Application of the Helium Ion Microscope to Biological Sciences

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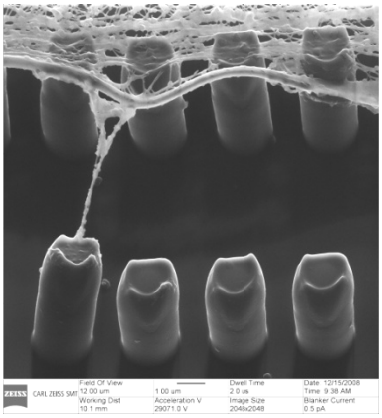
The Helium Ion Microscope (HIM) is a new imaging technology based on a high brightness and stable Gas Field Ion Source (GFIS). The GFIS employed exhibits a low energy spread (<1 eV), small virtual source size (< 0.3 nm) and a high brightness > 4 x 10^9 A/cm^2 sr [1]. This, in conjunction with the shallow escape depth (<1 nm) of the secondary electrons generated by the incident 30 keV helium ions, contribute to the HIM's primary advantage in the imaging of solid samples: its high spatial resolution (0.25 nm) [2]. We have applied this novel technology across a broad spectrum of multidisciplinary applications (from basic materials science to semiconductor applications) to assess its utility and possible advantages over alternative techniques. One area where our investigations have gained significant traction is in the imaging of biological specimens.

The utility of this instrument in addressing topics of the biological sciences is due in part to the HIM's high spatial resolution. However, in the context of biological specimens, it is the ability to image non-conductive samples without the application of a metal (or other conductive) overcoat and without the need of a background gas (both of which degrade resolution and surface details), which has proven to be a distinguishing attribute. With the HIM, sample neutralization is achieved with an integrated electron gun which is aligned to impinge a focused electron beam onto the ion scan field. The balance between the positive sample charging induced by the ion beam and the neutralization effects of the negatively charged electron beam can be tuned (by adjusting the electron beam focus, landing energy, and dwell time) so that a wide range of insulating samples (e.g. uncoated cells on glass cover-slips or PDMS) can be observed without degradation of resolution or loss of surface contrast. The HIM has a further advantage of a large depth of field. Together, these attributes open up a whole new range of biological problems that can be solved rapidly and with less risk of artifacts. In terms of scientific problems that are being investigated, we have already initiated studies in cellular biomechanics where we are exploring the detailed organization of the actin cytoskeleton in cell motility, Figure 1; in bacterial pathogenesis where we are studying the bacterial invasion of epithelial cells and how the bacteria bind to epithelial cells in the invasion process, Figure 2; and in health effects of engineered nanoparticles where we are tracking the transport and aggregation/disaggregation of nanoparticles within exposed rodents, Figure 3.

As with any imaging technique, there are limitations. In our case, the samples must be dry, either by critical point drying or by freeze drying. It is likely that samples frozen in vitreous ice are also viable, but we have not yet explored this option since our cold stage is not yet ready. Nevertheless, our initial studies have enabled us to image protein filaments within the cytoskeleton as small as 4 nm; we have observed that during the pseudomonas bacterial invasion of lung fibroblast cells, the 4 nm pilus structure extends at right angles from the end of the bacterium and that structure binds the bacterium to the cell surface; and in the rodent/nanoparticle toxicity studies we have observed pore sizes in the rat kidney as small as 7 nm in diameter. The detailed scientific findings from these investigations will be published elsewhere. Here, we use these investigations to guide our discussions on the technical issues (both promises and limitations) which we have experienced in the adaptation of this new technology to the biological sciences.

1. B. Ward, J. Notte, and N. Economou, J. Vac. Sci. Technol. B, Vol. 24, No. 6, Nov/Dec 2006
2. Application Note, Carl Zeiss SMT, "Ultra-High Resolution Imaging in ORION®PLUS", PI No. 0220-2008-ENG, Nov. 21, 2008



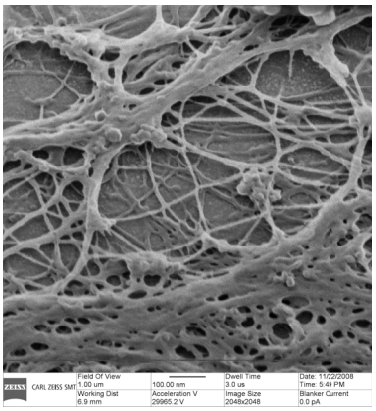
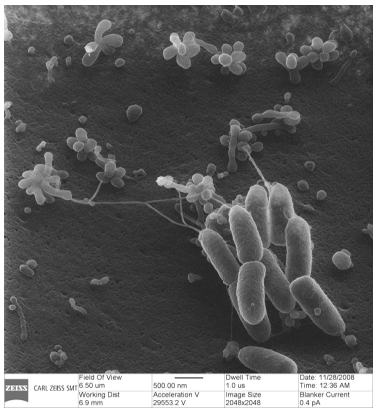


Figure 1a,b-Biomechanics: a) Cytoskeletons of cells spread on PDMS pillars showing force on a single pillar (FOV 12 μm). b) Detailed image of actin cytoskeleton of cell spread on glass coverslip (FOV 1 μm). Our experience with the He+ ion microscope has shown us that the system can image protein filaments as small as 4 nm with no metal coating.



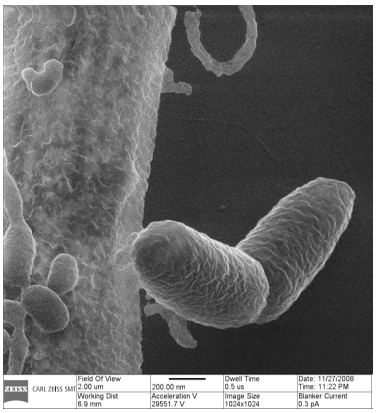
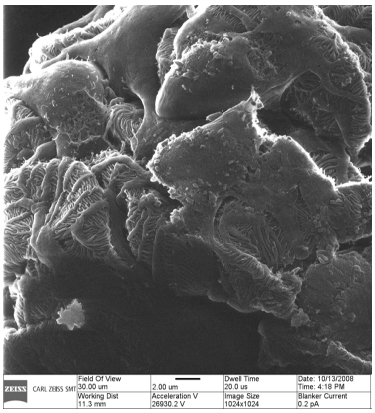


Figure 2a,b-Bacterial pathogenesis: a) A colony of Pseudomonas bacteria infecting lung fibroblast cells (FOV 6.5 μm). Multiple pili (and bifurcated pili) are observable and appear to have an affinity for the cell microvilli. b) A single Pseudomondas bacteria attaching to a lung fibroblast cell (FOV 2 μm). The response of the cell to this attack (depression in cell membrane) is evident.



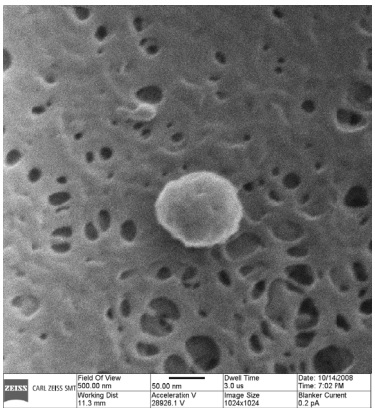


Figure 3a,b-Invasiveness of nanoparticles: a) a) The fine filter-like structure of a rat kidney (FOV 30 μm). b) An enlarged of a nanoparticle trapped within the kidney (FOV 500 nm). The fine structure of the kidney is evident, with pores as small as 7nm observable.