

The combination of DIUTHAME-IMS/FT-ICR conserves high mass accuracy and resolution over the DIUTHAME-IMS/TOFMS in the laser desorption/ionization imaging mass spectrometry

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Abstract

Introduction

Recently, a novel matrix-free ionization-assisting (laser desorption/ionization) substrates have been developed based on porous alumina membranes named DIUTHAME (Desorption Ionization Using Through-Hole Alumina Membrane). DIUTHAME has a two-dimensional ordered porous alumina membrane structure. It significantly reduces the sample-pretreatment time by just placing DIUTHAME on the sample to be analyzed by imaging mass spectrometry (IMS). The molecules in the sample rise to the DIUTHAME by capillary action without distortion of positional information of samples. Successful evaluation experiments have been conducted on the feasibility of that membrane for the ionization method using liquid samples. In our current study, we aimed at extending its potential application as an alternative to the matrix to see the molecular distribution of animal tissue through IMS.

Methods

C57BL/6J adult mice brain sections mounted on indium tin oxide (ITO) glass slides were coated with matrix (2, 5-dihydroxybenzoic acid, 40mg/ml in 50% methanol) or DIUTHAME, and subsequently measured by Solarix XR (Bruker Daltonics) in positive ion mode. Mass spectra ranging from the mass-to-charge ratio (m/z) 50 to 1200 were collected. Data acquisition and analysis were performed by ftmsControl 2.1 and flexImaging 4.1 software (Bruker Daltonics).

Results & Discussion

DIUTHAME-imaging mass spectrometry (DIUTHAME-IMS) showed a softness of the ionization process in the mice brain tissue. DIUTHAME-IMS were capable of detecting almost same molecular species as a matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) with high reproducibility and drastically less background noise. Data analysis showed that 86.78% spectra were identical in both cases. In most cases, the ion images obtained by DIUTHAME-IMS showed better spatial resolution compared to that of the MALDI-IMS. As a first study, here we applied the combination of DIUTHAME-IMS and Fourier Transform Ion Cyclotron Resonance (DIUTHAME-IMS/FT-ICR) mass spectrometer using mice brain tissue. This combination has great advantages on the mass accuracy and resolution over the combination of DIUTHAME-IMS and Time-of-flight mass spectrometry (DIUTHAME-IMS/TOFMS). In the case of TOFMS, especially, mass accuracy might suffer from the fluctuation of surface heights of the sample, (i.e. undulation of the alumina membrane). However, the combination of DIUTHAME-IMS/FT-ICR resolves this problem.

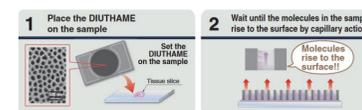
Novel Aspect

The novel combination of DIUTHAME-IMS/FT-ICR using mice brain tissue conserves high mass accuracy and resolution in the IMS.

Materials & Methods



DIUTHAME and conventional matrix application processes

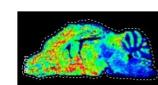


Sample rises to the DIUTHAME by capillary action

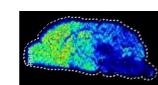


Solarix XR

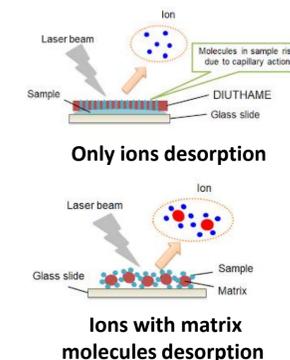
Molecular Assignment



DIUTHAME-IMS Spectra and ion images



MALDI-IMS Spectra and ion images



Results & Discussion

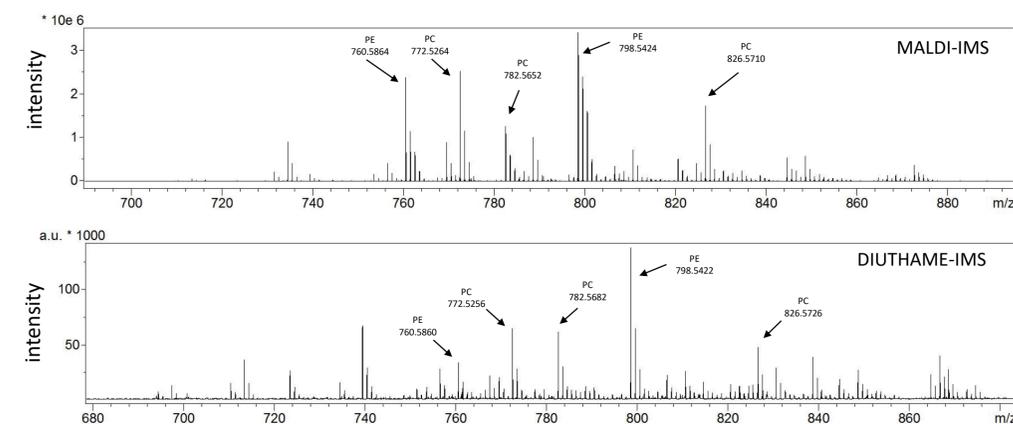


Fig 1: Spectra of most abundant molecular species from both MALDI-IMS & DIUTHAME-IMS

Table 1: Mass accuracy comparison between MALDI-IMS and DIUTHAME-IMS

	Molecular Species	Ion Form	Measured m/z	Theoretical m/z	Mass Accuracy (ppm)
MALDI-IMS	PE (37:1)	(M+H) ⁺	760.5864	760.5851	1.709
	PC (32:0)	(M+K) ⁺	772.5264	772.5253	1.423
	PC (34:1)	(M+Na) ⁺	782.5652	782.5670	2.300
	PE (37:1)	(M+K) ⁺	798.5424	798.5410	1.753
	PC (36:1)	(M+K) ⁺	826.5710	826.5723	1.572
DIUTHAME-IMS	PE (37:1)	(M+H) ⁺	760.5860	760.5851	1.183
	PC (32:0)	(M+K) ⁺	772.5256	772.5253	0.388
	PC (34:1)	(M+Na) ⁺	782.5682	782.5670	1.533
	PE (37:1)	(M+K) ⁺	798.5422	798.5410	1.502
	PC (36:1)	(M+K) ⁺	826.5726	826.5723	0.362

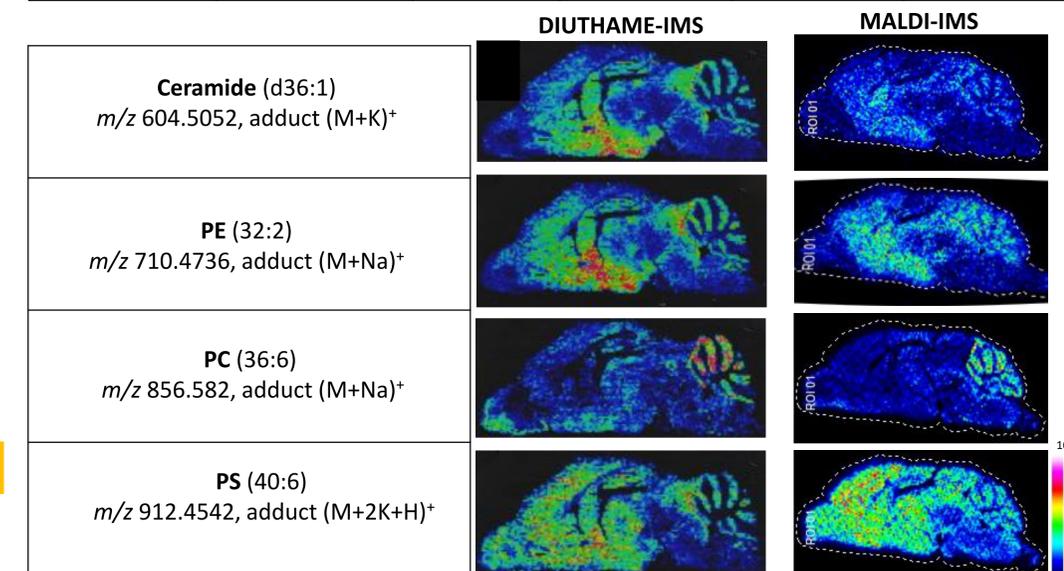


Fig 2: Representative ion images from both DIUTHAME-IMS and MALDI-IMS

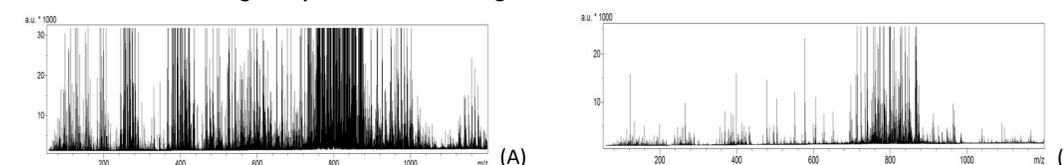


Fig 3: Ion spectra with background noise of both MALDI-IMS (A) and DIUTHAME-IMS (B)

Conclusion

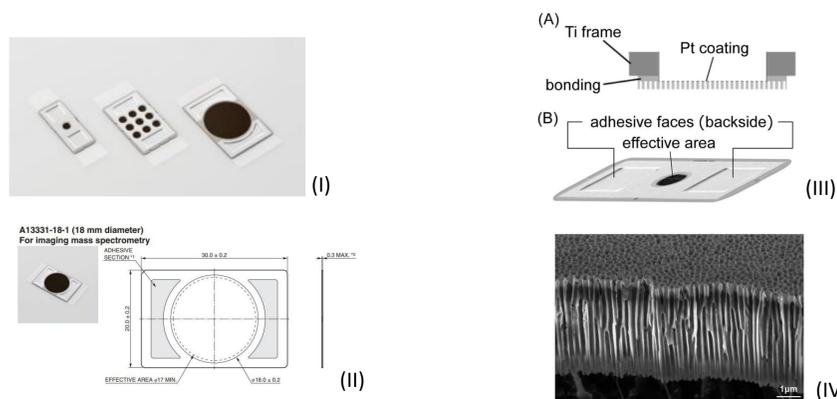
Our current findings suggest that the novel combination of DIUTHAME-IMS/FT-ICR mass spectrometer conserves high mass accuracy and resolution in the mice brain tissue and DIUTHAME might be used in the place of the matrix in the IMS.

Acknowledgements

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What is DIUTHAME?



Ionization-assisting substrates DIUTHAME series (I), Dimensional outline (II), Schematic view and photograph of the DIUTHAME chip in its final form (III), and the through hole porous alumina membranes observed by scanning electron microscopy (IV).

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