model versus age-matched controls (C57BL)

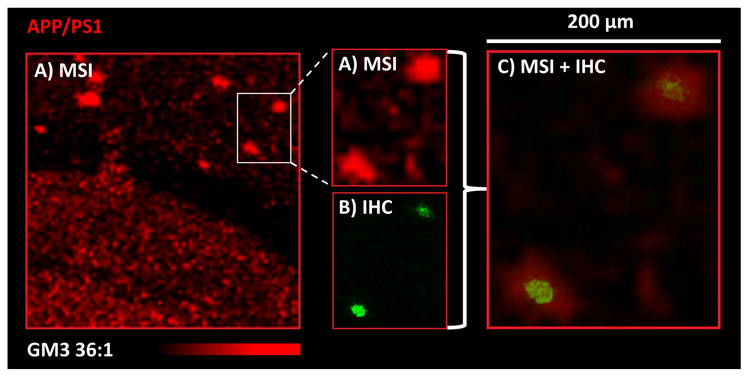
We used iMatrixSpray method mentioned above for deposition of 1,5 DAN and for study of lipid changes in APP/PS1 mouse model. The APP/PS1 mouse model at six months of age already exhibits Aβ deposition in the form of senile plaques.

Acquired data were studied using statistical software SCiLS Lab 2016b in the positive and negative MS mode for lipid changes. Receiver operator characteristic (ROC) analysis was used to identify changes in the m/z values between APP/PS1 mice and age-matched controls.

We found a higher concentration of phosphatidylinositols (PI 38:4, 36:4), gangliosides (GM2 36:1, GM3 36:1), lysophosphatidylethanolamine (LPE 18:0) and lysophosphatidylcholine (LPC 16:0, 18:0) compared to controls. In contrast, in the same site, we found a lower concentration of sulfatides (ST 18:0, 22:0, 24:0 and ST-OH 22:0, 24:0).



To confirm that the lipid changes were associated with plaques, we performed fluorescent immunohistochemical (IHC) staining of β-amyloid plaques after MALDI MSI measurement using a monoclonal Aβ antibody.



References

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Conclusions

- iMatrixSpray spraying of 10 mg/ml 1,5 DAN in 70% ACN gave the best results.
  - Deposited matrix was stable in the vacuum and measurement was reproducible.
  - Fast deposition of matrix.
  - Possible use of high spatial resolution, because of small matrix crystals (ca. 4 μm)
- The results suggest that the lipid changes are associated with neurodegenerative changes.
- Our optimized method of mass spectrometry imaging is appropriate for studying neurodegenerative changes in the brain.

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