Microfabricated mechanical biosensor with inherently differential readout

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We report measurements with a micromachined mechanical biosensor that inherently suppresses background effects by producing a differential signal with respect to a reference. The sensor comprises two adjacent cantilevers with interdigitated fingers between them that allow interferometric detection of the differential, i.e., relative bending. We demonstrate that differential detection can efficiently suppress unspecific chemical effects that result in cantilever bending. We show that the differential deflection noise is up to an order of magnitude lower than the absolute deflection noise in the low-frequency range of 0.0003–1 Hz, where many types of biologically relevant reactions occur. We also demonstrate the sensor's applicability to biological receptor–ligand systems by reporting experimental results on direct differential detection of biotin–streptavidin binding. © *2003 American Institute of Physics.* [DOI: 10.1063/1.1605238]

Intermolecular forces that result from adsorption of biomolecules can bend a micromachined cantilever and enable detection of nucleic acids and proteins without any prior labeling of target molecules.[1,2] Often, the tip deflection of the cantilever is detected using the optical lever method, i.e., by focusing a laser beam at the tip of the cantilever and measuring the changes in position of the reflected beam. The potential of cantilever-based sensors to detect numerous chemical and biomolecular interactions has already been demonstrated.[3,4] Researchers have also shown that by using the optical lever method to separately measure the bending of two identical cantilevers, the reliability of the signal resulting from the molecular binding reaction is improved by monitoring the relative, or differential bending.[2,4]

We developed an interferometric sensor that inherently measures the differential bending between two adjacent cantilevers, thereby eliminating the need for two separate optical setups and alignment steps.[5] The two cantilevers constitute a sensor-reference pair, whereby only the sensing surface is functionalized with receptors that are specific to the ligand to be detected. The two cantilevers have closely matched responses to background disturbances. Hence, disturbance-induced unspecific deflections are suppressed upstream, i.e., before the optical signal is measured. This scheme also has the potential of being packaged in a small volume. Loh *et al.* demonstrated that an accelerometer that uses the same principle for motion detection can be packaged into a 10 cm^3 volume.[6]

We have previously reported that in air, the resolution of the interferometric cantilever-based sensor at high frequencies (40–1000 Hz) is only limited by its subangstrom thermomechanical noise ($\sim 0.2\ Å_{rms}$).[5] However, at lower frequencies, the sensor exhibits a flicker or $1/f$-type behavior, which yields noise levels that are much higher ($\sim 10\ Å_{rms}$) than the thermomechanical noise. For biological applications of cantilever-based sensors, it is the low-frequency behavior in liquid that poses the detection limit. In this letter, we examine the low-frequency behavior of the sensor in liquid and demonstrate that it can be improved by differential detection. We also demonstrate the potential of the sensor to perform differential detection of protein binding.

Figure 1 shows a schematic of the device and the detection method. The two adjacent 1-μm-thick silicon nitride cantilevers are supported by 10-μm-thick L-shaped structures.[5] Interdigitated fingers between the supports and the cantilevers form diffraction gratings that allow interferometric detection of each cantilever's absolute deflection. Likewise, the fingers between the two cantilevers enable detection of the differential cantilever bending. The sections at the end of the cantilevers that accommodate the fingers are thicker than the flexible parts of the cantilevers. This prevents cantilever warping and also reduces the bending of the fingers. When the fingers are illuminated with a laser beam (1126P, JDS Uniphase), the reflected light forms a pattern composed of several modes whose intensities depend on the vertical distance between the two finger sets.[7] Bending is determined by measuring the intensity of a single mode with a silicon photodetector. Since the dependence of each mode's

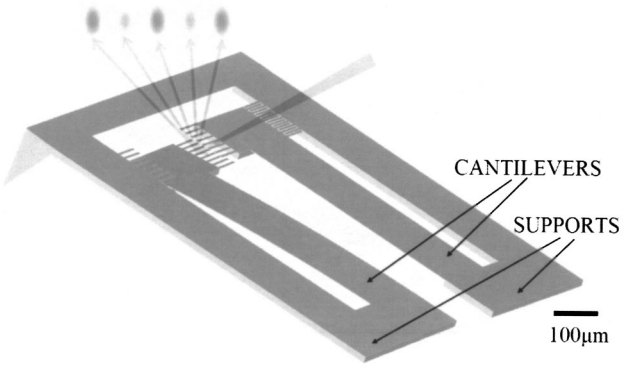

FIG. 1. Device schematic and optical detection principle. Interdigitated fingers between the two 1-μm-thick cantilevers are illuminated with a laser beam, and a diffraction pattern is formed. Intensities of diffraction modes change as one cantilever bends relative to the other. 10-μm-thick L-shaped supports connect the cantilevers to the substrate, and also allow insertion of cantilevers into pipettes for surface functionalization/preparation.

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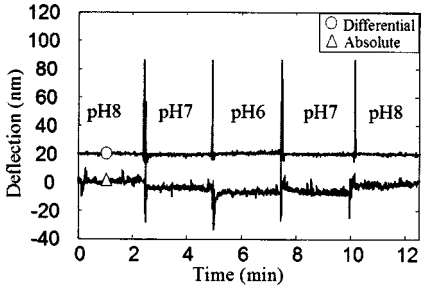

FIG. 2. Absolute and differential cantilever bending due to *p*H changes in aqueous environment. Spikes represent injections. Absolute response (triangle) of a single cantilever to *p*H changes is significantly reduced by the differential detection (circle). Differential response was intentionally plotted with a dc offset for clarity.

intensity on the relative displacement is known analytically [Eq. (1)], the photodetector output can be directly calibrated for deflection, and requires only the knowledge of the laser wavelength in the medium:[5]

$$I_1 \propto \sin^2\left(2\pi\frac{\xi}{\lambda}\right). \tag{1}$$

Here, I_1 is the intensity of the first diffraction mode, ξ is the relative cantilever deflection, and λ is the wavelength of the detection laser in solution.

The device has a thin layer of gold on one side that both improves reflectivity and enables thiol-based surface functionalization. The difference in the thermal expansion coefficients of gold and silicon nitride allows deflection of the reference cantilever upon being heated by a secondary laser beam (HL6501MG, Hitachi), which can be used to bias the interferometer to its most sensitive point, i.e., $\xi = m\lambda/8$, m being an odd integer.

We tested the device's ability to reject unspecific chemical disturbances by observing its response to *p*H variations. It is known that a silicon nitride surface reacts chemically to *p*H changes in the surrounding solution.[8] It has also been shown that this reaction generates a surface stress that can bend a micromachined cantilever that has different top (gold) and bottom (silicon nitride) surfaces.[9] To determine the sensor's response to changes in *p*H, we placed it in a fluidic chamber and injected phosphate buffer solutions (100 mM) with *p*H values 8, 7, and 6 into the chamber. Figure 2 shows the absolute and the differential cantilever bending in response to *p*H changes in the fluidic chamber. The differential detection significantly reduces the effect of *p*H changes, and results in a more stable response. Figure 2 shows that differential detection can also suppress the transients following the injection.

Most cantilever-based detection systems exhibit flicker, i.e., $1/f$-type noise at low frequencies.[5,10] For detecting biological reactions, many of which occur over minutes or tens of minutes, low-frequency noise becomes the limiting factor. We investigated the low-frequency noise of our sensor by monitoring its behavior in phosphate buffer over a period of 1 h. We used an analog low-pass filter (428, Keithley) at the output of the photodetector to attenuate the noise components above 1 Hz and sampled at a frequency of 20 Hz. Figure 3(a) shows the absolute and differential cantilever bending responses over time. The absolute response shows a

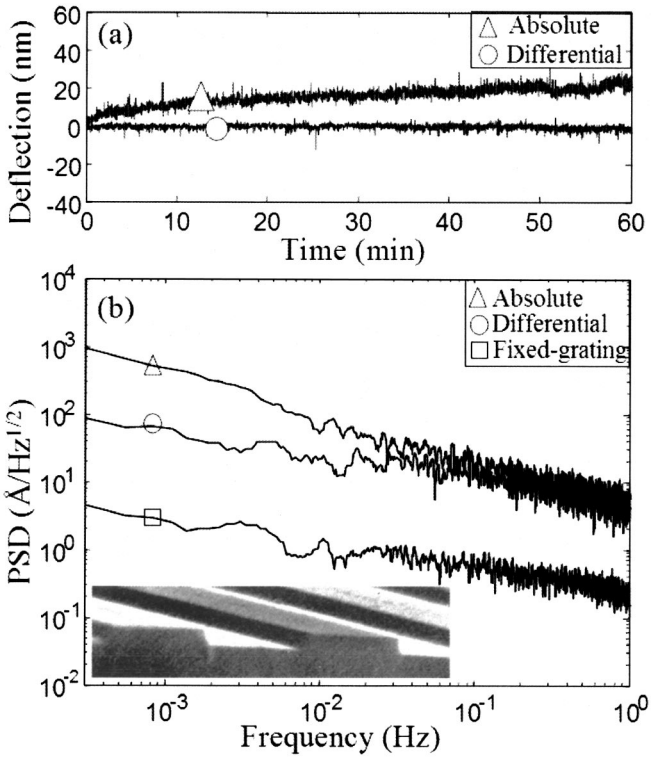

FIG. 3. (a) Absolute (triangle) and differential (circle) cantilever response in phosphate buffer over 1 h. (b) Power spectral density (PSD) of absolute (triangle) and differential (circle) cantilever bending in comparison with the deflection-equivalent PSD of a fixed diffraction grating response (square). The inset shows an image of the micromachined grating with a fixed depth of 210 nm.

substantial drift whereas the differential response exhibits almost no drift. Figure 3(b) shows the power spectral densities (PSD) of both the absolute and the differential response. The differential response revealed lower overall noise (10 $Å_{rms}$) than the absolute response (49 $Å_{rms}$), and at low frequencies, the differential detection reduced the noise by as much as an order of magnitude (at 0.0003 Hz).

Also shown in Fig. 3(b) is the signal from a diffraction grating with fixed depth in phosphate buffer. The grating was fabricated by patterning 210 nm of thermally grown oxide on silicon to have the equivalent geometry as the interdigitated diffraction grating of the sensor [inset of Fig. 3(b)]. The grating was coated with a 20 nm gold layer (1 nm titanium for adhesion layer) and placed in the fluidic cell. Since the grating has a fixed depth, its response excludes deflection noise. Here, possible noise sources include fluctuations in laser wavelength and intensity, and those in the refractive index of the solution within the grating. We calibrated the response of the grating for an equivalent deflection to enable comparison with the cantilever response. We performed the calibration by solving the argument of Eq. (1) for virtual variation in ξ that would cause an equal change in modal intensity to that caused by a small change in laser wavelength in the fluidic chamber:

$$\Delta\xi = \xi_0\frac{\Delta\lambda}{\lambda_0}. \tag{2}$$

Here, ξ_0 is the fixed depth of the grating (210 nm), λ_0 is the detection laser wavelength in water (476 nm), and $\Delta\lambda$ is a

perturbation in laser wavelength. We determined the dependence of the modal intensity on laser wavelength by sequentially injecting fluids with known refractive indices[11] (water and ethanol) and observing the photodetector output. We then recorded the signal as a function of time while the grating was immersed in phosphate buffer. The deflection-equivalent signal was calculated using Eq. (2) and by realizing that for a small perturbation Δn in the refractive index n_0:

$$\frac{\Delta\lambda}{\lambda_0} \cong \frac{\Delta n}{n_0}. \tag{3}$$

Again, the PSD of the resulting signal was calculated off-line. The low noise level of the grating in Fig. 3(b) indicates that most of the low-frequency sensor noise is due to cantilever deflection. The deflection-equivalent noise contributed by the optical detection system alone is only $0.5\ \text{Å}_{\text{rms}}$ over the frequency range 0.0003–1 Hz.

To demonstrate the application of inherent differential detection to a model biological receptor–ligand system, we performed a biotin–streptavidin binding experiment. Our experimental result resembles directly the differential response between a surface that allows the binding, and a neighboring one that does not. We passivated the gold-coated side of both cantilevers with thio-modified polyethylene glycol (PEG) (Rapp Polymere GmbH, Germany) to prevent protein adsorption to this surface. The cantilevers were passivated immediately after gold evaporation by inserting them into a PEG solution (1 mg/ml in water). The device geometry also allows fluidic delivery to each cantilever using commercially available pipettes, significantly facilitating the functionalization process. The nitride surface of the sensing cantilever was functionalized with biotin-labeled bovine serum albumin (bBSA, Sigma), by inserting the cantilever into a glass pipette containing the bBSA solution. Similarly, the nitride surface of the reference cantilever was blocked with bovine serum albumin (BSA, Sigma). Since both the top and bottom surfaces of the reference cantilever were blocked (with PEG and BSA), we expect no specific binding to this cantilever to occur. Both BSA and bBSA were dissolved in phosphate buffered saline (PBS, Sigma) at a concentration of 5 mg/ml.

Following the functionalization, the device was placed in the fluidic chamber and allowed to equilibrate in a BSA solution (100 μg/ml in PBS). Several BSA injections were performed to obtain a base line and verify the stability of the sensor. A streptavidin (SA) solution (700 μg/ml in PBS) was injected to initiate biotin–streptavidin binding on the sensor cantilever. Figure 4 shows the differential cantilever bending during the experiment. Injecting a BSA solution at time $=3.5$ min caused a negligible bending response (after the injection peak), while SA injection at time $=9$ min resulted in 92 nm of differential cantilever bending (~ 0.04 N/m of differential surface stress). The differential bending resulted from binding of SA to bBSA that was present only on one of the sensor cantilever's surfaces, and not on either surface of the reference cantilever. Using fluorescence microscopy, we verified that both BSA and bBSA adsorb to an identical silicon nitride surface, and that SA binds to bBSA and not to BSA. We also verified with ellipsometry that thio-modified PEG prevents the adsorption of BSA, bBSA, and SA to a gold surface (data not shown).

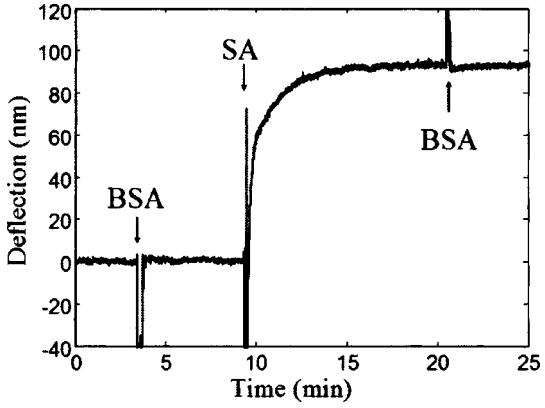

FIG. 4. Direct differential detection of biotin–streptavidin binding. Control injections (BSA) yield negligible differential bending. Streptavidin (SA) binds to biotin that is present only on one surface of the sensing cantilever, yielding a significant differential bending.

In conclusion, we have presented a microcantilever biosensor that generates an inherently differential signal and provides upstream suppression of unspecific effects. By enabling inherently differential detection, the sensor eliminates uncertainties that could be introduced by performing control experiments sequentially. The sensor enables the detection of protein molecules without the need of prior labeling, and its differential nature significantly enhances the stability, resolution, and reliability of label-free measurements. We anticipate that the minimal alignment requirements associated with the interferometer will be advantageous for point-of-use applications where a miniaturized detection setup is desirable.

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[1] H. J. Butt, J. Colloid Interface Sci. **180**, 251 (1996).
[2] J. Fritz, M. K. Baller, H. P. Lang, H. Rothuizen, P. Vettiger, E. Meyer, H.-J. Güntherodt, Ch. Gerber, and J. K. Gimzewski, Science **288**, 316 (2000).
[3] G. Wu, R. H. Datar, K. M. Hansen, T. Thundat, R. J. Cote, and A. Majumdar, Nat. Biotechnol. **19**, 856 (2001).
[4] Y. Arntz, J. D. Seelig, H. P. Lang, J. Zhang, P. Hunziker, J. P. Ramseyer, E. Meyer, M. Hegner, and Ch. Gerber, Nanotechnology **14**, 86 (2003).
[5] C. A. Savran, A. W. Sparks, J. Sihler, J. Li, W.-C. Wu, D. E. Berlin, T. P. Burg, J. Fritz, M. A. Schmidt, and S. R. Manalis, J. Microelectromech. Syst. **11**, 703 (2002).
[6] N. Loh, M. A. Schmidt, and S. R. Manalis, J. Microelectromech. Syst. **11**, 182 (2002).
[7] S. R. Manalis, S. C. Minne, A. Atalar, and C. F. Quate, Appl. Phys. Lett. **69**, 3944 (1996).
[8] D. L. Harame, L. J. Bousse, J. D. Shott, and J. D. Meindl, IEEE Trans. Electron Devices **34**, 1700 (1987).
[9] H.-F. Ji, K. M. Hansen, Z. Hu, and T. Thundat, Sens. Actuators B **72**, 233 (2001).
[10] J. Lai, T. Perazzo, Z. Shi, and A. Majumdar, Sens. Actuators A **58**, 113 (1997).
[11] R. C. Weast and M. J. Astle, *CRC Handbook of Chemistry and Physics*, 59th ed. (CRC, Boca Raton, FL, 1979).