

# Combining DIUTHAME and Stigmatic-Type Mass Microscope toward Cellular Scale Imaging Mass Spectrometry

HAMAMATSU  
PHOTON IS OUR BUSINESS



<sup>1</sup>The Graduate School for the Creation of New Photonics Industries (GPI), <sup>2</sup>Hamamatsu Photonics K.K.  
Otsuyoshi Hirao<sup>1,2</sup> • Yasuhide Naito<sup>1</sup>

## What's new?

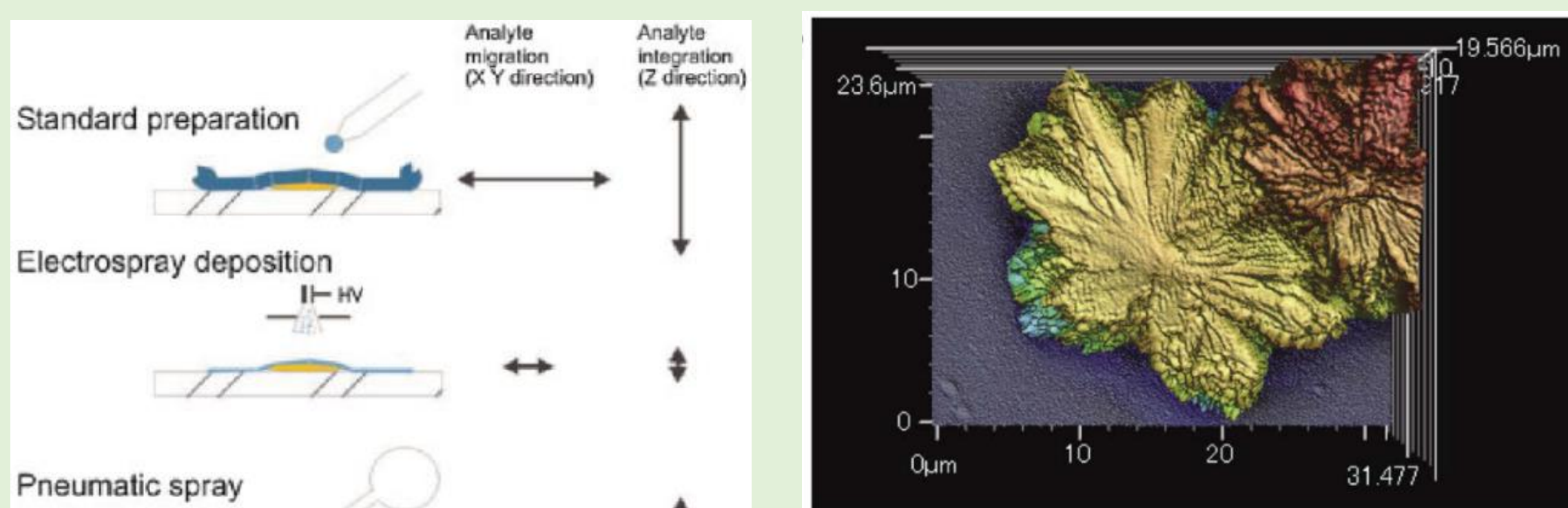
DIUTHAME × Stigmatic IMS  
= Ultimate high spatial resolution

## Overview

Purpose : Verifying a combined approach of DIUTHAME and stigmatic IMS for ultrahigh spatial resolution  
Methods : Mouse brain tissue and oral epithelial cells were used as trial samples for the verification  
Results : Distributions of PC head group fragment were observed with the spatial resolution of 1 μm

## Introduction

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-IMS) has been gaining the spatial resolution and used in a variety of applications. The spatial resolution of MALDI-IMS is restricted by laser scanning (spot diameter, movement pitch) and matrix coating (crystal size, homogeneity). To achieve a further high spatial resolution, these restrictions need to be overcome; however, they come close to their limits.



S. Shimma; *J. Mass Spectrom. Soc. Jpn.* **64**(5) 179-182 (2016).

The size and homogeneity of matrix crystalline depend on the compounds. 2,5-dihydroxybenzoic acid (DHB) is a typical matrix compound. Although it is versatile matrix particularly for MALDI-IMS of small molecules, DHB forms relatively large crystals whose needle-like shapes lead to inhomogeneous matrix coating layer.

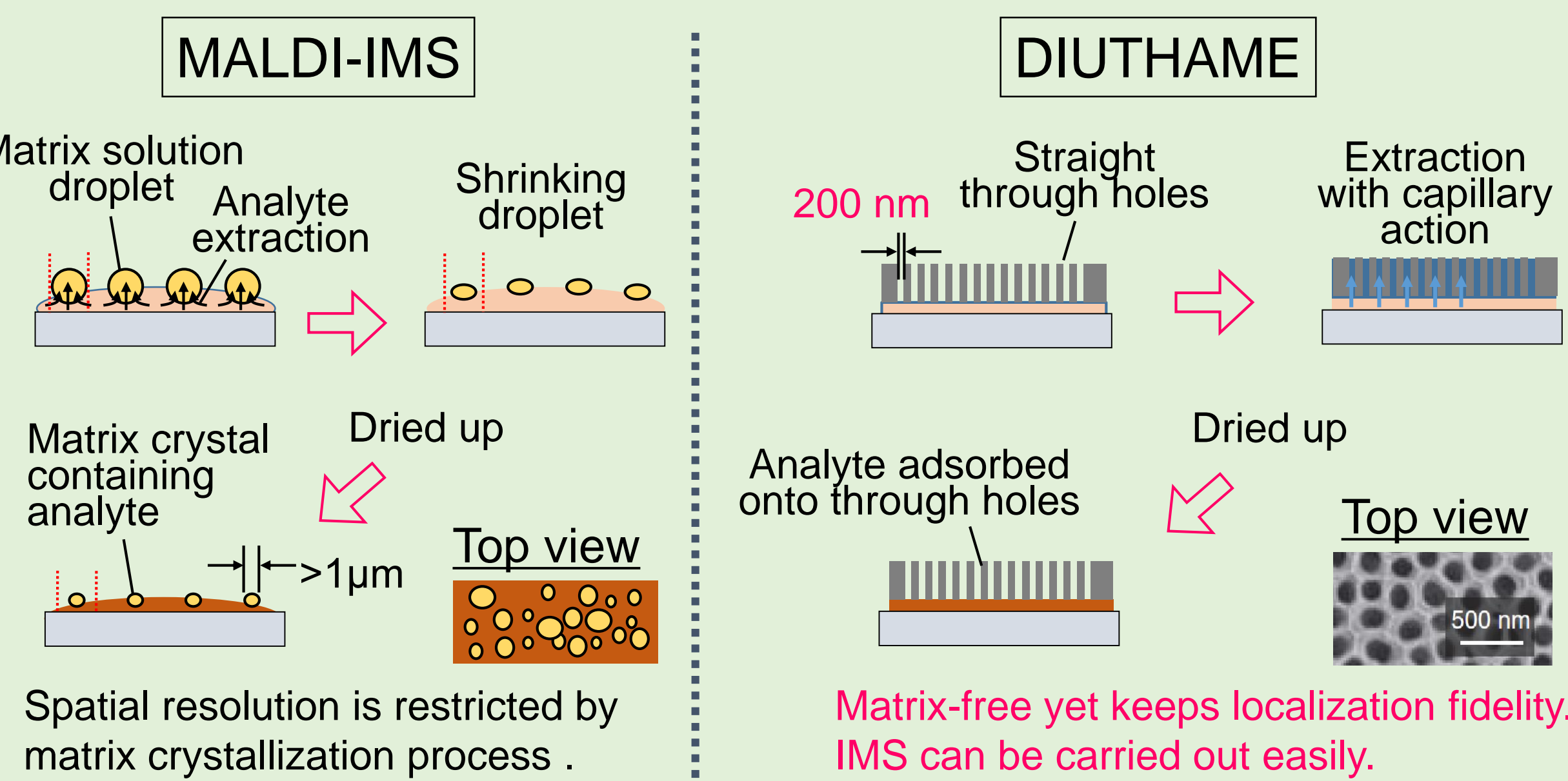
W. Bouschen, O. Schulz, D. Eikel, B. Spengler; *Rapid Commun. Mass Spectrom.* **24** 355-364 (2010).

Various methods for matrix application in MALDI-IMS have been developed. The spatial resolution and analyte extraction efficiency have a trade-off relationship in general.

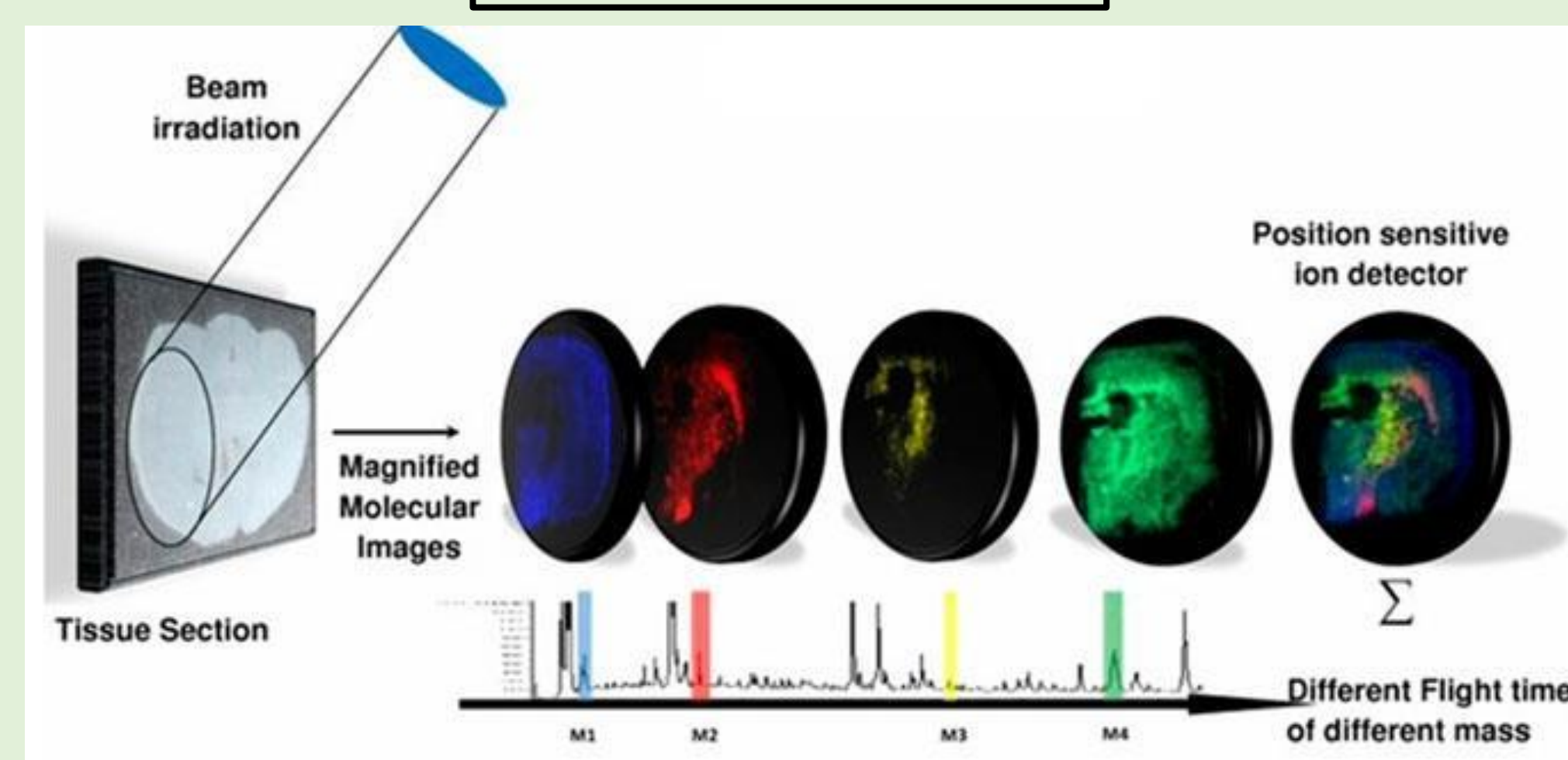
We aim to provide an IMS technology enabling a higher spatial resolution to user. In order to achieve the ultimate high spatial resolution, we propose a combined approach of a novel matrix-free soft laser desorption/ionization method (desorption ionization using through hole alumina membrane; DIUTHAME) and stigmatic-type IMS.

DIUTHAME: to overcome the limitation of matrix coating

Stigmatic-type IMS: to overcome the limitation of laser scanning



## Stigmatic-type IMS



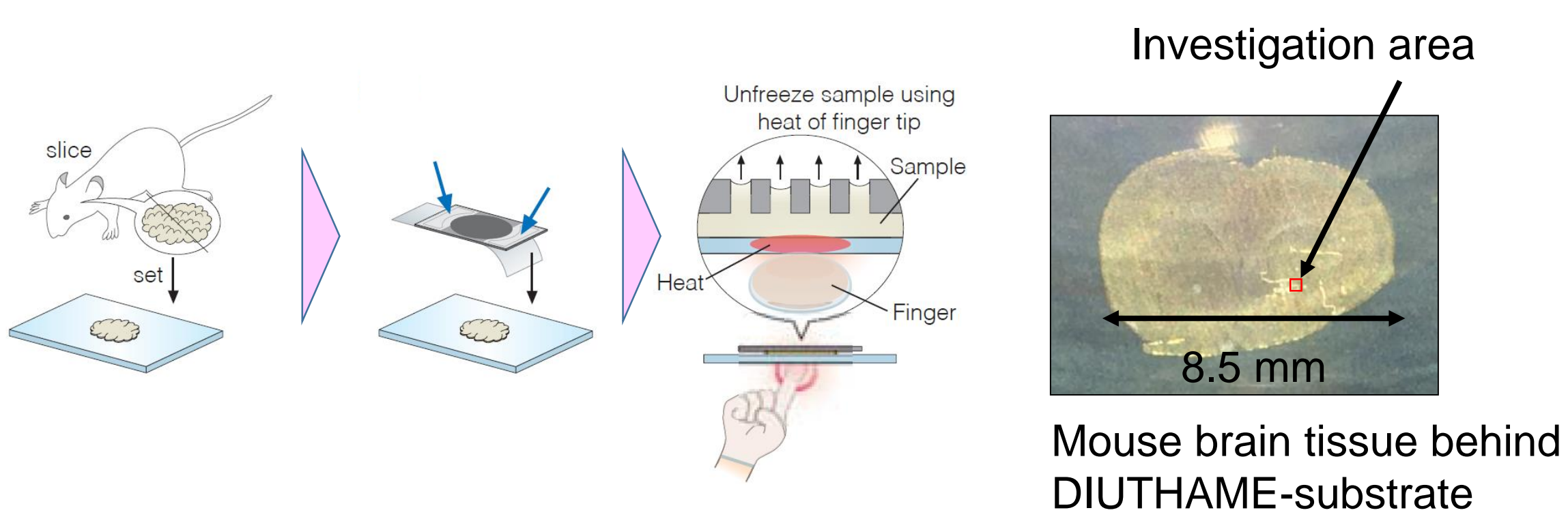
S.L. Luxembourg, T.H. Mize, L.A. McDonnell, R.M.A. Heeren; *Anal. Chem.* **76**(18) 5339-5344 (2004).

IMS instrumentations utilizing a stigmatic time-of-flight ion optics detect ion distributions of analytes within a laser spot and can provide a high spatial resolution irrespective of the focused laser beam diameter.

## Methods

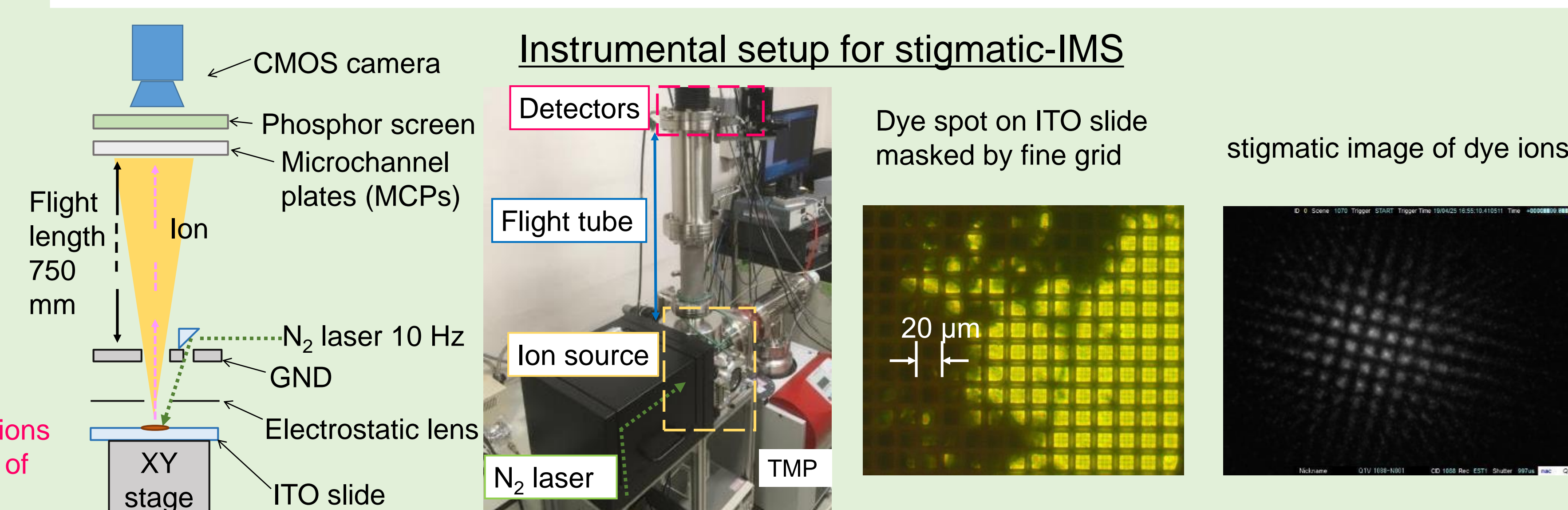
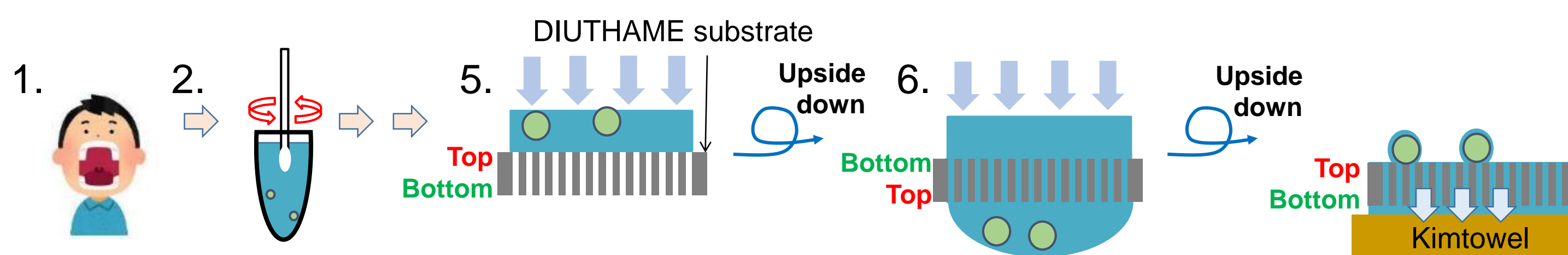
### 1. Mouse brain tissue

1. Dissect mouse and take out brain. Stock it in a deep freezer until use.
2. Make a thin-slice of frozen mouse brain with cryomicrotome.
3. Set the slice of frozen mouse brain on an ITO glass slide.
4. Place a DIUTHAME-substrate on the mouse brain slice before it thaws.
5. Unfreeze the sample using heat of finger tip on the bottom of the glass slide.
6. After the sample thaws, the components derived from the sample are soaked up by a capillary action.
7. After the sample dries, introduce it in the ion source and conduct stigmatic-IMS.

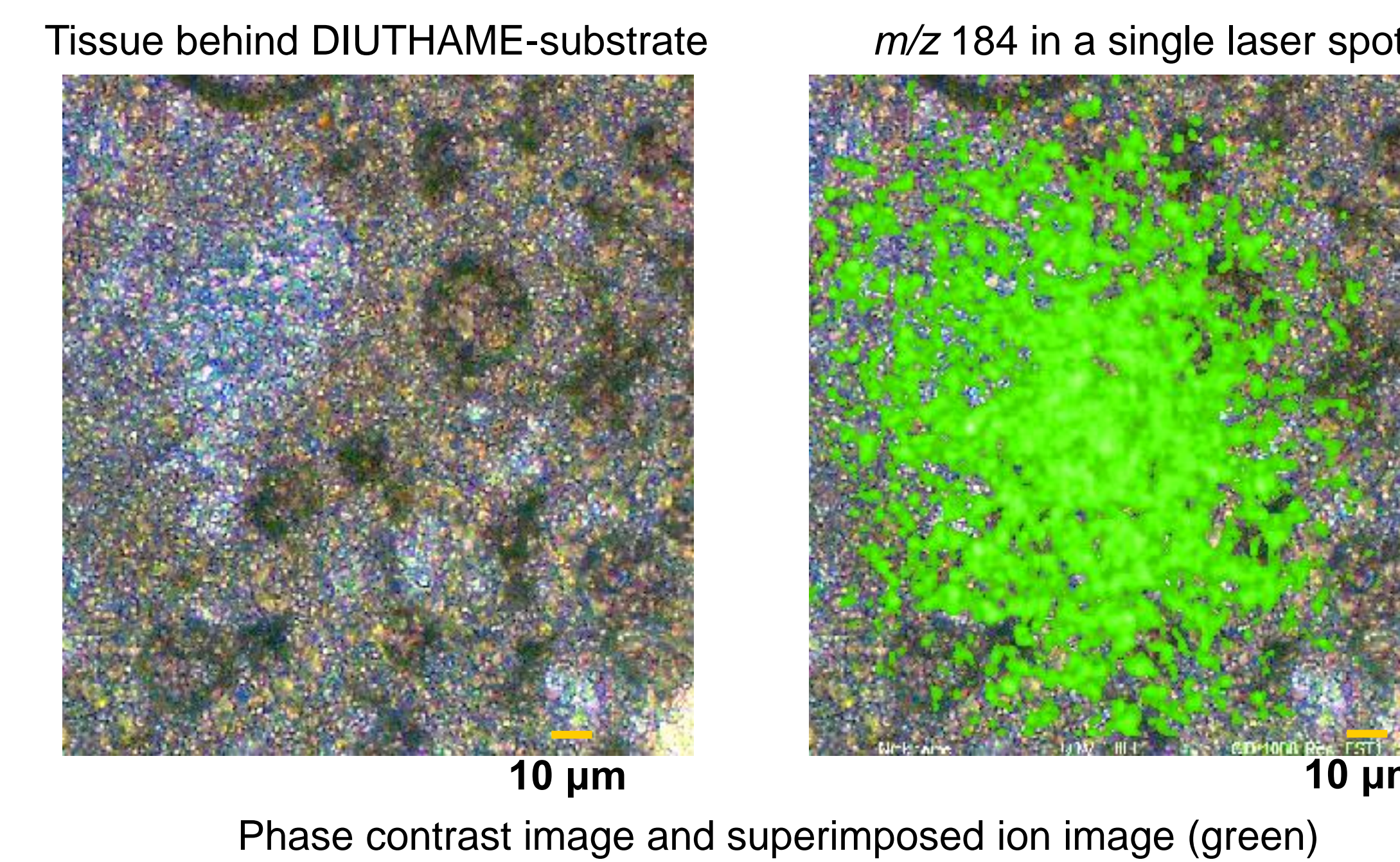
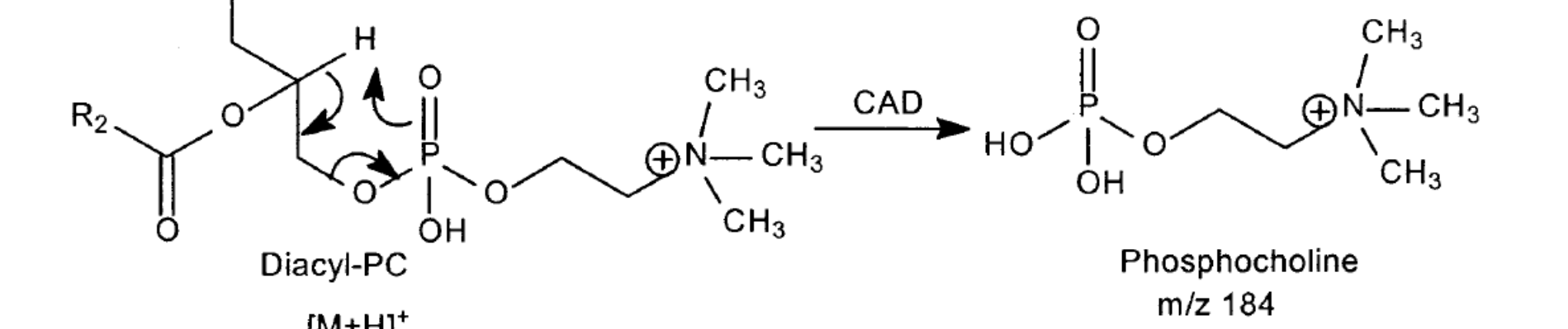
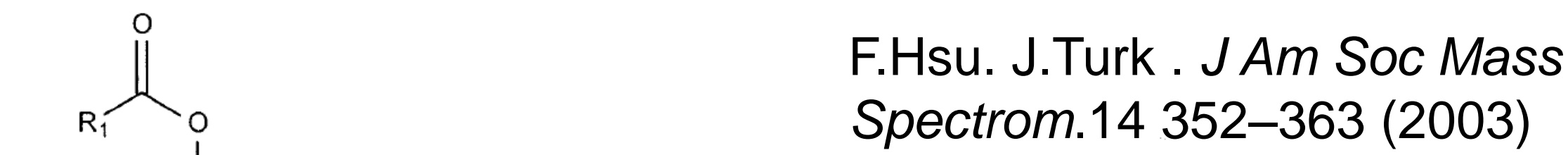


### 2. Isolated oral epithelial cells

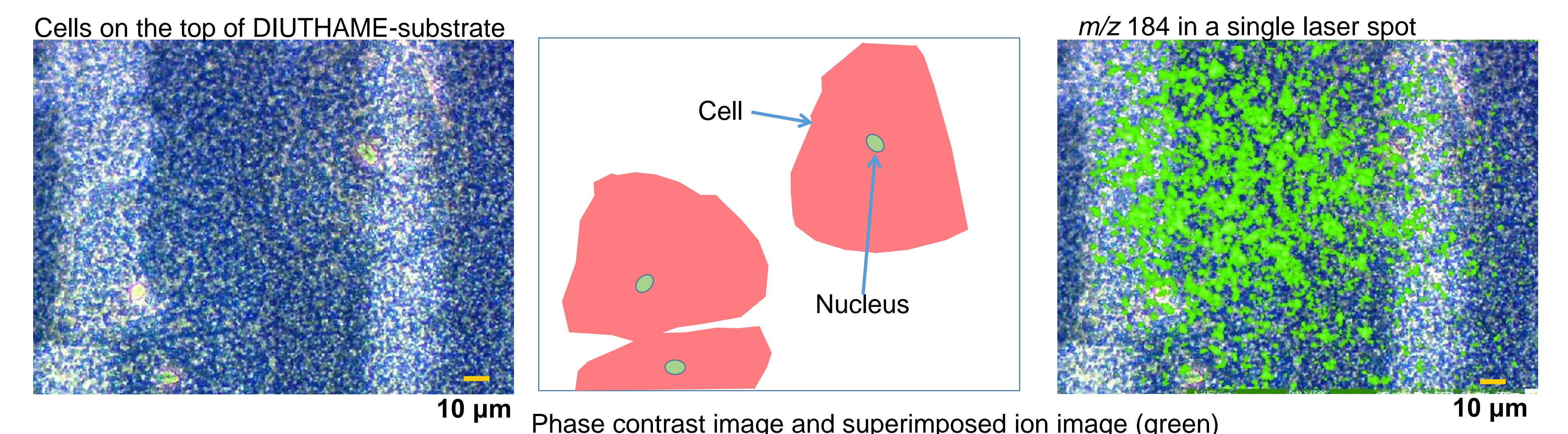
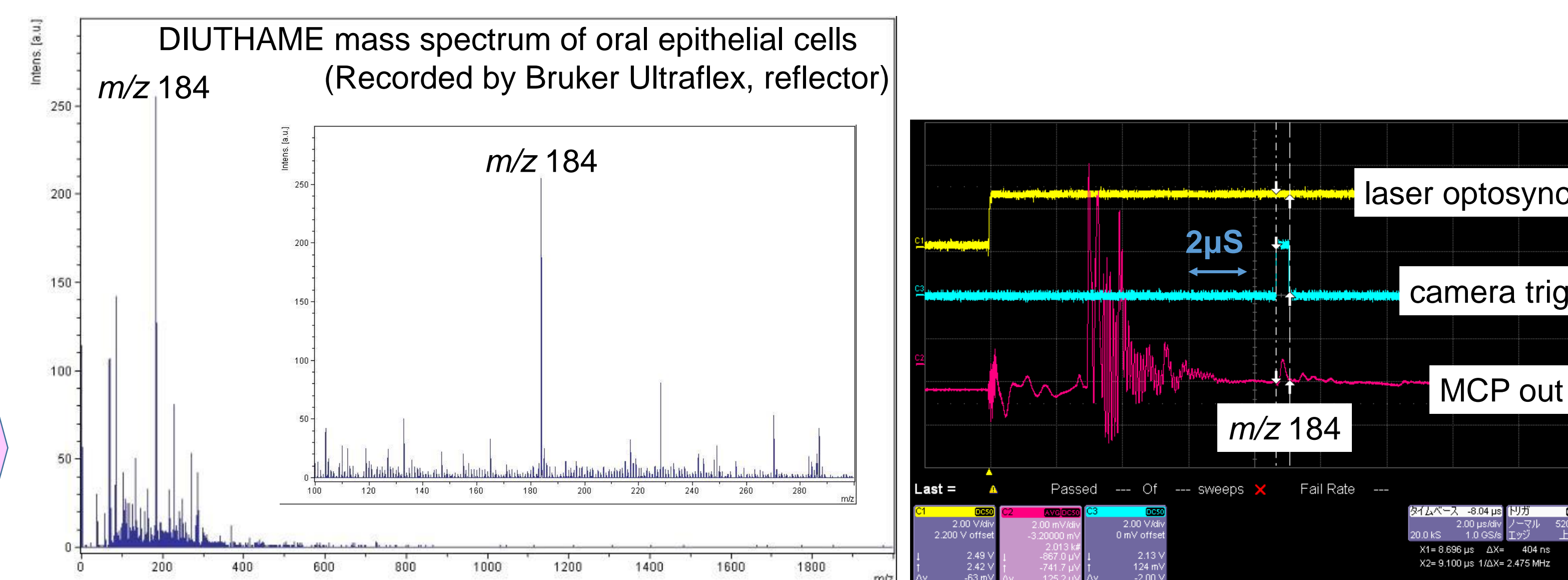
1. Rub mucous membranes with a cotton swab in mouth.
2. Immerse the cotton swab in pure water and release oral epithelial cells.
3. Centrifugation at 3000 G for 1 minute. Remove the water and replace to PBS.
4. Centrifugation at 3000 G for 1 minute. Remove PBS and replace to pure water.
5. Apply the cell suspension on a DIUTHAME-substrate.
6. Apply pure water on the DIUTHAME-substrate from the bottom side and absorb surplus water by Kimtowel.
7. Wash the DIUTHAME-substrate by pure water from both sides and absorb water by Kimtowel. Repeat this step 4 times.
8. After the sample dries, introduce it in the ion source and conduct stigmatic-IMS.



## Results



- Spatial distribution of phosphatidylcholine (PC) in a single laser spot on the brain tissue was visualized as a stigmatic image of the fragment ion  $m/z$  184 (polar head group).
- Although the correction between optical and ionic images is unclear, some consistency was found particularly in the region close to rim of the laser irradiation spot.
- The spatial resolution of the present data was roughly estimated to be 1 μm.



- Spatial distribution of PC head group ( $m/z$  184) in a single laser spot was visualized. The fragment ions were observed in anywhere, including extracellular regions.
- Although it was vague, the ion image showed a sign of the contours of cell membranes and nuclei.

## Conclusion

• The combination of DIUTHAME and stigmatic-IMS was examined for the first time. This combined approach was proved to be effectual for achieving ultimate high spatial resolution. In the present study, the spatial resolution is roughly estimated to be 1 μm.

• A high speed stigmatic imaging of  $m/z$ -selected ions became possible by capturing optical images on the phosphor screen with a triggered camera.

• It was suggested that the combined approach is applicable to investigate biological samples in the cellular scale.

## Future directions

• Stigmatic imaging of multiple molecular constituents will be established.

• An attachment unit with these technologies will be implemented in commercial MALDI-TOF mass spectrometers.

• The unit will be near commercialization by overcoming some problems in the present technology.

## Acknowledgement

○The Graduate School for the Creation of New Photonics Industries  
Dr. Minako Hirano, Dr. Hiroaki Yokota, Dr. Toshiyuki Kusumoto, Dr. Katsuhiko Ishii

○Hamamatsu Photonics K.K.  
Akira Tashiro, Masahiro Kotani, Takayuki Ohmura, Yuuya Washiyama  
Takayuki Nakamura, Minoru Kondo

○The National Institute of Advanced Industrial Science and Technology  
Dr. Hiroaki Sato

