A NOVEL SELF-SENSING PIEZOELECTRIC MICROCANTILEVER-BASED SENSOR FOR DETECTION OF ULTRASMALL MASSES AND BIOLOGICAL SPECIES

A Dissertation Presented

by

Samira Faegh

to

The Department of Mechanical and Industrial Engineering

in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

in the field of

Mechanical Engineering

Northeastern University

Boston, Massachusetts

June 2013
ABSTRACT

Nanotechnological advancements have made great contributions in developing label-free and highly sensitive biosensors. Development of biosensing tools has contributed significantly to high-throughput diagnosis and analytical sensing exploiting high affinity of biomolecules. Detection of ultrasmall adsorbed masses has been enabled by such sensors which translate molecular interaction into detectable physical quantities. More specifically microcantilever (MC)-based biosensors have caught a widespread attention for offering label-free, highly sensitive, and inexpensive platform for detection. MC-based systems with different applications are equipped with external devices and instruments for actuation and read-out purposes which makes the entire platform expensive and bulky. Although there have been a number of measurement techniques, a compact detection platform with the capability of miniaturization, low power consumption, cost effective, and yet sensitive methodology is highly desirable.

This dissertation presents a unique self-sensing piezoelectric MC-based sensor for the purpose of detecting ultrasmall masses and biological species. The entire developmental process is covered and presented which includes: development of comprehensive mathematical modeling framework, numerical simulation, designing, building and testing the sensor. In the beginning chapters of this dissertation, the main focus is on analytical studies investigating modeling and simulation of piezoactive MC-based systems with diverse applications along with the relative experimental verification. Sophisticated comprehensive mathematical modeling frameworks capable of describing static and dynamic behavior of MCs are presented. A unique self-sensing strategy utilizing direct and inverse piezoelectric properties was developed which eliminates the need for any bulky and expensive external equipment. The ability of the self-sensing platform to measure ultrasmall masses was mathematically modeled and simulated, and then experimentally tested. Similar experimental setup was built using optical-based equipments for comparison and verification of the self-sensing platform. High level of accuracy was achieved both theoretically and experimentally implementing the self-sensing platform for detection of adsorbed biological species over MC surface. High mode vibrational studies were conducted for sensitivity enhancement of the system. A new model of measurement was developed to overcome the challenges of mechanical measurements in different environment (e.g. both gaseous and
aqueous). The developed platform was further utilized to detect physiological concentrations of glucose as low as 500 nM in liquid media. The developed platform can be implemented for detecting gases, chemical compounds and biological species with embedded miniaturized actuator and sensor being capable of functioning both in gaseous and aqueous media with the simplest and most inexpensive equipments.
ACKNOWLEDGEMENTS

I would like to take this opportunity to thank those who have supported me during this chapter of my life. First of all, my sincerest appreciation goes to my advisor, Prof. Nader Jalili, for his guidance and inspiration at every step of this study. His wide knowledge and logical way of thinking have been of great value to me. His understanding, encouraging and personal guidance both in academical and non-academical aspects of life were substantial keys to make this journey possible and rewarding and make me feel grateful and blessed to have worked with him.

I also would like to offer my special appreciation to my co-advisor, Prof. Srinivas Sridhar, for his continuous support and guidance throughout this study. His great passion for research, willingness to help and availability at any time has enlightened the path of my research.

I give my sincere gratitude to my committee member, Prof. Sinan Müftü, for all his guidance, feedback and support during the process of completing my dissertation.

The financial supports of National Science Foundation through the IGERT fellowship program, NSF-DGE-0965843 is greatly appreciated as well.

In addition, I would like to specially thank Dr. Ali Marzban for providing such useful guidance and insights on my research and most importantly for making me believe that nothing is impossible.

Furthermore, I would like to offer my appreciation to my friends, colleagues and lab mates, Dr. Arman Hajati, Dr. Ozgur yavuzcetin, Dr. Sohrab Eslami and Nima Sarli for assisting me patiently with my experiments and their honest suggestions and feedbacks.

Last but not least, I would like to dedicate this work to my family and thank them for their everlasting love and support.
# TABLE OF CONTENTS

ABSTRACT ................................................................................................................................. i

ACKNOWLEDGEMENTS ................................................................................................................. iii

TABLE OF CONTENTS .................................................................................................................. iv

LIST OF FIGURES .......................................................................................................................... vii

LIST OF TABLES .............................................................................................................................. xi

CHAPTER 1. MOTIVATION AND PROBLEM STATEMENT ......................................................... 1

1.1. Problem Statement .................................................................................................................. 1

1.2. Contributions .......................................................................................................................... 2

1.3. Dissertation Overview .......................................................................................................... 3

CHAPTER 2. INTRODUCTION AND PRELIMINARIES .................................................... 6

2.1. ImmunoAssay Techniques .................................................................................................... 7

2.1.1. Enzyme-Linked ImmunoSorbent Assay (ELISA) .............................................................. 8

2.1.2. RadioAllergoSorben Test (RAST) .................................................................................. 10

2.1.3. RadioImmunoAssay (RIA) .............................................................................................. 11

2.1.4. Immunofluorescence ....................................................................................................... 12

2.1.5. Enzyme-Linked Immunosorbent Spot (ELISPOT) ............................................................ 12

2.1.6. Disadvantages of immunoassay diagnosis ...................................................................... 12

2.2. Diagnosis Based on Nanomaterial Immunoassay .............................................................. 14

2.2.1. Nanoparticle-based immunosensors ................................................................................ 14

2.2.2. Bio-Barcode technology for protein detection ............................................................... 16

2.2.3. Nanowire array for protein detection ............................................................................. 17

2.2.4. Carbon nanotube-based electrochemical immunosensor ................................................. 19

2.3. Electrochemical Immunosensors .......................................................................................... 20

2.3.1. Quartz Crystal Microbalance (QCM) ............................................................................ 22

2.3.2. Diagnosis with MC-based biosensors ............................................................................ 23

2.4. Key Challenges and Unique Opportunities ......................................................................... 25

CHAPTER 3. COMPREHENSIVE MATHEMATICAL MODELING OF PIEZOACTIVE
MICROCANTILEVER-BASED SYSTEMS ...................................................................................... 31

3.1. Introduction ............................................................................................................................ 31

3.2. Mathematical Modeling ......................................................................................................... 34

3.3. Piezoresistive Modeling ......................................................................................................... 39

3.4. Piezoelectric Sample Modeling ............................................................................................. 40
LIST OF FIGURES

Figure 2.1. Effect of interference of autoantibody and anti-reagent antibodies in sandwich immunoassay [Hoofnagle and Wener, 2009], with permission .............................................. 13

Figure 2.2. Mechanism of bio-barcode assay A) design of the assay, B) Detection of PSA and identification of DNA [Chen et al. 2009], with permission ............................................. 16

Figure 2.3. a) Immunoassay consisting of array of nanowires, b) set of array of three nanowires functionalized with antibodies specific for PSA, CEA, and mucin-1 over silicon nanowires 1, 2, and 3 respectively, c) plot of conductance versus time as a result of detection of PSA, CEA, and mucin-1 [Zheng et al. 2005, Chen et al. 2009], with permission ...................... 18

Figure 2.4. Label free electrochemical immunosensor based on array of microelectrodes modified with SWCNs which are functionalized through immobilization of antibodies specific for disease antigens [Okuno et al. 2007], with permission ........................................ 19

Figure 2.5. Microelectrode array on a silicon chip for detection of multiple analytes [Chen et al. 2009], with permission .............................................................. 21

Figure 2.6. a) Schematic of a quartz crystal as the main part of QCM(R2), b) a commercially available QCM(R3), with permission .............................................. 22

Figure 2.7. Schematic of disease diagnosis through MC-based biosensor ........................................ 23

Figure 2.8. Array of MCs with functionalized surfaces through biomolecules for disease biomarkers. Microchannels are used to bring sample to respective MC. The intermolecular binding between the disease biomarker and the immobilized biomolecules over cantilever surface induces differential stress thus deflects MCs. The amount of MC deflection can be measured through any readout device ......................... 24

Figure 3.1. Schematic of piezoresistive MC sensor ........................................................................... 33

Figure 3.2. Schematic of the proposed distributed-parameters modeling of the piezoresistive MC sensor, (sys. 1) .................................................................................................................. 35

Figure 3.3. Schematic of the proposed distributed-parameters modeling of the piezoresistive MC-based PFM, (sys. 2) ................................................................................................................. 35

Figure 3.4. a) tip deflection of the cantilever, w(L,t) in sys.1 b) output voltage, V0(t) in sys.1 and c) contact force, f(t) in sys.1 all in non-dimensional form, d) tip deflection of the cantilever, w(L,t) in sys.2 e) output voltage, V0(t) in sys.2. and f) tip force, Ftip(t) in sys.2, (Faegh and Jalili, 2011) .................................................................................................................. 42

Figure 3.5. a) Error of area under contact tip force, f; versus length of piezoresistive layer in sys. 1, b) System’s amplitude versus local spring constant of piezoelectric sample in sys. 2. c) System’s amplitude versus location of piezoresistive layer in sys. 2 ......................................................................... 43

Figure 4.1. Veeco Active Probe® with the self-sensing layer attached at the probe .............................. 47
Figure 4.2. Schematic representation of Veeco Active Probe with ZnO stack on top extended from 0 to $L_1$ (Salehi-Khojin et al. 2009c), with permission. 49

Figure 4.3. Numerical results: (a) tip deflection of microcantilever, $w(L,t)$, (b) shift in the first natural frequency as a result of functionalization, (c) the effect of added surface mass due to functionalization on the first natural frequency, (d) the effect of added surface mass on vibration amplitude as a result of functionalization. 53

Figure 4.4. Veeco active probe with ZnO stack on top extended from 0 to $L_1$. 56

Figure 4.5. Eigenfunction for the first mode of the rectangular cantilever plate, $W_{11}$. 60

Figure 4.6. (a) time response of microcantilever, $q_{11}(t)$, (b) Deflection of microcantilever at the tip of the MC in the middle, $w(L_1\frac{W_{b1}}{2},t)$, (c) FFT of the response of the system representing system’s first natural frequency and the effect of added absorbed mass in the shift of natural frequency (Faegh et al. 2013a). 63

Figure 4.7. MC mounted on a holder placed over a 3D stage positioned under laser vibrometer head. 64

Figure 4.8. (a) Decibel versus frequency, FFT of the output signal showing first resonance frequency at 56.1 kHz, (b) Amplitude ratio versus frequency. 65

Figure 4.9. Shift of the first resonance frequency as a result of: (a) GoX functionalization, (b) immobilization of Amin solution and enzyme solution consequently. 66

Figure 4.10. Quantification of frequency shift as a result of adsorbed mass exploiting mathematical modeling framework. 67

Figure 5.1. Veeco Active Probe® with ZnO self-sensing layer deposited on the probe. 71

Figure 5.2. Micrograph/photograph of a Veeco Active Probe with a ZnO stack on top extended from 0 to $L_1$ (Salehi-Khojin et al. 2009c), with permission. 73

Figure 5.3. (a) Pure capacitive bridge, and (b) Resistive-Capacitive (R-C) bridge (Gurjar and Jalili, 2006). 73

Figure 5.4. Numerical results: (a) tip deflection of microMC, $w(L,t)$, (b) Input voltage, $V_i(t)$, output voltage, $V_o(t)$, and self-induced voltage, $V_s(t)$, (c) FFT response of the system with 1st natural frequency highlighted, (d) the effect of added surface mass due to functionalization on the first natural frequency (Faegh et al. 2013a). 78

Figure 5.5. Sensitivity of the vibration amplitude of the tip of MC with respect to $C_1$. 79

Figure 5.6. Schematic of the adaptive self-sensing strategy (Faegh et al. 2010, 2013). 80

Figure 5.7. (a) Tip deflection of MC, $w_L(x,t)$, (b) FFT response of the system with 1st natural frequency highlighted. 81

Figure 5.8. The effect of $\theta$ on the calculation of self-induced voltage, $V_s(t)$. 82

Figure 5.9. Veeco Active Probe mounted on a holder (a) connected to the pure capacitive bridge for self-sensing implementation, (b) placed under laser vibrometer head. 83
Figure 5.10. (a) FFT of the response of the system using self-sensing bridge, (b) Input, output and self-induced voltages, (c) FFT of the response of the system using laser vibrometer

Figure 5.11. Shift in the first resonance frequency measured by (a) self-sensing bridge, (b) Laser vibrometer

Figure 5.12. Quantification of frequency shift as a result of adsorbed mass exploiting mathematical modeling framework

Figure 6.1. (a) Veeco Active probe® used in this study for modeling and experiment, (b) schematic of the beam used for modeling

Figure 6.2. Normalized Mode Shapes (MS) (a) MS 1-5, and (b) MS 4-7

Figure 6.3. FFT of the response of the system, \( w(x,t) \) where \( n=20 \), depicting a) first 10 and b) next 10 resonance frequencies of the system

Figure 6.4. Frequency shift as a result of different amount of mass immobilization on (a) 10th mode, (b) 11th mode, (c) 12th mode, (d) 15th mode, with \( n=20 \)

Figure 6.5. Shift in resonance frequency calculated for different mode numbers as a result of different amount of mass immobilization

Figure 6.6. Veeco Active Probe mounted on a holder (a) connected to the pure capacitive bridge mounted on a bread board for self-sensing implementation, (b) placed under laser vibrometer head

Figure 6.7. Resonance frequencies measured by (a) self-sensing method running the system in its tenth mode, (b) laser vibrometer running the system in its third mode

Figure 6.8. Shift in the resonance frequencies in the a) first mode, b) second mode, and c) third mode of vibration measured by self-sensing platform

Figure 6.9. Shift in the resonance frequencies in the a) first mode, b) second mode, and c) third mode of vibration measured by laser vibrometer

Figure 6.10. Increase in frequency shift with the first three modes of vibration measured with self-sensing platform and laser vibrometer

Figure 7.1. Veeco Active Probe® with ZnO self-sensing layer deposited on the probe

Figure 7.2. a) Self-sensing circuit for actuating and sensing the system (b) MC mounted on a holder placed over a 3D stage positioned under laser vibrometer head

Figure 7.3. Circuit model to find equivalent impedance, \( Z_{eq} \)

Figure 7.4. Schematic of a model of MC molecular probe interface biosensor including three capacitors in series (Faegh et al. 2013b)

Figure 7.5. Effect of values of (a) \( C_1 \) and \( C_r \) and (b) \( L \) on circuit’s sensitivity in detecting shift in resonance frequency

Figure 7.6. First resonance frequency of MC and shift in the resonance frequency in air as a result of GoX functionalization measured with (a) self-sensing circuit, and (b) laser vibrometer
Figure 7.7. Quantification of amount of adsorbed mass with respect to shift of mechanical resonance frequency of system utilizing comprehensive distributed-parameters mathematical modeling framework, (Faegh and Jalili, 2013, Faegh et al. 2013a).

Figure 7.8. Shift in the resonance frequency of the self-sensing circuit consisting of MC as a result of GoX functionalization over sensor MC surface.

Figure 7.9. Resonance frequency of the circuit consisting of sensor MC and reference MC and the shift in resonance frequency in liquid as a result of injecting (a) 0 glucose, (b) 500 nM glucose, (c) 1 μM glucose, (d) 100 μM glucose, (e) 200 μM glucose (Faegh et al. 2013b).

Figure 7.10. Differential Shift in the resonance frequency of the circuit with sensor and reference MC (Δfref – Δfsensor) as a result of injecting different concentrations of glucose (Faegh et al. 2013b).

Figure 8.1. The proposed diagnostic kit involving one reference and more than one sensor probes equipped with a compact fluidic setup, injection valve, and syringe pump.
## LIST OF TABLES

| Table 2.1. | An illustrative comparison between various immunoassay techniques and cantilever-based diagnosis. | 26 |
| Table 2.2. | MC-based measurement techniques | 27 |
| Table 3.1. | Numerical values used in the simulation | 41 |
| Table 4.1. | The system parameters used for modeling | 52 |
| Table 4.2. | Comparing the results obtained from mathematical modeling presented in parts I and II to the experimental results | 65 |
| Table 5.1. | The system parameters used for modeling | 76 |
| Table 5.2. | Comparing the results obtained from mathematical modeling presented in Sections 2 and 3 with the experimental results | 85 |
| Table 6.1. | Calculated resonance frequencies using different order model (n) | 96 |
| Table 6.2. | Shift in the resonance frequency as a result of mass immobilization (1 ng-10 μg) for all modes 1st-20th | 99 |
| Table 6.3. | Resonance frequencies running the system in its tenth mode calculated theoretically and measured experimentally | 99 |
| Table 7.1. | Quantification of adsorbed mass with respect to circuit’s resonance frequency calibrated by mechanical response of the system | 120 |
| Table 7.2. | Comparison of detection limit of measuring glucose concentration | 120 |
CHAPTER 1

MOTIVATION AND PROBLEM STATEMENT

1.1. Problem Statement

Nanotechnological advancements have significantly contributed to the development of Nano- and Micro- Electromechanical Systems (NEMS and MEMS). Label-free and highly sensitive methodologies for detection of ultrasmall masses and biological species have been discovered for detection and diagnostic purposes utilizing micro and nano scale environmental, gas, and biological sensors. High-throughput diagnosis and analytical sensing require advanced biosensing tools exploiting high affinity of biomolecules. There are a number of useful biosensing techniques such as electrophoretic separation and spectrometric assays. Electrophoretic separation operates based on spatiotemporal separation of analytes whereas changes in the mass or optical properties of target proteins are utilized in spectrometric assays.

One of the most promising methodologies developed for detection is utilizing high affinity of molecules. Identification and quantification of target molecules has been made possible based on molecular recognition which is transferrable to detectable physical quantities (Fritz et al. 2000). Therefore, two main elements determining the success of sensors include: i) sensitive molecular probe interacting with target molecules where recognition occurs, and ii) transducer which transforms the molecular recognition into a detectable physical quantity.

There are a number of instruments developed for mass sensing purposes which are equipped with these elements including quartz crystal microbalance (QCM), surface plasmon resonance (SPR), enhanced-Raman spectroscopy, field effect transistors (FET) and MicroCantilever (MC)-based
systems. MC-based systems have become very popular due to offering a simple, inexpensive and highly sensitive sensing platform with possible miniaturization capabilities.

Although MC-based biosensors have received a widespread attention for label-free detection, there are not enough analytical studies investigating modeling and simulation of piezoactive MC-based system along with the relative experimental verification. Therefore, there is still a need for a more comprehensive mathematical modeling framework capable of describing static and dynamic behavior of MCs. Along this line of reasoning, a very comprehensive mathematical modeling framework for a variety of piezoactive MC-based systems with diverse application is presented here. Numerical simulations at high vibrational modes as well as fundamental modes are performed. Relative experimental setup for each section is built and verified with mathematical modeling. Finally, a unique self-sensing piezoelectric MC-based platform is developed, both theoretically and experimentally, and tested for detection of ultrasmall masses and biological species. The platform is further utilized as a gas sensor for detection of alcohol vapors with high sensitivity.

1.2. Contributions

The major contributions of this dissertation can be summarized as:

- Development of comprehensive mathematical modeling for piezoresistive MC-based systems specifically implemented for Piezoresponse Force Microscopy (PFM) and as a biological sensor operating in contact mode.
- Development of an extensive modeling framework for piezoelectric MC-based sensor using both thin plate theory and Euler-Bernoulli beam theory and conducting free and forced vibration analyses to verify and compare beam and plate theories.
- Development of an extensive experimental setup for verification of the theoretical modeling frameworks in previous steps.
- Development of a unique self-sensing piezoelectric MC-based sensor for detection of ultrasmall masses and biological species. This design and implementation processes include:
  1) Development of analytical modeling framework for the entire platform,
  2) Conducting numerical simulations,
  3) Adopting an adaptive strategy to compensate for variations of piezoelectric material embedded in the structure of the sensor,
  4) Conducting high-mode vibrational analysis for sensitivity enhancement both theoretically and experimentally,
  5) Designing and building the sensor and verifying the capability of the self-sensing strategy for measurement by comparison to optical-based techniques,
  6) Implementing the developed sensor for detection of different concentrations of glucose in a sample solution and measuring the sensitivity of the system.

1.3. Dissertation Overview

In order to have a precise MC-based system, a very comprehensive modeling needs to be developed and utilized. In almost all of the studies regarding MC-based systems, simple lumped-parameters modeling was used which is not capable of describing the dynamics within the cantilever and the consequent sensing characteristics.

Along with this line, Chapters 3 and 4 are devoted to develop a comprehensive mathematical model for piezoactive (including both piezoresistive and piezoelectric) MC-based
nanotechnological systems. Different systems are investigated and extensively modeled and simulated which are:

- Piezoresistive MC sensor implemented for measuring intermolecular force in contact mode,
- Piezoresistive MC sensor implemented for Piezoresponse Force Microscopy (PFM),
- Piezoelectric MC used for mass sensing and detection modeled as Euler-Bernoulli beam,
- Piezoelectric MC used for mass sensing and detection modeled as non-uniform rectangular plate.

In Chapter 5, a unique self-sensing detection technique for piezoelectric MC-based sensor is developed. It provides a laser-free, portable and cost-benefit sensing platform for detection of ultrasmall masses and biological species. Direct piezoelectric property is used to sense the self-induced voltage generated in the piezoelectric layer as a result of beam deformation. At the same time, inverse property of piezoelectric material is used to generate deformation and bring the system into vibration as a result of applying a harmonic voltage to it.

Comprehensive mathematical modeling is developed and simulated. An experimental setup is built and tested. Theoretical results are compared to experiment and the entire setup is verified with optical-based measurement techniques.

High mode resonating MC has been investigated and implemented as an effective solution for sensitivity enhancement. However, there have not been any analytical distributed-parameters modeling for systems operating in their high modes. As a result, in Chapter 6, a comprehensive mathematical modeling for a piezoelectric self-sensing MC-based sensor operating at ultrahigh mode (e.g. 20th mode) is presented. The effect of adsorbed mass on the frequency shift are
investigated. An experimental setup is built implementing the systems at its higher modes and tested for mass sensing capabilities at different modes. Optical method is tested for verification as well.

Once the capability of the self-sensing strategy was verified both at high mode as well as fundamental mode, the developed platform was implemented as a biological sensor. One important factor determining the success of all biological sensors performing based on analytical sensing of high affinity of biomolecules is the ability of the sensor to operate in liquid media with high sensitivity. We have addressed this challenge by operating the proposed self-sensing biosensor in dynamic mode in liquid media by exciting the system at high frequency. In Chapter 7, glucose detection implementing the self-sending MC-based sensor is presented. Rapid, continuous, and highly sensitive measurement of molecular recognition was measured. The use of self-sensing circuit’s resonance frequency instead of MC mechanical resonance frequency is extensively discussed. Circuit modeling is developed and experimental setup is built to detect different concentrations of Glucose in liquid sample solution.

The same study was performed using interdigitated electrodes (IDE) as the sensing element. Self-sensing circuit was applied implementing the IDE as a capacitive-based biosensor. Change of capacitance of the sensing element as a result of molecular binding was measured and compared with the MC-based sensing platform.

Finally, concluding remarks and future work are discussed in chapter 8.
CHAPTER 2

INTRODUCTION AND PRELIMINARIES

Identifying signatures of disease also known as biomarkers is the main factor in disease diagnosis. The expression level of these biomarkers is related to a specific disease which forms the basis of monitoring different diseases. Most of the traditional methods of diagnosis rely on animal models experiments and relating the results to similar cases in human’s benefits. However, the inherent differences between animal’s and human’s immune system triggers new efforts and methodologies for studying human’s immune system directly. In order to achieve this purpose, short time process of multiple sample and measurement of a great number of parameters is necessary with the aid of technological advancements.

Speaking generally, different diagnosis techniques include:

- **ImmunoAssay Techniques**
  - Enzyme Linked ImmunoSorbent Assay (ELISA)
  - RadioAllergoSorbent Test (RAST)
  - RadioImmunoAssay (RIA)
  - ImmunoFluorescence
  - Enzyme Linked Immunosorbent Spot (ELISPOT)

- **Nanomaterial-based ImmunoAssays**
  - Nanoparticles
  - Bio Bar code technology
- Nanowire-array-based detection
- Carbon Nanotubes

- **Label Free Electrochemical Immunosensors**
  - Quartz Crystal Microbalance (QCM)
  - MC-based biosensors

These techniques are briefly discussed next. The advantages and disadvantages of each technique are disclosed and conclusive statements are presented. This comparative study and brief review would help the reader to better realize the motivation behind this dissertation.

### 2.1. ImmunoAssay Techniques

One of the commonly used methodologies for measurement of concentration of materials such as analytes in biological samples is ImmunoAssay technique. It is capable of quantitatively measuring the presence of biomarkers in sample liquids such as serum or urine. Molecular interaction of antibodies with specific antigens of particular disease forms the basis of immunoassay detection. The success of this methodology highly relies on the degree of specificity of the receptor to the corresponding analytes and creating specific interaction which should dominate the unspecific binding that might occur as a result of presence of other substances in the sample.

The main requirement of a detection technique is that it should be equipped with sufficient tools to recognize the specific binding that takes place between specific analytes and corresponding receptors and transducing the obtained signal into some detectable physical property. Changes in refractive index and light scattering have been used a lot for this purpose. Some labels that have been used for this purpose include: enzymes, coenzymes, selenium colloidal particles,
fluorescent, phosphorescent, etc. Intermolecular interaction can be recognized as the label-produced signal changes.

In immunoassay techniques, usually a reference sample is utilized which contains no analyte. Comparing the response of the sample containing the minimum detectable level of concentration of analyte with the reference sample provides a good source of quantitative measurement of biomarker concentration in the sample solution.

In general, there are two main categories of immunoassay techniques:

a) Competitive Immunoassay: In this technique, a few number of antigens in the sample solution are labeled which produce the binding signals. The obtained signal is inversely proportional to the concentration of the analyte contained in the sample which competes with the labeled analytes. Therefore, higher number of analytes in the sample would create lower signal produced by the labeled analytes.

b) Noncompetitive Immunoassay (Sandwich Assay): In this method, some antibodies are labeled. The labeled antibodies make a bound with the antigens in the sample which themselves interact with antibody site. Therefore, the response produced by the labeled antibodies reflects the amount of antigens in the sample.

There are a number of immunoassay techniques that are used for detection of the concentration of analytes in a sample. These techniques are discussed next:

2.1.1. Enzyme-Linked ImmunoSorbent Assay (ELISA):

This immunoassay technique is utilized as a method of diagnosis for measuring the concentration of an antibody or an antigen contained in a sample solution. The experimental procedure of

a) Immobilizing an amount of antigen (unknown) over a substrate (specifically or non-specifically),

b) Adding the detection antibody to make specific binding with the immobilized antigen,

c) Linking the detection antibodies to an enzyme or conjugating the detection antibodies to secondary antibody and then linking the secondary antibody to an enzyme,

d) Adding an enzymatic substrate which physical quality’s changes with the concentration of the antigen in the sample solution.

An important step that should take place in order to prevent nonspecific binding of antibodies or other substances is to use detergents to wash plate.

Several materials have been used to produce signals due to presence of antigens in a sample in this technique which include chromogenic, fluorogenic, and electrochemiluminescent signal producers which work based on changing the color of substrate. A reference solution is prepared containing a standard concentration of analyte of a sample disease. Signal produced from test samples containing an unknown amount of analytes can be compared to the reference solution and evaluated accordingly which forms the basis of detection in this immunoassay technique.

There are two typical formats of ELISA which are quantitative and qualitative. In qualitative ELISA, comparison of produced signal from the test sample to the reference sample would reveal positive or negative evaluation with positive meaning stronger signal thus higher concentration of analyte and vice versa.

There are three main types of ELISA which include indirect, sandwich, and competitive ELISA. In indirect ELISA, the solution containing antigens is added to a microplate. A sample with
unknown concentration of primary antibodies is brought into contact with the microplate which results in creation of specific interaction between primary antibodies and immobilized antigens. Enzyme-linked secondary antibody is added thus binding occurs between primary and secondary antibodies. This interaction changes the color of the enzyme substrate indicating the reaction between antigen and antibody thus the concentration of primary antibody. Passivation of microplate with non-reacting proteins would decrease unspecific binding.

In sandwich ELISA, a known concentration of antibody is immobilized over a substrate. Sample solution containing unknown amount of antigen is then added which binds to the immobilized antibodies. Enzyme-linked antibodies are brought into contact with sample which further interacts with the antigens. Adding enzyme-substrate, concentration of antigen can be evaluated from the detectable signal observed in the substrate.

In Competitive ELISA, a sample containing antigen bounded to its specific antibody is prepared and brought to an antigen immobilized well. The unbounded antibodies in the solution would then bind to the immobilized antigen on the well. Therefore, higher concentration of antigen in the sample would result in lower binding of antibody with to antigen in well. Enzyme-linked secondary antibody is added and finally is linked to a substrate which change of its properties can be a measure of concentration of antigen in the sample solution.

2.1.2. RadioAllergoSorben Test (RAST)

This immunoassay technique is used to determine the specific response of IgE which is the antibody associated with Type I allergic response. It evaluates the allergy of a person to a known allergen through the concentration of produced IgE against that specific allergen. In this technique, the sample solution containing antibody associated with a know allergen is added to an insoluble material where the allergen are immobilized. As a result, specific binding occurs
between IgE antibodies and allergens. Secondary antibodies which are radio-labeled are added and bind to primary antibodies. The concentration of antibodies in serum can be detected from the radioactive signal produced form interaction of secondary and primary antibodies. Stronger radioactive signal means higher concentration of IgE antibodies in the serum bounded to allergen, thus higher allergy of the person to that particular allergen. This method is suggested over the simple skin-prick testing especially when there is a widespread allergy, and high sensitivity of the patient to a specific allergen. However, it is not as sensitive and specific as the skin-prick test (Ten et al. 1995).

2.1.3. RadioImmunoAssay (RIA)

Radioimmunoassay is a very sensitive method for detection of concentration of antigens in a sample utilizing radioactive substances with high accuracy. In this technique, a solution is prepared with a known amount of antibodies. Specific antigens for that antibody are radio-labeled usually with gamma-radioactive isotopes and are brought into contact with the solution where specific bindings occur between labeled antigens and antibody (Acebedo et al. 1975, Yalow and Berson, 1960). The competitive assay takes place when the patient sample solution containing unknown amount of antigens is added; therefore, unlabeled antigens in the sample solution and radio-labeled antigens try to bind with the antibodies. The higher concentration of antigens in sample solution means the higher interaction with antibodies and the higher concentration of the remained unbounded radio-labeled antigens. Therefore, the radioactivity of the unbounded labeled antigens would be a good source of concentration of unknown antigens in the patient’s sample fluid. Colorimetric signals utilized in ELISA are sometimes implemented in RIA instead of radioactive signal in order to reduce the required precautions of dealing with radioactive materials.
2.1.4. Immunofluorescence

Immunofluorescence is widely utilized for detecting the location of antibodies through use of fluorophores. This technique is used in light microscopy for visualization of individual cells and distribution of proteins and small biomolecules to name a few. There are two main types of immunofluorescence methods which are direct and indirect.

In direct immunofluorescence, one antibody labeled with fluorophore binds to its receptor which can be visualized through microscope. This technique reduces non-specific binding thus background signal. However, in the indirect immunofluorescence, one antibody, which is unlabeled, targets its receptor and a secondary antibody which is labeled with fluorophore binds to the first antibody and can be visualized.

There is a certain limitation in using this technique in vivo. Challenges with labeling biomolecules and problems resulting from photobleaching are the main drawbacks of this technique.

2.1.5. Enzyme-Linked Immunosorbent Spot (ELISPOT)

This immunoassay technique is mainly used for detection of immune responses. It enables monitoring antigen-specific immune system response. Type of immune antibody and number of cells producing this response can be monitored implementing ELISPOT.

The technique is very similar to sandwich type of ELISA. A modified version of ELISPOT which utilizes multiple fluorescent anticytokines for detection is named FluoroSpot (Czerkinsky et al. 1983).

2.1.6. Disadvantages of immunoassay diagnosis
Even though immunoassay techniques have been widely used for detection, there are certain disadvantages accompanied with them. One of the main drawbacks of this technique is the lack of consistency between different immunoassay platforms. The main step in diagnosis of a disease is to detect proteins secreted from damaged tissues at very low concentration, and the main approach in immunoassay techniques is selecting proper antigen for this approach. However, the results obtained from one assay may vary to the other. As a result and in order to detect a particular analyte, different antibody targets different epitope in assays. Examples may include different detection results obtained from immunoassay techniques for thyroid stimulating hormone, and tumor biomarkers for pancreatic (Rawlins and Roberts, 2004, La’ulu and Roberts, 2007).

Another important challenge associated with immunoassay techniques is the interference of autoantibodies. This interference leads to a false results obtained from immunoassay platform due to the fact that autoantibodies target antigens that are recognized by reagent antibodies and interacts with them (Spencer et al. 1998, Spencer and Lopresti, 2008).

Non-specific aggregation caused by anti-reagent antibodies is also an important factor that has to be considered using immunoassay technique. Anti-reagent antibodies are capable of attaching to

Figure 2.1 Effect of interference of autoantibody and anti-reagent antibodies in sandwich immunoassay [Hoofnagle and Wener, 2009], with permission.
capture antibodies and then can be targeted by reagent antibodies thus leading to false evaluation (Dale et al. 1994, Kricka, 1999, Levinson and Miller, 2002, Sapin et al. 2007). The interference of anti-reagent antibodies has been reported to affect biomarkers of particular diseases (Morgan and Tarter, 2001, Preissner et al. 2003, Rotmensch and Cole, 2000, Willman et al. 1999).

The aforementioned interferences are demonstrated in Figure 2.1 where a sandwich bound takes place on a magnetic bead coated with streptavidin. Biotin binds to streptavidin as a result of high affinity between these two molecules and it further captures antibody which binds to the analytes. Reagent antibodies which are enzyme-labeled target the analyte and can be detected by various methods. However, as shown in Figures 2.1 B and C, the presence of autoantibodies and non-specific binding of anti-reagent antibodies distorts the sandwich immunoassay.

There is another phenomenon in immunoassay techniques known as high dose hook effect. It happens in sandwich immunoassays where false evaluation is obtained as the concentration of analyte in the sample solution increases higher than a certain amount. Analytically, increase in the concentration of analyte increases the response of the immunoassay platform. However, theoretically, when concentration of analyte reaches a specific value, the response shows a reverse effect and decreases which is not accurate. Some studies have demonstrated the high dose hook effect in patient samples containing high concentration of analytes (Fleseriu et al. 2006, Furuya et al. 2001, McCudden et al. 2009).

2.2. Diagnosis Based on Nanomaterial Immunoassay:

Nanomaterial Immunoassay techniques have a potential alternative to conventional immunoassay detection techniques. Different nanomaterial research and developments are discussed as follows.

2.2.1. Nanoparticle-based immunosensors
Nanoparticles have received a widespread attention in disease diagnosis during past few years for their unique potential in offering a suitable bioanalysis platform. Their unique characteristics such as high surface-to-volume ratio and capability of biomolecule immobilization make them a proper alternative for conventional clinical immunoassay techniques. Quantum dots, gold and magnetic nanoparticles have been utilized for developing immunoassays for detecting tumor markers.

A label-free nanoparticle based immunoassay has been developed consisting of five electrodes including a reference electrode integrated on a glass substrate. Each electrode contains NiFe$_2$O$_4$/SiO$_2$ nanoparticles with a different antibody immobilized on its surface. The interaction between antibody and antigen in the sample solution changes the electrode potential which consequently produces a detectable signal. Four tumor markers including AFP, CEA, CA 125, and CA 15-3 have been detected simultaneously implementing this nanoparticle-based immunosensor (Tang et al. 2007a). Gold Nanoparticles have also been used for detection of CEA tumor marker. Gold nanoparticles modified with a glutathione monolayer were employed for immobilization of CEA antibodies and the whole bioconjugate was integrated on Au electrode. Formation of CEA antibody-antigen complexes could be detected by changes in the resistance of the electrode (Tang et al. 2007b). The immunosensor enables detection in the range of 0.5-20 ng/mL with the resolution of 0.1 ng/mL.

Gold nanoparticles being characterized with electrocatalytic property has been used for signal amplification in electrochemical detections. Gold-nanocatalyst labels were demonstrated to enhance produced signal in detection of Prostate Specific Antigen (PSA) (Das et al. 2006).
2.2.2. Bio-Barcode technology for protein detection

![Diagram of bio-barcode assay](image)

**Figure 2.2** Mechanism of bio-barcode assay A) design of the assay, B) Detection of PSA and identification of DNA [Chen et al. 2009], with permission.
The bio-barcode technology has been proposed for detection of PSA biomarkers utilizing combination of gold and magnetic nanoparticles (Nam et al. 2003).

The system consists of magnetic microparticle with iron oxide core coated with polyamine with the diameter of 1 μm. The magnetic microparticle is functionalized with antibodies specific for a target protein such as PSA. On the other hand, gold nanoparticles are functionalized with DNA unique for that target protein plus antibodies capable of creating a sandwich with the target protein captured by the magnetic microparticle. After formation of sandwich, a magnetic field is applied which results in separation of magnetic microparticle and consequently dehybridization of bar-code DNA. Identifying the DNA sequence allows the determination of the presence of the target protein. The mechanism of bio-barcode assay is demonstrated in Figure 2.2. This technique provides a highly sensitive method for detection of protein markers due to the fact that a great number of bar-code DNA can be loaded on nanoparticle surface for detection of each protein marker. It is also capable of detecting multiple protein markers simultaneously (Nam et al. 2007, Stoeva et al. 2006).

One of the main limitations of this technique is the challenge associated with design and preparation of microparticle probe and nanoparticle. Silica nanoparticles have also been used for development of electrochemical immunosensors due to their unique properties such as being biocompatible, stable, and functionalized with bioreagents. Detection of PSA was reported through silica nanoparticle-based immunosensor (Qu et al. 2008).

2.2.3. Nanowire array for protein detection

Immunoassay nanodevices based on nanowires are also promised to be a suitable tool for protein detection due to unique properties of nanowires such as high surface-to-volume ratio and electron transportation properties. It consists of arrays of 1D semiconductor or conducting
polymer nanowire array. The nanowire arrays can be functionalized with a great number of capturing biomolecules such as antibodies. Having a high surface-to-volume ratio, nanowires create assays of multiple disease markers by immobilizing antibodies specific to disease antigen thus offering a highly selective and simultaneous detection nanostructure. Molecular interaction between immobilized antibodies over nanowire surface and disease antigens imposes surface perturbations on nanowire array thus changes its electronic conductance due to novel electron transportation properties of nanowires. Zheng et al. (2005) performed a study implementing real-time, label free, multiplexed immunoassay based on arrays of nanowires for detection of four cancer markers as shown in Figure 2.3. The immunoassay device consists of plenty of silicon nitride metal electrodes connected to nanowires. Figure 2.3b demonstrates array of three silicon-nanowires functionalized with antibodies specific for PSA, CEA, and mucin-1 on nanowires 1, 2, and 3 respectively. Intermolecular binding induces conductance change which is depicted in Figure 2.3c as a function of time.

**Figure 2.3** a) Immunoassay consisting of array of nanowires, b) set of array of three nanowires functionalized with antibodies specific for PSA, CEA, and mucin-1 over silicon nanowires 1, 2, and 3 respectively, c) plot of conductance versus time as a result of detection of PSA, CEA, and mucin-1 [Zheng et al. 2005, Chen et al. 2009], with permission.
Electrochemical alkaline phosphatase nanowire-based assay was implemented to detect lung cancer biomarkers (interleukin-10 and osteopontin) (Ramgir et al. 2007) and metal oxide nanowire-based immunoassay was implemented for detection of tumor marker proteins (Li et al. 2005).

### 2.2.4. Carbon nanotube-based electrochemical immunosensor

Carbon nanotubes and their utilization in electrochemical immunosensors have caught widespread attention due to their unique electrical and mechanical properties. Single Walled Carbon Nanotubes (SWNT) having a high aspect ratio and electron transfer property promises a suitable tool for electrochemical measurement (Okuno et al. 2007). Biosensors consisting of arrays of microelectrodes modified with carbon nanotubes have been utilized for detecting marker proteins (Okuno et al. 2007, Yu et al. 2006, Briman et al. 2007).

A label-free electrochemical immunosensor based on carbon nanotubes was developed for detection of cancer biomarker T-PSA (Okuno et al. 2007) as shown in Figure 2.4. It consists of arrays of microelectrodes modifies with SWNTs. Having a high aspect ratio, SWNTs offer immobilization of a great number of anti-T-PSA over their surface. Interaction occurs between PSA and anti-PSA immobilized over SWNT surface. Peak current as a result

![Figure 2.4](image-url)
of antigen-antibody binding produces the signal which can be a source of measurement of concentration of PSA. Sensitivity of 0.25 ng/mL was reported using this device (Okuno et al. 2007).

2.3. Electrochemical Immunosensors

Due to the fact that most cancers have more than one marker proteins, simultaneous detection of multiple analytes plays a crucial requirement in developing a label free and cost effective immunoassay devices. Performance of immunoassays is highly dependent on selection of antibodies considering crucial properties such as sensitivity, specificity, cross-reactivity and costs. Therefore, it asks for the development of new immunoassay techniques with higher sensitivity and specificity.

Immunosensors with the capability of dynamic analysis of immunoreactions have been implemented for detection of tumor markers. There are a number of immunosensor devices which include electrochemical (potentiometric, capacitive, amperometric, and impedimetric), optical (fluorescence, luminescence, refractive index), microgravimetric, thermometric, and immunosensors supplementing other techniques such as flow injection analysis (Chou et al. 2004, Fu et al. 2006, Nakamura et al. 2001, Zhang et al. 2007a). Protein chips-based electrochemical immunosensors with the capability of transducing molecular recognition into detectable electrical signals has caught a widespread attention for offering advantages such as low detection limit, small analyte volume, and integration in protein chips (Shi et al. 2006).

There are two main types of electrochemical immunosensor including 1) labeling detection techniques such as in fluorescence and electrochemical methods, and 2) label-free detection techniques such as Quartz Crystal Microbalance (QCM), and cantilever-based detections. In electrochemical immuneosensors, biomolecules such as proteins, peptides, oligonucleotides, and
others are immobilized in arrays on the substrate with the capability of retaining activity and remaining stable. These immobilized biomolecules over substrate also known as probes are then brought into contact with serums or cellular extracts where molecular recognition occurs. Electrochemical sensors enable miniaturization and developing a lab-on-a-chip device. Short assay time and high sensitivity is possible and enhance the detection of immunological reactions (Wang et al. 2001, Yakovleva et al. 2002, Zheng et al. 2005). One important factor that should be considered utilizing electrochemical immunosensors is that the immobilized biomolecules on the substrate should have a very high specificity with the biomarkers. Otherwise, unspecific interactions and cross-over to non-specific biomolecules immobilized at other spots may produce false signals and distorts the results obtained from the biosensor.

In many cancer diagnoses, detection of only one marker associated with the cancer is not enough since most cancers have more than one biomarker. Therefore, developing an immunosensor with the capability of detecting multiple analytes simultaneously is necessary. One approach to this strategy is developing multiple arrays of immobilized immunological biomolecules. Miniaturized arrays of microelectrodes on a silicon chip for multichannel
electrochemical measurement has been developed and used for detection of multiple analytes simultaneously as shown in Figure 2.5 (Chen et al. 2009).

There are other methods that can be incorporated into protein chips such as mass sensitive methods including QCM and MC-based biosensors which offer suitable tools for label-free biodetection. These techniques are briefly discussed next.

2.3.1. Quartz Crystal Microbalance (QCM)

The microgravimetric QCM has been utilize for biosensing applications and its capability in detection of DNA hybridization has been demonstrated (Zhou et al. 2000). It is capable of measuring sub-nanogram levels of mass changes. QCM is made of a thin quartz disc sandwiched between a pair of electrodes as shown in Figure 2.6. By applying an AC voltage across its electrodes, the crystal oscillates as a result of piezoelectric properties of crystal.

The mass absorbed to the crystal surface changes the resonance frequency of the crystal surface which forms the basis of QCM operation. It can be used in both vacuum and liquid environments. Surfaces functionalized with recognition sites can be used for determining the molecular interaction in QCM.

Micrcocantilever resonance-based detection is somehow similar to QCM in the vibration-working mode with some fundamental differences. These differences include:

1) MC based sensors are much smaller than QCM with the capability of miniaturization of the entire platform. As a result, lower amount of target molecules is required to produce a detectable signal.

2) High throughput analysis for detection of multiple analytes is possible using arrays of MCs and functionalizing each MC with a different receptor, therefore allowing for making simultaneous measurement with high efficiency, which is not the case with QCM.
3) Integration of QCM is difficult as a result of complicated structures and electronics, however, MCs can be integrated and therefore creating a simpler platform for detection.

### 2.3.2. Diagnosis with MC-based biosensors


![Figure 2.6](image1.png)

**Figure 2.6** a) Schematic of a quartz crystal as the main part of QCM\(^{(R2)}\), b) a commercially available QCM\(^{(R3)}\), with permission.

![Figure 2.7](image2.png)

**Figure 2.7** Schematic of disease diagnosis through MC-based biosensor.

Detection of PSA which is the marker of early detection of prostate cancer has been enabled implementing piezoresistive self sensing MC-based biosensors (Wu et al. 2001, Wee et al. 2005). Polyclonal anti-PSA antibody was immobilized over MC surface as a ligand. Specific interaction between this ligand and unbounded PSA in the sample target solution deforms MC which

Figure 2.8 Array of MCs with functionalized surfaces through biomolecules for disease biomarkers. Microchannels are used to bring sample to respective MC. The intermolecular binding between the disease biomarker and the immobilized biomolecules over cantilever surface induces differential stress thus deflects MCs. The amount of MC deflection can be measured through any readout device.
consequently changes surface stress. The induced surface stress can be read out through different devices thus enabling measurement of diagnostic PSA concentration range.

Level of Glucose in blood has been detected utilizing MC biosensors coated with enzyme (Subramanian et al. 2002). MC surface was coated with gold and functionalized with enzyme glucose oxidase. Interaction between glucose and glucose oxidase induces surface stress and causes the MC to deflect which can be measured by read-out devices.

Funtionalizing MC with anti-creatkin kinase and anti-myoglobin antibodies, cardiac biomarker proteins such as creatine kinase and myoglobin were detected utilizing this technique (Arntz et al. 2003). Detection of human leukocyte antigen sequences which contains single nucleotide polymorphisms utilizing piezoresistive MC arrays has been suggested for evaluation of susceptibility to autoimmune diseases (Adami et al. 2010). Detection of DNA and protein on the same array was also reported using MC-based platforms (Huber and Aktaa, 2003).

2.4. Key Challenges and Unique Opportunities

Although there have been a number of well-established detection techniques and other detection methodologies under development, MC-based systems have emerged as an outstanding tool for offering a label-free, simple, inexpensive, and yet highly sensitive detection platform (Tzeng et al. 2009, 2011, Delnavaz et al. 2009, Bradley et al. 2009, Mahmoodi et al. 2008, Afshari and Jalili, 2007). It has a number of advantages over other detection techniques. Table 2.1 shows an illustrative comparison between various commonly used immunoassay techniques and MC-based detection.
MC-based biosensors operate in two main modes; i) static mode and ii) dynamic mode. In static mode, deflection of MC from a stable baseline is indeed a measure of detection (Gupta et al. 2004, Yang et al. 2003); however in dynamic mode, the system is brought into excitation at or near its resonance frequency. The shift in resonance frequency as a result of mass absorption can be quantitatively related to the amount of adsorbed mass and species (Blake et al. 2012, Chen et al. 1995; Daering and Thundat, 2005, Gurjar and Jalili, 2007, Faegh et al. 2013a).

All MC-based techniques are equipped with read-out methodologies including optical, capacitive, and piezactive (piezoelectric and piezoresistive). Table 2.2 provides a list of the measurement techniques that MC-based techniques are equipped with.

Table 2.1 An illustrative comparison between various immunoassay techniques and cantilever-based diagnosis.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Immuno-diffusion</th>
<th>Enzyme-linked Immunosorbent Assay (ELISA)</th>
<th>Radio ImmunoAssay (RIA)</th>
<th>Fluoroscent ImmunoAssay (FIA)</th>
<th>Cantilever-based Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (ml)</td>
<td>3-20 mg</td>
<td>0.1-1.0 ng</td>
<td>0.1-1.0 ng</td>
<td>1.0 ng</td>
<td>In the order of picogram</td>
</tr>
<tr>
<td>Cost</td>
<td>Costly</td>
<td>Costly</td>
<td>Highly costly</td>
<td>Highly costly</td>
<td>Economical</td>
</tr>
<tr>
<td>Safety</td>
<td>Safe</td>
<td>Safe</td>
<td>Hazardous</td>
<td>Safe</td>
<td>Safe</td>
</tr>
<tr>
<td>Small diagnostic platform</td>
<td>Possible</td>
<td>Possible</td>
<td>Not possible</td>
<td>Not Possible</td>
<td>Possible</td>
</tr>
<tr>
<td>No. of steps</td>
<td>More</td>
<td>More</td>
<td>More</td>
<td>More</td>
<td>Less</td>
</tr>
<tr>
<td>Assay duration</td>
<td>4-5 days</td>
<td>2 hours</td>
<td>&lt;1 hour</td>
<td>2 hours</td>
<td>&lt; 30 min</td>
</tr>
<tr>
<td>Sample required</td>
<td>In ml</td>
<td>In ml</td>
<td>In ml</td>
<td>In ml</td>
<td>In μl</td>
</tr>
<tr>
<td>Personnel required</td>
<td>Highly skilled</td>
<td>Highly skilled</td>
<td>Highly skilled</td>
<td>Highly skilled</td>
<td>Average</td>
</tr>
<tr>
<td>Multianalyte sensing in a single step</td>
<td>Not Possible</td>
<td>Not Possible</td>
<td>Not Possible</td>
<td>Not Possible</td>
<td>Possible</td>
</tr>
</tbody>
</table>
The most common measurement technique is optical-based which is extensively used in AFM. It operates based on shining a laser beam over the surface and measuring the shift in the angle of the laser beam reflected from the surface. Although being very sensitive, this method has a number of disadvantages such as being bulky, expensive and having surface preparation requirement. Moreover, laser alignment and adjustment, high power consumption and the restriction of conducting the experiment in a transparent chamber have always been certain downsides to this technique. Refraction of the laser beam as a result of traversing liquid makes it a limitation of usage in aqueous media. Miniaturizing the detection platform is one of the key elements in developing a micro and nano sensor. The need for having an external lighting setup for sample illumination and photodetector for capturing the reflected laser beam off the surface makes it impossible to miniaturize the whole optical-based sensing platform.

Implementing optical based sensing in dynamic mode, there is always a need for actuating the system. Using external actuation is the most common methodology. However external actuators are bulky and expensive. Using piezoelectric excitation by applying voltage to a piezoelectric

### Table 2.2 MC-based measurement techniques.

<table>
<thead>
<tr>
<th>Measurement Technique</th>
<th>Downsides</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Optical</strong></td>
<td>Shift in laser beam reflected on the photodetector surface</td>
</tr>
<tr>
<td><strong>Capacitive</strong></td>
<td>Change of the capacitance of a plane capacitor</td>
</tr>
<tr>
<td><strong>Piezoelectric</strong></td>
<td>Change of voltage of piezoelectric layer over cantilever surface</td>
</tr>
<tr>
<td><strong>Piezoresistive</strong></td>
<td>Change of resistivity of piezoresistive layer over cantilever surface</td>
</tr>
</tbody>
</table>

Alternative methods are capacitive-based measurement where change of the capacitance of a plane capacitor is the base of measurement. However, it is not suitable for large displacements and measurement in electrolyte solutions. Piezoresistive read out methods have extensively been used which address some of the limitations of optical-based systems. It measures the change of resistivity of the piezoresistive layer embedded in the structure of the MC as a result of MC deflection. As a result, this allows for miniaturizing the system and saving the overall cost of the platform. However, it comes with complicated electronic circuit and the power consumption is still high. Moreover, it results in self-heating and drifting. Since the piezoresistive layer is employed for only reading out system’s response, there is still a need for actuating the system in dynamic mode. It is either provided by using an external actuator which is bulky and expensive or through depositing an extra piezoelectric layer and applying voltage to it.

Piezoresistive MC-based sensors with piezoelectric-based actuator have been built and used for imaging and sensing purposes. Piezoelectrically-driven MC with piezoresistive read-out was used in scanning probe microscopy operating in constant force mode. Piezoelectric patch on the MC provides excitation and also controls the distance between tip and sample. This concept is disclosed in “Cantilever for Scanning Probe Microscope including Piezoelectric Element and Method of Using the Same”, Minne et al. U.S. Patent No. 5742377 issued Apr. 21, 1998. and “Atomice Force Microscope for High Speed Imaging Including Integral Actuator and Sensor”, Minne et al., U.S. Patent No. 5883705 issued Mar. 16, 1999.

Another measurement technique is piezoelectric-based systems where a piezoelectric material is used in order to create voltage as a result of induced surface stress due to mechanical deformation of the beam. This technique provides a simple sensitive read-out mechanism.

Utilizing a single piezoelectric layer for both sensing and actuating purposes was introduced in MC sensing technology for the purpose of mass detection which was disclosed in “Apparatus and Method for Measuring Micro Mass Using Oscillation Circuit”, Lee et al. U.S. Patent No. 7,331,231 issued Feb. 19, 2008. and also for detection purposes as disclosed in “Self-Sensing Array of MicroCantilevers for Chemical Detection”, Adams, U.S. Patent No. 2006/0257286 issued Nov. 16, 2006. Even though, sensitive measurement can be perfomed using MC in air, detection of analytes in liquid media utilizing the shift of the fundamental resonance frequency of MC does not provide a suitable detection tool due to heavy hydrodynamic damping effects. Moreover, there is still need for bulky monitoring devices such as network analyzer.

Although there have been a number of measurement techniques, a compact detection platform with the capability of miniaturization, low power consumption, cost effective, and yet sensitive methodology is highly desirable. MCs with the purpose of detecting gasses, chemical compounds
and biological species with embedded miniaturized actuator and sensor being capable of addressing all deficiencies of the measurement techniques that was discussed is therefore desired. The measurement capability of the platform both in air and aqueous media with the simplest and most inexpensive actuation and sensing equipment is still required. This dissertation is focused on developing a MC-based sensor for the purpose of detecting ultrasmall masses (e.g., chemical compounds, biological species, gasses, etc). Two main studies are carried out in order to achieve this purpose which are: a) developing extensive mathematical modeling and simulation for MC-based systems and specifically MC-based sensing platform, and b) conducting relative experiments to verify the developed theory and to design, build, and test the sensing platform.
CHAPTER 3

COMPREHENSIVE MATHEMATICAL MODELING OF PIEZOEACTIVE MICROCANTILEVER-BASED SYSTEMS

3.1. Introduction


* The contents of this chapter may have come directly from our previous publication (Faegh and Jalili, 2011).
Two main applications of MC-based nanotechnology can be listed as:

**a) Piezoresponse Force Microscopy (PFM)** which is a powerful device for nanoscale imaging, spectroscopy, and characterization of local properties of piezoelectric and ferroelectric materials (Su et al. 2003, Felten et al. 2004, Guthner and Dransfeld, 1992, Gruverman et al. 1997, Salehi-Khojin et al. 2009a,b). High resolution imaging in nanometer level as a result of piezoelectric coupling in biomaterials has been enabled using PFM. PFM functions based on detecting bias-induced surface deflection and is complementary to Atomic Force Microscopy (AFM)-based imaging. An oscillatory electrical field applies between a MC conducting tip and the electrode attached to the piezoelectric sample. The applied voltage results in deformation of the piezoelectric sample which consequently oscillates MC. The amplitude of MC oscillation gives a good insight into the surface characteristics (Hidaka et al. 1996, Kalinin and Bonnel, 2002, Kalinin et al. 2004, Bashash et al. 2009).

**b) Biological Sensors** also known as biosensors for monitoring diseases by detecting the marker proteins relative to that specific disease. Measuring molecular binding force and detecting concentration of an antigen in a sample fluid has been enabled using arrays of MCs. There are a number of available read-out techniques in MC-based systems including piezoelectric, piezoresistive, capacitive, and optical laser-based systems. Piezoelectric-based MC sensors operate based on change of voltage in piezoelectric patch due to beam deflection. Two patches of piezoelectric material deposited over the surface of the MC makes it difficult to miniaturize the structure of the sensor. Moreover, complicated electronic circuit is required to process the signal. Capacitive-based MC sensors monitor capacitance change as a result of beam
deflection. There are some limitations accompanied with this type of sensor which include low resolution, complicated electronic circuits and fabrication processes. Optical force measurement which is a very powerful device in measuring small deflections is widely utilized in AFM. The inherent disadvantages of this technology are high cost, surface preparation, and optical alignment and adjustment requirement.

Piezoresistive force sensors work based on change of resistance in the piezoresistive layer when MC bends as a result of external tip force. The change of resistance can be measured utilizing the output voltage of the system. Piezoresistive MCs offer a great advantage over other types of MC sensors, especially the optical measurements where sample preparation and laser alignment and adjustment are serious limitations. The schematic of a piezoresistive sensor is shown in Figure 3.1. They have found their application in atomic data storage systems, AFM cantilevers, portable cantilever-based sensors, pressure sensors, and accelerometers (Hong et al. 2001). MC deflection and surface stress measurement has been enabled utilizing piezoresistive layer over MC surface (Harley and Kenny, 1999, Boisen et al. 2000).

In order to have a precise MC-based system, a very comprehensive modeling needs to be developed. In most of the studies regarding piezoresistive MC-based system, simple lumped-parameters modeling was used which is not capable of precisely describing the dynamics within the MC (Harley and Kenny, 1999, Boisen et al. 2000, Thaysen et al. 2001). This study is aimed at developing a comprehensive mathematical model for MC-based nanotechnological systems.
with specific implementations as described in a) and b) above. Therefore, two main sections are included in this study investigating:

I) **An extensive distributed-parameters modeling of MCs operating in contact mode (System 1):** Utilizing such a precise model, the output voltage of the piezoresistive layer can be obtained as a function of the slope of the beginning and end points of the piezoresistive patch over the MC surface. Moreover, the interaction forces between the MC tip and sample can be measured having the deflection of the MC. Therefore, it provides an inexpensive and portable read-out system.

II) **A distributed-parameters mathematical modeling of MC-based PFM implementing on piezoelectric sample which performs tip-excitation (System 2):** A mathematical model is proposed relating the response of the piezoelectric sample to the response of the MC and consequently the output voltage of the system which is the main source of the read-out equipment.

Having such precise mathematical modeling of piezoresistive MC-based sensors and force microscopy, any phenomenon occurring both at the MC tip and within the MC can be described which gives a thorough insight into the behavior of the system. Implementation of piezoresistive read-out technique provides information of the system eliminating the need for bulky expensive laser-based feedback and read-out equipment.

### 3.2. Mathematical Modeling

An analytical model is reported which describes the behavior of the piezoresistive MC. The piezoresistive MC is assumed to be an Euler-Bernoulli beam which is modeled as a distributed-parameters system. Figures 3.2 and 3.3 show the schematic of piezoresistive MC sensor (sys. 1)
and PFM (sys. 2), respectively. The MC beam is attached to a base with mass $m_b$ at one end which moves vertically. $S(t)$ represents the base motion. An unknown tip mass $m_e$ is attached at the other end of the MC. The beam is considered to have length $L$, thickness $t_b$, and volumetric density $\rho_b$. The piezoresistive layer over the top surface of the MC has a length of $L_2 - L_1$, thickness $t_p$, and volumetric density $\rho_p$. Both MC and piezoresistive layer are considered to have width $b$. $w(x,t)$ denotes the midplane deflection of MC with the equivalent tip deflection $w(L,t)$. MC deflection is assumed to be small and the system properties are taken linear in developing the equations of motion.

Kinetic and potential energies of sys. 1 can be written as:

$$KE = \frac{1}{2} m_b \dot{S}^2(t) + \frac{1}{2} m_e (\dot{S}(t) + \dot{w}(L,t))^2 + \frac{1}{2} \int_0^L \rho(x)(\ddot{S}(t) + \ddot{w}(x,t))^2 \, dx$$  \hfill (3.1)

$$PE = \frac{1}{2} \int_0^L EL(x) \left[w''(x,t)\right]^2 \, dx$$  \hfill (3.2)

whereas for sys. 2 are:

$$KE = \frac{1}{2} m_b \dot{S}^2(t) + \frac{1}{2} m_e (\dot{S}(t) + \dot{w}(L,t))^2 + \frac{1}{2} \int_0^L \rho(x)(\ddot{S}(t) + \ddot{w}(L,t))^2 \, dx$$  \hfill (3.3)
where $\rho(x)$ and $EI(x)$ are defined as

$$\rho(x) = \rho = \rho_b b t_b + \rho_p b t_p G(x)$$

(3.5)

$$EI(x) = \frac{1}{12} E_b t_b^3 b + E_p t_p b \left( \frac{t_b^2}{3} + \frac{t_b t_p}{2} + \frac{t_p^2}{4} \right) G(x)$$

(3.6)

with $G(x) = H(x-L_1) - H(x-L_2)$, and $H(x)$ being the Heaviside function. $E_b$ and $E_p$ represent the Young’s modulus of elasticity of beam and piezoresistive layer, respectively.

Virtual work for sys. 1 is given by

$$\delta W = \int_0^L (-B \dot{w}(x,t) - C \dot{w}'(x,t)) \delta w(x,t) dx + f_b(t) \delta S(t) + f_c(t) \delta w(L,t)$$

(3.7)

and for sys. 2 is:

$$\delta W = \int_0^L (-B \ddot{w}(x,t) - C \dot{w}'(x,t)) \ddot{w}(x,t) dx + f_b(t) \delta S(t) + f_c(t) \delta w(L,t)$$

$$-C_z \dot{w}'(L,t) \delta w(L,t)$$

(3.8)

which is a result of damping, base force, and contact tip force. $B$ and $C$ represent the coefficients of viscous and structural damping respectively (Duc et al. 2007, Dadfarnia et al. 2004). $K_z$ and $C_z$ denote spring constant and damping coefficient of the piezoelectric material, respectively.

Utilizing Extended Hamiltonian principle, equations of motion of the system are obtained as

$$\rho(x) \left( \dddot{w}(x,t) + \ddot{S}(t) \right) + EI(x) \dddot{w}(x,t) + B \ddot{w}(x,t) + C \dot{w}'(x,t) = 0$$

(3.9a)

$$\left( m_b + m_e + \rho(x)L \right) \ddot{S}(t) + \int_0^L \rho \ddot{w}(x,t) dx + m_e \ddot{w}(L,t) = f_b(t) + f_c(t)$$

(3.9b)

with the following boundary conditions

$$w(0,t) = \dot{w}(0,t) = \dddot{w}(L,t) = 0$$

(3.10a)

$$m_e \left( \dddot{w}(L,t) + \ddot{S}(t) \right) - E_b L_b \dddot{w}(L,t) = f_c(t)$$

(3.10b)
\[ m_e \left( \ddot{w}(L,t) + \ddot{S}(t) \right) - E_b I_b w'''(L,t) + K_z w(L,t) + C_z \dot{w}(L,t) = f_c(t) \]  

(3.10c)

Equations (3.10a) and (3.10b) apply to sys. 1 and Eqs. (3.10a) and (3.10c) to sys. 2. In order to solve the equations of motion of the system, the partial differential equations (PDEs) given by (3.9a,b) should be converted into ordinary differential equations (ODE).

For this reason, the obtained boundary conditions need to be homogenized utilizing the following change of variables so that the term \( f_c(t) \) is omitted in the boundary condition using standard discretization techniques (Jalili, 2010):

\[ w(x,t) = z(x,t) + f_c(t) g(x) \]  

(3.11)

with \( g(x) \) defined as (Dadfarnia et al. 2004)

\[ g(x) = \frac{-1}{9EI(x)L} x^4 + \frac{5}{18EI} x^3 - \frac{L}{6EI} x^2 \]  

(3.12)

Implementing the suggested change of variables, equations of motions can now be rewritten as

\[ \rho(x) \left( \ddot{z}(x,t) + \ddot{S}(t) \right) + EI(x)z'''(x,t) + B \dot{z}(x,t) + C \dot{z}'(x,t) = -(\rho g(x) \ddot{f}_c(t) + \) \[ Bg(x) \ddot{f}_c(t) + Cg'(x) \ddot{f}_c(t) + EI(x)g'''(x) f_c(t)) \]  

(3.13a)

\[ (m_b + m_e + \rho L) \ddot{S}(t) + \int_0^L \rho \ddot{z}(x,t) \, dx + m_e \ddot{z}(L,t) = f_b(t) + f_c(t) - \ddot{f}_c(t) \int_0^L \rho g(x) \, dx \]  

(3.13b)

with the homogenized boundary conditions

\[ z(0,t) = z'(0,t) = z''(L,t) = 0 \]  

(3.14a)

\[ m_e \left( \ddot{z}(L,t) + \ddot{S}(t) \right) - E_b I_b z'''(L,t) = 0 \]  

(3.14b)

\[ m_e \left( \ddot{z}(L,t) + \ddot{S}(t) \right) - E_b I_b z'''(L,t) + K_z z(L,t) + C_z \ddot{z}(L,t) = 0 \]  

(3.14c)

The new set of governing equations for MC can be solved numerically using Galerkin’s method by discretizing \( z(x,t) \) as follows:

\[ z(x,t) = \sum_{j=1}^{n} \phi_j(x) q_j(t), \quad j = 1,2, \ldots, n \]  

(3.15)
where \( \phi_j(x) \) and \( q_j(t) \) represent the clamped-free beam eigenfunction and generalized coordinates respectively. \( f_c(t) \), which appears in the equations of motion, represents the contact force between the tip of MC and the sample where in sys.1, it can be found from the following equation (Jalili et al. 2004)

\[
f_c(t) = \frac{4E^*\sqrt{R}}{3} (S(t) + w(L, t))^{3/2}
\]

(3.16)

where \( R \) denotes the radius of MC tip, and \( E^* \) denotes the reduced elastic modulus obtained from

\[
\frac{1}{E^*} = \frac{(1-v_s^2)}{E_s} + \frac{(1-v_T^2)}{E_T}
\]

(3.17)

with \( E_s \) and \( E_T \) being the elastic modules of the sample and MC tip respectively, and \( v_s \) and \( v_T \), the poison’s ratio of the sample and MC tip respectively. Implementing the change of variable suggested in equation (3.11), \( f_c(t) \) can be written as

\[
f_c(t) = \lambda(S(t) + z(L, t))^{3/2} \quad , \quad \lambda = \frac{4E^*\sqrt{R}}{3}
\]

(3.18)

with \( \dot{f}(t) \) and \( \ddot{f}(t) \) being the first and second derivative of \( f(t) \) as follows

\[
\dot{f}_c(t) = \frac{3}{2} \lambda (S(t) + \dot{z}(L, t))(S(t) + z(L, t))^{1/2}
\]

(3.19)

\[
\ddot{f}_c(t) = \frac{3}{2} \lambda \left[ \left( \dot{S}(t) + \ddot{z}(L, t) \right)(S(t) + z(L, t))^{1/2} + \frac{1}{2}\left( \dot{S}(t) + \ddot{z}(L, t) \right)^2 (S(t) + z(L, t))^{-1/2} \right]
\]

(3.20)

Substituting equations (3.18), (3.19), and (3.20) into the governing equations (3.13a,b), the nonlinear differential equations of the MC for sys.1 can be obtained as follows

\[
\rho(x) \left[ \ddot{S}(t) + \sum_{l=1}^{n} \phi_l(x) \ddot{q}_l(t) \right] +
\]

\[
\frac{3}{2} \lambda \rho g(x) \left[ \left( \dot{S}(t) + \sum_{l=1}^{n} \phi_l(L) \dot{q}_l(t) \right)(S(t) + \sum_{l=1}^{n} \phi_l(L) q_l(t))^{1/2} + \right.
\]

\[
\frac{1}{2}\left( \dot{S}(t) + \sum_{l=1}^{n} \phi_l(L) \dot{q}_l(t) \right)^2 (S(t) + \sum_{l=1}^{n} \phi_l(L) q_l(t))^{-1/2} \right] + B \sum_{l=1}^{n} \phi_l(L) \dot{q}_l(t) +
\]

\[\frac{1}{2}\left( \dot{S}(t) + \sum_{l=1}^{n} \phi_l(L) \dot{q}_l(t) \right)^2 (S(t) + \sum_{l=1}^{n} \phi_l(L) q_l(t))^{-1/2} \right] + B \sum_{l=1}^{n} \phi_l(L) \dot{q}_l(t) +
\]
The obtained equations were solved in MATLAB. As a result, deflection of MC, \(w(x,t)\) and base motion \(S(t)\) were obtained in both sys.1 and 2, from which the tip deflection can be calculated.

In order to observe the deflection \(w(x,t)\) in the piezoresistive MC system, the output voltage should be represented in terms of \(w(x,t)\). Therefore, a piezoresistive modeling framework is presented in the following section.

### 3.3. Piezoresistive Modeling

When MC tip is brought into contact with the sample, MC deflects as a result of contact force. MC deflection consequently results in the change of resistance of piezoresistive layer deposited over MC surface. Change of resistance of piezoresistive layer can be obtained from the following equation (Saeidpourazar and Jalili, 2009)

\[
\Delta R = \left( \frac{\partial w(L_2,t)}{\partial x} - \frac{\partial w(L_1,t)}{\partial x} \right) \times C_{pz}
\]

\[
C_{pz} = \left( -z \frac{\partial R}{\partial l_p} - \frac{zv\omega_p}{L_2-L_1} \frac{\partial R}{\partial b} - \frac{\rho E z (\pi x - v \pi y)}{(1-v^2)(L_2-L_1)} \frac{\partial R}{\partial r_p} \right)
\]

(3.22)
where $z$ is the distance between the geometrical surface of the piezoresistive layer and the neutral axis of the MC. $L_p (= L_2 - L_1)$ and $b$ denote the length and width of piezoresistive layer respectively. $r_p$ represents the resistivity of piezoresistive layer with $\pi_x$ and $\pi_y$ being the longitudinal and transverse piezoresistance coefficients. $C_{pz}$ was evaluated experimentally to be equal to $4.99571 \times 10^4$ (Saeidpourazar and Jalili, 2009).

Implementing the change of variables proposed in Eq. (3.11) results in

$$
\left( \frac{\partial w(L_2,t)}{\partial x} - \frac{\partial w(L_1,t)}{\partial x} \right) = \left( \frac{\partial z(L_2,t)}{\partial x} - \frac{\partial z(L_1,t)}{\partial x} \right) + f(t) \left( \frac{\partial g(L_2)}{\partial x} - \frac{\partial g(L_1)}{\partial x} \right)
$$

(3.23)

Having the change of resistivity in the piezoresistive layer and $R$, which is the resistance of the piezoresistive layer in a Wheatstone bridge, the output voltage of the system can be obtained by (Harley and Kenny, 1999)

$$
V_0 = \frac{1}{4} V_b \frac{\Delta R}{R}
$$

(3.24)

where $V_0$ and $V_b$ are the output voltage and supply voltage of the Wheatstone bridge, respectively. The deflection of the MC at any time can be obtained through the developed equations. As a result, the output voltage can be calculated.

The proposed approach in modeling the piezoresistive MC as a distributed-parameters system offers many advantages over lumped-parameters modeling such as describing the dynamics of the system at any location of the MC. The slope of the MC at the beginning and end point of the piezoresistive patch which is crucial in obtaining the output voltage of the piezoresistive layer can be found through distributed-parameters modeling. Whereas, the lumped-parameter modeling is capable of describing only the MC tip movements. Numerical simulations are performed to solve the equations of motion of the system and to demonstrate the capability of the proposed approach.

3.4. Piezoelectric Sample Modeling
The piezoelectric sample is characterized with piezoelectric and viscoelastic behavior in all directions. An electrode is attached to the rear side of the sample. An external electric field is applied between the sample and MC tip which causes the sample to undergo both piezoelectric and piezoviscoelastic deformations. The piezoelectric response of the sample can be modeled as an electromechanical force applied at the MC tip which is proportional to the applied voltage and material’s piezoelectric coefficient, i.e., \( f_c(t) = \gamma V(t) \).

The value of sample’s piezoelectric coefficient, \( \gamma \) is considered to be 2.54 nN/V in this study. The viscoelastic response of the sample can be modeled as a parallel spring and damper (Kelvin-Voigt viscoelastic model), (Dadfarnia et al. 2004, Salehi-Khojin et al. 2009a) as shown in Figure 3.3. Therefore, the total forces applied at the MC tip would be the combination of spring, damping, and electromechanical forces obtained as follows

\[
F_{\text{tip}} = -K_z \ddot{w}(L,t) - C_z \dot{w}(L,t) + f_c(t)
\]

### 3.5. Numerical Simulations

In order to demonstrate the effectiveness and accuracy of the proposed model, a set of numerical simulations is implemented. The equations of motion obtained were solved numerically in MATLAB. In sys.1, a sinusoidal base force of amplitude of \( 1 \times 10^{-3} \) N and frequency of \( 1.25 \times 10^3 \) Hz was applied at the base of the MC. However, in sys.2 a sinusoidal bias voltage of the amplitude of 10 V and frequency of 200 Hz was applied between the conductive MC tip and the surface. This

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>( L )</td>
<td>( 500 \times 10^{-6} )</td>
<td>m</td>
</tr>
<tr>
<td>( L_p )</td>
<td>( 375 \times 10^{-6} )</td>
<td>m</td>
</tr>
<tr>
<td>( \rho_b )</td>
<td>2330</td>
<td>kg m(^{-3})</td>
</tr>
<tr>
<td>( \rho_p )</td>
<td>7660</td>
<td>kg m(^{-3})</td>
</tr>
<tr>
<td>( t_b )</td>
<td>( 4 \times 10^{-6} )</td>
<td>m</td>
</tr>
<tr>
<td>( t_p )</td>
<td>( 4 \times 10^{-6} )</td>
<td>m</td>
</tr>
<tr>
<td>( E_b )</td>
<td>( 150 \times 10^9 )</td>
<td>Pa</td>
</tr>
<tr>
<td>( E_p )</td>
<td>( 160 \times 10^9 )</td>
<td>Pa</td>
</tr>
<tr>
<td>( m_b )</td>
<td>( 5 \times 10^{-6} )</td>
<td>kg</td>
</tr>
<tr>
<td>( m_e )</td>
<td>( 0.5 \times 10^{-6} )</td>
<td>kg</td>
</tr>
<tr>
<td>( V_b )</td>
<td>2.5</td>
<td>V</td>
</tr>
<tr>
<td>( R )</td>
<td>675</td>
<td>( \Omega )</td>
</tr>
<tr>
<td>( E_s )</td>
<td>1000</td>
<td>Pa</td>
</tr>
<tr>
<td>( E_T )</td>
<td>( 150 \times 10^9 )</td>
<td>Pa</td>
</tr>
<tr>
<td>( v_s )</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>( v_T )</td>
<td>0.3</td>
<td></td>
</tr>
</tbody>
</table>
introduces a new method of excitation (tip excitation), different from base excitation or excitation through piezoelectric layers deposited over MC surface. It can find its application in the mass sensing devices which eliminates the need for other commonly used methods of excitation.

The value of \( C_{pz} \) was found to be \( 4.99571 \times 10^4 \) from the experience (Johnson et al. 1985). Solving the equations of motion numerically, deflection of MC at any point in different times, \( w(x,t) \) and the movement of the base, \( S(t) \), are obtained from the contact force between the MC tip and sample. Consequently, the output voltage of the piezoresistive layer, \( V_0(t) \), can be calculated through Eq. (3.24) developed in piezoresistive modeling section 3.2. Numerical values of the system’s parameters utilized in simulation are listed in Table 3.1.

\[
C_{pz} = 4.99571 \times 10^4
\]
Simulation was performed using two modes. Temporal non-dimensionalization was implemented in sys.1 in order to save computational time and effort. Figure 3.4 a,b, and c show the tip deflection of the MC, \( w(L,t) \), contact force, \( f_c(t) \), and output voltage, \( V_0(t) \), in non-dimensional form respectively in sys.1. Figure 3.4 d, e, and f, on the other hand, show the tip deflection of the MC, \( w(L,t) \), tip force, \( F_{\text{tip}}(t) \), and output voltage, \( V_0(t) \), respectively.

It is observed from the results that utilizing piezoresistive MC, the output voltage of the system reveals the information of the MC deflection which can further be utilized in obtaining the contact force between the MC tip and the sample. Using larger number of modes in the distributed-parameters modeling would result in more precise results.

### 3.6. Sensitivity Analysis

In order to study the sensitivity of these systems, two cases were investigated. In sys. 1, the error of area under contact tip force was calculated versus the length of the piezoresistive layer, \( L_p \), over MC. A very nice trend was observed in the error of contact tip force versus \( L_p \). As depicted in Figure 3.5a, the error decreases with increasing \( L_p \). On the other hand, changes in system’s amplitude were monitored in sys. 2 while changing the location of the piezoresistive layer over MC surface.
The length of the piezoresistive layer was kept constant at 0.3 times the total piezoresistive length, $L_p$. Figure 3.5b demonstrates the effect of local spring constant of piezoelectric sample on the vibration amplitude. The value of $K_z$ was selected based on the proposed system identification method for evaluating the proper range of system parameters (Salehi-Khojin et al. 2009a). It shows that the amplitude of vibration increases almost linearly with spring constant of piezoelectric material. Figure 3.5c shows the change in amplitude versus the location of piezoresistive patch denoted by the ratio of the length of the beginning point of it, $L_1$, to the total MC length, $L$. It is observed that the location of piezoresistive patch affects system’s amplitude significantly while it does not have a noticeable influence on the shift in the resonance frequency of the system.

3.7. Chapter Summary

In this chapter, a distributed-parameters modeling framework was developed for MC-based biosensor (sys.1) and MC-based PFM (sys.2) equipped with piezoresistive read-out system. Hamiltonian Principle was used to obtain the equations of motion of the system. Sys.1 operates in contact mode where the contact force was modeled as a function of MC deflection and introduced into the equations of motion. Whereas in sys.2, MC tip was brought into contact with the piezoelectric sample and an external periodic electric field was applied between the conducting tip and the sample. The piezoelectric and piezoviscoelastic deformations of the sample served as the source of excitation of the system.

The obtained equations were simulated in MATLAB from which MC deflection as a function of time and space, $w(x,t)$, was obtained. The contact tip force, change of resistivity of the piezoresistive patch, and consequently output voltage of the system was calculated utilizing MC’s deflection. Simulation results have been presented and verified the capability of the
proposed distributed-parameters model. Sensitivity of the systems with respect to length and location of piezoresistive layer over MC and the value of local spring constant of piezoelectric sample were studied in sys.1 and sys. 2, respectively.

Compared to lumped-parameters modeling, the proposed model addressed the uncertainties and unmodeled dynamics which are required for a precise MC-based force sensor. The reported modeling framework can be utilized for predicting system’s behavior in many different aspects.
CHAPTER 4†

COMPREHENSIVE MATHEMATICAL MODELING OF PIEZOELECTRIC MICROCANTILEVER USED FOR ULTRASMALL MASS SENSING

4.1. Introduction

MC-based biosensors have become a good alternative in place of conventional mass sensing techniques such as surface plasmon resonance detectors (Nelson et al. 2002) and QCM (Bizet et al. 1998). Although MC-based biosensors have received a widespread attention for label-free bio-detection, there are not enough analytical studies investigating modeling and simulation of piezoactive MC-based biosensors. Most of the related studies are based on simple lumped-parameters system modeling the biosensor using Euler-Bernoulli beam theory (Yena et al. 2009, Boisen et al. 2000, Thaysen et al. 2001, Duc et al. 2007).


3D dynamic behavior of an eight-MC array structure was analyzed numerically by AFM showing good agreement in lower mode but not in higher modes (Reed et al. 2006). However, such systems (lumped-parameters modeling) and such numerical analysis are not capable of

† The contents of this chapter may have come directly from our previous publication (Faegh and Jalili, 2013).
describing all dynamics and phenomena occurring within the MC with any type of designs and geometries and in all vibrational modes. Therefore, there is still a need for a more comprehensive mathematical framework capable of describing static and dynamic behavior of MCs with any shape and design in both low and high modes. Having such a model is crucial for having a precise biosensing tool.

In this chapter, a comprehensive distributed-parameters modeling is proposed for piezoelectric MC. Veeco Active probe® is taken to be the MC which has the capability of self excitation through ZnO stack mounted at the base of the probe as shown in Figure 4.1. Other than being implemented on the Dimension AFM (Itoh and Suga, 1994, Itoh et al. 1996, Li et al. 1996, Jamitzky et al. 2006), high speed imaging (Salehi-Khojin et al. 2008, Senesac et al. 2003, Oden et al. 1996, Zhang et al. 2007b, Saeidpourazar et al. 2008b, Lee and Chung, 2004, Grbovic et al. 2006) and active control (Saeidpourazar and Jalili, 2008a,b, 2009, English et al. 2006, Lee, 2007), these probes can be used as biosensors. Therefore, the proposed comprehensive modeling helps to understand performance of these probes acting as actuator as well as biosensor.

This chapter is organized in three parts; the first two parts presents mathematical modeling of the piezoelectric MC-based biosensor, while the third part deals with experimental results carried out.
to verify the theoretical results presented in the first two parts. In the first part, the Euler-Bernoulli beam theory is used to derive the equation of motion along with the response of the system and natural frequencies. In second part, the same system is modeled as a nonuniform cross-section rectangular plate with a uniform piezoelectric layer on its surface. The equations of motions of the rectangular plate actuated by piezoelectric layer are derived. Free and forced vibration analyses are performed using estimated function and Galerkin’s method respectively in order to solve the equation of motion. Finally, in last part of this chapter, an experimental setup is developed and extensive testing is performed on Veeco Active probe® equipped with piezoelectric layer. The piezoelectric property of the active probe is used as an actuator in this study while a laser vibrometer is used to measure the response of the system. The results obtained from the experiment are compared and verified with the theoretical results obtained in the preceding two parts.

4.2. Beam Modeling

In this section comprehensive mathematical modeling framework followed by numerical simulation is presented.

4.2.1. Mathematical modeling

An analytical model is adopted assuming the Active Probe to obey the Euler-Bernoulli beam theory assumption. Distributed-parameters modeling is used to describe the behavior of active probes acting as biosensor. Figure 4.2 depicts the schematic of Veeco Active Probe with ZnO stack mounted on the base of the probe and extended close to the tip. The beam is considered to have length $L$, thickness $t_b$, and volumetric density $\rho_b$. The piezoelectric layer over the top surface of the MC has length $L_1$, thickness $t_p$, and volumetric density $\rho_p$. Both MC and piezoresistive layer are considered to have width $b$. $w(x,t)$ denotes the midplane deflection of MC
with the tip deflection as $w(L,t)$. Small deflection and linear system properties assumptions are taken into account. The Extended Hamilton’s principle is used in developing the equations of motion. The system is excited by applying a sinusoidal voltage to the piezoelectric layer with the frequency close to system’s first natural frequency and the amplitude of 5 Volts.

What it follows next, is a distributed-parameters modeling framework for the transverse deflection of the beam, $w(x,t)$. For this, the kinetic energy of the system is written as

$$KE = \frac{1}{2} \int_0^L \rho(x) \left[ \frac{\partial w(x,t)}{\partial t} \right]^2 dx$$  \hspace{1cm} (4.1)

where

$$\rho(x) = \rho A(x) = \rho_b b t_b + \rho_p b t_p G(x)$$  \hspace{1cm} (4.2)

with $G(x) = 1 - H(x-L_1)$, and $H(x)$ being the Heaviside function. Considering that beam only extends in the $x$-direction, potential energy of the system can be written as

$$\delta PE = \int_0^L \sigma_x \delta \varepsilon_x dx$$  \hspace{1cm} (4.3)

where the stress-strain relationship for beam and piezoelectric layer can be obtained from

$$\sigma_x^b = E_b \varepsilon_x$$  \hspace{1cm} (4.4)

![Figure 4.2 Schematic representation of Veeco Active Probe with ZnO stack on top extended from 0 to $L_1$ (Salehi-Khojin et al. 2009c), with permission.](image-url)
\[ \sigma_x^p = E_p \varepsilon_x + E_p d_{31} \frac{V(t)}{t_p} \]  

(4.5)

with \( E_b \) and \( E_p \) being beam and piezoelectric elastic moduli, respectively. \( V(t) \) is the applied voltage which is the input to the system, and \( d_{31} \) is the piezoelectric constant (Jalili, 2010, Mehta, 2009).

Strain in the \( x \)-direction is related to the transverse deflection of the beam by

\[ \varepsilon_x = -y \frac{\partial^2 w(x,t)}{\partial x^2} \]

which should be modified as \( \varepsilon_x = -(y - y_n) \frac{\partial^2 w(x,t)}{\partial x^2} \) when used for piezoelectric section as a result of shift in the neutral axis. \( y_n \) is defined as

\[ y_n = \frac{E_p t_p (t_p + t_b)}{2(E_p t_p + E_b t_b)} \]

(4.6)

Therefore, the virtual potential energy can be written as

\[ \delta PE = \int_0^L \frac{\partial^2}{\partial x^2} \left[ EI(x) \frac{\partial^2 w(x,t)}{\partial x^2} \right] dx + M_{p0} V(t) \int_0^L \frac{\partial^2 G(x)}{\partial x^2} dx \]

(4.7)

where \( M_{p0} \) is defined as follows

\[ M_{p0} = bE_p d_{31} \left[ \frac{1}{2} (t_b + t_p) - y_n \right] \]

(4.8)

The varying stiffness of the system \( EI(x) \) is

\[ EI(x) = E_b I_b(x) + E_p I_p(x) \]

\[ I_b(x) = \frac{1}{12} b t_b^3 + G(x) b t_b y_n^2 \]

\[ I_p(x) = \left[ \frac{1}{12} b t_p^3 + b t_p y_n^2 \left( \frac{1}{2} (t_b + t_p) - y_n \right) \right] b G(x) \]

(4.9)

The virtual work due to ever-present viscous and structural damping terms is given by

\[ \delta W = \int_0^L \left( -B \dot{w}(x,t) - C \dot{w}'(x,t) \right) \delta w(x,t) dx \]

(4.10)

where \( B \) and \( C \) represent the coefficients of viscous and structural damping, respectively (Dadfarnia et al. 2004). \( (\cdot)' \) denotes the partial derivative with respect to spatial coordinate \( x \),
while (\dot{\cdot}) represents temporal derivative. Utilizing Extended Hamilton’s principle, the equations of motion of the system can be obtained as

$$\rho(x) \frac{\partial^2 w(x,t)}{\partial t^2} + \frac{\partial}{\partial x} \left[ EI(x) \frac{\partial^2 w(x,t)}{\partial x^2} \right] + B \frac{\partial w(x,t)}{\partial t} + C \frac{\partial^2 w(x,t)}{\partial x \partial t} = -M_{p0}V(t)G''(x) \quad (4.11)$$

with the boundary conditions

$$w(0,t) = w'(0,t) = 0 \quad (4.12a)$$
$$w''(L,t) = w'''(L,t) = 0 \quad (4.12b)$$

4.2.2. Numerical simulations and results

The obtained governing equations of motion of the system are solved numerically using Galerkin’s method. The PDE (4.11) can be converted into ODE using the following discretization proposition

$$w(x,t) = \sum_{j=1}^{n} \phi_j(x) q_j(t), \quad j = 1, 2, \ldots, n \quad (4.13)$$

with \(\phi_j(x)\) and \(q_j(t)\) being the clamped-free beam eigenfunction and generalized coordinates, respectively. Therefore, the equation of motion can be represented as a function of time in a matrix form. The ODE for the system can now be represented as

$$M \ddot{q}(t) + D \dot{q}(t) + Kq(t) = K_V(t) \quad (4.14)$$

where

$$q = \{q_1, q_2, \ldots, q_i\}, \quad \dot{q} = \{\dot{q}_1, \dot{q}_2, \ldots, \dot{q}_i\}$$

$$M = \{M_{ij}\},$$

$$M_{ij} = \int_0^L \rho A(x) \phi_j(x) \phi_i(x) dx, \quad i, j = 1, 2, \ldots, n$$

$$D = \{D_{ij}\},$$

$$D_{ij} = B \int_0^L \phi_j(x) \phi_i(x) dx + C \int_0^L \phi'_j(x) \phi_i(x) dx$$
\[ K = \{ K_{ij} \}, \]

\[ K_{ij} = \int_0^L EI(x) \phi_j''(x) \phi_i''(x) \, dx \]

\[ K_v = \{ K_{ij} \}, \]

\[ K_{ij} = -M_p \int_0^L \phi_j'(x) \delta(x - L_1) \, dx = -M_p \phi_j'(L_1) \quad (4.15) \]

The ODEs represented by Eqs. (4.14) and (4.15) are solved in MATLAB using the numerical values given in Table 4.1. Forcéd vibration problem is solved with the input, the applied voltage to ZnO stack, being a sinusoidal function with the amplitude of 5 Volts and the frequency close to systems first natural frequency.

Selecting appropriate admissible functions\(^\dagger\), \(\phi_j(x)\) and using Eq. (4.13), the deflection of the MC at any location of the beam at any time can be obtained. The tip deflection of the MC, \(w(L,t)\), is then plotted in Figure 4.3(a). Taking the Fast Fourier Transform (FFT) of the response, the system’s first natural frequency is obtained to be 52.99 kHz as shown in Figure 4.3(b). The effect of adsorbed ultrasmall mass as low as 200 ng was calculated numerically. The added mass was modeled as surface mass over the active area of functionalization on MC surface \((0-L_1)\). As a result of the adsorbed mass, resonance frequency

\[ \text{Table 4.1 The system parameters used for modeling.} \]

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>(L)</td>
<td>486</td>
<td>(\mu m)</td>
</tr>
<tr>
<td>(L_1)</td>
<td>325</td>
<td>(\mu m)</td>
</tr>
<tr>
<td>(L_2)</td>
<td>360</td>
<td>(\mu m)</td>
</tr>
<tr>
<td>(W_{bl})</td>
<td>230</td>
<td>(\mu m)</td>
</tr>
<tr>
<td>(W_{b2})</td>
<td>40</td>
<td>(\mu m)</td>
</tr>
<tr>
<td>(W_p)</td>
<td>180</td>
<td>(\mu m)</td>
</tr>
<tr>
<td>(b)</td>
<td>50</td>
<td>(\mu m)</td>
</tr>
<tr>
<td>(t_b)</td>
<td>4</td>
<td>(\mu m)</td>
</tr>
<tr>
<td>(t_p)</td>
<td>4</td>
<td>(\mu m)</td>
</tr>
<tr>
<td>(\rho_b)</td>
<td>2330</td>
<td>(kg.m^{-3})</td>
</tr>
<tr>
<td>(\rho_p)</td>
<td>6390</td>
<td>(kg.m^{-3})</td>
</tr>
<tr>
<td>(E_b)</td>
<td>105</td>
<td>(GPa)</td>
</tr>
<tr>
<td>(E_p)</td>
<td>104</td>
<td>(GPa)</td>
</tr>
<tr>
<td>(v_b)</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>(d_{ij})</td>
<td>11</td>
<td>(pC/N)</td>
</tr>
<tr>
<td>(s_{12}^i)</td>
<td>(-4.05 \times 10^{-12})</td>
<td>(m^2/N)</td>
</tr>
<tr>
<td>(m_b)</td>
<td>5</td>
<td>(\mu g)</td>
</tr>
<tr>
<td>(C_s)</td>
<td>10</td>
<td>(Ns/m)</td>
</tr>
<tr>
<td>(K_s)</td>
<td>200</td>
<td>(kN/m)</td>
</tr>
</tbody>
</table>

\(^\dagger\) Simple functions that provide approximate solution to the structures with complicated geometries satisfying boundary conditions.
Reduction in the first natural frequency about 1 kHz occurs as a result. Figure 4.3(c) and (d) illustrate the effect of functionalization over MC surface on its first natural frequency and vibration amplitude, respectively.

4.3. Plate Modeling

This section presents a precise modeling framework for the same system modeled as a nonuniform rectangular thin plate. Free and forced vibration problems were solved. Numerical simulation results are presented.
4.3.1. Mathematical modeling

In this section, an analytical model is proposed assuming the Active Probe to be a rectangular plate with a piezoelectric layer on top. Distributed-parameters modeling is used to describe the behavior of active probes acting as biosensor and the equations of motion are derived using Hamilton’s principle. Figure 4.4 shows the schematic of Veeco Active Probe presented as a nonuniform plate with ZnO stack mounted on its base and extended close to the tip. The dimensions of the system are kept similar to beam section with $W_b$ and $W_p$ being the width of the beam and piezoelectric layers, respectively.

Neglecting the electrical kinetic energy, the kinetic energy of the system can be written as follows (Jalili, 2010)

$$T = \frac{1}{2} \int_0^L \int_0^W \rho(x, y) \left( \frac{\partial w(x, y, t)}{\partial t} \right)^2 \, dx \, dy$$

$$= \frac{1}{2} \int_0^{W_{b_1}} \int_0^{L_1} \rho_b t_b \left( \frac{\partial w(x, y, t)}{\partial t} \right)^2 \, dx \, dy + \frac{1}{2} \int_{e_1}^{e_1+W_p} \int_0^{L_1} \rho_p t_p \left( \frac{\partial w(x, y, t)}{\partial t} \right)^2 \, dx \, dy$$

$$+ \frac{1}{2} \int_0^{W_{b_1}} \int_{L_1}^{L_2} \rho_b t_b \left( \frac{\partial w(x, y, t)}{\partial t} \right)^2 \, dx \, dy + \frac{1}{2} \int_{e_2}^{e_2+W_{b_2}} \int_{L_2}^L \rho_b t_b \left( \frac{\partial w(x, y, t)}{\partial t} \right)^2 \, dx \, dy \quad (4.16)$$

where

$$\rho(x, y) = \rho_b t_b + \rho_p t_p G(x, y) \quad (4.17)$$

with $G(x, y) = [1 - H(x-L_1)][H(y-e_1) - H(y-(W_p+e_1))]$, and $H(x)$ being the Heaviside function.

The volumetric strain energy of the system including the strain energy of the plate and piezoelectric actuator can be written as

$$U = \iiint (\pi_b + \pi_p) dv \quad (4.18)$$

where $\pi_b$ and $\pi_p$ represent strain energy of plate and strain energy of piezoelectric layer, respectively defined as follows

54
\[ \pi_b = \frac{1}{2} \left[ (\sigma_{xx}\varepsilon_{xx})_b + (\sigma_{yy}\varepsilon_{yy})_b + (\sigma_{xy}\varepsilon_{xy})_b \right] \]

\[ \pi_p = \frac{1}{2} \left[ (\sigma_{xx}\varepsilon_{xx})_p + (\sigma_{yy}\varepsilon_{yy})_p + (\sigma_{xy}\varepsilon_{xy})_p \right] \]  

(4.19)

where the stress-strain relationship for the piezoelectric material can be obtained from the fundamental equation

\[ \varepsilon_p = s^E_{pq} \sigma_q + d_{ip} E_i \]  

(4.20)

with \( \varepsilon_p \) being mechanical strain, \( s^E_{pq} \) being the elastic compliance matrix, \( \sigma_q \) being the mechanical stress, \( d_{ip} \) being the piezoelectric charge constant, and \( E_i \) being the electric field vector. Eq. (4.5) can also be written in the following form

\[ \sigma_q = c^E_{pq} \varepsilon_p - e_{qj} E_j \]  

(4.21)

where \( c^E_{pq} \) represents elastic stiffness under constant electric field and \( e_{qj} = c^E_{pq} d_{qj} \).

Having the above fundamental equations accompanied with the plate equations, the stress-strain relationship for the plate with a piezoelectric layer can be obtained as (Mehta, 2009)

\[
\begin{bmatrix}
\sigma_{xx} \\
\sigma_{yy} \\
\sigma_{xy}
\end{bmatrix} =
\begin{bmatrix}
\frac{E_p}{1-\vartheta_p^2} & \frac{\vartheta_p E_p}{1-\vartheta_p^2} & 0 \\
\frac{\vartheta_p E_p}{1-\vartheta_p^2} & \frac{E_p}{1-\vartheta_p^2} & 0 \\
0 & 0 & \frac{E_p}{2(1-\vartheta_p)}
\end{bmatrix}
\begin{bmatrix}
\varepsilon_{xx} \\
\varepsilon_{yy} \\
\varepsilon_{xy}
\end{bmatrix} -
\begin{bmatrix}
\frac{E_p}{1-\vartheta_p^2} & \frac{\vartheta_p E_p}{1-\vartheta_p^2} & 0 \\
\frac{\vartheta_p E_p}{1-\vartheta_p^2} & \frac{E_p}{1-\vartheta_p^2} & 0 \\
0 & 0 & \frac{E_p}{2(1-\vartheta_p)}
\end{bmatrix}
\begin{bmatrix}
d_{31} \\
d_{32} \\
\varepsilon_p
\end{bmatrix}
\]  

(4.22)

with \( \vartheta_b \) being beam’s Poisson’s ratio and \( \vartheta_p \) the piezoelectric’s Poisson’s ratio which can be calculated as (Jalili, 2010)

\[ s^S_{11} = \frac{1}{E_p}, \quad s^S_{12} = -\frac{\vartheta_p}{E_p} \]  

(4.23)

in which \( s^S_{pq} \) represents piezoelectric’s compliance coefficient.

Based on Eqs (4.21-4.23), the total strain energy (Eq. 4.18) can be written as
The virtual work due to damping forces can be written as

$$
\delta W = \int_0^{W_b} \int_0^L \left[ -B \frac{\partial w}{\partial t} \delta w(x,y,t) \right] dx \, dy + \int_0^{W_b} \int_0^L \left[ -C \frac{\partial^3 w}{\partial x \partial y \partial t} \delta w(x,y,t) \right] dx \, dy \quad (4.27)
$$

where $D_1$ and $D_2$ are defined as

$$
D_1 = \frac{1}{12 \ 1-\sigma_b^2} \frac{E_p \mu_b}{\lambda_b}, \\
D_2 = \frac{1}{12 \ 1-\sigma_b^2} \frac{E_p \mu_b \mu_p}{1-\sigma_p^2} \frac{t_p + t_b - z_n}{2} \left( W_{b1} \right)^2 + \frac{E_p \mu_b}{1-\sigma_b^2} \frac{t_p + t_b - z_n}{2} \left( W_{b1} \right)^2 + \frac{E_p \mu_b}{1-\sigma_b^2} \frac{t_p + t_b - z_n}{2} \left( W_{b1} \right)^2 \quad (4.25)
$$

Since the combined thickness of the plate and piezoelectric in not constant as a result of piezoelectric layer on the surface, the neutral axis is shifted from the mid-section. This upward shift in the neutral axis due to non-uniformity in plate thickness can be given as (Mehta, 2009)

$$
Z_n = \frac{E_p t_p W_p (t_p + t_b)}{2 (E_p t_p W_p + E_b t_b W_{b1})} \quad (4.26)
$$

The virtual work due to damping forces can be written as

$$
\delta W = \int_0^{W_b} \int_0^L \left[ -B \frac{\partial w}{\partial t} \delta w(x,y,t) \right] dx \, dy + \int_0^{W_b} \int_0^L \left[ -C \frac{\partial^3 w}{\partial x \partial y \partial t} \delta w(x,y,t) \right] dx \, dy \quad (4.27)
$$
where $B$ and $C$ represent the coefficients of viscous and structural damping, respectively.

By evaluating the variations of kinetic and potential energies along with the virtual work and substituting them into the extended Hamilton’s principle

$$\int_{t_1}^{t_2} \delta(T - U + W)\,dt = 0,$$  \hspace{1cm} (4.28)

the following equation of motion can be obtained

$$\rho t(x) \frac{\partial^2 w(x,y,t)}{\partial t^2} + \frac{\partial^2}{\partial x^2} \left[ D_1 \left( \frac{\partial^2 w}{\partial x^2} + \vartheta_p \frac{\partial^2 w}{\partial y^2} \right) + D_2 \left( \frac{\partial^2 w}{\partial y^2} + \vartheta_b \frac{\partial^2 w}{\partial x^2} \right) \right] + \frac{\partial^2}{\partial y^2} \left[ D_1 \left( \frac{\partial^2 w}{\partial y^2} + \vartheta_b \frac{\partial^2 w}{\partial x^2} \right) + D_2 \left( \frac{\partial^2 w}{\partial x^2} + \vartheta_p \frac{\partial^2 w}{\partial y^2} \right) \right] +$$

$$C \frac{\partial^3 w(x,y,t)}{\partial x \partial y \partial t} = -M_{p1}V_1(t) \frac{\partial^2 g(x,y)}{\partial x^2} - M_{p2}V_2(t) \frac{\partial^2 g(x,y)}{\partial y^2}$$  \hspace{1cm} (4.29)

where $M_{p1}$ and $M_{p2}$ are given as

$$M_{p1} = \frac{E_p}{2(1-\vartheta_p^2)} \left( t_p + t_b - z_n \right) (d_{31} + \vartheta_p d_{32})$$

$$M_{p2} = \frac{E_p}{2(1-\vartheta_p^2)} \left( t_p + t_b - z_n \right) (d_{32} + \vartheta_p d_{31})$$  \hspace{1cm} (4.30)

By inspecting Eq. (4.29), it can be seen that the system’s input is the voltage applied to the piezoelectric layer which creates responses in both $x$- and $y$-directions.

**4.3.2. Free vibration analysis**

In order to solve the free vibration problem, eigenfunctions and eigenvalues need to be obtained. Eigenfunctions are the exact solution of the free vibration problem satisfying all the boundary conditions including both geometrical and natural boundary conditions. However, in complex and nonuniform problems, finding the exact eigenfunction solution is very tedious. In these cases, an approximate solution or what is referred to as admissible function is typically utilized. Since the plate under study here is nonuniform in thickness and cross-section, an approximate solution is desired with acceptable accuracy. Increasing number of modes in solving the forced-
vibration problem can also compensate for the approximation considered in solving the free-vibration problem.

A number of studies have investigated the exact free-vibration solution of MC plate exploiting different methods such as Rayleigh Ritz, superposition, and separation of variables (Gorman, 1976, 1982, 1984, 1995, Rao, 2007, Yu, 2009). In order to obtain an admissible function, symmetric and antisymmetric free vibration modes of MC plate was calculated using Gorman’s method of superposition (Gorman, 1982). Three building blocks were considered developing Levy-type solution for each building block and forcing the solutions to satisfy boundary conditions. Alternative to this method is to find the exact analytical solution to the free-vibration problem using separation of variables (Gorman, 1982). Both of these methods have been used; however, the exact analytical solution (Gorman, 1982) provided more accurate eigenfunctions and eigenvalues, and so this method is followed in this study.

In order to find the solution of the free, undamped vibration problem of a rectangular plate with total length of $L$ and width $W_{b,l}$ as depicted in Figure 4.4(a), the following equation needs to be solved [Rao, 2007]

\[ \rho t(x) \frac{\partial^2 w(x,y,t)}{\partial t^2} + \nabla^2 \left( D(x,y) \nabla^2 w(x,y,t) \right) = 0 \]  

(4.31)

The solution is assumed to take the following form utilizing the concept of separation of variables with respect to location and time

\[ w(x,y,t) = W(x,y)T(t) \]  

(4.32)

By substituting Eq. (4.32) into Eq. (4.31), the following two equations are obtained which are separated in time and position $(x,y)$ assuming constant plate stiffness, $D(x,y) = D$ and plate thickness $t(x) = t$,

\[ \frac{1}{T(t)} \frac{d^2 T(t)}{dt^2} = -\omega^2 \]  

(4.33a)
with \( \omega \) being the natural frequencies and \( \beta_l \) defined as \( \beta_l = \frac{D}{\rho e} \). The general solution of Eq. (4.33a) can be expressed in terms of harmonic functions as follows

\[
T(t) = A \cos(\omega t) + B \sin(\omega t)
\]  
(4.34)

while Eq. (4.33b) can be written as

\[
(\nabla^4 - \lambda^4)W(x, y) = (\nabla^2 - \lambda^2)(\nabla^2 + \lambda^2)W(x, y) = 0
\]  
(4.35)

with \( \lambda^4 = \frac{\rho t \omega^2}{D} \). The general solution of Eq. (4.33b), \( W(x, y) \), can be obtained by superposing \( W_1(x, y) \) and \( W_2(x, y) \) each of which satisfies the following equations

\[
(\nabla^2 + \lambda^2)W_1(x, y) = \frac{\partial^2 W_1}{\partial x^2} + \frac{\partial^2 W_1}{\partial y^2} + \lambda^2 W_1(x, y) = 0
\]  
(4.36a)

\[
(\nabla^2 - \lambda^2)W_2(x, y) = \frac{\partial^2 W_2}{\partial x^2} + \frac{\partial^2 W_2}{\partial y^2} - \lambda^2 W_2(x, y) = 0
\]  
(4.36b)

Each of \( W_1(x, y) \) and \( W_2(x, y) \) can be obtained in terms of harmonic functions as follows

\[
W_1(x, y) = C_1 \sin \alpha x \sin \beta y + C_2 \sin \alpha x \cos \beta y + C_3 \cos \alpha x \sin \beta y + C_4 \cos \alpha x \cos \beta y
\]  
(4.37a)

\[
W_2(x, y) = C_5 \sinh \alpha x \sinh \beta y + C_6 \sinh \alpha x \cosh \beta y + C_7 \cosh \alpha x \sinh \beta y + C_8 \cosh \alpha x \cosh \beta y
\]  
(4.37b)

where \( \lambda^2 = \alpha^2 + \beta^2 = \gamma^2 + \eta^2 \). Therefore, the general solution of \( W(x, y) \) is

\[
W(x, y) = W_1(x, y) + W_2(x, y)
\]  
(4.38)

In order to find a unique solution for \( W(x, y) \), the eight coefficients \( C_1 - C_8 \) need to be found which can be evaluated using the boundary conditions. The applied boundary conditions for the rectangular MC plate are clamped at one edge where \( x=0 \), and free at the other three edges (Fig. 4). Therefore, the eight boundary conditions can be written a

@\( x = 0 \): \( W(x, y) = 0 \), \( \frac{\partial W(x, y)}{\partial x} + \vartheta \frac{\partial W(x, y)}{\partial y} = 0 \)
\( @x = L: \quad \frac{\partial^2 W(x,y)}{\partial x^2} + \vartheta \frac{\partial^2 W(x,y)}{\partial y^2} = 0, \quad \frac{\partial^3 W(x,y)}{\partial x^3} + \vartheta \frac{\partial^3 W(x,y)}{\partial x^3} = 0 \)

\( @y = 0: \quad \frac{\partial^2 W(x,y)}{\partial y^2} + \vartheta \frac{\partial^2 W(x,y)}{\partial x^2} = 0, \quad \frac{\partial^3 W(x,y)}{\partial y^3} + \vartheta \frac{\partial^3 W(x,y)}{\partial x^3} = 0 \)

\( @y = W_{b1}: \quad \frac{\partial^2 W(x,y)}{\partial y^2} + \vartheta \frac{\partial^2 W(x,y)}{\partial x^2} = 0, \quad \frac{\partial^3 W(x,y)}{\partial y^3} + \vartheta \frac{\partial^3 W(x,y)}{\partial x^3} = 0 \) \quad (4.39a-d)

Introducing the eight boundary conditions into Eq. (4.38), the eigenvalues and eigenfunctions can be obtained. The eigenfunctions are calculated and plotted for the first mode as depicted in Figure 4.5.

**4.3.3. Numerical simulations and results**

The obtained equation of motion represented by (4.29) was solved numerically using MATLAB. For this, the partial differential equation (PDE) was converted to ODE discretizing system response, \( w(x,y,t) \), with respect to both spatial and temporal components exploiting Galerkin’s method as

\[
    w(x,y,t) = \sum_{n=1}^{N} \sum_{m=1}^{N} W_{mn}(x,y) q_{mn}(t)
\]  

\( q_{mn}(t) \)

**Figure 4.5** Eigenfunction for the first mode of the rectangular cantilever plate, \( W_{11} \).
For a clamped-free-free rectangular plate with continuous geometry, the equations of motion can be expressed in terms of function of time in the matrix form as follows

\[ M\ddot{q}(t) + D\dot{q}(t) + Kq(t) = K_{v_1}V(t) + K_{v_2}V(t) \]  

(4.41)

where

\[ M = \{M_{rsmn}\}, \]
\[ M_{rsmn} = \int_0^{W_{b1}} \int_0^L \rho(x,y)t(x)W_{rs}(x,y)W_{mn}(x,y)\,dx\,dy \quad r,s,m,n = 1,2,\ldots,N \]
\[ D = \{D_{rsmn}\}, \]
\[ D_{rsmn} = B \int_0^{W_{b1}} \int_0^L W_{rs}(x,y)W_{mn}(x,y)\,dx\,dy + C \int_0^{W_{b1}} \int_0^L W_{rs}(x,y) \frac{\partial^2 W_{rs}}{\partial x \partial y} \,dx\,dy \]
\[ K = \{K_{rsmn}\}, \]
\[ K_{rsmn} = \int_0^{W_{b1}} \int_0^L D(x,y)\nabla^2 W_{rs}\nabla^2 W_{mn}\,dx\,dy \]
\[ K_{v_1} = \{K_{v_1rs}\}, \]
\[ K_{v_1rs} = -M_{p1} \int_{e_1}^{W_p+e_1} \int_0^{L_1} \frac{\partial W_{rs}}{\partial x} \frac{\partial G(x,y)}{\partial x} \,dx\,dy = -M_{p1} \int_{e_1}^{W_p+e_1} \frac{\partial W_{rs}}{\partial x}(L_1,y)\,dy \]
\[ K_{v_2} = \{K_{v_2rs}\}, \]
\[ K_{v_2rs} = -M_{p2} \int_{e_1}^{W_p+e_1} \int_0^{L_1} \frac{\partial W_{rs}}{\partial y} \frac{\partial G(x,y)}{\partial y} \,dx\,dy = \]
\[ -M_{p2} \int_0^{L_1} \frac{\partial W_{rs}}{\partial y}(x,e_1) + \frac{\partial W_{rs}}{\partial y}(x,(W_p + e_1))dx \]  

(4.42)

The ODE represented by Eqs. (4.41) and (4.42) were solved in MATLAB using the numerical values given in Table 4.1. The piezoelectric material was assumed to be transversely isotropic. This assumption results in the piezoelectric constant \( d_{32} \) to be equal to \( d_{31} \) (Jalili, 2010). Forced vibration problem was solved with the input being the applied voltage to ZnO stack which was taken to be a sinusoidal function with the amplitude of 5 Volts and the frequency close to systems
first natural frequency of about 56 kHz. The time response of the MC, \( q_{11}(t) \) is obtained for 10 ms of operation of MC. Deflection of any point of MC, \( w(x,y,t) \), can be calculated having admissible function, \( W_{mn}(x,y) \), obtained from section 4.3. Multiplying \( W_{mn}(x,y) \) by the respective generalized coordinates, \( q_{mn}(t) \), the response of the system can be found at any particular location and at any time.

Figure 4.6 depicts the results obtained from solving the equation of motion based on the mathematical modeling framework presented. Time response of the MC for the first mode, \( q_{11}(t) \), is plotted in Figure 4.6(a).

Deflection of MC at an arbitrary point which is selected to be at the free end of the MC in the middle corresponding to \( x = L \) and \( y = \frac{W_b}{2} \) is calculated and plotted in Figure 4.6(b). Taking the FFT of the time response, the system’s first resonance frequency is observed to be 56.34 kHz as clearly seen in Figure 4.6(c).

The ultimate goal of this study is to quantitatively detect the ultrasmall absorbed mass on the surface of the MC with the intention of implementing the presented system as a highly sensitive biological sensor. Operating the presented MC in the dynamic mode, the shift in natural frequency was calculated giving a good insight into the amount of absorbed mass to the surface of MC. Figure 4.6(c) depicts the shift in the first natural frequency of the system as a result of absorbed mass as low as about 200 ng.

4.4. Experimental Verification

The experimental section of this study includes the measurement of ultrasmall added surface mass using MC and verifying the results with the mathematical modeling presented in the preceding sections. Veeco Active Probe® is used with the capability of excitation through the ZnO stack mounted at the base of each probe (see Figure 4.1).
The Active probe® was mounted on a holder which was fixed on a 3D stage and placed under the laser vibrometer as shown in Figure 4.7.

A sinusoidal input voltage with the amplitude of 1 Volt and excitation frequency of 50 kHz was generated through oscilloscope (Agilent InfiniiVision 2000 X-Series-sw Oscilloscope). The input voltage applied to the ZnO stack produces excitation to the system. The produced signal is read out as velocity by laser vibrometer (Polytec CLV-2534).

Figure 4.6 (a) time response of microcantilever, $q_{11}(t)$, (b) Deflection of microcantilever at the tip of the MC in the middle, $w(L, \frac{W_b}{2}, t)$, (c) FFT of the response of the system representing system’s first natural frequency and the effect of added absorbed mass in the shift of natural frequency (Faegh and Jalili, 2013).
4.4.1. Non-functionalized MC: verification with modeling

The first step of the experiment is performed on a non-functionalized MC. The excitation frequency is swept from 0 kHz to 100 kHz and the first natural frequency of the system is captured to be around 56 kHz which exactly matches the theoretical result of modeling the system as a rectangular non-continuous plate presented in Section 4.3. Figure 4.8(a,b) shows the FFT of the system’s response captured by optical measurement.

Comparing the results obtained from mathematical modeling with the experimental results, it is shown that mathematical modeling presented in both Part I and II, i.e., modeling the system as Euler Bernoulli and rectangular plate, respectively, predict the real situation with a great level of accuracy. Although the Euler Bernoulli modeling provided explanation of dynamics and behavior of the proposed platform in this case, it will not be sufficient for modeling other geometries of the similar platform. Since geometry of MC in biosensors dramatically influences the sensitivity of the system, there is always a need to optimize geometrical properties such as using shorter and wider MCs. Therefore, having a comprehensive modeling framework describing all geometries and designs of MC provides a powerful theoretical layout for such systems and explains the necessity of modeling complexity and effort. Table 4.2 compares the theoretical results with the experiment.

4.4.2. Detection of adsorbed mass

In the second step of the experiment, the Active Probe is used for detection of ultrasmall adsorbed mass. The MC operates in dynamic mode where it is brought to excitation close to its
first resonant frequency by applying a sinusoidal voltage to the piezoelectric layer with the amplitude of 1 Volt.

In this study, the detection of \(i\) Amino groups, and \(ii\) Glucose Oxidase (GoX) enzyme layer formed on top of MC surface is investigated. The absorbed mass is sensed as a result of shifted laser beam reflected from tip of the MC captured by laser vibrometer and monitored by the scope. Taking the FFT of the response of the system illustrates the change of system’s resonant frequency. In order to functionalize enzyme layer, the active part of the MC surface is used

![Graph](image)

**Figure 4.8** (a) Decibel versus frequency, FFT of the output signal showing first resonance frequency at 56.1 kHz, (b) Amplitude ratio versus frequency.

<table>
<thead>
<tr>
<th></th>
<th>First Resonance Frequency, (w_{n1}) (kHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theory 1: Beam Modeling</td>
<td>52.99</td>
</tr>
<tr>
<td>Theory 2: Plate Modeling</td>
<td>56.34</td>
</tr>
<tr>
<td>Experiment</td>
<td>56.1</td>
</tr>
</tbody>
</table>

**Table 4.2** Comparing the results obtained from mathematical modeling presented in parts I and II to the experimental results.
which is the extended electrode coated with gold. Gold is employed for immobilizing Gox enzyme which is itself a receptor for biomolecules such as Glucose.

**Materials:** Glucose Oxidase, Gox, 8.0% glutaraldehyde, 2-aminoethanethiol were purchased from Sigma. A 0.1M phosphate buffer solution was prepared. Its pH was adjusted to 7 using dilute HCl and NaOH. Deionized water was used for preparing solutions.

**Procedure:** Before starting functionalization, the Active Probe was washed in acetone and ethanol for 10 minutes. A Teflon chamber was designed in order to dip in the MC into a droplet of liquid such that it only wets the MC and does not proceed to the electronic circuits. A 3D stage with resolution of submicron was used in order to navigate the MC in x-, y-, and z-direction and place it into the droplet.

A 0.1M of aminoethanethiol solution was prepared by dissolving 2-aminoethanethiol powder into deionized water. A single layer of aminoethanethiol was formed on the gold surface by attachment of thiol groups to gold. The change in the first resonant frequency is measured and recorded.

![Figure 4.9](image-url)

**Figure 4.9** Shift of the first resonance frequency as a result of: (a) GoX functionalization, (b) immobilization of Amin solution and enzyme solution consequently.
Enzyme solution is then prepared by dissolving 0.2% glutaraldehyde and 0.1 unit/ml GoX into a pH 7.0 buffer solution. Dipping MC into the enzyme solution aldehyde groups of glutaraldehyde react with the Amino groups at one end and with GoX at other ends letting layer of enzyme to grow over the surface.

Once the enzyme functionalization is complete, new measurement is taken by exciting the MC and sweeping the frequency. Taking the FFT of the response of the system, the shift in resonance frequency as a result of formation of enzyme can be illustrated.

Figure 4.9 shows the shift in the first resonance frequency of the system as a result of immobilization amin groups and enzymes over the gold surface of MC. Measurement was performed eight times with the average of 49 kHz and standard deviation of 0.2098. Figure 4.9 (a) depicts a shift of 5.98 kHz as a result of GoX functionalization. Figure 4.9 (b) shows 4.47 kHz shift in resonance frequency as a result of Amin immobilization which is followed by 7.50 kHz as a result of higher concentration of GoX immobilization.

Exploiting the mathematical modeling presented in this study the amount of adsorbed masses can be quantified having the shifts in resonance frequency. Figure 4.10 shows the shift in the first resonance frequency as a result of adsorbed mass utilizing the mathematical modeling framework providing the relationship between the added mass and frequency change. Based on this method of quantification, the adsorbed mass as a result of Amino and GoX functionalization depicted in

![Figure 4.10](image-url)
Figure 4.9 (b) would be about 545 ng and 368 ng respectively.

4.5. Chapter Summary

Comprehensive distributed-parameters modeling framework was presented for piezoelectric MC-based biosensor with the purpose of detecting ultrasmall biological species. Two models of the system were exploited as either Euler-Bernoulli beam or a rectangular clamped-free-free-free plate. Performing extensive numerical simulations for both cases in dynamic mode, the effect of absorbed mass was modeled and illustrated. An experiment was also set up and performed on Veeco Active Probes being self-excited with piezoelectric layer. Laser vibrometer was used to measure system’s response which was further verified with the mathematical models presented in this study. Active probe was then implemented for detection of ultrasmall adsorbed mass. The immobilized biomolecules were detected operating the system in dynamic mode and quantified exploiting the proposed mathematical framework. Experimental results were further verified with the presented theory. It was shown that both Euler-Bernoulli beam theory and rectangular plate theory provide powerful modeling frameworks for predicting the dynamics of the proposed system. A high level of accuracy was achieved utilizing both modeling frameworks. Although the Euler-Bernoulli modeling also satisfied the explanation of dynamics and behavior of the proposed platform in this case, it will not be sufficient for modeling other geometries of the similar platform. Since geometry of MC in biosensors dramatically influences the sensitivity of the system, there is always a need to optimize geometrical properties such as using shorter and wider MCs. Therefore, having a comprehensive modeling framework describing all geometries and designs of MC provides a powerful theoretical layout for such systems and explains the necessity of modeling complexity and effort.
CHAPTER 5

SELF-SENSING ULTRASMALL MASS DETECTION USING PIEZOELECTRIC MICROCANTILEVER-BASED SENSOR

5.1. Introduction

MC sensors have generated widespread interest as a result of their sensitivity and capability in detecting small forces, mechanical stresses, and added adsorbed mass molecules (Tao and Yung, 2003). One of the most inspiring applications of MC sensors has been their implementation as an inexpensive, sensitive, label-free platform for real-time detection of biomolecules (Arntz et al. 2003, Pei et al. 2003, 2004, Shin et al. 2008, Shin and Lee, 2006, Sree et al. 2010a, b, Wu et al. 2001, Yang and Chang, 2010). Multiplexed detection of concentrations of antigens in a sample fluid has also been enabled utilizing arrays of MCs.


§ The contents of this chapter may have come directly from our previous publication (Faegh et al. 2013a).
as a result of molecular recognition yields a good insight into the amount of adsorbed mass (Johnson and Mutharas, 2012, Von Muhlen et al. 2010).

There are two main features determining the success of all biological sensors: first, the molecular binding between the receptor and the biomolecule of interest; second, the read-out system capable of transducing the molecular binding into detectable physical property. There are a number of read-out methodologies including optical-based, capacitive-based, piezoresistive-based and piezoelectric-based measurement techniques. The concept of these methods and the challenges associated with them was extensively discussed in Chapter 2.

In order to overcome the aforementioned challenges, a unique self-sensing piezoelectric-based MC sensor is reported in this chapter. In self-sensing MC sensors both direct and inverse properties of a piezoelectric material is utilized to play the role of both sensor and actuator. Direct piezoelectric property is used to sense the self-induced voltage generated in the piezoelectric layer as a result of beam deformation. At the same time, inverse property of piezoelectric material is used to generate deformation and bring the system into vibration as a result of applying a harmonic voltage to it. Therefore, a single piezoelectric layer embedded in the MC sensor is utilized to both actuate and sense the system exploiting a capacitance bridge network (Faegh et al. 2013a). This provides a simple and inexpensive platform for mass sensing and detection purposes with the opportunity of miniaturizing the platform. A Veeco Active Probe® is used here where a ZnO stack is used to implement the MC in self-sensing mode as shown in Figure 5.1.

As described in previous chapters, most of the available mathematical modeling targeting piezoactive MC-based systems includes lumped-parameters modelings which are not capable of
describing all dynamics and phenomena occurring within the MC with any type of designs and geometries and in all vibrational modes. This drives the need for a more comprehensive mathematical framework capable of describing static and dynamic behavior of MCs. Therefore, in the first part of this chapter mathematical modeling is developed for self-sensing piezoelectric-based MC followed by simulation results.

In the final part of the chapter, an experimental setup is developed and extensive testing is performed on Veeco Active Probe® equipped with piezoelectric layer functioning in dynamic mode. A capacitance bridge network is utilized to implement the active probe in self-sensing mode. Detection of adsorbed biological species, which is the covalent binding of thiol groups of Aminoethanethiol, was made possible through the proposed self-sensing piezoelectric-based MC sensor. Similar mass detection setup was built and performed utilizing optical-based method and the results were compared to the self-sensing methodology to verify the applicability of the proposed platform. Quantification of adsorbed masses was carried out and the sensitivity of the system was measured.

Piezoelectric properties at the nanoscale are sensitive to temperature and other ambient variations. In order to have a precise model of the actuation/sensing, an adaptation strategy needs
to be implemented in order to compensate for the variation of piezoelectric property (here ZnO stack). For this, a mathematical adaptation law is presented in Section 5.3 followed by simulation results and comparison with those of Section 5.2. The experimental results were verified with the theories presented in Sections 5.2 and 5.3. Based on the results, the accuracy of the proposed modeling frameworks is demonstrated.

5.2. Mathematical Modeling and Preliminaries

Precise modeling framework for the defined system is reported here followed by numerical analysis and results.

5.2.1. Beam modeling

A comprehensive mathematical modeling is proposed in this section using a distributed-parameters model. The system includes a Veeco Active probe® as a self-sensing MC in dynamic mode. The MC beam is assumed to obey the Euler-Bernoulli beam theory. The use of Euler-Bernoulli beam theory was proven to model the current system with a high level of accuracy compared to plate theory in Chapter 4 and (Faegh and Jalili, 2013). The self-sensing mode can be implemented through the ZnO stack mounted on the base of the probe extending close to the tip as shown in Figure 5.2. The MC beam is narrowed in the tip which adds to the sensitivity of the system. The MC is modeled as a nonuniform cross-section beam with the total length of $L$ and an active length (piezoelectric layer) of $L_I$ which is used for functionalization. Other system properties are the same as those described in Chapter 4, Section 4.2.1.
Figure 5.2 Micrograph/photograph of a Veeco Active Probe with a ZnO stack on top extended from 0 to $L_1$ (Salehi-Khojin et al. 2009c), with permission.

The following distributed-parameters modeling is proposed for the response of the system to the applied input. For this, the kinetic energy, potential energy and virtual work of the system were developed as presented in Chapter 4, Section 4.2.1.

Two main impedance bridges have been used to supply voltage and sense the induced voltage in the piezoelectric patch (Gurjar and Jalili, 2006, 2007). They are mainly pure capacitive and resistive-capacitive bridges as shown in Figure 5.3.

Figure 5.3 (a) Pure capacitive bridge, and (b) Resistive-Capacitive (R-C) bridge (Gurjar and Jalili, 2006).
The piezoelectric actuator is modeled as a capacitor and a voltage source in series as shown in the dashed box in Figure 5.3. $C_p$ represents the effective capacitance of the piezoelectric element and $V_s$ is the induced voltage in the piezoelectric patch. For the purpose of self-sensing, the piezoelectric actuator is connected in a bridge with other elements (i.e., the capacitors $C_1$, $C_r$ and/or resistors $R_1$, $R_2$). In this study, pure capacitive bridge network in employed as shown in Figure 5.3(a). $V_c(t)$ is the voltage applied across the capacitor bridge. Therefore, the voltage applied across the piezoelectric actuator can be written as:

$$V(t) = \frac{C_1}{C_1 + C_p} V_c(t) - \frac{C_p}{C_1 + C_p} V_s(t) \quad (5.1)$$

The self-induced voltage generated in the piezoelectric layer as a result of induced surface stress due to beam vibration can be written as (Gurjar and Jalili, 2006, 2007):

$$V_s(t) = \frac{1}{C_p} b E_p d_{31} \left( \frac{1}{2} (t_b + t_p) - y_n \right) [w'(L_1, t) - w'(0, t)] \quad (5.2)$$

Implementing Extended Hamilton’s principle, the equation of motion of the system can be derived as:

$$\rho(x) \frac{\partial^2 w(x,t)}{\partial t^2} + \frac{\partial^2}{\partial x^2} \left[ E I(x) \frac{\partial^2 w(x,t)}{\partial x^2} \right] + B \frac{\partial w(x,t)}{\partial t} + C \frac{\partial^2 w(x,t)}{\partial x^2} \frac{\partial}{\partial x} + \frac{M_p}{C_p + C_1} b E_p d_{31}
\left( \frac{1}{2} (t_b + t_p) - y_n \right) [w'(L_1, t) - w'(0, t)] G''(x) = -M_p V_c(t) G''(x) \quad (5.3)$$

with the boundary conditions:

$$w(0, t) = w'(0, t) = 0 \quad (5.4)$$

$$w''(L, t) = w'''(L, t) = 0 \quad (5.5)$$

The self-sensing nature appears in the equation of motion such that $V_c(t)$ appearing in the right hand side of the equation is employed for actuation, and at the same time, the sensing effect is
observed in the left hand side (with the extra term being a function of slope of the beginning and end points of piezoelectric layer).

That is, from Eq. (5.3), it is observed that the voltage generated in the piezoelectric layer, $V_s(t)$, is a function of the slope of the beginning and end point location of the piezoelectric layer which contain the information of the response of the system. In order to acquire this signal, its introduction into the output voltage of the capacitive bridge should be analyzed. For this, the bridge output voltage is expressed as (Gurjar and Jalili, 2007):

$$V_0(t) = \left[ \frac{C_p}{C_1 + C_p} - \frac{C_r}{C_1 + C_r} \right] V_c(t) + \frac{C_p}{C_1 + C_p} V_s(t)$$ (5.6)

In order to extract the induced voltage from the bridge output signal, the bridge should be balanced by selecting the appropriate bridge elements such as $C_1$ and $C_r$. Frequency analysis of the obtained self-induced signal would reveal information about the resonance frequencies of the system. Being able to have an insight into the resonance frequencies of the system, the effect of adsorbed mass on the MC surface can be analyzed running the system in dynamic mode.

### 5.2.2. Numerical simulations and results

In order to solve the obtained governing equations of motion, Eq. (5.3), it is discretized using Galerkin’s method (Gurjar and Jalili, 2007, Faegh et al. 2010). For this, the PDE (5.3) is converted into ODE using the following discretization:

$$w(x, t) = \sum_{j=1}^{n} \phi_j(x)q_j(t), j = 1,2, \ldots, n$$ (5.7)

with $\phi_j(x)$ and $q_j(t)$ being the clamped-free beam eigenfunction and generalized coordinates, respectively. Therefore, the equation of motion can be expressed as a function of time in a matrix form. The ODE for the system can be then represented as:
\[ M \ddot{q}(t) + D \dot{q}(t) + K q(t) = K_v V(t) \] (5.8)

where

\[ q = \{q_1, q_2, ..., q_i\} \rightarrow \dot{q} = \{\dot{q}_1, \dot{q}_2, ..., \dot{q}_i\} \]

\[ M = \{M_{ij}\}, M_{ij} = \int_0^L \rho A(x) \phi_j(x) \phi_i(x) \, dx, i, j = 1, 2, ..., n \]

\[ D = \{D_{ij}\}, D_{ij} = B \int_0^L \phi_j(x) \phi_i(x) \, dx + C \int_0^L \phi_j'(x) \phi_i(x) \, dx \] (5.9)

\[ K = \{K_{ij}\}, K_{ij} = \int_0^L EI(x) \phi_j''(x) \phi_i''(x) \, dx \]

\[ -\frac{M_{p0}}{C_1 + C_p} K_s [\phi_j'(L_1) - \phi_j'(0)][\phi_i'(L_1) - \phi_i'(0)] \]

\[ K_v = \{K_v_{ij}\}, K_v_{ij} = -M_{p0} \int_0^L \phi_j'(x) \delta(x - L_1) \, dx = -M_{p0} \phi_j'(L_1) \]

**Table 5.1** The system parameters used for modeling.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>(L)</td>
<td>486</td>
<td>(\mu m)</td>
</tr>
<tr>
<td>(L_1)</td>
<td>325</td>
<td>(\mu m)</td>
</tr>
<tr>
<td>(L_2)</td>
<td>360</td>
<td>(\mu m)</td>
</tr>
<tr>
<td>(b)</td>
<td>50</td>
<td>(\mu m)</td>
</tr>
<tr>
<td>(t_b)</td>
<td>4</td>
<td>(\mu m)</td>
</tr>
<tr>
<td>(t_p)</td>
<td>4</td>
<td>(\mu m)</td>
</tr>
<tr>
<td>(\rho_b)</td>
<td>2,330</td>
<td>kg m(^{-3})</td>
</tr>
<tr>
<td>(\rho_p)</td>
<td>6,390</td>
<td>kg m(^{-3})</td>
</tr>
<tr>
<td>(E_b)</td>
<td>105</td>
<td>GPa</td>
</tr>
<tr>
<td>(E_p)</td>
<td>104</td>
<td>GPa</td>
</tr>
<tr>
<td>(d_{31})</td>
<td>11</td>
<td>pC/N</td>
</tr>
</tbody>
</table>
The system parameters used for simulation are listed in Table 5.1. The forced vibration problem represented by ODE (5.8) was solved in Matlab with the input being the applied voltage to the ZnO stack mounted on active probes.

A harmonic voltage with the amplitude of 2.5 Volts and frequency close to system’s first natural frequency was applied and the system’s generalized coordinates for at least two modes, $q_1(t)$ and $q_2(t)$ were obtained. $\phi_i(x)$ was selected to be the admissible function of a clamped-free beam with the modified mass and stiffness properties of a beam with piezoelectric layer. The values of $C_l$ and $C_r$ were selected to be 30 pF. The deflection of MC with respect to location and time, $w(x,t)$, was calculated using Eq. (5.7).

Consequently, deflection of the tip of the MC, $w(L,t)$, output voltage, $V_0(t)$, and self-induced voltage, $V_s(t)$, were obtained and plotted in Figure 5.4(a,b). Taking the FFT of the system’s response, the first natural frequency of the system was obtained to be 52.99 kHz as illustrated in Figure 5.4(c). The effect of ultrasmall adsorbed mass was modeled as added surface mass over the gold-coated MC surface, length of $0-L_f$. The amount of adsorbed mass was assumed to be as low as 200 ng which resulted in the reduction of the 1st natural frequency to the amount of 1.8 kHz. The shift in natural frequency is depicted in Figure 5.4(d).

Sensitivity of vibration amplitude of the MC tip with respect to the selected $C_1$ was also studied and it was shown that the amplitude of tip vibration increased by increasing the value of $C_1$ as shown in Figure 5.5.

5.3. Adaptive Estimation

This section adopts an adaptation law to compensate for variation of piezoelectric material. Numerical simulations using this law is performed and presented as follows.
Figure 5.4 Numerical results: (a) tip deflection of microMC, \( w(L,t) \), (b) Input voltage, \( V_c(t) \), output voltage, \( V_0(t) \), and self-induced voltage, \( V_s(t) \), (c) FFT response of the system with 1st natural frequency highlighted, (d) the effect of added surface mass due to functionalization on the first natural frequency (Faegh et al. 2013a).
5.3.1. Adaptation law

Considering the fact that the properties of the piezoelectric materials vary with ambient temperature and time, compensating for these variations would dynamically improve the proposed self-sensing implementation. An adaptive compensatory self-sensing strategy (Law et al. 2003) is utilized in order to estimate the variations of the capacitance of piezoelectric material, $C_p$, with respect to time.

In order to compensate for time variation of $C_p$, a parameter called $\theta$ is defined which is the ratio of impedances in the bridge as follows:

$$\theta = \frac{C_p}{C_1 + C_p} \quad (5.10)$$

The estimation of the measured parameter $\theta$ is defined to be $\hat{\theta}$ which needs to be obtained. In order to find the estimated parameter, $\hat{\theta}$, a parametric error is defined as $\bar{\theta} = \theta - \hat{\theta}$ which should be driven to zero. Figure 5.6 shows the schematic of the adaptive self-sensing strategy. $\Psi(t)$ is a low power persistent excitation signal which is applied to measure $\theta$. It should be low enough such that it does not introduce vibration in the MC and contribute to the self-induced voltage.

![Figure 5.5](image-url) **Figure 5.5** Sensitivity of the vibration amplitude of the tip of MC with respect to $C_1$. 

[Image: Figure 5.5 showing the relationship between $C_1$ (pF) and Tip Amp. of Vibration (µm)]
Referring to Figure 5.6, the voltage of the upper branch of the bridge can be written as:

\[ V_1 = \theta \psi(t) \]  \hspace{1cm} (5.11)

with \( \hat{V}_1 \) being the estimation of \( V_1 \) as:

\[ \hat{V}_1 = \bar{\theta} \psi(t) \]  \hspace{1cm} (5.12)

Therefore, the bridge output voltage can be expressed as:

\[ V_0(t) = V_1(t) - \hat{V}_1(t) = (\theta - \bar{\theta})\psi(t) = \bar{\theta} \psi(t) = e(t) \]  \hspace{1cm} (5.13)

The proposed adaptation law for estimation of parameter \( \theta \) is as follows (Law et al. 2003):

\[ \dot{\bar{\theta}}(t) = -k_1 e_1 - P_1(t) \frac{\partial}{\partial \bar{\theta}} e_1^2 \]  \hspace{1cm} (5.14)

which can be further simplified as

\[ \dot{\bar{\theta}}(t) = -k_1 \psi \bar{\theta} - P(t) \psi^2 \bar{\theta} \]  \hspace{1cm} (5.15)

where \( k_1 \) and \( P(t) \) represent a constant gain and time-dependent adaptation gain, respectively. The time-varying adaptation gain can be replaced by a constant gain in order to simplify the calculation. Therefore, the update law can be simplified to
\[ \dot{\theta}(t) = -k_1 e_1 - P_0 \frac{\partial}{\partial \theta} e_1^2 \]  

(5.16)

and consequently

\[ \dot{\theta}(t) = -k_1 \psi \bar{\theta} - P_0 \psi^2 \bar{\theta} \]  

(5.17)

where \( P_0 \) represents the constant adaptation gain \((P_0 > 0)\). Available references (Gurjar and Jalili, 2007) and (Law et al. 2003) provide more information regarding the implementation of this adaptation law.

### 5.3.2. Simulation results for adaptive estimation

In this section, the equations of motion presented in Section 5.2.1. are simulated considering the estimated time-varying piezoelectric capacitance, \( C_p \) obtained through implementing adaptive estimation strategy. All other conditions are kept the same as those in Section 5.2.1. The system’s response along the beam at any time, \( w(x,t) \), is obtained. Consequently tip deflection and frequency response of the system are calculated and plotted as depicted in Figure 5.7.

![Figure 5.7](image)

**Figure 5.7** (a) Tip deflection of MC, \( w_L(x,t) \), (b) FFT response of the system with 1st resonance frequency highlighted.
It is shown that the first natural frequency of the system is obtained to be 51.6 kHz, which is about 1.3 kHz less than that obtained in Section 5.2.2. The contribution of the self-induced voltage in the bridge output signal is dependent on the unknown gain defined as $\theta$. A study was conducted to investigate the effect of the defined $\theta$ on the reasonable and maximum contribution of the $V_s(t)$. The result is depicted in Figure 5.8 demonstrating that by increasing $\theta$, the calculated self-induced voltage gets closer to the output voltage.

![Figure 5.8](image)

**Figure 5.8** The effect of $\theta$ on the calculation of self-induced voltage, $V_s(t)$.

### 5.4. Experimental Setup

In this section, the capability of the self-sensing strategy is validated experimentally and the results are compared with those obtained from the mathematical modeling presented in Sections 5.2 and 5.3. The same experiment was performed using a laser vibrometer as the read-out method to verify the self-sensing measurement technique.

A Veeco Active Probe® was utilized with the self-sensing capability. A pure capacitive bridge (Figure 5.3(a)) was used to send a harmonic voltage to the ZnO stack mounted at the base of each probe and at the same time receive the output voltage as a result of MC vibration. The Active Probe was mounted on a holder which was fixed on a 3D stage with submicron moving
capabilities in $x$-, $y$-, and $z$-directions. Figure 5.9(a) shows the experimental setup for implementing self-sensing strategy. The value of $\theta = 0.5$ was used experimentally. The same platform is placed under the laser vibrometer (Polytec CLV-2534) in order to measure MC vibrations through optical method as shown in Figure 5.9(b).

![Figure 5.9](image)

**Figure 5.9** Veeco Active Probe mounted on a holder (a) connected to the pure capacitive bridge for self-sensing implementation, (b) placed under laser vibrometer head.

### 5.4.1. Non-functionalized MC: verification with modeling

Measurement of the first resonance frequency of a non-functionalized MC was made in this section implementing both self-sensing strategy and optical read-out systems. In order to obtain the frequency at which the system resonates, the excitation frequency of system’s input was swept from 0 kHz to 100 kHz with resolution of 10 Hz. The amplitude was kept at 2.5 V. Taking the FFT of the output voltage obtained from the bridge, it was observed that the first resonance frequency of the non-functionalized MC was captured at 51.50 kHz which is in a great level of accuracy with the theoretical result obtained through implementing adaptive strategy. Having the input voltage, $V_c(t)$, and measuring the output voltage, $V_0(t)$, through the self-sensing bridge and
Figure 5.10 (a) FFT of the response of the system using self-sensing bridge, (b) Input, output and self-induced voltages, (c) FFT of the response of the system using laser vibrometer.
consequently calculating self-induced voltage, $V_s(t)$, relatively similar results were obtained compared to the theoretical part. Figure 5.10(a) shows the FFT of the response of the system while Figure 5.10(b) shows input, output and self-induced voltages. Performing the same experiment through a laser vibrometer, the resonance frequency of the system was captured to be 51.69 kHz which proves the capability of the self-sensing strategy with the precision of 99.63%. The obtained frequency response is depicted in Figure 5.10(c). Table 5.2 shows a comparison between the experimental results to theoretical ones obtained from Sections 5.2 and 5.3.

**Table 5.2** Comparing the results obtained from mathematical modeling presented in Sections 2 and 3 with the experimental results.

<table>
<thead>
<tr>
<th></th>
<th>First Resonance Frequency, $w_{n1}$ (kHz)</th>
<th>Precision (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theory Section 5.2: Self-sensing</td>
<td>52.9</td>
<td>97.48</td>
</tr>
<tr>
<td>Theory Section 5.3: Self-sensing, Adaptive estimation</td>
<td>51.6</td>
<td>99.82</td>
</tr>
<tr>
<td>Experiment: Self-sensing</td>
<td>51.50</td>
<td>99.63</td>
</tr>
<tr>
<td>Experiment: Laser vibrometer</td>
<td>51.69</td>
<td>—</td>
</tr>
</tbody>
</table>

**5.4.2. Functionalized MC: detection of adsorbed mass**

In this section, the main application of the developed platform is tested. Same active probe equipped with a self-sensing read-out mechanism was implemented to detect the adsorbed mass over the gold surface. The system was operated in dynamic mode where the MC was brought into excitation by applying a harmonic voltage to the self-sensing bridge with a frequency close to system’s first resonance frequency.

Thiol groups which attach to many biomolecules were immobilized over the MC surface by making a covalent binding to gold creating a self-assembled monolayer. The gold-coated surface
was washed in acetone, ethanol and DI water for 10 minutes. The main challenge in functionalizing the self-sensing active probe is the integrated electronics on the base of the probe. Therefore, washing and submerging it into any solution comes with the risk of damaging or destroying the whole platform. In order to address this issue, a Teflon chamber was designed such that creating any droplet of liquids over the chamber’s surface was made possible. The Active Probe was then mounted on a holder and placed over a 3D stage with resolution of submicron which was used to place MC tip into the droplet such that it does not wet any electronics in the vicinity of the probe.

A 0.1M of aminoethanethiol solution was prepared by dissolving 2-aminoethanethanol powder in deionized water. The tip of the MC was dipped into a droplet of the prepared solution. As a result, self-assembled monolayer of aminoethanethiol was formed over the gold surface by attachment of thiol groups to gold.

In order to find the frequency at which the system resonates after functionalization, the excitation frequency was swept between 0-100 kHz. The response of the system was measured by both self-sensing bridge and the laser vibrometer as shown in Figure 5.11. The amount of shift in the first resonance frequency of the system was observed to be equal to 3.98 kHz and 3.69 kHz as obtained from self-sensing bridge and laser vibrometer, respectively. Measurements were performed multiple times and a frequency sweep was carried out each time. The standard deviation was calculated to be 0.2098. The results obtained by the laser vibrometer reinforce those obtained by the self-sensing bridge. However, there are certain limitations with implementing laser vibrometer measurements in liquid media which can be addressed by adopting the self-sensing platform.
Figure 5.11 Shift in the first resonance frequency measured by (a) self-sensing bridge, (b) Laser vibrometer.

The amount of absorbed mass can be quantitatively calculated implementing the mathematical modeling framework presented in Sections 5.2 and 5.3. Comparison of the experimental results to those obtained from Sections 5.2 and 5.3 verifies the accuracy of the mathematical models. Adopting the mathematical modeling presented in this study the amount of adsorbed masses can be quantified having the shifts in resonance frequency. Figure 5.12 shows the shift in the first resonance frequency as a result of adsorbed mass utilizing the mathematical modeling framework providing the relationship between the added mass and frequency change.

The amount of adsorbed mass measured with self-sensing circuit and laser vibrometer was calculated to be 486.04 ng and 450.24 ng, respectively, utilizing this method of quantification. The sensitivity of the reported platform was measured to be about 122 pg/Hz.
5.5. Chapter Summary

A unique laser-less MC-based sensor which utilizes a Veeco Active Probe as a piezoelectric MC with self-sensing capabilities was proposed in this study. A pure capacitive bridge was designed to implement the detection platform in the self-sensing mode where the system was excited by applying a harmonic voltage to the piezoelectric layer which simultaneously produces output voltage as a result of the system’s response. Utilizing the proposed platform, one self-sensing bridge can be exploited for both exciting the system and measuring the response of the system, thus eliminating the need for bulky and expensive optical based detection techniques.

Three main sections were presented for proving the concept of self-sensing methodology and testing its capability to be used as a mass sensing platform. A comprehensive distributed-parameters modeling framework was proposed for the self-sensing MC biosensor performing in dynamic mode. Since piezoelectric properties of material vary at the nanoscale, an adaptation law was exploited in order to compensate for the changes of piezoelectric properties of the ZnO
stack embedded in the active probe. Numerical simulations were carried out in Matlab and presented. It was shown that the level of accuracy for measuring the fundamental resonance frequency of MC increases from 97.48% to 99.82% using adaptation strategy. In order to utilize the platform for mass sensing purposes, the capability of measurement system was compared and verified with optical-based read-out and a 99.63% accuracy was illustrated.

Implementing the proposed platform as a biological sensor, an extensive experimental setup was built to detect thiol groups immobilized over the MC surface. The shift in the first resonance frequency as a result of mass adsorption was obtained through both optical and self-sensing methods indicating the immobilization of mass over the MC surface.

The present study paves the way towards implementing such a system for detection of the concentration of any type of biomolecules and further developing a laser-less, cost-effective and portable diagnostic kit for any biomarker protein or biomolecule. It is planned to improve the proposed platform with higher sensitivity and selectivity for detection of smaller proteins such as PSA and myocardial infarction marker proteins, and also hybridization of DNA with the implementation of sensor and reference MC in the diagnostic platform.
CHAPTER 6**

IMPLEMENTATION OF SELF-SENSING PIEZOELECTRIC MICROCANTILEVER SENSOR AT ITS ULTRAHIGH MODE FOR MASS DETECTION

6.1. Introduction

The demand for detection of ultrasmall masses and biological species drives the need for developing ultrasensitive MC-based sensors. Sensitivity has been recognized as one of the main elements in the success of sensors. As far as sensitivity is concerned, several investigations have been carried out to enhance the functionality of MCs. Two common operational modes of MC-based techniques are static mode where changes in the surface stress is measured (Arntz et al. 2003, Pei et al. 2003, Wu et al. 2001, Huber et al. 2007, Shua et al. 2008, Stiharu et al. 2005, Álvarez and Tamayo, 2005, Thaysen et al. 2001, Grogan et al. 2002, Yena et al. 2009, Zhou et al. 2009, Cherian et al. 2005, Backmann et al. 2005, Zhang et al. 2006) and dynamic mode where differences in resonance frequency of MC are detected (Campbell et al. 2007, Ilic et al. 2001, 2004, Lee et al. 2004, Thundat et al. 1995, Gupta et al. 2004, Ono et al. 2003, Ekinci et al. 2004). It has been shown that operating MC in its dynamic mode provides higher sensitivity compared to measurement of surface stress change in static mode. Introducing stress concentration regions over MC surface has been proven to enhance sensitivity using finite element modeling (Amarasinghe et al. 2009). Employing nanoparticle-enhanced MCs (Chaa et al. 2009, Lee et al. 2009), assembling carbon nanotubes (He et al. 2006) and nanowires (Lee et al. 2007) over MC surface have been reported to dramatically improve sensing capabilities and

** The contents of this chapter may have come directly from our previous publication (Faegh et al. 2013c).
MC sensitivity. Geometrical modification of MCs has been proven both numerically (Finite element methods) and experimentally to substantially affect MC sensitivity (Fletcher et al. 2008, Lim et al. 2010, Loui et al. 2008, Morshed et al. 2009, Shin et al. 2008).

One of the most promising methods adopted for sensitivity enhancement was operating MC in modes other than its fundamental resonance flexural mode. Higher quality factor thus higher sensitivity was achieved resonating MC in its in-plane mode comparing to out-of plane resonance mode as a result of decreasing liquid drag force (Tao et al. 2011). Using torsional and lateral resonance of MCs was also reported to result in an order of magnitude higher mass sensitivity compared to conventional fundamental flexural mode (Sharos et al. 2004, Xie et al. 2007). Resonating MC in high modes generally has been proven both numerically and experimentally to increase quality factor thus sensitivity. Higher sensitivity was reported operating MC in its second (Jin et al. 2006) and fourth (Dohn et al. 2005) resonance bending mode for mass sensing applications. Operating MC in its seventh flexural mode resulted in two orders of magnitude increase in sensitivity compared to its fundamental flexural mode as reported by Zurich research laboratory group (Ghatkesar et al. 2007).

Although high mode resonating MC has been investigated and implemented as an effective solution for sensitivity enhancement, there has not been any analytical distributed-parameters modeling which addresses all dynamics and phenomenon of the system in its higher modes. Conventional lumped-parameter model has been used correlating mass change to frequency shift which is not capable of describing the behavior of system in its high modes. As a result, for the first time in this study, we are presenting a comprehensive mathematical modeling for a piezoelectric self-sensing MC biological sensor operating at its ultrahigh mode (20th mode). Effect of adsorbed mass on the frequency shift was investigated and reported analytically for
high modes as well as fundamental and lower modes. Mode convergence theory was adopted in order to get the best estimation of resonance frequencies at different modes.

An extensive experimental setup was developed operating the MC sensor at different resonance modes. Veeco Active Probe® equipped with piezoelectric layer was used operating in dynamic mode. A capacitance bridge network is utilized to implement the active probe in self-sensing mode. Detection of ultrasmall adsorbed biological species, which is Amino groups of aminoethanethiol solution, was made possible through the proposed self-sensing piezoelectric-based MC sensor. Operating MC in high resonance mode and detecting the shift in high mode resonance frequency, the quality factor was estimated and reported. Similar mass detection setup was built and performed utilizing optical-based method comparing and verifying the capability of the self-sensing platform for mass detection.

6.2. Mathematical Modeling

Distributed-parameter mathematical modeling is presented in this section using Extended Hamilton’s principle for describing spatiotemporal behavior of self-sensing MC sensor. The MC utilized is Veeco Active Probe® with extended piezoelectric layer embedded in its structure which is adopted to implement the system in self-sensing mode. Figure 6.1 shows the MC used.

Figure 6.1 (a) Veeco Active probe® used in this study for modeling and experiment, (b) schematic of the beam used for modeling.
being hold on a base with piezoelectric (ZnO) layer which is gold coated on the surface. As seen in the figure, MC is non-uniform in its cross section and depth which is all accounted for in the comprehensive modeling presented. Euler Bernoulli beam theory is adopted in developing the model. Using Euler Bernoulli beam theory was proven to model the current system with high level of accuracy comparing to plate theory (Faegh and Jalili, 2013). Small deflection and linear system properties are assumed. Other system properties and geometry are the same as those presented in Chapters 4 and 5. Equation of motion of the system is derived using Extended Hamilton’s principle. Using distributed-parameter modeling and assuming that beam only extends in x-direction, kinetic and potential energies and virtual work of the system were derived as shown in Eqs. (4.1-4.10).

Using the pure capacitive bridge (Figure 5.3a) and exploiting the piezoelectric self-induced voltage and ultimately output voltage of the system (Eqs. 5.1 and 5.2), the following equations of motion for the self-sensing piezoelectric MC was derived

\[ \rho(x) \frac{\partial^2 w(x,t)}{\partial t^2} + \frac{\partial^2}{\partial x^2} \left[ E I(x) \frac{\partial^2 w(x,t)}{\partial x^2} \right] + B \frac{\partial w(x,t)}{\partial t} + C \frac{\partial^2 w(x,t)}{\partial x \partial t} - \frac{M_p0}{c_p+c_t} b E_p d_{31} \]

\[ \left( \frac{1}{2} (t_b + t_p) - y_n \right) [w'(L_1, t) - w'(0, t)] G''(x) = -M_p0 V_c(t) G''(x) \]  

with the boundary conditions

\[ w(0, t) = w'(0, t) = 0 \]  

\[ w''(L, t) = w'''(L, t) = 0 \]  

This equation is numerically solved and simulated at high modes in the following section.

6.3. Numerical Simulations and Results

Galerkin’s method (Gurjar and Jalili, 2007) is used to solve the obtained governing equations of motion (Eq. 6.1). ODE is obtained using the following descretization
\[ w(x,t) = \sum_{j=1}^{n} \phi_j(x) q_j(t), \quad j = 1, 2, \ldots, n \]  

(6.3)

where \( \phi_j(x) \) and \( q_j(t) \) are the clamped-free beam eigenfunction and generalized coordinates, respectively. Using the above discretization in time and space, ODE for the system can be expressed as

\[ M\ddot{q}(t) + D\dot{q}(t) + Kq(t) = K_pV(t) \]  

(6.4)

where \( M, D, K, \) and \( K_p \) were defined in Eq. (5.9).

System parameters used for simulation are provided in Chapter 5. Forced vibration problem represented by ODE (6.3) was solved in Matlab with the input being a harmonic voltage applied to the ZnO stack mounted on active probes. \( \phi_i(x) \) was selected to be the admissible function of a clamped-free beam with modified mass and stiffness properties of a beam with piezoelectric layer. Values of \( C_l \) and \( C_r \) were selected to be 30 pF considering the effect of stray capacitances in experiment.

The equation was simulated for different modes as high as 20\textsuperscript{th} mode and system’s response, deflection of MC with respect to location and time, \( w(x,t) \) was calculated using Eq. (6.14).

**Figure 6.2** Normalized Mode Shapes (MS) (a) MS 1-5, and (b) MS 4-7.
In order to analyze the sensing characteristics of the system being implemented in dynamic mode, resonance frequencies of the system should be obtained. Taking Fast Fourier Transform (FFT) of system’s response, $w(x,t)$, the resonance frequencies were obtained. Normalized mode shapes were calculated and are partially (1st - 7th modes) depicted in Figure 6.2. FFT plot illustrating first twenty resonance frequencies of the system is shown in Figure 6.3. Mode convergence study was conducted calculating responses of the system in different modes and monitoring the convergence of the resonance frequencies as number of modes, $n$, increases. Table 6.1 shows the calculated resonance frequencies using $n=12-28$ order model illustrating the convergence of resonance frequencies as increasing the model order. Based on convergence theory, the type and number of trial functions influence the convergence of the approximate solution to the actual one. If the trial function is appropriately selected (not a simple polynomial), then it can be expected that the first $\frac{n}{2}$ solutions are accurate if running the system for $n^{th}$ order model. Therefore, in this study, the simulation runs for $20^{th}$ order model providing converged and accurate solution for the first ten eigenvalues.

Figure 6.3 FFT of the response of the system, $w(x, t)$ where $n=20$, depicting a) first 10 and b) next 10 resonance frequencies of the system.
Simulation was performed for different amount of masses immobilized over MC surface on its active area which is gold coated (length \(0-L_1\)). Masses in the range of 10 pg-10 μg were assumed to be immobilized and simulation was carried out for 20\(^{th}\) mode which have been proven to produce converged results. The shift in the resonance frequency as a result of each amount of mass immobilization was calculated for each mode running the system for \(n=20\). Figure 6.4 shows the frequency shift plots versus different immobilized mass for some modes.

Table 6.2 shows the frequency shift as a result of mass immobilization for all modes 1\(^{st}\)-20\(^{th}\). It is clearly shown that sensitivity of mass detection increases with the number of modes. In order to

Table 6.1. Calculated resonance frequencies using different order model (\(n\)).

<table>
<thead>
<tr>
<th>Mode</th>
<th>(n=12)</th>
<th>(n=14)</th>
<th>(n=15)</th>
<th>(n=16)</th>
<th>(n=17)</th>
<th>(n=18)</th>
<th>(n=20)</th>
<th>(n=22)</th>
<th>(n=24)</th>
<th>(n=26)</th>
<th>(n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>53</td>
<td>53</td>
<td>53</td>
<td>53</td>
<td>53</td>
<td>53</td>
<td>53</td>
<td>53</td>
<td>53</td>
<td>53</td>
<td>53</td>
</tr>
<tr>
<td>2</td>
<td>231</td>
<td>231</td>
<td>230</td>
<td>230</td>
<td>230</td>
<td>230</td>
<td>229</td>
<td>230</td>
<td>227</td>
<td>227</td>
<td>227</td>
</tr>
<tr>
<td>3</td>
<td>394</td>
<td>394</td>
<td>393</td>
<td>393</td>
<td>393</td>
<td>393</td>
<td>391</td>
<td>391</td>
<td>390</td>
<td>390</td>
<td>390</td>
</tr>
<tr>
<td>4</td>
<td>929</td>
<td>929</td>
<td>928</td>
<td>928</td>
<td>928</td>
<td>927</td>
<td>927</td>
<td>927</td>
<td>927</td>
<td>927</td>
<td>927</td>
</tr>
<tr>
<td>5</td>
<td>1482</td>
<td>1474</td>
<td>1474</td>
<td>1474</td>
<td>1474</td>
<td>1472</td>
<td>1471</td>
<td>1464</td>
<td>1463</td>
<td>1456</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2041</td>
<td>2031</td>
<td>2028</td>
<td>2028</td>
<td>2028</td>
<td>2026</td>
<td>2024</td>
<td>2023</td>
<td>2020</td>
<td>2019</td>
<td>2016</td>
</tr>
<tr>
<td>7</td>
<td>3002</td>
<td>2990</td>
<td>2971</td>
<td>2969</td>
<td>2964</td>
<td>2956</td>
<td>2951</td>
<td>2946</td>
<td>2946</td>
<td>2945</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3975</td>
<td>3930</td>
<td>3909</td>
<td>3895</td>
<td>3895</td>
<td>3890</td>
<td>3886</td>
<td>3881</td>
<td>3875</td>
<td>3873</td>
<td>3866</td>
</tr>
<tr>
<td>9</td>
<td>5145</td>
<td>4994</td>
<td>4990</td>
<td>4987</td>
<td>4978</td>
<td>4971</td>
<td>4970</td>
<td>4967</td>
<td>4962</td>
<td>4960</td>
<td>4956</td>
</tr>
<tr>
<td>10</td>
<td>6714</td>
<td>6357</td>
<td>6231</td>
<td>6216</td>
<td>6200</td>
<td>6143</td>
<td>6116</td>
<td>6081</td>
<td>6077</td>
<td>6074</td>
<td>6073</td>
</tr>
<tr>
<td>11</td>
<td>8481</td>
<td>7901</td>
<td>7634</td>
<td>7548</td>
<td>7548</td>
<td>7519</td>
<td>7489</td>
<td>7470</td>
<td>7469</td>
<td>7467</td>
<td>7463</td>
</tr>
<tr>
<td>12</td>
<td>10240</td>
<td>9755</td>
<td>9488</td>
<td>9328</td>
<td>9251</td>
<td>9238</td>
<td>9224</td>
<td>9212</td>
<td>9212</td>
<td>9210</td>
<td>9207</td>
</tr>
<tr>
<td>13</td>
<td>11930</td>
<td>11680</td>
<td>11390</td>
<td>11050</td>
<td>10770</td>
<td>10690</td>
<td>10570</td>
<td>10550</td>
<td>10530</td>
<td>10530</td>
<td>10530</td>
</tr>
<tr>
<td>14</td>
<td>14150</td>
<td>13960</td>
<td>13540</td>
<td>12990</td>
<td>12570</td>
<td>12380</td>
<td>12330</td>
<td>12310</td>
<td>12300</td>
<td>12300</td>
<td>12300</td>
</tr>
<tr>
<td>15</td>
<td>16160</td>
<td>15850</td>
<td>15420</td>
<td>15080</td>
<td>14690</td>
<td>14630</td>
<td>14580</td>
<td>14580</td>
<td>14580</td>
<td>14570</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>18420</td>
<td>18210</td>
<td>17900</td>
<td>17050</td>
<td>16430</td>
<td>16380</td>
<td>16290</td>
<td>16290</td>
<td>16290</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>21020</td>
<td>20790</td>
<td>19520</td>
<td>18620</td>
<td>18510</td>
<td>18440</td>
<td>18420</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>23510</td>
<td>22480</td>
<td>21640</td>
<td>21160</td>
<td>21120</td>
<td>21030</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>25810</td>
<td>24910</td>
<td>23710</td>
<td>23410</td>
<td>23320</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>29190</td>
<td>28270</td>
<td>26720</td>
<td>26010</td>
<td>25940</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
better visualize the increase of sensitivity with mode number, shift in the resonance frequency versus mode number is plotted in Figure 6.5 for different amount of mass immobilization.

6.4. Experimental Setup and Results

In this section, an experimental setup was built in order to i) verify the functionality of self-sensing method to measure high mode responses of the system, and ii) implement the self-sensing platform to detect ultrasmall adsorbed mass over MC surface. Veeco Active Probe® equipped with piezoelectric layer (ZnO stack) was utilized to operate the system in self-sensing mode (self-excitation/self-sensing) through using a capacitance bridge network. The active probe was mounted on a holder which was fixed on a 3D stage. Figure 6.6(a) shows the experimental setup for implementing self-sensing strategy. The same platform is placed under laser vibrometer (Polytec CLV-2534) in order to measure MC vibrations through optical method as shown in Figure 6.6(b).

The excitation frequency was swept between 1 kHz to 10 MHz and the resonance frequencies were captured using both self-sensing and optical methods. Resonance frequencies up to tenth mode were measured using self-sensing method. On the other hand, resonance frequencies were measured using optical method running the system up to its third mode. Higher resonance modes could not be captured due to the limitations of the available version of laser vibrometer.
Figure 6.4 Frequency shift as a result of different amount of mass immobilization on (a) 10th mode, (b) 11th mode, (c) 12th mode, (d) 15th mode, with $n=20$.

Figure 6.5 Shift in resonance frequency calculated for different mode numbers as a result of different amount of mass immobilization.
Table 6.2. Shift in the resonance frequency as a result of mass immobilization (1 ng-10 μg) for all modes 1st-20th.

<table>
<thead>
<tr>
<th>Mode#</th>
<th>1 ng</th>
<th>2 ng</th>
<th>5 ng</th>
<th>10 ng</th>
<th>20 ng</th>
<th>50 ng</th>
<th>100 ng</th>
<th>10 μg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>79</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>133</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>7</td>
<td>15</td>
<td>71</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>7</td>
<td>14</td>
<td>177</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>6</td>
<td>15</td>
<td>29</td>
<td>430</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>8</td>
<td>21</td>
<td>43</td>
<td>92</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>11</td>
<td>27</td>
<td>52</td>
<td>432</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>7</td>
<td>14</td>
<td>34</td>
<td>68</td>
<td>764</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>8</td>
<td>16</td>
<td>39</td>
<td>78</td>
<td>186</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>12</td>
<td>24</td>
<td>60</td>
<td>119</td>
<td>688</td>
</tr>
<tr>
<td>12</td>
<td>2</td>
<td>4</td>
<td>7</td>
<td>14</td>
<td>28</td>
<td>70</td>
<td>138</td>
<td>1148</td>
</tr>
<tr>
<td>13</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td>12</td>
<td>24</td>
<td>60</td>
<td>118</td>
<td>301</td>
</tr>
<tr>
<td>14</td>
<td>2</td>
<td>4</td>
<td>10</td>
<td>21</td>
<td>40</td>
<td>99</td>
<td>195</td>
<td>851</td>
</tr>
<tr>
<td>15</td>
<td>2</td>
<td>5</td>
<td>12</td>
<td>25</td>
<td>49</td>
<td>122</td>
<td>242</td>
<td>974</td>
</tr>
<tr>
<td>16</td>
<td>3</td>
<td>6</td>
<td>14</td>
<td>28</td>
<td>55</td>
<td>135</td>
<td>266</td>
<td>1628</td>
</tr>
<tr>
<td>17</td>
<td>4</td>
<td>7</td>
<td>17</td>
<td>35</td>
<td>69</td>
<td>170</td>
<td>334</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>4</td>
<td>8</td>
<td>21</td>
<td>43</td>
<td>86</td>
<td>215</td>
<td>423</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>5</td>
<td>11</td>
<td>26</td>
<td>52</td>
<td>104</td>
<td>256</td>
<td>504</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>6</td>
<td>12</td>
<td>30</td>
<td>60</td>
<td>120</td>
<td>297</td>
<td>583</td>
<td></td>
</tr>
</tbody>
</table>

Table 6.3 Resonance frequencies running the system in its tenth mode calculated theoretically and measured experimentally.

<table>
<thead>
<tr>
<th>Resonance Frequencies (kHz)</th>
<th>Theory</th>
<th>Experiment Self-Sensing</th>
<th>Experiment Optical</th>
<th>Quality Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>$w_{r1}$</td>
<td>53</td>
<td>48.16</td>
<td>48.31</td>
<td>126.94</td>
</tr>
<tr>
<td>$w_{r2}$</td>
<td>229</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$w_{r3}$</td>
<td>391</td>
<td>320.6</td>
<td>321.1</td>
<td>144.5</td>
</tr>
<tr>
<td>$w_{r4}$</td>
<td>927</td>
<td>908.6</td>
<td>910.9</td>
<td>180.55</td>
</tr>
<tr>
<td>$w_{r5}$</td>
<td>1472</td>
<td>1123</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$w_{r6}$</td>
<td>2024</td>
<td>1935</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$w_{r7}$</td>
<td>2951</td>
<td>2840</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$w_{r8}$</td>
<td>3886</td>
<td>3611</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$w_{r9}$</td>
<td>4970</td>
<td>4803</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$w_{r10}$</td>
<td>6116</td>
<td>5667</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$w_{r11}$</td>
<td>7489</td>
<td>6908</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 6.6 Veeco Active Probe mounted on a holder (a) connected to the pure capacitive bridge mounted on a bread board for self-sensing implementation, (b) placed under laser vibrometer head.

Figure 6.7(a) shows the resonance frequencies running the system in its tenth mode while measuring system’s response through self-sensing bridge. Similar study was conducted using laser vibrometer measuring resonance frequencies up to third mode, as shown in Figure 6.7(b) which verifies the results obtained by self-sensing method. Table 6.3 shows a comparison between the calculated resonance frequencies using distributed-parameters modeling and measured resonance frequencies experimentally (self-sensing/optical). It was observed that the proposed mathematical model was able to approximately predict the resonance frequencies measured experimentally with a reasonable level of accuracy. Quality factor was calculated for the first three modes measured optically as listed in Table 6.3. Higher quality factor was observed with increasing the number of modes therefore higher sensitivity is expected at higher modes.
Once the capability of the self-sensing platform to measure system’s response was verified both with optical method and theoretical result, the platform was implemented as a mass sensor to detect added ultrasmall adsorbed mass. For this purpose, Amino groups of aminoethanethiol was immobilized over MC on the gold surface. 2-aminoethanethiol was purchased from Sigma.

Active Probe was first washed in acetone and ethanol for 10 minutes. A Teflon chamber was designed in order to dip the MC into a droplet of liquid such that it only wets the MC and does not proceed to the electronic circuits. A 3D stage with resolution of submicron was used in order to navigate MC in x-, y-, and z-direction and place it into the liquid.

A 0.1M of aminoethanethiol solution was prepared by dissolving 2-aminoethanethiol powder into deionized water. A single layer of aminoethanethiol was formed on the gold surface by attachment of thiol groups to gold. The change in resonance frequencies is measured both by self-sensing and optical methods and recorded. Figures 6.8 and 6.9 illustrate the change in resonance frequencies as a result of mass immobilization over MC surface measured by self-

**Figure 6.7** Resonance frequencies measured by (a) self-sensing method running the system in its tenth mode, (b) laser vibrometer running the system in its third mode.
sensing circuit and laser vibrometer respectively. A high level of accuracy was observed comparing the resonance frequency shifts measured by self-sensing and optical methods.

It was observed that the shift in resonance frequency as a result of a definite amount of mass immobilization increases with increasing number of modes. Figure 6.10 illustrates the amount of increase in frequency shift for the first three modes of vibration indicating the accuracy of the reported self-sensing platform to detect absorbed mass over MC surface. It is clearly shown that the sensitivity of measurement in detection of frequency shift increases with the number of modes. Correlating the amount of frequency shift obtained experimentally to the theoretical results, the amount of immobilized mass can be estimated to be about 1-2 µg.

Figure 6.8 Shift in the resonance frequencies in the a) first mode, b) second mode, and c) third mode of vibration measured by self-sensing platform.
Figure 6.9 Shift in the resonance frequencies in the a) first mode, b) second mode, and c) third mode of vibration measured by laser vibrometer.

Figure 6.10 Increase in frequency shift with the first three modes of vibration measured with self-sensing platform and laser vibrometer.
6.5. Chapter Summary

Sensitivity enhancement of MC-based systems being one of the key elements in success of any sensor has been investigated extensively. Different studies were conducted to enhance the sensitivity of any type of MC systems including geometry modification, exploiting nanoparticles and carbon nanotubes in the structure of the system, and resonating MCs in vibration modes other than flexural mode such as lateral and torsional modes. Resonating MCs in high modes has been one of the most promising approaches in sensitivity enhancement through increasing quality factor.

A comprehensive mathematical model was presented in this study which extensively describes the dynamics and behavior of MC operating at its ultrahigh mode. Distributed-parameters modeling using Extended Hamilton’s principle was developed for MC-based sensor being implemented in self-sensing mode. Mode convergence theory was used to accurately estimate the resonance frequencies of the system at high modes. Extensive numerical simulations using Matlab were carried out for the proposed model and also to investigate the effect of mass immobilization over MC surface.

A complete experimental setup was built in order to verify the theoretical modeling framework. Laser vibrometer was utilized in order to optically measure the response of MC up to its third mode. The results were compared to self-sensing methodology thus verifying the capability of self-sensing method to characterize system’s behavior at high modes. The system was then implemented as a biosensor for detection of ultrasmall mass which was Amino groups of aminoethanethiol solution being immobilized over MC surface. The shift in the resonance frequencies were measured and plotted and the amount of mass adsorption was then estimated
utilizing the mathematical modeling framework. It was proved that resonating MC at modes higher than its fundamental mode would clearly increase the sensitivity of the system to detect the adsorbed mass as a result of increase in quality factor of the system.
CHAPTER 7††

DETECTION OF GLUCOSE IN A SAMPLE SOLUTION USING THE DEVELOPED
SELF-SENSING PLATFORM

7.1. Introduction

Reducing the dimensions of electromechanical systems to micro- and nano-scale has enabled the identification of biological molecules utilizing mechanical biosensors. High-throughput diagnosis and analytical sensing require advanced biosensing tools exploiting high affinity of biomolecules. There are a number of useful biosensing techniques such as electrophoretic separation where spatiotemporal separation of analytes is possible. Another important technique is identifying the changes in the mass or optical properties of target proteins using spectrometric assays. Identification and quantification of target biomolecules due to high affinity which is based on molecular recognition has been known as one of the most reliable biosensing mechanisms.

There are two main elements in a biosensor which are i) sensitive biological receptor probe which interacts with target proteins and ii) transducer which transforms the molecular recognition into detectable physical quantity. There are a number of instruments equipped with these elements developed for biodetection such as quartz crystal microbalance (QCM), surface plasmon resonance (SPR), enhanced-Raman spectroscopy, field effect transistors (FET) and MC-based biosensors. Among these techniques, MC-based biosensors have emerged as an outstanding sensing tool for being highly sensitive, label-free, and cost effective (Arntz et al.††).

†† The contents of this chapter may have come directly from our previous publication (Faegh et al. 2013b).
2003, Pei et al. 2003, 2004, Shin and Lee, 2006, Shin et al. 2008, Sree et al. 2010a, b, Wu et al. 2001, Yang and Chang, 2010). Detection of proteins and pathogens, physical parameters, (Corbeil et al. 2002, Lee, C., and Lee, G., 2003), and biochemical agents, (Pinnaduwage et al. 2004, Tang et al. 2004, Ji et al. 2000, 2001, Ilic et al. 2001, Zhang and Feng, 2004, Gupta et al. 2004), has been enabled utilizing this type of sensors. All MC-based sensors are equipped with a read-out device which is capable of measuring the mechanical response of the system. There are a number of conventional read-out systems among which optical based measurement is the most commonly used. They have been widely used in AFM and measure the mechanical changes of the system by calculating the difference of the angle of laser beam reflected from the surface of the MC. Even though being sensitive, there are certain limitations with this technique which are mainly high cost, being bulky and surface preparation requirement. Moreover, laser alignment and adjustment and the requirement of the sample solution and liquid chamber to be transparent imposes serious challenges for adopting such a method as a read-out device in molecular sensing tools. In order to address all the aforementioned challenges, we are proposing a unique self-sensing technique where a single piezoelectric layer deposited over MC surface performs as both an

![Image](image_url)

**Figure 7.1** Veeco Active Probe® with ZnO self-sensing layer deposited on the probe.
actuator and sensor. Direct piezoelectric property is used to sense the self-induced voltage generated in the piezoelectric layer as a result of beam deformation. At the same time, inverse property of piezoelectric material is used to generate deformation and bring the system into vibration as a result of applying a harmonic voltage to it. Therefore, a single piezoelectric layer embedded in the MC sensor is utilized to both actuate and sense the system through implementing a resonating circuit. This provides a simple and inexpensive platform for mass sensing and detection purposes with opportunity of miniaturizing the platform. The piezoelectric MC used is Veeco Active Probe with a ZnO stack embedded in MC providing the self-sensing capability as shown in Figure 7.1.

There are two main operational modes of MC-based sensors which are i) static and ii) dynamic modes. Most of the studies regarding identification of molecular affinities have been performed in the static mode where the induced surface stress as a result of deflection of MC from a stable baseline is used to measure molecular binding, (Pei et al. 2003, 2004, Wu et al. 2008, Shua et al. 2008, Stiharu et al. 2005, Thaysen et al. 2001, Grogan et al. 2002, Yena et al. 2009, Zhou et al. 2009). Arrays of MCs have been used for high-throughput measurements, (Arntz et al. 2003, Huber et al. 2007, A´ lvarez and Tamayo, 2005, Thaysen et al. 2001, Cherian et al. 2005, Backmann et al. 2005, Zhang et al. 2006). On the other hand, in dynamic mode, the system is brought into excitation at or near its resonance frequency, (Ruzziconi et al. 2012). The shift in the resonance frequency as a result of molecular recognition yields a good insight into the amount of adsorbed mass. Different studies have been conducted enhancing the sensitivity of MEMS, (Faegh et al. 2013c, Jin et al. 2006). In a study by Zhang and Turner, (2005a,b), parametric resonance-based mass sensing was reported measuring dc offset instead of frequency shift resulting in 1-2 orders of magnitude sensitivity enhancement.
One important factor determining the success of all biological sensors performing based on analytical sensing of high affinity of biomolecules is the ability of the sensor to operate in liquid media with high sensitivity. However, high dampening and viscous effect of solutions indeed imposes a burden on the performance of biological sensor in liquid environment. Some approaches have been developed to overcome this challenge by i) operating the system in humid gas-phase media, (Lee et al. 2009), and ii) dipping the sensing probe in the solution, and then removing and desiccating it and finally doing the measurement, (Oliviero et al. 2008). However these methods deviate from the reality, increase the interference of unspecific biomolecules, and prohibit real-time and continuous monitoring.

This challenge is addressed in this study by operating the reported self-sensing biosensor in dynamic mode in the liquid media by exciting the system in high frequency.

A self-sensing circuit is used to apply the voltage to MC. Circuit’s resonance frequency and the shift of the resonance frequency as a result of the change in the capacitance due to molecular binding is measured while operating the system in liquid, therefore allowing for rapid, continuous, and highly sensitive measurement of molecular recognition.

In this study, the reported diagnostic kit is implemented for detection of concentration of glucose in sample solution. An extensive experimental setup is built including a reference MC and a sensor MC. Active surface of the sensor MC is functionalized with the receptor biomolecule which is glucose oxidase (GoX) in this study. MCs are then exposed to different level of glucose concentration and the limit of sensitivity is determined.

7.2. Materials and Methods

The reported diagnostic kit includes a reference MC to compensate for all background noises and undesired interferences by allowing for measurement of differential response. One or more
sensor MC involve depending on the number of analytes to be measured. The MCs are mounted in series and dipped in the Teflon chamber that is designed such that only MCs be exposed to the solution without wetting the probe base with electronics attached.

In order to functionalize MC by enzyme layer, the active part of the MC surface is used which is the extended electrode coated with gold. Gold is employed for immobilizing GoX enzyme which is itself a receptor for biomolecules such as Glucose.

*Materials:* Glucose Oxidase (GoX), 8.0% glutaraldehyde, 2-aminoethanethiol were purchased from Sigma. Deionized water was used for preparing solutions.

### 7.2.1. Immobilizing GoX over MC surface

Sensor MC was washed in acetone, ethanol and DI water consequently. A Teflon chamber was designed in order to dip in the MC into a droplet of liquid such that it only covers the MC and does not proceed to the electronics attached to the probe base. A 3D stage with resolution of submicron was used in order to navigate the MC in x-, y-, and z-direction and place it into the droplet.

A 0.1M of aminoethanethiol solution was prepared by dissolving 2-aminoethanethiol powder into deionized water. MC was dipped into a droplet of the prepared solution for self-assembled monolayer of aminoethanethiol to form on the gold surface by attachment of thiol groups to gold.

An enzyme solution was then prepared by dissolving a definite amount of GoX into DI water which was 5 mg/mL. 0.2% glutaraldehyde was used as a cross linking reagent being capable of binding to both the enzyme and Amino groups of aminoethanethiol monolayer already formed on the gold surface. Dipping MC in enzyme solution, the aldehyde groups of glutaraldehyde
react with the Amino groups at one end and with GoX at other ends letting layer of enzyme grow over the surface.

*Binding Detection:* Veeco Active Probe is used as the self-sensing MC with the capability of self-excitation through the ZnO stack mounted on the base of each probe (Figure 7.1).

### 7.2.2. Detection in Air

Fundamental resonance frequency of the MC is measured employing two different measurement systems which are *i)* laser vibrometer (Polytec CLV-2534, Figure 7.2(b)), and *ii)* self-sensing circuit (Figure 7.2(a)). A harmonic voltage was generated through oscilloscope (Agilent Infinii Vision 2000 X-Series-sw Oscilloscopes). The shift in the resonance frequency as a result of molecular binding is then measured with both measurement systems and compared.

This process of detection serves two purposes which are: *i)* prove the capability of the self-sensing circuit to detect the change of frequency as a result of adsorbed mass, and *ii)* calibrate the mass detection in liquid by correlating the amount of adsorbed mass calculated from

![Diagram of MicroCantilever](image)

**Figure 7.2** (a) Self-sensing circuit for actuating and sensing the system (b) MC mounted on a holder placed over a 3D stage positioned under laser vibrometer head.
mechanical resonance frequency shift to the circuits frequency shift as a result of variation of capacitance of the molecular interface.

7.2.3. Detection in liquid

Even though the ultrasmall masses functionalized over MC surface could be detected through self-sensing circuit with ultrahigh sensitivity, measuring the shift in mechanical resonance frequency of MC does not provide an effective tool for detection of marker proteins in liquid environment due to high dampening effect. Instead, another sensitive method using the capacitance of the gold electrodes was used. The circuit consisting of capacitor and inductor with the MC element modeled as a capacitor and a voltage source resonates at a certain frequency. The theoretical modeling for finding the resonance frequency of such a system can be developed by calculating the equivalent impedance of the system.

In order to find the equivalent impedance of the circuit from the output port, the circuit shown in Figure 7.2a is turned into the circuit illustrated in Figure 7.3 with $V_x$ being an imaginary source of voltage, $Z_c$ the impedance as a result of induced stray capacitance ($C_c$) and resistance ($R_c$) from the connecting cable, $Z_p$ and $Z_r$, the impedance resulting from other elements of the circuit including capacitors ($C_l$ and $C_r$) and inductor ($L$). Each of these impedances can be calculated as follows

$$Z_p = \frac{1}{C_1 w j + C_p w j}$$  \hspace{1cm} (7.1)

$$Z_r = \frac{1}{C_1 w j + \frac{1}{L w j}}$$  \hspace{1cm} (7.2)

$$Z_c = \frac{1}{R_c + C_c w j}$$  \hspace{1cm} (7.3)
\[ Z_{eq} = \frac{1}{\frac{1}{z_c} + \frac{1}{z_p+z_r}} \] (7.4)

with \( w \) being the frequency of the circuit. Based on the above equations, \( Z_{eq} \) can be calculated which is a complex function. Setting the imaginary part of \( Z_{eq} \) to zero, the following equation is obtained

\[
[AF + EB] \times [CG - DH] - [AE - FB] \times [CH + DG] = 0 \tag{7.5}
\]

where A-H is given as follows:

\[
\begin{align*}
A &= -C_1R_c w - R_c(C_1 + C_p)w + [1 - C_1Lw^2]wC_c R_c^2 - (C_1 + C_p)L C_c R_c^2 w^3 \\
B &= R_c(1 - C_1Lw^2) - R_c(C_1 + C_p)Lw^2 + C_1C_c R_c^2 w^2 + C_c R_c^2 w^2(C_1 + C_p) \\
C &= -(C_1 + C_p)(1 - C_1Lw^2)(w + w^3 C_c R_c^2) \\
D &= -C_1(C_1 + C_p)(w^2 + w^4 C_c^2 R_c^2) \\
E &= C \\
F &= D \\
G &= -(C_1 + C_p)(1 - C_1Lw^2)R_c w + C_1(C_1 + C_p)w^3C_c R_c^2 - C_1w - (C_1 + C_p)w - C_1 C_c^2 R_c^2 w^3 \\
&\quad - (C_1 + C_p)w^3 C_c R_c^2 \\
H &= -R_c C_1(C_1 + C_p)w^2 + (C_1 + C_p)(1 - C_1Lw^2)w^2 C_c R_c^2 + (1 - C_1Lw^2) - (C_1 + C_p)Lw^2 + \\
&\quad (1 - C_1Lw^2)w^2 C_c^2 R_c^2 - (C_1 + C_p)w^4 L C_c^2 R_c^2 \tag{7.6}
\end{align*}
\]

Solving Eq (7.5) for \( w \), the resonance frequency can be obtained which is a function of the varying capacitance \( C_p \).

Molecular affinity that occurs over the surface of MC resulting in the binding between receptor and target biomolecule changes the capacitance of the MC element thus affects the value of \( C_p \) in the circuit. The model presenting the effect of binding on the change in the total capacitance of this element was shown by Tsouti et al. (2011). The total capacitance of the MC element shown in the circuit can be modeled as three main capacitors in series including the capacitance of the
insulating layer, $C_{\text{ins}}$, functionalization layer, $C_{\text{bind}}$, and diffuse layer, $C_{\text{dif}}$, as shown in Figure 7.4. Therefore, total capacitance, $C_p$ can be written as

$$\frac{1}{C_p} = \frac{1}{C_{\text{ins}}} + \frac{1}{C_{\text{bind}}} + \frac{1}{C_{\text{dif}}} \quad (7.7)$$

When binding occurs, the capacitance of the functionalization layer ($C_{\text{bind}}$) varies thus the total capacitance $C_p$ changes. The change in the capacitance of MC produces a detectable shift in the resonance frequency of the circuit which can be calculated adopting the circuit modeling presented in this study therefore providing qualitative and quantitative insight into the amount of binding and consequently the concentration of target biomolecule in the solution.

The effect of different values of circuit’s elements which are capacitors and inductors ($C_I$, $C_r$, and $L$) on the sensitivity of the circuit to measure the change in resonance frequency was also investigated. It was observed that decreasing the values of $C_I$, $C_r$ and $L$ obviously increases circuit’s sensitivity. Figure 7.5 (a) and (b) illustrates the effect of $C_I$, $C_r$ and $L$ on circuits sensitivity to measure shift in resonance frequency respectively. In order to optimize circuit’s
response, the values of circuit’s elements were chosen such that they fall in the sensitive region based on the results illustrated in Figure 7.5.

7.3. Results and Discussions

One sensor and one reference MC were used in this study. The sensor MC was functionalized with receptor biomolecule which was GoX while the reference MC was left unfunctionalized in order to compensate for all non-specific binding, background noises and unwanted interferences.

7.3.1. Immobilized mass detection in air (Laser vibrometer and Self-sensing circuit)

The capability of the self-sensing circuit was first verified with the laser vibrometer measuring

![Figure 7.5](image1)

**Figure 7.5** Effect of values of (a) \( C_1 \) and \( C_r \) and (b) \( L \) on circuit’s sensitivity in detecting shift in resonance frequency.

![Figure 7.6](image2)

**Figure 7.6** Fundamental resonance frequency of MC and shift in the resonance frequency in air as a result of GoX functionalization measured with (a) self-sensing circuit, and (b) laser vibrometer.
the shift in the fundamental resonance frequency of MC as a result of GoX-functionalization. The first resonance frequency of MC was measured with both self-sensing circuit and laser vibrometer by applying a sinusoidal voltage with a sweeping frequency of 0-100 kHz. It was measured to be 44.50 and 44.30 kHz by laser vibrometer and self-sensing circuit respectively. The shift of 12.5 kHz was measured with both laser vibrometer and self-sensing circuit as a result of GoX-functionalization. Figure 7.6 shows the FFT of the response of MC at its fundamental resonance.

Utilizing a comprehensive distributed-parameters mathematical modeling framework that was presented in Faegh and Jalili, (2013), Faegh et al. (2013a). the amount of mass immobilization can be quantified having the shift in the resonance frequency. Adopting the mathematical modeling and simulation illustrated in Figure 7.7 the frequency shift of 12.5 kHz correlates to the mass immobilized over the surface of the amount of 1531 ng. This amount of mass detection was further correlated to the shift of circuit’s resonance frequency which was measured in liquid media. Implementing such a comprehensive modeling framework was advantageous in calibrating the mass detection in liquid when electrical response of the system is utilized.

7.3.2. Immobilized mass detection in liquid (Self-sensing circuit’s resonance)

Even though the reported self-sensing method is capable of measuring small adsorbed masses

![Graph showing linear relationship between adsorbed mass (ng) and frequency shift (kHz).](image)

**Figure 7.7** Quantification of amount of adsorbed mass with respect to shift of mechanical resonance frequency of system utilizing comprehensive distributed-parameters mathematical modeling framework, (Faegh and Jalili, 2013, Faegh et al. 2013a).
with ultrahigh sensitivity, it does not produce an effective method to detect target proteins in liquid media due to high viscoelastic damping and losses in the surrounding liquid. As a result, the resonance frequency of the circuit consisting of MC was monitored instead of the mechanical resonance of MC. The reported circuit consisting of capacitors and inductor resonates at a certain frequency which was modeled calculating the equivalent impedance of the whole system. This resonance frequency was measured and recorded while putting both sensor and reference MCs in DI water. The shift in the resonance frequency as a result of GoX functionalization over the sensor MC was measured to be 2.612 MHz using the resonance frequency of the circuit as shown in Figure 7.8.

7.3.3. Detection of marker protein in liquid (Self-sensing circuit’s resonance)

Different concentrations of glucose ranging from 500 nM to 200 μM was injected in DI water and the resonance frequency of the circuit with both sensor and reference MCs was measured after each injection while exciting MCs inside the sample solution. It was shown that increasing the amount of glucose concentration in the liquid results in higher amount of shift in the resonance frequency of the circuit with sensor MC. On the other hand the resonance frequency of the circuit with reference MC does not change significantly. Figure 7.9 (a-e) depicts the resonance frequency of the circuit for both sensor and reference MC as a result of glucose injection.
It is shown that the resonance frequency of the circuit with reference MC stays within 9.102-9.106 MHz when implementing the system in solutions with different concentrations of glucose.

**Figure 7.9** Resonance frequency of the circuit consisting of sensor MC and reference MC and the shift in resonance frequency in liquid as a result of injecting (a) 0 glucose, (b) 500 nM glucose, (c) 1 μM glucose, (d) 100 μM glucose, (e) 200 μM glucose (Faegh et al. 2013b).
On the other hand, detectable changes were observed in the circuit with GoX-functionalized MC. Figure 7.10 shows the differential shift of circuit’s resonance frequency between sensor and reference MC with respect to glucose concentration. No significant change of resonance frequency was observed injecting concentration of glucose higher than 200 μM indicating the saturation of functionalized surface of sensor MC.

The main and most dominant nature of the nonlinearity between glucose concentration and frequency shift arises from the saturation of the sensing element. The response is the highest for the first and second injection and then it saturates as more injections take place. Adopting the theoretical circuit model presented in the previous section, the corresponding change of capacitance as a result of molecular binding over the surface was calculated having the amount of shift in circuit’s resonance frequency as is depicted in Figure 7.10.

Calibrating the system with the mechanical response obtained in the above section the mount of mass adsorption was quantified and presented in table 7.1. Considering the fact that physiological level of glucose in blood is about 4-20 mM, the present platform is capable of detecting even lower amount of glucose with very high sensitivity.

Comparing to glucose studies where the amount of glucose concentration was measured mechanically using MC in static mode, (Pei et al. 2003, 2004) and electrically using modified
**Table 7.1** Quantification of adsorbed mass with respect to circuit’s resonance frequency calibrated by mechanical response of the system.

<table>
<thead>
<tr>
<th>Circuit’s resonance freq. Shift (kHz)</th>
<th>Amount of mass adsorption (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>17.08</td>
</tr>
<tr>
<td>35</td>
<td>20.62</td>
</tr>
<tr>
<td>37</td>
<td>21.79</td>
</tr>
</tbody>
</table>

**Table 7.2** comparison of detection limit of measuring glucose concentration.

<table>
<thead>
<tr>
<th>Detection of Glucose</th>
<th>Sensitivity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC-static mode, using optical based read-out</td>
<td>0.2 mM</td>
<td>Pei et al. Oak ridge national lab. (2003, 2004)</td>
</tr>
<tr>
<td>polytyramine-modified gold electrode</td>
<td>1 μM</td>
<td>Labib et al. (2010)</td>
</tr>
<tr>
<td>AuNanoparticle modified electrode</td>
<td>180 μM</td>
<td>Shan et al. (2010)</td>
</tr>
<tr>
<td>Glucose Oxidase–graphene–chitosan modified electrode</td>
<td>0.02 mM</td>
<td>Kang et al. (2009)</td>
</tr>
<tr>
<td>MC, present self-sensing method</td>
<td>0.5 μM</td>
<td>Current study.</td>
</tr>
</tbody>
</table>
gold electrodes, (Labib et al. 2010), utilizing the self-sensing circuit provides a very simple, laser-free, and cost effective MC-based platform with the capability of detection of glucose level lower than its physiological limit with high sensitivity. Table 7.2 shows a comparison of the amount of sensitivity utilizing the reported self-sensing technique to the other studies detecting glucose.

There are certain limitations with the reported detection platform including the low dynamic range which results from saturation of receptor biomolecules over the surface of MC. To address this limitation two approaches are considered for the future work which are i) increasing the surface area of the molecular interface resulting in higher number of immobilized receptor which can be accomplished by utilizing a different molecular probe such as interdigitated electrodes or depositing nanoparticles over MC surface, and ii) using a chemical solvent which only rebounds GoX-glucose, and not the functionalized receptor molecules over the surface, therefore, making MC reusable for a higher number of steps.

7.4. Chapter Summary

A unique piezoelectric MC-based biological sensor for detection of molecular binding was reported in this study. Implementing a self-sensing circuit, the system was performed in dynamic, self-sensing mode by exciting the piezoelectric MC and sensing its response simultaneously. Utilizing the reported circuit, the need for bulky and expensive optical based system was eliminated. Two MCs, one sensor and one reference MCs were implemented. The sensor MC was functionalized by receptor enzyme, GoX, while the reference MC was left unfunctionalized to compensate for all undesired interactions. In the first step of this study the capability of self-sensing circuit to detect the functionalized mass (Amino groups and GoX) was verified by comparing it to optical based measurement (laser vibrometer).
A high level of accuracy and sensitivity was observed monitoring the shift in the fundamental mechanical resonance frequency of sensor MC. In order to detect the target molecules (glucose) the system had to be operated in aqueous media. Therefore, the resonance frequency of the circuit consisting sensor and reference MC was measured and monitored separately. Dipping both MCs in solutions containing a certain level of glucose, binding occurs over the surface of functionalized MC changing its capacitance thus shifts the measured resonance frequency obtained from the circuit. On the other hand, the resonance frequency of the circuit consisting of unfunctionalized reference MC does not change significantly.

A detectable shift in the resonance frequency of the circuit with sensor MC was measured and reported when injecting different amount of glucose (500 nM-200 μM) in DI water. At the same time, negligible changes in resonance frequency of the circuit with reference MC was reported indicating the capability of the sensor to detect the molecular binding.

As a result, the reported biological sensor provides a very simple, cost-effective, and highly sensitive platform aiming at being implemented as a diagnostic tool. Increasing the level of sensitivity, testing selectivity, and operating the sensor in greater dynamic range with the reported platform are under study.
CHAPTER 8

CONCLUSIONS AND FUTURE WORKS

8.1. Concluding Remarks

This dissertation presented the entire developmental process of a unique piezoelectric MC-based sensor. Although MC-based biosensors have received a widespread attention for label-free detection, there are not enough analytical studies investigating modeling and simulation of piezoactive MC-based systems along with the relative experimental verifications. Most of the studies implementing MC-based systems in specific applications exploited simple lumped parameters modeling frameworks. On the other hand, the available sophisticated analytical studies are not complementary with the relative experimental verifications. Therefore, in this dissertation, an extensive investigation has been conducted on the piezoactive MC-based sensors both on theoretical and experimental aspects. The whole developmental process of the sensor that was presented in this dissertation includes the following important steps and developments.

1) Extensive mathematical modeling of piezoactive MC-based systems with different applications,

2) Comparison of Euler-Bernoulli beam modeling and plate modeling of piezoelectric MC-based sensors with experimental verification,

3) Reporting a unique self-sensing piezoelectric MC-based sensor for detection of ultrasmall masses and biological species and comparison with optical based methods,

4) Exploiting adaptation strategy to compensate for variations of piezoelectric material,
5) Implementing the system at high modes for sensitivity enhancement including the simulation and experimental results

6) Implementing the self-sensing platform for detection of different concentrations of glucose,

7) Implementing the self-sensing platform as a gas sensor for detection of ethanol and water vapors.

In Chapter 3, two piezoactive-based systems were investigated. Sys. 1 was defined to be piezoresistive MC-based sensor operating in contact mode; whereas, Sys. 2 was a MC-based PFM functioning on piezoelectric sample with tip excitation. An external periodic electric field was applied between the conducting tip and the sample. The piezoelectric and piezoviscoelastic deformation of the sample served as the source of excitation of the system.

These two systems were investigated extensively. Comprehensive mathematical modeling framework were developed and simulated for the aforementioned systems. Extended Hamiltonian’s principle was used and system’s response, deflection of MC, was obtained from which contact tip force, change of resistivity of the piezoresistive patch, and consequently output voltage of the system was calculated utilizing the developed model. Moreover, the effect of length and location of piezoelectric layer over MC on the sensitivity of Sys. 1 was simulated. On the other hand, the sensitivity of Sys.2 with respect to local spring constant of piezoelectric sample was studies and presented. It was shown that the amplitude of vibration increased almost linearly with spring constant of piezoelectric material. Moreover, it was observed that the location of piezoresistive patch affects system’s amplitude significantly while it does not have a noticeable influence on the shift of the resonance frequency of the system. The presented
modeling frameworks addressed the uncertainties and unmodeled dynamics which are required for precise MC-based systems compared to lumped-parameters modeling.

One of the main areas of application of MC-based systems is their implementation as sensors. Detection of ultrasmall masses and marker proteins has been made possible using MCs due to their tremendous advantages including low cost, simplicity and sensitivity. The main focus of this dissertation has been on development of a unique self-sensing piezoelectric MC-based sensor for the purpose of detecting ultrasmall masses and biological species. The entire developmental process is presented in this dissertation. A comprehensive mathematical modeling framework was developed for the sensing platform. In the first step along that line, different modeling methods were adopted and compared.

In Chapter 4, the piezoelectric MC-based system was modeled as a non-uniform rectangular thin plate and also as an Euler-Bernoulli beam. Distributed-parameters modeling using Extended Hamilton’s principle was adopted developing the equations of motions of the system. Free and forced vibration problems were solved and simulated. The system was performed in dynamic mode by self-excitation through applying voltage to piezoelectric layer. Fundamental resonance frequency of the system was measured. The capability of the proposed system in detection of ultrasmall masses was tested by measuring the shift in the resonance frequency as a result of absorbed mass over MC surface. The amount of 2.33 kHz shift in resonance frequency was observed as a result of adsorption of ~ 200 ng surface adsorbed mass illustrating a satisfying level of mechanical sensitivity. Relative experimental setup was built to verify the theoretical modeling frameworks. Veeco Active probe® was used to measure absorption of thiol groups over MC surface. It was observed that both Euler-Bernoulli beam theory and plate theory were adequate to predict the current system’s behavior with high level of accuracy.
Although the Euler-Bernoulli model also satisfied the explanation of dynamics and behavior of the proposed platform in this case, it will not be sufficient for modeling other geometries of the similar platform. Since geometry of MC in biosensors dramatically influences the sensitivity of the system, there is always a need to optimize geometrical properties such as using shorter and wider MCs. Therefore, having a comprehensive modeling framework describing all geometries and designs of MC provides a powerful theoretical layout for such systems and explains the necessity of modeling complexity and effort.

The main concept of developing a laser-free self-sensing MC-based sensor was discussed in Chapter 5. A MC with a single piezoelectric layer embedded in its structure along with a pure capacitive bridge was used to implement the system in self-sensing mode. Inverse piezoelectric property was used to actuate the system by applying voltage to it. Simultaneously, system’s response was sensed through direct piezoelectric property by measuring output voltage of the bridge. As a result, the need for bulky and expensive external actuator and read-out systems was eliminated resulting in an inexpensive, simple platform with miniaturization capability.

In order to have a thorough insight into the dynamics of the self-sensing mechanism, two approaches were taken. First, a comprehensive distributed-parameters mathematical modeling framework was developed for the aforementioned mechanism. The system was simulated and solved in Matlab. Second, an adaption law was exploited to compensate for the variations of piezoelectric property of the material used in MC with respect to temperature or other environmental interferences. The system was again simulated using the adaptation strategy. Finally, an extensive experimental setup was built to test and prove the capability of the self-sensing mechanism. A pure capacitive bridge was built and attached to the piezoelectric MC (Veeco active probe®). The system was performed in dynamic mode. A harmonic voltage was
applied to the bridge and at the same time, the output voltage of the bridge was measured and monitored. Fundamental frequency of the system was measured taking FFT of system’s response captured by self-sensing mechanism. The same procedure was repeated measuring system’s response optically through laser vibrometer. A 97.50% precision of accuracy was observed comparing the experimental results with those obtained from mathematical modeling. It was shown that exploiting adaptation law, the precision of accuracy was improved to 99.98%. The capability of the proposed self-sensing method was therefore proved with the theoretical results and moreover, it was compared to optical based measurement. Comparing the measurements from optical method to those from self-sensing technique, a 99.74 % precision of accuracy was illustrated.

Sensitivity enhancement of the developed platform was extensively studied in this dissertation. Sensitivity, being one of the most important elements determining the success of each sensor has been investigated using different methods. Both numerical and experimental studies were conducted for increasing sensitivity in MC-based systems. Techniques such as geometry modification, exploiting nanoparticles and carbon nanotubes in the structure of the system, and exciting MCs in vibration modes other than flexural mode (e.g. lateral and torsional modes) were investigated. Resonating MCs in high modes has emerged as one of the most promising approaches in sensitivity enhancement through increasing quality factor. Although being investigated, there have not been enough analytical high fidelity models describing all dynamics and behavior of MCs operating in high modes along with experimental verifications.

In Chapter 6 of this dissertation, a comprehensive mathematical modeling framework for piezoelectric self-sensing MC operating at its ultrahigh mode (20th mode) is presented. Changes in resonance frequencies as a result of added mass is calculated for high modes as well as
fundamental and lower modes. Accurate level of estimation for resonance frequencies was made adopting mode convergence theory. Extensive experiment was carried out operating MC at its high mode using both self-sensing and optical measurement methodologies. The obtained results are compared and verified with theoretical results. The same platform is used to detect immobilized ultrasmall mass. Amino groups of aminothenethaiol solution are immobilized over MC surface by covalent binding to gold. Shift in resonance frequencies in higher modes are measured and the quality factor is calculated for each mode proving the fact that sensitivity of MC to detect adsorbed masses was enhanced as the number of modes increased.

The ultimate goal of developing the self-sensing piezoelectric MC-based sensing platform was to implement it as a biological sensor for detecting ultrasmall biological species in a sample solution. Accomplishing the extensive analytical and numerical studies and proving the capability of the self-sensing platform to perform accurately for measurement in Chapters 3-6, the final step of the development which was the real implantation of the platform for detection was precisely discussed in Chapter 7. The sensing platform involved two MCs. One MC was implemented as the reference which was left unfunctionalized in order to compensate for all unspecific interactions and background noises.

On the other hand, the sensor MC was functionalized with the receptor molecule specific to target molecules to be detected. Detection of glucose was tested as the target molecule using glucose oxidase as the receptor enzyme which was proved to have high affinity with glucose. First, detection of functionalized receptors which were Amino groups and glucose oxidase was reported using self-sensing platform and compared to the results measured optically by laser vibrometer. Performing the system in dynamic mode, the shift in the fundamental mechanical resonance frequency of sensor MC was measured with high level of accuracy comparing to
optical-based method. The system ought to operate in aqueous media for the second step of measurement which was measuring different concentrations of glucose in a sample solution. Due to high dampening effect and viscoelastic behavior of the surrounding media, the mechanical responses of MCs did not provide a sufficient tool for this step of measurement. To overcome this challenge, the resonance frequency of the circuit consisting of sensor and reference MCs were monitored. Variation of circuit’s resonance frequency as a result of change of capacitance due to molecular binding was studied following the model introduced by Tsouti et al. 2001. Dipping both MCs in solutions containing a certain level of glucose, binding occurs over the surface of functionalized MC changing its capacitance thus shifted the measured resonance frequency obtained from the circuit. On the other hand, the resonance frequency of the circuit consisting of unfunctionalized reference MC did not change significantly.

A detectable shift in the resonance frequency of the circuit with sensor MC was measured and reported when injecting different amount of glucose (500 nM-200 μM) in DI water. At the same time, negligible changes in resonance frequency of the circuit with reference MC was reported indicating the capability of the sensor to detect the molecular binding. Extensive circuit modeling was presented correlating the amount of frequency shift to the change of capacitance and consequently to the added adsorbed mass.

As a result, a compact detection platform with the capability of miniaturization, low power consumption, cost effective, and yet sensitive methodology is developed and reported in this dissertation. The measurement capability of the platform both in air and aqueous media with the simplest and most inexpensive actuation and sensing equipments was presented both theoretically and experimentally.
8.2. Future Works

There are certain improvements on the developed sensing platform for future investigations which are discussed as follows.

I- Sensitivity enhancement using geometrical modifications of MC such as decreasing the size of MC provides a certain improvement in the functionality of the sensing platform. Testing selectivity and operating the sensor in greater dynamic range are other important improvements to be considered for future investigations.

II- The entire research on the self-sensing piezoelectric MC that was presented in this dissertation was performed on Veeco Active Probe® with the piezoelectric layer that was embedded in the structure of the MC. These probes were designed for AFM applications and were not optimized for sensing purposes. However, the developed sensing platform was optimized using Veeco active probes in order to remove the fabrication process and save time, effort and financial resources for other aspects of developments which included: analytical study, numerical simulation, designing and testing the platform. As a result, one important direction for future improvements would be fabrication of MCs with designs and geometries modified and optimized for the self-sensing platform and sensing applications.

III- Moreover, High throughput analysis can be performed using arrays of MCs each of which functionalized with a different receptor that is specific to different marker proteins. Therefore, fabrication of an array of MC with any piezoelectric layer with an output port that is attached to the circuit is necessary.
Figure 8.1 The proposed diagnostic kit involving one reference and more than one sensor probes equipped with a compact fluidic setup, injection valve, and syringe pump.
IV- Developing a portable and compact microfluidic setup equipped with an inlet valve for injection of sample solutions and a syringe pump to withdraw the solution at a certain rate is highly desirable. The Figure 8.1 provides the schematic of the proposed fluidic setup including one reference probe and an optional number of sensor probes depending on the number of analytes that needs to be measured.

V- Another important feature determining the success of the reported sensing platform is developing a high quality factor resonating circuit. The higher the quality factor of the self-sensing circuit accompanied with the molecular probe, the simpler and more sensitive the detection of frequency shift. Using high quality factor crystals and microresonators is strongly suggested.

VI- Testing the improved sensing platform on different analytes would be a major direction for future investigations. Analyzing gene expression at the genomic and proteomic level is the main source to understand cell responses to changes in their environment. A number of methodologies have been developed for analyzing gene expression which includes Enzyme-Linked ImmunoSorbent Assays (ELISA), Surface Plasmon Resonance (SPR), 2D electrophoresis, and DNA microarrays. Microcantilever-based biosensor technology allows for label-free fast detection of transcription factors, does not require cloning, scaling up the number of microcantilevers on an array in not a limit, provides analysis of multiple transcription factors in a single step, and provides higher sensitivity compared to all other techniques. Therefore, utilizing the proposed improved self-
sensing platform for detection of DNA hybridization with specific selection of DNA sequences would be very promising.

VII- Implementing the sensing platforms on applications other than biosensor is another major direction. Exploiting this platform, different areas of application can be targeted which includes:

a) Environment as an environmental sensor: enables detection of toxic chemicals and biological agents. Screening potential environmental contaminants such as endocrine disrupting chemicals or detection of microbial pathogens in water and other environmental samples would have a great impact in monitoring and saving environmental resources.

b) Shipping industry, customs and border patrol and homeland security as a gas sensor: enables screening high explosive gases and toxic chemicals. Detecting tiny masses in air and differentiating particles based on a signature would be revolutionary since current real time instrumentation cannot differentiate between engineered and incidental nanoparticles.
REFERENCES
Baller, M., Lang, H.P., Fritz, J., Gerber, Ch., Gimzewski, J.K., Drechsler, U., Rothuizen, H.,


(R2) http://www.pharmaceutical-int.com/categories/qcm-technology/quartzcrystal-microbalance-qcm.asp

(R3) http://www.masscal.com/instrument.html