PROSPECTS FOR ATOM-PROBE ANALYSIS IN BIOLOGY AND MEDICINE

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The atom-probe field ion microscope may be the ultimate microanalytical tool because a single atom, chosen from its neighbors at the discretion of the experimenter, can be visualized in atomic resolution and then identified by its mass-to-charge ratio. Although the analysis procedure is destructive, the lateral and depth resolution of the atom-probe is impressive, exceeding 0.5 nm under favorable conditions. Despite these attributes, atom probe analysis has been largely confined to problems in the materials sciences. The atom probe has made no impact in biology or medicine, largely because of restrictions imposed by the technique on the preparation, imaging, and analysis of biological samples. Recent developments in each of these areas has made atom probe analysis of biological samples a more viable prospect.

Biological Sample Preparation

The atom-probe technique places severe restrictions on the type of sample that can be analyzed. Field ionization is used to image a surface in atomic resolution, and field desorption provides a source of ions for analysis. These processes occur with a high probability only in electric fields greater than 100 MV/cm. Electric fields of this magnitude (and the nature of the imaging process) require the use of a needle-like substrate, known as a field-emitter "tip". Ions are generated at the highly curved apex of the tip where the electric field is enhanced. Practical constraints on the uniformity of the field restrict the field-of-view to ~40° from the tip axis. The magnification and the resolution of the ion image are affected by the curvature of the tip apex and limit the maximum tip radius to ~200 nm. A tip is usually formed from the material to be analyzed. Since biological material cannot be formed into a tip, a sample of the material must be placed on the tip apex for analysis. If the presence of the biological material distorts the electric field the ion image will be impossible to interpret. These constraints suggest that a biological sample suitable for atom probe analysis must be very thin (a small fraction of the tip radius), and must be uniform and isotropic over the tip apex within an angle of 40° from its axis. Although it is unlikely that biological objects of cellular dimension will meet these requirements, isolated biological objects of molecular size will be suitable for atom-probe analysis.

Molecules of biological interest (proteins or DNA) and isolated virus particles are suitable for atom-probe analysis and can be placed on the apex of a metallic tip by deposition from aqueous solution. A routine deposition protocol was established using the transmission electron microscope to image the tip apex. Biological objects are easily seen as contrast variations along its contour, particularly after they have been stained with uranyl acetate or rotary shadowed with tungsten (as shown in Fig. 1 and Fig. 2). Immunologic binding to the tip apex has also been successful. The small size and the shape of a field-emitter tip raises the prospect of using an immunologically active tip to acquire, in vivo, presellected antibody or antigen for analysis.

Biological Imaging in the Atom-Probe

The electric field strength generated at the tip apex during ion imaging is the greatest obstacle to successful imaging of biological samples in the atom-probe. The atom-probe technique requires stable imaging conditions in order to select an object from the tip apex for analysis. An outward-directed electrostatic pressure, \( P_L = e_0F^2/2 \approx 30 \text{ tons/in}^2 \), is established at the tip apex under normal imaging conditions. Individual molecules or pieces of a biological sample (which are insulators) can be redistributed on the tip apex or torn from the surface before the field strength required for ion imaging is reached. The field strength at which this phenomenon occurs can be estimated by noting that surface tension forces produce similar effects when a biological sample is moved through an aqueous interface and dried in air. The pressure exerted on a biological sample of characteristic dimension, \( d \), as it dries in air is \( P_L = 2\gamma d \gamma = 0.073 \text{ Nm}^{-1} \). Suppose that isolated biological molecules (\( d \approx 10 \text{ nm} \)) are examined in the atom-probe. \( P_L = \gamma d \) at a field strength:

\[ F = (2\gamma d)^{1/2} = (4\gamma d)^{1/2} = 18 \text{ MV/cm} \]

Imaging at even lower field strengths seems advisable to minimize field-induced damage of a biological sample.
Clever, but unsuccessful attempts to image biological objects at lower field strengths attest to the severity of the imaging problem. In principle, field-emission tunneling could be used for imaging at fields of 10 MV/cm, but experience has shown that molecular images generated by electron tunneling are notoriously difficult to interpret. Fortunately, another imaging solution exists. It is called "Field Ion Tomography", and has produced the first stable ion images of protein monolayers on metal surfaces (Fig. 3). During the tomographic imaging process, biological objects on the tip apex are embedded within a layer of benzene condensed onto the tip surface at 80 K from the gas phase. As the field is increased, the layer is removed as cluster ions: (C_{14}H_{2n})^{+} (n = 1,2). The ion image replicates and records the three-dimensional morphology of objects on the tip apex. Cryofixation techniques have been recently developed to preserve biological objects within a layer of vitreous water ice at the tip apex. Procedures have also been implemented to transfer the tips cryogenically into the atom probe at 80 K where their apex can be imaged. In principle, the vitreous ice layer can be replenished from the gas phase, providing a quasicontinuous image for object selection during atom-probe analysis.

Biological Analysis in the Atom-Probe

The most common (time-of-flight) configuration of the atom-probe is ideally suited for the analysis of biological samples because all ionization events initiated by a high-voltage or laser pulse will be detected simultaneously. The ionization event is expected to be gentle, and offers all of the advantages of field ionization mass spectroscopy which tends to preserve the identity of the parent molecule. If complete ionization occurs it would not be unreasonable to expect the Imaging Atom-Probe technique to provide spatially resolved elemental maps that document the composition of isolated biological molecules with nanometer resolution.

References

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