Piconewton Regime Measurements of Biomolecular Interactions by Nanomechanical Force Gauge

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Abstract — A piconewton regime measurement of biomolecular interactions in an aqueous solution by a novel Nanomechanical Force Gauge (NFG) is presented in this paper. A highly sensitive nanoscale cantilever with a spring constant, which is thousand times smaller than that of an atomic force microscope (AFM) microcantilever, is fabricated by a batch process. The NFG has a capability of direct reading without any optical amplification. The control of nanoscale thickness of a single crystal silicon cantilever is done by a thermal oxidation process. The deflection of the cantilever, corresponding to piconewtons is directly measured by reading the tick movements in the reading scale of the NFG under the microscope. The spring constant of the NFG is calculated by identifying the natural frequency using electrostatic force excitation, and the minimum value of the designed device was 78.6 pN/μm. As an example of the biomolecular applications, the dissociation between a biotinylated bead and avidins is measured, and the mean is 636 pN. The NFG has the potential of 1 pN/μm sensitivity through the nanofabrication technology as well as serving as an inexpensive and powerful substitute for an atomic force microscope in studying bio-molecular interactions.

I. INTRODUCTION

Highly sensitive microprobes such as atomic force microscopy (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, which consist 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 cantilever-based sensors for the detection of physical phenomena and chemical reactions [1, 2, 3]. However, most of the sensors require extra optical components and detectors. In addition, the laser optical alignment is often a cumbersome and time-consuming task for the optical amplification of signals.

Unlike an AFM or other microprobes, the NFG introduced in this paper has a capability of direct reading without any laser, detector, and optical amplification. It is a simple and robust device to install and measure. In addition, the NFG is one of the most inexpensive ways to measure the interaction between biomolecules in the piconewton regime since it is fabricated with a similar cost of an AFM tip. The NFG is easy to set up on microscope and can be measured by the direct reading of the tick movements through the objective lens as illustrated in Fig. 1. Simultaneous force measurement and epifluorescence detection can be also performed directly in an epifluorescence microscope without optical amplifications.

This paper reports the fabrication, calibration, and demonstration of a NFG with a highly sensitive nanoscale cantilever. The microfabrication of a nanoscale cantilever with lateral flexibility is described using thermal oxidation. The calibration of the device is carried out through measuring the natural frequency by an electrostatic force excitation source. As an example of the biomolecular applications, the measurement of the dissociation force of proteins is demonstrated with the NFG.

Fig. 1. An apparatus of piconewton measurement of biomolecular interactions by a Nanomechanical Force Gauge (NFG) with a nanoscale cantilever.
II. OPERATION AND FABRICATION

A. Principle of Operation

The NFG consists of a freestanding ultra thin nanoscale cantilever with a sample holding stage at the free end. The constrained end of a microcantilever is anchored to a stationary frame, which includes reading scale with 13 ticks spaced 3 \( \mu m \) apart to measure the displacement of the cantilever relative to the stationary frame as shown in Fig. 2. This device is anchored to a stainless steel rod and constrained to have no rotation, which is connected to a three-axis manipulator. Once the sample holding stage contacts a sample target, which is bound onto the surface by molecular interactions such as protein association, the small force at the piconewton level for dissociating the molecular interaction is applied to the sample target by precisely positioning the NFG. The cantilever in the NFG, which has a spring constant of thousand times smaller than that of an AFM microcantilever in the AFM, is deflected by \( \delta_G \) as described in Fig. 3. The displacement (\( \delta_G \)) is directly measured by reading the deflection of a microcantilever relative to the frame with the tick changes in the reading scale through the objective lens of a microscope. The exact force for each tick movement can be simply calculated by multiplying the number of ticks of a sample holding stage at the time by the spring constant of a cantilever.

![Fig. 2. Nanomechanical force gauge consist of a sample holding stage, a nanoscale cantilever, a reading scale with 13 ticks, a frame, and a tether.](image)

Fig. 4. Batch nanofabrication procedure of the NFG.

B. Batch Nanofabrication Procedure

The outline of the batch nanofabrication procedure of the NFG is shown in Fig. 4. First, 30 \( \mu m \) deep trenches are made in a silicon substrate by deep reactive ion etching (Step 1). The trenched structure is covered with 1 \( \mu m \) thick oxide by the first thermal oxidation (Step 2). In order to make the 30 \( \mu m \) deep single crystal silicon structure with a microcantilever, the wafer is wet etched in TMAH, until light comes through the thermal oxide layer on the front side (Step 3). Finally, the nanoscale thickness (290 \( nm \)) of a single crystal silicon cantilever is precisely controlled by the second oxidation (Step 4). The thermal oxide layer on the structure is completely removed in HF (Step 5). The structure is still anchored to the substrate with silicon tether, which is designed large enough to see by eye. By cutting a tether with a tweezer, the NFG is set free from the substrate. The gauge is carefully glued on the surface of a silicon block, which is mounted on the end surface of a stainless steel rod with silver epoxy.

![Fig. 3. The measurement principle of a NFG, where \( \delta_G \) is the relative deflection of a lateral microcantilever in a reading scale.](image)
Table 1. The dimensions, natural frequency, and spring constant of two cantilevers with different length.

<table>
<thead>
<tr>
<th></th>
<th>$L$</th>
<th>$t$</th>
<th>$d$</th>
<th>$M$</th>
<th>$m^*$</th>
<th>$\omega_0$</th>
<th>$k$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(nm)</td>
<td>(nm)</td>
<td>(nm)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(Hz)</td>
<td>(pN/\mu m)</td>
</tr>
<tr>
<td>NFG I</td>
<td>744</td>
<td>0.29</td>
<td>30</td>
<td>0.3</td>
<td>3.67</td>
<td>714</td>
<td>78.7</td>
</tr>
<tr>
<td>NFG II</td>
<td>395</td>
<td>0.29</td>
<td>30</td>
<td>0.3</td>
<td>1.95</td>
<td>2360</td>
<td>518.7</td>
</tr>
</tbody>
</table>

C. Natural Frequency Measurement

The sensitivity of the NFG as a force sensor is determined by the spring constant of the nanoscale cantilever. The cantilever is considered as an end-loaded beam of rectangular cross section, since it has a mass of the sample holder at the end. The spring constant of the cantilever is given by

$$k = \frac{E t^3 d}{4 L^2}$$  \hspace{1cm} (1)

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. Since the spring constant is proportional to the elastic modulus, the variation of the elastic modulus is critical to determine the spring constant. Even though the elastic modulus is not exactly known, the spring constant can be determined by the dimensions and natural frequency of the cantilever using Eq. (2) [4].

$$k = (2 \pi \nu)^2 (m^* + M)$$  \hspace{1cm} (2)

where $\nu$ is the natural 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, which means a mass of the sample holding stage in a NFG. The dimensions of the NFG can be measured by a scanning electron microscope (SEM). The masses, which are $m_b$ and $M$, were calculated by using the dimensions of the cantilever and the bulk density of silicon, 2.32 g/cm$^2$.

The natural frequency was measured by recording the deflection amplitude of the cantilever with an electrostatic excitation method. Fig. 5 shows the large deflection of the cantilever due to the electron charging effect on the surface captured by the fast scanning mode in a scanning electron microscope.

![Fig. 5. The deflection of microcantilever due to the electron charging effect on the surface captured by the fast scanning mode in a scanning electron microscope.](image)

Fig. 6. Experimental results of natural frequency measurements of two different NFGs (different length) by the electrostatic force excitation.

III. MEASUREMENT IN PICONEWTON REGIME

As an example of the biomolecular applications using the NFG, the dissociation between streptavidin and biotin on polystyrene latex microspheres with 10.9 µm diameter was measured by reading the deflection of a nanoscale cantilever (dimension: 290 nm x 20 µm x 744 µm, spring constant: 77.6 pN/µm) as demonstrated in Fig. 7. We prepared the samples on a single crystal silicon wafer deposited with 3000 Å thick silicon nitride. A transparent slide glass can also be used to test with the inverted microscope, which allows manipulating the NFG without the restriction of the working distance. The wafer was then diced into approximately 1 cm x 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.
buffer saline (PBS) solution. The samples were incubated with avidin from egg white for twenty minutes at room temperature and then washed several times with a fresh PBS buffer solution. Both the BBSA and the avidin were purchased from Sigma. Biotinylated polystyrene latex beads of 10.9 \( \mu \text{m} \) in diameter (Bangs Laboratories, Fishers, IN) were added immediately afterwards and allowed to bind to the substrate with avidin. After the preparation of the sample, the NFG was set up under 25 x objective lens and manipulated by three-axis micropositioner in order to touch a biotinylated bead. Fig. 7 shows the cantilever of a NFG is deflected by seven ticks in a reading scale due to the protein dissociation. Since the spring constant of the cantilever is 78.7 \( pN/\mu\text{m} \) and the tick-to-tick distance is 3 \( \mu\text{m} \), the force corresponding to the deflection of seven ticks is calculated to be 1,653 \( pN \). Fig. 8 shows that several measurements on the sample with avidin concentration of 1 \( \text{mg/ml} \) were made on the different beads and the mean value of the deflection is 2.7 ticks, which corresponds to 638 \( pN \).

**IV. CONCLUSION**

In this work, a piconewton regime measurement of biomolecular interactions in an aqueous solution by the NFG is presented. The NFG has a capability of direct reading without any optical amplification. Using a novel batch fabrication, a highly sensitive nanoscale cantilever with a spring constant, which is thousand times smaller than that of AFM cantilever, is fabricated. The control of nanoscale thickness of a single crystal silicon cantilever is done by a thermal oxidation process. The optimization of the design and fabrication will provide the NFG with the sensitivity of 1 \( pN/\mu\text{m} \). It will allow to study single molecule biophysics as well as clinical applications. We believe the NFG will provide a useful tool for quantifying folding and unfolding nanomechanism of individual proteins and binding of single ligand receptor pairs.

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**REFERENCES**


