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Peak force tapping AFM, state of the art tool for quantitative nanomechanical mapping

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This study highlights the capacity of Peak Force Tapping[1], a novel AFM imaging mode, for investigating nanomechanical properties of different regions in marine diatoms, providing high resolution quantitative maps. In Peak Force Tapping, the probe and sample are intermittently brought together (similar to tapping mode) to contact the surface for a short period, eliminating lateral forces. Unlike Tapping Mode, in which the feedback loop keeps the cantilever vibration amplitude constant, Peak Force Tapping controls the maximum force (Peak Force) applied by the tip. Peak Force Tapping is a technique that generates force/distance curves at each pixel Information concerning different mechanical properties (elasticity, deformation, adhesion, dissipation) can be extracted during the course of scanning the entire cell using built-in software. The data points (high resolution nanomechanical maps) have the same resolution as the topographic image.

Marine diatoms are eukaryotic, unicellular algae that are ubiquitously present in almost every water habitat on earth. Apart from diatom ecological significance, diatoms are mainly known for the intricate geometries and spectacular patterns of their silica-based walls. These patterns are species specific. Understanding of how the organic component is associated with the silica structure silica structure is providing an important insight into biomineralization process and patterning on the cellular level.

We have selected *C. closterium,* strain CCNA1, isolated from northern Adriatic Sea[2], in order to study structure and morphology of its cell wall by probing the nanomechanical properties of the two compositionally and morphologically different regions, namely valve and girdle bands, using a novel AFM imaging mode (Peak Force Tapping). The unique characteristics of *Cylindrotheca spp.* cell wall are regions (valve) which are believed to be completely unsilicified. That makes them ideal candidates for studying structure and organization of organic and inorganic domains of cell walls.

The fibulae were the stiffest (200 MPa) and the least deformable (only 1nm) cell wall region. Girdle band region appeared as series of parallel stripes and was characterized by two sets of values of Young modulus and deformation: one for silica stripes (43.7 MPa and 3.7 nm) and the other between the stripes (21.3 MPa, 13.4 nm). The valve region was more complex, with high standard deviation obtained for elasticity and deformation. Average Young modulus (29.8 MPa) and deformation (10.2 nm) did not differ significantly from the girdle band values. Complexity of *C. closterium* cell wall was further confirmed by performing acid treatment on the cells attached to mica substrate prior to AFM imaging. In the valve region we identified silica particles of approximately 15 nm that appeared to be incorporated in organic matrix. This study[3] highlights the capacity of Peak Force Tapping AFM for investigating nanomechanical properties of different regions in marine diatoms, providing high resolution quantitative maps.

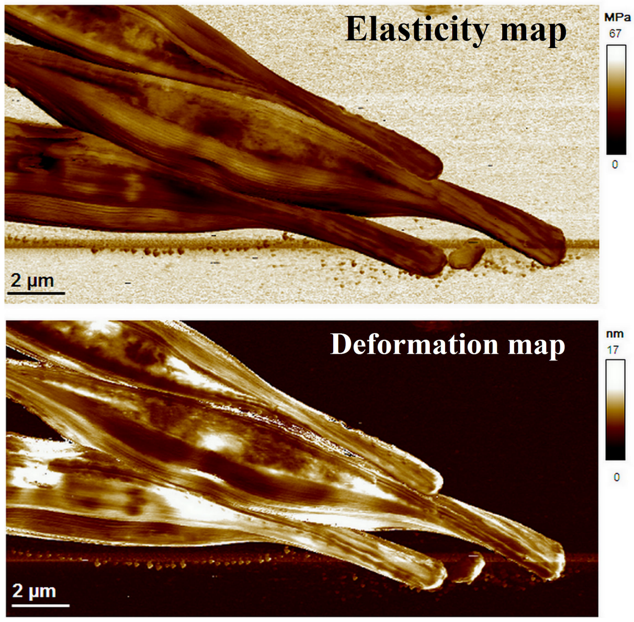

Figure 1. Nanomechanical (elasticity and deformation) maps of diatom cells.

[1] Pittenger, B., Erina, N. & Su, C. 2010. Quantitative mechanical property mapping at the nanoscale with Peak Force QNM. *Veeco Application Note* AN128, Rev. AO:1-12.
[2] Pletikapić, G., Mišić Radić, T., Hozić Zimmermann, A Svetličić, V., Pfannkuchen, M., Marić, D., Godrijan, J. & Žutić, V. 2011. Extracellular polymer release AFM imaging of extracellular polymer release by marine diatom *Cylindrotheca closterium* (Ehrenberg) Reiman& J.C. Lewin. *J. Mol. Recognit.* 24:436-445.
[3] Pletikapić, G., Berquand, A., Mišić Radić, T., Svetličić, V. 2011 Quantitative nanomechanical mapping of marine diatom in seawater, *J.Phycol.* (accepted for publication)