31st Annual International Conference of the IEEE EMBS
Minneapolis, Minnesota, USA, September 2-6, 2009

An Ultra-sensitive ΔR/R Measurement System for Biochemical Sensors using Piezoresistive Micro-Cantilevers

Sudip Nag, Nitin S. Kale, V. Ramgopal Rao, Dinesh K. Sharma
Electrical Engineering Department, Indian Institute of Technology Mumbai, India

***Abstract*— Piezoresistive micro-cantilevers are interesting bio-sensing tool whose base resistance value (R) changes by a few parts per million (ΔR) in deflected conditions. Measuring such a small deviation is always being a challenge due to noise. An advanced and reliable ΔR/R measurement scheme is presented in this paper which can sense resistance changes down to 6 parts per million. The measurement scheme includes the half-bridge connected micro-cantilevers with mismatch compensation, precision op-amp based filters and amplifiers, and a lock-in amplifier based detector. The input actuating sine wave is applied from a function generator and the output dc voltage is displayed on a digital multimeter. The calibration is performed and instrument sensitivity is calculated. An experimental set-up using a probe station is discussed that demonstrates a combined performance of the measurement system and SU8-polysilicon cantilevers. The deflection sensitivity of such polymeric cantilevers is calculated. The system will be highly useful to detect bio-markers such as myoglobin and troponin that are released in blood during or after heart attacks.**

***Keywords*— ΔR/R measurement, Polymeric micro-cantilever, piezoresistor, lock-in amplifier, probe station, deflection sensitivity, myoglobin.**

I. Introduction

Micro-cantilever based sensors are gaining popularity as biochemical detectors due to distinct advantages of improved sensitivity and ease of use as compared to existing counterparts. The design of measurement platform using such sensors is of great interest due to possible wide range of applications, such as for cardiac markers' detection in blood due to heart attacks.

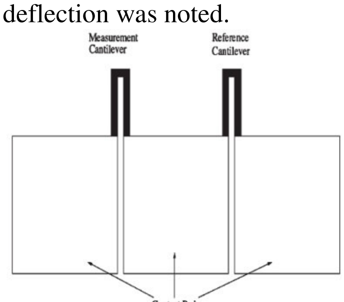
In cross-section, the micro-cantilever may consist of a structural layer, piezoresistive layer and an immobilization layer. We may consider polymeric micro-cantilevers that encapsulate a polysilicon piezoresistor [1][3]. The base resistance of piezoresistor depends on its dimensions and doping. SU8 is used as a flexible polymeric structural layer material. The top surface may be coated or immobilized with anti-bodies which are sensitive to certain antigens only. The target antigen molecules bind onto the anti-bodies which causes surface stress (Fig. 1). The flexible micro-cantilever bends upto few micrometers causing the base resistance (R) of the polysilicon piezoresistor to change by few parts per million (ppm). Here we refer the antigens as cardiac markers in blood sample and anti-bodies as anti-myoglobin, anti-troponin among others.

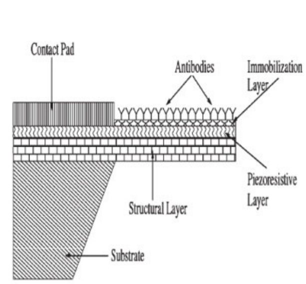
The change in deflection may be detected in a variety of ways. Electrical detection schemes are often preferred over optical methods due to portability, easy adjustments and user friendliness. Electrical schemes also make measurements possible in opaque media. In electrical detection schemes the change is often extremely small for standard instruments to reliably register. A highly sensitive, precision, customized and reliable electronic instrument is therefore necessary.

We present the design and demonstration of an ultra-sensitive instrument, to detect minuscule electrical changes in piezoresistive cantilever micro-structures while deflected. The electrical measurement of deflection characteristics is possible in dc and ac modes. However, dc mode, such as with Wheatstone bridge configuration, has the drawback of high flicker noise as in [2]. The ac mode has a reduced flicker noise problem but is sensitive to the parasitic components of the sensor and the layout. We prefer to use the ac mode for piezoresistive micro-cantilevers with minimal capacitive components.

A twin-cantilever structure in a single die was considered where one is the reference and other is the measurement cantilever (Fig. 1(a)). Only the measurement cantilever will be coated with bio-sensing molecules (Fig. 1(b)). During our experiments, the micro-cantilevers were connected to the instrumentation system and only the measurement cantilever was deflected using micromanipulators in a probe station; while the measurement system registered the miniscule changes (ΔR). The system provides an adjustment mechanism to compensate the base resistance mismatch up to a limit in micro-cantilevers.

The system output is in the form of a voltage. The calibration was done in two steps. First, known ΔR/R versus output voltages were noted and instrument sensitivity was calculated. Second, experiments on micro-cantilever deflection versus millivolt outputs were performed and the deflection sensitivity of piezoresistive micro-cantilevers was calculated. Therefore a relation between ΔR/R and cantilever deflection was noted.





(a) (b)

Figure 1: (a) Schematic plan view of the cantilever die. (b) Schematic cross-section of the measurement cantilever. [3]

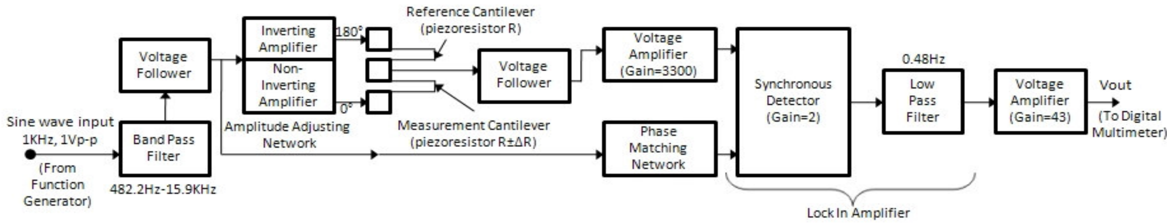


Figure 2: Functional block diagram of the instrumentation system for piezoresistive micro-cantilevers.

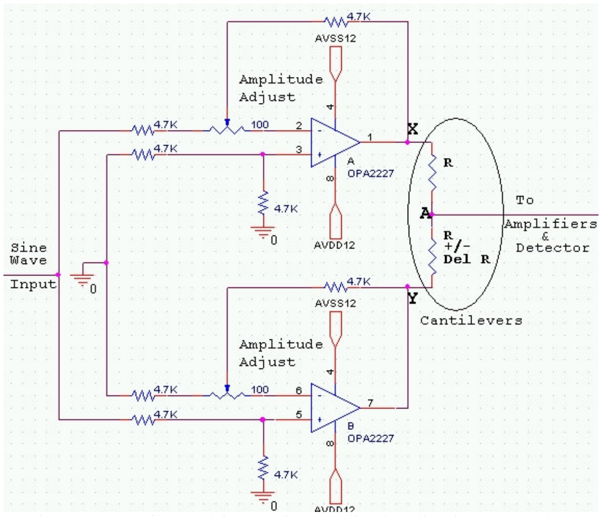


Figure 3: Cantilever drivers with amplitude adjusting network.

II. INSTRUMENTATION SYSTEM

In this section we describe the principles, system architecture, calibration and important considerations for such high performance instrumentation system.

A. Principles of Measurement

Two piezoresistors are connected in half bridge between nodes *X* and *Y*, and are fed with sine waves drivers (Fig. 3), that are 180° phase apart for each piezoresistor. The voltage divider node *A* sets at 0mV if the resistors are identical. Due to ΔR changes in the measurement cantilever in deflected conditions, a small ac voltage appears at the node *A*. This signal is amplified and applied to the signal input of a lock-in amplifier (LIA). The reference input to the LIA is the same input sine wave, through a phase matching network. The magnitude of the measured signal at *A* ranges from nanovolts up to a few microvolts. This is relatively weak as compared to ambient noise and necessitates the use of an LIA to reliably recover the small signal [4][5]. The output of LIA is a DC voltage which is proportional to the resistance variation (ΔR) in the measurement cantilever.

The LIA consists of a synchronous detector followed by a low pass filter. A synchronous detector is a signal multiplier, whose output is expressed as,

$$V_{SYN} \propto V_s V_r[\cos\{(\omega_s-\omega_r)t+\theta_s-\theta_r\} - \cos\{(\omega_s+\omega_r)t+\theta_s+\theta_r\}] \quad (1)$$

where, V_s and V_r are the peak amplitude of measurement and reference signals, ω_s and ω_r are angular frequencies, θ_s and θ_r are the corresponding phase angles. Subscript '*s*' and '*r*' refer to sensing/ measurement and reference inputs. Assuming, $\omega_s=\omega_r$ and $\theta_s=\theta_r=0$, the above equation simplifies to,

$$V_{SYN} \propto V_s V_r[1-\cos(\omega_s+\omega_r)t] \quad (2)$$

(a)

(b)

Figure 4: (a). Instrumentation set-up. (b). Analog PCB.

A large time constant low pass filter (LPF) at the output removes the cosine term and causes the LIA output to be:

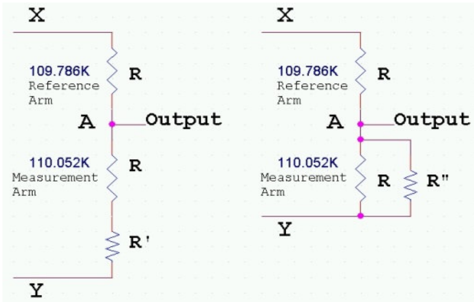
$$V_O \propto V_s V_r \quad (3)$$

The output of synchronous detector is thus a function of *amplitude*, *frequency* and *phase difference* of the signal and the reference sine waves. Considering the frequency and phase are identical at both the inputs, the output of a synchronous detector is therefore proportional to the signal amplitude variation only.

B. System Architecture

The key to measure ppm level changes in resistance are: (i) *isolation of all the cantilever connections* from *the system power rails*; and, (ii) use of *ac mode actuation* along with a *lock-in amplifier* based detection scheme.

The functional block diagram of the analog circuit module is shown in Fig. 2 and the instrument set-up in Fig. 4(a). A 1KHz, $1V_{p-p}$, 0mV offset signal is applied to the input from a function generator (Agilent AFG 33220A). The input stage consists of a first order resistor-capacitor (R-C) band pass filter (482.2Hz-15.9KHz) to reject out-of-band input noise, and a unity gain voltage follower to avoid loading. The buffered output is fed to externally adjustable non-inverting and inverting amplifiers which act as differential cantilever drivers. These drivers are also used as amplitude adjusting network, as depicted in Fig. 3, to compensate mismatch in cantilevers' base resistance values. The cantilever voltage divider node *A* is connected to a voltage follower to avoid loading. A high gain voltage amplifier (Gain=3300) is connected at the output of voltage follower. The output of this amplifier is connected to the signal input of the synchronous detector. The reference input of synchronous detector is fed with the same input sine wave through a phase adjusting or R-C network [6] due to drivers. The gain of the synchronous detector's output is 2. The signal is then passed through a low pass filter (LPF) (f_o=0.48Hz) for high



(a) (b)
Figure 5: Resistor arrangements during instrument calibration.

frequency rejection. A final dc voltage amplifier (Gain=43) boosts the output further. A clean dc voltage at the output indicates the change in resistance ((ΔR)) of the measurement arm. The output voltage was displayed using 7.5 digit digital multimeter (Keithley DMM 2000). The measurement circuit was powered using batteries.

The filters and amplifiers were realized using OPA2227 precision operational amplifiers (Burr Brown). The synchronous detector was implemented using AD630 balanced modulator/ demodulator integrated circuit (IC) (Analog Devices). Small outline integrated circuit (SOIC) packaged ICs and 0603 sized surface mount passive components were used on the printed circuit board (PCB). Surface mount devices were preferred due to smaller dimensions and reduced parasitic components. Fig. 4(b) shows the analog PCB.

C. Calibration Method

The quasi-static instrument calibration was performed by using standard 1% tolerance resistors in series-parallel combinations. Since the resistors on our cantilevers had a nominal value of 110KΩ, similar value resistors were considered. The nominal values of the reference and measurement arms were 109.786KΩ and 110.052KΩ. Fig. 5 shows the half bridge connected resistors during calibration process. The values of ΔR were introduced either by connecting the resistors *R'* in series (Fig 5(a)), or by *R"* in parallel to the measurement arm (Fig. 5(b)).

The sensitivity of the instrument was calculated by dividing the measured voltage by a known change in resistance. Table 1 shows the system performance over a range of ΔR.

D. Important Considerations

1) Electrical Considerations: High signal-to-noise ratio is a challenge while acquiring weak signals. The ac actuation was preferred over dc to avoid 1/f noise. The cantilevers were driven in differential mode and thus isolated from direct power supply connections in order to avoid drift due to supply noise. Low noise op-amps (3nV/√Hz) and high precision ICs were used in the circuit. 1KHz actuating sine signal is optimum for the synchronous detector IC and thus was used. Decoupling capacitors were placed close to the power supply pins of all ICs. Low noise voltage regulators were mounted on the same analog PCB. The layout was realized on high dielectric constant glass epoxy PCB substrate (FR4 grade) with 0.5mm wide and 35µm thick copper tracks. Ground plane was provided for noise immunity. Precision ten-turn potentiometers (Bourns) were used for external amplitude and phase adjustments. Gold plated bayonet coupling (BNC) connectors and low leakage teflon cables were used to connect the cantilevers. The unit was housed inside a double shielded box to protect the circuit from undesired electro-magnetic interferences and temperature drift. The batteries were placed inside the shielded box. High input impedance Keithley 2000 multimeter was used for calibration/ measurement purposes.

TABLE I
CALIBRATION DATA

ΔR	Known Values of |ΔR|	ΔR /R (Measurement Arm)	Measured Output Voltages	Instrument Sensitivities
R'	0.99 Ω	9.08 ppm	0.006 V	0.6948 mV/ppm
R'	13.70 Ω	124.48 ppm	0.086 V	0.6948 mV/ppm
R'	25.61 Ω	232.72 ppm	0.162 V	0.6961 mV/ppm
R'	49.85 Ω	453.00 ppm	0.315 V	0.6971 mV/ppm
R"	50.07 MΩ	2192.79 ppm	1.551 V	0.7073 mV/ppm
R"	40.18 MΩ	2731.28 ppm	2.017 V	0.7384 mV/ppm
R"	30.11 MΩ	3640.17 ppm	2.750 V	0.7554 mV/ppm
R"	20.05 MΩ	5457.29 ppm	4.193 V	0.7683 mV/ppm

2) Cantilever mismatch: The cantilevers should ideally be matched between the reference and measurement arms. Such assurance is practically impossible due to fabrication process variation. It was observed experimentally that a nominal value mismatch of upto 5% was reliably accommodated by the potentiometer based adjustments in the amplitude matching network. Otherwise a finite non-zero output offset values were present in each measurement and the sensitivity was affected as the mismatch increases.

3) Mechanical considerations: The micro-cantilever based experiments may be performed on a vibration isolated platform. All the cables were held static with no dangling part during measurements.

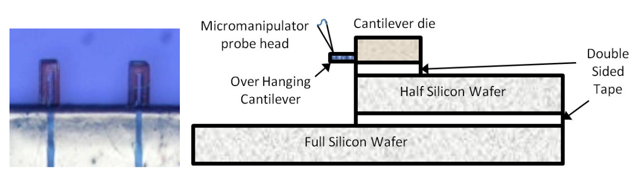
4) Thermal considerations: The actuation voltage was kept low in order to reduce the possibility of thermal stress on the devices. A 4.5µA peak current was forced through 110KΩ cantilever bridge during experiments.

III. EXPERIMENTS

Deflection sensitivity of micro-cantilever structure is an important performance parameter. It is determined as a relative change in resistance (ΔR) with known cantilever deflection. An experiment was designed to measure such parameter. The micro-cantilevers' fabrication is discussed in [3] and the polymeric test devices are shown in Fig. 6(a).

A. Experimental Set-up

A 'test device' was prepared by sticking the cantilever die on a 2 inch half wafer, which was further mounted on 2 inch full wafer using double sided tapes. Fig. 6(b) shows the schematic cross-section of the 'test device'. The maximum



(a) (b)
Figure 6: (a). Micro-cantilevers used in measurements. (b). Schematic cross-section of the 'test device' [1][3].

possible deflection was about 325µm (275µm wafer edge + 50µm thick double sided tape). The 'test-device' was mounted in Karl Suss PM8 probe station during experiments. The probe station provides the following advantages [3][5]: (i) gold contact pads on cantilever die can be viewed easily under the built-in microscope; (ii) probing of the pads was easy; (iii) the tungsten probe (tip diameter of 7µm) can be accurately placed on the measurement cantilever for deflection; (iv) the 'test device' can be firmly held on the chuck with the aid of vacuum. The cantilevers were fabricated in half-bridge connection; three micromanipulators were thus used to probe the gold contact pads. A calibrated micromanipulator was used to deflect the measurement micro-cantilever. The least count of micrometer screw gauge of the micromanipulator was 10µm. The entire assembly was kept inside a Faraday shield and placed on a vibration resistant table. The function generator, digital multimeter and the instrumentation box were placed outside, but near the Faraday shield of the probe station.

B. Experimental Procedure

The 'test device' was placed inside the probe station and micromanipulators were pre-adjusted for probing. The instrumentation box, function generator and digital multimeter were powered and allowed to attain a stable operating temperature. Nominal resistances of cantilevers were measured. The instrumentation box was then connected to the probe station connectors (BNC). The output was approximately set to 0mV by adjusting the potentiometers in the amplitude and phase adjusting network when the cantilevers were not deflected.

The measurement cantilever was deflected with the calibrated micromanipulator probe. The reference one remained undisturbed. The voltage readings were noted at each deflected position. A settling time of 20 seconds was allowed for each reading.

IV. RESULTS

The characterization experiments on piezoresistive micro-cantilevers were performed using our custom instrumentation block. A repeatable output voltage shift was observed as the measurement cantilever was deflected. A graph showing ΔR/R versus tip deflection is depicted in Fig. 7. The slope of the curve reduces at larger deflections. This may be due to non-linear resistance variation in the cantilever. The 10µm deflection in micro-cantilever causes a 40mV change in output. Considering the sensitivity of the instrumentation block as 0.6948mV/ppm, the ΔR is approximately 57.57ppm. The deflection sensitivity is therefore 5.757ppm/µm. The measurements were repeated to check the consistency. A smaller ΔR can always be registered using a better micromanipulator with a higher resolution. ΔR variation below 6ppm may be difficult to note due to the output voltage fluctuation of up to 4mV. The reference and measurement cantilevers may be interchanged in the half bridge, but the readings will only have the opposite polarity without affecting the sensitivity.

The present system is useful for cantilevers with resistive component only. The polymer based cantilevers are usually free of capacitance problems unlike silicon based cantilevers. Compensation of reactive components due to capacitance in is not addressed in this paper.

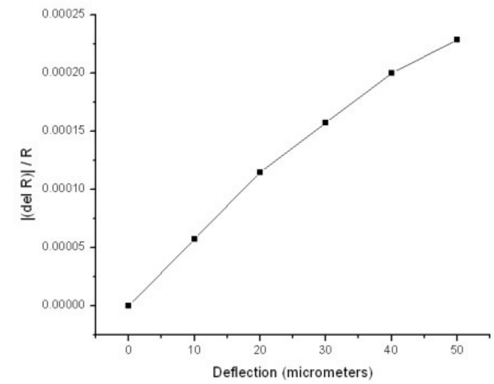


Figure 7: Tip deflection versus ΔR/R.

TABLE II
SPECIFICATIONS OF ΔR/R MEASUREMENT SYSTEM

Parameters	Values
Cantilever Resistance Range (R)	10KΩ - 1MΩ
Change in Resistance Range (ΔR)	6ppm - 3600ppm
Sensor Actuation Voltage	1Vp-p Sine Wave
Sensitivity	0.6948 mV/ppm
Signal-to-Noise Ratio	56db max.
Settling Time	15 seconds
Supply Voltage / Current	±12V / 30mA

V. CONCLUSION

A highly sensitive ΔR/R measurement block for piezoresistive cantilevers is demonstrated with a sensitivity of 0.6948mV/ppm. The instrument calibration technique is discussed and quasi-static experiments were performed to determine the deflection sensitivity of the micro-cantilever structures. The sensitivity is nearly constant up to 2200ppm. The performance can even be improved by placing the cantilever die onto the analog PCB and using an on-board low-noise oscillator. The analog output may be interfaced to high resolution analog-to-digital converters (ADC) with full scale input range of ±2.5V. The system is targeted towards measurement of ΔR/R, in affinity cantilevers for Bio-MEMS applications, which are of quasi-static nature.

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