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Electromechanical and Elastic Probing of Bacteria in Cell Culture Medium

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Abstract

Rapid phenotype characterization and identification of cultured cells, which is needed for progress in tissue engineering and drug testing, requires an experimental technique that measures physical properties of cells with sub-micron resolution. Recently, band excitation piezoresponse force microscopy (BEPFM) has been proven useful for recognition and imaging of different types of bacteria in pure water. Here, the BEPFM method is performed for the first time in physiologically-relevant electrolyte media, such as Dulbecco's phosphate-buffered saline (DPBS) and Dulbecco's modified Eagle's medium (DMEM). Distinct electromechanical responses for *Micrococcus lysodeikticus* (Gram-positive) and *Pseudomonas fluorescens* (Gram-negative) bacteria are demonstrated in DPBS. The results suggest that mechanical properties of the outer surface coating each bacterium, as well as the electrical double layer around them, are responsible for the BEPFM image formation mechanism in electrolyte media.

Keywords

bacteria; electromechanical coupling; double layer; PFM; SPM

1. Introduction

The use of atomic force microscopy (AFM) for measurement of functional properties of biological systems (*e.g.*, expression of surface moieties, mechanotransduction, electromechanical coupling, etc.) is rapidly expanding. AFM-based techniques allow not only measurements of macroscopic properties of biological objects, but also provide an opportunity to study those properties at the molecular level [1–3]. Previously, AFM has been utilized for quantifying passive mechanical properties of soft biological matter [4–7], active mechanochemical coupling within cells [8–12], and for probing the physiological role of electromechanical coupling of whole cells on the macroscopic scale [13–15]. Force-distance methods are well-established for determination of mechanical properties, typically by fitting a Hertzian model for elasticity to the force curve. However, when force is measured via quasi-static deflection, there is a lack of sensitivity of smaller forces, and mapping is time-consuming and lacking in pixel resolution. Oscillatory techniques are more sensitive to small changes in deflection than static ones and usually are quicker for the same resolution. Several implementations of oscillatory techniques for mechanical properties measurements

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exist, for example, HarmoniX and Peak-force from Veeco Instruments (now Bruker-AXS, Inc.) [16]. Both of those techniques suffer from ambiguity of data processing when mechanical properties of materials are calculated from measured contrast.

The other option of utilizing oscillatory technique is to measure mechanical response to electrical stimulus. This technique is called piezoresponse force microscopy (PFM). Recently, attempts to resolve differences in responses within the cell were made using single-frequency resonance piezoresponse force microscopy (PFM) [17]. Although PFM images of a culture of breast cancer cells showed contrast in the piezoresponse phase, there was a lack of contrast in the piezoresponse amplitude. Thus, the origin of the PFM contrast (piezoelectricity associated with the cells, difference in mechanical properties of the cells, etc.) could not be determined. The studies described above were done in cell culture media, which potentially facilitated a weakened response because of associated double layers. However, contrast in phase and not in amplitude suggests convolution of the signal by the topological and mechanical properties of the cells.

Capturing all parameters of tip-surface resonance (amplitude, frequency, quality factor and phase) helps to distinguish between materials contrast from variation in piezoelectric constants and from variation in contact mechanical properties [18]. Band excitation piezoresponse force microscopy (BEPFM) is one of the most convenient methods for such analysis and has been applied, for example, to characterization of nanoscale domains in ferroelectric thin films [19, 20]. In this paper, we use BEPFM to study the nature of electromechanical responses of bacteria (*Micrococcus lysodeikticus* [*ML*] and *Pseudomonas fluorescens* [*PF*]) adhered to a poly-L-lysine (PLL)-coated mica substrate in a physiologically relevant, electrolyte-containing cell culture medium, and to demonstrate distinct responses among living Gram-positive (*e.g.*, *ML*) and Gram-negative (*e.g.*, *PF*) bacteria and damaged bacteria, suggesting the application of BEPFM for Functional Recognition Imaging (FRI) [21, 22]. AFM-based FRI techniques provide alternative, *in situ*, single-cell identification methods based upon tip-cell interactions (*e.g.*, electromechanical, elastic, chemical, etc.) to the traditional fluorescence-based methods (*e.g.*, fluorescence-activated cell sorting, FACS [23]) that rely upon exogenous markers and can require perturbation of the cells. In the case presented herein, identification of cells is based on electromechanical and mechanical properties of the surface coating, which will provide information relevant to the impact of environmental and exogenous factors on the surface coating and cell integrity in future studies.

2. Results and discussion

In order to distinguish bacteria based on their electromechanical signature, by an understanding of the contrast formation mechanism in BEPFM experiments, a typical distribution of electromechanical properties for each type of bacterium is required. Thus, our studies of electromechanical response of *ML* and *PF* deposited on PLL-coated mica were structured as following:

1. Contrast formation mechanism during BEPFM experiments was derived from single-point frequency spectra on *ML* and *PF* in different media (water, Dulbecco's modified Eagle's medium (DMEM), and Dulbecco's phosphate-buffered saline (DPBS)) [section 2.1.].
2. Distribution of electromechanical parameters (amplitude, resonance frequency and quality factor) was measured for *ML* and *PF* in different media (water, DMEM and DPBS) [section 2.2.].
3. Identification of the bacteria was performed based on electromechanical signature in DMEM.

2.1. BEPFM image formation mechanism in electrolyte media

BEPFM spectra were measured by repeated scanning of a single line (slow scan disabled, Supp. Fig. 2) and spectra were averaged over an area on *ML*, *PF* and *PLL*. The amplitude of electrical excitation changed from 10 V to 100 V, and dependence of the Fourier-transformed, frequency-domain electromechanical response spectra on the voltage applied from the tip were recorded for Millipore water ($18 \text{ M}\Omega\cdot\text{cm}$) and electrolyte media, DPBS and DMEM. In water one contact resonance peak on *PLL* appears at $\sim 38 \text{ kHz}$. The other contact resonance peak develops around 100 kHz at 60 V ; then it shifts to lower frequencies as the tip bias increases (Fig. 1b). Previous considerations of the dynamics of PFM as a contact mode method [20, 24–26] discuss how the resonance frequencies are independent of relative electrostatic and electromechanical effects while providing information on voltage-dependent, local elastic properties of the sample (via contact stiffness) and on topography. The overall positive charge of *PLL* in water at the pH of the experiment results in a large Debye length with a highly diffuse electrical double layer (EDL), which permits an increase in probing volume with increasing bias, and a softening of the contact. Stiffness of probe – sample contact correlates with EDL thickness in two other solvents (DMEM and DPBS) used in the experiment (Fig. 1e, 1h). As EDLs shorten and contact stiffens with increasing ionic strength, DMEM exhibits the next highest f of $\sim 60 \text{ kHz}$, and f is $\sim 70 \text{ kHz}$ in DPBS (Fig. 1e, 1h; Table 1).

The major peak on the *ML* bacterium for all three liquid environments appears near the resonance frequency of 50 kHz , and all peaks are broad, spanning 20 to 100 kHz . Applying Gaussian fits to peaks smoothed by adjacent averaging of 75 points (as in Fig. 1) provided maximum amplitudes, which are plotted against applied biases for *ML* bacteria (Fig. 2), revealing a quadratic increase in amplitude with increasing bias in water, and linear relationships of response amplitude with bias in DPBS and DMEM (Fig. 2 zoomed areas). In water, the nonlinear relationship of amplitude with voltage occurs because of electrostriction [27] and variations in electromechanical dissipation [28]. The linear relationships for DPBS and DMEM indicate that the predominant contribution to the signal comes from electromechanical coupling in the system. Although the linearity of response versus bias is similar for DPBS and DMEM, the differences in the respective spectra in different electrolyte solutions indicate that the EDL is responsible for a portion of the response signal, while similarities in amplitudes and peak resonances emphasize the contributions from the dynamical properties of the cantilever-tip-sample system.

Electromechanical response of the *ML* bacterium on *PLL* is a convolution of the *ML* bacterium's response to electrical excitation, the *PLL* response to electrical excitation, and the tip transfer function (*i.e.*, system dynamics). In liquid environments, the tip transfer function is very complicated, and the ratio of the convoluted response (assuming multiple layers of *ML*-with-*PLL*) to *PLL* electromechanical responses should be, to the first order approximation, independent of the tip transfer function. Also, taking the ratio of *ML*-with-*PLL* to *PLL* electromechanical responses removes the multiplicative component of the *PLL* response from the spectrum. Such a ratio shows that in DMEM, the *ML*-with-*PLL* resonance curve is independent of excitation amplitude within a wide range of the higher voltages. Such behavior suggests an absence of electromechanical response from *ML*, and image formation is dominated by the mechanical transfer function of *ML*. In water and DPBS, a convolution of electromechanical and mechanical properties of the *ML* bacterium is measured. Thus, the electromechanical response of *ML* changes depending on the solvent (Fig. 1a, d, g). This solvent dependency becomes even more pronounced (Fig. 1c, f, i) when there is compensation for the electromechanical response of *PLL*.

2.2. Electromechanical contrast between bacteria and PLL substrate in electrolyte media

Fitting a damped harmonic oscillator (DHO) model to the spectra from each voxel of the BEPFM data set generates maps of four parameters: peak amplitude (A), resonance frequency (f), quality factor (Q), and phase (ϕ) [29]. The frequency ranges of the peaks that were fit are given by the ranges of the color bars in the figures. Each of the four maps from BEPFM show contrast between an isolated *ML* cell and the underlying PLL-coated mica in the three liquid environments tested: water (Fig. 3a), DPBS (Fig. 3b), and DMEM (Fig. 3c). The amount of contrast is highest in water, lower in DMEM, and lowest in DPBS. It is noted here that contrast decreases yet is maintained at lower applied biases, and similar results have been obtained with other AFM tips and separately-prepared samples of the same type of bacteria on PLL-coated mica in the same conditions (ref. [9] and data not published). The ionic strength, pH, osmolarity and main components of DPBS and DMEM are presented in Supplemental Table 1. Although the inorganic components and ionic strengths for DPBS and DMEM are similar, DMEM contains a host of organic components, mainly glucose and including various amino acids and vitamins, which can affect the biological activity of the bacteria [30, 31] as well as BEPFM image formation. The contrast observed in DHO fits of BEPFM images can consist of contributions from the electromechanical properties of the sample material (per the discussion in Section 2.1), from the EDLs present between the tip and sample [18, 32], from viscous effects of the liquid on the cantilever [4], and from heterogeneity of mechanical properties within varying tip-surface contact area [24, 33], especially at the edges between the bacteria and substrate surface.

2.3. Comparison of Gram-positive and Gram-negative bacteria

One goal of this paper is to distinguish Gram-positive (e.g., *ML*) and Gram-negative (e.g., *PF*) bacteria based on electromechanical response, for the first time in biologically relevant media, which is accomplished by comparison of response spectra. *ML* and *PF* demonstrated distinctive BEPFM spectra while the PLL surface adjacent to each bacterium elicited similar responses, especially frequency responses. The results shown (Fig. 4, again normalized to PLL as in Fig. 1c,f,i) were obtained at 50 V. The spectra from water and DPBS that are presented were obtained with slow scan disabled and were averaged from five successive lines (as in Supp. Fig. 2). Averaged areas did not include edge effects, which arise from rapid transitions in tip-surface contact mechanics and are observed especially in maps of f . Results for DMEM were obtained from a single image as discussed in the next paragraph. Immediately evident in Figure 4 are some differences in the frequency domain. In water, *ML* has a f around 60 kHz, whereas *PF* has a peak centered about 80 kHz. DPBS attenuates f for *ML* more than for *PF*, while the relative amplitude decreases more for *PF*. Because the amplitude-frequency spectra in water and DPBS are a convolution of electromechanical and mechanical properties (Section 2.1), the amplitude change suggests that the more negatively charged *PF* experiences more shielding from the EDL in DPBS, yet retains stiffer contact than for *ML* in DPBS per the shift in f . Although most types of bacteria demonstrate relatively long-term survivability in water and PBS [34], *ML* and *PF* are non-spore-forming bacteria that tend to convert into dormant, cyst-like forms with altered ultrastructures [35, 36], whereas in DMEM similar bacteria maintain a natural form for a longer time because of the presence of glucose in the solution. Also, the possibility of distinguishing between *ML* and *PF* in pure water has already been shown by Nikiforov *et al.* [21]. Here, large scale mapping of co-seeded *ML* and *PF* bacteria was performed in DMEM.

Capturing both *ML* and *PF* within the same image in DMEM allows for direct comparison between the cells in the electrolyte culture medium (Fig. 5). The AFM height image confirms that the bacteria speculated to be alive are 400 nm to 600 nm tall, as expected (Fig. 5a). Bacteria that appear damaged or dead (*D-PF*) are clearly discerned in the deflection image (Fig. 5b), which also contains what seems to be *PF* reproducing and undergoing

binary fission in the upper left corner. Groups of *ML* are in the center of the image, while a *ML* and a *PF* adjacent to one another are in the lower left corner. Figure 5c shows the first principal component of the BEPFM amplitude image, which again shows contrast between bacteria and the PLL surface. Principal component analysis (PCA) of the BE data [37] serves as the initial analytical step in performing artificial neural network analysis (ANN) for functional recognition imaging (FRI) [21] of each type of bacteria on the PLL. However, FRI using ANN analysis does not distinguish readily between intact *ML* and *PF*. Functional recognition of bacteria also is possible by fitting with a DHO model to the two peaks within the spectra, resulting in two maps each of amplitude, f and Q (Supp. Fig. 3b). By selecting several spectra from voxels on intact *ML*, intact *PF*, D-*PF* and PLL (Fig. 5d), average bacteria spectra divided by the PLL spectrum are shown to differ mainly in amplitudes, especially for the speculatively damaged cells (Fig. 5e). Histograms compiled for each response parameter (Supp. Fig. 3a) show that the distribution of responses is similar for *ML* and *PF*, yet it is apparent that damaged cells and PLL have distinguishing responses. Notably, each spectrum in DMEM exhibits similar resonances and peak shapes (Fig. 5e), suggesting that any electromechanical response is attributable mainly to the constituents of the liquid environment and similar EDL interactions, while the majority of the response depends upon mechanical transfer function of the system, as investigated further in the next section.

2.4. Effect of contact mechanics on BEPFM of bacteria in electrolyte solution

Changing applied force by means of the deflection set point in DMEM on *ML* during slow-scan disabled BEPFM (Fig. 6) supports the interpretation that electromechanical response is weakly dependent upon contact area mechanics. Again, *ML*-with-PLL spectra are divided by PLL spectra. Off-surface, the spectrum loses observable, dominant peaks (Supp. Fig. 4), thus confirming the limits of BEPFM at low biases as a contact mode technique. An effect from indentation is observed by the values of amplitude for each step in applied force. At increased strains [38] and greater deflection values of 162 nm (9.26 nN) and 204 nm (11.6 nN), relative amplitudes decrease with the increased contact stiffness, whereas at lower indentation forces when contact is still retained (at 0 nN to -0.468 nN), the relative amplitudes increase. When retracting from the surface in the attractive regime at -2.32 nN, which was much lower than the contact set point during approach, the response of *ML* is relatively the same as for PLL. At the contact point where the force is -0.926 nN, the relative response is largest, consistent with electrostatic coupling in an EDL [32]. Although resonance peaks do not change noticeably, a minor contribution from mechanical properties of the samples and from stiffening of the contact [24] or surface penetration upon increased indentation cannot be discounted without a complete consideration of the complex, heterogeneous mechanical, dielectric and piezoelectric properties of the systems.

The relative structures of Gram-positive (*e.g.*, *ML*) and Gram-negative (*e.g.*, *PF*) bacteria cell walls (Supp. Fig. 5) provide insight into the results presented here; furthermore, results obtained with other methods in the literature qualitatively agree with the preceding analysis of BEPFM data. Using electrophoretic mobility, Sonohara *et al.* [39] found Gram-positive *S. aureus* to possess a surface that was less rigid and less negatively-charged than Gram-negative *E. coli*. However, Vadillo-Rodriguez *et al.* [40] performed AFM force-curve creep relaxation experiments to show that the entire Gram-positive *B. subtilis* cell is stiffer and more viscous than an *E. coli* cell, in accordance with the relatively thicker peptidoglycan cell wall layers of the Gram-positive bacteria (Supp. Fig. 5a). Our data from BEPFM in liquids of differing ionic strengths corroborates with the surface characteristic results of Sonohara *et al.* in that the Gram-positive *ML* displays a lower f in water and DPBS suggesting a less rigid surface. The smaller changes in f for *PF* in different liquids are attributed to the stiff lipopolysaccharide (LPS) layer on their surface, as compared to the

more compliant, charged “brushy” coating of *ML* that makes it more susceptible to changes in overall cell stiffness with changes in ionic strength of the liquid because of turgor pressure [41]. Decreases in amplitudes with increasing ionic strength, if normalized to an average PLL spectrum, are greater for *PF* than *ML*, consistent with the more dramatic screening of the more negatively charged *PF*. In DMEM, the spectra for each type of cell are dominated by the tip transfer function and are nearly the same because the organic components of DMEM can coat the tip and interact strongly with the surface molecules of the bacteria.

For comparison, a quasi-static force-volume map of Young’s modulus for the same group of bacteria in DMEM with the same tip was acquired at different force set points (Fig. 7). Although each map took approximately the same amount of time to acquire as the BEPFM image (~10 min.), the resolution is obviously lower. This makes identification of the bacteria based on force-distance data difficult. The Young’s modulus quantity can be calculated from force curves given accurate knowledge of contact geometry and Poisson’s ratio of the sample, yet this requirement cannot be met here because the contact locations on each bacterium cannot be discerned (*e.g.*, the tip could be interacting with the side of a cell) and Poisson’s ratios are generally unknown. However, the apparent Young’s modulus values calculated by the Hertzian model (assuming a Poisson’s ratio of 0.5) of approximately 0.5 MPa to 1.2 MPa are reasonable for the high strain rates of 50 $\mu\text{m}/\text{sec}$ and the large strain gradients induced by a sharp tip [38] and fall within a range seen in the literature [42, 43]. Also as expected, the apparent Young’s moduli of the bacteria increased as the tip delved closer to the underlying PLL surface. Yet, more accurate results require better resolution of the probed locale, and imaging techniques such as multi-harmonic AFM [44] and BEPFM, which are based upon different physical mechanisms and offer unique information sets, can provide this complement.

3. Conclusions

The application of BEPFM in liquid electrolyte media is demonstrated for the first time. In a previous publication we utilized BEPFM in pure water to perform FRI of Gram-positive *ML* and Gram-negative *PF* on the same PLL-coated mica substrate using PCA of BEPFM images and ANNs [21]. Here, we show that *ML* and *PF* can be distinguished readily using a similar approach in physiological electrolyte solution (DPBS) but not in cell culture media (DMEM). Supplemental explanation of the basis for functional recognition is gleaned from this study using different liquid media. Comparative responses observed during electromechanical imaging of the two types of bacteria are presented in the amplitude-frequency spectra from BEPFM and are consistent with differences in surface elasticity and associated electrical double layers (EDLs) of the cells, as caused by differences in their cell wall composition and structure (Supp. Fig. 5). The semi-crystalline order of the charged, outer lipopolysaccharide layer of *PF* imbues a greater resistance to liquid flow near its surface compared to that of *ML*. These structural properties are evidenced in the BEPFM data by the lower resonance frequency of *ML* in water and DPBS. Based on analysis of BEPFM image formation mechanisms in water, DPBS and DMEM, electrostriction dominates the responses in water above 50 V, whereas the EDLs on the tip and surface dominate the response in the water below 60 V and in electrolyte media at all voltages. The EDLs in electrolyte media exhibit shorter Debye lengths, on the order of ~1 nm, and alleviate adverse electroosmotic flow and allow for closer tip-surface interaction, although the amplitude of the electromechanical response is reduced in large part because of the double layers [18]. Future investigations will benefit from detailed analysis of EDLs using BEPFM, which can then be combined with other AFM modes and instrumentation to better characterize active cellular responses.

Experiment

Band excitation instrumentation

Line-mode Band Excitation PFM was implemented 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. During the trace raster, a constant-voltage excitation band ranging approximately 16.5 – 145 kHz (increasing chirp) was applied to the microscope tip at each voxel; each BEPFM data set consists of 128×256 voxels. The excitation voltage amplitude was amplified by a factor of ten (FLC Electronics F10A voltage amplifier) to a maximum of 100 V. The mechanical response of the system was recorded by measuring and digitally storing the motion of the tip, taking the Fourier transform of the response during the retrace raster. The amplitude, resonance frequency, phase, and quality factor were extracted from individual voxels using a damped harmonic oscillator model. A gold-coated, pyramidal tip (Olympus, TR400PB) with a measured stiffness of 0.057 N/m and an inverse optical lever sensitivity (InvOLS) of 42 nm/V in the aqueous solutions was used for the experiments [45]. The cantilever had a triangular shape with 13.4 μm width of each leg, 100 μm total length and 0.4 μm thickness.

Sample preparation

Gram-positive bacteria *M. lysodeikticus* (Sigma-Aldrich # M3770) and Gram-negative bacteria *P. fluorescens* (ATCC # 11150) were grown in Trypticase Soy Broth (BD # 211768) and Difco™ Nutrient Broth (BD # 234000), respectively, for at least 24 hrs at 37°C. The suspensions of bacteria were purified by centrifugations at $1000\times g$ for 5 minutes, and then the bacteria were resuspended in Millipore water. For bacteria immobilization we used mica coated with poly-L-lysine (PLL, Sigma-Aldrich #P4707) – 50 μl of a sterile 0.01% PLL solution was incubated on freshly cleaved mica (EMS #71851-05) at room temperature for 30 minutes and then washed with Millipore water. Then 50 μl of each suspension of bacteria in water was adsorbed simultaneously onto adjacent spots on the PLL-coated mica for 15 minutes, followed by washing with copious amounts of Millipore water.

Experimental setup for imaging

A closed fluid cell (Asylum Research) consisting of a round glass slide screwed into a poly(ether ether ketone) PEEK holder with a Viton rubber O-ring was used for all imaging. The mica sample was epoxied onto the glass slide, and 2.5 mL of liquid was placed into the fluid cell. The metal stage was separated from the tip-electrode by approximately 1.5 cm of Millipore water (18 $\text{M}\Omega\cdot\text{cm}$) and 4 mm PEEK (10^{10} $\text{M}\Omega\cdot\text{cm}$ [46]).

Liquid imaging

The same sample and same tip were used for acquisition of all the data presented here except for in Figure 1b (a full BEPFM image set of *M. lysodeikticus* in DPBS), for which the same type of sample and tip were used. Slow scan disabled voltage steps in DPBS used the same sample and tip as all other data. BEPFM responses are typically similar for multiple cells of the same type imaged with the same tip, as averaged spectra from a group of *ML* on PLL in water exhibit similar variation to that of a single *ML* bacterium in water. Repeatability of BEPFM in liquid was revealed by slow scan disabled images of *ML* in water, which retained characteristic spectra with repeated scanning, although some instrumental drift or movement of the soft sample resulted in large standard deviations across any single column of voxels. The bacteria sample was prepared three days prior to BEPFM imaging in Millipore water. For the first 24 hours, the bacteria were immobilized on the PLL surface in Millipore water, and for the next 48 hours they were in Dulbecco's

phosphate buffered saline (DPBS). Then, immediately before imaging in the open fluid cell, the sample was washed with copious amounts of Millipore water and imaged in 2.5 mL Millipore water for several hours. The same day the sample was rinsed with copious amounts of DPBS and imaged in 2.5 mL DPBS for 1.5 days, after which the sample was rinsed with copious amounts of Dulbecco's modified Eagle's medium (DMEM) and imaged for 2 days in 2.5 mL DMEM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Amplitude-frequency spectra representing electromechanical responses were averaged over the separate areas of voxels for *M. lysodeikticus* (a,d,g), PLL (b,e,h), and the ratio of ML to PLL (c,f,i) in water (a,b,c), DPBS (d,e,f), and DMEM (g,h,i) during steps in applied bias.

The applied voltages are designated by the colors as follows: 100 V – black, 90 V – red, 80 V – blue, 70 V – magenta, 60 V – green, 50 V – dark blue, 40 V – violet, 30 V – cyan, 20 V – brown, 10 V – dark yellow.

Fig. 2.

Applied bias versus BEPFM response amplitude at resonance frequency of Gaussian fits of the peaks for *M. lysodeikticus* in water (■), DMEM (●, red) and DPBS (▲, blue) and for PLL in DMEM (★, cyan) and DPBS (◆, green). The zoomed inset shows a linear relationship for DPBS and DMEM.

Fig. 3.

Simple harmonic oscillators (SHO) fit to the spectra of each voxel of BEPFM data form an image displaying contrast between different *M. lysodeikticus* bacteria and the underlying PLL-coated mica substrate in (a) water, (b) DPBS, and (c) DMEM. Fits were performed on peaks of 54 to 60 kHz at 50 V for water, 60 to 80 kHz at 100 V for DPBS, and 46 to 48 kHz at 100 V for DMEM. The images have dimensions of 2.5 μm by 2.5 μm in water and 3 μm by 3 μm in DPBS and DMEM. AFM images acquired simultaneously with BEPFM data show morphologies as observed in the BEPFM images, and the bacteria are 400 nm at maximum height in water and 600 nm maximum heights in DPBS and DMEM.

Fig. 4. Comparison of electromechanical response spectra in water (a) and DPBS (b) of each bacterium and their PLL substrate is presented by dividing the former by the latter.

Fig. 5.

M. lysodeikticus (ML), *P. fluorescens* (PF), and speculatively damaged or dead PF (D-PF) on PLL in DMEM within the same area and image are shown in the trace (a) height, (b) deflection, and (c) 1st principal component of the BEPFM amplitude channel. Note that there is a PF cell undergoing cell division in the upper left of the image. The spectra from selected areas on each type of bacterium, ML, PF and D-PF, were averaged and smoothed using adjacent averaging over 75 points. The averaged spectrum for ML (■, black), PF (●, red), and D-PF (◆, green) was divided by the averaged spectrum for PLL (e), all from selected areas as indicated in

Figure 6.

Steps in applied force from -2.32 nN to 11.6 nN on ML divided by PLL in DMEM were performed an excitation amplitude of 100 V with slow-scan disabled and result in the spectra shown in (a).

Fig. 7.

Force volume maps of *M. lysodeikticus* (*ML*), *P. fluorescens* (*PF*), and speculatively damaged or dead *PF* (*D-PF*) on PLL in DMEM – the same as in Figure 5 – are calculated using the Hertzian model on the approach curve with an force set point of (a) 35 nm or 2 nN and (b) 165 nm or 10 nN. At lower set points, less contrast between bacteria and the PLL surface was observed.