Ambient molecular water accumulation on silica surfaces detected by a reflectance interference optical balance

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Water is a persistent background in virtually all biosensors, yet is difficult to quantify. We apply an interferometric optical balance to measure water film accumulation from air onto several types of prepared silica surfaces. The optical balance uses in-line common-path interferometry with balanced quadratures to measure the real-time accumulation of molecular films. The accumulated water thickness is sensitive to ambient conditions, with thicknesses that vary from picometers up to nanometers, even on hydrophobic silanized surfaces. These results demonstrate that water adsorption contributes an excess signal in dry label-free protein microarray optical biosensors and presents a fundamental limit to assay sensitivity. © 2010 American Institute of Physics.

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Water is the most fundamental and ubiquitous constituent of biological processes. It participates at all levels of biochemistry, from the dynamics of protein folding, to the chemical configurations of DNA and antibodies in biosensors.¹ Water plays a particularly crucial role on silica surfaces that are the most common platform for gene and protein microarray production. At room temperature, in air, water spontaneously deposits on silica surfaces as an ultra-thin film and can form hydroxyl groups bound to silicon.^{2,3} These hydroxyl groups participate in the functionalization of silica surfaces to covalently immobilize proteins or DNA to the surface. Water film, as a constituent of the biochemical analytes or as a dielectric layer, may contribute background signals to mass-sensitive biosensors which detect label-free biomaterials on microarrays, especially when the detection is performed after the sample has been dried.^{4–6} Therefore, the quantitative measurement of water on biosensor surfaces is essential to understand how water affects the sensitivity and accuracy of mass-sensitive biosensors. However, this measurement is a challenging metrology problem because the water film is usually extremely thin and is present on all surfaces. Studies of adsorbed water⁷ using x-rays,^{3,8} IR absorption,^{9,10} nonlinear optical methods,¹¹ or ellipsometry¹² have measured saturated films, and scanning tunneling microscopy requires special conditions.^{13,14} Although several chemical approaches have been used for water detection on silica,^{15–17} they use irreversible chemical reactions that cannot be performed as real-time measurements. Label-free optical biosensors¹⁸ have high precision to detect adsorbed layers^{19–21} but to capture the pervasive presence of water that attaches to all surfaces requires an accurate differential measurement to remove drift and bias, as provided by our interferometric optical balance.²²

The land-contrast²² BioCD (Ref. 23) uses self-referencing interferometry to directly and nondestructively sense thin film deposition on surfaces. The land-contrast approach uses two adjacent areas of an oxidized silicon chip that have equal reflectance (optical balance) but reflection coefficients that have opposite signs. When molecules attach

to the surfaces, the reflectance changes, and the additional molecular film increases the reflectance for one area but decreases the reflectance for the other. Therefore, the accumulation of molecular films tips the optical balance, making the film visible to scanning lasers or to imaging systems. The contrast increases as the film deposits on the substrate [shown in Fig. 1(a)]. The important feature of this interferometric approach is that it locally self-references and performs common-mode rejection of fluctuations, making it highly sensitive.

To create the two conjugate surfaces on a single chip, we fabricated spot-shaped mesas (to mimic a conventional microarray geometry) on silicon thermal oxide by photolithography and plasma etching [Fig. 1(b)]. The SiO₂ on the land (the background of the substrate) was etched to 77 nm, leaving 140 nm for the 150 μ m diameter mesas (the spot regions).²⁴ The reflection coefficients are $\pm 0.38i$ under normal incidence of 633 nm wavelength, and both reflectances are equal at $R=0.147$. The size of the chip was 3×4 mm². Each chip contained 64 spots and was labeled by a unique

FIG. 1. (Color online) The principle of land-contrast interferometry on the BioCD. (a) Reflection coefficients are $-0.383i$ on 77 nm SiO₂/Si (land) and $0.383i$ on 140 nm SiO₂/Si (mesa), and $R=0.147$ for both. A 1 nm water film changes the reflection coefficients by -0.0017 and $+0.0017$ on the land and the mesa, respectively. (b) Spot-shaped mesas were etched on silicon thermal-oxide silicon chips by photolithography. The contrast between the spot and the land is sensitive to water accumulation, because the local contrast is self-referenced with common-mode rejection of system fluctuations.

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barcode. As water accumulates on the chip, the reflectance of the mesas increases, while the reflectance of the land decreases by an equal amount. We acquired the reflectance image of the chips, measured the contrast between the mesa and the land, and then calculated the accumulated water film thickness. Two scanning mechanisms were adopted in this work. The spinning disk interferometric (SDI) BioCD scanning system uses a 633 nm wavelength laser as the probe and an avalanche photodiode to acquire the reflectance image of the LC chip by spinning the sample. The other system uses molecular interferometric imaging (MI2),²⁵ which acquires the reflectance image of the chip under a microscope equipped with a 630 nm photodiode as the light source. Both systems acquire the reflectance image under normal incidence and using an in-line common-path interferometric configuration. One of the fundamental differences between the two scanning systems is that the sample moves (spins) on the SDI system while the sample remains static on the MI2 system, which has an important effect on the kinetics of water accumulation.

Land-contrast chips were prepared to study the water accumulation on the following four different surface chemistries on thermal oxide: (1) bare silica incubated in 90 °C water vapor for 2 h; (2) bare silica dehydrated by baking at 150 °C for 30 min; (3) silanized silica by soaking in 30 mM chlorodimethyl-octadecylsilane in toluene for 8 h to make the surface hydrophobic; (4) silanized silica subsequently printed with a 2.5 nm protein layer (by soaking in 50 $\mu\text{g}/\text{ml}$ rabbit IgG solution for 30 min) on the surface. Sample 3 and sample 4 were dried by a dry nitrogen stream, which is a standard protocol step for protein array production. The SDI scanning system scanned the samples by attaching the chips to the edge of a 100 mm diameter disk and spinning at $40\pi/\text{s}$ angular speed in ambient air. Scans were performed on four chips at 30 min intervals with an overall observation time of ten hours (25 °C room temperature) at several relative humidities. Before a final round of scans, the four chips were baked at 90 °C for 30 min to desiccate the surfaces. The relative humidity was 40% for the final scans. The reflectance contrast of the chips increased during the exposure in air, and was converted to thickness shown in Fig. 2(a), with the rate dependent on the relative humidity.

The curves in Fig. 2(a) demonstrate different affinities of the selected surface treatments to capture water molecules. For reference, the lattice constant of a full layer of water ice is 370 pm,²⁶ which is consistent with x-ray data of water on hydrophilic surfaces.⁸ In the first round of scans, the dehydrated silica gained 340 pm water, while the silanized (hydrophobic) silica gained 290 pm water. The vapor-treated silica gained 120 pm water film in the air flow, indicating that the 90 °C vapor treatment did not fully saturate the surface. The protein-on silica gained 660 pm of water after long-term spinning. Water is captured substantially more by protein than by silica because protein molecules, especially IgG antibody molecules, tend to have hydrophilic amino acid residues on the exterior while sequestering the hydrophobic residues on the interior to assist in the globular folded structure. The mean time of water adsorption on the spinning chips was about 3.5 h under 40% relative humidity.

We compared the water accumulation on silica surfaces for static versus spinning chips. Two protein-coated silica LC chips were prepared by soaking the silanized LC chips in 50 $\mu\text{g}/\text{ml}$ rabbit IgG solution for 30 min and dried by a dry

FIG. 2. (Color online) Water film accumulation on bare and chemically-treated silica surfaces under differing humidities. (a) The four surface preparations were dehydrated silica, hydrated silica, silanized silica (chlorodimethyl-octadecylsilane), and protein coated silica. The chips were baked after 1800 min and scanned again. (b) Water accumulation on protein-coated chips is 3 $\times$ larger on a chip spinning at 5 m/s compared to a static chip. The chips were baked after 600 min and scanned again.

nitrogen stream. One sample was scanned by the SDI system which measured the reflectance image by spinning the sample. The linear speed was 5 m/s with a corresponding Bernoulli pressure of 15 Pa or 1×10^{-4} atm and a Reynolds length of 1×10^3 m. The other sample was imaged by the MI2 system which imaged the reflectance of the static sample. The measurements on both samples were performed simultaneously in the same laboratory room. The two samples initially were dried by nitrogen stream and then observed over 10 h. From Fig. 2(b), it is evident that both samples adsorb water with different adsorption rates. The spinning sample (scanned by the SDI system) adsorbed water at a rate about three times larger than for the static sample. Both samples were dried by baking them at 90 °C to desiccate the surfaces, after which water again accumulated on the surfaces during the subsequent scans at threefold different rates.

To compare the land-contrast results with protein spots on a BioCD (Ref. 23) biosensor, we directly measured the protein signal on a BioCD chip. Eight rabbit IgG spots were printed by pipette on a silanized silica surface using 1 μl rabbit IgG solution (100 $\mu\text{g}/\text{ml}$ in phosphate buffered saline buffer) and incubated for 30 min. Subsequently, the protein array was washed with water and dried by a pure nitrogen stream. Scans were performed for 8 h, and the average height

FIG. 3. (Color online) Eight protein spots (rabbit IgG) were printed on a 140 nm thick silica-on-silicon surface and dried by nitrogen. The sample was scanned by the SDI system exposed to air. Each image frame was acquired every 20 min. The thickness increased over 8 h by approximately 320 pm of water on the protein spots relative to the silanized (hydrophobic) land.

of the protein spots was calculated for each scan. The apparent protein height increment (water height being interpreted as protein height) is shown in Fig. 3. The initial average thickness of the protein layer was 3.15 nm. After exposure to air for 8 h at 40% relative humidity, the average protein thickness gained about 220 pm. For water, with a refractive index of $n=1.33$, this corresponds to about 320 pm of water relative to the silanized surface, which is nearly a monolayer of water. Within the first hour (a typical slow-scan BioCD read time) the equivalent protein thickness gain (caused by water adsorption) was 80 pm, which is comparable to the spot-to-spot protein variability on BioCD measurements²⁷ and hence sets the practical limit on assay sensitivity between 50 to 100 pm, which is far above the metrology sensitivity of 2 pm for the technique.

In conclusion, we have applied land-contrast interferometry for quantitative and real-time measurements of water accumulation on silica surfaces in air with a height sensitiv-

ity of 2 pm. The binding time to equilibrium is about 3 h. The amount of adsorbed water is different for protein-coated surfaces and bare surfaces and therefore can contribute a 320 pm thickness to a protein layer with an original thickness of 3 nm. Similarly, we tested a protein array exposed in air at 40% relative humidity and acquired an 80 pm excess signal in the first hour. This excess signal of a dry label-free biosensor caused by humidity had not been previously calibrated, but it represents a fundamental limit to such assays independent of the underlying metrology sensitivity of the technique.

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