Affinity cantilever sensors for cardiac diagnostics

Manoj Joshi, Nitin Kale, S Mukherji, R Lal & V Ramgopal Rao*

Micro-fabrication Laboratory, Indian Institute of Technology Bombay, Mumbai 400 076

* E-mail: rrao@ee.iitb.ac.in

Cardiac problems are on the rise in the Indian sub-continent due to the changing life styles and food habits. Acute Myocardial Infraction (AMI) is becoming a major concern. In this paper, we report the development of affinity cantilever based sensors for the detection of AMI with optical and electrical readouts. We designed, simulated and fabricated these cantilevers with various micro fabricated materials such as silicon dioxide, silicon nitride and SU-8. Thin films such as silicon nitride and *p*-type polysilicon are deposited using hot wire CVD technique. For electrical detection, *p*-type polysilicon was used as a piezoresistive layer. The mechanical and electrical performance parameters of these cantilevers were investigated using Atomic Force Microscope (AFM) set-up. The top surface of these cantilevers was selectively immobilized with the antibodies. Following this, antibody specific antigens were allowed to react with the cantilever surface in the liquid cell of AFM set-up for the optical detection. For electrical signal detection, microcontroller based signal conditioning and digital readout circuit was developed.

Keywords: Cantilever sensors, Cardiac diagnostics, Myocardial infraction, Piezoresistive layer, Atomic force microscope

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1 Introduction

Cardiac markers are basically biomolecules (proteins/enzymes), which are released from the cardiac muscle after the onset of myocardial infraction. Pattern of release of different cardiac markers[1] with respect to time are as shown in Fig. 1. Antibody-antigen attachments on either top or bottom surface of the microcantilever surface generates differential surface stresses at the opposite faces of the cantilever, which leads to a bending of the cantilever (Fig. 2), which can be detected optically using a standard AFM set-up. For the detection of change in stress within the piezoresistive affinity cantilevers, a highly sensitive electrical readout circuit is developed.

Fig. 1 — Plot of the appearance of cardiac markers in the blood versus time after onset of the acute myocardial infraction symptoms

2 Experimental Details

2.1 MEMS material development

Hotwire CVD was chosen as the technique for depositing thin films of doped microcrystalline silicon (mSi:H) and silicon nitride. Using this technique one can deposit thin films at low substrate temperature, thus enabling the usage of polymers as structural material. Hotwire CVD deposits films at high deposition rate, and films so deposited are free from plasma damage.
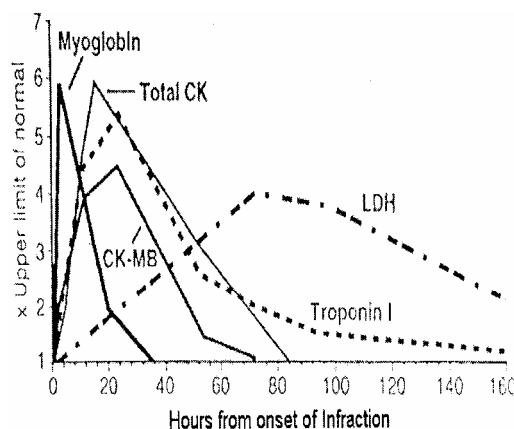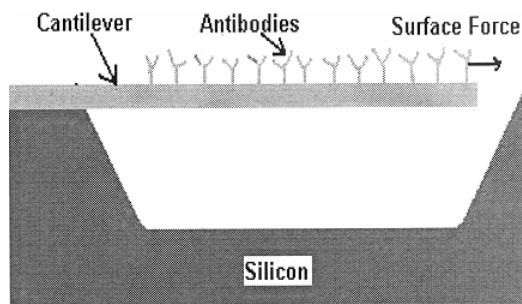

Fig. 2 — Cantilever with immobilized antibodies which develop a surface stress

Silicon nitride films were deposited at a substrate temperature of 300°C, at different gas pressure values, and also with or without nitrogen dilution. The films were characterized for their deposition rate, refractive index and etch rate in buffered hydrofluoric acid (BHF). The deposition rate was between 1.2 A/s to 1.7 A/s. Refractive index, which indicates stoi-chiometry, was measured using ellipsometer and was between 1.78 and 2.2. The etch rate depends on the gas pressure and usage of nitrogen dilution. Etch rate varied between 1 and 100 nm/min for these deposition parameters. Low etch rate films are desired for a better selectivity over silicon dioxide. We optimized the deposition parameters for low etch rate as:

(i) SiH_4 : NH_3 flow rate ratio = 1 : 20; (ii) gas pressure = 50 mTorr; (iii) no usage of nitrogen dilution.

Doped *p*-type microcrystalline silicon films were developed for achieving a high gauge factor. The films were deposited at different substrate temperature and hydrogen dilution. Substrates used were Corning 7059 glass or SU-8 coated Corning glass.

The deposition rates of these films were measured to be between 3.6 to 4.0 A/s. These films have a grain size of between 15 to 30 nm. Grain size measurements were made using intensity versus 2 theta XRD plots and Scherrer's formula. We have achieved a gauge factor of above 20 for films deposited at a substrate temperature[2] of 150°C. Residual stress in mSi:H films was measured using XRD sin2psi technique and it was observed to vary with film thickness and hydrogen dilution. The measured residual stresses[3] are in the range 200-400 MPa.

3 Fabrication of Cantilevers to Fit in AFM Nose

For the detection of bending of affinity cantilever optically, either we need to develop optical detection system or one can use the standard AFM set-up. We decided to use the AFM set-up with liquid cell accessories for this measurement. The main bottleneck in this experiment was to fabricate cantilevers, which would fit in the AFM set-up. Fig. 3 shows the prototype and the pictures of the fabricated silicon dioxide and SU-8 cantilevers which can fit in the AFM nose.

4 Antibody Immobilization on Affinity Cantilevers

Fabricated cantilevers need to undergo surface modification followed by antibody immobilization. It is extremely essential to selectively immobilize the antibodies only on top or bottom surface of the cantilever. The detailed study of selective immobilization is demonstrated in Ref. 4. For the immobilization of antibodies on various materials, different protocols were developed and charac-terized[5-8]. Using these immobilization protocols, the top surfaces of the cantilevers were immobilized with the antibodies. The surface was investigated using a fluorescent microscope. One example of antibody immobilization on oxide affinity cantilever used for optical detection and SU-8 cantilever used for piezoresistive detection is shown in Figs 4 and 5,

Fig. 3 — Highly sensitive affinity cantilevers fabricated at IIT Bombay for optical detection. These cantilevers have been fabricated to fit into a commercial AFM nose (a) Prototype picture (b) SEM image of silicon dioxide cantilever (c) Optical image of SU-8 cantilever
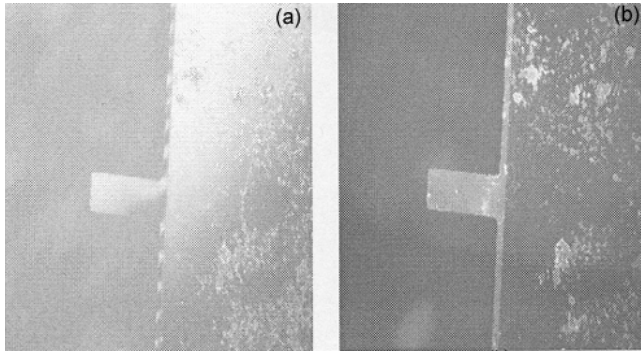
Fig. 4 — Micrograph of silicon dioxide cantilever used for the optical detection with incubation of HIgG followed by FITC tagged goat anti HIgG observed under (a) optical microscope (b) fluorescent microscope
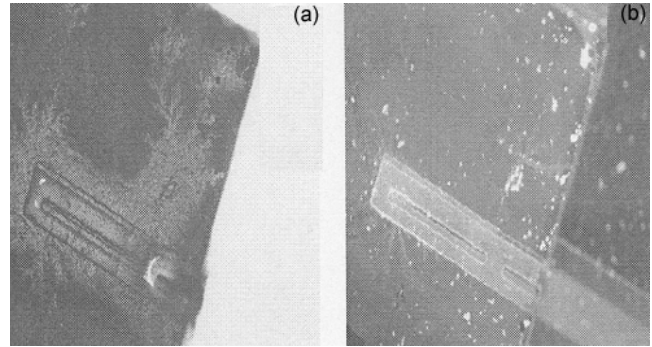

Fig. 5 — Micrograph of SU-8 cantilever used for the optical detection with incubation of HIgG followed by FITC tagged goat anti HIgG observed under (a) optical microscope (b) fluorescent microscope
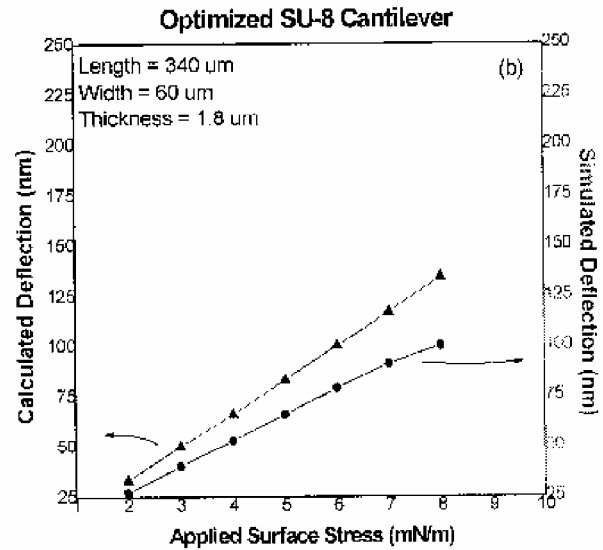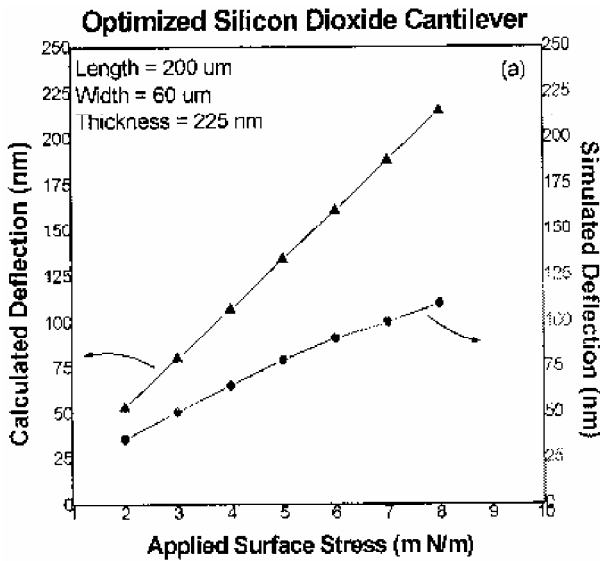

Fig. 6 — Applied surface stress versus deflection of affinity cantilevers for optical detection (a) silicon dioxide cantilever (b) SU-8 cantilever

respectively. The top surface of these cantilevers was modified to enable it for the antibody immobilization which was followed by the immobilization of human immunoglobulin (HIgG) and FITC tagged anti-HIgG. These cantilevers were further observed under normal optical and fluorescent microscope.

5 Simulation Studies

The performance of the affinity cantilever mainly depends on the properties of material used and its geometrical dimensions. In our study, we proposed silicon dioxide, silicon nitride and SU-8 cantilevers for optical and electrical detection. In electrical detection *p*-Type polysilicon was used as the piezoresistive material. The prototypes of such cantilevers was shown in Fig. 3.

Mathematical equations for the analytical calculations of performance parameters of affinity cantilevers are reported in literature[9-13]. The magnitude and the nature of the stress developed on cantilever surface depends on the type of biomolecules immobilized. The affinity cantilevers demonstrated in this paper are proposed for the detection of a cardiac marker called myoglobin. The surface stress developed on the cantilever surface due to immobilization of such proteins[12] is of the order of few mN/m. For detecting such biomolecules, sensitivity of affinity cantilevers should be high. A change in the surface stress has to be induced by changing liquid medium around the cantilever. Preferably this should be done *in-situ* in a liquid cell having flow through attachment, which allows us to
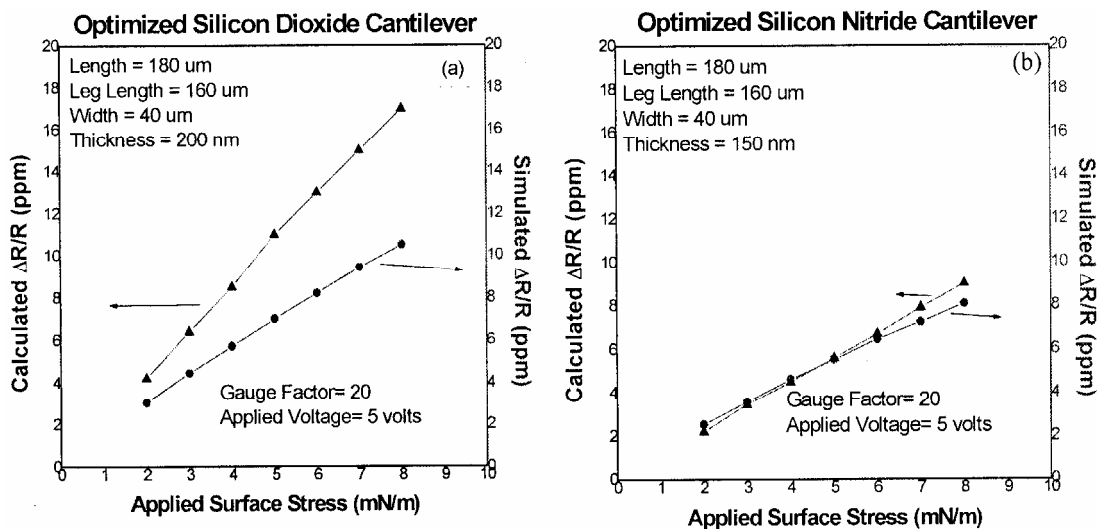
Fig. 7 — Applied surface stress versus deflection of affinity cantilevers for piezoresistive detection (a) silicon dioxide cantilever (b) silicon nitride cantilever
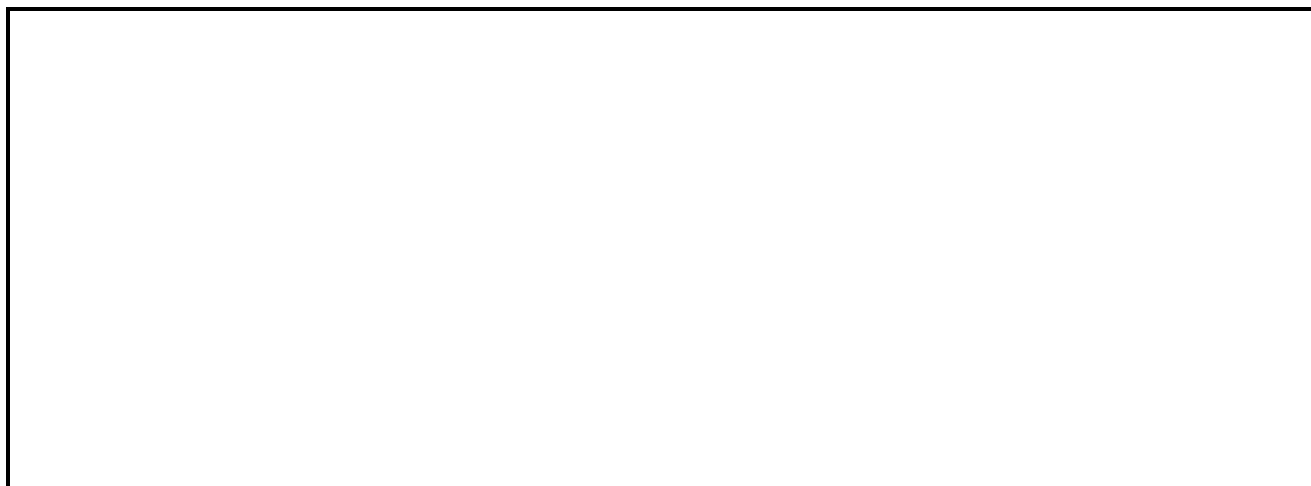

Fig. 8 — Piezoresistive affinity cantilevers fabricated at IIT Bombay with the total stack thickness below 300 nm (a) schematic diagram (b) SEM images of silicon dioxide cantilever (c) SEM images of silicon nitride cantilever with a polysilicon piezo-resistor

monitor change in surface stress and hence bending of the cantilever continuously.

Fig. 6 shows the surface stress versus deflection of silicon dioxide and SU-8 cantilevers. The mismatch in the curves of analytically calculated and simulated deflection is due to the non-linear behaviour of the both cantilever. Similar type of simulation studies were performed for the piezoresistive affinity cantilevers. The simulations were carried out using the simulator Coventorware.

Fig. 7 shows the surface stress versus $\Delta R/R$ obtained from silicon dioxide and silicon nitride cantilevers. In these cantilevers, the mismatch in the analytically calculated and simulated $\Delta R/R$ curve is also due to the non-linearities presents within the cantilevers.

6 Fabrication of Piezoresistive Cantilevers and Immobilization

In Fig. 8, we show the prototype and the fabricated cantilever structures that can be used for piezoresistive detection. The structures have contact pads for electrical probing. They are a part of a Wheatstone bridge. One of them will act as the sensing element while the others act as reference elements. The cantilever stack was etched by employing reactive ion etching (RIE). The devices were released using TMAH.
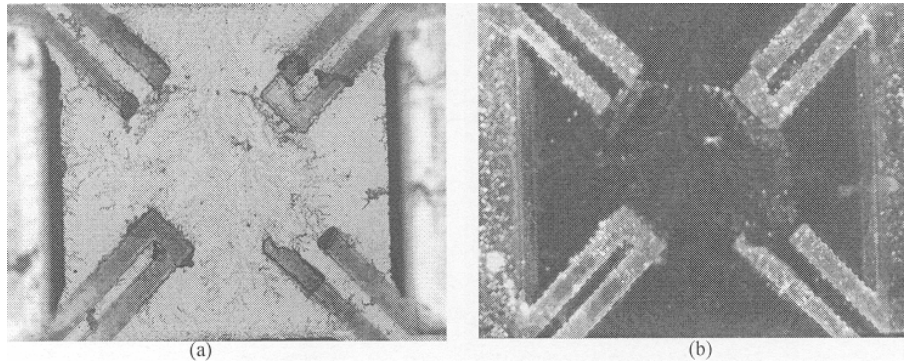
(a)   (b)

Fig. 9 — Micrograph of piezoresistive silicon nitride cantilevers used for the electrical detection with incubation of HIgG followed by FITC tagged goat anti HIgG observed under (a) optical microscope (b) fluorescent microscope
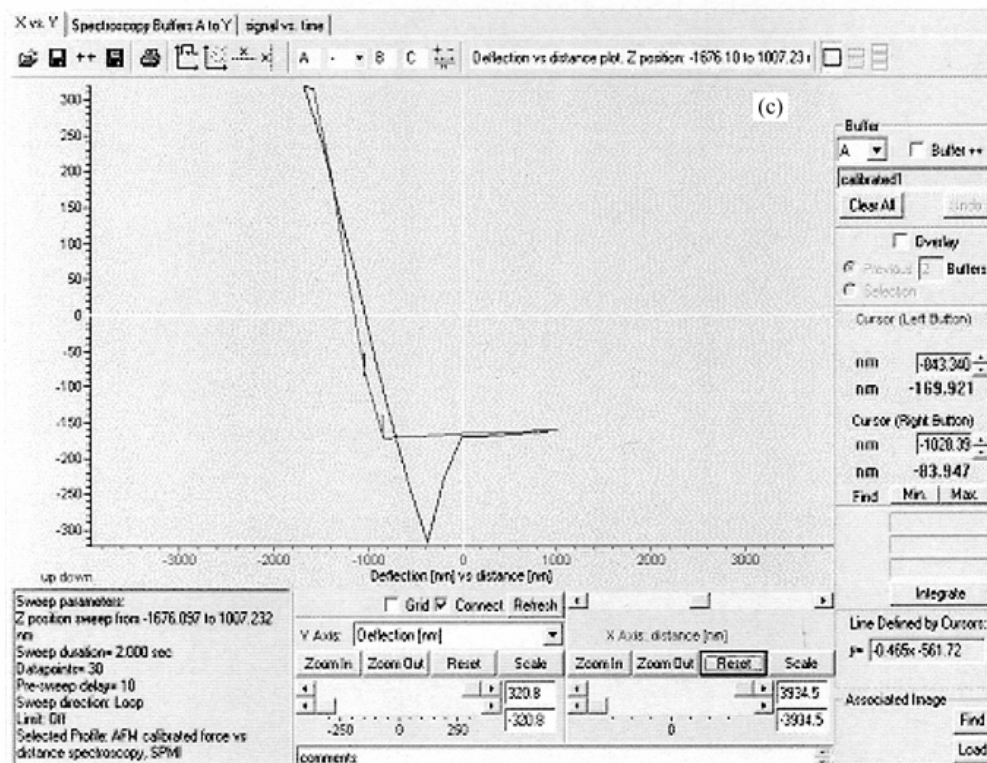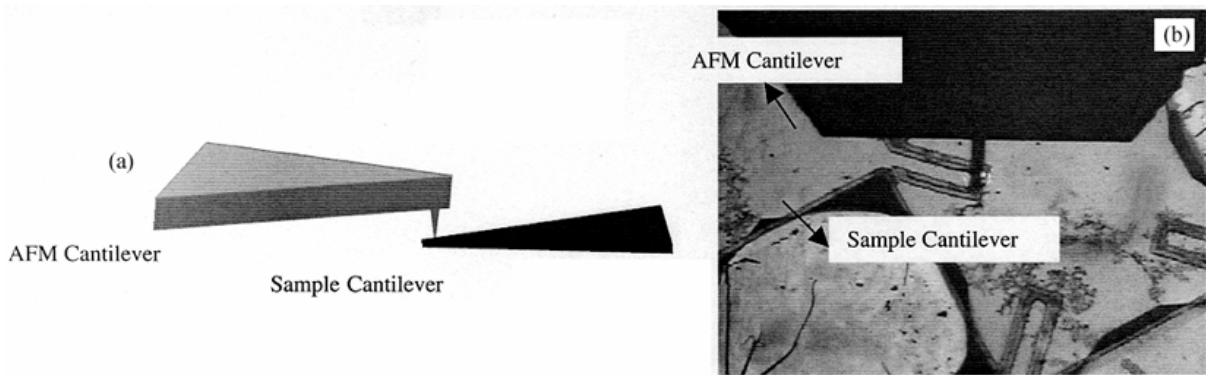

Fig. 10 — AFM cantilever deflecting sample cantilever (a) principle used for spectroscopy (b) Actual picture taken from the CCD camera (c) Force-distance curves

In Fig. 9, the images of cantilevers whose top surface is immobilized with antibodies are shown.

6.1 Cantilever Characterization

Sensitivity of the affinity cantilever depends on its mechanical and electrical performance parameters. Hence before using the fabricated cantilever for the bio-sensing application, it is essential to investigate these parameters Fig. 10.

(a) *Mechanical characterization* — Force-distance spectroscopy of AFM was used to extract the spring constant of a silicon nitride cantilever[14]. From the measured spring constant and the dimensions of the cantilever we calculated the Youngs modulus of silicon nitride. In this method, the tip of the AFM cantilever is rested on the tip of the sample cantilever. A known amount of sweep is applied to the AFM cantilever in the Z-direction. Since the sample cantilever is in contact with the AFM cantilever, it will also deflect. Deflection can be measured using a PSD and force can be calculated using the known spring constant of AFM cantilever. The AFM cantilever had a spring constant of 0.11 N/m, measured spring constant of sample cantilever was 94.6 mN/m.

Ansys 6.0 was used to simulate the silicon nitride cantilever. Structure was created using SOLID 8 Node brick element. Initially default value of Young's modulus was used. Keeping the base fixed, a point force is applied at the tip of the structure and the deflection was observed. This deflection is used to calculate spring constant. This procedure is iterated several times by changing the default value of the Young's modulus. Finally a match is obtained between measured and simulated Youngs modulus. Using this method we calculated the Young's modulus of silicon nitride, deposited by Hotwire[15] CVD, as 207 GPa.

(b) *Electrical characterization* — In case of piezoresistive affinity cantilevers under study, the expected change in the resistance is of the order of few parts per million (ppm). To detect such a small change in resistance and the associated electrical signal, sensitive signal conditioning and display circuits were developed[16]. The microcontroller MSP430f161x was used to achieve a high resolution. When the signal is very weak, higher signal to noise ratio is always desired. Faraday's cage was used to eliminate the noise within the measurement set-up. The circuit details are beyond the scope of this paper. However, change in resistance as low as 10 ppm was measurable with the set-up.

7 Conclusion

We have employed affinity cantilevers to detect cardiac markers. These cantilevers bend due to surface stresses that are created by the immobilization of target molecule. We have measured the etch rates of microcrystalline silicon and silicon nitride. We have measured the gauge factor of microcrystalline silicon to be above 20. The Young's modulus of silicon nitride was measured to be 207 GPa. Cantilever structures for optical detection technique were fabricated so as to fit in an AFM's nose. Piezoresistive cantilevers were also fabricated. Immobilization protocols were developed for antibody immobilization on surfaces like silicon dioxide, silicon nitride, SU-8. A measurement set-up has been fabricated to detect a resistance change of the order of 10 ppm. Further work is on to carry out the liquid cell experiment.

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