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Measurement and Simulation Techniques for Piezoresistive Microcantilever Biosensor Applications

Aan Febriansyah[1], Ratno Nuryadi[2], Djoko Hartanto[3]
[1]Electrical Engineering, State Manufacturing Polytechnic of Bangka Belitung
Jl. Timah Raya Airkantung Sungailiat-Bangka Belitung, Indonesia, Phone/Fax:+62 71793586/93585
[2]Center for Material Technology, Agency for the Assessment and Application of Technology (BPPT)
Jl. MH. Thamrin No. 8 Jakarta, Indonesia, Phone/Fax: +62 213169893/3169873
[3]Department of Electronics Engineering, Faculty of Engineering – Indonesia University
Kampus UI Depok-Jawa Barat, Indonesia, Phone/Fax: +62 217270078/7270077
e-mail: aanfebriansyah@yahoo.com, ratnon@gmail.com, djoko@elektro.ui.ac.id

Abstrak

Aplikasi mikrokantilever sebagai biosensor mulai banyak digunakan dalam dunia kesehatan, biologi, kimia dan lingkungan. Riset ini akan membahas perancangan teknik pengukuran dan simulasi aplikasi piezoresistive mikrokantilever sebagai biosensor, meliputi pembuatan rangkaian Wheatstone bridge sebagai detektor obyek, simulasi perubahan frekuensi resonansi berbasis Persamaan Euler-Bernoulli Beam sebagai deteksi keberadaan obyek, dan simulasi gerak mikrokantilever dengan menggunakan program software COMSOL Multiphysics 3.5. Tipe piezoresistive mikrokantilever yang digunakan adalah produk Seiko Instrument Technology (Jepang) dengan panjang 110 µm, lebar 50 µm, dan tebal 1 µm. Massa dari mikrokantilever 12.815 ng. Contoh obyek yang dideteksi adalah bakteri EColi, dimana massa untuk satu bakteri diasumsikan 0,3 pg. Hasil simulasi pada saat pendeteksian terjadi, untuk satu massa obyek bakteri akan menyebabkan nilai defleksi sebesar 0,03053 nm dan nilai frekuensi resonansi sebesar 118,90 kHz. Sedangkan untuk empat obyek bakteri akan menyebabkan nilai defleksi sebesar 0,03054 nm dan nilai frekuensi resonansi sebesar 118,68 kHz. Dari data tersebut terlihat bahwa bertambahnya massa bakteri akan menyebabkan naiknya nilai defleksi dan turunnya nilai frekuensi resonansi.

Kata kunci*: biosensor, COMSOL, defleksi, frekuensiresonansi, mikrokantilever*

Abstract

Applications of microcantileversas biosensors have been explored by many researchers for the applications in medicine, biological, chemistry, and environmental monitoring. This research discusses a design of measurement method and simuations forpiezoresistivemicrocantileveras a biosensor, which consist of designing Wheatstone bridge circuit as object detector, simulation of resonance frequency shift based on Euler Bernoulli Beam equation, and microcantilever vibration simulation using COMSOL Multiphysics 3.5. The piezoresistivemicrocantilever used here is Seiko Instrument Technology (Japan) product with length of 110 µm, width of 50 µm, and thickness of 1 µm. Microcantilever mass is 12.815 ng, including the mass receptor. The sample object in this research is bacteria EColi. One bacteria mass is assumed to 0.3 pg. Simulation results show that the mass of one bacterium will cause the deflection of 0.03053 nm and resonance frequency value of 118.90 kHz. Moreover, four bacterium will cause the deflection of 0.03054 nm and resonance frequency value of 118.68 kHz. These data indicate that the increasing of the bacteria mass increases the deflection value and reduces the value of resonance frequency.

Keywords*: biosensor, COMSOL, deflection, microcantilever, resonance frequency*

1. Introduction

A simple detection system with a high sensitivity is desired in the fields of biotechnology and medical science. The basic principle of biologically-based sensor (called as biosensor) is an interaction between bio-molecules, especially antigen-antibody and deoxyribonucleic acid (DNA)-DNA.Such bio-molecules interaction is generally measured by using gas chromatography mass spectrometry (GCMS), a quartz crystal oscillator and a surface Plasmon resonance. However, these methodshave some disadvantages as follows [1]. First, GCMS has a high mass resolution of about 1 pg, but it takes long time to measure a sample. Second, the

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quartz crystal oscillator method has a low sensitivity of about 30 pg/Hz, and third, the Surface Plasmon Resonance method is complex and expensive

Micro-machined cantilever is a good candidate for sensor.This was first used as force probes in Atomic Force Microscopy (AFM). The extreme sensitivity to several environmental factors, such as noise, temperature, humidity and pressure was immediately evident. In 1994, research teams in Oak Ridge National Laboratory and IBM have converted the mechanism causing interference into a platform for a novel family of biosensors [1]. Some of characteristics of microcantilever are as follows:

a) small size (in range micrometer)
b) high sensitivity (scale in *attogram*, 10^{-18} gram)
c) low cost relatively
d) low power consumption
e) detectable of various object in one platform
f) simple fabrication
g) integrated in microarray form.

The microcantilever based sensors areapplicable for various fields in medicine and biology field and in chemistry & environmental monitoring [2], such as detection of DNA and protein, detection of myoglobin, detection of temperature and heat changes, detection of Escherichia Coli bacteria, detection of humidity, etc.This paper discusses a design of measurement technique and simuations for piezoresistive-typedmicrocantilever as a biosensor, including the design of Wheatstone bridge circuit as object detector, simulation of resonance frequency shift based on Euler-Bernoulli Beam equation, and microcantilever vibration simulation using COMSOL.

2. Research Method

In this research, the measurement techniques and simulations of microcantilever-based biosensor are discussed, especially the changes in deflection ($\Delta\delta$) and the change in resonance frequency (Δf) ofmicrocantileverdue to the changes of object mass (Δm) absorbed on the microcantileversurface. The measurement techniqueswere designed using a Wheatstone bridge circuit and operational amplifier of INA 128. The microcantilever used here is piezoresistivetype made by Seiko Instrument Technology (Japan). Microcantilever surface will be coated by a material receptor that serves as a catcher and selector that only react with the selected object. The microcantilever size is 110 μm in length, 50 μm in width, and 1 μm in thickness. Microcantilever mass is 12.815 ng, including the mass receptor.The object used here is Escherichia Coli bacteria, which has the mass of0.3 pg. Simulation of the resonance frequency is performed based on Euler-Bernoulli Beam Equation, and the microcantilever motion simulation is done by using COMSOL Multiphysics 3.5.

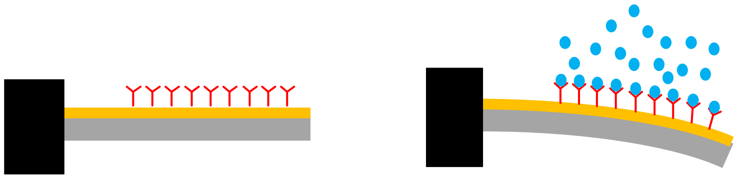
2.1. Microcantilever Structure

Figure 1 describes a simple structure of the microcantilever before and after deflection. In order to use the micro cantilever as a biosensor, it must be placed bio-receptor as functionalization layer on the surface of microcantilever and placing the electronics devices to detect the deflection of the microcantilever. Figure 2 shows a biosensor microcantilever (before (a) and after (b) deflections), where functionalization layer is coated on the microcantilever surface.

In case of the bacteria object detection, the microcantilever needs to be coated by antibody layer on its surface which only can react with bacteria. The mass detected here is the reaction result between both antibody and bacteria mass which is represented by BSA (Bovine Serum Albumin). BSA and the antibody wereimmobilized on the microcantilever surface using physicaladsorption as follows. First, the microcantilevers were cleaned using piranha solution (H_2O_2:H_2SO_4=1:1) and then immersed in ethanolbefore critical point dried (CPD). Second, the microcantilevers werethen rinsed for around 30 s in deionized (DI) water and treated with BSA at a concentration of 2 mg/ ml for 15 min. The resonance frequency of the microcantileveris then measured after both the antibody and BSA are attached tothe surface [3]. Commercial microcantilever dimensions used here is 50-200 μm in length, 10-40 μm in width, and 0.3-3 μm in thickness. Such microcantilever size is possible to detect attogram scale (10^{-18} gram) object mass [4].

(a) Before deflection (b) Deflection condition

Figure 1. Structure of Microcantilever



(a) Before deflection (b) Deflection condition

Figure 2. Biosensor microcantilever system

2.2. Microcantilever Deflection Modes

Microcantileverbiosensors can operate in two modes (see Figure3), i.e., static and dynamic modes. In the static mode, themicrocantileverbiosensor operates based on the difference ofsurface stress generated between the top and bottom surfacesduring binding or adsorption of target molecules on afunctionalized surface. The change of surface stress causes the cantilever to bend. This bending is known as deflection (*δ*).

On the other hand, in the dynamic mode, the microcantilever is oscillated at a certain frequency andthe change in oscillationfrequency due to mass change (Δm) loading is measured on its surface. Deflection values can be calculated using the two methods, i.e., distributed load and pointed loadas shown in Figure 4.

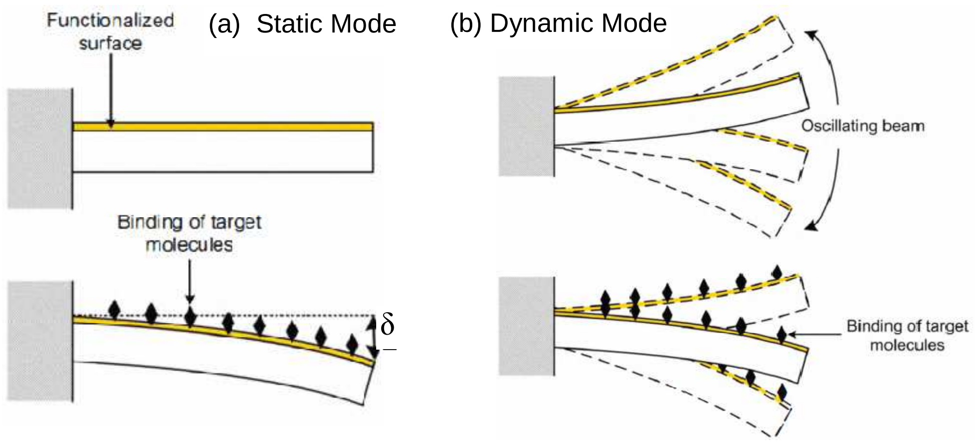
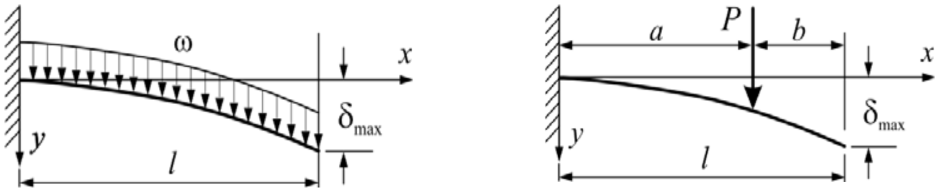


Figure 3. Microcantilevermode [5]



a. Distributed load *b. Load at any Point*

Figure 4.Microcantilever deflectionmethod

The calculation of deflection values in static mode for uniformly distributed load method usesequation (1), while the calculation of deflection values for pointed load method usesequation (2) [6]:

$$\delta = \frac{\omega l^4}{8EI} \tag{1}$$

$$\delta = \frac{Pa^2}{6EI}(3l - a), \tag{2}$$

where ***E*** isYoung's Modulus of material, ***P*** is the force in microcantilever beam, ***l*** is length of microcantilever, ***a*** is the object distancefromfixed end,and ***I*** is InertiaMoment ofmicrocantilever, which is calculated by the equation(3) [7].

$$I = \frac{wt^3}{12}, \tag{3}$$

where***w***and***t***arewidth and thickness of microcantilever, respectively. To determine the amount of deflection in the dynamic mode, we use the Euler-Bernoulli Beam Equationwith uniformly distributed load method [7] as follows,

$$EI\frac{\partial^4 \delta}{\partial x^4} + \rho A \frac{\partial^2 \delta}{\partial t^2} = P(x,t), \tag{4}$$

wheren ***ρ*** is the density of material, and ***A*** is cross section area ($\boldsymbol{A} = \boldsymbol{w} * \boldsymbol{t}$). To change the partial differential equations into differential equations on the value of x only, we assume a harmonic input force applied to the system with the radial frequency (ω), and then$P(x,t) = Pe^{i\omega t}$and$\delta(x,t) = \delta e^{i\omega t}$. Equation (4) can be then simplified by replacing the factor$e^{i\omega t}$, becomes [8]:

$$EI(1 + i\gamma)\frac{\partial^4 \delta}{\partial x^4} + \rho A \delta \omega^2 = P, \tag{5}$$

where$(\boldsymbol{1} + \boldsymbol{i\gamma})$ is a constant value of structural damping factor at the time of the vibrations and strains that occur in the microcantilever.

The value of resonance frequency is influenced by the mass and strain value factor (spring constant) of microcantilever [9].

$$f = \frac{1}{2\pi}\sqrt{\frac{k}{M+m}} \tag{6}$$

$$k = \frac{3EI}{l^3} = \frac{3Ewt^3}{12l^3} = \frac{Ewt^3}{4l^3} \tag{7}$$

where ***M*** is object mass on microcantilever surface and ***m*** is effective mass of microcantilever ($m = \rho Al = \rho wtl$) [10]. By adding the mass, the resonance frequency changes (Δf) will reduce the value of the frequency and then mass change (Δm) can be determined. The resonance frequency change can be seen in the following equation [11].

$$\Delta f = f\left(\sqrt{\frac{1}{\frac{\Delta m}{m}+1}} - 1\right) \tag{8}$$

3. Results and Discussions

3.1. Measurement Results of ΔV and ΔR

Here, the measurement of microcantileverdeflection was done using a Wheatstone bridge circuits which consists of potentiometers and resistors components (Figure 5). Potentiometer can be considered to replace the piezoresistor inmicrocantilever. From the data obtained in this experiment, we can calculate the resistance value changes and voltage changes.

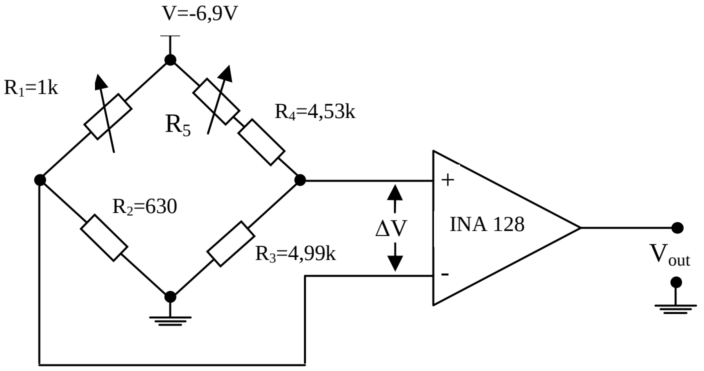
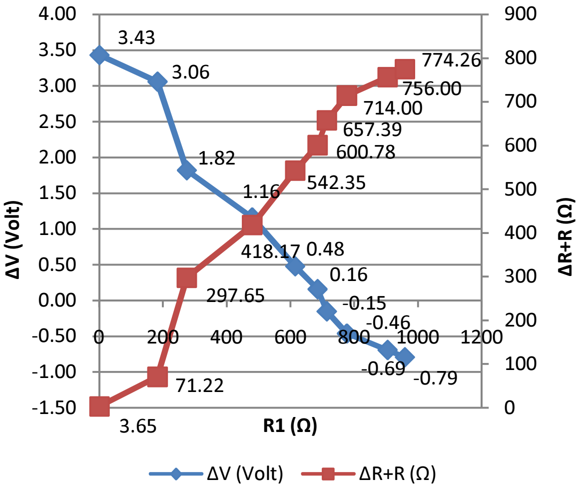


Figure 5. Wheatstone bridge circuit and operating amplifier INA128

R_1 and R_2 components are piezoresistors within the piezo resistive microcantilever. Components R_3, R_4 and R_5 arean external resistors. The amplifier INA128 is used to enhance the value of ΔV. In the experimental procedure, value R_1 is set close to the value R = 630 Ω and Vout is set to zero. Once the value of V_{out} = 0 is obtained, then the value of V_{out} is measured for all values of R_1 as shown in Table 1. The measurementof ΔV values for all values of R1 in Table 1 are shown in Figure 6.

Table 1.Experimental result of ΔV measurement

R_1 (Ω)	**V_{out} (Volt)**	**ΔV (Volt)**	**ΔR (Ω)**	**ΔR+R (Ω)**
1	14.26	3.43	-626.35	3.65
183	13.88	3.06	-558.78	71.22
275	9.45	1.82	-332.35	297.65
480	6.02	1.16	-211.83	418.17
615	2.50	0.48	-87.65	542.35
685	0.00	0.16	-29.22	600.78
714	-1.04	-0.15	27.39	657.39
777	-2.42	-0.46	84.00	714.00
905	-3.61	-0.69	126.00	756.00
959	-4.11	-0.79	144.26	774.26



igure 6. Graphs of experimental Resultsof ΔV and ΔR+R as function of R1

For comparison, we performed the calculation of ΔV and ΔR using equation (9) and equation (10) [12].

$$\Delta V = \frac{R_1R_3 - R_2R_x}{(R_3 + R_x)(R_1 + R_2)} E \qquad (9)$$

$$\Delta V = \frac{\Delta R}{2R} E \qquad (10)$$

The calculation results are shown in Table 2. The graphs of ΔV and ΔR+R as function of R1 from Table 2 can be shown in Figure 7.

Table 2. Calculation Results of ΔV and ΔR+R

R1 (Ω)	Vout (Volt)	ΔV (Volt)	ΔR (Ω)	ΔR+R (Ω)
1	24.289	3.520	-642.798	-12.798
183	13.647	1.978	-361.178	268.822
275	9.897	1.434	-261.922	368.078
480	3.776	0.547	-99.930	530.070
615	0.846	0.123	-22.385	607.615
685	-0.437	-0.063	11.555	641.555
714	-0.929	-0.135	24.580	654.580
777	-1.928	-0.279	51.026	681.026
905	-3.706	-0.537	98.072	728.072
959	-4.370	-0.633	115.646	745.646

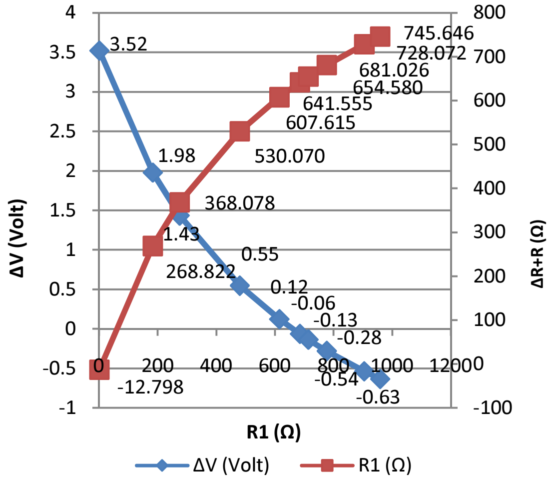


Figure 7. Calculation resultsof ΔV and ΔR+R as function of R1

3.2. Simulation Results of The Dynamic Mode

In this simulation, mass of bacteria as object is assumed to be 0.3 pg. We use Euler-Bernoulli Beam equation approach to simulate the deflection and resonance frequency. The simulation is done using Matlab software and the result is shown in Figure 8.

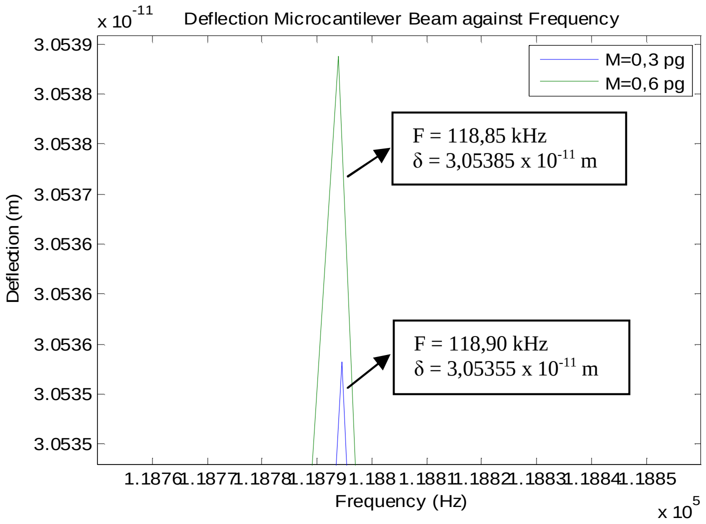


Figure 8. Deflection and amplitude changes for bacteria masses of 0.3 pg and 0.6 pg

To see the deflection shift (Δδ) and the change of resonance frequency (Δf), we will simulate two bacteria masses, i.e., 0.3 pg and 0.6 pg. Figure 8 shows that the changes of objectmass (Δm) causes the changes of frequency (X axis) and the changes of deflection value (Y axis).

For bacteria mass is 3 pg, the resonance frequency value (f) is 118.90 kHz and the deflection value (δ) is 30.536 pm. On the other hand, for bacteria mass of 6 pg, the resonance frequency value (f) is 118.85 kHz and the deflection value (δ) is 30.539 pm. It shows that the change of the bacterial mass from 3 pg to 6 pg results in a decrease in the resonance frequency value of 0.05 kHz and an increase in the deflection value of 0.003 pm.

3.3. Simulation of Microcantilever Motion

It is well known that the deflection of microcantilever is influenced by several factors related to physical geometries such as length, width and thickness. Therefore, in order to see the difference in the deflection value, the simulation is done by using COMSOL Multiphysics 3.5 software [14-17]. Here, the dimensions of the microcantilever are the length of 110 μm, a width of 50 μm and a thick of1 μm. The results of this simulation can be seen in Figure 9.The values of frequency and microcantilever deflection can be seen.

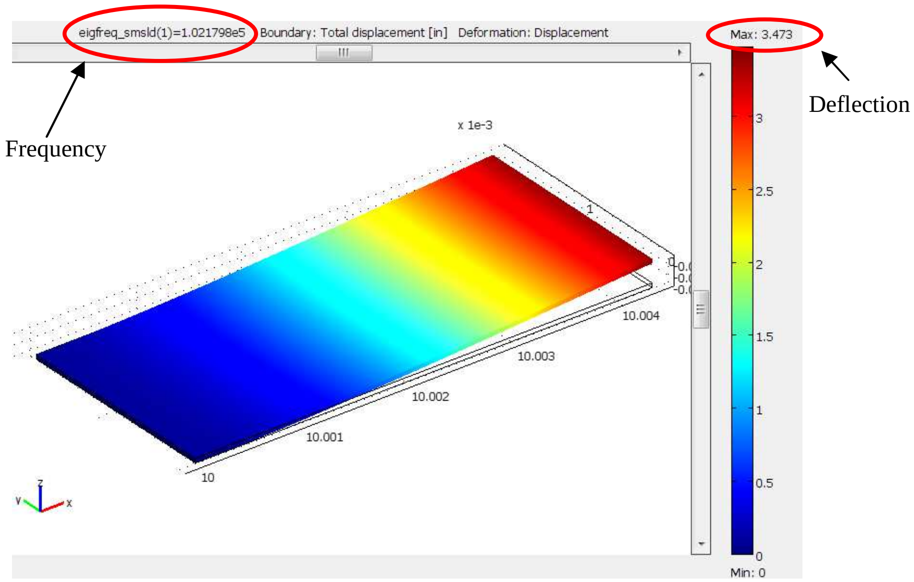


Figure 9.Simulation of microcantilevermotion by COMSOL.

When we increase the length of the microcantilever, the value of deflection increases and the value of resonance frequency decreases.This indicates that the increasing of microcantilever dimension will cause a change in microcantilever mass and also the changes in the value of deflection and resonance frequency.The detail of simulation results for several bacterial masses can be seen in Table 3.

Table 3.The comparison of the resonancefrequencyand deflection for different values of bacteria masses

Microcantilever mass (gram)	The detected object mass (gram)	Deflection (m)	Resonance frequency (kHz)
12,815 x 10^{-9}	3 x 10^{-13} (1 bacterium)	3,05355x10^{-11}	118,90
	6 x 10^{-13} (2 bacteria)	3,05385x10^{-11}	118,85
	9 x 10^{-13} (3 bacteria)	3,05415x10^{-11}	118,74
	12 x 10^{-13} (4 bacteria)	3,05445x10^{-11}	118,68

From the result in Table 3, it can be seen that the increasing of bacteria masses on the microcantilever surface will decrease the value of the resonance frequency and increase the deflectionvalue of microcantilever.

4. Conclusion

The main results of this research as follows:

1. In static mode deflection, the increasing of object mass causes an increase in the value of the Wheatstone bridgevoltage change (ΔV).
2. In dynamic mode deflection, one bacteria will cause the deflection value of 0.30535 pm and resonance frequency value of 118.90 kHz. Four objects of bacterium will cause the deflection value of 0.30544 pm and resonance frequency value of 118.68 kHz. It means that increasing of the object mass causes an increase in the value of deflection and decrease in the resonance frequency value.

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