# Establishing better laboratory protocols for desorption ionization using through hole alumina membrane (DIUTHAME)

Yasuhide Naito[1] · Masahiro Kotani[2] · Takayuki Ohmura[2] （The Graduate School for the Creation of New Photonics Industries (GPI)[1] · Hamamatsu Photonics K.K.[2]）

**OVERVIEW**

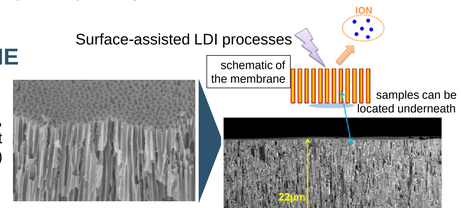
Purpose : A matrix-free soft laser desorption/ionization method, *DIUTHAME*, was tested for ability to conduct high throughput screening.
Methods : Acetylcholinesterase (AChE) enzyme functional assays were performed and monitored by MALDI and DIUTHAME.
Results : AChE reaction time course was successfully monitored by DIUTHAME with several advantages over MALDI.

## Introduction

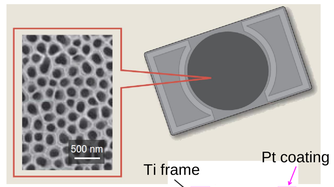
The unique characteristics of desorption ionization using through hole alumina membrane (DIUTHAME) not only enable imaging mass spectrometry (IMS) but also are potentially suited for applications relying on robotic processing, such as high throughput screening (HTS). This expectation was confirmed by monitoring protein functional activity shown in DIUTHAME spectra. Acetylcholinesterase (AChE) enzyme reaction assays were performed then analyzed by using DIUTHAME.

### Basic concept & device of DIUTHAME

No branches, no side holes, only vertically aligned straight through holes (i.d. ~ 200 nm) are formed in the membrane




Vertical cross-sectional SEM image

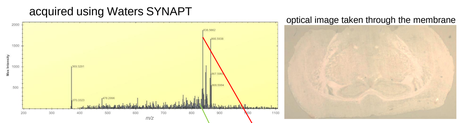


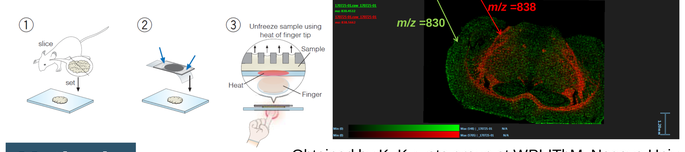


DIUTHAME-substrate has been commercialized as ready-to-use chips

### DIUTHAME-IMS of mouse brain

DIUTHAME has shown abilities for imaging mass spectrometry (IMS)




Obtained by K. Kuwata group at WPI-ITbM, Nagoya Univ.

## Methods

AChE assays
- Acetylcholinesterase (AChE, Sigma-Aldrich, C3389), 2.5 mU in 40 mM Tris-Cl
- Acetylcholine chloride (ACh, Sigma-Aldrich, A6625), 25 μM in 40 mM Tris-Cl

Mixed 2.5 μL of each aliquoit to make enzyme reaction assays (no inhibitor was added)
Left to stand for the predefined reaction times then quenched by 1% Formic Ac

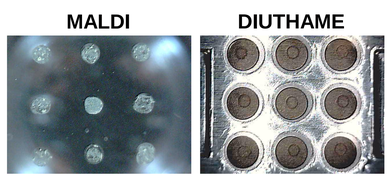
MALDI measurements
Matrix: CHCA, 7 mg/mL in MeOH/TFA 0.2% 7:3 (v/v)
Targets were deposited by sandwich method (matrix/sample/matrix, 0.5 μL each)

DIUTHAME measurements
Applied the assays on the top of DIUTHAME-substrate (9ch-type, 0.5 μL each)

All MS measurements were conducted by using Bruker Ultraflex TOF/TOF equipped with a nitrogen laser (50 Hz), in reflector mode

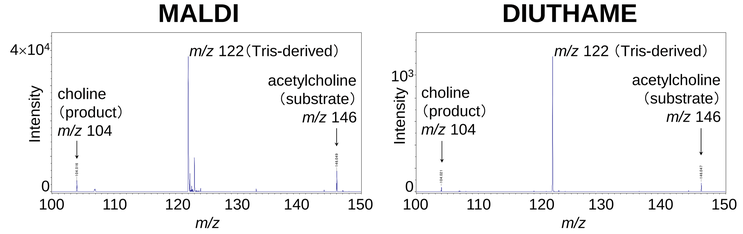
For DIUTHAME experiments, the assays were applied on the top (hydrophobic) surface of a 9ch DIUTHAME-substrate

Reaction time
0 min 2 min 4 min
6 min 8 min 10 min
14 min 18 min 22 min



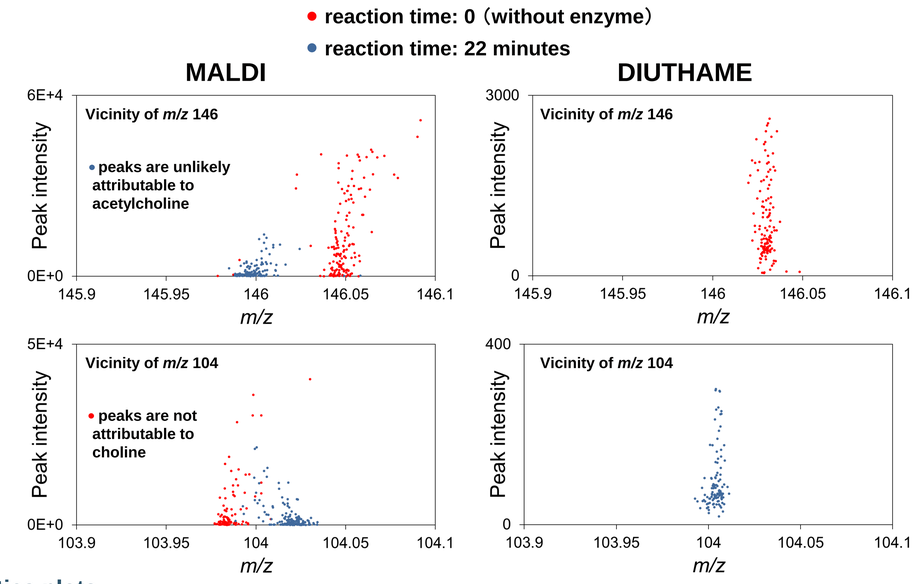

## Results

### Mass spectra obtained from AChE assays（reaction time: 2 minutes）



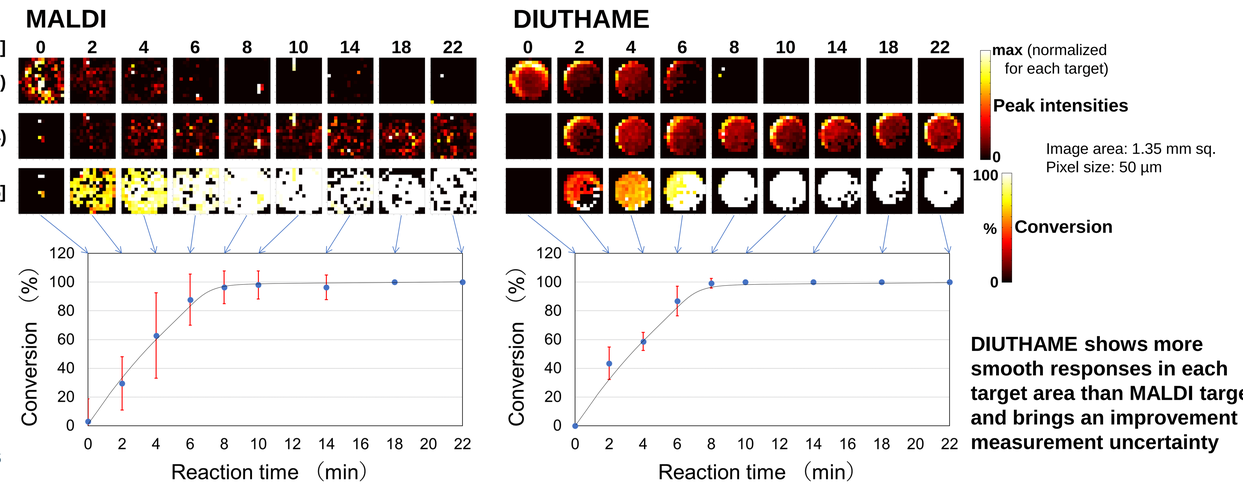

**Despite of weak signals, DIUTHAME provides good signal-to-noise ratios**

### Scatter charts of peak values in the vicinities of *m/z* 146 and *m/z* 104




**DIUTHAME is interference-free**

### Monitoring AChE reaction time course: Heat map representations of target responses and kinetics plots




**DIUTHAME shows more smooth responses in each target area than MALDI targets and brings an improvement in measurement uncertainty**

### Conversion

$$\text{Conversion} = \frac{I_{Ch}}{I_{Ch} + A \times I_{ACh}}$$

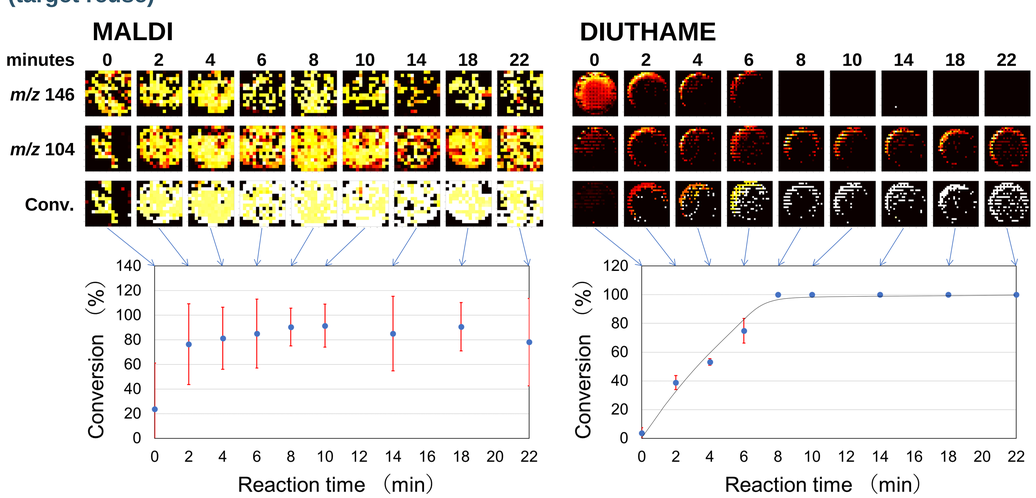
$I_{Ch}$: **Peak intensity of choline (*m/z* 104)**

$I_{ACh}$: **Peak intensity of acetylcholine (*m/z* 146)**

***A*: Correction factor for ionization efficiencies**
Compensate a lower ionization efficiency for choline than that for acetylcholine
In MALDI, *A* = 0.16 has been proposed.[1]
In this study, *A* = 1 to make comparison between MALDI and DIUTHAME

[1] C. Haslam, et. al.; *J. Biomol. Screen.* **21**(2) 176-186 (2016).

### Remeasurements of the same targets (target reuse)




**DIUTHAME targets have no hysteresis and can repeat measurements (with shifted irradiation spots)**

## Conclusion

The enzyme reactions of *acetylcholinesterase* were successfully monitored as conversion of the substrate acetylcholine to the product choline with the reaction time course by using DIUTHAME. It was found that DIUTHAME has several advantages (interference free, reduced uncertainty in measurement, no target hysteresis) over MALDI in the measurements of the enzyme reaction assays. Because of its high tolerance for additives such as detergents, DIUTHAME is highly promising for HTS applications.

## References

1. Y. Naito, M. Kotani, T. Ohmura; A novel laser desorption/ionization method using through hole porous alumina membranes; *Rapid Commun. Mass Spectrom.* **32** 1851-1858 (2018) Open access article: https://doi.org/10.1002/rcm.8252
2. DIUTHAME product datasheet, Hamamatsu Photonics K.K. Download site: https://www.hamamatsu.com/jp/en/product/type/A13331-3-1/index.html