Research Article

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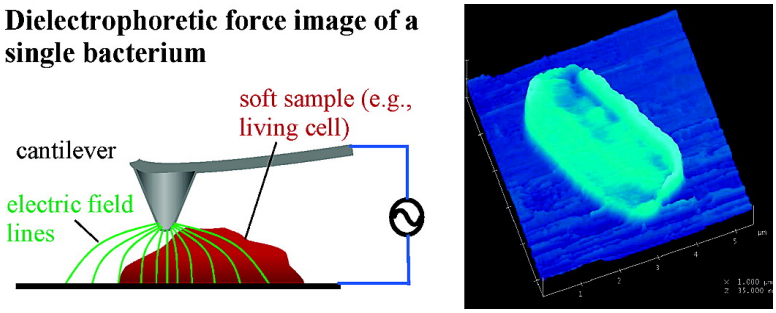
Langmuir, **2005**, 21 (4), 1436-1440• DOI: 10.1021/la0484366 • Publication Date (Web): 19 January 2005



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Received June 24, 2004. In Final Form: November 1, 2004

A novel scanning probe microscopy technique has allowed dielectrophoretic force imaging with nanoscale spatial resolution. Dielectrophoresis (DEP) traditionally describes the mobility of polarizable particles in inhomogeneous alternating current (ac) electric fields. Integrating DEP with atomic force microscopy allows for noncontact imaging with the image contrast related to the local electric polarizability. By tuning the ac frequency, dielectric spectroscopy can be performed at solid/liquid interfaces with high spatial resolution. In studies of cells, the frequency-dependent dielectrophoretic force is sensitive to biologically relevant electrical properties, including local membrane capacitance and ion mobility. Consequently, dielectrophoretic force microscopy is well suited for in vitro noncontact scanning probe microscopy of biological systems.

Introduction

First defined by H. A. Pohl, dielectrophoresis (DEP) describes the force on a polarizable particle within a nonuniform electric field.[1] The net DEP force along the direction of the field gradient is attractive for a particle that is more polarizable than the medium in which it is suspended, and vice versa. The time-averaged dielectrophoretic force (F_{DEP}) on a particle of volume V can be summarized in the following equation:[2]

$$\langle F_{\mathrm{DEP}}(\omega)\rangle = ({}^3/_2)\epsilon_1 V\,\mathrm{Re}[K(\omega)]\nabla E^2 \qquad (1)$$

In eq 1, ϵ_1 is the real part of the permittivity of the surrounding medium, V is the volume of the particle, Re[$K(\omega)$] is the real component of the Clausius−Mossotti factor (K) of the system as a function of the alternating current (ac) frequency (ω), and E is the root-mean-square (rms) magnitude of the ac electric field.

DEP has a rich history of use in characterizing and sorting cells and biological molecules.[3−6] The DEP forces acting on a biological system are related to electrical properties such as membrane capacitance and surface charge.[2] Consequently, DEP-based techniques have been developed for separating different species of bacteria[7] and for separating viable yeast cells from nonviable yeast cells.[8] The dielectrophoretic properties of cells and viruses can also change significantly upon exposure to chemicals that activate biochemical changes, including drugs to induce apoptosis,[9] toxicants,[10] and K^+ channel ionophores.[11] Recent studies have allowed dielectric characterization of single living cells, either through single-cell electrorotation[12] or by performing localized dielectric spectroscopy measurements with microscale electrodes.[13]

Virtually all previous DEP studies of biological systems have been performed using fixed electrodes with the DEP forces used to manipulate and measure mobile particles. In the studies described in this work, the measurement configuration is reversed. The particles themselves are effectively immobile, and the equal and opposite force on a mobile electrode is measured. Unlike direct current (dc) and quasi-dc (i.e., for $\omega \leq \sim$5 kHz) scanning probe methods for characterizing local electrical properties, dielectrophoretic force microscopy (DEPFM) as defined in this work is explicitly a sensitive function of the ac frequency. In studies closely related to dielectrophoresis, scanning polarization force microscopy (SPFM) has emerged as a means of imaging the variation in dc polarizability of a sample as a function of position.[14−18] However, the tip−sample forces that are measured by these techniques still arise primarily from effectively electrostatic interactions, rather than the ac electrokinetic DEP forces. The majority of SPFM and voltage-modulated electric force microscopy techniques have been performed with either dc or ac frequencies less than a few kilohertz.[16,18−20]

The goal of the present work is to demonstrate DEPFM as a viable means to probe the ac electrokinetic properties of nanoscale surface structures and cells at liquid interfaces in the frequency range from ∼10 kHz to ∼10 MHz. By measuring the ac-field-induced force on a scanning probe tip as a function of position, DEPFM allowed for the imaging of the surface ac polarizability with nanoscale spatial resolution. DEPFM measurements were first

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10.1021/la0484366 CCC: $30.25
Published on Web 01/19/2005

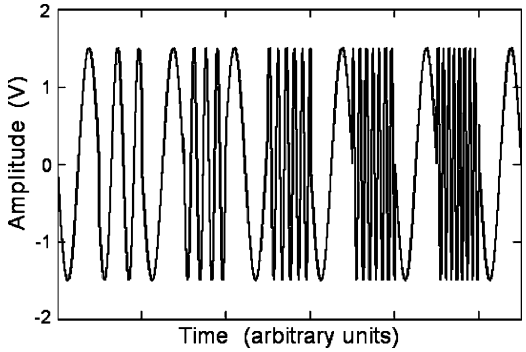

Figure 1. Schematic representation of the waveform applied to the cantilever to generate the dielectric spectra. The ac waveform was toggled between a reference frequency and a test frequency as the test frequency was swept over the desired range. The modulation rate ($^1/_{50}$ of the scan rate) was much slower than the resonance frequency of the cantilever.

performed for semiconductor surface structures with predictable ac electrokinetic properties, followed by DEPFM imaging of single *Escherichia coli* cells.

Experimental Methods

Atomic Force Microscope (AFM). AFM measurements were acquired with a Dimension 3100 atomic force microscope (Veeco). Imaging was performed in tapping mode in high resistivity (~17 MΩ cm) water (NANOpure, Barnstead/Thermolyne Corp) with titanium/platinum-coated silicon nitride AFM tips (μMasch) having spring constants of ~1 N/m and typical resonance frequencies of 6−8 kHz in water. The cantilever holder was modified to allow for the application of an electrical potential directly to the AFM tip from a function generator. Artifacts arising from reflections in the unterminated cable can be reasonably assumed to be negligible because the cable lengths were always much less than the wavelength of electromagnetic radiation used (~1 m compared to ~200 m, respectively).

An Agilent 33250A function generator was used to apply an ac potential across the AFM tip and the grounded underlying silicon substrate. During imaging, the waveform output of the function generator was applied while the tip was scanning every other scan line of the raster pattern. Separate images of topography and topography combined with changes in apparent height from DEP forces were generated by separating the two alternate-line images and interpolating the removed data with average values of adjacent lines. Images of DEP force maps alone were then generated by subtraction of topography from the images containing both topography and DEP forces. This subtraction approach had the advantage of simultaneously providing images of topography and DEP forces in both height and phase each time the tip completed a single raster scan. In general, the DEP images exhibited slight offsets within the plane of the image. All reported images were adjusted for optimal topographic alignment before subtraction. Force curve measurements were performed at a scan rate of 1 Hz with the AFM in contact mode.

Silicon Well Sample. A silicon wafer with a uniform, ~200 nm silicon oxide overlayer was prepared by photolithography with a series of ~6 μm diameter wells in which a large portion of the oxide layer was removed, leaving a region with an oxide layer of only ~2 nm. The underlying silicon substrate was connected to electrical ground. The resulting silicon well sample was imaged with an 8 V_{pp}, 10 MHz ac signal applied to the tip every other line.

***E. coli* Sample.** A native strain of *E. coli* was used in all measurements. To enhance bacterial adhesion, the silicon wafer substrate was immersed in "piranha" solution (1:1 ratio of H_2SO_4 and 30% H_2O_2) for ~30 min, washed with deionized water, and dried under nitrogen gas (*caution: piranha solution is a strong oxidant*). A drop containing a suspension of *E. coli* was then placed on the silicon surface for ~10 min. The surface was then washed thoroughly with deionized water and allowed to dry in air. DEPFM images in high resistivity water were then acquired using a 5 V_{pp}, 100 kHz ac field.

Frequency-Dependence Measurements. Dielectric spectra were obtained by applying a frequency varying ac field to an AFM tip and recording the changes in apparent height. Dielectric spectra were acquired while the AFM was scanning a 10 nm by 10 nm area of a silicon wafer with an oxide overlayer of ~200 nm. Typically over 200 individual spectra were obtained for each scan. The frequency dependence of the dielectrophoretic effect was tested by applying a 3 V_{pp} waveform that was modulated between a 25 kHz base frequency and a swept test frequency between 25 kHz and 1.975 MHz (generated by linked Agilent 33250A and 33220A function generators). A schematic depiction of this waveform is shown in Figure 1.

Results

Topographic and dielectrophoretic images of a microfabricated silicon well structure are shown in Figure 2. This structure consisted of a Si substrate with an ~200 nm thick oxide overlayer. The oxide overlayer was removed in ~6 μm diameter circular regions, leaving a native oxide thickness of ~2 nm. The acquired map of the field-induced change in apparent height in Figure 2c indicates an increase of ~20 nm over the region inside the well relative to the surrounding region. In the absence of an applied field, the apparent height difference for the two different regions was on the order of the instrumental noise (i.e., 4 Å), shown in Figure 2e and f.

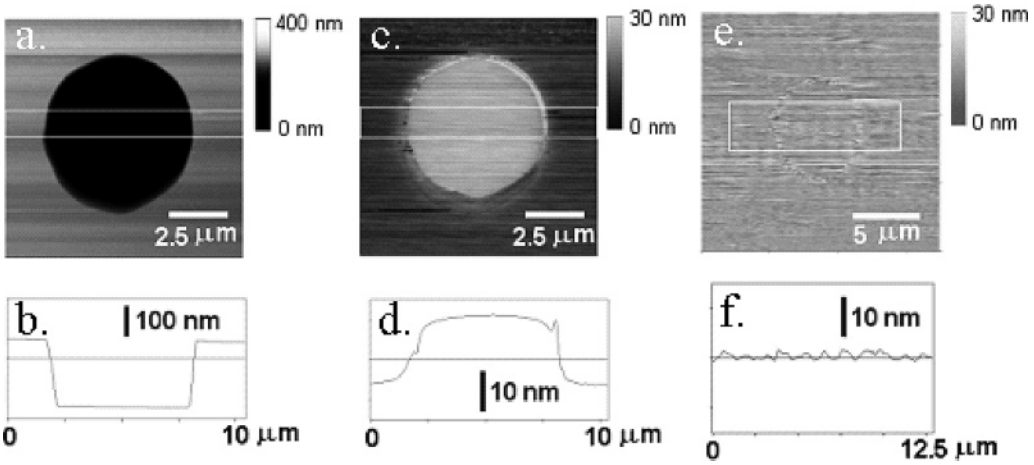

Figure 2. Dielectrophoretic force microscopy of a water/SiO_2/Si test structure. Topography (a) and cross section (b) of a silicon oxide surface with an ~200 nm deep well with an ~6 μm diameter. Dielectrophoretic force map (c) and cross section (d) of a silicon well with an 8 V_{pp}, 100 kHz field applied to every other line. Control DEP force image (e) and cross section (f) with no applied electric field. DEP forces are manifested as changes in apparent height.

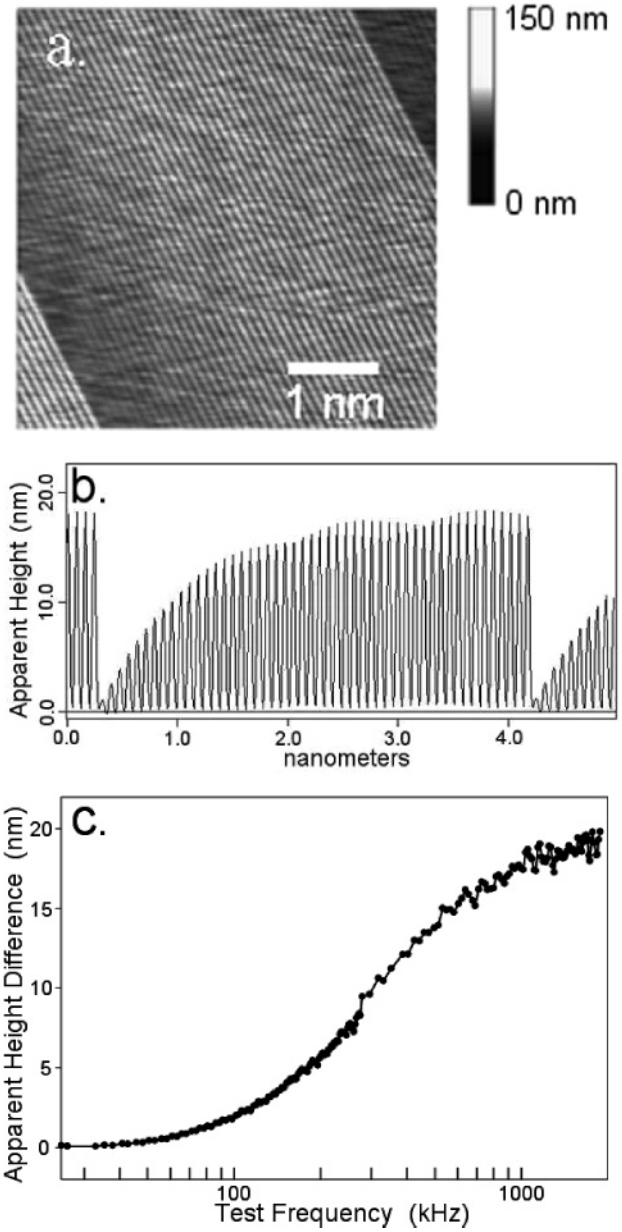

Figure 3. Dielectric spectroscopy of a 10 nm × 10 nm region of the water/SiO_2/Si interface: raw image consisting of the apparent topography measured at different ac frequencies, appearing as ripples (a); diagonal cross section of the water/SiO_2/Si interface imaged as the ac frequency of the voltage across the tip that was modulated between a reference frequency of 25 kHz and a test frequency (3 V_{pp}) swept from 25 to 1975 kHz (b); the difference in DEP force relative to the force of the reference frequency, measured as a change in apparent height (c).

The frequency dependence of the apparent height change upon the application of an ac field is shown in Figure 3. Figure 3a contains a raw image illustrating the general procedure used to obtain dielectric spectra. In brief, the ac field was modulated between a fixed reference frequency and a variable test frequency as the test frequency was swept over the desired range. The "ripples" in Figure 3a indicate frequency-dependent differences in the DEP force. A slight timing mismatch between the scan rate and the sweep cycle was introduced in order for the ripples to appear at an angle with respect to the slow-scan axis. Figure 3b contains the averaged cross section of ~250 spectra similar to those shown in Figure 3a. The dielectric spectrum shown in Figure 3c was acquired from analysis of the differences in apparent height as a function of frequency. Using this approach, a complete dielectric spectrum containing ~50 data points and spanning the full range of desired frequencies was obtained every ~$^1/_2$ s with nanometer-scale spatial resolution.

Representative images of the topography and the corresponding DEP force map of a single *E. coli* cell are shown in parts a and c of Figure 4, respectively. The DEP force map indicates an apparent height increase of ~15 nm between the *E. coli* and the surrounding substrate.

Force curve measurements were performed with and without the application of a 7 V_{pp}, 1 MHz ac field, and they are presented in Figure 5. Values for the tip−sample interaction force were inferred from the acquired deflection data by assuming a cantilever spring constant of 1 N/m.

Discussion

An understanding of the nature of the image contrast that arises in the presence of an inhomogeneous ac field is a prerequisite for interpreting the DEP force maps shown in Figures 2−4. All scanning probe images were acquired in tapping mode, in which the amplitude of cantilever oscillation provided feedback for imaging. The DEP force maps shown in Figures 2−4 are images of the *difference in apparent height* in the presence of the ac field. In brief, the addition of a steeply distance-dependent ac-field-induced force on a damped, driven cantilever results in a change in the quality factor of the resonator and an increased damping in the amplitude of the cantilever oscillation.[21] The instrument responds by increasing the distance between the tip and the interface to maintain constant amplitude, and this field-induced change in apparent height gives rise to the contrast observed in the images. From numerical solutions of a differential equation describing the motion of a damped, driven oscillator subjected to a spatially varying DEP force (not shown), the change in apparent height scales linearly with the magnitude of the DEP force under the conditions expected in these experiments. When the tip is scanned over regions with different polarizabilities, the field-induced contributions to the image contrast appear as changes in both apparent height and phase.

The total polarization force arising between an AFM tip and a polarizable sample generally has two main contributions: nonlocal forces acting on the cantilever, arising from interactions that are relatively far away from the surface (15−20 μm), and local forces from interactions immediately adjacent to the probe tip (e.g., within a few angstroms from the surface).[17] As a result, the force on the cantilever contains one bulk component that is relatively insensitive to the local topography and polarizability (appearing as an overall offset in apparent height) and one component that is sensitive to the local topography and polarizability, yielding image contrast. The total force change acting on the AFM cantilever induced upon application of the electric field was roughly 100 nN, as determined from the force curve measurements shown in Figure 5. The size of this interaction force is due to the fact that the force comprises both local (immediately adjacent to the cantilever tip) and nonlocal (e.g., from capacitive interactions with the rest of the cantilever) interactions.

The description above provides a context for interpreting the contrast in the DEPFM force maps of the silicon well sample shown in Figure 2. Intuitively, the DEP force is

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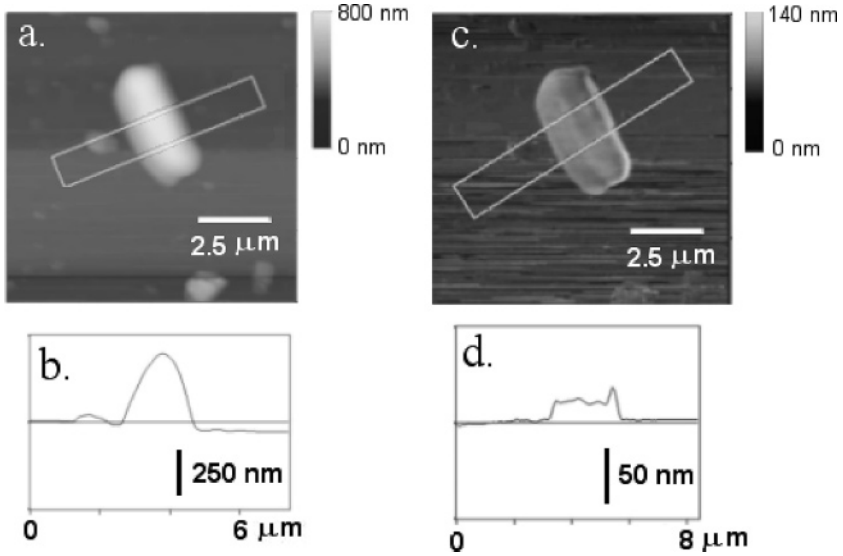

Figure 4. Dielectrophoretic force microscopy of *E. coli*. Topography (a) and cross section (b) of *E. coli* on a Si/SiO_2 substrate in water. Dielectrophoretic force image (c) and cross section (d) of *E. coli* with a 5 V_{pp}, 100 kHz ac field.

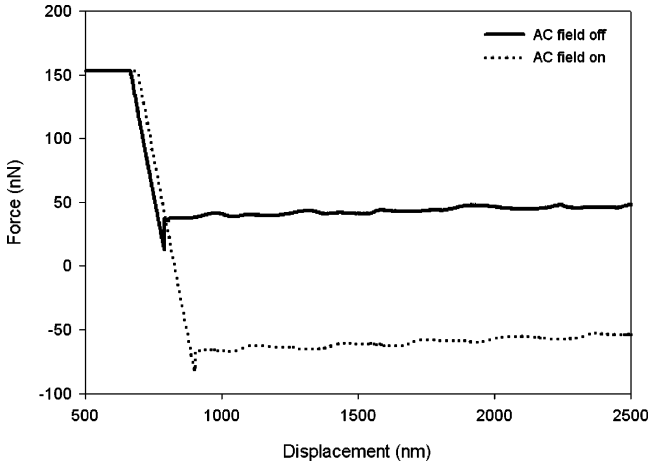

Figure 5. Force curves (retraction) obtained for the water/SiO_2/Si in the presence (dotted line) and absence (solid line) of a 1 MHz, 7 V_{pp} ac potential applied between the tip and the silicon substrate. The reported absolute force in nanonewtons has an error of $\sim \pm 50\%$ arising from uncertainties in the cantilever spring constant.

expected to be greater when the oxide layer between the tip and the silicon substrate is minimized, since silicon is significantly more polarizable than silica. Inspection of Figure 2 reflects this trend. In the presence of an ac field, the apparent height increased by ~20 nm in the regions with a thin native oxide. This change in apparent height was on the same order of magnitude as the average total amplitude of oscillation of the cantilever during tapping-mode imaging in water. Removal of the applied field resulted in nearly perfect removal of image contrast in the DEP force map (Figure 2c). In the control experiment, the average height change between the inside of the well and the outlying regions was only ~0.2 nm (i.e., comparable to the rms noise of the instrument). It is reasonable to conclude that the observed height changes in the DEP force maps shown in Figure 2 can be attributed to field-induced interactions and not to potential artifacts related to changes in topography.

Unlike electric force microscopy and polarization force microscopy, in which the applied fields are either dc or slowly varying ac fields (e.g., a few kilohertz),[16,18−20] DEP forces arising from radio frequency electric fields are well established to be sensitive functions of the ac frequency.[2,22] Consequently, DEPFM provides a means for performing dielectric spectroscopy with nanoscale spatial resolution, an example of which is shown in Figure 3 for the water/SiO_2/Si interface. The sigmoidal shape of the frequency spectrum is consistent with the presence of a "Maxwell−Wagner" resonance at ~300 kHz, in which the relative conductivities of water and the thin SiO_2 surface film dominate the Clausius−Mossotti factor at low frequencies, while the permittivities dominate at high frequencies.[2]

The studies of semiconductor/oxide/water interfaces provide a context for interpreting the local measurements of the DEP forces arising within different regions of a single *E. coli* cell, shown in Figure 4c. The apparent height increase of ~15 nm in the region above of the cell is consistent with an increased local polarizability relative to the local polarizability for a bare Si/SiO_2/water interface. The DEP forces were relatively constant across the cell and did not track the topography, as shown in Figure 4a. These results are consistent with the uniformity in dielectric properties of single-celled organisms predicted by simple models in which cells are treated as uniform spheres of cytoplasm surrounded by insulating cell walls.[2,22] The frequency range from ~10 kHz to ~10 MHz is easily accessible using this general approach and covers key resonances related to membrane capacitance and charge mobility in studies of living systems.[10,11,23−26] Measurements at lower frequencies were problematic due to interference from the mechanical resonances of the cantilevers, while effects associated with electronic reflections can complicate localized dielectric spectroscopy measurements for frequencies > ~20 MHz. To our knowledge, these are the first nanoscale measurements of the DEP forces arising within different regions of a single cell.

The overall magnitudes of the field-induced changes in apparent height suggest that DEPFM allows for noncon-

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tact imaging of aqueous interfaces. Observed apparent height changes of ∼50−80 nm were routinely measured in DEPFM experiments while still maintaining feedback, yielding image contrast of ∼10−20 nm from differences in local polarizability. Because the amplitude of oscillation for a tip undergoing tapping-mode motion in water is ∼50 nm, it is reasonable to conclude that DEPFM is a noncontact AFM technique. Few alternative methods are currently available for simple and reliable noncontact scanning probe imaging of aqueous interfaces.[27]

The direct relationship between the DEP forces and ion mobility at and across biological interfaces suggests that DEPFM is particularly well suited for the study of biological systems.[2] For example, membrane capacitance can differ substantially between lipid bilayers of differing thicknesses and/or compositions.[28] Additionally, chemical activation of biochemical pathways has been shown to affect the dielectrophoretic properties of assemblies of cells and viruses.[10,11] DEP measurements can be carried out under physiological conditions,[29] and the noncontact nature of DEPFM reduces the likelihood of sample damage and/or deformation due to physical contact with the AFM tip. These capabilities suggest that DEPFM can be a useful and broadly applicable technique for in vitro imaging of cells, viruses, and membranes.

Acknowledgment. The authors gratefully acknowledge financial support from the Showalter Trust Organization and Purdue University. We acknowledge Mr. Siyu Koswatta and Prof. David Janes for the silicon well test sample, Ms. Jessica Anderson and Prof. Christine Hrycyna for providing the *E. coli* colonies, and Dr. Alexander Ribbe of the Purdue Laboratory for Chemical Nanotechnology.

LA0484366

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