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Atom Probe Tomography of Mammalian Cells: Advances in Specimen Preparation

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Atom Probe Tomography (APT) is an emerging technology used to analyze the three-dimensional structure of a variety of substances at resolutions in the sub-nanometer range. A strong electric field (~20 V/nm) coupled with a sharp needle-shaped specimen (~100 nm diameter end-form) and a two-dimensional detector create a point-projection microscope whereby ions are field-evaporated from the surface and diverge to the detector creating a highly-magnified projection of the specimen surface [1]. The specimen preparation challenge is to form prospective materials into a sharp-needle geometry that also allows them to maintain structural integrity while exposed to an extreme electric field. The development of Focused-Ion-Beam-based TEM lift-out techniques and subsequent transfer of these techniques to APT has made it possible to ion-mill microscopic regions from a variety of solid bulk materials into a geometry appropriate for APT [2]. Unfortunately, not all materials with the correct shape survive application of the required electric field without premature failure, and not all needle geometries provide the same results [3]. Nevertheless, evaluation of the potential to analyze new materials with APT must start with the manufacture of specimen tips followed by assessment of analysis yield and data quality. Variation of tip shape, tip composition, and analysis conditions ultimately affect the ability of these specimens to survive analysis and yield interpretable data.

The desire to apply APT to biological systems has had a long history [4]. The advent of commercial Pulsed-Laser APT systems has enabled analysis of electrically insulating materials such as silicon dioxide and sapphire [5]. A broad range of biological entities such as proteins, viruses and cells have structural and chemical compositions that are potentially amenable to APT, but methods to prepare needle-shaped specimens suitable for analysis by APT have not yet been reported. Here, we have evaluated the feasibility of analyzing biological specimens preserved under near-native conditions using these principles. We report the successful biopsy and manufacture of APT specimens from lyophilized mammalian cells, allowing for the possibility of high-resolution three-dimensional mapping of cells in a near-native state. Because cell interface adhesion was a concern for specimen survivability, HeLa cells grown on silicon substrates were manufactured in top-down [2], backside [6], and cross-section [7] orientations for purposes of comparative analysis (Fig. 1). Mass spectra recorded from a typical specimen are presented in Fig. 2, demonstrating detection of ions including hydrogen, carbon, nitrogen and oxygen, and several peaks with higher masses. Our results show that biological specimens prepared in cross-section orientation allow for controlled field-evaporation, allowing us to demonstrate the first 3D chemical map of a mammalian cell at nanometer resolution obtained using APT.

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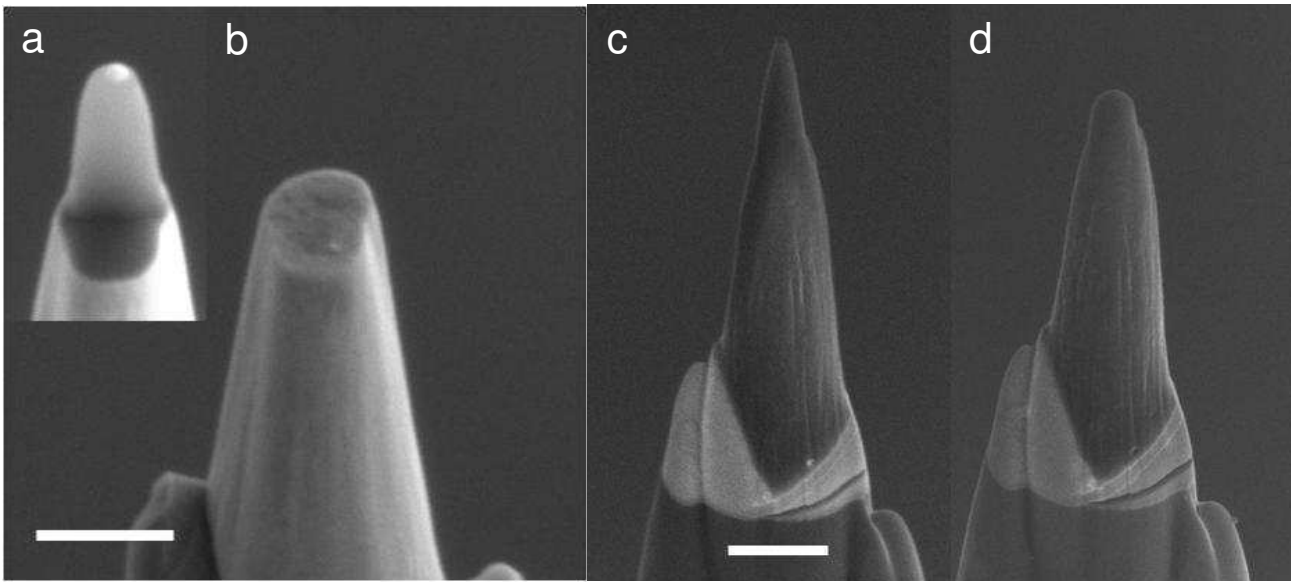

FIG. 1. SEM images (a) and (b) show a top-down prepared specimen before analysis and after failure. This particular specimen yielded ~100 thousand ions before failing at the cell/substrate interface. SEM images (c) and (d) show a specimen prepared using the cross-section method before analysis and after the removal of ~7.7 million ions. The reconstructed volume is consistent with the change in the imaged volume. The resulting mass spectrum is shown in Fig. 2. Scale bars 500 nm.

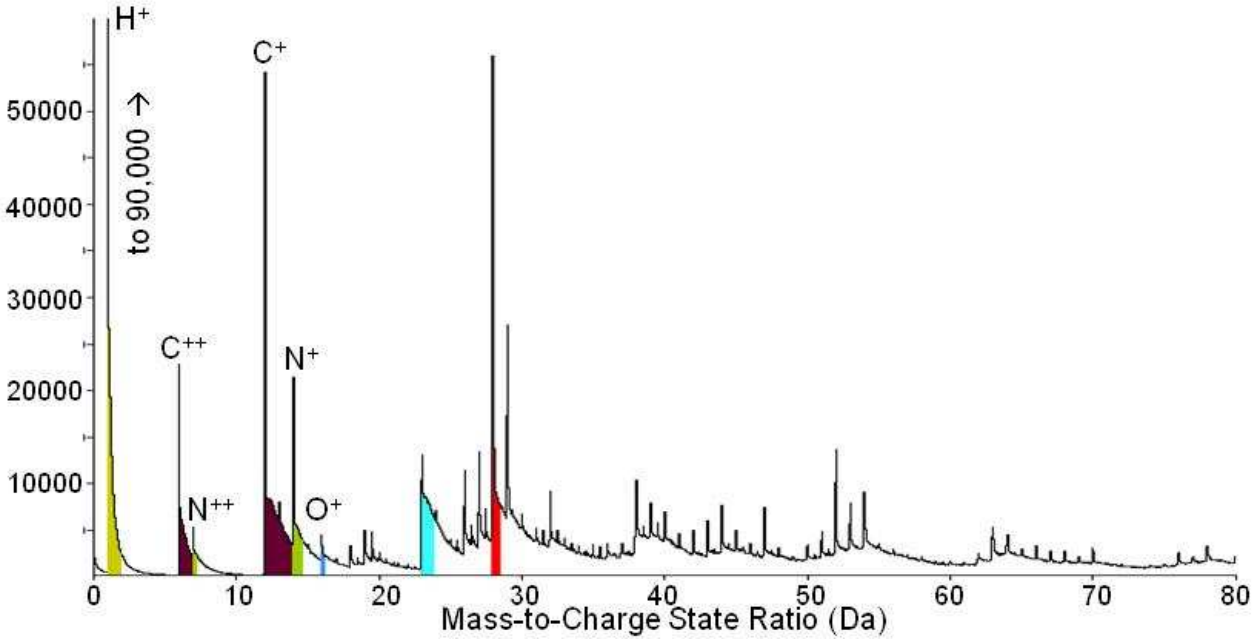

FIG. 2. APT mass spectrum from a lyophilized mammalian cell.

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