UNIVERSITÉ DE MONTRÉAL

CANTILEVER ARRAY PLATFORM FOR QUANTITATIVE BIOLOGICAL ANALYSIS

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CANTILEVER ARRAY PLATFORM FOR QUANTITATIVE BIOLOGICAL ANALYSIS

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DEDICATION

To my family who have always believed in me and supported me through everything

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RÉSUMÉ

L'objectif de ce projet est de développer un réseau de microcapteurs pour collecter des données biologiques quantitatives. Ces types de données peuvent être utilisés dans divers domaines, notamment pour l'analyse cellulaire et moléculaire, la détection d’interactions biologiques spécifiques, la surveillance de maladies et la découverte de médicaments.

Les capteurs proposés possèdent des réseaux de « cantilevers » qui convertissent les interactions biologiques en variations mécaniques et électriques. Ces capteurs peuvent avoir une sensibilité élevée et ont montré leurs efficacités dans diverses applications. De plus, leur utilisation permet de concevoir un système à haut débit pour la détection en temps réel de diverses paramètres.

Afin de développer ces capteurs, un logiciel multiphysique (COMSOL) a été utilisé pour modéliser les « cantilevers » et plusieurs simulations électromécaniques ont été réalisées pour atteindre une conception appropriée.

Deux méthodes de lecture, piezorésistive et capacitive, ont été choisies pour être utilisées avec les capteurs. Les deux capteurs ont été fabriqués par le biais de CMC Microsystems; le processus PolyMUMPs a été employé pour la fabrication de réseaux de capteurs capacitifs, et les capteurs piézorésistifs, quant à eux, ont été développés par le processus de MetalMUMPs. Enfin, les capteurs fabriqués ont été caractérisés suivant différentes étapes incluant l’interferometrie afin d'assurer leur fonctionnalité.

Sur la base des résultats de simulation et de caractérisation obtenus, ces capteurs peuvent être utilisés pour élaborer une plateforme haut débit à bas coût pour diverses applications biologiques.

ABSTRACT

The objective of the present project is to develop an array of microsensors for gathering cellular and molecular quantitative biological data. Such data can be used in various fields including cellular and molecular analysis, detection of specific biological interactions, monitoring diseases, and drug discovery.

The proposed sensing platform in this project can convert biological interactions into mechanical variations and subsequently converts the mechanical variations to electrical ones. This platform offers the advantage of high sensitivity, real time measurement, high throughput sensing array suitable for fundamental studies as well as clinical applications.

We modeled the operation of cantilevers using COMSOL multiphysics software. These simulation techniques can efficiently be used to choose the suitable design and dimensions of cantilevers. Two readout methods, piezoresistive and capacitive, have been chosen to be used along with sensors. Both sensors were fabricated through CMC Microsystems; PolyMUMPs process was employed for fabrication of capacitive sensor array and piezoresistive sensors were developed by MetalMUMPs process. The functionality of cantilevers and their incorporated sensors were characterized through different techniques including interferometry.

Based on these simulation and characterization results, the proposed sensors can be good candidate for developing a low cost, high throughput platform for various biological applications.

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LIST OF SYMBOLES AND ABBREVISATIONS

α-hGH	human Growth Hormone antibody
AFM	Atomic Force Microscopy
ASIC	Application Specific Integrated Circuit
CCD	Charge Coupled Device
CMOS	Complementary Metal Oxide Semiconductor
CRP	C-Reactive Proteins
DNA	Deoxyribonucleic Acid
E.Coli	Escherichia Coli
GST	Glutathione-S-Transferase
HBsAg	Hepatitis B surface antigen
HbA1c	Glycated hemoglobin
hGh	human Growth Hormone
IgG	Immunoglobulin G antibody
ISFET	Ion Sensitive Field Effect Transistor
LD	Laser Diode
MBM	Molecular Biomechanical
MEMS	Microelectromechanical system
MOSFET	Metal Oxide Semiconductor Field Effect Transistor
MUMPs	Multi User MEMS Processes
PCB	Printed Circuit Board
PD	Photo Detector
PDMS	Polydimethylsiloxane
PSA	Prostate Specific Antigen

PSD	Position Sensitive Detector
PZT	Lead Zirconate Titanate
SIO_2	Silicon Dioxide
SOI	Silicon on Insulator
ssDNA	Single Strand DNA
TSMC	Taiwan Semiconductor Manufacturing Company
VCSEL	Vertical Cavity Surface Emitting Laser
Zno	Zink oxide
t	Thickness
L	Length
E	Young modulus
ρ	density
f	Resonance Frequency
ε_0	Dielectric Constant
ε_r	Relative Permittivity
F	Force
A	Effective area of Capacitor
d	Distance between two plates of Capacitor
Δv	Output Voltage of Wheatstone Bridge
V_i	Applied Voltage to Wheatstone Bridge
π_1	Longitudinal Piezoresistive Coefficient
ξ	Correction Factor
σ	Stress
$\{\pi\}$	Piezoresistive tensor

Δz	Displacement of the cantilever beam
υ	Poisson ratio
β	Expansion Coefficient
λ	Thermal Conductivity
P	Total generated power
n	Geometrical parameter
w	Width
k	Spring Constant
C	Capacitance
R	Resistance
$\Delta\varepsilon$	Strain variation

INTRODUCTION

The optical methods are widely used in biological laboratories using labeling techniques. These methods require bulky and costly setup along with expensive chemical and biological reagents.

Developing a system that gives us quantitative biological data and enables us to analyze the samples without using labeling methods can be useful for many applications. Such a system can be used in biological research, for instance to analyze several parameters like cell growth, extracellular matrix interactions, and DNA analysis. Hospitals and laboratories can also benefit from this device for various clinical purposes including the detection of cancer markers in patient's sample.

Nowadays, considerable efforts are made for designing label-free techniques. However, most of these platforms contain small number of sensors, or they rely on using expensive measurement instruments. High throughput sensing platforms incorporated with appropriate readout systems can overcome these limitations and will be very useful for many life science applications. The primary step toward developing such a platform is the selection and modeling of sensors.

In order to propose a sensor for a biological analysis platform, different parameters should be taken into account. One of the first steps is to choose the type of sensor. Several studies have demonstrated the advantages of microelectromechanical systems (MEMS) for biological applications [1-3]. These devices are called BioMEMS. By using a large number of MEMS sensors in a platform it would be possible to monitor multiple parameters in real time. BioMEMS are implemented using different structures like micro-needles, membranes, cantilevers, plates, and etc. Among all the above-mentioned structures, cantilevers show a good potential for measuring various parameters with high sensitivity. Cantilevers are composed of a suspended beam that can convert biological interactions into mechanical and electrical variations. These sensors function based on the adhesion of cells or molecules on their top surface. Due to the variation in surface stress after adhesion, the beam will deflect and it is possible to relate the amount of deflection to the biological interaction that causes the displacement. One of advantages of the cantilever structure is that its fabrication is supported in several standard MEMS processes. Using a standard fabrication process makes it possible to develop many sensors at low cost. Another advantage of using cantilevers as sensors is that they have the potential to be integrated along with readout circuits on one chip.

Next step toward developing a sensing platform is to choose a suitable readout method for the detection of final output. Several methods can be used to measure the displacement of cantilevers among which optical, capacitive, piezoresistive, and piezoelectric readout are the most common techniques. Each of these methods has its own advantages and disadvantages. In order to select a good readout technique, one should consider various parameters including cost, portability, simplicity, and sensitivity.

Finally, in order to design an efficient system, it is important to model the sensors before the fabrication process and analyze the effect of different parameters on them. For modeling structures like cantilevers that deal with mechanical and electrical variations, it is essential to consider both physics in the modeling. Multiphysical simulations have already been used for several biological engineering applications and it has been shown that the results from these simulations can help in understanding the sensing process [4-5]. By considering all the relevant physics in modeling of cantilever sensors, it would be possible to reach a suitable design before starting the fabrication step.

In this project we have developed two arrays of cantilever sensors for quantitative biological analysis. Piezoresistive and capacitive techniques have been chosen as readout methods and the sensors have been modeled with COMSOL Multiphysics software. The multiphysical simulation methods enable us to create a link between electrical and mechanical characteristics of cantilever. Each array of sensors has been developed with a standard MEMS fabrication process through CMC Microsystems and several characterizations have been done to ensure their functionality.

This thesis is organized in 5 chapters. In chapter 1, the fundamentals of cantilever biosensors will be introduced and different biological applications of these types of sensors will be presented. Chapter 2 contains the information regarding the first proposed sensor array that uses piezoresistive measurement for detection of displacement. The content of this chapter is submitted as journal paper to IEEE Sensors Journal. Chapter 3 focuses on the second proposed cantilever array using capacitive readout method following by the detailed information of simulation, fabrication and characterization processes. Chapter 4 discusses the challenges of developing these sensors along with practical considerations. Finally, the project will be concluded in chapter 5.

CHAPITRE 1 FUNDAMENTALS OF CANTILEVER BIOSENSORS

Nowadays developing small and inexpensive systems for monitoring various biological parameters is one of the growing demands in life science. Handheld devices have attracted the attention of researchers in both industrial and academic sectors to develop miniaturized biological analysis systems that have high sensitivity and can be used for drug discovery or for monitoring health conditions. One of the important steps in developing all these devices is the design of sensors. Several research groups are working on developing different types of micro scale sensors that can be used for various biological and medical applications. Among all different types of sensors, cantilevers have shown a good potential for biological studies. Cantilever is a beam which is fixed in one end and will deflect due to application of force on its surface. These sensors can have high sensitivity, good resolution and they can be fabricated with low-cost fabrication processes.

Originally, the idea of using a cantilever structure as a sensor emanated from atomic force microscopy (AFM) [6]. In AFM, a cantilever with a sharp tip is being used for measuring different parameters like the interactions between antibody-antigen [7-8], surface imaging [9] or manipulating small particles [10]. Figure 1-1 presents a common cantilever used in AFM [11]. In 1996, Butt reported one of the first applications of a single cantilever as a biosensor [12]. Since that time, many research groups become interested in using this structure for different chemical and biological measurements.

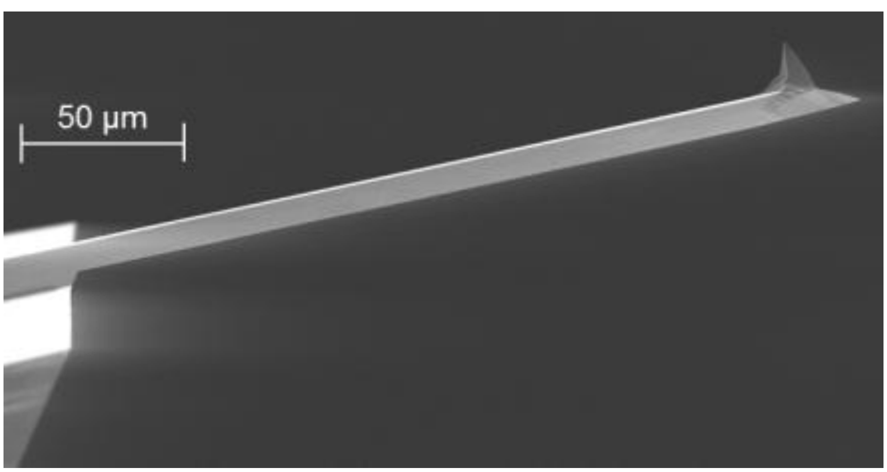


Figure 1-1: AFM Cantilever (Image provided by Geiss and Hurley, NIST, Boulder, Colorado, USA) [11]

Although single cantilever can have good performance in different applications, in order to have a high throughput system, it is necessary to increase the number of sensors. High throughput systems enable us to have several measurement points and they can be used in a variety of applications where multiple parameters or particles need to be detected simultaneously. In addition, by having more than one cantilever in a platform, it would be possible to calibrate and analyze the output based on a reference point. For example, in molecular detection applications, this reference point can be one of the cantilevers in the array which is exposed to the same environment, but it is not coated for binding with specific molecules. In this case, the signals from the coated beam will be compared with the reference cantilevers, thus it would be possible to remove the noise from the output and achieve more accurate measurements. Figure 1-2 presents a platform with sensing and reference electrodes.

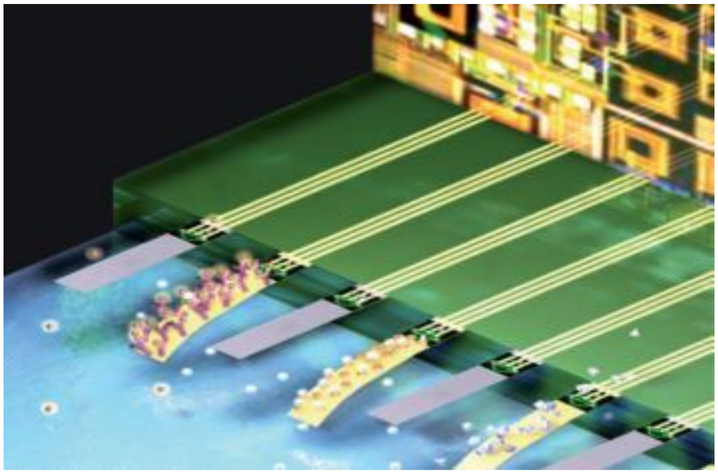


Figure 1-2: Cantilever array platform with sensing and reference cantilevers [13]

In 1997, a group in Switzerland developed one of the first cantilever array platforms which was used for gas and vapor identification [14]. One of the primary projects that showed high potential of using cantilever array in biological studies was reported in 2000 by IBM-Zurich laboratory [15]. In their project, they developed a platform for DNA analysis and translated the variation of surface stress due to DNA hybridization and molecular binding into mechanical response of cantilever. Since then, all the researches which have been done in the domain of cantilever sensors demonstrated that sensor platform which consists of cantilever arrays can provide many advantages for various studies.

More recently, cantilever-based biosensors have been used for different biological applications including the detection of pH [16-17], label-free detection of molecules [18], monitoring temperature [19], DNA hybridization [15,20], interaction between antibody-antigen [21], etc. Usually in this type of sensors, application of force on top of the cantilever or variation of temperature or mass will displace the suspended beam and this displacement can be detected using different readout methods.

Cantilever biosensors are typically made from polymers, silicon, silicon nitride, or Polysilicon. However, in most biosensing applications, it is essential to cover the surface of the tip of cantilever with some specific layer of material. This process is known as the functionalization of cantilever sensors. The materials used to develop cantilevers depend on the application. For example, if the sensor is employed for detection of antigen, this layer can be a special type of antibody [22]. Sometimes a microfluidic structure should also be integrated with cantilevers in order to prepare the samples prior to the sensing step [23].

Cantilevers can be categorized in different groups based on several factors like their readout method and their operational mode. In the following sections of this chapter, further discussions and detailed information regarding each of these groups will be provided and then different reported applications of cantilever biosensors will be discussed.

1.1 Modes of cantilever operation

In general, when a cantilever operates as a sensor, two signals can be monitored. These two signals are the displacement (or bending of beam), and the change in the resonance frequency response. Cantilever's operation can be categorized based on the type of variation used in the sensing process. Three modes defined for operation of cantilevers are static, dynamic, and heat modes.

In the static mode (figure 1-3 (a)), variation of surface stress will cause static bending of the beam. This surface stress variation is usually due to the attachment of target substances on the surface of cantilevers. Several studies have used cantilevers in this mode for sensing. For example Weeks et al used cantilevers in static mode as a pathogen detector with high sensitivity [24] and Pei et al presented a glucose biosensing system based on using cantilevers in this mode [25]. In static mode, the direction of bending depends on the type of the force being applied on the surface. If the tensile stress is applied on top of the cantilevers, the beam will have upward bending and compressive stress will bend the beam toward down side [26-27].

Cantilevers used as biosensors can also operate in the dynamic mode (figure 1-3 (b)). This mode is usually used for monitoring mass variations; however any changes in surface stress can also be detected in this mode.

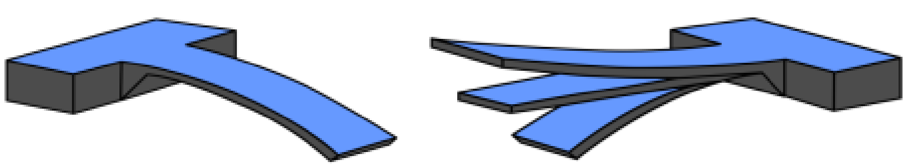
With biosensor cantilevers in the dynamic mode a shift of their resonance frequency is detected. When cantilevers are used in the dynamic mode, the beam will be excited externally at its resonance frequency and due to variation of the mass of the sensing target on its surface (e.g. due to attachment of particles) the resonance frequency will change. The relation between resonance frequency and mass can be described by the following equation [28]:

$$\Delta m = k(4n\pi^2)^{-1}(f_1^{-2} - f_0^{-2}) \tag{1.1}$$

In equation 1.1, resonance frequency before and during the sensing process is defined by f_0 and f_1 respectively, k represent spring constant of the cantilever, Δm is change in mass, and n is a geometrical factor.

Different studies have shown that by monitoring the variation of resonance frequency it would be possible to gather data about specific attached particles. For example, Detzel et al used variation of cantilever's resonance frequency for assessment of E.Coli [29] and Hwang et al also used a cantilever in resonance mode for analyzing prostate-specific antigen (PSA) [21].

Dynamic cantilevers are usually not suitable for application in which the sensors should be put in aqueous environment. This is because the damping effect in solution can affect cantilever's resonance frequency. In this case, in order to have a sensing system with high resolution, it might be essential to have a very complicated readout setup [30].



(a) (b)

Figure 1-3: (a) Static and (b) dynamic modes of cantilever

The third mode for operation of cantilevers is called bimetallic or heat mode [30-31]. In heat mode, deflection of cantilever is due to bimorph effect. Cantilevers which are used in this mode can be fabricated by two layers of material with different thermal expansion coefficient factors. Usually in these types of cantilevers, one layer is metal and the other is silicon. By varying the temperature, the difference in the expansion coefficient result in deflection of the beam and it would be possible to detect small changes in temperature with high sensitivity.

In chemical and biological studies, the variation of temperature can be due to some reactions which are happening in the sample or it can be a part of characteristics of the material which is being sensed. For example, Berger et al used this type of cantilever as a calorimeter [32] and Gimzewski et al used a bimetallic cantilever for observing chemical reactions [33].

Equation 1.2 shows the relation between the bending of bimetallic beam as the function of the properties of materials which are used in the fabrication of cantilever [30]:

$$\Delta z = \frac{5}{4}(\beta_1 - \beta_2)\frac{t_1+t_2}{t_2^2 k}\frac{l^3}{(\lambda_1 t_1+\lambda_2 t_2)w}P \tag{1.2}$$

In equation 1.2, Δz is the deflection of the cantilever, β_1, β_2, λ_1, and λ_2 represent the expansion coefficients, and thermal conductivities of two layers of materials respectively, t_1, t_2 are the thicknesses of the layers, w is the width of the beam, P is the total generated power on the cantilever, and n is a parameter related to the geometry of beam.

1.2 Cantilever’s readout methods

Several methods can be used for detecting the deflection of cantilever. It is possible to categorize all these methods into two main groups- electrical and optical methods. Electrical methods convert the deflection of cantilevers into a change of electrical signals. The main readout techniques associated with cantilever structures are piezoresistive, piezoelectric and capacitive. On the other hand, for the cantilever systems in which detection is based on optical methods, the displacement of cantilevers will be monitored by using optical equipment.

Each of these techniques has advantages and disadvantages. In order to choose the best technique, one should consider different parameters like sensing environment, required resolution, dimensions of cantilever, minimum and maximum estimated deflection, etc. In the following sections, principles of detection based on each of these techniques will be presented and the advantages and disadvantages will be discussed.

1.2.1 Optical

Optical readout technique was first introduced in 1988 [34]. Optical sensors usually work based on emitting light to the surface of cantilever with a laser and analyzing the position of reflected light to measure the beam displacement. Figure 1-4 illustrates the optical readout method.

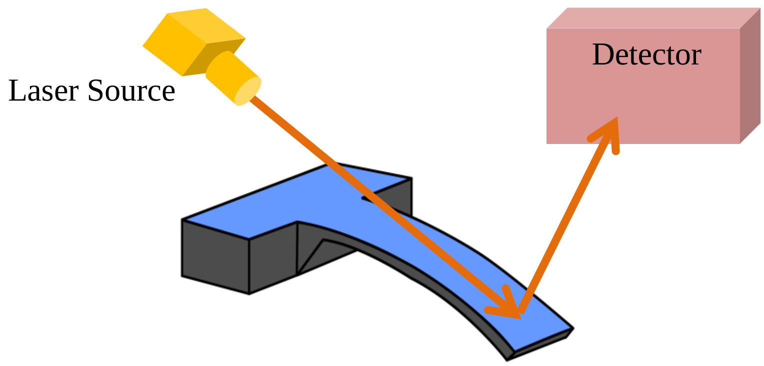


Figure 1-4: Optical detection for cantilevers

Cantilevers that use optical readout are covered with reflective layers like metals and they follow a mechanical relation between deflection and the applied force that can be described by the Stoney formula [35]:

$$\Delta z = 3\Delta\sigma(1-\upsilon)\left(\frac{L}{t}\right)^2/E \tag{1.3}$$

In equation 1.3, $\Delta\sigma$ is the surface stress, υ is the Poisson ratio, E is the Young modulus, and L and t are the length and thickness of the cantilever respectively.

Optical systems have high sensitivity but require high complexity setup. In addition, whole optical readout setup is usually very bulky and it might not be suitable for handheld devices. However, some methods have been proposed to integrate the optical setup in a small space. For example, in 2006 a group proposed the integration of an optical readout method [36]. In that project, the cantilever was employed as a waveguide and its output light was being monitored.

Another disadvantage of optical readout is the complexity of laser alignment. Usually, in most optical readout setups, the laser should be precisely focused on the tip of the cantilever and the alignment process can be very time consuming and costly. Moreover, optical sensors suffer from limitations related to the environment where the sensing is performed. If the sensor is used in a liquid environment, refraction can affect the final output read by an optical system [37].

In addition, optical readout is not suitable for reading large array of sensors and it requires a time consuming and difficult process. Usually in order to use optical readout for a high throughput platform containing N sensors, there should be the same number of light sources and detectors in order to detect the variation of all cantilevers.

However, some studies proposed using multiplexing method to reduce the number of sources and detectors [14,38-39]. For example in 2002 a group developed a system for monitoring 8 cantilevers [40]. This setup consists of 8 VCSELs (Vertical-cavity surface-emitting laser) and one photo detector. Also it is possible to use one CCD (Charge coupled device) image screen as a detector for all sensors. For example, Yue et al used one collimated beam as a light source for hundreds of cantilever and detect their variation with a CCD plane [41].

Besides having high sensitivity and good resolution, another advantage of optical readout is the simplicity of the fabrication process for sensors which are going to be used with an optical setup. Because there is no need to integrate electronic structures in the sensor, the fabrication process is simpler in comparison with other sensors and it needs fewer fabrication steps. However, since detection in these sensors will be done based on light's reflection from a surface, it is important to have a uniform surface to avoid light scattering [42].

1.2.2 Piezoresistive

Piezoresistive cantilever biosensors can measure surface stress variations which is usually due to the attachment of specific molecules on top of a cantilever beam. In this type of sensors, a piezoresistor is incorporated on the cantilever and the displacement of the beam will change its bulk resistance. Usually, the piezoresistive layer should be placed at a highly stressed point of the beam, close to the fixed end of the cantilever. For a simple rectangular beam, maximum stress (σ_{Max}) can be expressed by equation 1.4 [43]:

$$\sigma_{Max} = \frac{3Et}{2L^2}\Delta z \tag{1.4}$$

In this case, the resistance variation is demonstrated in [43]:

$$\frac{\Delta R}{R} = \pi_1 \sigma_{Max} = \xi \frac{3Et\pi_1}{2L^2}\Delta z \tag{1.5}$$

In equation 1.5, π_l is the longitudinal piezoresistive coefficient, and ξ is a correction factor between 0 and 1.

Usually, a Wheatstone bridge circuit is used to monitor the output of piezoresistive cantilever sensors. As can be seen in figure 1-5, this circuit contains four resistors and the variation of their resistance will affect the output voltage.

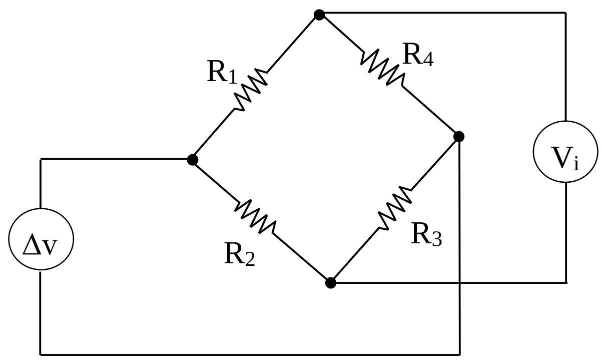


Figure 1-5: Wheatstone bridge

The relationship between the output voltage variation (Δv), the applied voltage (V_i), and the resistance can be explained by equation 1.6 [44]:

$$\Delta v = \frac{R_1R_3 - R_2R_4}{(R_1+R_2)(R_3+R_4)} V_i \tag{1.6}$$

Assuming the resistance on the cantilever is one of the resistors, and keeping the other 3 at the same value we will have [44]:

$$R_1 = R + \Delta R \tag{1.7}$$

$$R_2 = R_3 = R_4 = R \tag{1.8}$$

$$\Delta v = \frac{2R\Delta R + \Delta R^2}{(2R+\Delta R)^2} R \tag{1.9}$$

And since usually the resistance variation is very small compared to R, we can estimate that [44]:

$$\Delta v = \frac{\Delta R}{2R} V_i \tag{1.10}$$

Therefore, as shown in equations 1.10, it would be possible to follow the variation of resistance by monitoring the output voltage of Wheatstone bridge.

Piezoresistive cantilevers are good candidates for high throughput applications. Since there is no need for bulky, expensive optical equipment, it is possible to integrate sensor and readout circuit in the same chip. Therefore, using the piezoresistive cantilever concept, it is possible to design an inexpensive handheld device with hundreds of sensors.

However there are some disadvantages in using cantilevers based on the piezoresistive method. The most important drawback of this type of sensors is their low resolution compared to optical sensors. In addition, the output signal of these sensors depends on temperature variations [45]. Also since the current will pass through piezoresistive layer, there will be heat dissipation and thermal drifts in the sensor [46].

In order to solve these issues, several studies suggest a reference cantilever in the sensing platform [42]. This technique can decrease the effect of environmental changes and noise. In this case, one functionalized cantilever will act as a sensor and the other which is not functionalized will be placed in the same sensing environment and act as a reference. By performing the differential measurement based on two cantilevers, it will be possible to increase the sensitivity and the accuracy of the sensing system. Figure 1-6 presents an illustration of the differential measurement technique.

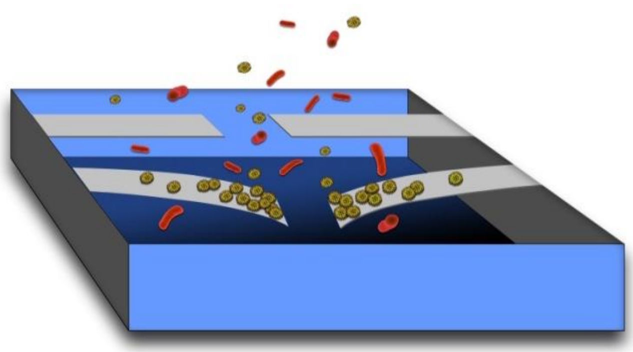


Figure 1-6: Differential measurement in cantilevers; sensor beams will deflect due to attachment of particles while reference beams will have no or very small deflection

One of the challenges in piezoresistive cantilevers is the complexity of their fabrication process compared to optical sensors. Usually in the fabrication of these sensors, some extra steps should be applied in order to deposit the piezoresistive layer embedded in between two insulator materials in order to avoid the contact between the sample solution and the electrical active components. In general, packaging complexity is another drawback of these sensors. Since in biological applications, sensors are usually immersed in the solution, it is important to have a suitable package to cover all the electrical connections.

Another challenging issue for piezoresistive cantilevers is to search for a suitable method or material to increase their sensitivity. Among many proposed methods, it has been shown that is possible to use materials with low Young modulus to increase sensitivity [37]. In addition, the advantages of adding stress concentration regions into cantilever structures have been demonstrated in some studies [47]. In order to increase the stress in specific regions, some holes can be incorporated in the beam and simulations demonstrated that the number and shape of these holes can improve the output signal of cantilevers [47].

Unlike optical sensors, piezoresistive cantilevers are not limited by the sensing environment, and it is possible to do the measurement in an opaque media. Also, compared to optical sensors, the calibration step is usually very easy and it can be done automatically on a chip.

1.2.3 Piezoelectric

Like piezoresistive cantilevers, in piezoelectric sensors, mechanical variations will be translated into electrical signals. Generally when piezoelectric materials are under stress, voltage will develop across their boundaries and conversely, when a voltage is applied to them, they will deform. The piezoelectric effect was first discovered in the 1880s and since then it has been used in many different applications [48]. For example Hwang et al used this type of sensors for quantitative analysis of prostate specific antigen (PSA) [21], and McGovern et al developed a piezoelectric cantilever platform for label free detection of Bacillus anthracis [49].

In piezoelectric cantilevers, the sensing and actuating structure can be incorporated on the beam by depositing a piezoelectric material like Lead Zirconate Titanate (PZT) and Zink Oxide (ZnO) [50]. In these sensors, attachment of target substances on the cantilever will result into variation of resonance frequency. Equation 1.11 can be used to describe the relation between the resonance frequency and the cantilever characteristics [51]:

$$f = 0.162(\frac{t}{L^2})\sqrt{\frac{E}{\rho}} \tag{1.11}$$

where t and L are the thickness and length of cantilevers respectively, E is its Young modulus, and ρ is the density. Piezoelectric sensors can be used in the development of high throughput systems and they have no limitations regarding the sensing environments. For example, unlike other sensors, piezoelectric cantilevers can operate in liquids and damp environments [52].

One of the disadvantages of both piezoresistive and piezoelectric sensors is the dependency of their output on the variation of temperature [45]. In addition, since these sensors also need electrical connections, it is important to follow a suitable protocol for their packaging.

Another drawback of this type of sensors is that the piezoelectric structure requires a thick layer of material in order to generate signal with appropriate amplitude. Having a thick layer of material will decrease the mechanical variation of the beam and consequently decrease the sensitivity [47]. Also, usually piezoelectric sensors are not efficient for low frequency applications or in situations when the variation of the cantilever position is very slow [53].

1.2.4 Capacitive

Capacitive sensors can be used to detect different variables like pressure and conductivity. Any parameters which affect the value of capacitance can be measured by capacitive sensors. In general, capacitance is equal to the division of the charge by the applied voltage between two conductive plates, and it can also be defined by equation 1.12:

$$C = \varepsilon_0 \varepsilon_r \frac{A}{d} \tag{1.12}$$

where ε_0 and ε_r are the dielectric constant and relative permittivity of the material between the plates respectively, A is the effective area and d is the distance between two plates. Different applications of capacitive sensors are based on changes of one of these parameters.

One of the main methods which is often used in capacitive cantilever sensors is to exploit the variation of the space between two electrodes. In this case, the beam will act as one electrode and the second electrode will be a fixed plate, which is placed underneath the beam. Since this variation has a direct effect on capacitance, cantilevers can detect very small forces that can make minute deflections.

Different studies have shown that the output signal of MEMS capacitive sensors is stable and independent of temperature variations [54-56]. This is one of the important advantages of capacitive sensors compared to piezo cantilevers. Another advantage of capacitive cantilevers compared to other electrical methods is their low-power consumption [54].

It is important to note that capacitive sensors cannot be used in applications requiring a large distance between the beam and bottom electrode. The reason is that usually the distance between two electrodes is very small and the deflection will be limited by this space. Another drawback of capacitive cantilevers is their low sensitivity compared to optical sensors [57].

The capacitive sensor based platforms can be used in high throughput applications when it is essential to monitor arrays of sensors. Up to now capacitive sensors have been used in various fields. For example, in 1992, Brugger et al used these types of sensors as atomic force microprobe with integrated readout circuits [58]. Also several capacitive cantilever platforms have been developed for mass detection with high sensitivity [59-60]. In recent years some systems based on capacitive sensors have been proposed for different biological applications. One of the new proposed platforms is introduced in 2013 by Sangeetha et al for Tuberculosis detection [61].

It should be noted that there is one major drawback in the cantilever platform using capacitive sensors and it is the generation of faradic currents between two plates when the solution is an electrolyte [57]. In addition, the packaging and encapsulation of electrical connections is another practical issue when the sensors are used in a liquid environment. These characteristics, limit the application of cantilever sensors operating in the capacitive mode.

1.3 Applications of cantilever biosensors

Until now, cantilever sensors have been employed in several biological domains including DNA studies [62-65], drug discovery [66], cellular analysis [67-68], and biomarker detection [69-71]. Results from many projects which use cantilever platforms have shown that these sensors can demonstrate good sensitivity and high resolution.

Some of the developed systems based on the single cantilever or an array of cantilevers have been demonstrated in table 1-1. As it can be seen in this table, cantilevers with different readout techniques have been used for various applications.

Table 1-1: Existing Cantilever sensor platforms

Application	Readout method	Ref
Water molecular adsorption	piezoresistive	[72]
Biochemical sensing	piezoresistive	[73]
Antibody-based protein detection	piezoresistive	[74]
Continuous label free detection of cardiac biomarker proteins	optical	[40]
Biochemical sensor	piezoresistive	[75]
Enzymatic detection	optical	[76]
Real time detection of Liposome	piezoresistive	[77]
Intermolecular force measurement	Piezoresistive	[78]
Detection of label-free disease marker proteins	piezoresistive	[18]
Glucose sensing	optical	[79]
Consolidated bioprocess monitoring (simultaneous Glucose and Ethanol detection)	piezoresistive	[80]
Pathogen detection (salmonella enterica)	optical	[24]
Detection of specific carbohydrate-protein interaction with Pico molar sensitivity	optical	[81]
Multiple label-free bio detection and quantitative DNA-binding assay	optical	[26]
Immunoassay of prostate-specific antigen	Piezoelectric	[82]
Label free protein recognition	optical	[83]
Label-free detection of amyloid growth	optical	[84]

In the following sections, three applications in which cantilevers have been proved to be useful will be discussed.

1.3.1 Label free detection of molecules

Cantilever sensors have been widely used for studying antibody-antigen interactions and/or for detection of different molecules in an analyte. These sensors enabled diagnosing different diseases and following up the treatments process. The most important advantages of cantilevers for these applications is that they are using label free techniques. Labeling is the most common approach which is being used for biological analysis. In this method, special chemical particles called probe molecules can be put in contact with samples. These particles can attach to molecules of interest, interact with them, and exhibit different behaviors after attachment. This difference can be in various forms include variation of color, emission of radioactive waves or light which can be detected with special equipment. Although labeling method can have good sensitivity, but it is usually very costly and time consuming [85]. Moreover, this method needs to be practiced by experienced persons in special facilities like hospitals, laboratories or clinics. Developing a platform based on cantilever sensors will reduce the cost and time of experiments and it can eliminate the risk of alteration of molecules during the labeling process [86]. One of the most common applications of cantilevers in this domain is the detection of specific biomarkers (e.g. cancer). Several groups have proposed different systems that can be used for screening and early stage diagnosis of different types of cancers. In these types of applications, specific antibody or antigen will be immobilized on the surface of beam and when the sensors are put in the sample, the target molecules will be attached to the immobilized layer and cause a deformation in the cantilevers.

Both optical and electrical readout methods have been used for developing systems for label free detection of molecules. For example, Zhou et al presented a piezoresistive cantilevers for the detection of P53 antibody which is a cancer biomarker [87]. Based on several studies, P53 gene is involved in different types of cancers including breast, lung, ovarian, prostate, and melanoma therefore having a platform which can detect this gene in a sample or monitor its level during a treatment can be very beneficial. In their platform, they have fabricated piezoresistive cantilevers with thickness of 650 nm. Usually piezoresistive cantilevers are fabricated by polycrystalline silicon. However, in their system, instead of using polycrystalline silicon, they have used single crystalline silicon layer of SOI (Silicon On Insulator) wafer for their piezoresistive structure to improve the sensitivity. In this platform, they have employed a differential measurement technique and the output was being monitored by a Wheatstone bridge. Based on their results, they were able to detect antibody in the range of 20 ng/ml to 20 µg/ml and they have monitored surface stress variation up to 0.12 N/m for this range of antibody concentration [87].

Another platform based on piezoresistive cantilever array is presented by Dauksaite et al who used this system for the detection of Glutathione-S-transferase (GST) [74]. They have used Cantion-NanoNord A/S nanomechanical cantilever technology platform that consists of four cantilevers (CantiChip4) and its readout system (CantiLab). The length, width and thickness of cantilevers were 120 µm, 50 µm and 480 nm respectively. During their experiments, GST antibodies were immobilized on the beam and the results showed that the system was able to detect 40 nM of GST protein with an average signal of 4-5 µV in a differential measurement scheme [74]. Wee et al also developed a piezoresistive sensor and they tested that sensor for the detection of C-reactive proteins (CRP) and prostate specific antigen (PSA), which are markers of cardiac disease and prostate cancer respectively [18]. They have fabricated their sensors and used

PDMS and polycarbonate to create a microfluidic structure for performing the biological tests. They have also employed Wheatstone bridge and differential measurement for reading the output signal.

In another project, Lee et al presented a system for the detection of PSA [82]. They have used PZT (Lead Zirconate Titanate) cantilever in their system and monitor the deflection of cantilever due to interaction between immobilized PSA antibody and PSA by using piezoelectric effect. The detection is being performed by monitoring the shift in resonance frequency and they have done their experiments with 2 different dimensions of cantilever beam (300 μm×100 μm×2.26 μm and 150 μm×50 μm×2.26 μm). Using different dimensions enabled them to observe the effect of beam dimension on minimum detectable sensitivity. They have reported an increase in resonance frequency change for smaller cantilever which indicates an increase in minimum detectable sensitivity and they proposed that this change might be also due to the smaller interaction area on these cantilevers. Their experimental results showed sensitivity as low as 10 pg/ml [82].

Another system that used piezoelectric method for antibody-antigen studies is presented by Lee et al [88]. They have a piezoelectric cantilever platform for measuring the concentration of Hepatitis B surface antigen (HBsAg). HBsAg is a part of surface of Hepatitis B virus and is being used as a biomarker for the detection of Hepatitis B infection. They have also employed PZT as their piezoelectric layer and they used standard micromachining process for fabrication of sensors. In their platform, monitoring the variation in resonance frequency was done with a computer based measurement device. By immobilizing anti-HBsAg on their cantilever and observing the resonance frequency shifts they were able to detect mass of HBsAg in range of 0.1-100 ng/ml [88].

In addition to silicon based cantilevers, several sensors have been developed by using polymer materials. SU-8 is one of the polymers which is commonly being used for cantilever fabrication and its low Young modulus makes it possible to design sensors with high sensitivity. Calleja et al are one of the groups that used SU-8 for their sensor fabrication [89]. Their system is based on single SU-8 cantilever and optical readout and they have used it for monitoring the interaction between human growth hormone (hGH) and its antibody (α-hGH). Based on their presented results, they were able to detect surface stress as low as 1 mN/m with their platform. In their project, they compared the results of polymer based cantilevers with silicon nitride beam coated with gold layer and they showed that using polymer for fabrication of cantilevers will eliminate the environmental limitations which usually exist in silicon based sensors like the dependency on pH variations [89].

Although the detection of a single antibody or antigen might provide informative data, however in practice it might be essential to detect the concentration of various target molecules (e.g. antibody/antigen). By taking advantage of multifunctional measurements using cantilever arrays, Arntz et al developed a platform enabling the detection of 7 different antibody-antigens [40]. They used an optical method for detecting the displacement of the beam. Although the optical method often limits the number of sensor array and makes readout a complicated task, it enabled high accuracy measurement (0.1 nm). Dimensions of their beams were 500 μm×100 μm×500 nm and the pitch between them was 250 μm. They have performed the experiment for detection of Creatin kinase, and Myoglobin which are cardiac biomarker proteins. In order to decrease the effect of thermal drift and other undesirable effects of the sensing environment, they have used differential measurement and based on their report they were able to reach sensitivity below 20 μg/ml for myoglobin detection [40]. Figure 1-7 presents the platform that they have used in their studies.

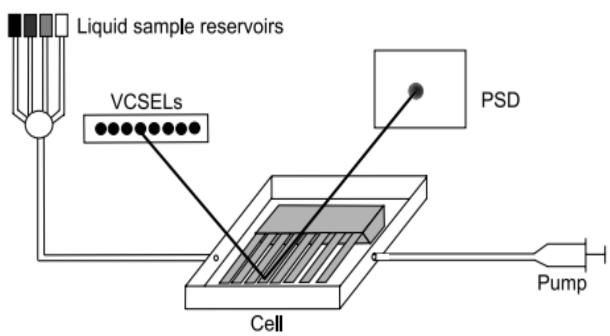


Figure 1-7: Sensing platform for monitoring multiple cantilevers; VCSEL (Vertical cavity surface emitting lasers), PSD (Position sensitive detector) [40]

Another platform that utilized high accuracy optical readout technique is presented by Sharma et al [90]. This group has developed a cantilever platform for detection of diabetic marker called Glycated hemoglobin (HbA1c). The detection level of HbA1c is very important for the diagnosis of diabetes as part of its treatment modalities. Usually the methods used for detecting HbA1c are chromatography, electrophoresis, colorimetric, spectroscopic, etc. But these methods are normally time consuming and expensive. In their system, they have utilized commercialized cantilever with dimensions of 500 μm×100 μm×1 μm and they used optical system with accuracy of 0.1 nm for detection of cantilever deflection. By using this platform they were able to reach a dynamic range of sensitivity located between 0.147 pM and 1.47 pM [90].

1.3.2 DNA analysis

Another application of cantilever biosensors is in field of DNA analysis. DNA (Deoxyribonucleic acid) is a macromolecule which carries genetic information of living creatures. DNA analysis plays an important role in variety of biological process such as detection of genetic diseases. In fact, DNA analysis can result into detection of new treatments for different diseases or discovery of the fundamental problems in living organisms.

DNA molecules are usually in the form of double helix which is composed of two long strands of nucleotides and each nucleotide has sub units of sugar, phosphate and nitrogenous bases. The strands are made by formation of the bond between sugar and phosphate of each two different nucleotides and two strands are linked together by hydrogen bonds between bases of two nucleotides. Melting process will result into separation of two strands of DNA and formation of ssDNA (Single strand DNA). Melting can occur in specific situations with high temperature, low salt, and high pH [91].

Usually DNA analysis is performed by using optical equipment and using labeling methods. DNA detection can also be performed by using ISFET transistors; however its accuracy of detection is influenced by pH value and temperature [92].

Several studies reported that cantilever biosensors can be a good candidate for DNA analysis. In these applications, in order to detect specific single strand DNA (ssDNA) in a solution, the hybridization process is often used. During the hybridization process, each single strand of DNA will be hybridized with its complementary pair. DNA hybridization can be useful for monitoring different gene expressions. In addition, the hybridization process can be used for screening of specific target molecules in a sample, monitoring a specific diseases and assessing steps of a treatment.

For this process, the surface of a beam is coated with special ssDNA probe and these probes will hybridize with their complementary ssDNA in the solution. Usually coating of the surface will be done by covering the beam with a layer of gold and using thiol linker at the end of ssDNA [93].

Different papers have shown that ssDNA adsorption on cantilever will result into the variation of surface stress around 30-50 mN/m [94]. Several experiments have shown that only the presence of complementary strand will result into deformation of cantilever and uncomplimentary ones will not have effect on the beam displacement [95-98] and it is because of the strong bond which will be formed between the complementary strand and the probe molecule on top of the cantilever.

Another application of this type of cantilever-based biosensors is the detection of DNA mutation which is responsible for several types of diseases. During mutation a change will happen in nitrogen base of DNA. Four types of nitrogen bases in nucleotides are Adenine (A), Thymine (T), Guanine (G), and Cytosine (C). As mentioned previously, there is a link between nitrogen bases in DNA. In this link, Adenine always paired with Thymine, and Guanine and Cytosine are always connected together. Figure 1-8 demonstrates the structure of DNA.

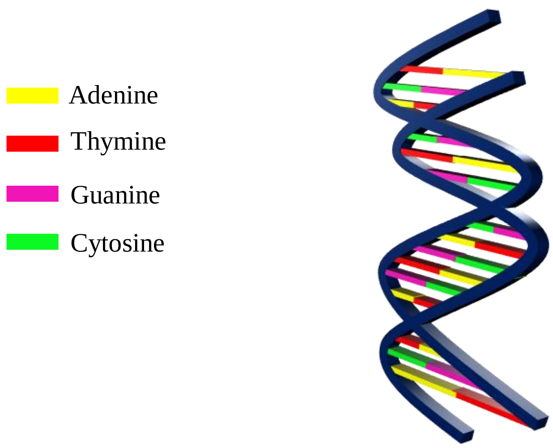


Figure 1-8: DNA structure

Some of the changes that can cause mutation are variations of the base, insertion of extra base pairs in DNA sequence, or loss of one or more of the bases. Some mutations might not have high impact on the whole living organism, but the others might lead into severe malfunction of proteins and cells. Mutation can result into genetic disorder and can cause disorders like anemia [99], and hemophilia [100-101]. Diseases like cancer can also result from series of mutations [102-105].

Many groups propose cantilever-based platform for performing DNA studies on mutations. For example, Hansen et al developed a cantilever-based system for detecting DNA single-Nucleotide mismatches [106]. In their platform, they used optical method for the detection of cantilever deflection which was caused by hybridization on surface of the beam. Based on their results, the deflection of cantilever in their experiments was in the nano meter range. In their study, they used triangular silicon AFM cantilever with length of 180 μm and they did the necessary surface functionalization for immobilization of probe DNA. Their label free platform can be used for monitoring and treatment of diseases, which are due to this type of mutation [106].

Also an optical cantilever made from SU-8 was developed by Calleja et al [107]. In order to increase the sensitivity of their sensors, they used the SU-8 polymer instead of silicon. Their experiments showed that sensitivity of their system is six times higher than commercial silicon nitride cantilevers. They employed cantilevers with different geometry (L=100, and 200 μm, W=20, 30, and 50 μm, t=1.3-2 μm) and their results showed nano meter deflection during the experiments. They have tested performance of the device by monitoring the adsorption of ss-DNA on the gold surface of the beams and reported that surface stress variations as small as 60 μN/m can be detected by SU-8 cantilevers [107]. Fritz et al also used an array of cantilevers with optical setup and showed that it is possible to detect single based mismatch between two 12-mer oligonucleotides (short ssDNA with 12 nucleotides) [15]. They have used a differential measurement technique in order to get very precise signals and their experimental results revealed that hybridization of 12-mer oligonucleotides can produce 5 mN/m compressive surface stress [15].

McKendry et al presented cantilever in array format to study DNA hybridization and detect DNA sequences [26]. They have used array of 8 cantilevers (L=500 μm, W=100 μm, t=1 μm) provided by IBM Zurich Research Laboratory and employed optical method for detecting displacement of the beams. Their experimental results showed that DNA hybridization produces average differential displacement signal of 9.8 nm which is equal to surface stress of 2.7×10^{-3} N/m.

Yang et al are one of groups that used cantilevers with electrical readout method for hybridization detection [108]. Their experiments showed that for 3×10^{11} ssDNA molecules on cantilever, surface stress will be 0.15 N/m. Their sensor which has been fabricated with CMOS technology has dimension of 125 μm×60 μm×0.75 μm with a Polysilicon layer incorporated as a piezoresistive material and its sensitivity was 3.5×10^{-5} m/N. In their platform, they employed bridge circuit for piezoresistive measurements and they used parallel sensing with reference electrode to avoid thermal noise. Another piezoresistive cantilever platform for DNA detection application was proposed by Huang et al [109]. They have presented a fully integrated DNA detection platform which has been fabricated in 0.35 μm CMOS Bio-MEMS technology. Their whole die area was 30.4 mm^2 and the dimension of cantilever beam was 180 μm×80 μm×1.5 μm. The difference between their system and other piezoresistive cantilever sensors was in their readout circuit. Since bridge circuit for piezoresistive measurements usually have offset problem, they chose a structure based on a ring type oscillator and convert the small resistance variation to frequency shifts. They have shown that there is 130 kHz frequency variation between matched and mismatched DNA samples and their detection range was between 100 pM to 1 μM [109]. Adami et al also developed a DNA detection platform that consists of array of piezoresistive sensors and a readout Application Specific Integrated Circuit (ASIC) which has been implemented in 0.35 μm CMOS technology [110]. They have built an array of very thin single crystal beams and used cantilevers in the static mode. In their paper, they have also compared the performance of two other types of piezoresistive cantilevers; SIO_2 beam with Polysilicon piezoresistor and SU-8 beam with gold strain gauge. Based on their report, although polymer cantilevers usually have better sensitivity because of their low elastic modulus but the performance of the sensor can be limited since gold strain gauge has high stiffness.

1.3.3 Cell studies

One of the hot research areas in this field is analyzing the interactions between cells and extracellular matrix or substrate which can be helpful in several biological domains like tissue engineering or characterizing disease like cancer and leukemia [111-115]. Also the data which can be gathered by this type of analysis will provide critical information on cell motility, migration and survival.

During the interaction between cells and extracellular matrix or substrate, special protein bonds so called focal adhesions are formed and cells can physically be connected to extracellular matrix or substrate through these bonds (Figure 1-9). These protein bonds will also provide a pathway for signaling. The force which will be applied on a substrate through these focal adhesions called traction force and it can be affected by different factors like stiffness of substrate and type of cells [116-120]. Having quantitative information about these types of forces can lead into new discoveries in different fields.

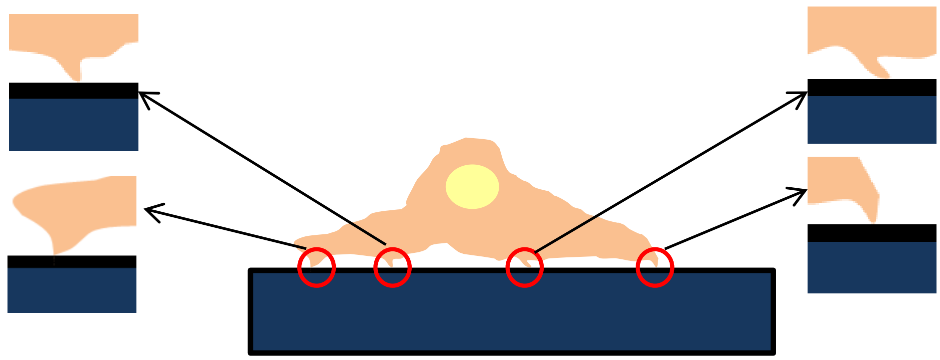


Figure 1-9: Attachment of cell to substrate through focal adhesions

Since cellular forces are very small, their measurement is a challenging task. Based on literature, the focal adhesion forces are in order of 10 nN [121-124]. Different structures and methods have been proposed for measurement of focal adhesion forces, understanding their formation and effect of their alteration on cell behaviour. The most common method which is being used for measurement of the traction force of cells is “traction force microscopy” [125-126]. This method involves using an elastic substrate that will deform due to the applied traction forces from cells and its deformation will be monitored by using imaging techniques. Some other systems which have been proposed for study of cell forces involve using micro-needles [127], micro pillar arrays [128] or thin membranes [129-131]. Another structure which has been proposed in 2010 by Klein et al is microstructure scaffold [132]. In addition to all these methods, cantilever platform can also be used for measurement of cellular forces. In 1997, a system composed of cantilevers and optical readout setup has been proposed by Galbraith and Sheetz and it was being used for monitoring the traction force during migration of chicken embryo fibroblast cell [133]. However, the system which was proposed by this group could only determine the force in one direction and was only measuring the force in specific locations.

Park et al also developed a system for real time measuring of contractile force of Cardiomyocytes which can be used in studying heart failure problems [134]. Cantilevers in this study were made from PDMS because of its low Young modulus and sensitivity to small amount of force. In addition, PDMS is a suitable material for biological applications since its transparency allow the use of optical techniques and it is nontoxic to cells. During their experiment, they have cultured Cardiomyocytes on the beams and displacement of the sensors has been monitored by optical method. Cantilevers had 20 µm thickness and they had been fabricated in 5 different widths (50, 100, 150, 200, and 300 μm). The length of each beam in all sensors was 5 times of its width. Sensitivity of sensors with length of 250, 500, and 750 μm was 6.97×10^{-4}, 1.74×10^{-4}, and 7.74×10^{-5} MPa/μm respectively. Based on their results, they were able to monitor variation of stress from cell in range of 2 to 5 nN/μm^2 which is consistent with other types of measurements. The minimum detectable stress in this platform was 7.74×10^{-5} MPa/μm.

Another platform based on cantilever sensor was developed by Antonik et al for monitoring the variations in mechanical behaviour of living cells [135]. Their project was based on the known fact that several parameters can change characteristics of cells. These parameters can be a signal such as hormone that can cause movement in some cells or viral infection that can change cytoskeletal activity of a cell. Their platform can be used in different applications like drug screening, pollution or biological weapon detection. In addition, it can be considered as a platform for studying mechanical properties of a living cell which is an important step in several biological studies. During their experiments, they have grown Madine Darby Canine Kidney cells on a commercialized tipless V shaped AFM-type cantilever and used optical methods for detection of beam displacement. Each chamber, designed for doing the tests, could have one or two cantilevers as a sensor. After growth of cells on the beam, they have observed its deflection in presence of two toxic substances (lytic bee venom melittin, and respiratory inhibitor sodium azide).

Yin et al also proposed a system for probing single cancer cell mechanics [136]. Their device composed of microfluidic channel and an array of piezoresistive cantilevers and they have used microelectrodes for applying dielectrophoresis and trapping human breast adenocarcinoma on the beam. This device can be used for analyzing the mechanical process that controls the invasion of cancer cells. Based on one report which has been published in Biophysical journal in 2008, there is a relation between contractile force of a cancer cell and its invasiveness; and cancer cells might become more invasive by becoming more contractile [137-138]. Their device was fabricated in TSMC 0.35 μm and the microfluidic structure had been prepared by PDMS molding process. Also some post processing steps have been done in order to prepare cantilevers for biosensing applications. Cantilever dimensions in their platform were 50 μm×30 μm×2 μm and they have used Wheatstone bridge as their readout circuit.

Besides using cantilevers for cellular force measurement, it is possible to use this structure for measuring cellular mass. Microscopic techniques are commonly being used for these types of studies but as it has been mentioned earlier, these methods are not useful for high throughput applications and usually they are very costly. One of the first systems which was being used for measurement of single cell mass without using any optical equipment was proposed by Park et al [139]. Their system composed of array of silicon cantilever (L=25-40 μm, W=10 μm, t=240 nm) enclosed in a PDMS channel and could be used for measuring the mass of adherent cell in a solution. In order to do the experiments, they used Hela cells and they have directed the cells toward cantilevers by using dielectrophoresis. The displacement measurements have been done by monitoring the resonance frequency shift of the beams. This platform was suitable for studying mass of the cells over a time frame and enables scientists to study living single cell in a non-invasive manner (without detaching them from surface).

As seen in the previous sections of this chapter, cantilevers have already demonstrated an excellent potential in a variety of applications including cellular and molecular biology. For various applications, several techniques have been developed using cantilevers in static or dynamic modes. However, to date, less attention has been paid to development of high throughput cantilever array. In fact, most of the developed cantilever based platforms are based on optical readout techniques which limit the number of sensors in the system. Furthermore, the electrical sensing based platforms are limited by the fabrication process. The proposed platform in this project can overcome the above mentioned drawbacks and can enable us to perform high throughput measurements and gather real time quantitative biological data.

CHAPITRE 2 PIEZORESISTIVE CANTILEVER ARRAY PLATFORM

As was mentioned in previous chapters, cantilever can be used in different operation modes and several readout techniques can be employed to monitor its variations. In this project two cantilever array platforms have been proposed for quantitative biological analysis applications and both sensors are being used in their static mode. In the first proposed array, the piezoresistive readout method is chosen for monitoring the output of sensors. The following section is the journal article which has been submitted to IEEE Sensor Journal on July 18th, 2013 with the title of "A cantilever array platform dedicated to quantitative biological analysis"; and the authors are: B. Gholamzadeh, E. Ghafar-Zadeh, F. Awwad, and M. Sawan.

2.1 Journal ARTICLE 1: A Cantilever Array Platform Dedicated to Quantitative Biological Analysis

B. Gholamzadeh, E. Ghafar-Zadeh, F. Awwad, and M. Sawan; **IEEE Sensor Journal; submitted on July 18th, 2013**

Abstract- Understanding characteristics of individual cells and molecules can help us to discover new drugs and more efficient methods of treatment for current diseases. As a long term objective, we aim to develop an innovative platform for assessment of biomechanical forces of cells and molecules in order to be able to analyze their behavior. Based on the simulation results which have been obtained by COMSOL multiphysical software, we can use these sensors for measuring small forces with high sensitivity. In this paper, as the first step toward this very crucial and particular need, we present a force measurement method using an array of 30 cantilevers implemented through standard MEMS foundry process (MetalMUMPs). Herein, we discuss and demonstrate design, fabrication and experimental results of proposed device using electrical and interferometry techniques.

2.1.1 Introduction

Recent advances of micro- and nanotechnologies have attracted the attention for highly precision cellular and molecular analysis related to clinical applications or fundamental biological researches. Among these, analyzing the molecular biomechanical forces (MBM) have received less attention due to the complexity of structures for the measurement or manipulation of mechanical forces in comparison to other applications requiring, for instance, electrical or magnetic manipulation. A quantitative study of MBM forces can offer valuable data related to mechanotransduction inside the cells. MEMS sensors may enable us to develop a platform, which is suitable for these types of studies. One of the most common MEMS structures for sensing applications is the cantilever. Cantilever is a beam, which is fixed in one end, and it can be used for monitoring different parameters. The principle of using a cantilever is based on the attachment of biomaterials (cells or molecules of interest) to the surface of cantilever beams. The binding of target cells (or molecules) with the attached biomaterials will result into a variation in the surface stress and subsequently into a deflection in the beam. By analyzing this displacement, it would be possible to study different biological parameters. Until now, cantilever sensors have been used in various biological studies like Glucose sensing [25], DNA hybridization [26], peptide analysis [140] cells studies [141], and cancer marker detection [142].

It is possible to categorize cantilevers based on their operation mode into two main groups of static and dynamic sensors. In dynamic mode the variation of resonance frequency (or quality factor); and in static mode, the displacement of cantilever can directly be measured using various techniques including capacitive or piezoresistive methods. Therefore it would be possible to detect the presence (or concentration) of associated molecules causing the deflection or frequency change in the static or dynamic modes respectively. Cantilever sensors can be designed for measuring the changes in both modes.

The most common sensing methods for cantilever sensors are optical [143], piezoresistive [144], piezoelectric [145], and capacitive [57]. Optical sensors usually work based on emitting a laser spot on the surface of the sensor and analyzing the reflected light. In piezoresistive, piezoelectric and capacitive sensors, the electrical properties of sensors are used in cantilevers. It is worth mentioning that temperature variations can also significantly change some mechanical factors (e.g. surface stress) of cantilevers. Therefore cantilevers can be used as precise sensors to measure temperature variations or indirectly measure the biological materials changing temperature variations. In order to increase the sensitivity of cantilevers, it is required to use two materials with two different expansion coefficients in their structures.

Among all these sensing methods, piezoresistive measurement has several advantages. Compared to optical cantilevers, they require a low complexity electrical measurement setup instead of lasers, photo detectors and optical analyzers. Furthermore, a large number of micro-scale piezoresistive cantilevers can be fabricated on the same chip for different applications. It will also be possible to fabricate sensors and readout circuits, all embedded on one chip by using different IC technologies like CMOS. Compared to capacitive cantilevers, they can accurately operate even in conductive mediums [57]. Beside all aforementioned advantages, unlike piezoelectric materials such as PZT, deposition of piezoresistive layers like Polysilicon is usually supported with standard technologies and that enables us to fabricate large arrays of uniform sensors at low cost. It is worth mentioning that there are some minor difficulties to implement the piezoresistive cantilever array using standard technologies. Based on our experience in using MetalMUMPs (see section 2.1.5), the bonding wires between the piezoresistive cantilevers and readout circuitry requires an extra post-processing step that should be performed with great attention during the packaging process. The other essential packaging process is to isolate the conductors from liquid solution. Another drawback associated with piezoresistive sensors is a physical phenomenon called thermal drift which is due to joule heating. This extra heating is generated by flow of current in piezoresistive structure [50]. In this project, a differential measurement technique is used to solve this problem. In this method one cantilever, which is not exposed to external forces due to cellular and molecular activities (in this paper, the mimicking mechanical forces) will act as the reference cantilever along with another sensing beam. The differential readout method is expected to offer the advantages of noise reduction and high accuracy.

In this paper, the design process of a biochip platform consisting of 30 piezoresistive cantilever sensors is presented. To our knowledge, this type of platform is among the first high throughput systems which have been proposed for quantitative biological analysis. This system can be used for real time, simultaneous monitoring of multiple cells or molecules in biological studies. A simplified illustration of the proposed system is demonstrated in figure 2-1.

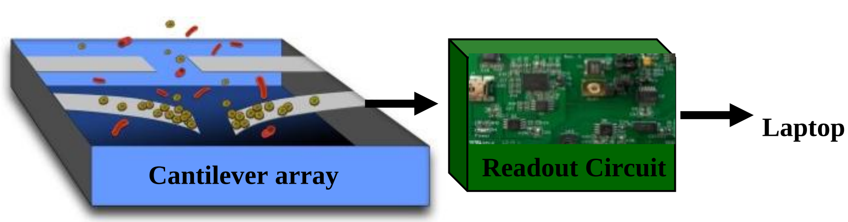


Figure 2-1: Illustration of the proposed cantilever array platform for quantitative biological analysis

As a primarily step toward developing this system, in this paper we demonstrate and discuss the design and implementation of cantilever array by using mechanical forces instead of biological ones. In this process a low complexity method using focused air pressure is efficiently used to apply forces on cantilever. The microfluidic packaging and biological test of this novel embedded biosystem is underway. The remaining parts of this paper are as follows; section 2.1.2 presents the cantilever design and implementation, then a theoretical overview of sensors and their simulations will be presented in Section 2.1.3. Finally the characterization of cantilever sensors will be demonstrated in Section 2.1.4 which will be followed by a discussion in section 2.1.5.

2.1.2 Cantilever design and implementation

In this project, the design and fabrication of an array of piezoresistive cantilever is described using MetalMUMPs technology. To our best knowledge, to date, this technology has not been used for high throughput applications. For this, a brief introduction about this standard MEMS process along with the related fabrication steps of the sensors is presented. Thereafter, we will address the cantilever design using this technology.

2.1.2.1 MetalMUMPs technology

MetalMUMPs is a part of Multi-User MEMS processes (MUMPs) program which is offered by MEMSCAP Inc. Similar to other standard microfabrication technologies such as Complementary-Metal-Oxide-Semiconductors (CMOS), there is a bunch of design rules that should be respected for the design of structures and preparation of layout.

In the first step of MetalMUMPs process, a silicon substrate will be covered by 2 µm isolation oxide (Sio2). In the next step, 0.5 µm PhosphoSilicate Glass (PSG) layer (oxide 1) will be deposited as a sacrificial layer and it will be patterned by the first mask. After patterning oxide 1, the first layer of Nitride with the thickness of 0.35 µm will be deposited and it will be covered with a 0.7 µm Polysilicon layer. Patterning of Polysilicon layer will be performed by the second mask using reactive ion etching (RIE). Another layer of Nitride with the thickness of 0.35 µm will thereafter be deposited. At this level, both Nitride layers will be patterned and etched using the third mask and RIE technique subsequently. In the next step, the second layer of sacrificial oxide layer will be deposited and patterned using the 4th mask. A chemical wet etching technique is applied to form the desired structures. By using metal deposition and liftoff process, a thin layer of Chromium (Cr) and Platinum (Pt) will be formed as an anchor (or adhesive interface layer) metal and then 500 nm of Copper (Cu) and 50 nm Titanium (Ti) will provide a plating base. The desired patterns for patterning the base metal layer will be formed using 5th mask. Then, 20 µm Nickel will be electroplated which will be followed by 0.5 µm gold layer. Finally, by using the 6th mask and electroplating process, sidewalls will be covered by metals. In the last step the structures are released following a series of chemical wet etching [146]. Figure 2-2 (a-i) show the fabrication steps of sensors in our platform. As seen in these figures, the resistor structure (RED) is fabricated by Poly layer and it is sandwiched between two Nitride layers (Light and dark brown colors), which form the main cantilevers. Also an anchor metal is used to create a very small and thin pad (Black) as polished adhesive layer for biological substances on the tip of cantilevers.

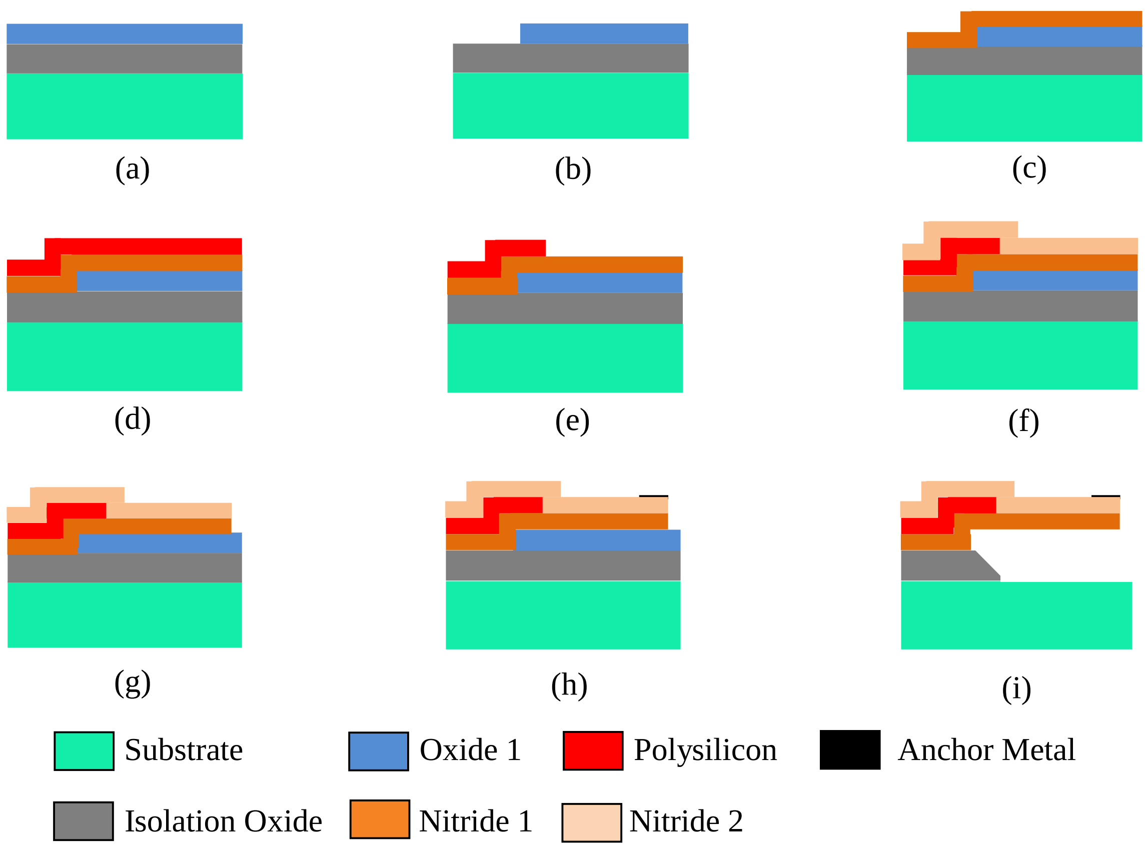
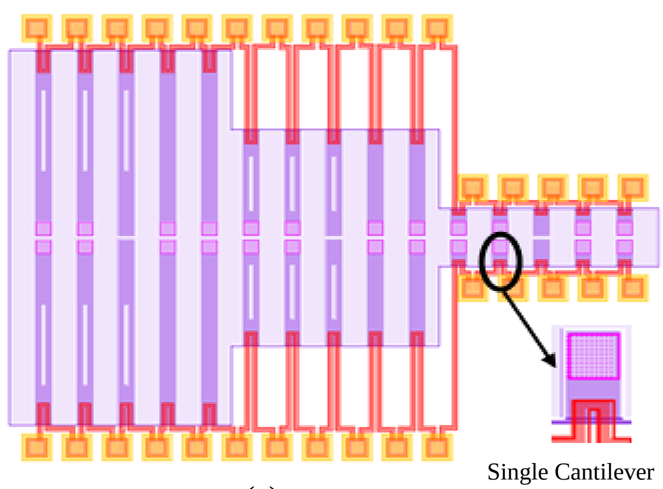


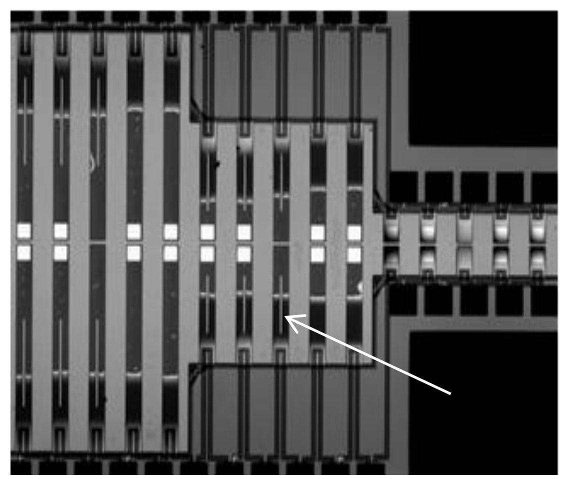
Figure 2-2: Fabrication steps of cantilever sensors. (a) deposition of oxide layers, (b) oxide etching, (c) deposition of first layer of Silicon Nitride, (d) deposition of first layer of Polysilicon, (e) patterning of Polysilicon, (f) deposition of second layer of Silicon Nitride, (g) patterning of Silicon Nitride, (h) deposition of Anchor metal, (i) Final etching steps

2.1.2.2 Cantilever Array

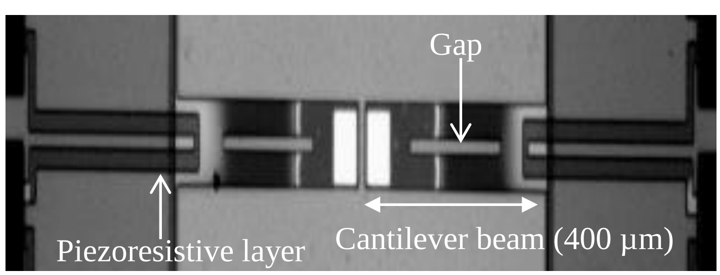
Two different designs of cantilevers have been used in this work. In the first structure, the cantilevers have a simple rectangular shape and in the second structure, we incorporated a gap in shape of rectangular hole in the beam. Based on simulations, this gap can increase the dynamic range of sensor and subsequently can increase the sensitivity of cantilever. In fact, in this structure, we expect the application of minute load on the tip of cantilevers results in a significant deflection of sensors. Another advantage of creating a gap in cantilever is the possibility of developing wider cantilever beams. In general, the less surface area of cantilever, the more effective etching process is performed to remove the sacrificial layer. The creation of gap in cantilevers can decrease the surface area. In this work, the cantilevers with different lengths have been implemented to search for suitable lengths. Figure 2-3 (a-c) show the schematic and microscopic images of cantilever array.



(a)



(b)



(c)

Figure 2-3: Piezoresistive sensor chip containing 30 cantilevers: (a) Schematic, (b) Microscopic image, (c) Zoomed image of two cantilever sensors

All the beams have the same width (60 µm) with different lengths (100, 400, and 700 µm) to study the effect of aspect ratio on the accuracy and resolution of force measurement. As shown in figure 2-3(a), the cantilevers have different lengths and designed in two different structures (with and without the gap). For those beams with larger lengths, the gap is embedded in the middle of structure to decrease the fracture risk of cantilevers. In this figure, top view of piezoresistive sensors with the first nitride layer (Violet) and Polysilicon (Red) is demonstrated.

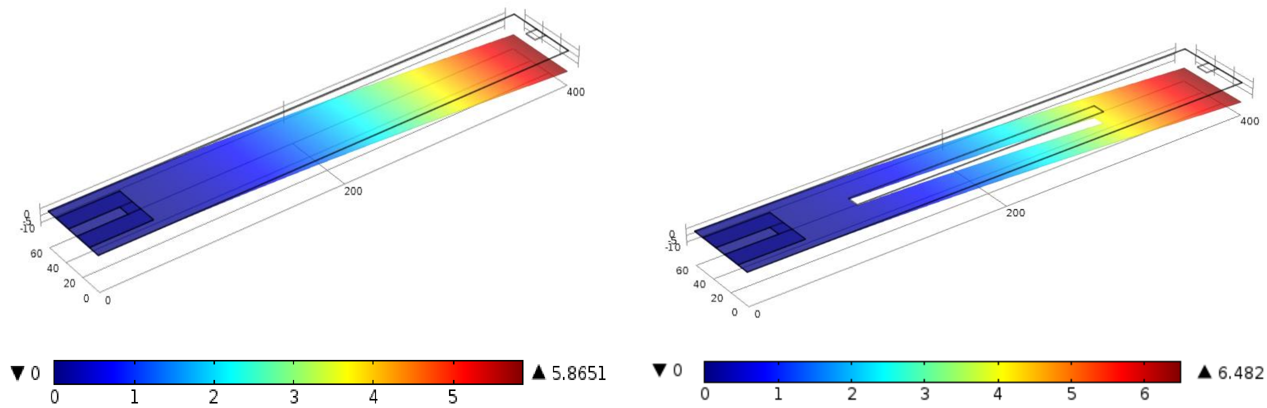
2.1.3 Multiphysical modeling

In this section, the modeling and simulation results are discussed. Herein we have studied mechanical properties of cantilever, including deflection versus applied forces and the effect of aspect ratio of sensors on their output. In these simulations, a base force as the traction force of cells is considered to be equal to 2.84 micro Newton which is equal to traction force of Hela cell [147]. This base force has been applied on top of the cantilever and then augmentation of this force by thousands of 20 pico-farad steps has been studied. In addition, for analyzing the effect of mechanical properties of sensors on their sensitivity and resolution, different range of surface stress has been applied on top of sensors and variation of their output have been studied. It is worth mentioning that the simulations have been done in MEMS module of COMSOL 4.3 by applying force on tip of cantilevers in an area of 10 µm×10 µm. The parameters, which have been used in simulations, are presented in table 2-1.

Table 2-1: Simulation parameters

Poisson Ratio	Polysilicon = 0.22 Silicon Nitride = 0.23
Young modulus [Gpa]	Polysilicon=160 Silicon Nitride=245
Length of Cantilevers [µm]	100-400-700
Width of Cantilevers [µm]	60

Two cantilever models have been simulated in COMSOL and the simulation results are shown in figure 2-4. In this figure, a cantilever with a length of 400 µm is subjected to a surface stress equal to 96 nN. Due to this stress, a deflection is created as shown in color codes. Based on these results, the creation of gap in cantilever can significantly increase the deflection.



(a) (b)

Figure 2-4: Displacement of cantilever sensors due to surface stress of 96 nN for rectangular cantilever beam, (a) without and (b) with the gap

In order to analyze the sensitivity versus aspect ratio, another simulation has been conducted for three different lengths of cantilevers (100, 400, and 700 µm). The deflection of cantilevers due to the application of different surface stresses ranging from 1 pN to 100 pN with the step size of 5 pN has been analyzed by COMSOL and demonstrated as shown in figure 2-5.

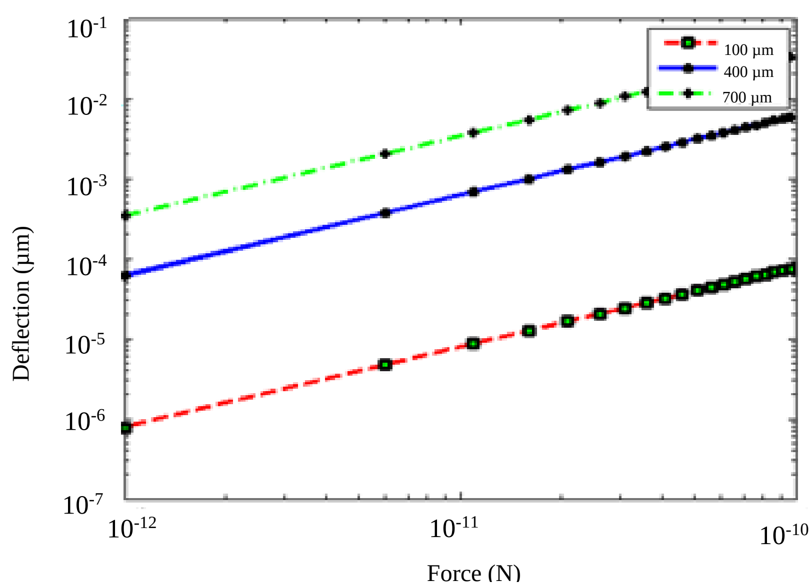


Figure 2-5: Deflection of cantilever (L=100, 400, and 700 µm) versus applied forces

Figures 2-6 and 2-7 present the exact value of deflection due to application of specific force in pico Newton range on two cantilevers with different lengths.

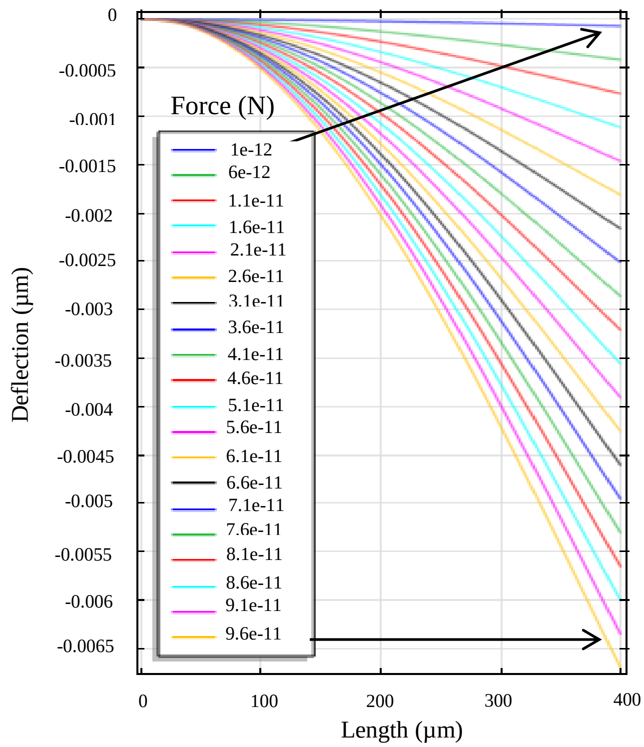


Figure 2-6: Displacement of sensor with length of 400 µm due to application of force in pico Newton range

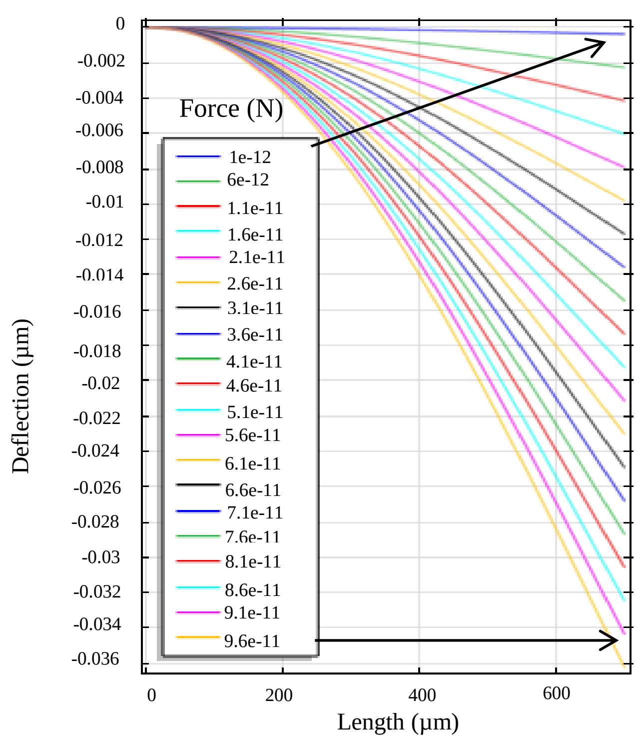


Figure 2-7: Displacement of sensor with length of 700 µm due to application of force in pico Newton range

Based on these simulation results shown in figures 2-6 and 2-7, it can be concluded the longer beams, the more deflection is generated. Therefore a low complexity readout system likely with lower resolution could be used for detection of resistance changes in piezoresistive sensor.

However it should be mentioned that by increasing the length of cantilevers, the Von Mises's stress (figure 2-8) on the fixed end of cantilever will increase. Thus, there will be a higher risk of fracture during the fabrication process or during the application of forces. Despite of aforementioned comment, this high stress end of cantilever can efficiently be used for placing piezoresistor structure.

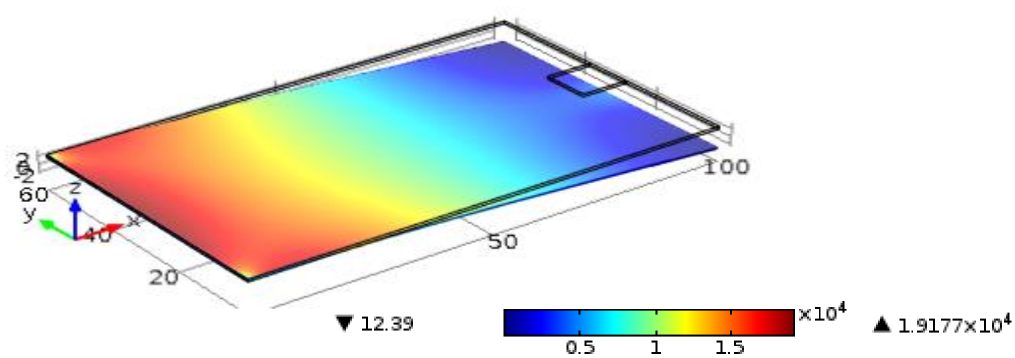


Figure 2-8: Von Mises's stress distribution (N/m2) on cantilever beam for 1 nN force

The maximum stress at this point can be calculated by equation 2.1 where *F* is the applied force and *L*, *w*, and *t* define the length, width and thickness of the beam respectively [148].

$$\sigma_{max} = \frac{6LF}{wt^2} \tag{2.1}$$

This stress can result into a resistance change in piezoresistor sensor. $\Delta R/R$ depends on piezoresistive tensor of the material which is defined by $\{\pi\}$ in equation 2.2 [148]:

$$\frac{\Delta R}{R} = \{\pi\}\sigma \tag{2.2}$$

By substituting maximum stress from equation 2.1 into the above formula, the variation of resistance versus the applied forces can be calculated. The simulation results show a linear relationship between the conductance of sensors versus the applied force when potential of 5 volt was applied on piezoresistive structure. Figure 2-9 shows the variation of conductance for the application of force between 2.84 µN to 10.84 µN on a cantilever with length of 100 µm.

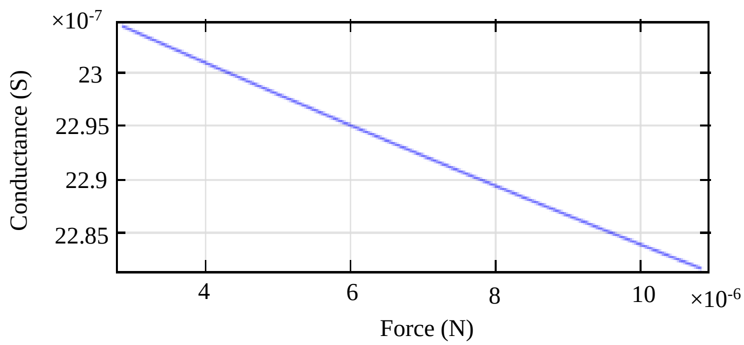


Figure 2-9: Variation of conductance for piezoresistive sensor (Length of cantilever=100 µm, Length of piezoresistive structure=20 µm)

2.1.4 Characterization of sensors

Mechanical and electrical characterizations have been examined to ensure the functionality of proposed sensing device. As the first step, we used interferometry method to investigate the relationship between the resonance frequencies of cantilevers with their lengths. This relationship is a function of various physical parameters including the length of cantilevers as shown in equation 2.3 [51].

$$f = 0.162 \frac{t}{L^2}\sqrt{\frac{E}{\rho}} \tag{2.3}$$

In this equation, E is the young modulus, ρ is the density, and t and L are the thickness and length of the cantilever respectively. Based on this equation the cantilevers with different lengths will have different resonance frequencies. In order to find the resonance frequency of the fabricated cantilevers, several tests have been done with a customer made setup as shown in figure 2-10.

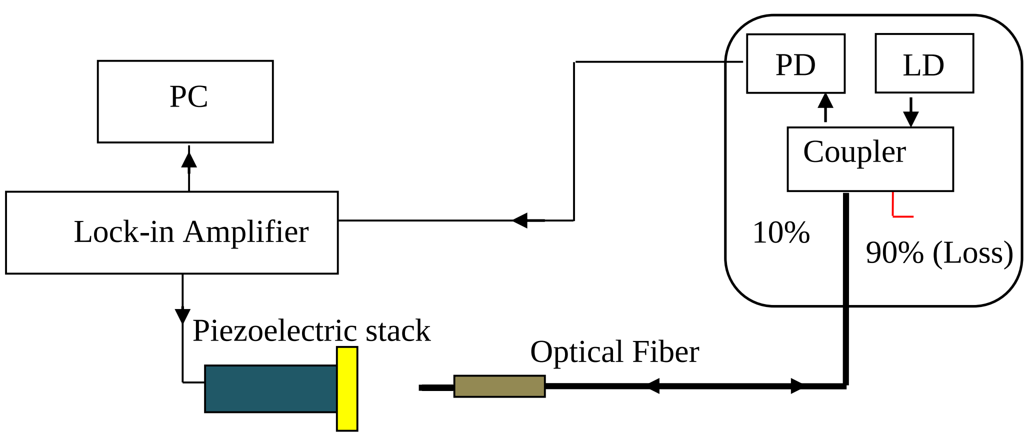


Figure 2-10: Block diagram of resonance frequency measurement setup; LD (Laser diode), PD (Photo detector)

In this set-up, the cantilever array was mounted on a piezoelectric stack and an optical fiber was fixed on top of the sensors. In this system, two reflections will happen. The first reflection will occur inside the optical fiber and the second one will be due to reflection of the light from surface of cantilever sensor. After the reflections pass through the coupler, the signal will be detected by photo detector. Finally, the frequency sweep with lock-in amplifier can be used for detection of the resonance frequency peaks. Figure 2-11 shows a photograph of mounted chip.

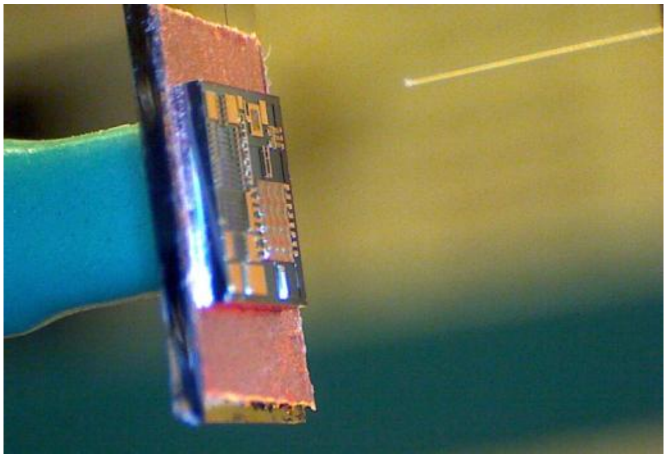


Figure 2-11: Interferometry setup for resonance frequency measurements

Based on the experimental results (figure 2-12), there is a peak in the frequency spectrum of cantilever showing the resonance frequency response of sensor.

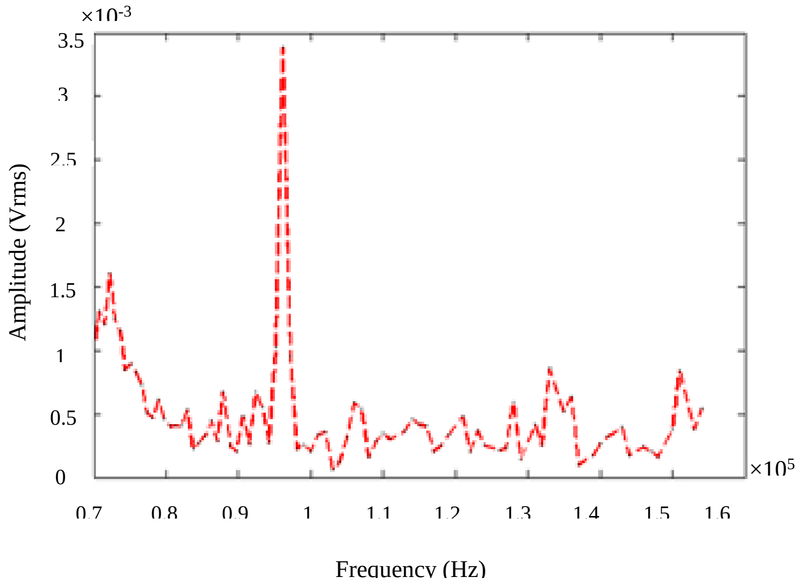


Figure 2-12: Frequency response of cantilever (Length=100 µm)

Figure 2-13 shows the comparison of experimental and simulation data for piezoresistive cantilevers. As seen in this figure, the experiments have been performed on two cantilevers with different lengths. On the other hand, in order to demonstrate the relation between cantilever's length and its resonance frequency we have done simulations for 7 different beam lengths.

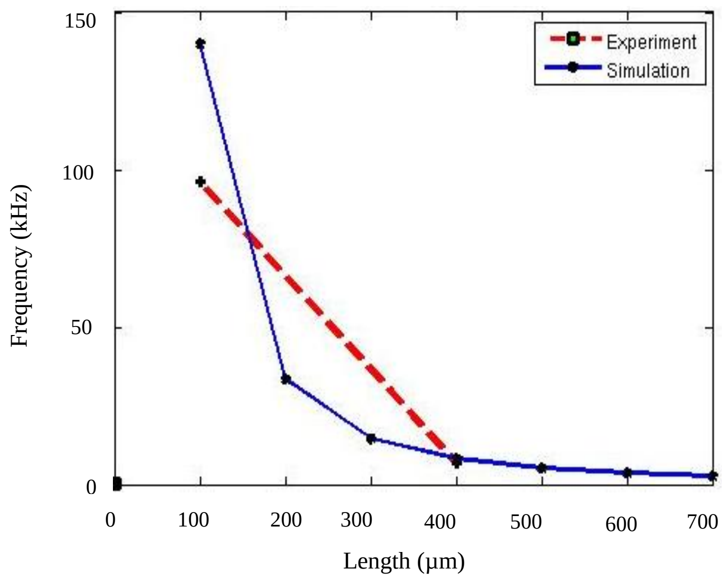


Figure 2-13: Comparison of resonance frequency

Moreover, electrical characterizations have been done for piezoresistive sensors. As shown in figure 2-14, we have applied air pressure using a handheld syringe-shape device on top of the sensors; the measurement of resistance was performed simultaneously and data transferred to computer.

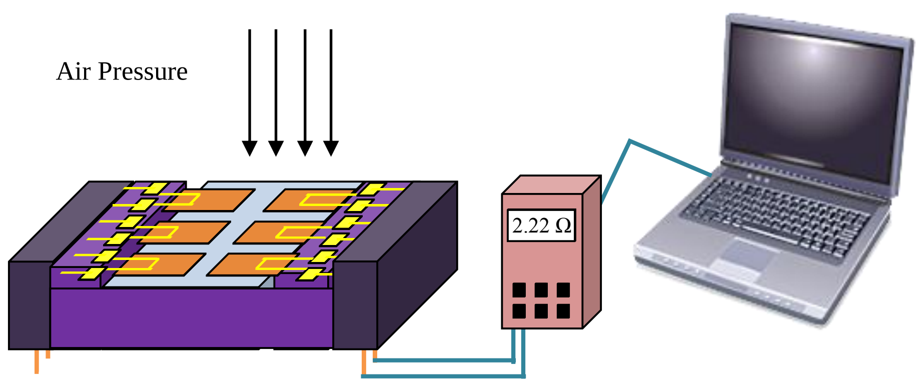


Figure 2-14: Electrical measurement setup

Figure 2-15 shows the variation of piezoresistive resistor as function of time in the presence and absence of applied forces. As it can be seen in this figure, when air is forced on top of the sensors, the beam will deflect and there is a sudden increase in resistance value.

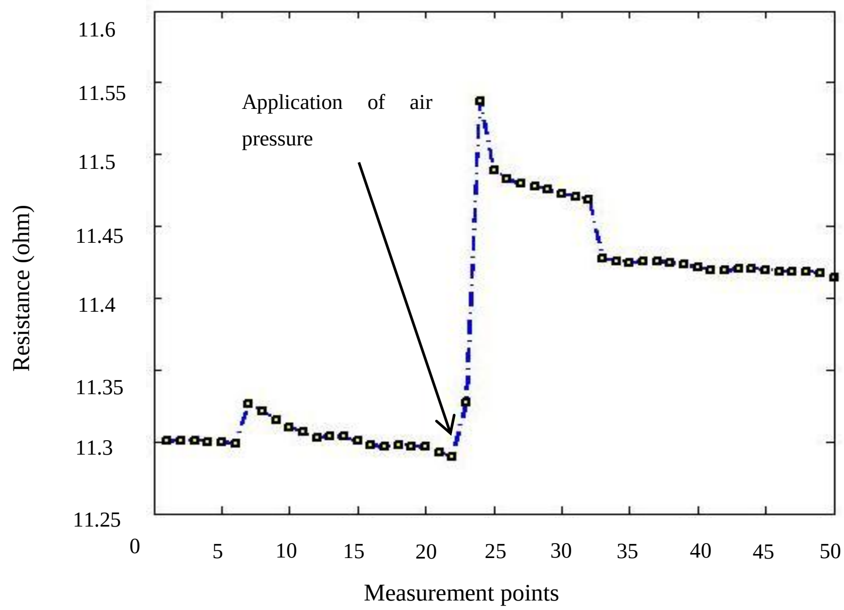


Figure 2-15: Piezoresistive sensing change due to applied mechanical force

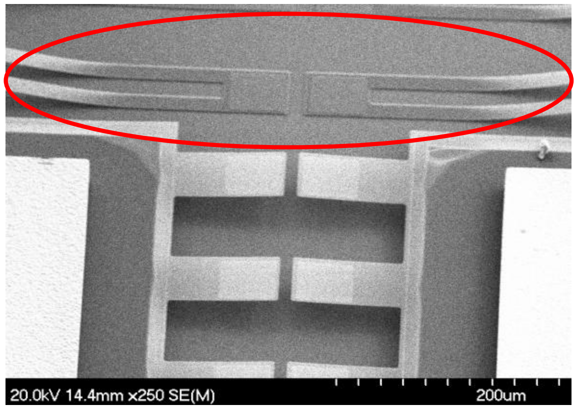
2.1.5 Discussion

In this section, we put forward the main bottlenecks associated with the fabrication process of cantilevers.

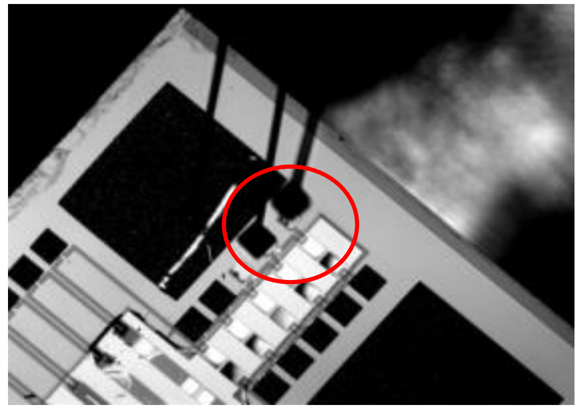
We should report the stiction problem observed in some of cantilevers (Figure 2-16(a)). Stiction problem usually exists when wet etching step is being used in fabrication process. It is likely due to drying process after the wet-etching. In fact, the capillary forces during the drying stage pull down the structure toward the substrate.

As expected, this problem was not observed in short-length cantilevers. In general, in order to avoid such a problem, an anti-stiction layer should be used along with a special drying process. However, since we are limited to a specific fabrication technology, the easiest solution to avoid this problem is to design shorter beams.

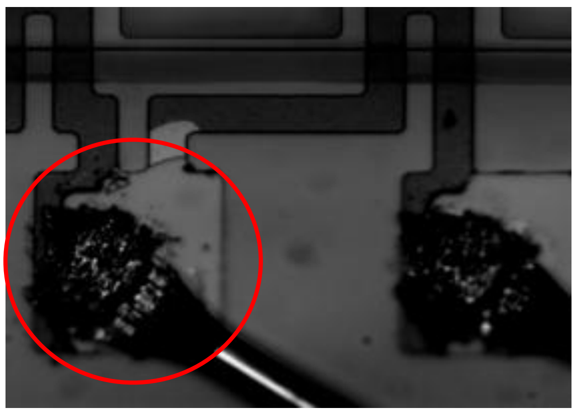
The second problem that we should report is the failure of wire-bonding process between the sensors and pads for some of cantilevers. It seems the pads were displaced and likely destroyed their underneath Polysilicon layer. Figure 2-16 (b-d) demonstrates damages to the sensor array during the wire-bonding process.



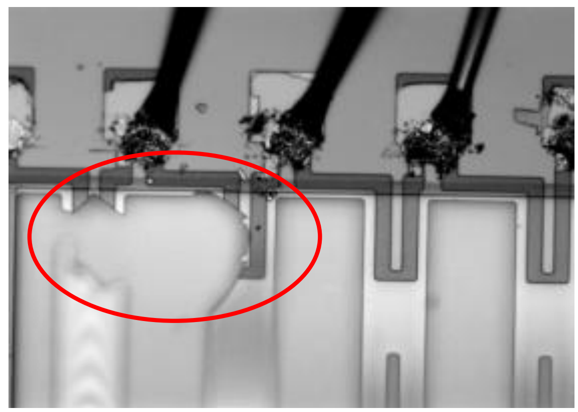
(a)



(b)



(c)



(d)

Figure 2-16: Practical considerations (a) Stiction of long cantilevers, (b) Displacement of the pads, (c) Removal of Polysilicon layer, (d) fractured cantilevers

Based on the technical discussion with technology provider, CMC Microsystems, the wire-bonding problem might be due to small anchor metal layer which is placed underneath the metal pads. This is why using the larger metal layer and anchor metal may be a solution to avoid the problem.

2.1.6 Conclusion

The ability to analyze biomechanical forces is the key step to quantitatively analysis of MBM. In this paper, we put forward a new sensing platform featuring an array of cantilevers integrated with piezoresistive sensors. These sensors are implemented in the fixed end of cantilever to measure the variation of surface stress. These surface stresses are generated by cells or molecules in proximity of the floating end of cantilever. The simulation results demonstrated that these sensors can detect forces in range of pico Newton with high sensitivity. The interferometric and electrical characterization techniques were used to successfully demonstrate the functionality of the proposed sensing platform. This platform can play an essential role for fundamental studies of MBMs and it can also be used for different biological applications like cancer marker detection, DNA analysis, or drug discovery.

2.1.7 Acknowledgment

Authors would like to thank Professor Peter Grutter, and Dr. Yoichi Mayahara for their help with mechanical characterizations. They would also thank CMC Microsystems for technical support.

CHAPITRE 3 CAPACITIVE CANTILEVER ARRAY PLATFORM

The second proposed cantilever array platform in this project uses capacitive method for monitoring the displacement of sensors. In this platform, each cantilever beam acts as the top electrode while the second electrode of capacitive sensor formed underneath the beam (figure 3-1).

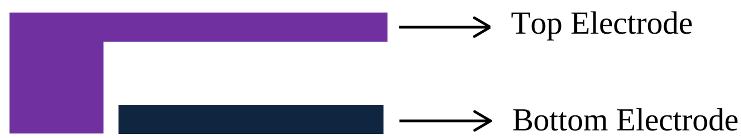


Figure 3-1: Structure of Capacitive sensor

PolyMUMPs process is used as standard technology to develop the proposed device. In the following of this chapter, first a brief overview of the PolyMUMPs fabrication process to develop capacitive sensors is presented and then we discuss the design, simulation, implementation and characterization results.

3.1 PolyMUMPs process

In this section, brief information regarding the fabrication steps which have been used for developing the sensors with PolyMUMPs technology will be presented in order to be able to get an idea about the structure of sensors. In addition to fabrication procedure, the fabricated sensors will be demonstrated at the end of this section.

PolyMUMPS is a part of MUMPs (Multi-User MEMS Process) which is offered by MEMSCAP through CMC Microsystems. Seven layers are being used in this process; three Polysilicons, two oxides, one metal, and one Silicon Nitride layer. The Process starts with covering the substrate by low stress Silicon Nitride and then first layer of Polysilicon with 500 nm thickness is deposited. This Polysilicon layer is patterned by first mask and reactive ion etching and then the first oxide layer with thickness of 2 µm is added.

In this stage two masks are used on oxide layer. The first one creates dimples and the second one allows us to create desired patterns. In the next step, 2 µm Polysilicon is deposited and it is followed by deposition of thin sacrificial layer. In order to define structures in second Polysilicon layer, a forth mask is applied and then a second layer of oxide with thickness of 0.75 µm covers all the structures.

After applying photoresist, the fifth mask is being used to pattern oxide layer and also create via holes. In order to define the contact regions between last layer of Polysilicon and substrate, patterning both oxide layers is necessary. This step is being done by using sixth mask. After defining the patterns, a second layer of Polysilicon with thickness of 1.5 µm is deposited and patterned by using seventh mask. As the last step, metallic structures are formed by using lift off method and last mask. Finally in order to release the structure, the chip is immersed in Hydrofluoric acid solution. More detailed information about this technology is available in PolyMUMPs design handbook [149].

Although PolyMUMPs process includes deposition of seven layers, but not all of them are being used for fabrication of our cantilever sensors. The sensors in this project are fabricated with the second Polysilicon layer and the first layer of Polysilicon is being used as the fixed electrode underneath the movable beam. The distance between two electrodes is defined by the thickness of first sacrificial oxide layer which is 2 µm. The Metal layer is not being used in the sensor structure, but it is the main layer in the pads.

In addition, we have used dimple mask in order to create a sharp structure on the bottom surface of the beam. Adding dimples to the beam will reduce the risk of stiction of the beams to substrate during the etching process. Stiction is one of the main problems in fabrication of suspended structures. When wet etching is being used for removal of sacrificial layer, if special techniques for avoiding the adhesion between microstructures are not being used, capillary forces can pull down the beam toward substrate. Based on the suggestion from CMC Microsystems, we have incorporated more than one dimple in large beams in order to decrease the chance of losing the cantilevers due to adhesion.

Also, in order to avoid the contact between dimples and the fixed electrode on the substrate, in case of application of heavy load, we have isolated the area underneath the dimples from the rest of conductive layer underneath the beam. Figure 3-2 shows the fabrication steps of cantilever sensors in PolyMUMPs process.

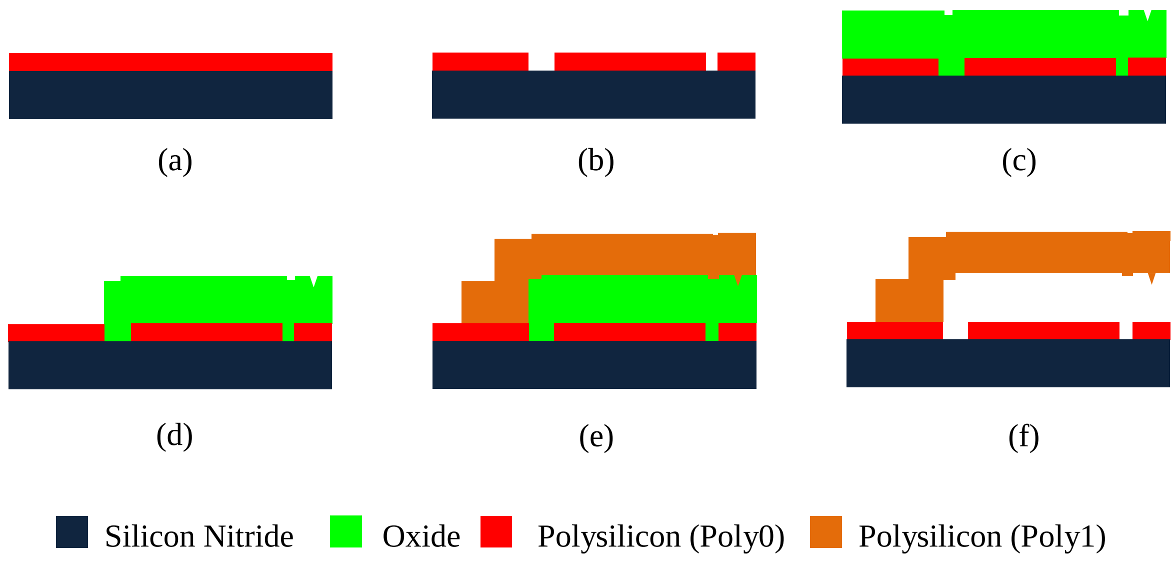


Figure 3-2: Fabrication steps of cantilever sensors in PolyMUMPs process;(a) Deposition of first layer of Polysilicon (Poly0), (b) Patterning Poly0, (c) Oxide deposition and formation of dimple, (d) Patterning the oxide layer, (e) Deposition and patterning of second layer of Polysilicon (Poly1), (f) Removal of sacrificial layer

Figure 3-3 demonstrates the layout of the chip featuring 74 sensors in an area equal to 5mm×5mm which was prepared by Coventor Software (Figure 3-3).

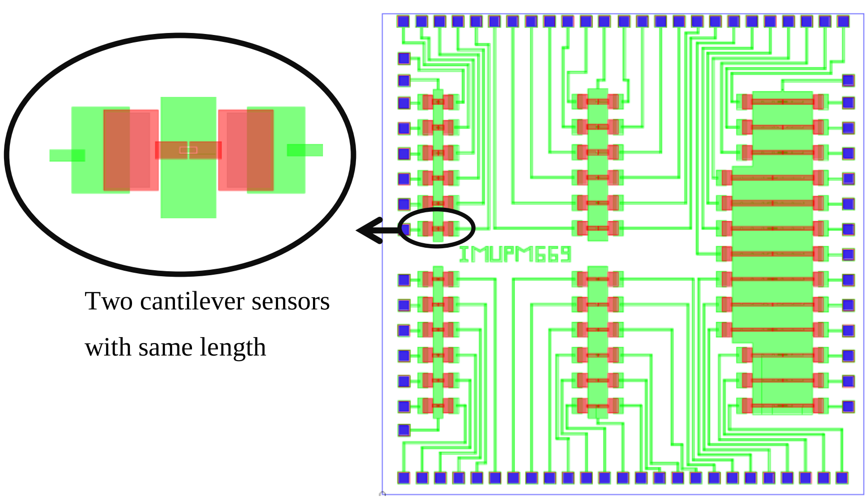


Figure 3-3: Layout of capacitive sensors

Figure 3-4 shows the fabricated chip through CMC Microsystems.

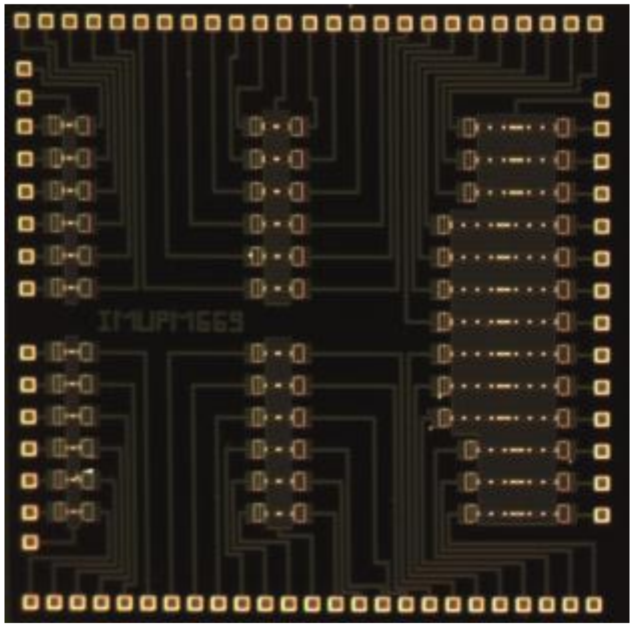


Figure 3-4: Fabricated sensors

In this structure, we can remove the base capacitance value of the sensors by comparing the output of two beams in differential mode and only monitor the capacitance variations due to application of forces.

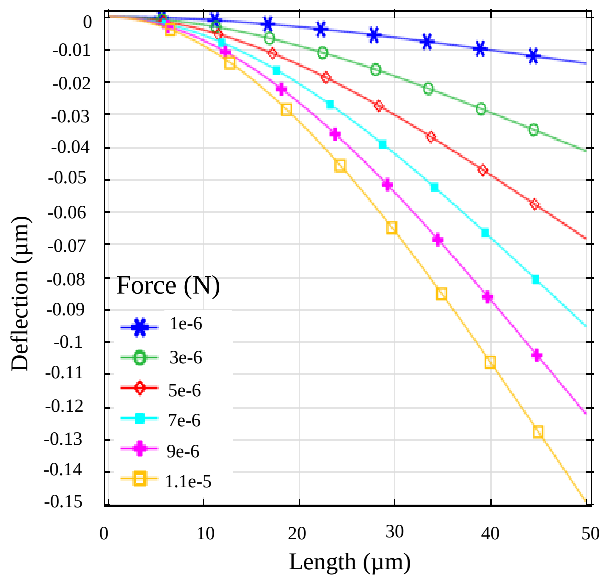
3.2 Simulations

Capacitive cantilever has been modeled in MEMS module of COMSOL software. By using this module it would be possible to apply the electrical and mechanical analyzes on one model. As the first step, the deflection of cantilever as a function of applied force is studied. As described in the last section, 2 µ space between the capacitive sensing electrodes is considered as a limitation factor to design cantilever sensor in static mode. For this reason, the multiphysical model of cantilever is essential to search for suitable dimensions to achieve the maximum sensitivity. Table 3-1 shows the main parameters for simulation of the cantilever.

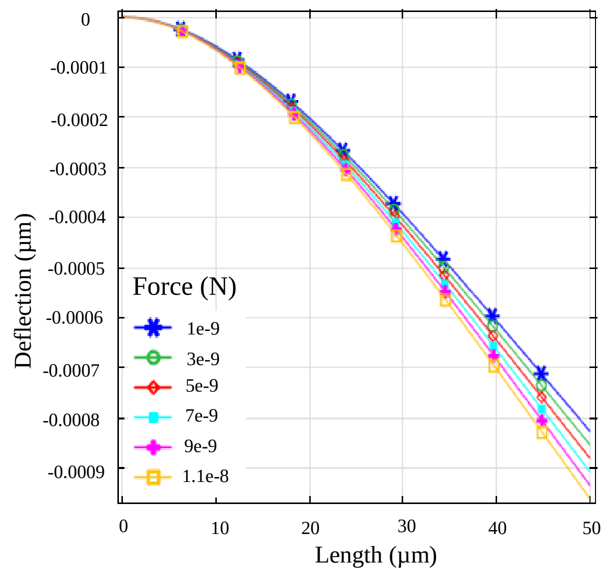
Table 3-1: Simulation parameters

Beam's material	Density(Kg/m^3)	Young's modulus (Gpa)	Poisson's ratio
Polysilicon	2320	158	0.22

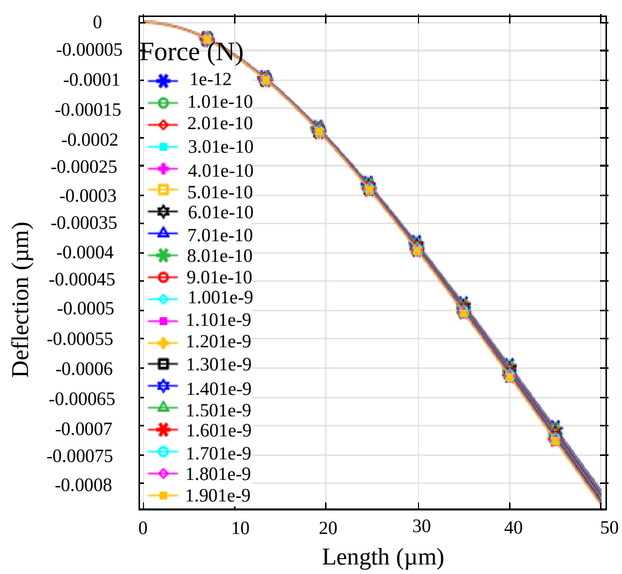
Figures 3-5 and 3-6 show the displacement of cantilevers with two different lengths due to the application of force in range of pN-µN. The width of all sensors is equal to 50 µm in order to assure the removal of sacrificial layers underneath the beam. As mentioned, the dimensions of cantilever significantly affect the functionality and accuracy of device. We put further on this discussion by modeling the device and applying the force on an area of 10 µm×10 µm on the tip of the beam.



(a)



(b)



(c)

Figure 3-5: Displacement of 50 µm beam for various ranges of force, (a) Micro Newton, (b) Nano Newton, (c) Pico Newton

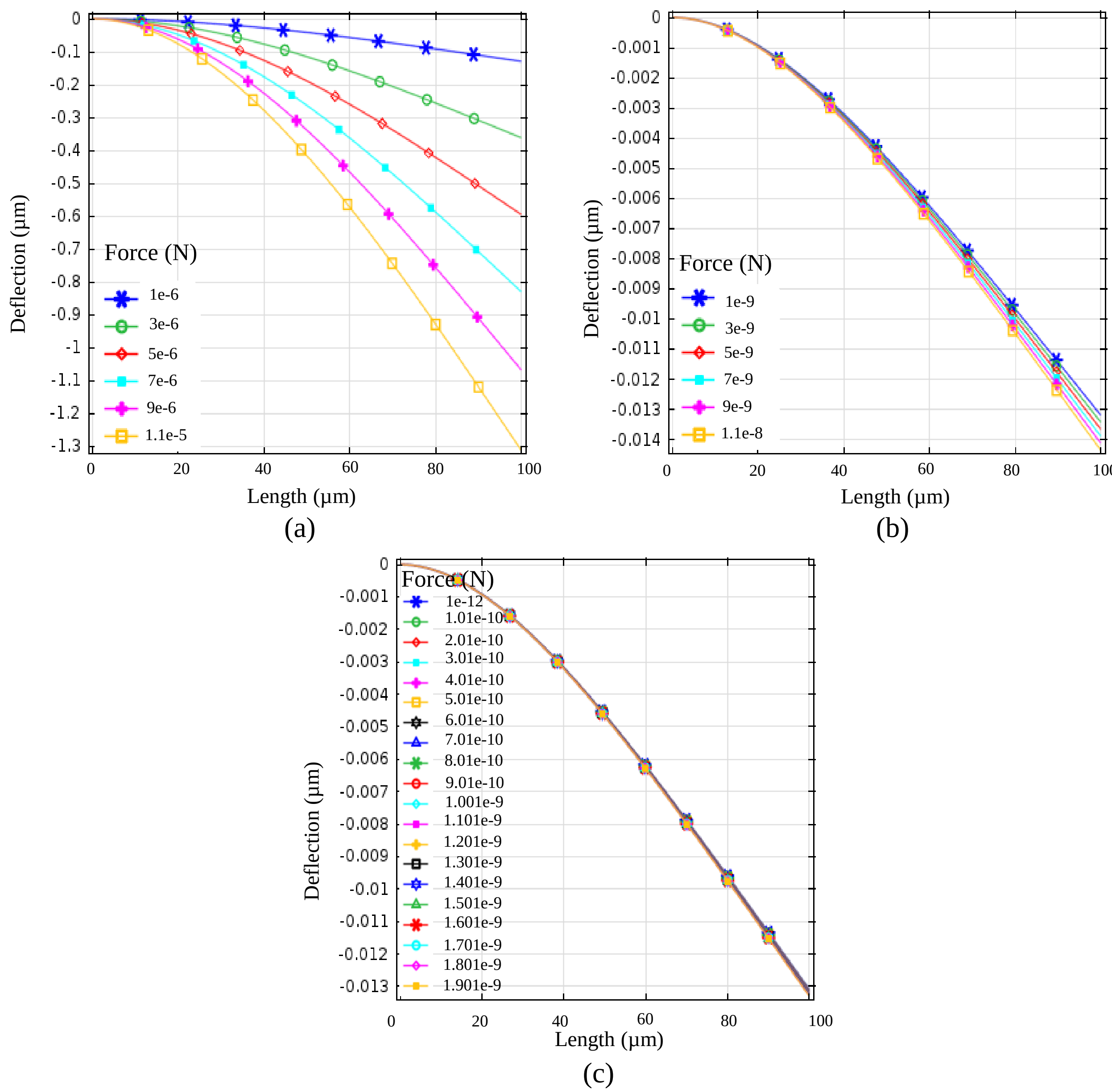


Figure 3-6: Displacement of 100 μm beam for various ranges of force, (a) Micro Newton, (b) Nano Newton, (c) Pico Newton

As it can be seen in figures 3-5 and 3-6, by reducing the applied force, the range of displacement significantly decreases. For example, based on these simulations, 1 μN to 10 μN variations in the applied force on 50 micrometer cantilever result in 0.13 μm displacement; and for 1 nN to 10 nN variations in force, the sensors will displace 1.35×10^{-4} μm. It is worth mentioning that the biologically relevant forces vary from one application to another one. Also by comparing figures 3-5 and 3-6, one can observe the effect of beam length on the final output of the sensors. The simulation results, showing the maximum deflection of 50 and 100 µm beams due to application of specific forces, are compared in figure 3-7.

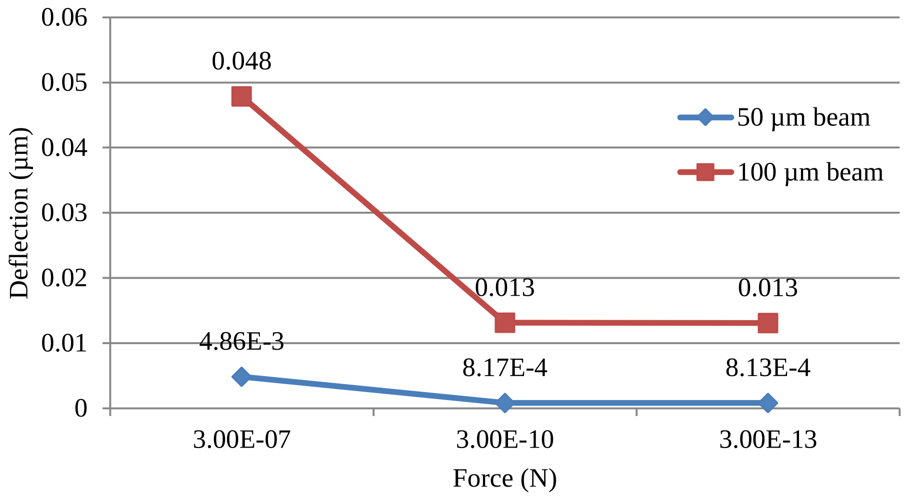


Figure 3-7: Comparison of deflection for two cantilevers

Larger displacement is more suitable since it will cause higher output signal; however, it is important to consider the limitation of space between two electrodes. For example, in figure 3-8 the results of application of force in range of micro Newtons on top of 200 µm beam are presented. In this case, the maximum displacement is 2.4 µm (for 1 µN) which is bigger that 2 µm space between two electrodes. So if this sensor is being used in an application that deals with 1 µN force, the top electrode will attach to the conductive layer on the substrate and the functionality of sensors will be compromised.

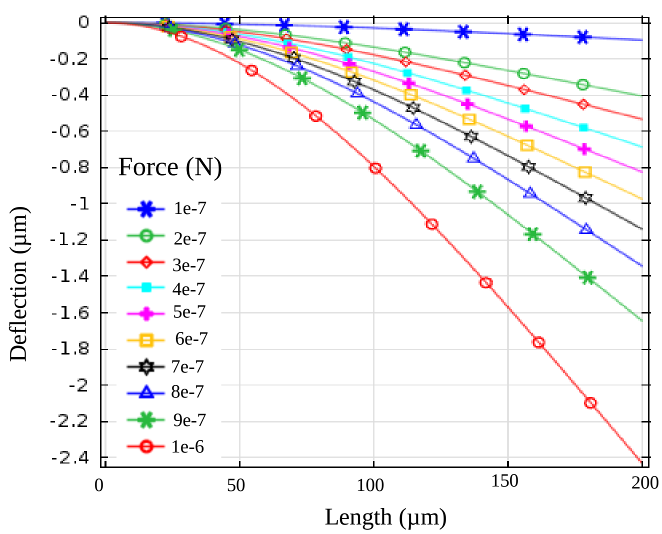


Figure 3-8: Displacement of 200 µm beam for micro Newton forces

Based on the above simulation results and the design rules of fabrication technology, we have chosen 100 µm as a maximum length of sensors. Longer cantilevers can be used for measuring much smaller forces but the risk of fracture of beam during the fabrication and operation will be higher. However, we have incorporated some beams with higher length on the chip in order to examine the possibility of fabrication of longer sensors with this technology. Dimensions of all cantilevers on the chip are presented in table 3-2.

Table 3-2: Dimension of cantilevers on the chip

Length (µm)	Width(µm)	Thickness(µm)
50,100 ,300,400	30,40,50	2

By applying 5 volts electrical voltage between the sensing electrodes of cantilever, the electrical analysis of the sensors was performed and the distribution of electric field between two electrodes is presented in figure 3-9.

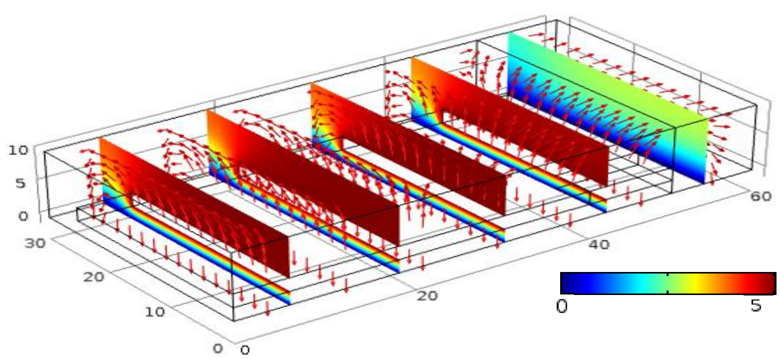
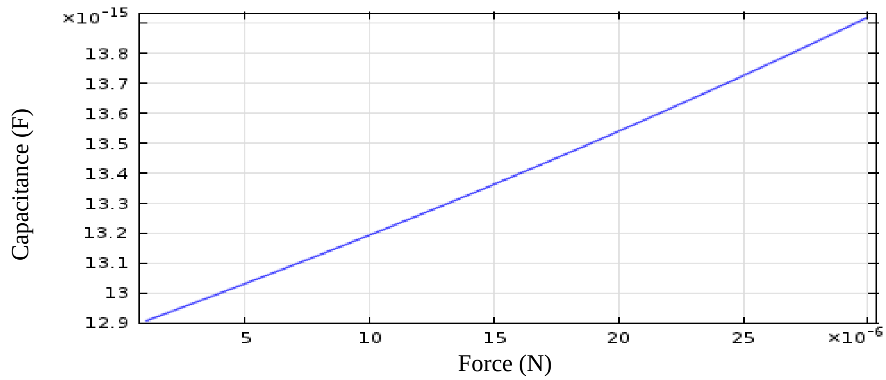
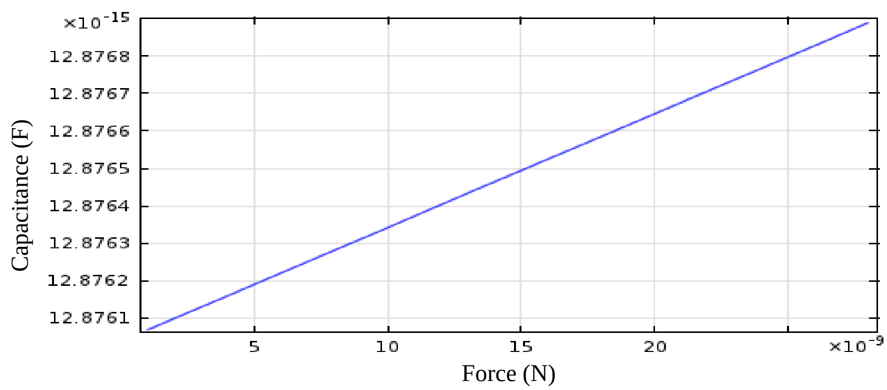


Figure 3-9: Distribution of electrical voltage (V) and Electric field (Arrows) on cross sectional plans for a beam with 50 µm length

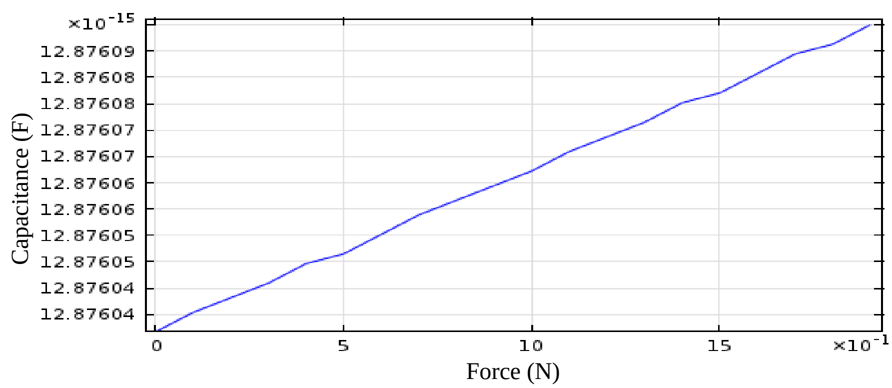
Another step of simulation is monitoring the variation of capacitance as a result of application of force on top of the sensors. Simulations have been done based on the selected values for width and length of the sensors and the applied force varies between micro and pico Newtons. Following results (figures 3-10 and 3-11) show the variation of capacitance of sensors with 50 µm and 100 µm length.



(a)

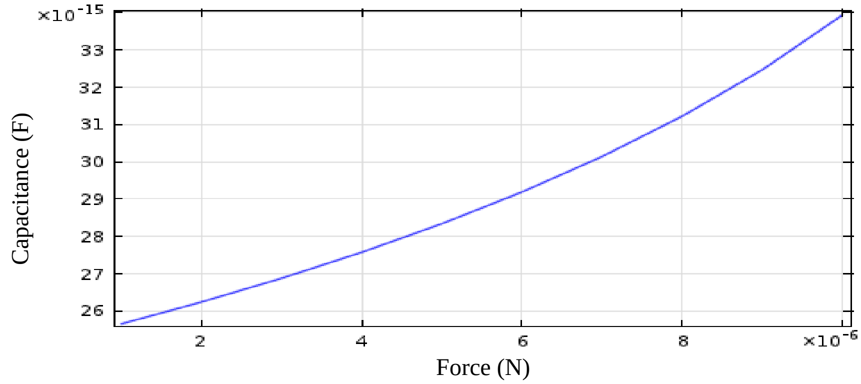


(b)

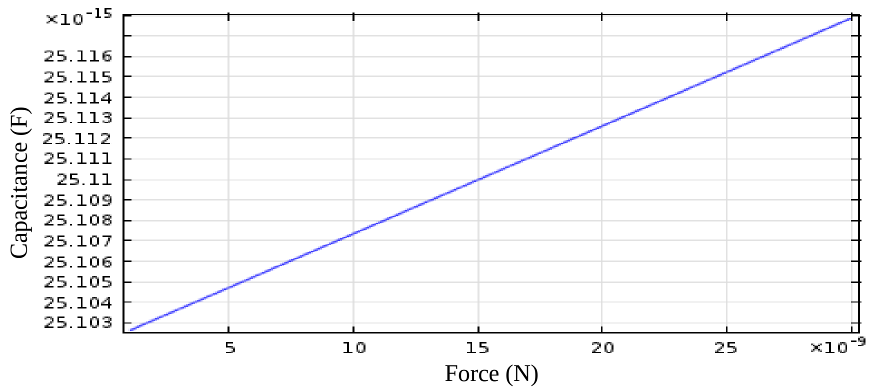


(c)

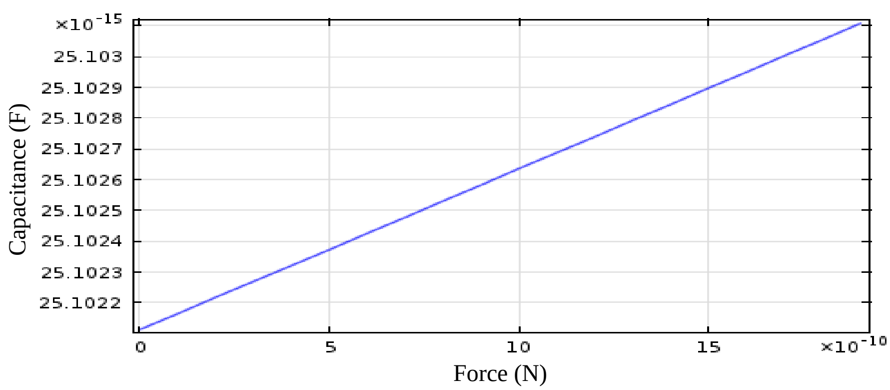
Figure 3-10: Variation of capacitance for 50 µm beam due to various ranges of force, (a) Micro Newton, (b) Nano Newton, (c) Pico Newton



(a)



(b)



(c)

Figure 3-11: Variation of capacitance for 100 µm beam for various ranges of force, (a) Micro Newton, (b) Nano Newton, (c) Pico Newton

As already mentioned, the resonance frequency is an important factor for characterization of beam. COMSOL can efficiently be employed to model this factor using the frequency domain analysis. We have also calculated the resonance frequency by using equation 2.3 and table 3-3 presents the results from simulation and theoretical calculations. As it can be seen in this table, both values are in good agreement with each other.

Table 3-3: Resonance Frequencies of Cantilever sensors

Length (μm)	Resonance frequency (Hz)	
	Simulation Results	Theoretical calculations
50	1.09×10^{6}	1.07×10^{6}
100	2.70	2.67×10^{5}
200	6.72×10^{4}	6.68×10^{4}
300	2.98×10^{4}	2.97×10^{4}
400	1.67×10^{4}	1.67×10^{4}

3.3 Characterization and measurements

Preliminary inspection was done using a microscope and a probe station to confirm removal of the sacrificial layer underneath the sensors. Herein, the sharp tip of a micro-probe was placed on top of the sensors to touch the cantilever in order to cause the deflection, thus proving the removal of the sacrificial layer during the etching process. In a second step, an experiment was done to find out the resonance frequency of the beams. The same interferometry method which has been discussed in the previous chapter is being used for this measurement (Figure 3-12).

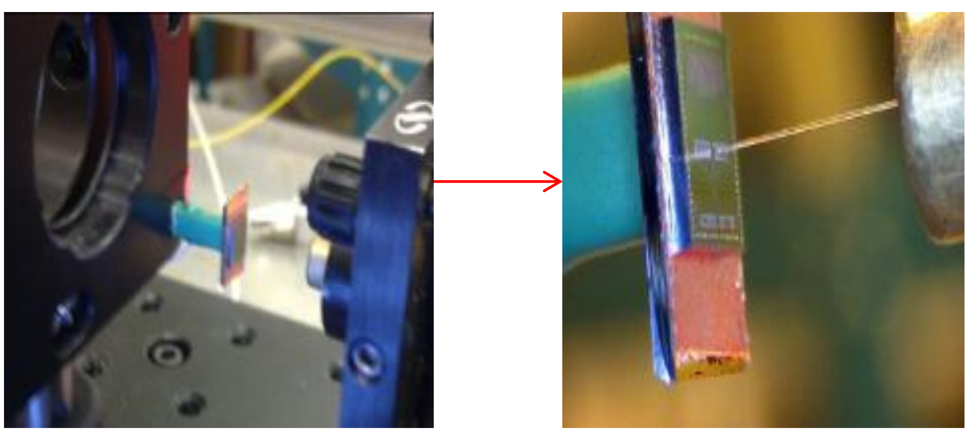
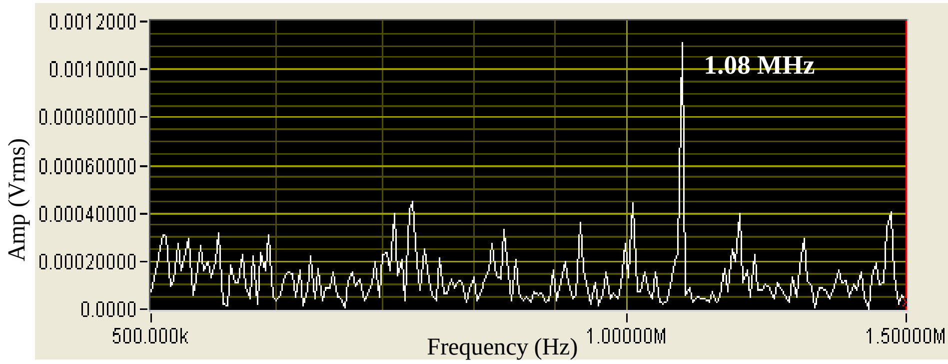


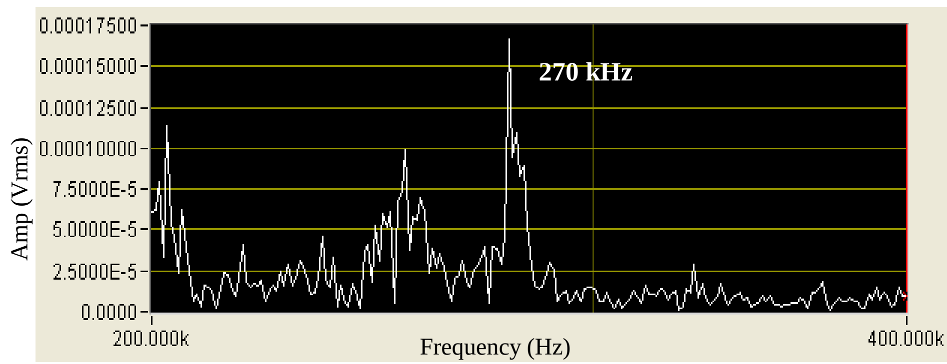
Figure 3-12: Interferometry setup

In order to find the resonance frequency of all the beams the optical fiber has being moved manually and placed closed to the tip of cantilevers with different dimensions and microscope was used in order to make sure about position of the fiber.

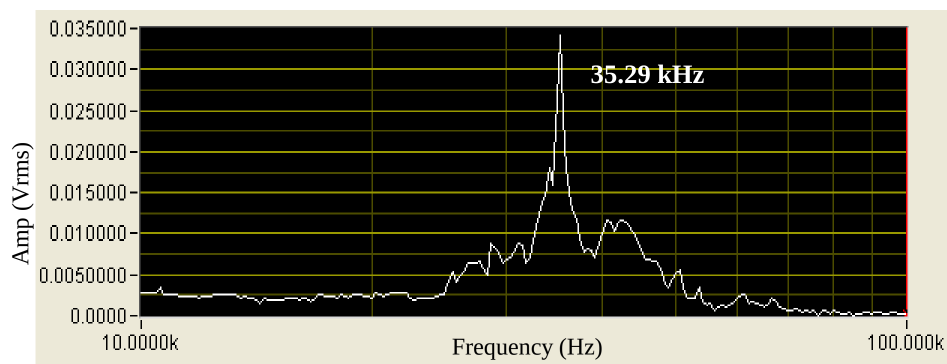
Figure 3-13 (a-d) shows the results of frequency sweep. In these figures, the peak defines the resonance frequency of cantilevers.



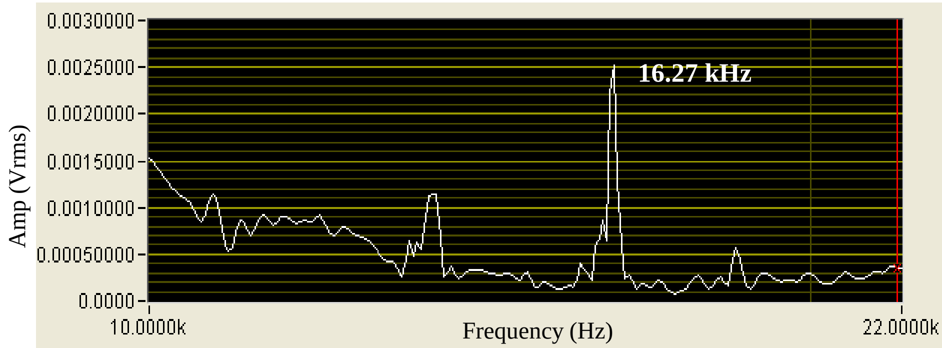
(a)



(b)



(c)



(d)

Figure 3-13: Resonance frequency measurements; (a) 50 µm beam, (b) 100 µm beam, (c) 300 µm beam, (d) 400 µm beam

In figure 3.14, the values of resonance frequency from experiment are being compared with the simulation results. As it can be seen from this figure, simulation and experimental values are very close to each other

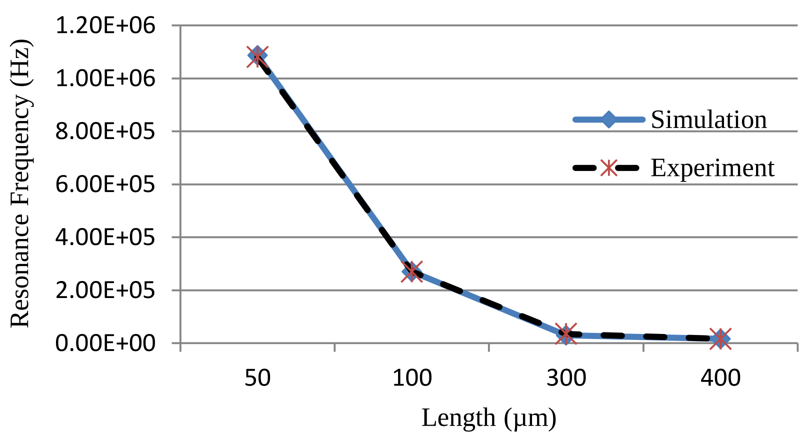
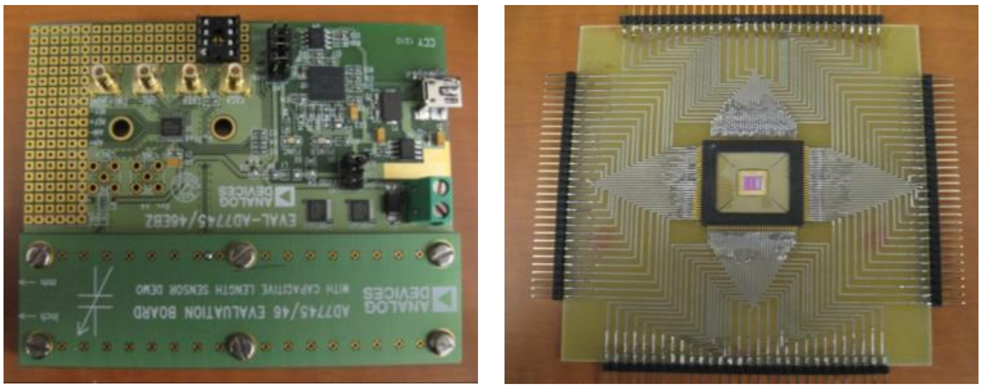


Figure 3-14: Comparison of simulation and experimental results for resonance frequency

In the next step of testing the sensors, electrical measurements were performed to make sure that sensors are functional. In order to do the test, the same procedure which has been mentioned in previous chapter has been used and variation of capacitance due to application of air pressure on top of the sensors was monitored.

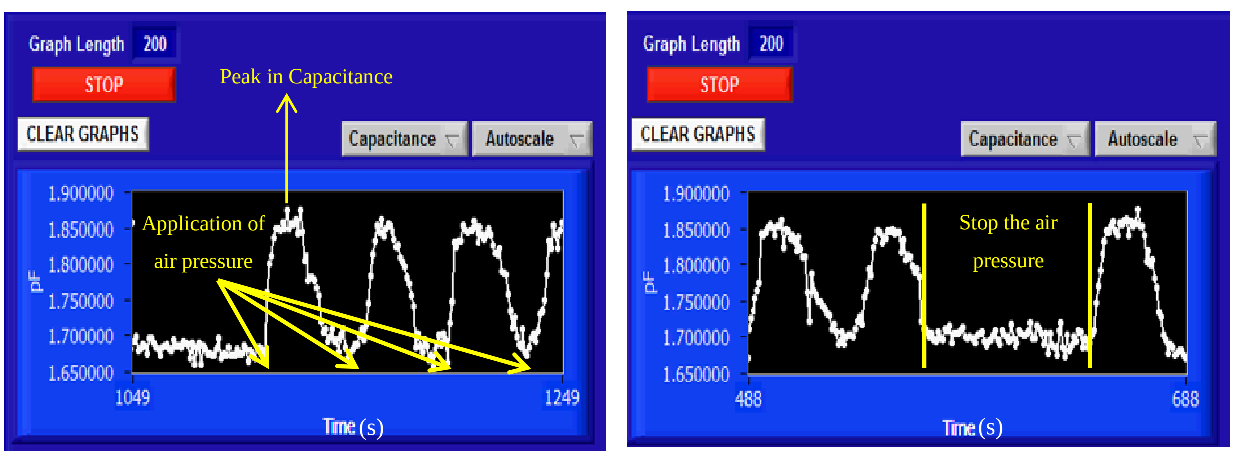
In this step, a capacitive to digital converter board (AD7746) was used as a readout system for monitoring the output capacitance. This device is a high resolution 24-bit capacitance to digital converter that has two input channels. It is possible to directly connect the sensors to the board and monitor the capacitance variation by using the specific software which comes with the board. In order to do the measurements, the packaged chip has been mounted on the PCB and two electrodes of a sensor are connected to the converter board. Figure 3-15 shows the board and the PCB which have been used for the test.



(a) (b)

Figure 3-15: Setup for electrical measurements; (a) Capacitive to digital convertor board, (b) Wire bonded chip on PCB

In these measurements, the fixed electrode on the substrate is connected to ground and 5 V is applied to cantilever beam (movable electrode). Figures 3-16 (a-b) show the variation of capacitance when the air is applied on top of 50 µm cantilever and deflect the beam.



(a) (b)

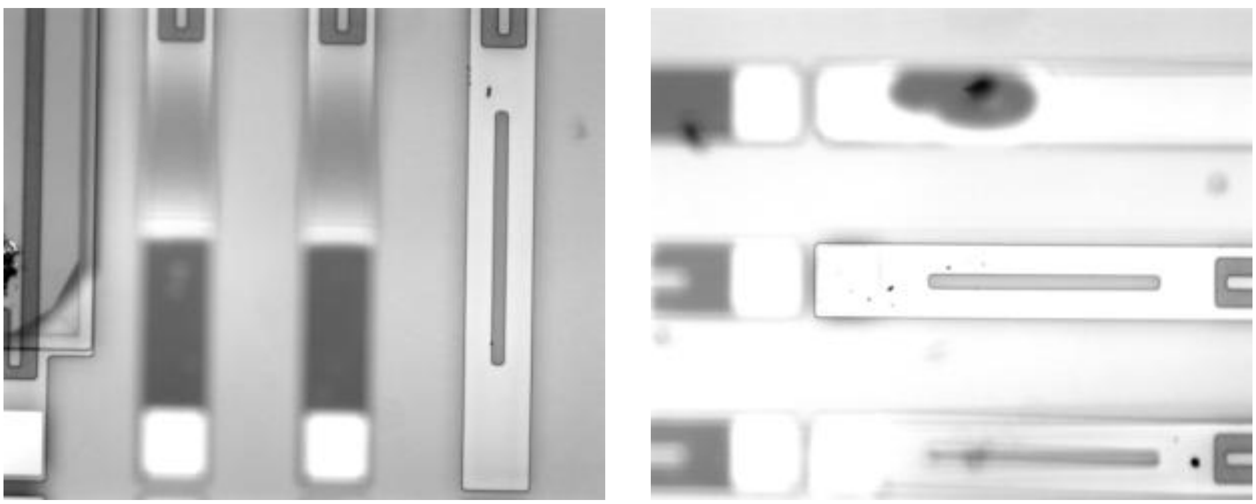
Figure 3-16: Variation of capacitance due to application of force

As shown in figure 3-16, there is a peak in value of capacitance when the air displaces the sensor. In order to reach to the above signals, the syringe was filled with air and air pressure was applied on top of the sensors for multiple times. In this case, the peaks were repeated when the air was applied and it disappeared when we stop the air pressure. Since we have a suspended structure which is sensitive to small amount of forces, there are fluctuations in the output signals. However, it is possible to remove the effect of these unwanted variations by using differential measurements. This issue will be explained with more details in the discussion chapter. The values from mechanical and electrical variations have shown that sensors are functional and they are ready to be tested in biological solutions.

CHAPITRE 4 GENERAL DISCUSSION

In this chapter the challenges and difficulties regarding the different steps of this project will be summarized and the proposed solutions will be discussed.

One of the most important tasks which need to be discussed is related to the fabricated sensors. In fact, long cantilevers fabricated with PolyMUMPs and MetalMUMPs technologies suffer from adhesion. After testing the sensors with probe station and microscope, it has been noticed that some of the longer beams are attached to substrate. This dysfunction exists for some of the 300, and 400 µm capacitive beams and 400, and 700 µm piezoresistive sensors. Figures 4-1 (a-b) shows some of the beams that adhere to substrate.

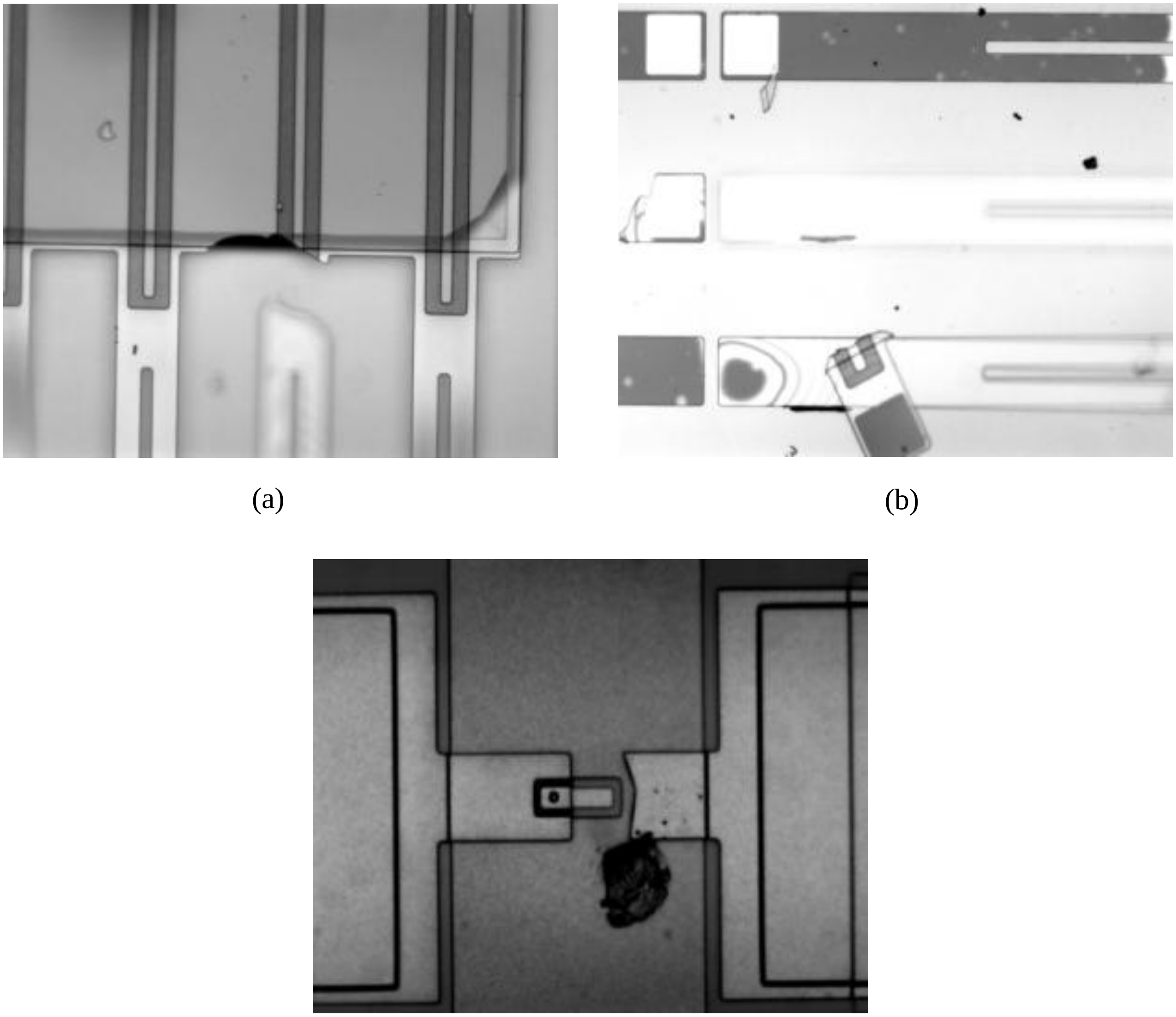


(a) (b)

Figure 4-1: Adhesion of the beams to the substrate due to stiction problem

In future design, it is important to consider the effect of stiction and limit the maximum length of sensors to smaller values in order to have more functional sensors.

In addition to the adhesion, since the sensors have suspended structure, they are very sensitive to forces and the possibility of their fracture during the wire-bonding, packaging, and tests are high. Because of this characteristic of sensors, some of the beams have been lost during the tests. Figure 4-2 (a-c) demonstrates some of the damages due to the fragile structure of sensors.



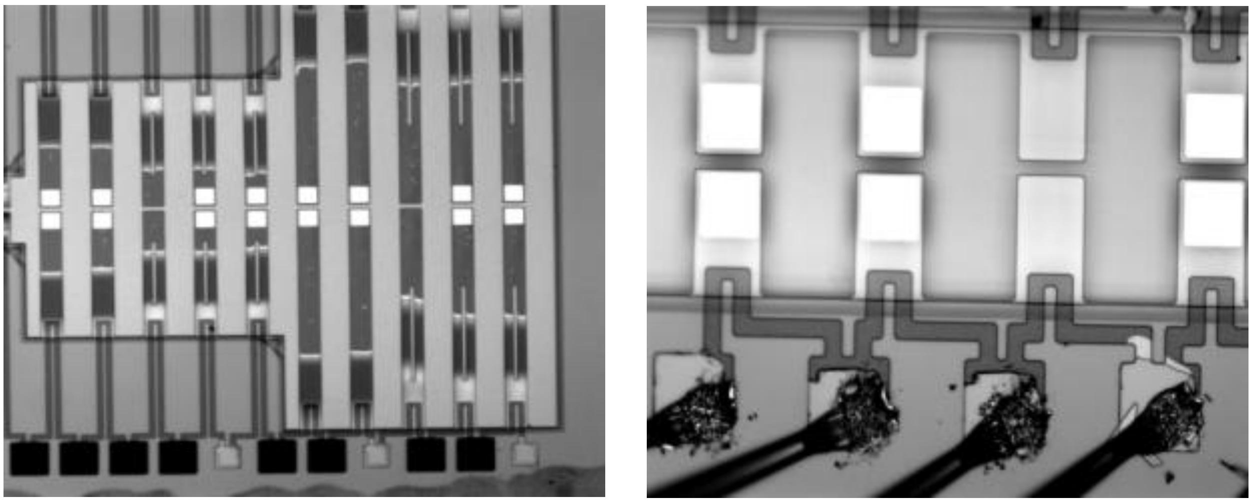
(a) (b)

(c)

Figure 4-2: Fracture of beams during the wire-bonding and tests

As it has been mentioned previously, another important challenge during this project was the unstable pads of piezoresistive sensors. During the wire-bonding procedure of piezoresistive chip, we have noticed that some of the pads are not fixed in their place and wire-bonding resulted into removal of layers in pad structure or displacement of them. In order to find out the origin of this instability we have contacted CMC Microsystems and went through the design rules again. Based on the discussions, it seems the Polysilicon layer in the pads was attacked during the etching. Although there is not any restriction regarding the anchor metal layer of the pads in the design handbook but this problem is probably due to having small area for this layer. CMC

Microsystems has mentioned that they previously had received some reports regarding this issue in other chips and they are going to add some design rules related to pad design. They have also suggested us to use larger anchor metal on the pads for future fabrications in order to have stronger connections. Figure 4-3 (a-b) shows the removal of pad layer in piezoresistive chip.



(a) (b)

Figure 4-3: Removal of pad layer during wire-bonding

Another challenge in this project is related to the characterization procedure and testing of the sensors. As shown in figure 3-16, the output signal contains some noises and it is important to propose a technique to remove the unwanted signals from the main output. Since this problem has been foreseen, the structure of sensors is designed in a way that it would be possible to use differential measurement technique. In each array of sensors several identical beams are placed near each other so it would be possible to use one of them as reference and the others as sensing beam. When one of the sensing beams displaces due to adhesion of biological substances, the reference beam will only have the unwanted variation of signals due to the environmental effects. By comparing the output of these two beams, the noise can be removed from the output signal and only the variations due to biological changes will be monitored.

The other challenging part in this project is providing more accurate mechanical characterizations. In this project, an air pressure through syringe was applied for deflecting the sensors. This type of characterization can ensure us that the sensors are functional and ready to be used in next step. However, this method might not give us detailed information regarding the amount of force which has been applied on top of the sensors. In order to solve this issue, two techniques have been proposed as future step of this project. The first technique involves using AFM for application of force on cantilevers. In this method, a special setup should be designed which can be used along with AFM machine for mounting the chip. By bringing the AFM tip in contact with the cantilevers it would be possible to analyze deflection of the beam due to specific force. The second proposed system composed of air pomp, which is connected to a chamber that contains sensors, and deliver specific air pressure to the enclosed space. The pressure of the air can be monitored by an air pressure sensor inside the chamber. This method enables us to find out the relation between the amount of pressure and output of sensors.

CHAPITRE 5 CONCLUSION

In this master thesis a sensor platform for collecting quantitative biological data has been developed. This platform consists of two chips containing microcantilever sensors. These sensors were fabricated through standard MetalMUMPs and PolyMUMPs technologies. The first chip featured 30 cantilevers integrated with piezoresistive structure and the second chip consisting of 70 cantilevers which use the capacitance measurement technique.

Both types of sensors, capacitive and piezoresistive, were modeled with COMSOL multiphysics software using electrical and mechanical analysis that were performed in order to search for suitable designs. In the second step toward the fabrication, the layout masks of sensors were developed with Coventor software and then fabrication process was done through CMC Microsystems.

Fabricated sensors were analyzed under microscope and with probe station in order to make sure that the suspended structures have been formed and then several tests were performed on the sensors to ensure their functionality. During the characterization step, the interferometry setup has been used to determine the resonance frequency of the sensors. In addition, focused air pressure was applied on top of the sensors, and the electrical output signals were monitored. Based on the obtained results, the resistance in piezoresistive sensors and the capacitance in the capacitive cantilevers vary each time the pressure was applied on top of the beams and caused the deflection.

The results from simulations and experiments confirm that these sensors may be good candidate for different biological applications including biomarker detections and cellular and molecular analysis. The sensors are now ready to be used in the next steps which involve mounting a microfluidic structure on top of the chip and further preparations for biological tests.

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