Optical microscopy and AFM as complimentary tools in detecting signs of life in ocean world analog samples

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Overview: Nanomechanical properties as novel biosignatures

This study is motivated by the need to recognize signs of life in ocean worlds (ice-covered water worlds) of the outer solar system, which are thought to hold the highest potential of sustaining life beyond earth. Here, we are evaluating both atomic force microscopy (AFM) and optical microscopy as tools for co-locating and detecting physical signs of life. AFM is not only capable of non-destructively mapping sample topography at nm-µm resolution but also measuring nanomechanical properties such as modulus (stiffness), adhesion, energy dissipation and motion. Beyond having potential for detecting physical signs of life beyond earth, AFM also has been coupled with various microscopy, spectroscopy and spectrometry techniques, further broadening its applications in space missions as a high spatial resolution chemical analysis instrument.

Introduction: Force data for identification of particle types

Atomic force microscopy is a tool which uses a scanning probe at the end of a microscopic cantilever (Fig. 1 & 2). Changes in the cantilever's position are tracked by a laser reflected onto a photodiode. AFM has space-flight heritage in which it was used on two missions for particle (soil and dust) imaging (NASA's Phoenix Mars Lander and ESA's Rosetta Space Probe to Comet 67P), and is a potentially viable and sensitive technique for identifying physical signs of life.

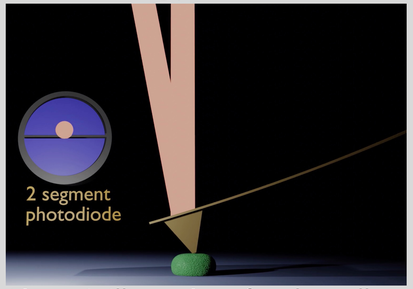


Figure 1. Illustration of probe, cell, laser and photodiode. Source: Sandor Kasas, EPFL, Switzerland

Nanomechanical data are collected by approaching, contacting and retracting the AFM probe from the sample and data are plotted in approach and retraction force curves. The combined, co-located property detection features of AFM have promising novel biosignature applications to identify potential life.

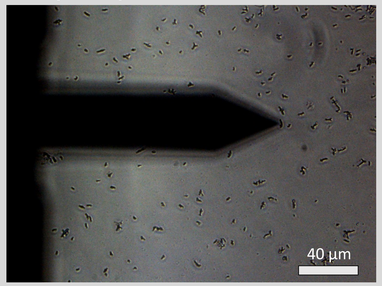


Figure 2. AFM probe on a cell sample

Methods: Six known particles, a mixture and an unknown

Different micron-scale particle types (abiotic and biotic) were prepared in aqueous suspensions (quantities adjusted for uniform coverage) and stained with DAPI (4',6-diamidino-2-phenylindole), a DNA-binding fluorescent stain (biotic-indicator), then rinsed and dried on glass substrate (Fig. 3 & 4). To avoid influencing the natural nanomechanics of cell membranes, fixatives were not used. Duplicate sample preparation allowed for oil immersion fluorescence microscopy (358 nm excitation). Following particle characterization, mixtures of the 6 particles and Antarctic glacial ice will be evaluated as an environmental unknown mix of abiotic and biotic particles and ocean world analog sample.

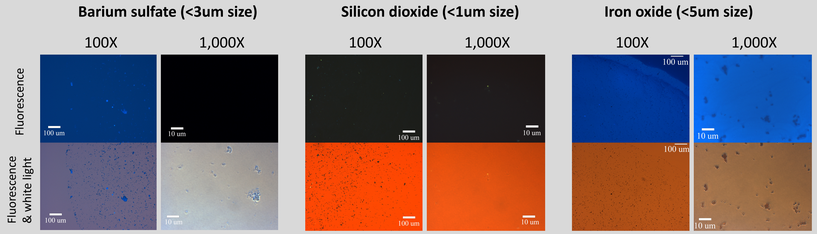


Figure 3. DAPI stained abiotic particle types at 100X & 1,000X magnification. To display abiotic particles, brightfield with fluorescence is also shown.

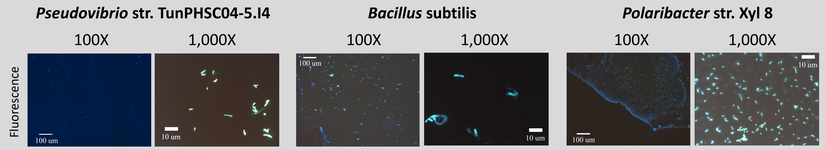


Figure 4. DAPI stained biotic particles (cells) at 100X & 1,000X magnification.

NanoWizard BioAFM (JPK-Bruker) measurements were taken using AC (tapping) mode of the cantilever across a region of particles. Individual force curve measurements were then collected across a consistent grid pattern of the region to randomize individual nanomechanical sample points (Fig. 5). Glass substrate data (confirmed by z-axis topography) act as negative control measurements.

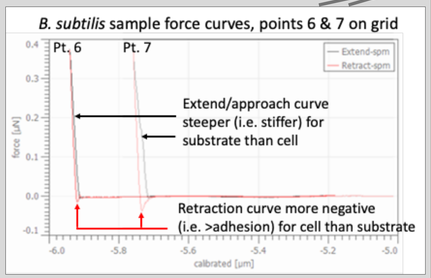
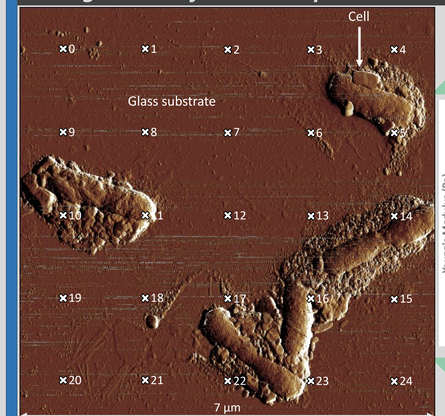
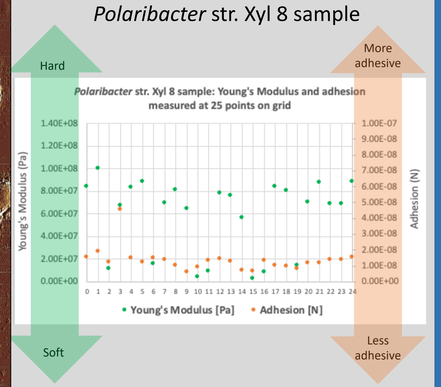


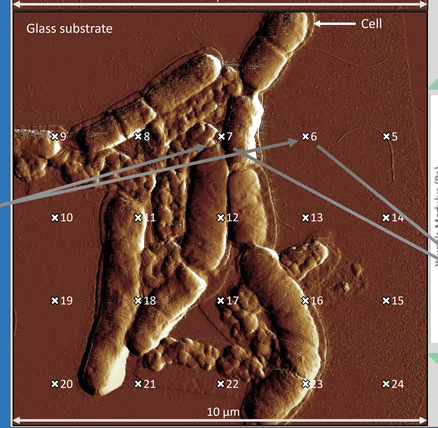
Figure 5. Approach & retraction curves

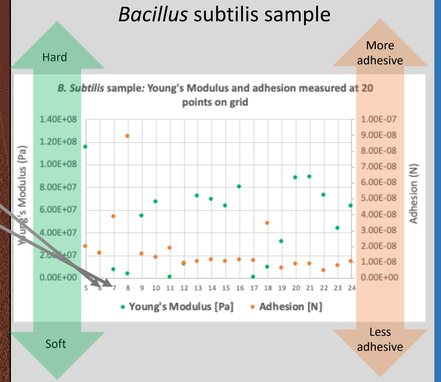
Preliminary results: *B. subtilis* and *Polaribacter* str. Xyl 8 modulus (stiffness) and adhesion.

Low modulus values (i.e. soft), approx. <2x10^7 Pa, visually correlate with biotic material (cells, extracellular polymeric substances, etc.), whereas glass substrate modulus values are higher. In this case, adhesive properties are less indicative of material type. ***We hypothesize nanomechanical properties of abiotic particles will be distinguishable from biotic particles co-located with optical microscopy.***









Acknowledgements: This material is based upon work supported by the Nevada NASA Space Grant Fellowship under Grant No. 80NSSC20M0043 to N. Ernster and the National Aeronautics and Space Administration COLDTech award#: NNZ17AF49G to A.E. Murray. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors(s) and do not necessarily reflect the views of the National Aeronautics and Space Administration. We thank S. Kasas at EPFL for use of his AFM animation.