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Microcantilever-based Biosensor using Frequency Modulation Detection Method in Liquids

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Abstract–We have developed a microcantilever-based biosensor using the frequency modulation (FM) detection method in liquids, which is capable of detecting the resonance frequency shift of the cantilever induced by the adsorption of the biomolecules on the cantilever. Since we found that the frequency fluctuation of the cantilever is strongly dependent on the noise-equivalent displacement in the displacement sensor, we decreased the sensor noise down to the level which is close to the shot noise limit. We employed an intensity-modulated laser light source to excite the cantilever oscillation, instead of using the piezoelectric actuator in order to avoid excitation of the mechanical resonances of the liquid cell. We successfully detected the adsorption of the DNA oligomer molecules on the gold-coated cantilever using the FM detection method.

1. Introduction

Microcantilever-based biosensors have been widely studied as one of the promising label-free biosensors, where deflection or resonance frequency shift of the cantilevers due to the change of surface stress or mass are detected, induced by specific interactions between biomolecules such as hybridization of nucleic acids and antigen-antibody reaction. However, it has been quite difficult to realize real-time sensing of the resonance frequency (f_0) of the self-oscillated cantilever in liquid due to extremely low mechanical Q-factor of the cantilever. Moreover, the coupling of the mechanical resonance frequency of the cantilever to those of liquid cell makes reliable frequency tracking difficult when the piezoelectric actuator is used to excite the cantilever.

2. Instrumentation

The minimum detectable mass in the microcantilever-based mass sensor δm is determined as

$$\delta m = \frac{2m}{f_0}\delta f \quad ,$$

where m and δf are the effective mass of the cantilever in the medium and the minimum detectable resonance frequency shift of the cantilever. In high-Q environments such as in vacuum, the fluctuation of the oscillator frequency can be ignored and δf is determined by the noise of the deflection sensor. However, in low-Q environments, one has to consider the contribution of the fluctuation of the oscillator frequency in addition to that of the deflection sensor noise.

The self-oscillation frequency of the resonator is the frequency at which the phase criterion,

$$\theta_{\mathrm{cantilever}}(f) + \theta_{\mathrm{circuit}}(f) = 2n\pi \, ,$$

is met, thus the self-oscillation frequency is determined by the phase response of the resonator. Therefore, the oscillation frequency fluctuates if the phase response of the resonator fluctuates. In other words, the phase noise of the resonator turns into the frequency noise with the factor of the slope of the phase versus frequency curve at the oscillating frequency with the following equation,

$$\left.\frac{d\theta}{df}\right|_{f=f_0} = -\frac{2Q}{f_0} \, .$$

Since the phase noise is greatly amplified in proportion to the frequency derivative of the phase shift in the resonator, as shown in Fig. 1,

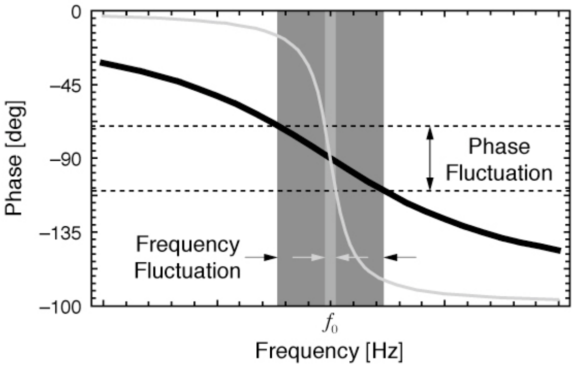

FIG 1: Schematic of the evolution of the phase fluctuation to the frequency fluctuation. Gray and black curves show the phase response of the high-Q and low-Q cantilevers, respectively.

Therefore the minimum detectable frequency shift of a low-Q cantilever becomes inversely proportional to the Q-factor, which can be described by the following equation,

$$\delta f = \frac{f_0}{\sqrt{2} Q A_0} n_{\mathrm{ds}} \sqrt{B} ,$$

where n_{ds} is the noise-equivalent displacement in the displacement sensor[1]. A_0 and B are the oscillation amplitude of the resonator and the measurement bandwidth, respectively. Therefore it is very important to reduce the phase noise (displacement sensor noise) at the frequency range close to the resonance frequency. Therefore we developed an ultra-low-noise optical beam deflection (OBD) sensor by refining optics and electronics. The noise-equivalent displacement is on the order of 10fm/sqrtHz.

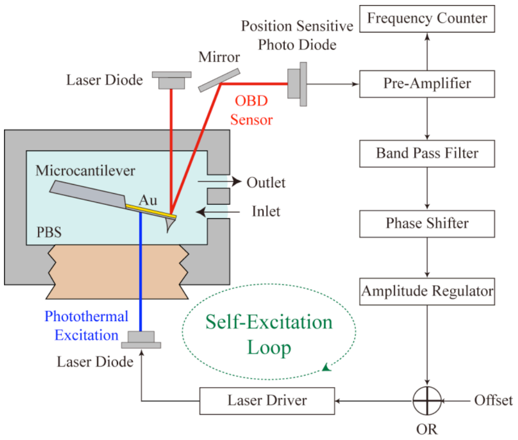

FIG 2: Schematic of an experimental setup for the microcantilever-based biosensor using the photothermal excitation method.

When the cantilever is oscillated at its resonance frequency by a piezoelectric actuator, it is difficult to avoid the excitation of the mechanical resonance frequency of the liquid cell. In order to avoid the self-oscillation at the spurious resonance frequency caused by the mechanical resonance of the liquid cell, we used a band-pass filter in the positive feedback electronics. We also employed the photothermal excitation method, in which the cantilever vibration was induced by the focused intensity-modulated blue-violet laser light.

An ideal frequency response free from the effect of the mechanical resonance of the liquid cell was observed by using the photothermal excitation method.

3. Experiment

We used gold-coated cantilevers immersed in 100 mM phosphate buffer saline. A phosphate buffered saline containing thiol-terminated single-stranded DNA oligomer (20-mer) was injected from a microsyringe using a syringe pump at a flow rate of 100 ul/min. The resonance frequency shift of the cantilever of about 30 Hz was observed after the injection, which can be explained by the mass loading due to chemical adsorption of thiol-terminated single-stranded DNA molecules onto the gold surface, as shown in Fig. 3.

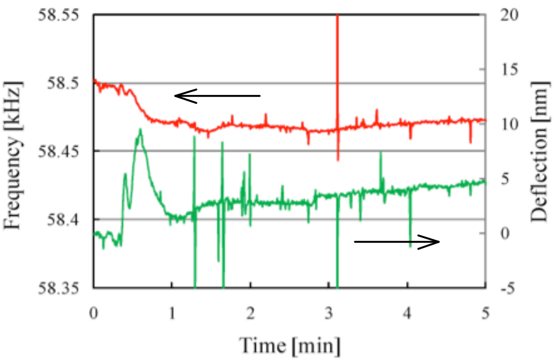

FIG 3: Plot of the resonance frequency shift after the injection process of a PBS containing thiol-terminated DNA oligomer molecules.

References

[1] K. Kobayashi, H. Yamada, and K. Matsushige, “Frequency noise in frequency modulation atomic force microscopy”, *Rev. Sci. Instrum.*, vol.80, 043708, 2009.