ToF-SIMS dual beam depth profiling and imaging of human HeLa cells

J. Brison, D.S.W. Benoit, P.S. Stayton, L.J. Gamble and D.G. Castner
National ESCA and Surface Analysis Center For Biomedical Problems, Departments of Chemical Engineering and Bioengineering, Box 351750, University of Washington, Seattle, WA 98195.

Statement of Purpose: Time-of-flight secondary ion mass spectrometry (ToF-SIMS) is increasingly used to study biological samples and image single cells [1,2]. The technique offers high molecular specificity with submicron lateral and depth resolution. However, the complex and fragile biological materials require special sampling handling so the ToF-SIMS 3D images acquired under high vacuum conditions can be related to the biological state. Also, processing the large ToF-SIMS dataset is not straightforward because of the complex sputtering and ionization processes. In this work, the influence of the sample preparation and experimental parameters on the ToF-SIMS cell images was studied. For this purpose, human HeLa cells were prepared by different methods (chemical fixation in paraformaldehyde, simple wash-and-dry with ammonium acetate, freeze-drying, freeze-etching, etc.) and characteristic signals from the cells were recorded and compared using various sputtering parameters (e.g., different Bi_3^+ and C_{60}^+ doses).

Methods: HeLa cells, human cervical carcinoma cells (ATCC CCL-2), were maintained in minimum essential media (MEM) containing L-glutamine (Gibco), 1% penicillin-streptomycin (Gibco), and 10% fetal bovine serum (FBS, Invitrogen) at 37 °C and 5% CO_2. After trypsinizing, the HeLa cells (12,000 cells/cm^2) were seeded onto surfaces (either PET transwell insert membranes (Corning, 0.4 µm pores) or Si surfaces) and allowed to adhere overnight. The cells were then prepared for ToF-SIMS analysis according to the different methods described below.

ToF-SIMS experiments were performed using an ION-TOF TOF.SIMS 5-100 (ION-TOF GmbH, Münster, Germany). 3D secondary ion images were collected in the dual beam mode (imaging alternating with etching). Images with submicron lateral resolution were acquired by rastering a pulsed 25 keV Bi_3^+ beam across the sample surface. Etching was done with a 20 keV C_{60}^{++} beam to minimize damage in etched sample. The imaging area was typically 150 x 150 µm^2 and was centered inside the 400 x 400 µm^2 C_{60} crater.

Results: Fig. 1 shows the positive total ion images of the HeLa cells fixed on PET in 4% paraformaldehyde in PBS and rinsed with water. The image in Fig. 1a shows that HeLa cells are not readily apparent before C_{60} etching. This limitation is due to the extreme surface sensitivity of ToF-SIMS and improper sample preparation. The uniformity of all signals and the low potassium to sodium ratio (not shown) indicate that the chemical integrity of the cell was not preserved during the sample preparation [3]. However, it can be seen in Fig. 1b that the surface contamination can be removed by etching for 15 seconds with C_{60} (i.e., 3.9 x 10^{13} ions per cm^2), revealing the HeLa cell morphology. This result indicates that C_{60} can be used to remove surface contamination with low chemical damage to the cell, allowing chemical state imaging of the cells despite the poor sample preparation method [4].

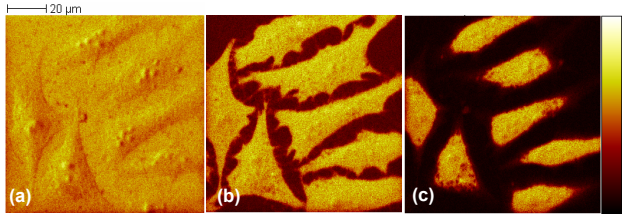

Figure 1. Positive total ion images of the HeLa cells (a) before C_{60} etching, (b) and (c) after 15 and 170 seconds of C_{60} etching, respectively.

After a higher C_{60} etching time/dose (Fig. 1c), the apparent cell size starts to decrease, indicating that the cytoplasm is being removed and the interface with the substrate is reached.

Fig. 2a shows HeLa cells on a Si substrate prepared by a simple wash-and-dry method [5]. The cells were washed for 15 seconds with ammonium acetate and dried in air before introduction into the ToF-SIMS instrument and etched with C_{60}.

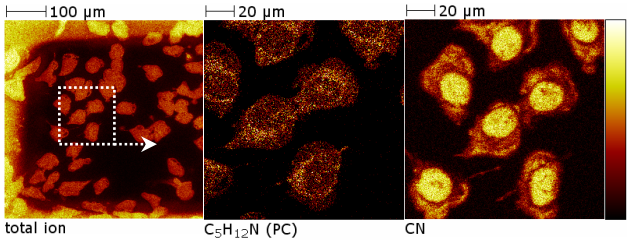

Figure 2. (a) Positive total ion image of the HeLa cells after C_{60} etching, (b) positive phosphocholine fragment at m/z = 86 and (c) negative CN signal at m/z = 26.

Fig. 2b and 2c show that subcellular features can be imaged after etching with 5 x 10^{13} C_{60} per cm^2. Location of phosphocholine fragments is shown in Fig 2b. Fig. 2c indicates the regions of higher nitrogen concentrations, which can be associated with the presence of DNA in the cells nuclei.

Conclusions: Human HeLa cell ToF-SIMS images were successfully acquired using a Bi_3^+ analysis beam and a C_{60}^{++} etching beam. Our results show that etching with a 5 x 10^{13} C_{60} dose improves the quality of ToF-SIMS images and allows subcellular chemical mapping of the HeLa cells, independent of the sample preparation method used.

References: 1. Winograd N, Anal. Chem. 2005;77,143A 2. Chandra S, Ana. Chem 2000;72,104A 3. Chandra S, Appl. Surf. Sci. 2008 *in press* 4. Kurczy ME, Appl. Surf. Sci. 2008, *in press* 5. Berman E, J. Am. Soc. Mass Spectrom. 2008, 19, 1230.