

Metabolic Profiling of Single Live Cells by Laser Ablation Electrospray Ionization MS

Bindesh Shrestha and Akos Vertes, *The George Washington University, Washington, DC 20052*

Introduction

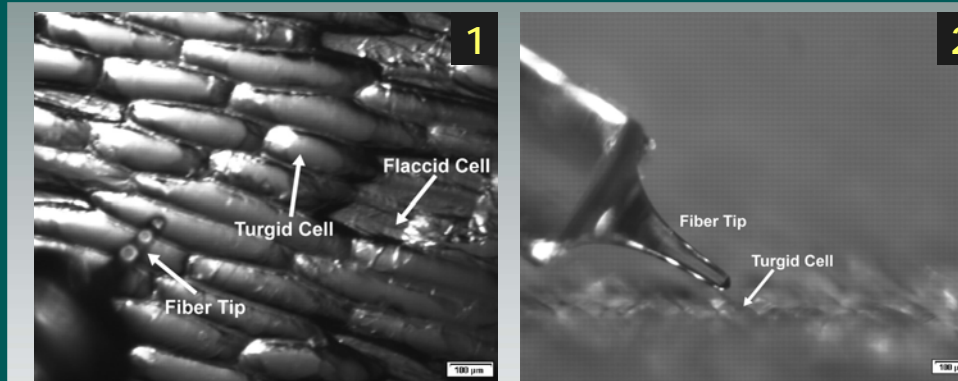
- Biochemical processes within a single cell are linked to the cell cycle, disease states, and ecological effects. Thus even cells of the same type exhibit diverse metabolic makeup depending on their age and interactions with the environment.
- In situ* analysis of metabolites in a single cell is challenging because of the limited size and complexity of the sample.
- Laser ablation electrospray ionization (LAESI) mass spectrometry (MS)¹ was employed to obtain metabolic profiles of single epidermal cells at ambient conditions.

Methods

- Mid-IR laser (Nd:YAG-OPO) radiation at 2.94 μm is readily absorbed by biological cells due to their high water content.
- The infrared ablation (~30 μm) of a cell with an etched optical fiber tip produces a plume from the cytoplasm in the form of vapor and small droplets.
- LAESI-MS is obtained after the ablation plume from the cell coalesces with electrospray droplets to produce ions, which are analyzed by a mass spectrometer (Q-TOF).
- Long-distance video microscopes are utilized to spot the cells for ablation and to control and distance between the fiber tip and sample surface.

[1] Nemes, P.; Vertes, A., "Laser Ablation Electrospray Ionization for Atmospheric Pressure, *In Vivo*, and Imaging Mass Spectrometry," *Anal. Chem.*, 2007, 79, 8098.

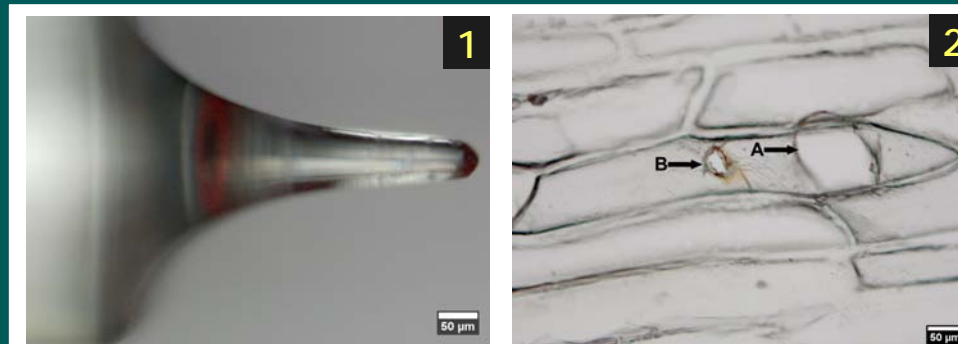
Cell Visualization



(1) The selection of a cell for analysis was achieved by aligning the fiber tip over the cell (image captured from the cell spotting microscope).

(2) The distance between the tip and the cell was adjusted to get the maximum ablation energy without any direct contact. Contact could result in breaking the tip, rupture of the cell wall, and interference with the laser ablation plume.

Single Cell Ablation

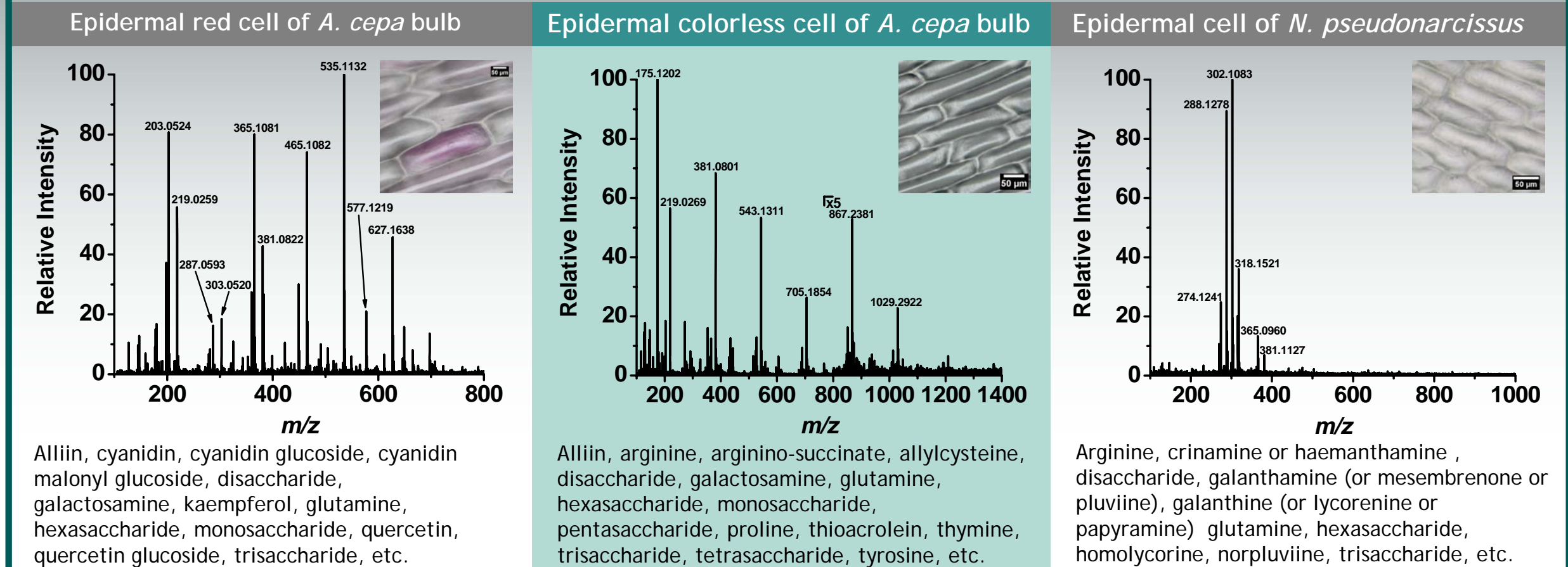


(1) The transmission of visible HeNe laser light through the etched fiber tip indicates that the leakage of energy is limited to the high curvature areas.

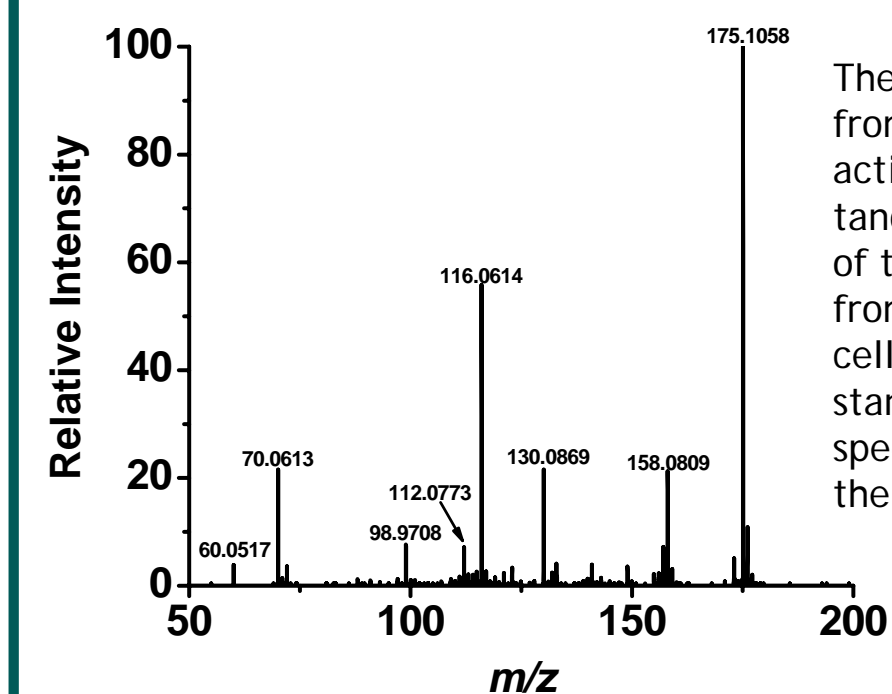
(2) Initial ablation (A), on a turgid *A. cepa* epidermal cell resulted in the phase explosion of cytoplasm inside the cell that led to the bursting of the cell wall and the ejection of the enclosed material for LAESI-MS. After the second ablation (B), on the same cell, when it was already flaccid due to the loss of the cytoplasm, only a slight degradation of the cell wall was observed.

Metabolic Profile of Single Cells at Ambient Conditions

LAESI mass spectra of single epidermal cells after subtraction of background ions from the electrospray are presented below with the list of a few assigned metabolites.



Tandem MS at Single Cell Level



The eight fragments from the collision activated dissociation tandem mass spectrum of the ion at m/z 175 from a single *A. cepa* cell is similar to the standard tandem mass spectrum of arginine in the NIST MS database.

Future Directions

- Reducing the ablation spot size while obtaining a meaningful mass spectrometric signal from smaller single cells and cell organelles.
- Analysis of biological tissues cell-by-cell, with the ultimate goal of molecular imaging based on cells as the natural voxels.
- Adjustment of laser parameters depending on cell types to perform single-cell analysis from intact multilayered cell populations.

The authors acknowledge the financial support from the W. M. Keck Foundation (041904), the Department of Energy (DEFG02-01ER15129), and the George Washington University Research Enhancement Fund for this work.