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HTML ABSTRACT + LINKS

Direct force measurements of biomolecular interactions by nanomechanical force gauge

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Without using the laser and optical detection method of an atomic force microscope (AFM), direct force measurements of biomolecular interactions in biological solution are accomplished by a nanomechanical force gauge. The device consists of integrated nanoscale single-crystal Si cantilever and reading scales, which allow the direct measurements of biomolecular interactions by reading the deflection of the cantilever with an optical microscope. The spring constant of the cantilevers was calibrated by measuring the resonant frequency under electrostatic force excitation, and the minimum value among the designed devices shows 80 pN/μm. The piconewton regime dissociation force between a biotinylated bead and streptavidins in an aqueous solution was directly measured with this device. © *2005 American Institute of Physics*. [DOI: 10.1063/1.1906332]

Highly sensitive microprobes such as atomic force microscope (AFM), fluorescence detection, and optical trapping have been recently introduced as proper tools to quantitatively characterize molecular interactions at the piconewton level such as single molecular mechanics, cell adhesion, or dissociation strength between biomolecules. In particular, an AFM consisting of a microcantilever, laser, optical apparatus, and detector, has been used for the studies of the mechanical behavior of biomolecules or living cells in both air and liquid environments. Many efforts have been put into the development of microcantilever-based sensors such as atomic force microscope for the detection of physical phenomena and chemical reactions.[1–3] However, most sensors require extra optical components and detectors. In addition, the laser optical alignment is often a cumbersome and time-consuming task.

Unlike an AFM or other microprobes, a nanoscale single-crystal Si cantilever with reading scales called nanomechanical force gauge (NFG) is an alternative to having a capability of direct reading without laser amplification. The force gauge is also cost effective to measure the interactions between biomolecules in the piconewton regime since it is fabricated with a similar cost of an AFM tip. The device can be easy to set up with an optical microscope and the simple and direct force measurement can be conducted by reading the lateral deflection of a highly sensitive nanoscale cantilever through microscope objective.

This article presents the fabrication, calibration, and demonstration of nanomechanical force gauges. The microfabrication of a nanoscale cantilever with lateral flexibility was based on thermal oxidation of single-crystal silicon. The calibration of the devices was carried out by measuring the resonant frequencies under an electrostatic force excitation. As an example of the biomolecular applications, the direct measurements of the dissociation strength of proteins in an aqueous solution were demonstrated.

The NFG consists of a freestanding ultra thin nanoscale cantilever with a sample holding stage at the free end as shown in Fig. 1. The constrained end of the cantilever is anchored to a stationary frame, which includes a reading

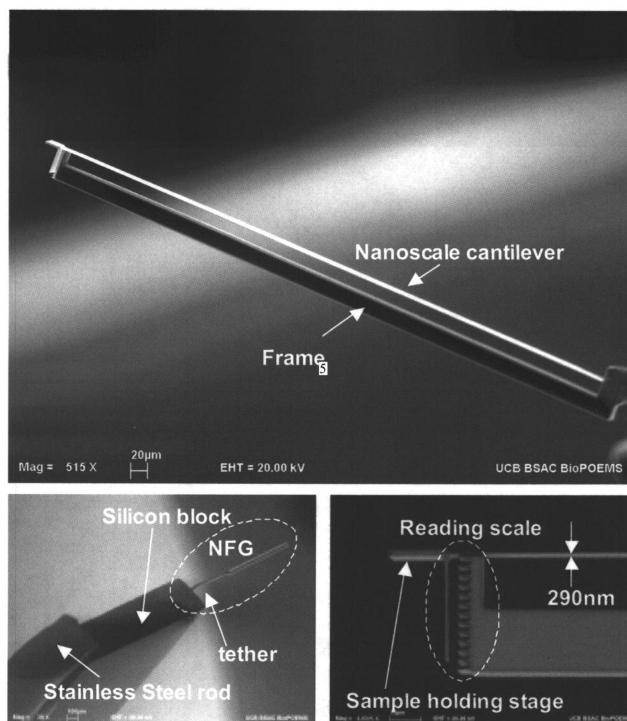

FIG. 1. SEM images of a nanomechanical force gauge consisting of a sample holding stage, a nanoscale cantilever, a reading scale with 13 ticks, a frame, and a tether.

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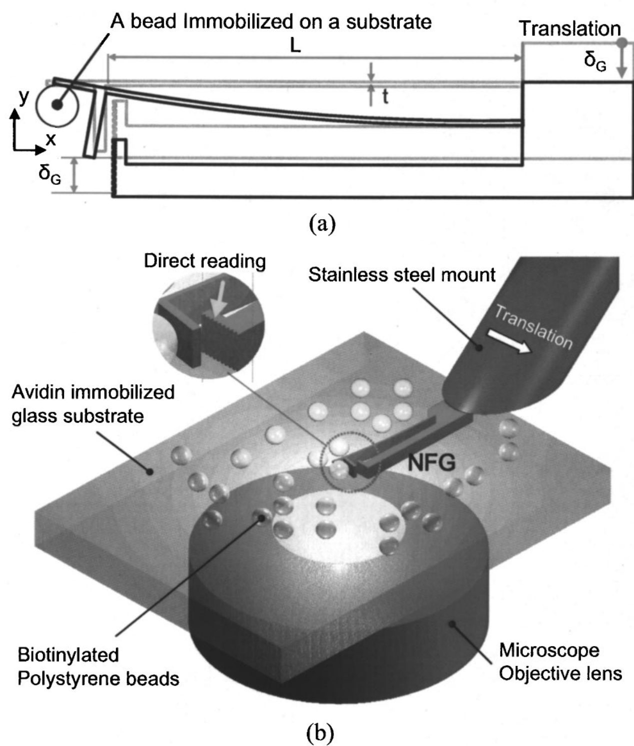

FIG. 2. Principle of measurement in a NFG: (a) top view, where δ_G is translation and (b) an experimental setup on a optical microscope.

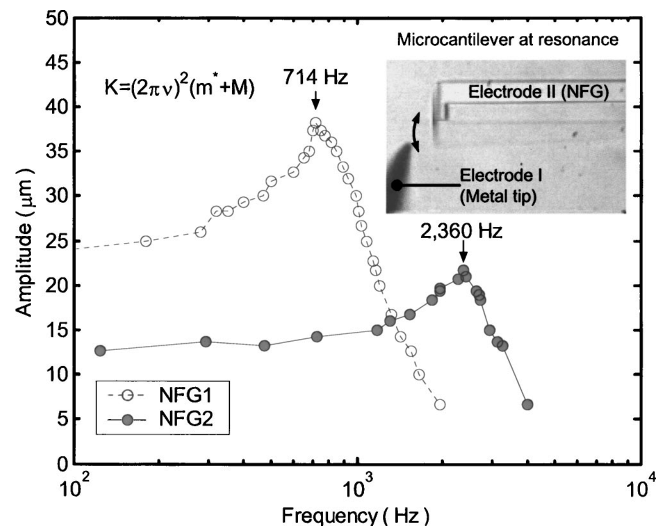

FIG. 4. Calibration of the spring constants through resonant frequency measurements of two different NFGs (different length) by electrostatic force excitation.

scale with 13 ticks spaced 3 μm distance apart to measure the displacement of the cantilever relative to the stationary frame. This device is glued with a stainless steel rod and then operated with a three-axis manipulator. Once the sample holding stage contacts a target sample, which is bound onto the surface by biomolecular interactions such as protein association, small force at the piconewton level for dissociating the molecular interaction is applied to the sample target by precisely translating a NFG with a three-axis manipulator. The cantilever in the NFG is deflected until biomolecular interactions are dissociated at δ_G as shown in Fig. 2(a). The deflection (δ_G) is directly measured by reading the tick changes in the reading scales relative to the frame through a microscope objective as illustrated in Fig. 2(b). The exact force for each tick movement can simply be calculated by multiplying the number of ticks of a sample holding stage by the spring constant of a cantilever. In order to increase the stability of the device during the operation and preventing the cantilever from being bent out of plane, the cantilever features a rectangular area with a high aspect ratio of over 100 to 1 (0.29 μm $\times$ 30 μm). The sensitivity of a NFG can be improved by either increasing the length of the cantilever or thinning the width, and the resolution is determined by the distance between ticks in reading scales.

The outline of the batch microfabrication procedures of the NFG is shown in Fig. 3. First, 30 μm deep trenches were made in a silicon substrate by deep reactive ion etching (Step 1). The trenched structure was covered with a 1 μm thick oxide by the first thermal oxidation (Step 2). In order to make the 30 μm deep single-crystal silicon structure with a cantilever, the wafer was wet-etched in tetramethyl ammonium hydroxide (TMAH), until the illuminating light came through the thermal oxide layer on the front side (Step 3). Finally, the nanoscale thickness (290 nm) of a single-crystal silicon cantilever was precisely controlled by the second oxidation (Step 4). After the thermal oxide layer on the structure was completely removed in HF (Step 5), the structure was still anchored to the substrate with a silicon tether, which was designed large enough to see by eye. The NFG was set free from the substrate by breaking the tether. The gauge was carefully glued on the surface of a silicon block that was

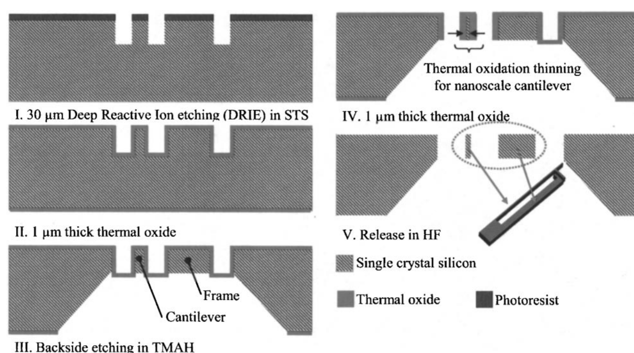

FIG. 3. Microfabrication procedures.

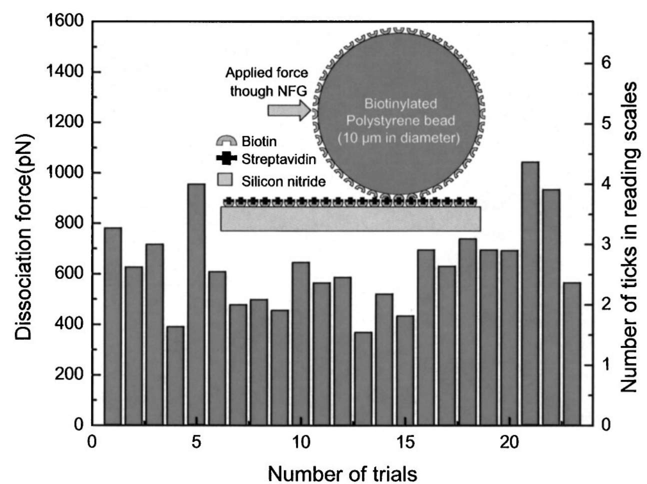

FIG. 5. Dissociation force (636 ± 176 pN, 2.7 ± 0.8 ticks) of streptavidins-biotins measured by a NFG with the spring constant of 80 pN/μm and each reading tick of 236 pN.

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TABLE I. Physical dimensions of nanomechanical force gauges and a comparison between the measured and calculated spring constants.

	Dimensions of nanoscale cantilever					Measured resonant frequency	Spring constant	
	$l(\mu m)$	$t(\mu m)$	$d(\mu m)$	$M(pg_m)$	$m^*(pg_m)$	ω_n(Hz)	Measured k(pN/μm)	Calculated k(pN/μm)
NFG 1	744	0.29	30	0.3	3.67	714±10	80±2	71
NFG 2	395	0.29	30	0.3	1.95	2,360±50	494±20	526

mounted on the end surface of a stainless steel rod with silver epoxy.

The sensitivity of a NFG is determined by the spring constant of the nanoscale cantilever. The cantilever is considered as an end-loaded beam of a rectangular cross section, since it has a mass of the sample holder at the end. The spring constant estimated by using beam theory is given by $k=Et^3d/4l^3$, where E is the elastic modulus of single crystal silicon, t is the thickness, d is the depth, and l is the length of the cantilever. After the measurement of the mass and resonant frequency of the cantilever, the spring constant is given by $k=(2\pi v)^2(m^*+M)$, where ν is the resonant frequency of the cantilever, m^* is the effective mass ($m^*=0.24\ m_b$, where m_b is a mass of the cantilever), and M is a mass added at the free end corresponding to the sample holding stage in a NFG.[4] The masses (m_b and M) were calculated from the measured geometry of the cantilever and the bulk density of silicon (2.32 g/cm^3). The resonant frequency was measured with an electrostatic excitation method. DC bias (16 V) and ac voltages (20 V) between electrode I (metal tip) and electrode II (NFG) were applied and then the resonance of the cantilever was clearly observed in air as shown in Fig. 4. The spring constants measured by resonant frequencies reasonably corresponds to the estimated values within ten percents as shown in Table I.

The measurement of the dissociation force between streptavidin and biotin on polystyrene latex microspheres with 11 μm diameter was demonstrated using a NFG with a nanoscale cantilever (NFG 1). The samples were prepared on a single-crystal silicon wafer deposited with 3000 Å thick silicon nitride. Alternatively a transparent slide glass can also be used to test with an inverted microscope, which allows manipulating the NFG without the restriction of the working distance. The wafer was diced into approximately 1 cm $\times$1 cm dies. The dies were soaked overnight in a biotin bovine serum albumin (BBSA) solution. The next day, the solution was removed and each die was washed twice with a fresh phosphate buffer saline (PBS) buffer. The samples were incubated with avidin from egg white for 20 min at room temperature and then washed several times with a fresh PBS buffer solution. Both the BBSA and the avidin were purchased from Sigma-Aldrich (St. Louis, MO). Biotinylated polystyrene latex beads of 11 μm in diameter (Bangs Laboratories, Fishers, IN) were added immediately afterward and allowed to bind to the substrate with avidin. After the sample preparation, NFG was set up under 25$\times$ objective lens and manipulated by a three-axis positioner to pick a target bead among the randomly distributed beads. Several measurements on the sample with avidin concentration of 1 mg/ml were made on the different beads. As shown in Fig. 5, the mean value and standard deviation of the deflection were 2.7±0.8 ticks, which correspond to 636±176 pN.

In this work, a piconewton regime measurement of biomolecular interactions in an aqueous solution by the NFG was presented. The NFG has a capability of direct reading without any optical amplification. The optimization of the design and microfabrication can offer the NFG with the sensitivity of 1 pN/μm. We believe the device may provide a simple and useful tool for quantifying folding and unfolding nanomechanism of individual proteins and binding of single ligand receptor pairs.

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