Imaging Bacterial Colonies and Phage-bacterium Interaction at Sub-nanometer Resolution Using Helium Ion Microscopy

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We present a novel method to study interactions between bacteria and their viruses bacteriophages in-situ on agar plates using helium ion microscopy (HIM). HIM has advantages with its sub-nanometer resolution and the possibility to image non-conductive samples. The first HIM-images of phage-bacterium interactions at different stages of the infection as they occur on an agar plate are presented in the Figure 1.

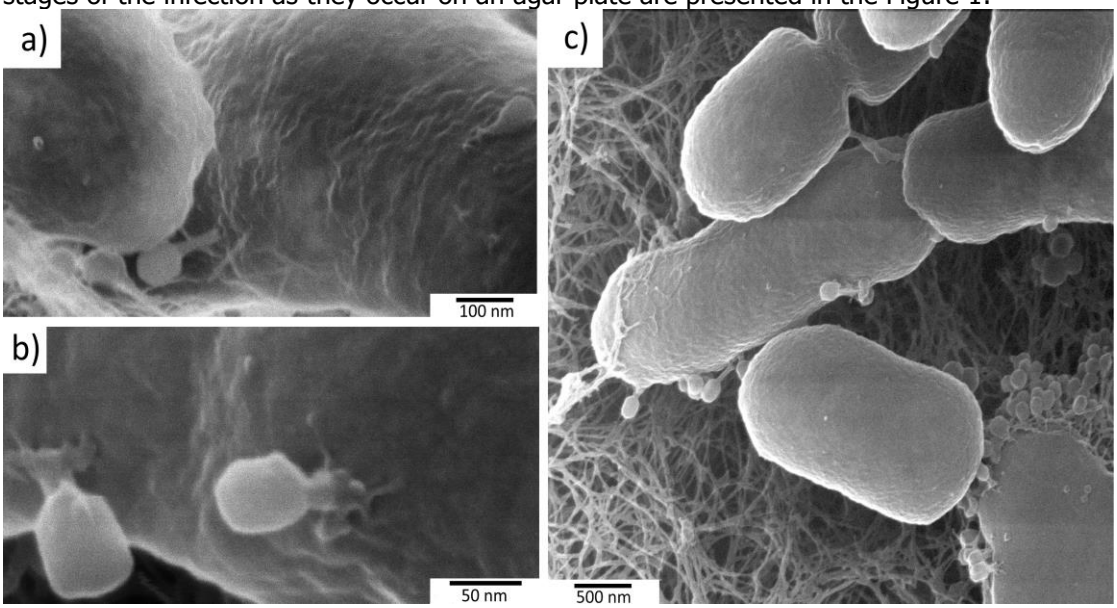

Figure 1: a) Phage attached to the bacteria surface before infection b) Phage with contracted tail indicating ongoing infection c) Burst out of new phage particles

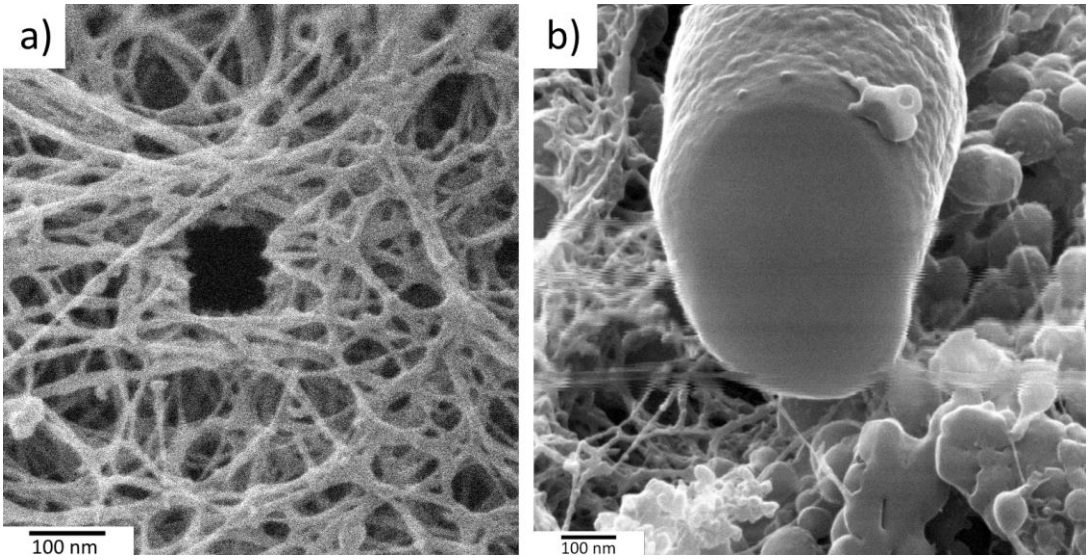

Figure 2: a) 100 x 100 nm area milled to the agar substrate b) He-milled bacteria showing up cut-off surface and a half-away cut phage particle on top of it. Milling was done from the 45 angle and imaging after 180 rotation.

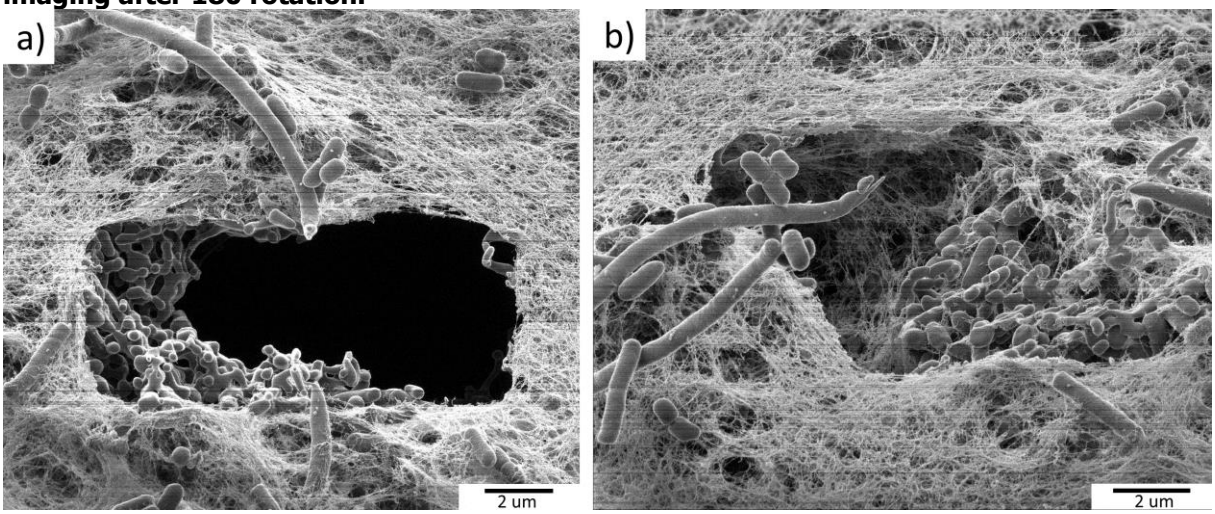

Figure 3: a) A slot milled to the agar substrate from an 45 angle with neon and imaged from 90 degree angle. b) The same milled area imaged with a 45 tilt and a 90 rotation.

Feasibility of the helium milling to reveal structure inside bacteria and bacteria colonies after cross sectioning was studied in the Figure 2. There a hole cut in the agar seen in the Fig. 2a, and cut bacteria with cut virus particle in the Fig. 2b. When using the Neon gas as the ion source, large scale milling to reveal under the surface bacteria colonies is possible as demonstrated in the Figure 3. We conclude that helium ion microscopy offers great opportunities to advance the studies of microbial imaging, in particular in the area of interaction of viruses with cells.

This work was supported Academy of Finland and Jane and Aatos Erkko Foundation. We would like to acknowledge technical guidance by K. Arstila.

References

[1] *M. Leppänen, L.-R. Sundberg, E. Laanto, G. M. de Freitas Almeida, P. Papponen, I. J. Maasilta* , Advanced Biosystems, **1**, 1700070 (2017).