Measuring of Electromechanical Properties of Bacteria Using Band-Excitation Piezoresponse Force Microscopy.

V. Reukov[1], G.L Thompson[1], M. Nikiforov[2], S. Jesse[2], S. Kalinin[2], A. Vertegel[1].
[1]Clemson University, Dept. Bioengineering, [2]Oak Ridge National Laboratory, Center for Nanophase Materials Science.

Statement of Purpose: Over the past years, different variations of scanning probe microscopy (SPM) have emerged as a powerful tool for imaging the surface of microbial cells under physiological conditions with nanometer resolution. Here, we used Band-Excitation Piezoresponse Force Microscopy (BE-PFM), which is a novel SPM technique in which the tip is electrically biased and the mechanical response is recorded over a band of frequencies simultaneously rather than at a single frequency as in conventional SPM's, for imaging and characterization of bacteria M. lysodeikticus and P. fluorescens under native-like conditions.

Methods: BE-PFM was performed using an Asylum Research (Santa Barbara, CA) MFP-3D Atomic Force Microscope (AFM) with an in-house developed MATLAB/LABVIEW data acquisition and control system. The MFP-3D tip holder allowed the tip to be directly biased in liquid. Measurements were performed using Au-coated SiN tips (Olympus TR800PB, 40 nm nominal tip radii).

Bacteria samples, M. lysodeikticus (Sigma-Aldrich # M3770) were grown in Trypticase Soy Broth (BD # 211768) for 24 hrs at 30°C, P. Fluorescens (ATCC # 11150) were grown in Difco™ Nutrient Broth (BD # 234000) for 24 hrs at 30°C. The bacteria suspension was purified by centrifugations at 1500×g for 5 minutes and then resuspended in 3 mL of Millipore water. For bacteria immobilization we used poly-L-lysine (PLL, Sigma-Aldrich #P4707) coated mica – 50 µl of sterile 0.01% PLL solution was dried-out on freshly cleaved mica (EMS #71851-05) at room temperature. Then 50 µl of bacteria suspension was adsorbed on PLL-coated mica for 15 minutes, followed by washing with copious amounts of Millipore water. Imaging was performed in 1 mL Millipore water in a static fluid cell.

Results/Discussion: Topography, PFM amplitude and PFM phase imaging of bacteria was performed using AC-voltage with 10 Vpeak-to-peak amplitude and frequency 160 kHz. Strong response from the bacterial contour in PFM amplitude and phase was observed (Fig.1). In single frequency PFM it is difficult to differentiate piezo-response of the material from topographical cross-talk. BE-PFM provides us an experimental method for decoupling topographical cross-talk from piezo-properties of the materials. There are four outputs in BE-PFM: piezoresponse amplitude, resonance frequency, piezoresponse phase and quality factor (Q). For M. lysodeikticus (Fig. 2) and P. fluorescens in Millipore-grade water, the resonance frequency decreases on the bacteria compared to the mica substrate, as would be expected for the softer biological material. The piezoresponse amplitude is higher upon the bacteria than on the mica.

Here, a novel technique BE-PFM was applied for imaging of bacteria M. lysodeikticus and P. fluorescens in aqueous media. Outstanding contrast on PFM phase and amplitude image has been found. Application of BE-PFM helped to avoid topographical cross-talk. Piezoresponse Force Microscopy (PFM) has been developed to the point that biological materials may be imaged in liquids and result in qualitative evaluation of biological material properties, especially by utilizing BE-PFM. The biological materials that have been imaged range from protein fibers to living cells. Further developments towards quantitative analysis of the data lie in the plane of separating mechanical contributions from the electromechanical response to the signal.

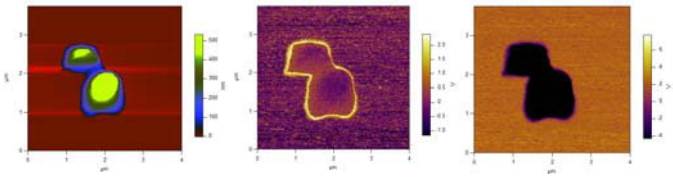

Figure 1. Topography and PFM amplitude and phase images of *Micrococcus lysodeikticus*. Scan size is 4 um x 4 um. Imaging was performed at 10 V_{app} with a frequency 160 kHz.

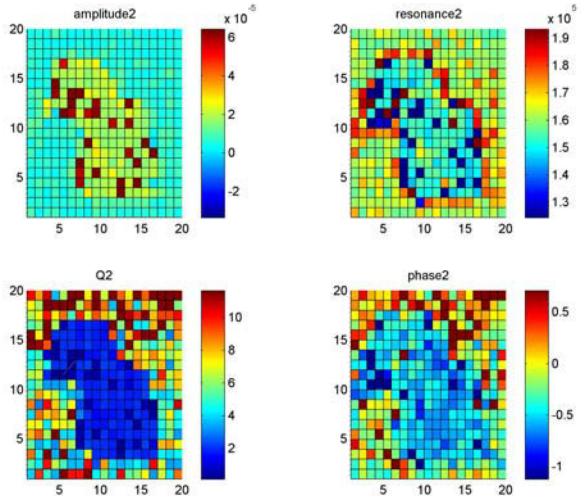

Figure 2. BE-PFM images of *Micrococcus lysodeikticus*. Scan size is 2.4 um x 2.4 um with 120 nm steps. Imaging was performed at 10 V_{app} with a 320 kHz bandwidth centered at 160 kHz.

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