

Differential surface stress sensor for detection of chemical and biological species

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We report a sensor consisting of two micromachined cantilevers (a sensing/reference pair) that is suitable for detection of chemical and biological species. The sensing strategy involves coating the sensing cantilever with receptors that have high affinities for the analyte. The presence of analyte is detected by determining the differential surface stress associated with its adsorption/absorption to the sensing cantilever. An interferometric technique is utilized to measure the differential bending of the sensing cantilever with respect to reference. Surface stress associated with hybridization of single stranded DNA is measured to demonstrate the unique advantages of the sensor. © 2008 American Institute of Physics. [DOI: 10.1063/1.2996411]

Microcantilever based sensors are increasingly being investigated to detect the presence of chemical and biological species in both gas and liquid environments. Thundat *et al.*¹ reported the static deflection of microcantilevers due to changes in relative humidity and thermal heating, and thus opened a myriad of possibilities for the use of atomic force microscopy (AFM) cantilever deflection technique for chemical and biological sensing. Cantilever based sensors have been demonstrated for alkanethiol self-assembled monolayers, proteins, antibodies and antigens, and nucleic acids (DNA/RNA).²⁻⁴ In the majority of the current state of art sensors, molecule absorption induced surface stress change is inferred from the deflection of a single or multiple laser beams reflected from the sensing surface. A large optical path is required between sensitized surface and position sensitive detectors to achieve high sensitivity in surface stress measurement. As a result, it is difficult to implement the sensing scheme into a single microfabricated device. In the current paper, we report a differential surface stress sensor that utilizes a single-mode fiber based Mach-Zehnder interferometer for measuring cantilever deflection and consequently, the detection of chemical and biological species. The interferometric technique is amenable to miniaturization and may facilitate the integration of all components of sensors into a single microfabricated chip. Surface stress associated with DNA hybridization is investigated to demonstrate the sensor's performance.

The differential surface stress sensor consists of two adjacent cantilevers, a sensing/reference pair, where only the sensing surface is activated for adsorption of chemical or biological molecules. Absorption/adsorption of analyte species on the sensitized surface is expected to induce differential bending and deflection between the sensing and reference cantilevers. The microcantilevers and a pair of microlens arrays (MLAs) are arranged in the optical arrangement shown schematically in Fig. 1 to measure the differential displacement between sensing and reference cantilevers. In this optical configuration, the incident laser beams at points A and C always arrive to points B and D, respectively,

regardless of their incident angle; and the differential bending produces a change in path length difference between the beams reflected from the two cantilevers.

After reflecting from the sensing and reference surfaces, the two beams accumulate a path length difference, l , equal to twice the differential displacement between sensing and reference surface. The beams are interfered to measure the path length difference and the differential surface stress ($\Delta\sigma$) between the two cantilevers is determined using Stoney's formula⁵

$$\Delta\sigma = \left[\frac{E}{3(1-\nu)} \right] \left(\frac{t}{L} \right)^2 l,$$

where E is the elastic modulus, ν is Poisson's ratio, L is the length, and t is thickness of the cantilevers. Measurement of the differential surface stress ensures that detected signal is proportional to the specific absorption of analyte species on the sensing cantilever and eliminates the influence of environmental disturbances such as nonspecific adsorption, changes in pH, ionic strength, and especially the temperature. Deflection of two laser beams reflected each from sensing and reference cantilevers may also be used for differential surface stress measurement but that setup will suffer from the following drawbacks: measurement sensitivity will

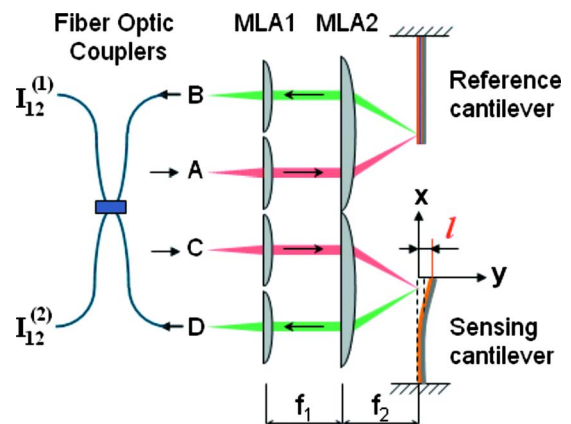


FIG. 1. (Color online) Principle of differential surface stress measurement.

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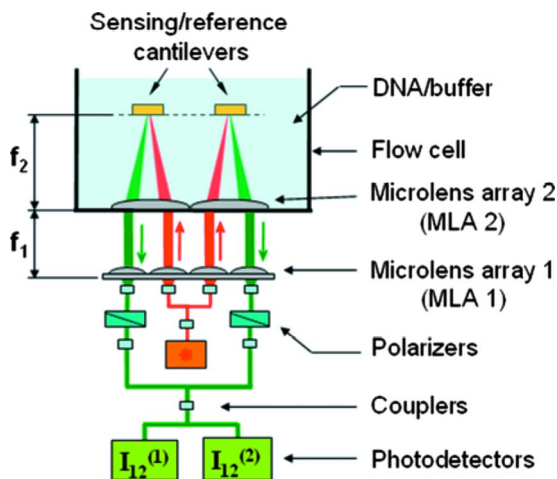


FIG. 2. (Color online) Optical circuit of differential surface stress sensor. MLA1 (diameter of $240\ \mu\text{m}$ and pitch of $250\ \mu\text{m}$) collimates beams and delivers to MLA2 (diameter of $900\ \mu\text{m}$ and pitch of $1\ \text{mm}$). Bidirectional couplers were used to split the reflected beams and direct one component toward photodetectors to measure the intensity of reflected beams.

again be proportional to the distance between cantilever and photodetector; and measured response will be determined by subtracting the two signals, which may lead to resolution losses. The unique advantages of the setup reported here are as follows: the sensor response is insensitive to environmental disturbance due to differential measurement of surface stress ensures and the resolution is independent of optical distance between cantilevers and photodetectors.

An optical circuit shown in Fig. 2 is utilized for assembling the surface stress sensor. In the system, two adjacent rectangular-tipless AFM cantilevers were used as a sensing/reference pair. The sensing and reference cantilevers are chemically modified and subsequently, mounted on a single column to ensure easy alignment of the optical setup. A pair of MLAs with lenses of 240 and $900\ \mu\text{m}$ diameter and pitches of 250 and $1\ \text{mm}$, respectively, was used to direct the beams toward the sensing/reference pair. Motorized and manual actuators were used to align the MLAs and to position the sensing/reference cantilevers in focal plane of second MLA2 (as shown in Fig. 2). A bidirectional coupler was applied to split the beam from a $633\ \text{nm}$ fiber coupled laser source and deliver it to MLA1 at a $50/50$ ratio. The reflected beams from the cantilevers were interfered using the second bidirectional coupler and the intensity of interfered beam was monitored using photodetectors. The polarization plane of the reflected beams was matched and common mode rejection was utilized to ensure maximum fringe visibility in the interfered beams. An isolation box covered all fiber couplers as well as sensor components to eliminate acoustic and vibrational noise from the system.

Silicon cantilevers used in the sensor realization are nominally $500\ \mu\text{m}$ long, $100\ \mu\text{m}$ wide, and $1\ \mu\text{m}$ thick (Nanoworld, Switzerland) with a top side coating of $5\ \text{nm}$ titanium and $30\ \text{nm}$ gold film. The cantilevers are batch produced with large variation of dimensions and mechanical properties from the manufacturer's quote.^{6,7} In order to accurately measure surface stress development, the thickness of each cantilever is calculated based on the experimentally measured spring constant with the material constants.⁶ Using contact mode atomic force microscopy, mean square roughness of the gold surface was found to be $2.07 \pm 0.23\ \text{nm}$ for

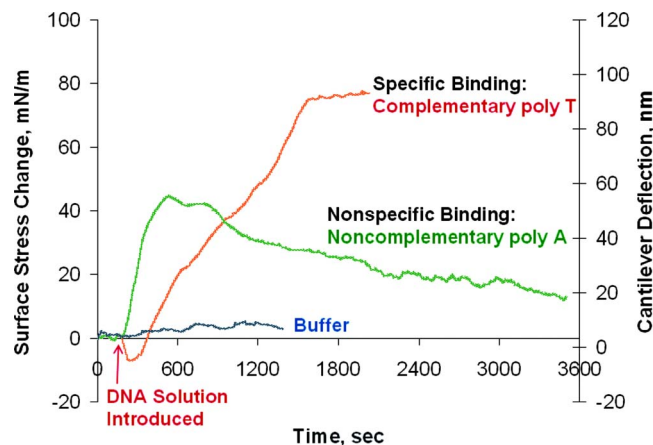


FIG. 3. (Color online) Differential surface stress change and corresponding cantilever deflections measured for the introduction of hybridization buffer with no oligonucleotide (buffer); $0.5\ \mu\text{M}$ poly(T) in hybridization buffer (specific binding); $0.5\ \mu\text{M}$ poly(A) in hybridization buffer (nonspecific binding).

the $750\ \text{nm}$ scan size and grains were found to be equiaxed with a size of $40 \pm 10\ \text{nm}$.

The surface stress change associated with hybridization of a surface immobilized $30\ \text{nt}$ polydeoxyriboadenosine [poly(A)] with its complementary $30\ \text{nt}$ polydeoxyribosymidine [poly(T)] was investigated to demonstrate the sensor performance. Oligonucleotides with the following sequences thiolated poly(A): $5'\text{-HS-(CH}_2\text{)}_6\text{-(A)}_{30}\text{-}3'$ and poly(T): $(\text{T})_{30}$ were purchased from Integrated DNA Technologies (Coralville, Iowa) and stored at $-20\ ^\circ\text{C}$ prior to the experiments. In preparation for the experiments, all cantilevers were cleaned by piranha solution (70% H_2SO_4 and 30% H_2O_2) for $30\ \text{min}$ and rinsed in de-ionized water and dried in the gentle N_2 flow. The sensing and reference cantilevers were incubated for $4\ \text{h}$ in $20\ \mu\text{M}$ of thiolated poly(A) in binding buffer ($50\ \text{mM}$ triethylammonium acetate, 25% ethanol, $\text{pH}\ 7.4$) to ensure that the poly(A) is only immobilized on the gold-coated surfaces. The reference cantilevers were then immersed for a further $4\ \text{h}$ in a solution of poly(T) in $1 \times \text{SSC}$ hybridization buffer ($0.15\ \text{M}$ NaCl and $0.015\ \text{M}$ sodium citrate, $\text{pH}\ 7.4$) (Ref. 4) in order to hybridize the poly(T) with the poly(A) coated on the gold film. As a result, the gold-coated surfaces of the sensing cantilevers were coated with single stranded poly(A) while the reference cantilevers were covered with hybridized DNA.

Three different experiments were carried out to demonstrate the sensor performance and to measure the surface stress associated with hybridization of surface immobilized poly(A) to complementary poly(T) oligonucleotides. The sensing and reference cantilevers were mounted in the sensor realization shown in Fig. 3 and the changes in phase difference between the reflected beams were monitored to determine the differential surface stress development. In the first experiment, only the hybridization buffer without poly(T) was introduced into the sensor flow cell to determine the influence of environmental conditions on sensor performance. In the second experiment, the two cantilevers were submerged in a $0.5\ \mu\text{M}$ poly(T) in hybridization buffer to measure the surface stress development due to DNA hybridization on the sensing cantilever. In the final experiment, a $0.5\ \mu\text{M}$ solution of noncomplementary poly(A)₃₀ in hybrid-

ization buffer was introduced to measure the surface stress due to nonspecific binding.

Surface stress changes observed during the three different experiments are plotted in Fig. 3. Introduction of hybridization buffer without any oligonucleotide does not produce a significant change in the surface stress signal. Hydrodynamic disturbance, index of refraction and temperature changes induced due to injection of hybridization buffer have no influence on sensor response due to the differential measurement. This result clearly demonstrates that use of sensing/reference pair ensures the rejection of common mode and makes the sensor response insensitive to environmental disturbances.

Hybridization of the complementary strands on the sensing cantilever produces a compressive surface stress change. On introduction of the complementary strands, the sensor undergoes an initial tensile surface stress change followed by a rapid build-up of compressive surface stress that reaches saturation value of 76 ± 2 mN/m in approximately 20 min. The magnitude of the surface stress changes measured in the experiments compares well with previous reports.^{3,8} The surface stress change during the hybridization has been reported to be both compressive⁴ and tensile.⁸ Compressive surface stress is hypothesized to occur due to binding of negatively charged complimentary strands and corresponding increase in negative charges on the surface and consequently greater repulsion between the bound surfaces species.⁴ The tensile surface stress change during hybridization is attributed to reduction in steric hinderances between single stranded DNAs (ssDNAs) due to transformation from a flexible single strand random coil to stiff hybridized double stranded DNA.⁸ In the current experiments, the initial tensile stress change may be due to the reduction in steric hinderances but as the hybridization of DNA continues, the Coulombic repulsion between the surface bound chains leads to the development of compressive surface stress.

Injection of noncomplementary ssDNA produced the most interesting results, differential surface stress signal rapidly built up to a value of 40 ± 5 mN/m during the first 10 min and then slowly decayed back to a value close to zero over the next 1.5 h. Similar changes in surface stress on introduction of noncomplementary strands have been previously reported⁹ and have been attributed to hydrodynamic effects induced due to the injection. The results of the first experiment clearly indicate that the sensor response is not influenced by such effects and the measure response may be due to transitory adsorption of the ssDNA on the gold or silicon surface,^{8,10} reversed-Hoogsteen T·AT triplex forma-

tion on the reference cantilever,¹¹ or sampling interactions involving reverse Hoogsteen or other configurations between the poly(A) chains on sensing cantilever. We are currently conducting further investigations to identify the mechanisms responsible for rapid stress build-up followed by the slow decay on introduction of noncomplementary ssDNA strands.

Surface stress measurements associated with DNA hybridization clearly demonstrate the unique advantages of the differential surface stress sensor. Measurement of differential bending of sensing cantilever with respect to reference cantilever ensures that sensor response is independent of environmental disturbances. Sensitivity of sensor measurement is not dependent on distance between the sensing surface and detector, as a result, surface stress sensor is amenable for miniaturization and array of sensors can be integrated with other systems on a single microelectro mechanical system device.

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