Supplementary Material (ESI) for Analytical Chemistry

Supporting Information

Semi-transparent nanostructured films for imaging mass spectrometry and optical microscopy

Jay G. Forsythe, Joshua A. Broussard, Jenifer L. Lawrie, Michal Kliman, Yang Jiao, Sharon M. Weiss,* Donna J. Webb,* and John A. McLean*

Departments of Chemistry, Vanderbilt Institute of Chemical Biology, Vanderbilt Institute for Integrative Biosystems Research and Education, Biological Sciences, Cancer Biology, Interdisciplinary Graduate Program in Materials Science, and Electrical Engineering and Computer Science, Vanderbilt University, Nashville, TN 37235

Email: sharon.weiss@vanderbilt.edu, donna.webb@vanderbilt.edu, and john.a.mclean@vanderbilt.edu.

Abstract: Semi-transparent porous silicon substrates have been developed for pairing nanostructure-initiator mass spectrometry (NIMS) imaging with traditional optical-based microscopy techniques. Substrates were optimized to generate the largest NIMS signal while maintaining sufficient transparency to allow visible light to pass through for optical microscopy. Using these substrates, both phase-contrast and NIMS images of phospholipids from a scratch-wounded cell monolayer were obtained. NIMS images were generated using a spatial resolution of 14 μm. Coupled with further improvements in spatial resolution, this approach may allow for the localization of intact biological molecules within cells without the need for labeling.

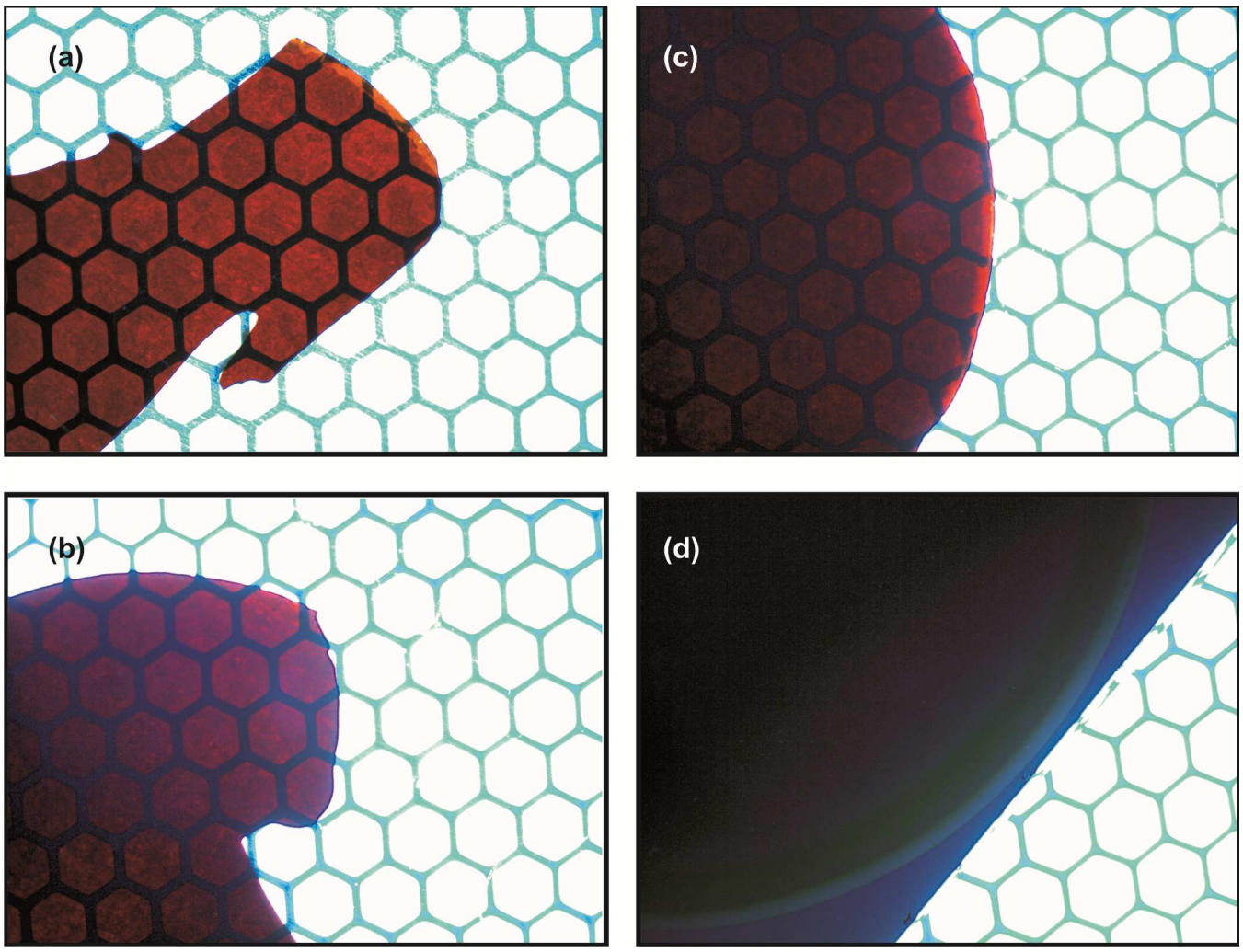

Figure S-1. Backlit CCD photographs of NIMS substrates above a metal grid. Semi-transparent NIMS films have thicknesses of (a) 10 μm, (b) 13 μm, and (c) 19 μm. Substrates for parts a-c are attached to ITO-coated glass slides. (d) Traditional NIMS surfaces on silicon wafers are not transparent.

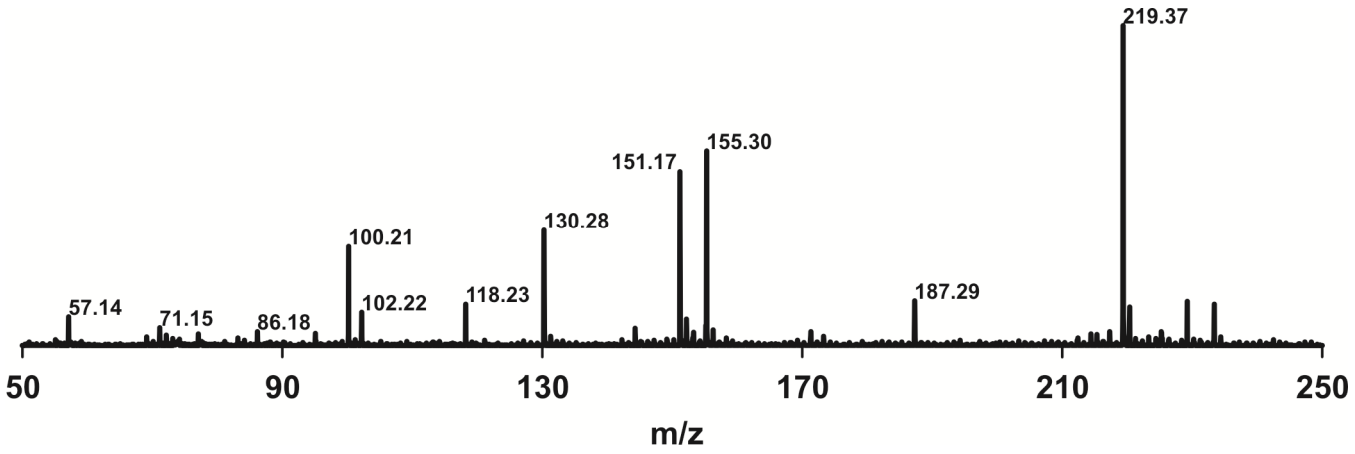

Figure S-2. Low *m/z* spectrum of an analyte-free region on NIMS film obtained using high laser energy. Several peaks (86, 102, 130, 155 Da) correspond to those described in Wen et. al. (reference 38 in main text), which were suggested to be alkylammonium salts used in the silicon fabrication process.

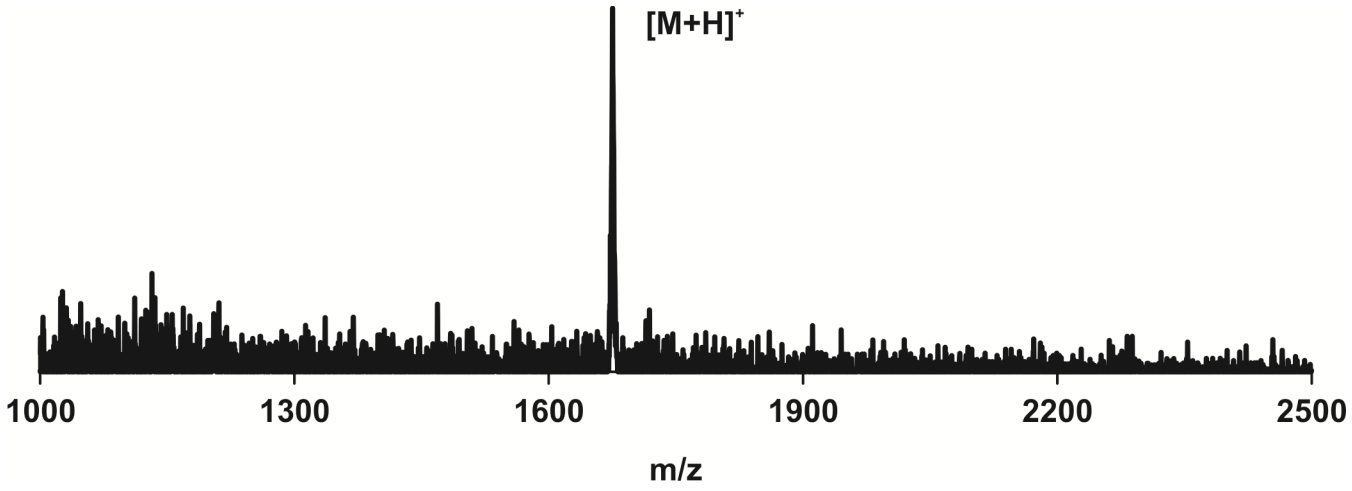

Figure S-3. Detection of 10 femtomoles of neurotensin peptide ($[M+H]^+$ = 1673 Da). Spectrum was obtained from 3-month-old substrate stored at room temperature and air.

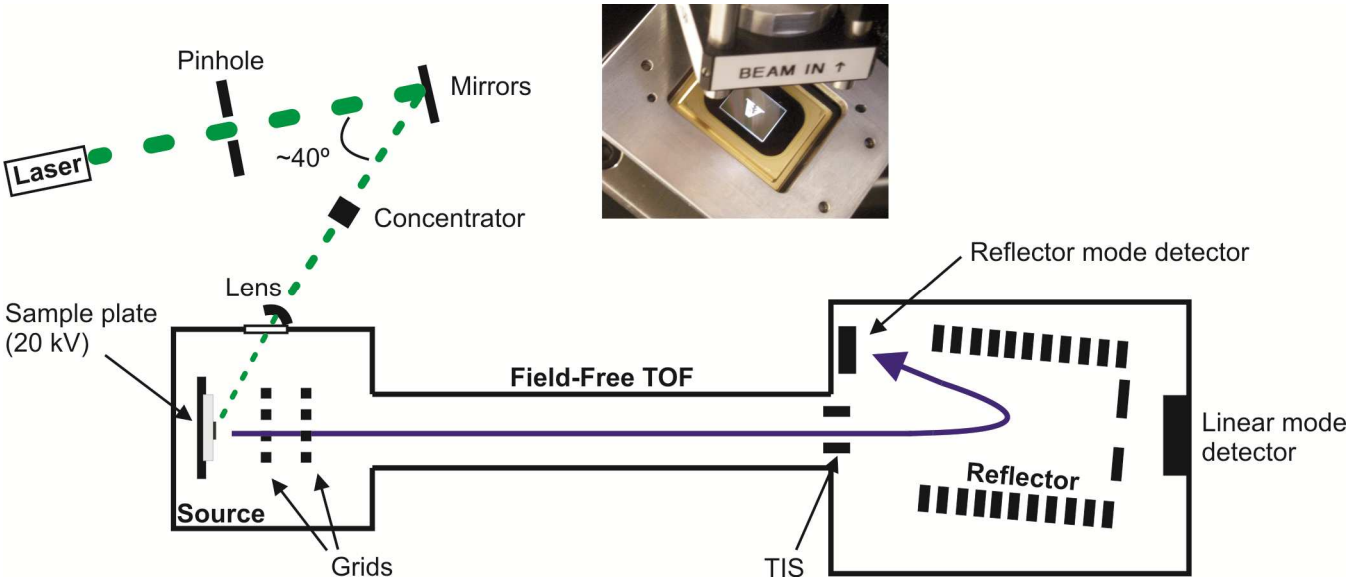

Figure S-4. Instrumentation used for NIMS imaging. A frequency-tripled Nd:YAG laser (355 nm) is fired onto a digital micromirror device (*inset*), which redirects a small, selected portion of the beam towards the Voyager DE-STR mass spectrometer. The mass spectrometer is operated in either linear or reflector TOF mode. The timed ion selector (TIS) can be used to isolate *m/z* values for PSD fragmentation.

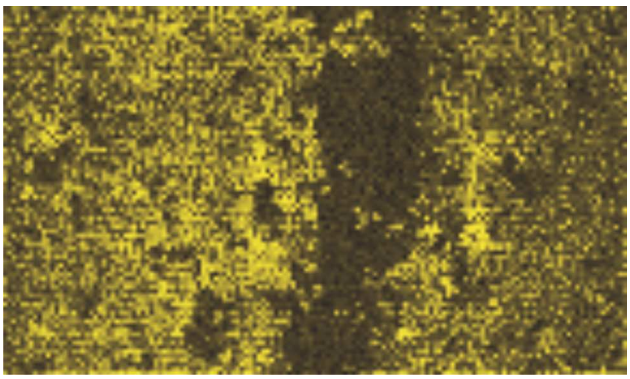

Figure S-5. Raw NIMS image of *m/z* 788.5 (PC 36:1) from scratch-wounded HT1080 cell monolayer. Image was acquired using MALDI MS Imaging Tool software. Individual pixels are 14 x 14 μm. Image covers a total area of 2.1 mm by 1.2 mm.