Subcellular Analysis of Cell Organelles with Micro-dissection on Nanophotonic Desorption Ionization Platforms

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Introduction

- Laser desorption ionization (LDI) from silicon nanopost arrays (NAPA) has been used to analyze small biomolecules in single cells and subcellular compartments.
- Microdissection enables the isolation, extraction and modification of subcellular organelles with high precision.
- Microinjection into single cells can be used to perturb the metabolic network.
- LDI from NAPA is used to analyze organelles from dissected cells and follow compositional changes in a cell after microinjection.

Microdissection of nucleus

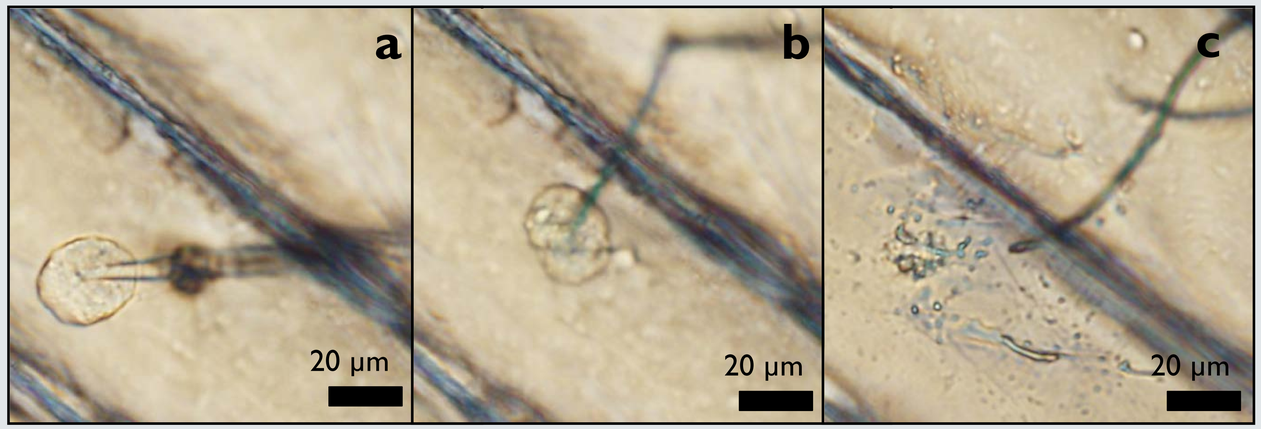

Figure 2. (a) Monolayer of *A. Cepa* epidermal cells with a stained nucleus and a 1 μm diameter capillary piercing the nuclear envelope. (b) Nuclear contents (nucleoplasm, chromosomes, etc.) were extracted into the capillary. (c) Nuclear contents were removed from the cell for MS analysis.

Nanopost arrays

- NAPA exhibits nanophotonic interactions with the laser pulse and ions are produced from the adsorbates with high efficiency.
- On nanoposts with subwavelength diameter, ion yield resonances are observed at specific aspect ratios.[1]
- Resonant structures show ultralow limits of detection (800 zmol for verapamil) and wide dynamic range of quantitation (see Figure 3).[2]
- In the absence of matrix peaks the spectral interferences are minimized enabling the detection of metabolites and xenobiotics.

Methods

- Dissection of tissue embedded cells can be initiated by a microneedle cutting the cell wall and exposing the cellular content (see Figure 1a).
- A microcapillary (Femtotip) is used for the removal of specific organelles or for the direct delivery of reactants.
- Entire or partial cell compartments can be extracted and deposited on the NAPA platform (see Figure 2).
- LDI mass spectrometry of the deposited cell or cell compartment provides insight into subcellular metabolic makeup and compositional changes following stimulus (see Figure 1b).

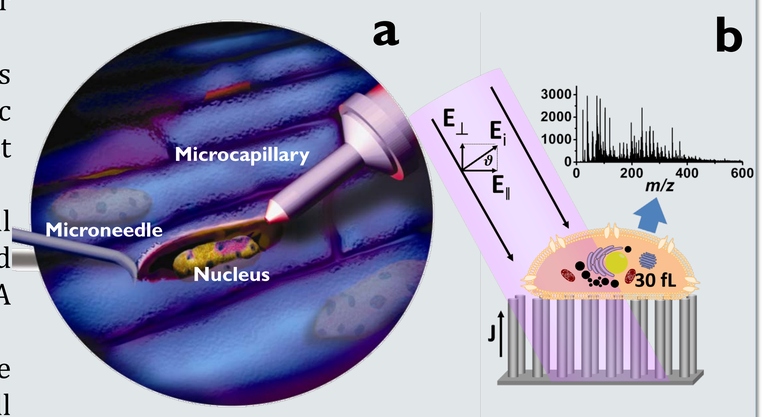

Figure 1. (a) Schematic of microdissection and microcapillary insertion. (b) LDI of subcellular content from NAPA for analysis by mass spectrometry.

NAPA figures of merit

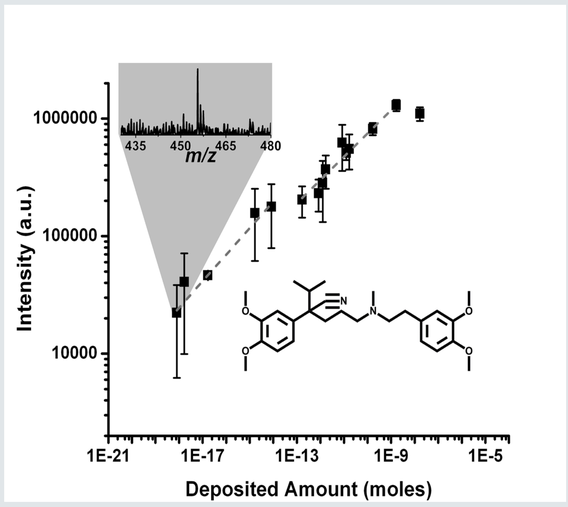

Figure 3. Verapamil molecular ion intensities as a function of the sample amount deposited on the NAPA chip. The limit of detection is 800 zmol.[2]

Future Projects

- The combination of cell dissection, microinjection and mass spectrometric analysis by LDI from NAPA helps to uncover compositional and functional changes in subcellular compartments with minimum interference from the rest of the cell.
- Direct introduction of xenobiotics into a cell or cell compartment followed by analysis can reveal the response of the metabolic network.
- Creating a micro-platform for culturing manipulation and analysis of cells.

(1) Walker, B. N.; Stolee, J. A.; Pickel, D. L.; Retterer, S. T.; Vertes, A. *Journal of Physical Chemistry C* **2010**, *114*, 4835-4840.

(2) Walker, B.N.; Stolee, J.A.; Vertes, A. *Anal. Chem.*, **2012**, *84*, in press.

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