LIQUID-PHASE BIOCHEMICAL SENSING WITH DISK-TYPE RESONANT MICROSENSOR

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Abstract: The application of a resonant microsensor platform based on disk-type microstructures vibrating in an in-plane resonance mode for biochemical sensing in liquid environment is described. Based on the measured short-term frequency stabilities of 1.1 10^{-8} in air and 2.3 10^{-6} in water, low femtogram and sub-picogram detection limits can be achieved in air and in liquid, respectively. The feasibility of liquid-phase biosensing using the disk resonators is demonstrated experimentally by detecting anti-β-galactosidase antibody using covalently immobilized β-galactosidase enzyme.

Keywords: Resonant sensor, Mass-sensitive sensor, Biosensor, β-galactosidase enzyme

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1. INTRODUCTION

Resonant mass-sensitive microsensors have generated increasing interest in recent years as possible platforms for chemical and biochemical sensing [1-4]. These sensors assess analyte concentrations by measuring the frequency change of a resonating structure. The sorption of analyte molecules to a (bio)chemical recognition layer deposited on top of the microresonator changes its oscillating mass, thus shifting the mechanical resonance frequency of the resonator. The frequency output of resonant sensors is insensitive to variations in the sensor signal amplitude and can be easily digitized with a counter. High mass sensitivity, wide dynamic range, fast response time, and small size are additional advantages of resonant microsensors.

Spurred by extensive research on the fabrication and characterization of probes for atomic force microscopy (AFM), microcantilevers have been most extensively used as microfabricated mass-sensitive transducers for chemical and biological sensing [1-4]. If operated in an amplifying feedback loop, the resolution of mass-sensitive sensors is given by the product of the sensitivity and the minimal detectable frequency change, which is closely related to the Q-factor of the resonance. While cantilever sensors reach Q-factors around 1,000 in air [2,5], their out-of-plane vibration modes are strongly damped in liquids, typically lowering their Q-factor to values of 20 or less [6].

In this work, a resonant microsensor platform based on disk-type microstructures vibrating in an in-plane resonance mode was used to improve the sensor resolution in a liquid environment. The performance of the resonator as a biological sensor in liquid was evaluated experimentally by detecting anti-β-galactosidase antibody with covalently immobilized β-galactosidase enzyme.

2. DISK RESONATOR

The disk-shape microstructure (radius: 120-150 μm, thickness: 6-10 μm) shown in Fig. 1 is operated in a rotational in-plane mode with typical resonance frequencies between 300 and 650 kHz [7]. By shearing instead of compressing the surrounding fluid, the damping is reduced and Q-factors up to 5,800 in air and 94 in water were measured. The resonators feature on-chip electrothermal excitation elements and a piezoresistive Wheatstone bridge, sensitive only to the desired in-plane rotational vibration mode for vibration detection.

For the application as a mass-sensitive sensor, the disk resonator was incorporated as frequency-determining element in a self-oscillating amplifying feedback loop. The short-term frequency stability of the resonators, which limits the minimal detectable frequency change and thus

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the sensor resolution, was measured both in air and in water using the Allan variance method [8]. With an integration time (gate time) of 1 sec, short-term frequency stabilities as low as 1.1 10^{-8} (4.6 mHz) in air and 2.3 10^{-6} (0.83 Hz) in water were measured (see Fig. 2). Considering the sub-microgram mass of the disk resonator itself, the measured frequency stabilities yield mass detection limits in the low femtogram and sub-picogram range in air and in liquid, respectively.

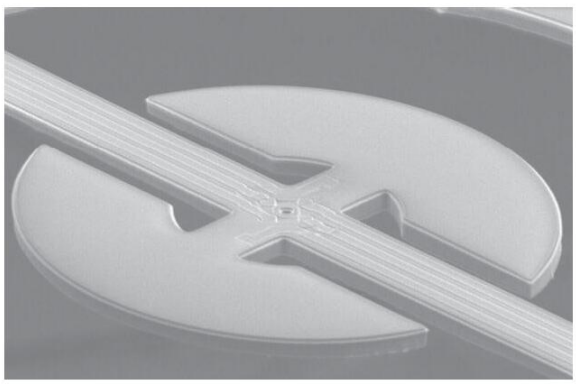

Fig. 1: SEM image of a rotational in-plane mode disk resonator.

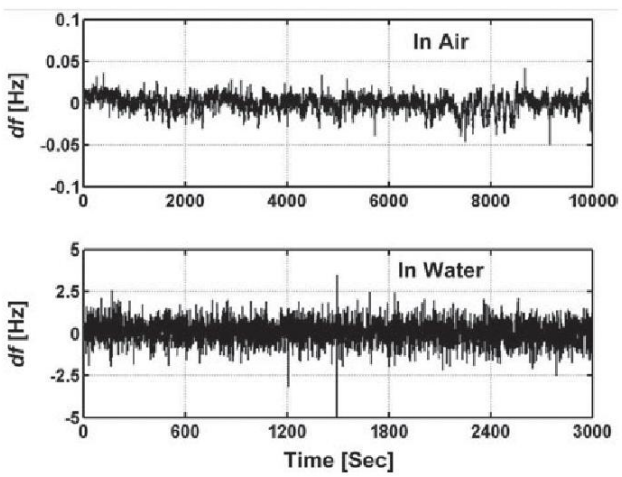

Fig. 2. Allan variance σ measurements with 1 s gate time: frequency change (f_{n+1} - f_n) vs. time in air ($\sigma = 1.1\ 10^{-8}$, $f_0 = 426$ kHz) and in water ($\sigma = 2.3\ 10^{-6}$, $f_0 = 321$ kHz).

3. SURFACE FUNCTIONALIZATION

Covalent binding of proteins to sensing surfaces is widely used because it can provide a highly irreversible surface loading and is relatively resistant to the operating conditions [9-11]. For the immobilization of β-galactosidase enzyme on the surfaces of the disk-type microstructures, the protocol suggested by Williams and Blanch [10] was modified and enzyme loading to the silicon dioxide and silicon nitride surface was characterized. Surface loadings of 0.045 IU (0.37 μg) were obtained on 5 mm × 5 mm silicon samples coated with the same passivation layers as the microresonators [12]. The immobilization steps and the chemical reactions involved are summarized in Fig. 3.

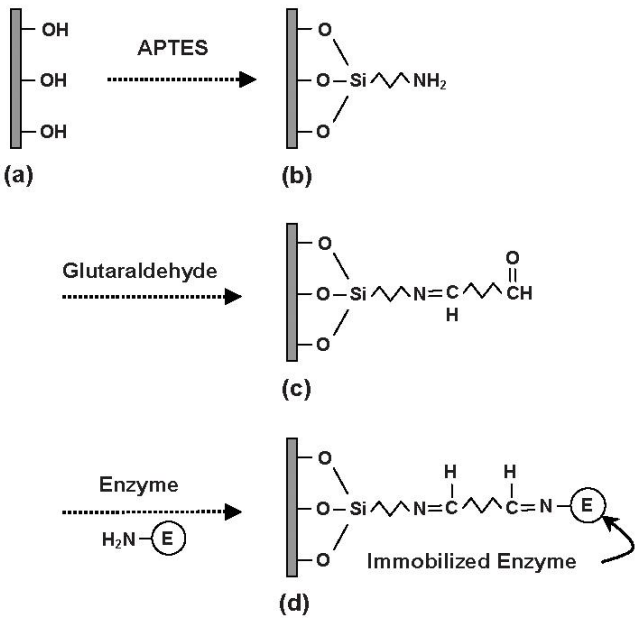

Fig. 3: β-galactosidase enzyme (Sigma G2513) immobilization protocol (from [10]): (a) Activation of surface silanol groups (SiOH) on the PECVD silicon nitride surface using 10 % nitric acid; (b) Formation of reactive amino groups (NH_2) on the resonator surface by treatment with 10 % aminopropyl triethoxysilane (APTES) solution (Fluka), (c) Application of 15 % glutaraldehyde solution (Sigma G5822) in pH 7, 200 mM sodium phosphate buffer (PBS) solution as cross-linker, (d) Immobilization of enzyme with 0.01 mg/ml enzyme solution in 25 mM PBS solution.

4. MEASUREMENT SETUP

The computer-controlled experimental setup consists of the resonator mounted in a dual-in-line package, a circuit board with the feedback circuit,

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a PMMA (polymethylmethacrylate) flow cell, two syringe pumps, and a frequency counter, as shown in Fig. 4. The antibody concentration and flow rate were adjusted using two computer-controlled syringe pumps, one filled with a diluted anti-β-galactosidase antibody solution, the other one with water. The liquids from both pumps were mixed at a tee-junction and supplied to the flow cell through PEEK (polyetheretherketone) tubing. The resonators were operated in an amplifying feedback loop using a custom made circuit board, and the resonance frequencies were measured with an Agilent 5331 frequency counter.

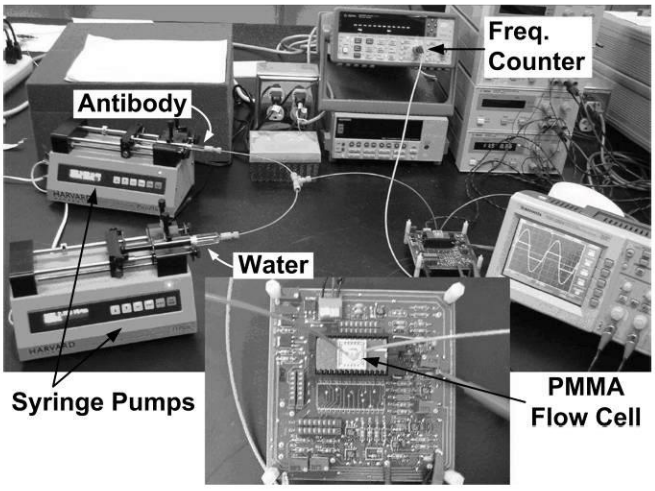

Fig. 4: Test setup for biochemical sensing with syringe pumps and flow-cell (see also close-up) mounted on top of packaged microresonator.

5. RESULTS AND DISCUSSION

A resonator design with a radius of 150 μm was used for the experiments. A typical open-loop frequency response of the resonators in air before and after enzyme immobilization is shown in Fig. 5. The resonance frequency decreased approx. 2.2 kHz or 0.63 % (the initial frequency of the in-plane rotational mode is 347 kHz) due to the mass loading of the immobilized enzyme.

However, the frequency changes of the resonators before and after enzyme immobilization showed substantial variation from sample to sample and from batch to batch, as shown in Fig. 6. This might indicate that the immobilized enzyme is not a monolayer but multiple layers of protein and that a significant quantity of enzyme molecules are adsorbed instead of covalently bonded on the sensor surface. Adsorbed enzymes, which might remain due to insufficient washing of the micro-resonators, are less stable than the covalently immobilized enzymes [10,12] and can cause unstable and irreproducible sensor response. Further optimization of the immobilization procedure could provide a substantial improvement in the reproducibility of the micro-resonators.

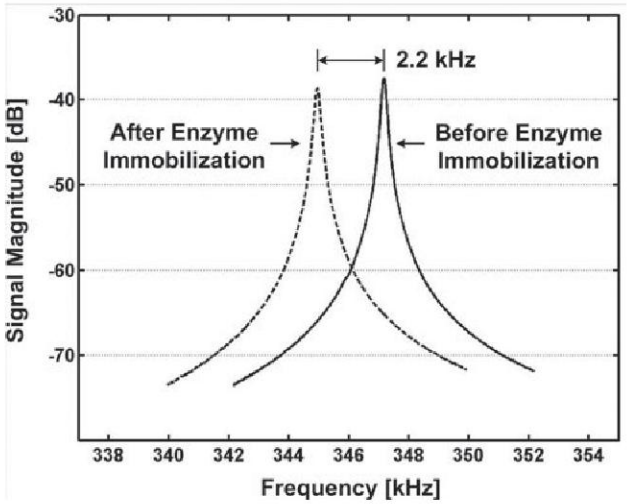

Fig. 5: Resonance frequency change of a disk resonator due to mass loading by the immobilized β-galactosidase enzyme.

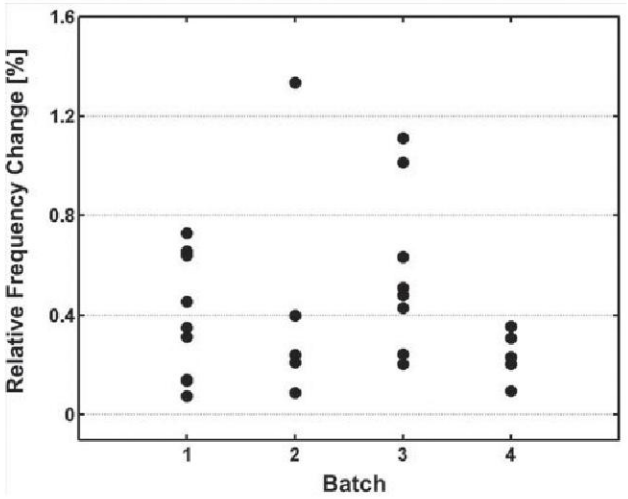

Fig. 6: Relative frequency change of 26 resonators from 4 coating batches after enzyme immobilization.

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A 0.73 μg/ml anti-β-galactosidase antibody solution was prepared by diluting the original 2.2 mg/ml antibody solution (Promega Z3781) with water by 1:3,000. The enzyme immobilized resonator, attached to the PMMA flow cell, was operated in water for several hours until its frequency signal stabilized at a constant flow rate of 8 μl/min. After the frequency of the resonator stabilized in water, 0.5 μl antibody solution was injected every 10 minutes at a flow rate of 0.5 μl/min, while maintaining a constant total flow rate of 8 μl/min through the flow cell.

Fig. 7 shows the resonance frequency over time. A distinct frequency change occurs about 2~3 minutes after the antibody injection into the flow cell. The combined volume of the tubing and the sample chamber inside the flow cell causes this time delay. After the first antibody injection, the resonance frequency decreases by approx. 1 kHz while the frequency drop is approx. 400 Hz after the second antibody injection. After the third injection, the frequency change is barely visible, indicating the gradual saturation of the antibody binding sites on the enzyme coated resonator surface.

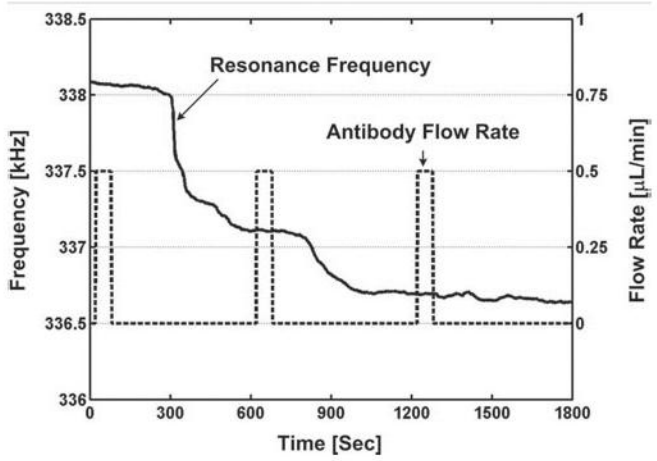

Fig. 7: Resonance frequency of disk resonator as a function of time while periodically injecting antibody solution.

6. CONCLUSION

The application of a disk resonator vibrating in a rotational in-plane mode as a biological sensor in liquid environment was demonstrated by specific binding of anti-β-galactosidase antibody to β-galactosidase enzyme immobilized on the resonator surface. With short-term frequency stabilities of the disk resonators as low as 1.1 10^{-8} in air and 2.3 10^{-6} in water, mass detection limits in the low femtogram and sub-picogram are expected in air and in liquid, respectively.

7. ACKNOWLEDGEMENT

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