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High throughput, high spatially resolved MALDI-MSI comparative analysis of an Alzheimer's disease mouse model with wild-type mice

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1. Highlights

- Mass Spectrometry Imaging generated at high spatial resolution (≤ 5 µm).
- High throughput imaging ~50 pixels/sec while maintaining good sensitivity.
- Unique integration of a wide-field camera and optical microscope with Atmospheric Pressure MALDI source.
- Full imaging solution to analysis of biological sample (Wild type and AD mouse model).

2. Introduction

The study presented herein aims to create a mass spectrometry imaging (MSI) method that enables high throughput, high spatially-resolved MS images to be obtained routinely without compromising sensitivity and spectra quality. The optimized methods ware applied to compare lipid profiles of wild-type (WT) and various mouse models of Alzheimer's disease at a single-cell resolution. Insight on lipid distribution and how it varies from WT to 5xFAD will give invaluable information on the effect of the disease on the brain molecular composition and distribution.

3. Methods

Sample: Brain tissues were collected from age-matched female mice of wild type (WT) and transgenic Alzheimer's models (5xFAD; JAX, B6J, ABCA7 HO, CLU h2kB KI HO, 5xFAD; HEMI, 5xFAD; ABCA7 HO, 5xFAD; CLU-h2kB KI HO). Tissue sections at 10 µm thickness were placed on an indium tin oxide (ITO) coated glass slide. Comparative analysis among AD models, age, and gender-matched 5 AD mouses tissue sections were collected for analysis. All tissue sections were kept at -80 C until use.
Matrix deposition: Brain tissues were coated with 9-Amino Acridine (98% pure), and a 0.9 µm thick layer was deposited onto the sample slide using an automated vapor deposition system (iMLayer, Shimadzu Corporation).
MALDI Acquisition: Optical images and mass spectra were acquired using an AP-MALDI QTOF (iMScope QT – Imaging Mass Microscope, Shimadzu Corporation) at a 5 µm laser diameter and variable stage step down to 10 µm. For comparative analysis among AD models, the laser diameter was set to ~20 µm with a 25 µm step size. Various laser repetition rates up to 20 kHz were used, and 150 laser shots were accumulated per pixel.
Data analysis: ImageReveal MS (Shimadzu corporation), a multifunctional software equipped with PCA, PLS, image classification, etc., was used to analyze data.

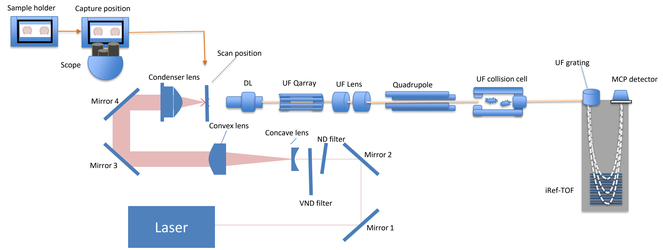
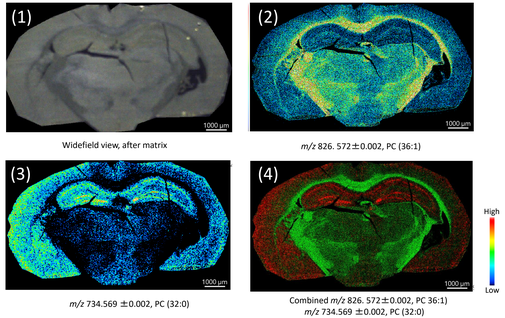


Fig. 1: Schematic of the build of the iMScope QT-QTOF system.

Main features:
a. ≤ 5 µm to 200 µm laser spot size
b. Integrated microscopic and widefield camera
c. Up to 50 pixels/second scan speed

4. Results

4.1. Full-length tissue analysis at 5 µm pixel size, 2 kHz laser speed experiment successfully generated images. A sharp lipid distribution, especially phosphatidylcholines (PC) reveals many details of the tissue areas like the corpus callosum and granular cell layer.



Widefield view, after matrix
m/z 826. 572±0.002, PC (36:1)
m/z 734.569 ±0.002, PC (32:0)
Combined *m/z* 826. 572±0.002, PC 36:1)
m/z 734.569 ±0.002, PC (32:0)

Fig. 2: Wide field camera image of tissue slice after matrix deposition (1) . Intensity distribution detected at *m/z* 826.572/ PC(36:1) (2) and *m/z* 734.569/PC (32:0) (3). Combined overlay of both intensity maps (4).

4.2. The iMScope QT can capture images using two optical devices: a wide-field camera and an optical microscope. The wide-field camera can capture images of up to 25 x 25 mm size objects. The microscopic camera has 3 lenses enabling 5x, 10x, and 40x zoom onto a surface of interest. For instance, the 5x lens can capture images of 4x3 mm size. This area decreases with increased zoom capability.

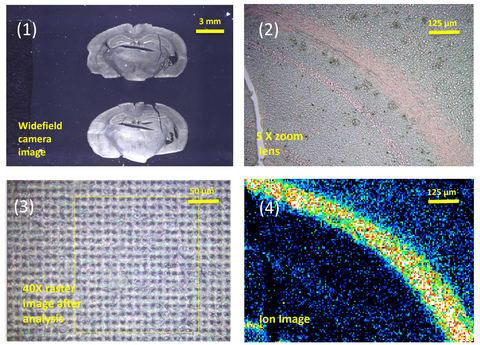


Fig. 3: Optical images captured using (1) Wide-Field camera. (2) 5x lens optical microscope (3) 40X lens of the analyzed area. (4) Resulting lipid (*m/z* 826. 572, PC (36:1)) distribution

4.3. High throughput MSI optimization was performed on a limited area of 5xFAD tissue slices. Several experiments were performed, and the combination of 5 µm and 20 kHz parameters gave a signal that was only 10-15% lower than the 5 µm and 2kHz combinations while offering a significant gain in experimental time. Approximately 90K data points were collected and compared. The increase in laser repetition rate dropped acquisition time to 36 minutes from 2.3 hours at 2 kHz.

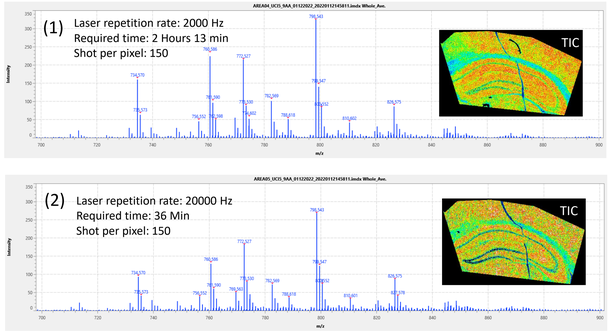


Fig. 4: Mass spectra obtained at different laser repetition rates [(1) 2 KHz ; (2) 20 KHz]. Comparison of the TIC images shows a similar pattern with negligeable variation in intensity.

4.4. Comparison of various target distributions in the region of interest of the 5xFAD mouse model. Increasing the speed from 12 pixels/s (2KHz) to 43 pixels/s (20 KHz) did not affect the image quality.

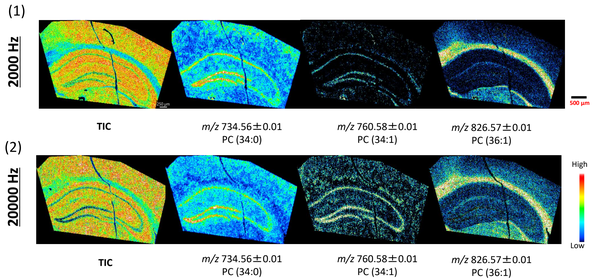


Fig.5: TIC and target ion distributions images at 2 KHz (1) and 20 KHz (2).

4.5. Early comparative analysis of full-length tissue section showed the total ion abundance is higher in the 5XFAD model mouse compared to the age-matched WT mouse. In the figure (6), selected lipids at *m/z* 798.54±0.05, PC (34:1) show distribution pattern differences between the AD and the model. AD models show high abundance at caudate-putamen and corpus callosum, but WT mice show negligible ions.

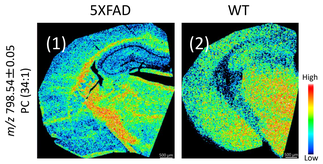


Fig.6: lipids at *m/z* 798.54±0.05, PC (34:1)shows high abundant at caudate-putamen and corpus callosum (1). Negligible ions at caudate-putamen and corpus callosum (2).

4.6. Image cluster analysis using ImageReveal MS shows inter-AD-models differences. Ion-images generated from *m/z* 760.58±0.05/PC (34:1) shows different in ion abundance.

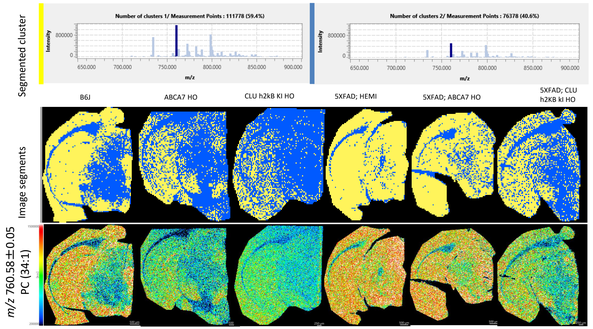


Fig. 7: Segmented cluster. Color scale indicate the cluster. 1 (a) and 1 (b), Two color segments (2), and particular ion shows ion intensities variations (3) .

5. Conclusions

- High resolution (≤ 5 µm) mass spectrometry images were obtained using a newly released AP-MALDI QTOF: iMScope QT - Imaging Mass Microscope
- Integrated wide field and microscopic cameras were used to set up experiments and imaging areas of interest.
- Higher acquisition speed did not affect data quality, and the drop in sensitivity was negligible.
- Early data review of duplicate runs showed a difference in lipid intensity response between WT and AD models. Statistical analysis performed via the "Image segmentation" agreed with this statement.
- Further investigation into lipid distribution differences and identification is needed.