GAS-LIQUID SEPARATED RESONATOR FOR BIO-CHEMICAL APPLICATION

H. Hida[1], M. Shikida[1], M. Okochi[2], H. Honda[2], and K. Sato[1]
[1]Dept. of Micro-Nano Systems Engineering, Nagoya University, JAPAN
[2]Dept. of Biotechnology, Nagoya University, JAPAN

ABSTRACT

We have developed a novel type of gas-liquid separated micro-resonator for measuring the multifunctional characteristics of biological cells. The resonator consists of an area for the resonator/sensor (gas-phase) and one for the biochemical substance (liquid-phase), with a water-shedding wall separating them. The specimen support extends from the sensor body and passes through the water-shedding wall to the inside of the sample chamber We fabricated the comb-drive resonator on a silicon-on-insulator (SOI) wafer by using a deep reactive ion etching process and resonated it at around 2.5 kHz. Experimental results confirmed that the water-shedding wall was able to prevent solution leakage at the gap between the wall and the support.

INTRODUCTION

Recently, various types of biochemical analysis devices have been developed by using microelectromechanical system (MEMS) technology. Two analysis methods are usually applied for evaluating the characteristics of living biological cells. One is a static measurement method, in which a micro-cantilever is used as a tool for measuring generative force [1-2]. In this method, the cells are placed on the end of the cantilever, and the generative force is detected by measuring the cantilever's elastic deformation value. This method is advantageous in that it is quite simple in terms of structure and principle. The static mode, however, generally makes it difficult to detect the generative force value with high resolution. For this reason we used the other usual method, i.e., a dynamic method in which a micro-resonator is applied as a detection tool, to take our measurements. The most famous resonators used in biochemical analysis are quartz crystal microbalance (QCM) resonators [3], in which the mass change caused by a biochemical reaction is detected by measuring the changes in resonance characteristics. This method is able to detect mass changes with high resolution; however, it is difficult to use it to evaluate generative force measurements. Another disadvantage to this method is that with it a cell manipulation function cannot be incorporated into the device chip.

With most MEMS resonators, e.g., electrostatic comb-drive actuators, it is possible to integrate both a multifunctional detection mechanism and handling tools on the same chip. However, there are still two problems involved in applying them as tools for biochemical analysis.

The first concerns high salt buffer solutions: With such solutions, the living cells in them must be suspended to maintain their activity. Therefore, it is difficult to directly immerse the resonator into a buffer solution due to the solution's high electrical conductivity.

The second involves liquid-phase conditions in general. As stated above, living cells must be suspended in liquid-phase conditions. Consequently, the solution's high damping force causes the Q-factor at the resonator to reduce. This makes it difficult to directly apply a comb-drive actuator used as a sensing tool in cases where solutions are involved.

To overcome these problems, we have developed a

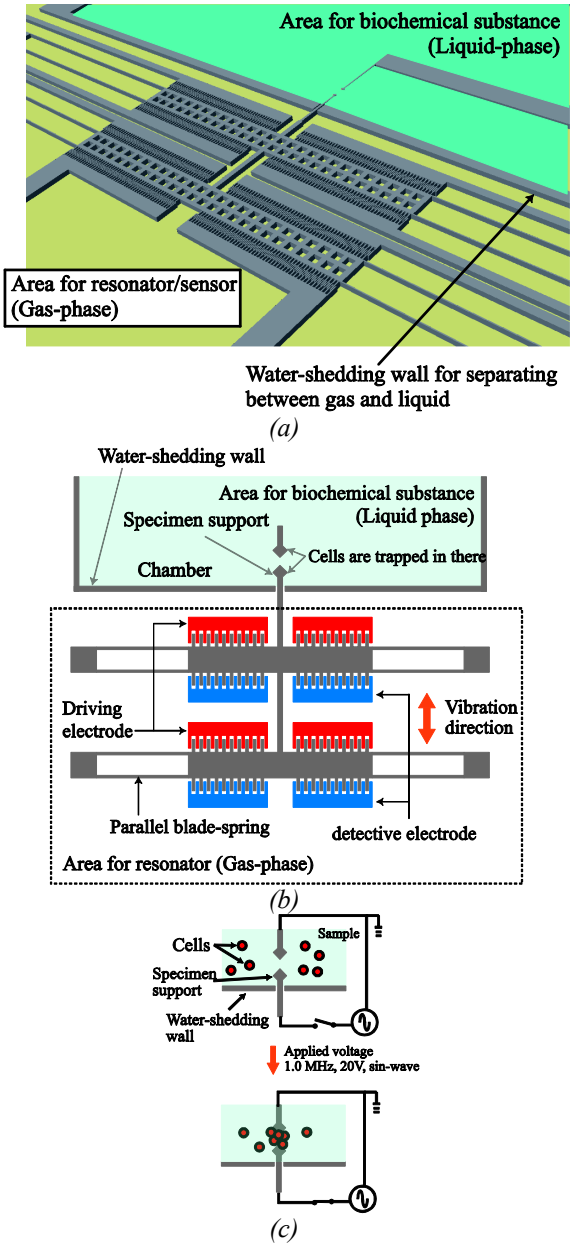

Figure 1: Schematic view of gas-liquid separated resonator and cell positioning mechanism onto specimen support. (a) Schematic view of gas-liquid separated resonator. (b) Detailed view of gas-liquid separated resonator; and (c) Cell positioning on support by dielectrophoresis force.

novel gas-liquid separated resonator in which a water-shedding wall separates the areas for the sensor itself and for the biochemical substance.

GAS-LIQUID SEPARATED RESONATOR

Principle

The gas-liquid separated resonator we have developed is shown in Fig. 1. The device comprises an area for the resonator /sensor (gas-phase) and one for the biochemical substance (liquid-phase). The comb-drive resonator has excitation and detection functions and is used as the sensing element (Fig. 1(b)). The resonator's moving parts are suspended by four parallel blade springs. The specimen support extends from the sensor body and passes through the water-shedding wall to the inside of the sample chamber. The buffer solution including the biochemical substances is placed inside the chamber. Applying a water-shedding surface onto the wall prevents leakage at the gap between the wall and the support. The bio-chemical substances, e.g., cells, are positioned at the end of the support by using a dielectrophoresis force, as shown in Fig. 1(c).

The mass change and the generative force in the cells positioned on the support by the bio-chemical reactions are detected by measuring changes in the resonance characteristics. The resonator's mechanical resonant frequency f_0, is given by

$$f_0 = \frac{1}{2\pi}\sqrt{\frac{k}{m}} \quad (1),$$

where k is the spring constant and m is the resonator's effective mass. When the cells are placed in the resonator, the resonant frequency is decreased by mass change (Δm).

$$f_{mass} = \frac{1}{2\pi}\sqrt{\frac{k}{m+\Delta m}} \quad (2)$$

Additionally, when the generative force in the cell is applied to the resonator, the changed resonant frequency f_{force}, is given by

$$f_{force} = \frac{1}{2\pi}\sqrt{\frac{k+\Delta k}{m}} \quad (3)$$

where Δk is an equivalent spring constant of the force applied by the cells. Using (1), (2) and (3), we can measure the mass change and the generative force in the cells by detecting the frequency difference. The resonant frequency change caused by the generative force is given by

$$\Delta f_{force} = f_{force} - f_0 = \frac{\sqrt{k+\Delta k}-\sqrt{k}}{2\pi\sqrt{m}} \quad (4)$$

This equation shows that reducing the spring constant can increase the frequency difference Δf_{force}, thus improving the resonator sensitivity.

Table 1: Mechanical properties of the designed resonator.

Length l [μm]	Spring constant k [N/m]	Resonant frequency f_0 [kHz]
250	234.4	17.012
500	97.8	5.938
750	62.5	3.089
1000	45.7	1.966

Mechanical properties

In designing the micro-resonator, we first determined its mechanical properties (i.e., spring constant and resonant frequency). The parallel blade springs were designed to have four values of length l, i.e., 250, 500, 750, and 1000 μm. We set their width w and thickness b at 4 μm and 20 μm respectively. The spring constants and resonant frequencies were calculated by using Coventorware finite element modeling (FEM) analysis. Table 1 shows the relationship between the parallel beam springs' mechanical properties and length.

Electrostatic driving force

We then designed the electrostatic comb-drive resonator and calculated its excitation/detection properties. When a DC voltage V is applied to the resonator, the generative electrostatic force F_{drive}, and the force displacement are given by

$$F_{drive} = \frac{2\varepsilon v V^2}{g-h}N = k\Delta x \quad (5)$$

where ε (= 8.86×10^{-12} F/m) is the permittivity of the air, b is the device thickness, g is the gap between the electrodes, h is the electrodes' thickness, N is the number of the comb structure's fundamental units, and Δx is the resonator displacement. We determined that the values of b, g, h, and N were 20 μm, 8 μm, 4 μm, and 200 respectively. The relationship between the applied voltage and the resonator's generative electrostatic force is given by

$$F_{drive} = 1.77\times10^{-8}V^2 \quad (6)$$

Sensitivity

The resonator's vibration behavior can be measured by detecting the capacitance. The resonator displacement can be related to the capacitance change ΔC by the following equation:

$$\Delta C = \frac{4\varepsilon b\Delta x}{g-h}N \quad (7)$$

Using the dimensional values given above, the relationship between the capacitance change and the resonator displacement is given by

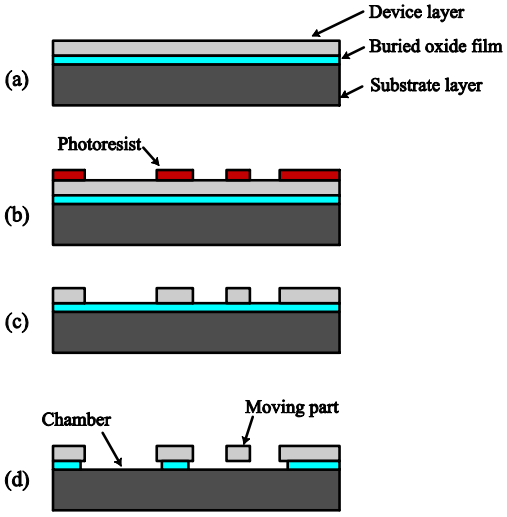

Figure 2: Fabrication process of the gas-liquid separated resonator. (a) Silicon on insulator water. (b) Patterning of photoresist. (c) Si etching by D-RIE; and (d) Removing the oxide film by wet etching process.

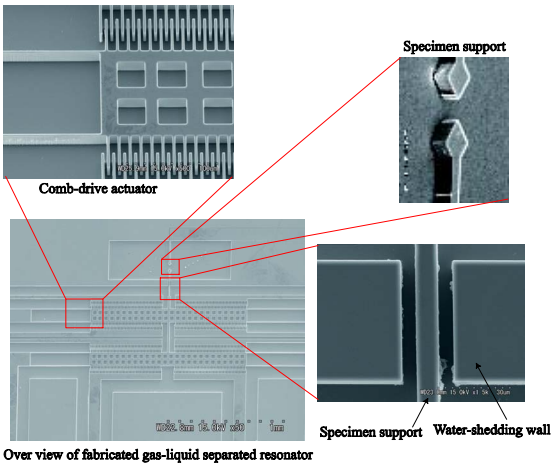

Figure 3: Fabricated gas-liquid separated resonator.

$$\Delta C = 3.54 \times 10^{-8} \Delta x \quad (8)$$

Water shedding wall

We also designed a water-shedding wall to separate the excitation/detection and biochemical substance areas. The wall thickness and that of the gap between the wall and the support were set at 50 µm and 5 µm respectively. We believe that the wall is able to suppress leakage not only due to its high aspect ratio but also due to the hydrophobic character of its silicon surface.

Fabrication

We fabricated the resonator on a silicon-on-insulator (SOI) wafer as shown in Fig. 2. First, a photoresist was applied to the wafer's device layer and patterned by using an exposure system. The resonator and chamber structures were then formed by the deep reactive ion etching (D-RIE) process. After the process was completed, the wafer was cut into dices. The resonator's moving parts were then released to selectively remove the buried silicon dioxide layer through an HF etching process. The device was then immersed in isopropyl alcohol and dried in the air to decrease the surface tension acting between the moving parts and the silicon substrate. Figure 3 shows an overview of the fabricated resonator.

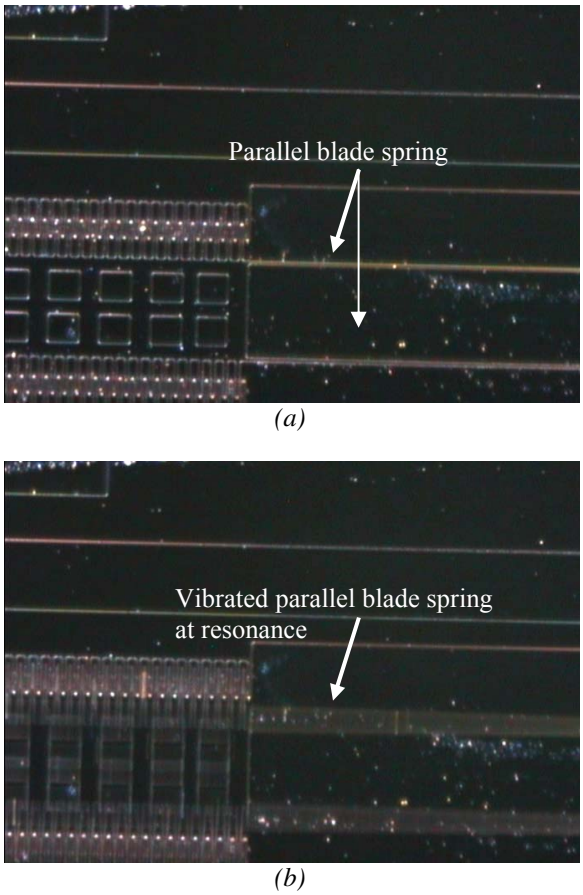

Figure 4: Resonated com-drive actuator at around 2.5 kHz. (a)Before applied voltage; and (b) after applied voltage.

RESONATOR PERFORMANCE

Frequency response

We evaluated the resonator's resonant frequency in air. First, the AC voltage required for excitation was amplified with a power amplifier and then applied to the fixed electrodes. The moving parts and substrate were grounded. At this time, we observed the resonator's vibration behavior with an optical microscope. We applied AC voltage of 50 V amplitude with various frequencies. The parallel springs' length and the theoretical resonant frequency were 750 µm and 3.09 kHz respectively. Figure 4 shows the resonator excited at around 2.5 kHz resonant frequency. The maximum displacement in air at this frequency was 20 µm. We found that the experimentally obtained resonance frequency coincided with the designed value.

Sample positioning in liquid environment

We also attempted to manipulate sample beads in a liquid environment. First, we selectively placed a liquid solution inside the chamber by using a pipette and tested

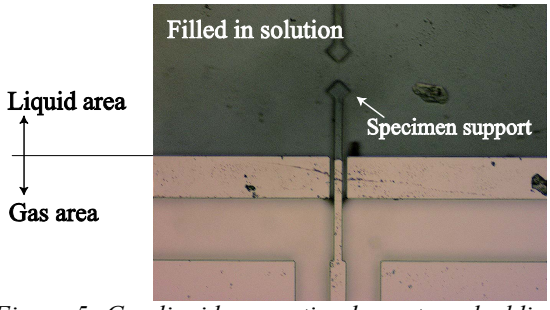

Figure 5: Gas-liquid separation by water- shedding wall.

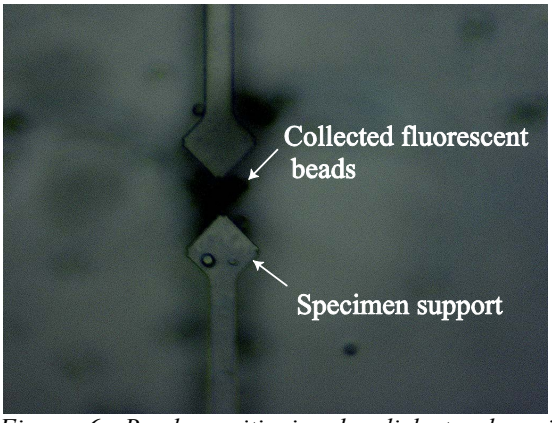

Figure 6: Beads positioning by dielectrophoresis force.

the resonator for leakage. As Fig. 5 shows, we confirmed that the water-shedding wall was successfully able to prevent solution leakage at the wall-support gap.

Finally, as a first cell-positioning trial, we used fluorescent beads 4.5 μm in diameter in place of a cell, and suspended them in water. A high-frequency voltage (1.0 MHz, 20V) was applied between the specimen support and the opposite pole to apply dielectrophoresis force to the beads. We found that the force successfully manipulated the beads so that they could be collected at the gap, as shown in Fig. 6.

CONCLUSION

We designed and fabricated a gas-liquid separated resonator for evaluating the multifunctional characteristics of biological cells. The micro-resonator was fabricated on an SOI wafer through the use of an D-RIE process. The resonator was resonated in air at around 2.5 kHz. In a cell-positioning trial, we also manipulated sample beads as cell substitutes by applying dielectrophoresis force to them in water and obtained very positive results. In the future, we will attempt to refine our gas-liquid separated resonator as follows:

First, by evaluating its resonant frequency in detail: We will try to evaluate the resonator's resonant frequency by using a chamber filled with liquid. In addition, we will also construct a circuit that can detect capacitance change in it.

Second, by determining the position and culture of actual living cells: We have succeeded in selectively positioning cell-substitute beads in water. The next challenge will be to ascertain the position and culture of living cells in buffered solutions to measure the cell-bonding force between them.

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