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In Situ Probing Biological Structures by Combining Focused Ion Beam and Atomic Force Microscopy

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Understanding the heterogeneity of biological structures at the micro/nano scale can offer insights valuable for multidisciplinary research in cell biology and biomimicry designs. Here we propose to combine nanocharacterization tools, particularly Focused Ion Beam (FIB) milling and Atomic Force Microscopy (AFM) for probing the mechanical modulus and chemical signatures of biological structures. This proposed strategy overcomes the physical limit of AFM sampling depth, and allows probing the interior of biological samples and acquiring data previously inaccessible.

To demonstrate the proposed approach, bacterial sample *K. pneumoniae* ATCC 13883 were treated with 2 mg/L polymyxin B for 24 hrs after cultivation. The bacterial cells were sliced at grazing angle ion beam at 9.7 pA to expose the cytoplasm and nucleoid regions for AFM probing (Figure 1) [1]. Figure 2a shows the Young's moduli of the bacterial cell interior measured in rehydrated condition. A large soft region was identified in the central region, which was surrounded by stiffer materials. A higher resolution map of stiffness (Figure 2b) was obtained on the surface of the sectioned cell with ~20 nm scanning intervals. The boundary between the cell and biofilm was distinct, and a number of soft regions were present and surrounded by stiffer features which presumably to be multi-protein complexes.

Also, another study was conducted on an important sensory system: rat whisker. For an individual whisker sample, two cuts were milled by FIB first (Figure 3a). The handles of a microgripper in FIB/SEM were utilized to enter both sides of the "disk" followed by milling off the remaining attached part for free transfer (Figure 3b). The flat side of the disk sample representing the cross section of rat whisker was positioned on the substrate, and FIB platinum deposition was used to secure the sample. Figure 4a-c present the acquired AFM images of adhesion for the cortex-medulla, cortex and cuticle regions of the rat whisker interior cross section, while distinct nano/microscale morphologies could be observed. Representative distributions of modulus acquired from different regions of rat whisker interior are presented in Figure 4d, suggesting cortex medulla and cortex are softer than cuticle regions. Based on the acquired information, a complete 3D elastic modulus model could be constructed for simulating and studying the performance of rat whiskers [2].

References:
[1] B Liu, MH Uddin, TW Ng, DL Paterson, T Velkov, J Li and J Fu Nanotechnology, **25(41)** (2014), 415101.
[2] VR Adineh , B Liu, R Rajan, W Yan and J Fu, Multidimensional characterization of biomechanical structures by combining atomic force microscopy and focused ion beam: a study of the rat's whiskers. (under review)
[3] This work was performed in part at the Melbourne Centre for Nanofabrication (MCN) in the Victorian Node of the Australian National Fabrication Facility (ANFF), and Monash Centre for Electron Microscopy (MCEM).

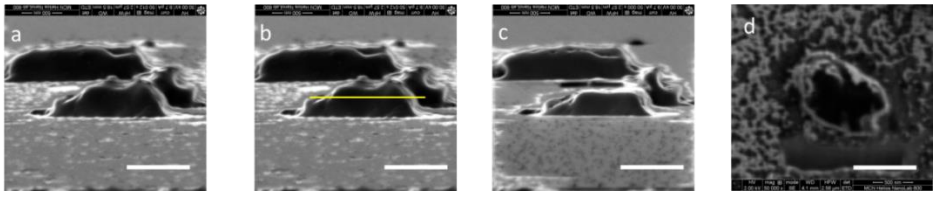

Figure 1. FIB milling of bacterial cells. (a) Tilt sample stage to grazing angle. (b) Slice the cell with FIB from the illustrated yellow line. (c) The cell after slicing with (d) top view. Scale bar: 1 μm.

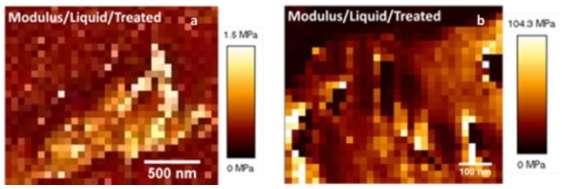

Figure 2. (a) Modulus map of bacterial cell interior and (b) with higher resolution.

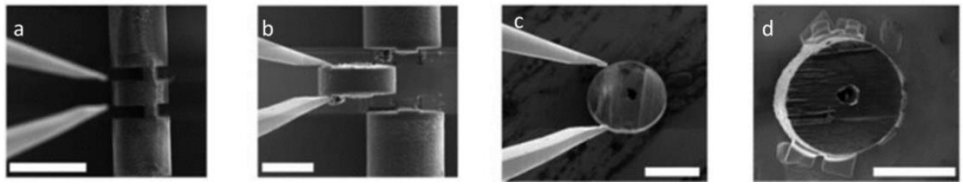

Figure 3. Retrieving and positioning rat whisker cross sections on the substrate using microgripper. The disk sample was cut off (a) and pinched (b), followed by being positioned on substrate with preferred orientation (c) and secured by platinum deposition (d). Scale bars: a) 100 μm; b)-d) 50 μm.

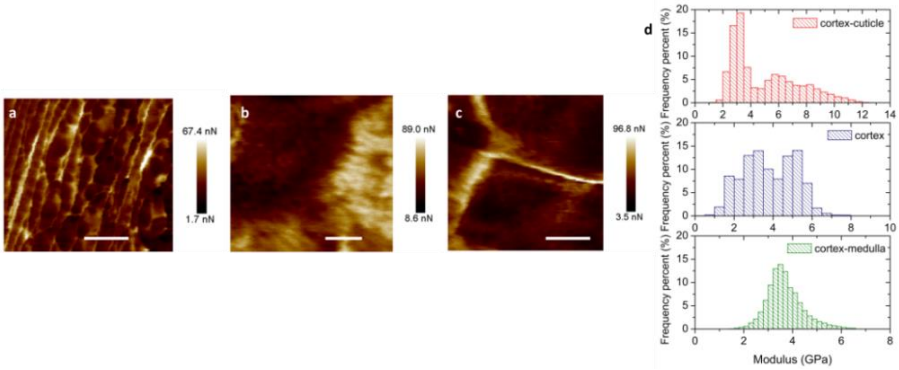

Figure 4. Adhesion maps of rat whisker cross section after FIB milling including (a) cortex-medulla, (b) cortex and (c) cuticle regions. (d) Histogram s of the modulus distributions. (scale bar: 200 nm)