DIUTHAME enables matrix-free mass spectrometry imaging of frozen tissue sections of Alzheimer's disease (AD) brain

○**Masaya Ikegawa[1], Yumiko Toyama[1], Yume Mukasa[1], Inori Shintani[1], Takayuki Ohmura[2] , Masahiro Kotani[2], Akira Tashiro[2], Takashi Nirasawa[3], Ryo Kajita[3], Nobuto Kakuda[1], Shigeo Murayama[4], Yasuhide Naito[5]**

1 Doshisha University, Kyotanabe City, Japan
2 HAMAMATSU PHOTONICS K.K., Iwata City, Shizuoka Pref., Japan
3 Bruker Japan K.K., Yokohama, Japan;
4 The Brain Bank for Aging Research, Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology, Tokyo, Japan
5 The Graduate School for the Creation of New Photonics Industries, Hamamatsu, Japan

Introduction

Neuropathology of Alzheimer's disease (AD) is characterized by the accumulation and aggregation of Amyloid β (Aβ) peptides into extracellular plaques of the brain. Increasing lines of evidence support a direct involvement of cholesterol in AD pathogenesis as well. For the rapid and reproducible analysis of multifactorial autopsy brains, a novel matrix - free laser desorption/ionization method based on porous alumina membranes was applied. Here, we applied a novel protocol of Desorption Ionization Using Through Hole Alumina Membrane (DIUTHAME) in combination with conventional MALDI-IMS on human autopsy brain tissues to obtain a comprehensive molecular mapping.

Methods

Human cortical specimens for IMS were obtained from those brains that were removed processed at the Brain bank at Tokyo Metropolitan Institute of Gerontology. Frozen tissue sections were cut on a cryostat at a 20 to 30 μm thickness onto ITO glass slides. DIUTHAME system is composed with the porous alumina membranes, a two - dimensional ordered structure consisting of closely aligned straight through holes that are amenable to mass production by industrial fabrication processes. The membranes were coated with platinum on a single side that was exposed to the laser.

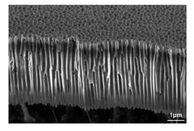


FIGURE 1 An example of the through hole porous alumina membranes observed by scanning electron microscopy (SEM). The scale bar indicates 1 μm

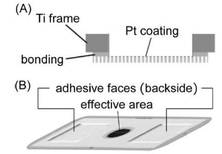
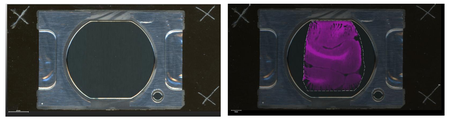


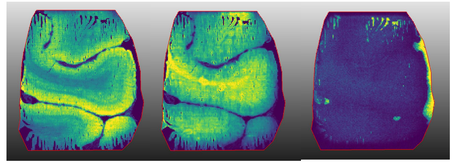
FIGURE 2 Schematic view A, and photograph B, of the DIUTHAME chip in its final form



Spectra were acquired using the rapifleX MALDI Tissuetyper, whereas ions were detected with spatial resolution of 50 μm. Obtained mass spectra were visualized with flexImaging and SCiLS Lab 2019 Software.

Results and Discussions

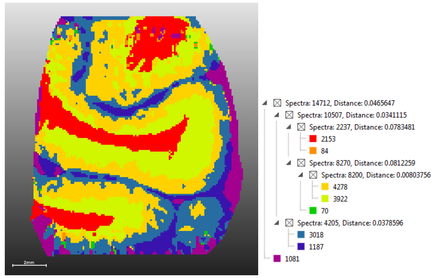
We have succeeded in obtaining low molecular imaging with DIUTHAME system for AD in contrast with non-pathological aged brains. Mass spectra were between 10 to 1000 m/z and underling white matter, grey matter and leptomenigeal vascular spaces were precisely visualized with low molecular lipid species. From AD and non-pathological brain section, m/z at 147 for grey matter dominant and m/z at 184 for white matter dominant. These can be annotated to cholesterol fragment and phosphatidylcholine fragments, respectively.



m/z 147.034 *m/z* 184.115 *m/z* 388.004

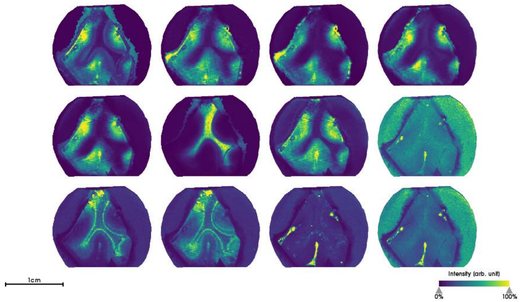
Lipid class	Assignment	*m/z*	Grey matter			White matter		
			$(H_2O)Ar_{2000}$	$(H_2O)_{5000}$	Ar_{2000}	$(H_2O)Ar_{2000}$	$(H_2O)_{5000}$	Ar_{2000}
Sterol Lipids	Cholesterol fragment C_8H_{15}	109.1	✓	✓	ND	✓	✓	✓
	Cholesterol fragment $C_{11}H_{19}$	147.1	✓	✓	ND	✓	✓	✓
	Cholesterol fragment $C_{13}H_{17}$	161.1	✓	✓	ND	✓	✓	✓
	Cholesterol $C_{27}H_{45}$ $[M-OH]^+$	369.3	✓	✓	✓	✓	✓	✓
	Cholesterol $C_{27}H_{45}O$ $[M-H]^+$	385.3	✓	✓	✓	✓	✓	ND
	Chol.-2mer $(C_{27}H_{45}O)_2Na$	795.6	✓	✓	✓	✓	✓	ND
	Chol.-3mer $(C_{27}H_{45}O)_3H$	1160	✓	ND	✓	✓	✓	ND
Glycerophospholipids	PC fragment $C_5H_{14}NO$	104.1	✓	✓	✓	✓	✓	✓
	PC fragment $C_5H_{13}NPO_3$	166	✓	✓	✓	✓	✓	✓
	PC fragment $C_5H_{15}NPO_4$	184	✓	✓	✓	✓	✓	✓
	PC fragment $C_5H_{14}PO_4Na$	206	✓	✓	✓	ND	ND	ND
	PC fragment $C_5H_{15}NPO_4$	224.1	✓	✓	ND	✓	✓	ND
	PC $[M+Na]^+$ sodiated fragment	463.2	ND	ND	ND	✓	✓	✓
	PE fragment	508.6	ND	ND	ND	✓	✓	ND

Automatic segmentation map based on low molecules obtained from DIUTHAME were also visualized according to the cortical brain structures. This is highly comparable with segmentation data at proteomic level obtained by ourselves with 20 μm resolution in another study.



Limitation of the current technology is also noticed from the current experiments; thickness of the tissue samples is sometimes resistant to be evaluated with conventional HE staining after mass spectrometric observations. However, by accumulating these evidences, then it will be able to apply into rapid, an intra surgical biopsy analysis in a various clinical settings of the neurological diseases.

	DIUTHAME	MALDI-IMS
Background noise	low	high
Handling	easy	difficult
Reproducibility	good	limited
Spatial Resolution	good	limited
Molecular Range	limited	good



Single mass images obtained from DIUTHAME for Alzheimer's Disease (AD) brain. From upper left to right, m/z = 185.014, 203.039, 206.997, 245.952, (middle left) 251.972, 264.269, 307.059, 498.136, (lower left) 546.205, 568.199, 616.123, 639.103. Bar = 1cm.

Conclusions

- Our strategy highlight the utility of DIUTHAME as a rapid and simple method to study neuropathology of AD.
- As DIUTHAME strategy is extremely rapid, simple and reproducible, it must be feasible to apply this technology into generating mass spectrometry library for the studies of neurological diseases.

Imaging Mass Spectrometry