Real-Time Mass Sensing by Nanomechanical Resonators in Fluid

Murali K. Ghatkesar [1&]*, V. Barwich[1&], T. Braun[2&], A.H. Bredekamp[1], U. Drechsler[3], M. Despont[3], H. P. Lang[1,3], M. Hegner[1], and Ch. Gerber[1,3]

[&]Equally contributed

[1]Institute of Physics, University of Basel, Switzerland

[2]Maurice E. Müller Institute, Biozentrum, University of Basel, Switzerland

[3]IBM Zurich Research Laboratory, Rüschlikon, Switzerland

Abstract

We have developed a sensitive method for real-time mass sensing in a fluid using a microfabricated array of nanomechanical cantilevers actuated at their resonance frequencies. The sensor platform consists of a streptavidin layer immobilised onto gold-coated cantilevers and interacts with biotin-labeled latex beads. The addition of mass involved with this process decreased the resonance frequency. By monitoring frequency spectra of higher harmonics we measured a mass sensitivity of 3.3 Hz/pg. Our data demonstrate that the sensitivity can be increased by operating the cantilever at higher harmonics.

Keywords

Cantilever Sensor, Bio-Sensor, Mass measurement

INTRODUCTION

The concept of using cantilevers for sensing applications has added a new dimension to the field of sensors [1], [2]. These mechanical sensors can be operated in both static and dynamic mode. In the static mode, the deflection of the cantilever is produced because of a change in surface stress. In the dynamic mode, the resonance frequency change occurs owing to a change in mass or/and spring constant of the cantilever. In the decade since its inception, researchers have demonstrated its sensitivity of single base pair mismatch detection in DNA hybridization [3] in static mode, and of single airborne virus particle [4] mass detection in dynamic mode. Until now mass detection using dynamic mode has been developed to work in gaseous environment. Bio-sensing requires the sensor to operate in aqueous environment. However, dynamic mode in a fluid still remains a challenge. Owing to the heavy hydrodynamic damping on a resonating cantilever, the quality factor Q of the resonance peaks drops to much lower values than in air. Fluid is dragged along with the resonating cantilever, adding to the oscillator's mass, thus lowering the resonance frequency [5, 6].

In this article we report real-time mass measurement of latex beads using the biotin streptavidin interaction in a fluid environment. The frequency is monitored in real time as the individual biotin-labeled latex beads bind to the streptavidin, which is immobilized on the cantilever. We actuate our cantilevers at higher harmonics, taking advantage of higher Q-factors, which lead better sensitivity even with long cantilevers.

*corresponding author, Email: murali.ghatkesar@unibas.ch

THEORY

The resonance frequencies of the different harmonics of a cantilever sensor vibrating in flexural mode in vacuum are expressed as [7]

$$f_n = \frac{\alpha_n^2}{2\pi}\sqrt{\frac{Et^2}{12\rho l^4}}, \tag{1}$$

where the α_n are related to the different eigenvalues of the harmonics ($\alpha_1 = 1.875$, $\alpha_2 = 4.694$, $\alpha_3 = 7.854$, $\alpha_4 = 11.000$, $\alpha_{5\ldots n} = \pi(n - 0.5)$), ρ is the mass density, E is the modulus of elasticity, l is the length, and t is the thickness of the cantilever, respectively.

A good approximation to describe resonance frequencies for a rectangular cantilever beam immersed in fluid is the well-known inviscid model [8]. It assumes the liquid to be incompressible in nature. With this model, the resonance frequencies in a fluid, f_{nl}, are given by

$$f_{nl} = f_n\left(1 + \frac{\pi\rho_l w}{4\rho_c t}\right)^{-1/2}, \tag{2}$$

where ρ_l and ρ_c are the density of the fluid medium and cantilever, respectively.

The spring constant k for a rectangular cantilever beam is defined as

$$k = \frac{Et^3 w}{4l^3}, \tag{3}$$

where w is the width of the cantilever. Expressing Eq. (2) in terms of the spring constant k and substituting $\rho = \frac{m_c}{lwt}$ yields

$$f_{nl} = \frac{\alpha_n^2}{2\pi}\sqrt{\frac{k}{3m_c}}\left(1 + \frac{\pi\rho_l w}{4\rho_c t}\right)^{-1/2}, \tag{4}$$

where m_c is the mass of the cantilever. To account for an uniformly loaded mass Δm on the surface of the cantilever, and assuming that the spring constant does not change, Eq. (4) can be modified as

$$f'_{nl} = \frac{\alpha_n^2}{2\pi}\sqrt{\frac{k}{3(m_c + \Delta m)}}\left(1 + \frac{\pi\rho_l w}{4\rho_c t}\right)^{-1/2}. \tag{5}$$

For $\Delta m \ll m_c$

$$f'_{nl} \approx f_{nl}\left(1 - \frac{1}{2}\frac{\Delta m}{m_c}\right) . \quad (6)$$

The mass load Δm in terms of the frequency shift Δf is

$$\Delta m = \frac{2m_c \Delta f}{f_{nl}} , \quad (7)$$

where $\Delta f = f_{nl} - f'_{nl}$.
The sensitivity of a mass-loaded cantilever can be defined as

$$S = \frac{\Delta f}{\Delta m} = \frac{f_{nl}}{2m_c} , \quad (8)$$

indicating that the sensitivity increases with the harmonic of the cantilever vibration.

EXPERIMENT

Micromechanical cantilever arrays consisting of eight silicon cantilevers of 500 μm length, 100 μm width and 1 μm thickness (Fig. 1) were fabricated at the IBM Zurich Research Laboratory. The spring constant of the cantilevers in the fundamental mode was 0.03 N/m.

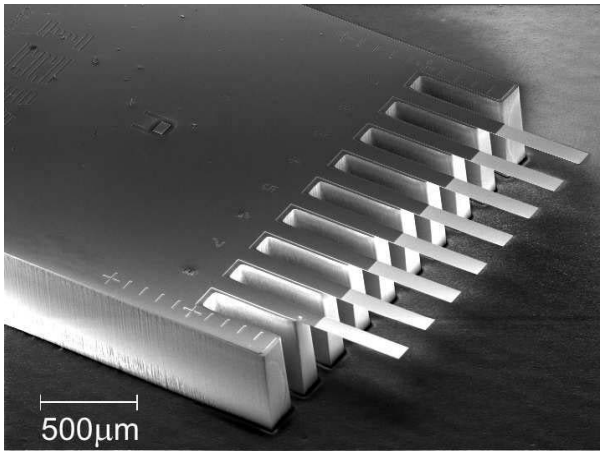

Figure 1. Scanning electron microscopy (SEM) image of the cantilever array.

Measurement Setup.

The principle of the optical deflection detection system used is shown in Fig. 2. The measurement setup consists of an array of vertical cavity surface-emitting lasers (VCSEL, Avalon Photonics, Switzerland), collimating optics, a position-sensitive detector (PSD, SiTek, Sweden), a syringe pump (Kent Scientific Corporation, USA) and a home-made fluid cell having a volume of 6 μl. A constant fluid flow rate in the fluid cell was maintained by the syringe pump. The cantilever array was excited by a sinusoidal signal applied to a piezoelectric actuator placed below the cantilever chip body. This method of excitation resulted in clean cantilever resonance peaks, avoiding vibrations from the fluid and the fluid cell as would occur if the entire fluid cell were vibrated [9]. The

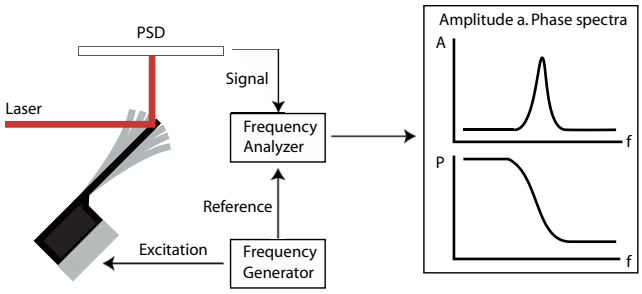

Figure 2. Measurement setup.

laser beam reflected from the tip of the vibrating cantilever was captured on the PSD. The resonance frequencies were determined from the PSD output using a frequency analyzer (HP 3589A) while sweeping the frequency range of interest. Typical amplitudes and the corresponding phase spectra obtained in the frequency analyzer are shown schematically in Fig. 2.

Cantilever Functionalization.

Piranha (H_2O_2:H_2SO_4 = 1:1) cleaned arrays were coated with a 2-nm-thick chromium adhesion layer followed by 30-nm-thick gold layer using sputtering (Baltec SCD 050 for Cr, Baltec MED 020 for Au) on both sides. On the freshly gold-coated cantilevers, home-made crosslinker dithiobis(succinimidyl undecanoate) (DSU) [10] was used (see Fig. 3) to immobilize streptavidin.

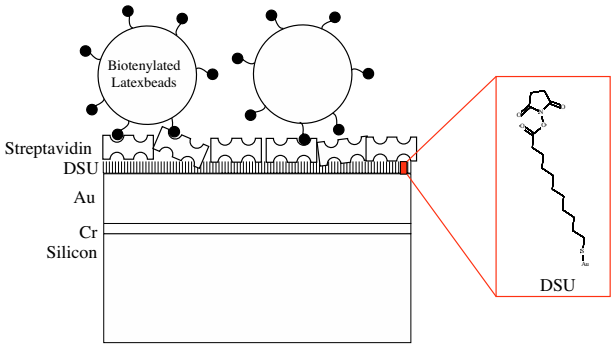

Figure 3. Schematic illustrating the binding of biotin latex beads immobilized on the streptavidin-functionalized cantilever surface.

Binding assay.

A buffer solution containing 20 mM KP_i pH 7.4, 100 mM $NaCl$and 0.01% NaN_3 was used during the experiment. Carboxylate-modified biotin-labeled polystyrene latex-beads (mean diameter: 250 nm) suspension in 10 mM PBS (Sigma-Aldrich) was used. It contains 1% solids in 0.4 ml PBS. The solution was diluted to 15 μl/ml in buffer before injection.

Measurement Procedure.

The experiment was started by recording the resonance spectra in a buffer flow for 45 min (baseline). A constant flow of 17 μl/min was maintained during the experiment. Then the buffer with biotin-labeled latex-bead suspension was injected for another 45 min. It is expected that, because of the biotin streptavidin interaction, the biotin-labeled latex beads bind to the immobilized streptavidin on the vibrating cantilever during this stage. Later the fluid cell was flushed with the buffer for another 45 min. This step removed all unbound biotin-labeled latex beads from the fluid cell.

Data Processing.

Each resonance peak was gauss-fitted to obtain the resonance frequency and Q-factor using IGOR PRO [11]. A plot of resonance peak values for each harmonic as a function of time yielded the frequency shifts observed with the mass loading of the latex beads on the cantilever.

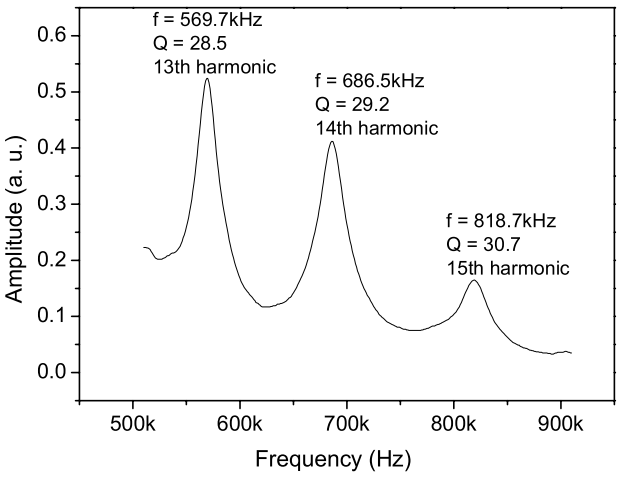

Figure 4. Frequency spectrum.

RESULTS AND DISCUSSION

In air, the fundamental resonance frequency for the cantilever used was 6 kHz with a Q-factor of 20. The separation between the harmonics increased with the harmonic number according to Eq. (1). A total of seven harmonics were observed in a frequency span of 1 MHz. The Q-factor of the peaks also increased with the harmonic number.

In buffer, owing to heavy hydrodynamic damping, the fundamental frequency value dropped to 1 kHz, with a Q-factor of 1. All the harmonics were more closely spaced than those in air. A total of 15 harmonics was observed in a span of 1 MHz. Peaks were clearly visible and their frequency value approximately matched with the values calculated using Eq. (2). According to Eq. (8), we choose to operate at higher harmonics for better sensitivity.

The applied input frequency was swept in a range covering three higher harmonics of the cantilever. A typical frequency spectrum obtained is shown in Fig. 4. The resonance frequencies 569.7, 686.5 and 818.7 kHz correspond to the 13^{th}, 14^{th} and 15^{th} harmonic of the cantilever, and the corresponding Q-factor values are 28.5, 29.2, and 30.7 respectively. During the experiment, the spectra were recorded with a time interval of 24 s.

The plots of frequency values of various harmonics as a function of time are shown in Fig. 5. The lower curve belongs to the 13^{th}, the middle to the 14^{th} and the upper to the 15^{th} harmonic, respectively. The corresponding negative frequency shifts of 2.1, 2.7 and 3.1 kHz are also shown. The shift in the frequency increased with the harmonic number proving Eq. (8).

According to the three steps of injection, the plots consists of three sections of about 45 min each indicated by vertical dashed lines (Fig. 5). In the first section, during the buffer injection, the resonance frequencies were quite stable with a maximum fluctuation of 300 Hz. In the second section, with the injection of buffer with latex beads, the frequencies started to shift. The frequency values are highly scattered. In the last section, while flushing the fluid cell with buffer, the frequencies became quite stable immediately with a maximum fluctuation of 200 Hz.

During the injection of buffer with latex beads, the frequency spectra obtained were very noisy and the gauss-fit of these spectra had large statistical errors. Hence the frequency values are highly scattered. Noise in the spectra is attributed to heavy light scattering by latex beads inside the fluid cell and/or collisions of the latex beads with the cantilever. The permanent decrease in the frequency after flushing the fluid cell with buffer indicates that the latex beads are permanently bound to the cantilever surface generating a mass load on the cantilever.

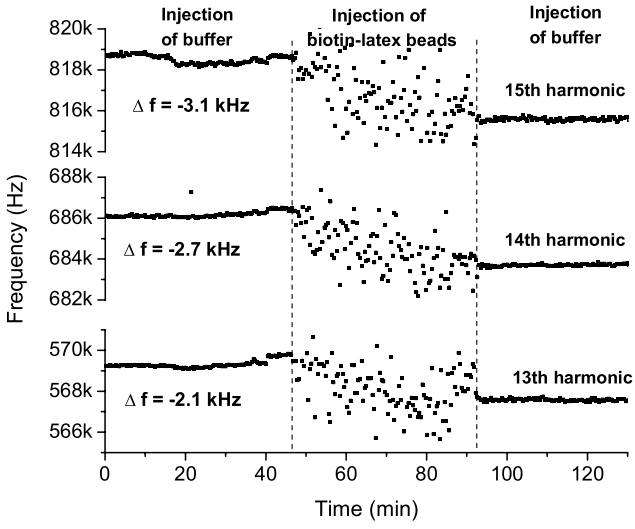

Figure 5. Frequency shifts due to biotin-streptavidin coupling of latex beads to the cantilever surface.

The mass m_c of the cantilever was 116.5 ng [12]. The

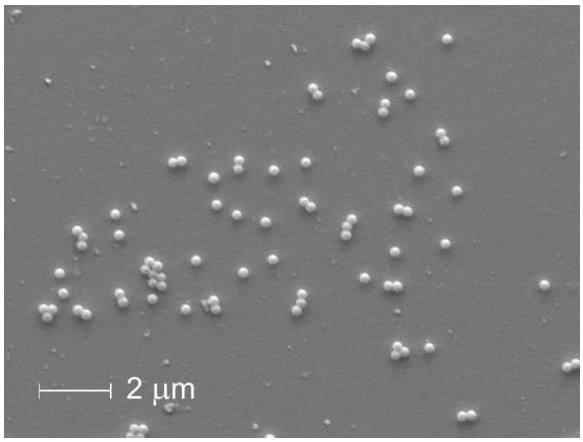

Figure 6. SEM Image of the cantilever with latex beads.

Table 1. Comparison of frequency shift observed in various harmonics and corresponding calculated mass.

Harmonic	Δf_{exp} (kHz)	Δm_{exp} (ng)
13^{th}	2.1	0.89
14^{th}	2.7	0.92
15^{th}	3.1	0.88

values of the mass increase obtained according to Eq. (7) for the 13^{th}, 14^{th} and 15^{th} harmonic were 0.89, 0.92 and 0.88 ng, respectively, within an error of ± 0.08 ng. The results are summarized in Table 1. This mass load corresponds approximately to an average coverage of about 1 latex bead per $\mu\mathrm{m}^2$ yielding 0.86 ng.

After the experiment, the cantilever surface was characterized by scanning electron microscopy (SEM). The spheres in the SEM image (Fig. 6) indicate the latex beads on the cantilever. The latex beads do not cover the entire cantilever. This could be due to nonuniform functionalization of the cantilever or because some of the beads might have been washed away during the cleaning procedure prior to SEM imaging. The cleaning procedure in nano-pure water was done to remove salt crystals (if any) on the cantilever.

CONCLUSIONS

Real-time mass addition on a cantilever in fluid through molecular recognition is demonstrated using biotin-streptavidin coupling of latex beads onto a gold-coated cantilever surface. It is shown that the frequency shifts are higher at higher harmonics for the same mass addition on the cantilever. Our method allows biochemical processes to be tracked in real time to a mass accuracy of better than 80 pg under physiological conditions.

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[12] The mass of the silicon cantilever was calculated by $m_c = \rho_{Si} lwt$, whereas $\rho_{Si} = 2330 kg/m^3$ is the density of silicon and l, w, t are the length, width and thickness of the cantilever, respectively.