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Imaging and Ion-Beam Milling of Biological Specimens with the Helium-Ion Microscope

Matthias Schmidt[1], Chaturanga Bandara[1], Marc Tamisier[1], Ilari Maasilta[2] and James Byrne[3]

[1]Helmholtz-Centre for Environmental Research GmbH - UFZ, Leipzig, Sachsen, Germany, [2]University of Jyväskylä, Jyväskylä, Finland, [3]University of Bristol, Bristol, England, United Kingdom

The last decade has seen several spectacular studies focusing on medical research, plants and small animals, microbiology and geomicrobiology using helium-ion microscopy (HIM) as a tool for high-resolution imaging and, to a lesser extent, nano-fabrication of biological objects [1]. HIM in many aspects resembles scanning electron microscopy (SEM), however, its high surface sensitivity, the possibility to image insulating specimens without having to coat them with a conductive material and the capability of gentle milling with a beam of highly focused light ions (He or Ne) render the technique preferable over SEM for many applications. In terms of resolution, HIM is closer to that of a standard, non-aberration-corrected transmission electron microscope (TEM) which enables imaging of some the tiniest structures in nature without thin lamella preparation. A recent, particularly spectacular example of this was the first HIM images of SARS-CoV2 viruses [2].

In the first part of this presentation we give a brief overview of the available literature on HIM imaging of biological samples and review some highlights that demonstrate the particular capabilities that the technique has to offer. We touch on the importance of sample preparation on the obtained micrographs and discuss whether HIM could benefit from well-established techniques used in electron microscopy, further outlining a protocol which we have developed to prepare bacterial samples [3]. It has several parameters to adapt to different experimental requirements and was successfully tested with different bacterial strains. We will briefly elaborate on the different contrast mechanisms that can be used in HIM provided the availability of suitable detectors. So far, bio-imaging has mostly relied on secondary electron detection, however, detecting transmitted or back-scattered ions, iono-luminescense or employing one of the recently developed and commercialised secondary ion mass spectrometers for the HIM is extremely promising for future life-science studies For example, these new detectors could help greatly advance the investigation of the interactions of nano-particles with cells or tissues.

In the second part of the presentation we will provide a brief overview of the application of HIM for ion-beam milling of biological objects including nematodes, bacteria, bacteriophages and dragonfly wings. Thereafter, we present some of our latest results on ion-beam milling of insect wings, microbes and microbial-induced corrosion crusts. We observed that successful milling strongly depends on controlling the heat damage of the sample due to the temperature increase under the beam [4]. Therefore, once the choice of the ion and its landing energy is made, the combination of beam current, dwell-time and spacing has to be optimised for the particular material lest it deteriorate or even melt. We systematically determined such a parameter set for insect wings [5] consisting mostly of chitin and lipids and applied SRIM simulations [6] and physical arguments to estimate the local temperature. With these optimised parameters it was possible to mill a line of about 35nm thickness through the nano-pillars on the wing of a dragonfly.

Overall, in this presentation we can only highlight just some of the exciting studies on bio-imaging with the HIM during the past decade and show a few of our latest results . However, the full potential of the HIM is far from being fully exploited and we hope that this powerful method will establish a firm place amongst the standard characterisation tools for bio-imaging and become more frequently used for nano-fabrication of biological objects during this decade.

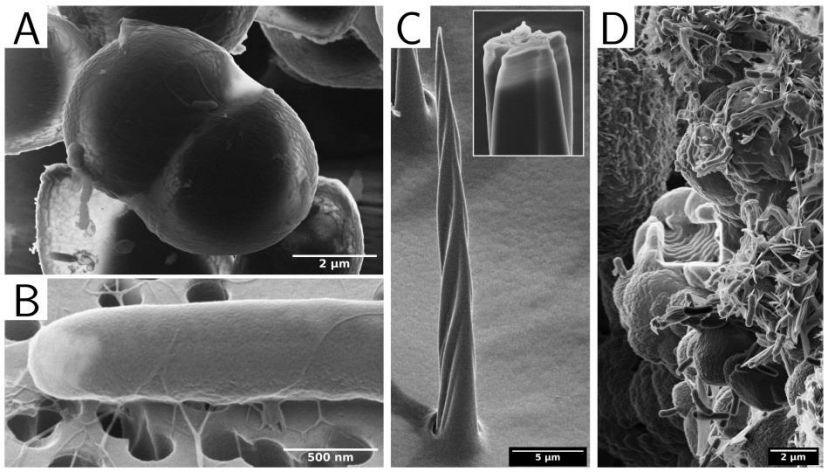

Figure 1. A) HIM images of A) a Chlorella microalgae (sample prepared by J.-H. Moreno-Osorio within the work of [5]) and B) a bacterial sample of Pseudomonas putida with flagella (sample prepared by F. Calabrese and N. Said). Ion-beam milling of C) a wing-hair of a bee (miling carried out by R. Dittrich) and D) into the crust on a microbially corroded steel coupon.

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