A simultaneous sensing cum actuating microcantilever transducer for determination of single cell' s mechanical properties

Rezanda, Felicia

2010

Rezanda, F. (2010). A simultaneous sensing cum actuating microcantilever transducer for determination of single cell' s mechanical properties. Master' s thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/41865>

<https://doi.org/10.32657/10356/41865>

A SIMULTANEOUS SENSING CUM ACTUATING MICROCANTILEVER TRANSDUCER FOR DETERMINATION OF SINGLE CELL'S MECHANICAL PROPERTIES

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A thesis submitted to the
Nanyang Technological University
in partial fulfilment of the requirements for the
Degree of Master of Engineering

2010

ACKNOWLEDGMENT

The author would like to give her sincere gratitude for her supervisor, Prof. Ling Shih-Fu for providing the topic and direction of the research. His advice, guidance, and support are significant for my research.

Gratitude is also given to Dr. Yin Yan Ling and Dr. Wang Deyang for their previous researches which became an important step stone for me to start my own research. Their help and advice had given me insight during the research. Thanks also to the technicians in Centre for Mechanics of Micro-System Laboratory, Ms Halimatun Bte Ma'arof and Mr. Chua Hock Leong, who allowed and patiently taught me to use most of the equipments there. For my fellow students, Ivan Tanra, Liu Dan, Lian Kar Fong, Hossein Moussavi Hondori, and Wong Yoke Rung, their help in conducting the experiments has been invaluable.

Many thanks also for some of the Chemical and Bioengineering School's top researchers. Thank you for A/P Li Changming for his advice and permission to approach some of his PhD students: Lu Zhisong, Guo Chunxian, Gan Ye, and Xinting. I am really grateful for their help, patience, and time in the AFM introduction and coating experiments.

Many thanks also to Dr. Ju Bingfeng from Zhejiang University for his counsel and expertise in AFM technology.

I am also grateful to Nanyang Technological University, School of Mechanical and Aerospace Engineering for the financial support and all the necessary facilities and resources.

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SUMMARY

Cell mechanics is the study of cell mechanical properties. Many techniques have been developed to study cell mechanics. One of the widely used techniques is an imaging technique called Atomic Force Microscopy (AFM). A new type of AFM microcantilever model had been developed by employing the piezoelectric effects. This piezoelectric device can perform as an actuator (inverse effect) and sensor (direct effect). This new cantilever is made by coating a layer of piezoelectric material on top of the cantilever. No external piezoactuators needed to oscillate the probe which makes it more compact and cheap.

This study aims to prove the possibility of using simultaneous Sensing cum Actuating (SSA) in calibrating this new type of cantilever and using it for finding the mechanical properties of single cells.

SSA applies a transduction matrix theory to define the measurement system. Using this matrix, the mechanical impedance of the output port can be calculated from the electrical impedance of the input port. Because of this, an actuator can act as a sensor also. This will minimize the difficulty in setting up the equipments and inaccuracy due to sensors.

Transduction functions of the cantilever transducer were predicted using ANSYS simulation. The transduction functions were then verified using electrical impedance measured in laboratory experiment. It was found that the transduction functions from ANSYS simulation can't model the electrical impedance result to be exact due to the design of the transducer. This transducer has large area of piezoelectric layer on top of unmoving/ stable die substrate which will affect the electrical impedance value greatly. Besides that, the piezoelectric layer also has a significantly high loss factor.

A calibration was done in laboratory experiment using a set of cantilever beams. It was found that the transduction functions differed from what were calculated using ANSYS. This might be caused by a combination of several factors. A measurement condition in laboratory could not be ideal and should be affected by instrument's

error/ resolution. The instrumental error materializes in .major proportion if the electrical sensing signal was low due to the low value of piezoelectric strain coupling of ZnO material. Because of that, the transduction function found will not only be unique to the transducer itself but also to the instrument used for measuring. The cantilever transducer can measured the mechanical impedance magnitude of around or lower than $5\text{e-}06$ Ns/m.

The transducer coated with insulating layer using electropolymerization method was capable to work in liquid environment during tests. It was found that SSA/transduction method can't predict the mechanical impedance due to liquid's hydrodynamic drag accurately. This was because SSA method can only predict point mechanical impedance at the output tip whereas hydrodynamic drag is a distributed force along the body of the transducer. Because of that, it was found that the objective to use this transducer and SSA method to determine mechanical properties of biological cells can't be achieved. Some recommendation to improve sensing capability of this type of transducer was mentioned.

Chapter 1

Introduction

1.1 Background

Cell mechanics is the study of mechanical properties of biological cells. As Lim et al. [1] mentioned, there are several reasons why cell mechanics has become one of the popular research studies. Firstly, our human body is constantly exposed to physical stress and strain. They can come from external physical forces or physiological environment inside the body. Apart from mechanical loadings, some drugs and chemicals are known to affect the properties of the cells. For example, Fabry et al. [2] in his investigation tried to find the effect of drugs such as histamine and Cytochalasin D to fibroblasts.

Secondly, biomechanical investigation can provide quantitative study on the change in the physical properties of cells and biomolecules with the progression of certain human diseases such as malaria and cancer. That way, it can potentially be used to quantitatively reflect the state of the person's health. This will lead possible application in clinical diagnostics and therapeutic treatment for some diseases such as investigation done by Suresh et al [3] on the effect of cancer to cell mechanics. It was found that for human epithelial cells that are cancerous the stiffness tend to decrease.

Thirdly, stress and strain have also been known to produce certain biological and biochemical responses in cells such as cell motility, differentiation and apoptosis. Hence, the study of cells and biomolecular mechanics make the understanding of mechanotransduction process, the active response of living cells to mechanical signals, possible [1,4].

Cell can be categorized as a viscoelastic material [5]. Viscoelastic materials are materials having viscous and elastic properties [6]. An elastic material has the

capacity to store mechanical energy without dissipation of energy. A pure viscous material can only dissipate energy and not store it. Viscoelastic material has the capacity to both store and dissipate energy.

Because of this, the modulus of viscoelastic material is a complex function. It consists of real part which is the storage modulus and imaginary part which is the loss modulus. Storage modulus is the measure of energy saved by the elastic part to push back. Loss modulus is the energy dissipated by the viscous part.

The stress and strain of viscoelastic material are functions of time. To find more accurate material properties of viscoelastic material, dynamic testing analysis should be done. This test is designed to find out the relation between the dynamic mechanical properties and some external variables such as time, frequency, and force. As it was mentioned before, viscoelastic material properties change with testing time which means it will also change with frequency. The dynamic properties of this viscoelastic material can be found by exciting this material at different frequencies.

Many techniques have been developed to study cell mechanics, either static test or dynamic test. There are several biomechanical tests and imaging methodologies available for the purpose of studying mechanical properties of cells and biomolecules. These are some of the widely used techniques: micropipettes [1, 7-12], Magnetic Twisting Cytometry [7-8, 13-22], and Atomic Force Microscopy (AFM)[1,7-8, 23-51].

Atomic Force Microscopy (AFM) is one of the most widely used instruments for cell mechanics research nowadays. This is due to its simple method and good accuracy. AFM is actually an imaging method and developed from scanning probe microscopy. In AFM, the images are not generated via reflection or transmission of photon or electron. They are created by locally applying mechanical force by a probe tip across a sample surface in a spatial dimension. A position sensitive photodiode monitors the displacement of the tip. The force was measured based on equation $F = -kx$. The value

of stiffness, k , is obtained from calibration. Based on this principle, AFM can be used not only for imaging but also for force displacement measurement. . There are many studies used this static mode to generate force displacement curve for many kinds of cells such as MDCK cells, smooth muscle cells, fibroblast, osteoblasts, and red blood cells[23-51].

There are two major types of AFM operational mode: contact mode and intermittent contact (IC)/tapping mode. In contact mode, the probe tip is pressed again the scanned surface and kept in constant contact with it. The disadvantage of this method is that if the material under investigation is very soft then the cantilever should have a low stiffness so that it can be bent. Not only that, it can also stick to the surface. In tapping mode, the probe cantilever is oscillated by piezoelectric actuator at or near its resonant frequency which is several hundred kilohertz. The probe is only lowered until it slightly touches the surface at the lower end of its movement

As it was mentioned before, the dynamic response of the cell would be a more accurate method to show the properties of the cells. Using tapping mode, the dynamic response of the cells can be gained. The piezoactuator is oscillating the cantilever at a range of frequencies. When the cantilever probe touches the material surface, there will be a change in amplitude and phase of the vibration. By processing the input signal and result signal, the force and deflection signal can be gained. Mahaffy et al. [34-35] and Smith et al. [37-38] are some researchers who have applied this method to find the dynamic properties of cells.

The conventional AFM makes use of an external piezoactuator to actuate the cantilever and optical sensor to detect its movement [23-28]. This means that the reliability of the system is affected by the reliability of the external devices. Not only that, the price of the AFM is expensive due to the laser, optical device, and the piezoactuators. This problem has been known and many researchers try to come with a novel solution to create a sensing cum actuating cantilever [54-60]. That way, the efficiency of the system can be improved.

This new self sensing/ actuating cantilever probes model can be created by adding a piezoelectric material on the cantilever itself. Piezoelectric material can be used for actuating and sensing because it has a reversible physical effect. In a reversible effect, a device can convert an electrical energy to mechanical energy and vice versa. There are two types of piezoelectric effect: direct and inverse effect. In a direct effect, a force given to the piezoelectric crystal will generate an electric charge. In inverse effect, a voltage will cause the system to vibrate with a velocity value. In other word, a piezoelectric device can perform as an actuator (inverse effect) and sensor (direct effect). This new cantilever is made by coating a layer of piezoelectric material on top of the cantilever. This cantilever applies the piezoelectric effect to make sure the cantilever can actuate and sense the force and displacement. An electrical voltage will be inputted to the piezo layer and generate displacement. This means no external piezoactuators needed to oscillate the probe which makes it more compact and cheap.

Liang et al [88-89] introduce a method called impedance method for structural health monitoring application. According to Liang, the mechanical impedance (force and velocity) of a host structure with an active material (e.g.: piezoelectric material) can be calculated from the electrical impedance (voltage and current) of the active material. Based upon this, Simultaneous Sensing cum Actuating (SSA) method is developed by Ling et al as a method to detect the dynamic mechanical impedance of a material or structure [90-95]. This method can calculate the mechanical impedance (force divided by velocity) of the system by measuring its electrical impedance (voltage and current) using a transduction matrix. The relationship between electrical voltage and current at its input port and the mechanical force and velocity at the output port can be described as 2x2 transduction matrix which consists of four transduction functions. This transduction functions is unique to the transducer system and can be found through a calibration process.

SSA technique incorporated in the piezoelectric-coated cantilever can be a new dynamic measurement technique for cells and biomaterials.

1.2 Objectives

This objective of this research is to discover the possibility of using a cantilever transducer to measure dynamic mechanical properties of biological cells based upon simultaneous sensing cum actuating method (SSA). Using this SSA technique, an actuator would act as a sensor also which will reduce any error caused by sensor and increase the environmental adaptability of the measurement device.

The main objectives are:

1. To determine the transduction functions of the cantilever transducer system
2. To modify the cantilever transducer for use in a liquid environment.
3. To measure the dynamic mechanical properties of cells.

The transduction functions are a unique representation of the transducer system. By knowing the transduction function, the mechanical impedance sensed or happened at the output point/tip can be calibrated.

Because most cells would need to be immersed in a medium solution to stay alive for a duration of time, the transducer would need to be able to work in a liquid environment. Some modification need to be done to insulate the conductive area on the transducer.

1.3 Organization of Thesis

The scope of this study encompasses the theoretical basis, the methods, and the possible use of SSA technique to characterize cells. A finite element model of a micro cantilever with piezoelectric layer coated on top of it will be created. A simulation was then done to determine the transduction functions of it using ANSYS simulation. A calibration process in a laboratory environment will also be done using the real piezoelectric transducer to find the transduction functions. This cantilever,

then, would be used to characterize cells specimens in a laboratory environment. Next paragraphs will discuss the organization of the thesis in details.

Chapter 1 provides an introduction to this thesis. It states the background, objective and the organization of this thesis.

In Chapter 2, Literature Review, relevant knowledge on cells structures, cells as viscoelastic materials, cells models, experimental technique in studying cell mechanics, AFM in details, piezoelectric effect, and Sensing Cum Actuating method. Cell has a complex structure and its mechanical properties depend upon the structural members such as cytoskeleton, ECM, cytoplasm, nucleus, cell membrane. It is known that cell is a viscoelastic material so the viscoelastic properties of cell is needed to be known. Researchers have been developing cell mechanical models to explain cell mechanics. Some of these cell models developed by other researchers are presented. Current research in cell mechanics is flourishing with the rapid development of measurement techniques out there. Some of these techniques used in characterization of cell mechanics are reviewed and compared. AFM is one of the popular measurement techniques for measurement of cell mechanics and imaging the cell surface. AFM technique has been used for static and dynamic measurement of cell properties. With the fast advance of MEMS technology, an idea of incorporating AFM cantilever and piezoelectric effect emerged. Using piezoelectric material, the probe can act simultaneously as sensor and actuator. Lastly, the basic principle and relevant research work about simultaneous sensing cum actuating method developed by Ling et al. are explained.

In Chapter 3, the SSA technique is discussed in details and a simulation of the cantilever using ANSYS is used to determine the transduction matrix of the system

In chapter 4, the cantilever transducer experimental calibration would be discussed in detail. This chapter will explain more about the calibration theory, experimental details, results found and some discussion regarding the results.

In chapter 5, the possibility of using the transducer in liquid environment would be discussed. Insulator coating method and the results of it will be shown. Besides that, the effect of distributed force such as hydrodynamic drag force to the transducer and transduction method results was explained.

Lastly, chapter 6 would explain the recommendation for future research investigation to improve the cantilever transducer's capability so as to measure mechanical properties of biomaterials and cells.

Chapter 2

Literature Review

Structural components of cells are firstly introduced in this chapter. Mainly, cell mechanics are depend upon different components that build cells such as cytoskeleton, cytoplasm, ECM, nucleus, and cell membrane. Cells themselves can be considered as viscoelastic material. So some fundamental details about viscoelasticity and cell's viscoelastic properties are discussed. In understanding cell mechanics, various models to represent cell has been developed by researchers. Some of this models are presented. Besides that, in cell mechanics analysis various measurement methods have been used to collect the cell mechanical properties. Some of the existing cell mechanics testing methods are reviewed and compared. A more in depth discussion about AFM is done. With the emergence of idea in developing AFM cantilever based upon piezoelectric effect, piezoelectric material and its sensing cum actuating properties are discussed. Lastly, the visibility of sensing cum actuating method that is developed by Ling et al. [90-95] in material characterization is explained.

2.1 Structural components involved in cell mechanics

Cells are complex organisms formed by an enormous amount of different molecules and organelles. The structure, organization, and nature of these components determine the different cell types, their function, their communication with the surrounding environment, their live cycle, and, as described in this section, their mechanical behavior.

2.1.1 Cytoskeleton

Cytoskeleton (CSK) can be translated as skeleton of the cell.[4] CSK indeed has a significant role in maintaining and organizing the structural morphology of the cell just as human skeleton. Besides acting as a static framework, CSK also regulates the musculature and circulatory system of the cell, being responsible of cell crawling, cell contraction, intracellular trafficking, or cell division. The cytoskeleton is thought to be

the main responsible of cellular mechanics. The CSK is formed by three main kinds of protein fibers bound by a series of intermolecular linkers and molecular motors, and connected to the extracellular environment and neighboring cells by membrane receptors. Those three protein fibers are actin filaments, microtubules, and intermediate filaments.

Figure 2.1 Cytoskeleton protein fibers. Figure cited from [3]

A. Actin filaments

Actin filaments (AF) or *microfilaments* have shown to be the major determinants of cytoskeletal mechanics [4]. Actin filaments are formed by polymerization of the

monomeric globular form of the protein (G-actin) into flexible helical filamentous polymers (F-actin) with two ends: barbed and pointed ends. Microfilaments, with a diameter of 6~8 nm, have a Young's modulus (E) of 1.3-2.5 GPa. It is important to note that individual actin filaments are $\sim 10^6$ times stiffer than actin gels and typical animal cells, $E \sim 100$ -1000 Pa. Their relatively short persistence length, $\sim 15 \mu\text{m}$, reflects their suitability to support tension stresses. In a typical animal cell, actin filaments are localized at the peripheral region of the cell (0.2-0.5 μm beneath the plasma membrane) forming a three-dimensional network cross-linked mainly by α -actinin and filamin called the actin cortex. Actin is also found throughout the cytosol in both its soluble and polymerized form.

Actin has high concentration in the majority of eukaryotic cells (5-10%). In some cell types, such as muscle cells the concentration even higher accounting for $\sim 20\%$. This shows that actin is a primary structural component of most cells and it has major significance in the CSK mechanics. Not only that, the dynamics of actin polymerization and depolymerization is critical to cell migration. Most of the movements of the cells are controlled by actin and its associated molecular motors. The polymerization of actin is indeed a form of movement and is regulated by the hydrolysis of energy. The difference in growth rate of the two ends of AF, existing a pointed end (more static, lower growth rate), and a barbed end (more dynamic, faster growth rate) is supposed to be responsible of filopodia (cell's finger protrusion) formation [61]. Actin can develop forces, leading to cellular contraction or cytoskeletal reorganization, being also able to support compression [16] when actin filaments form dense, rigid bundles cross-linked with the molecular motor myosin. Under this configuration, they are usually referred to as stress fibers.

Morphological changes in the actin CSK owed to pharmacological or mechanical stimulus commonly parallel variations in the global or local mechanical behavior of cells [46-48]. Alteration of the polymerization kinetics of actin by Latrunculin A or Cytochalasin D leads to CSK disruption and to a significant decrease in cellular stiffness, reducing also the ability of cells to move and contract. On the other hand, activation of the contractile apparatus, *i.e.* enhancement of actomyosin cross-bridge formation and actin polymerization, with chemicals such as thrombine and histamine

has shown to induce an increment in cellular stiffness in a number of cell types [2, 19, 33,37, 47-48,62]

Research on different type of cancer cells has been done using different types of instrument. The first example deals with the deformability of nonmalignant and malignant human breast cancer cells and of the latter malignant cells chemically modified to increase metastatic efficiency. The cancerous MCF 7 cells are more deformable than the normal MCF 10, and that the metastatic Mod MCF 7 cells are even more deformable than MCF 7 cells. The increased deformability of cancer cells with modified metastatic competence appears to be accompanied by a further reduction in structural strength compared with that of the non-metastatic cancer cells. These reductions in elastic rigidity appear to arise from a reduction in F-actin concentration of as much as 30% caused during the malignant transformation of the cell.

B. Microtubules

Microtubules (MT) are the second major constituent of CSK [8]. MTs are formed by polymerization of monomers of tubulin α - and β - tubulin arranged in a helical arrangement of ~25 nm outer diameter and ~15 nm inner diameter. Using atomic force microscopy and taking into account their tube-like structure, the Young's modulus of microtubules has been recently estimated to be ~1.9 GPa. Their structure and stiffness lead to a persistence length of ~6mm, much larger than typical cellular dimensions. [3]. Microtubules do not have sufficient shear or tensile stiffness to impart significant mechanical integrity to CSK. However, they can act in concert with other filamental biopolymer to stabilize CSK. They have a high bending stiffness and thus more suitable to withstand compression stresses [15]. They also can form of long slender structure such as cilia and flagella.

Although MTs are present in most of animal and vegetal cells, their concentration is much lower than that of Actin. MTs are dynamically unstable and their halflife is only several minutes. The polarized structural nature and the polymerization dynamics of MT strongly determine their organization within the cell. The so-called *plus* end exposes β -tubulin, and tubulin dimers are added more rapidly than to the *minus* end,

which exposes α -tubulin. The minus end is usually anchored to an amorphous matrix of filaments located near the nucleus called centrosome from which MT radiate out. The plus end abruptly changes from phases of linear growth to shrinkage in a random manner. As they form and disassemble, MT can find a preferable orientation if their plus ends are capped, usually near cell borders.

Drugs such as nocodazole or colchicine cause disruption of MT monomers addition so that a slight drop in cell stiffness and an increment in the inward or contraction forces happened [15,33]. Based upon these findings, it can be said that MT has significant effect in cellular mechanics and might be the responsible structures for supporting compression forces. In addition, MT have a key role in cellular trafficking. Molecular motors such as kinesin or dynein transport proteins and even whole vesicles and organelles move along MT to the different regions of the cell.

Another important function of MT is to move chromosomes and mitotic spindles to opposite sites of the cell during mitosis, leading to strongly differentiated MT organization depending on the cell phase. This movement is generated by both MT polymerization and motor proteins. Cilia and flagella are molecular machines that generate force and movement in certain cell types, and are mainly formed by a thick core of MT. In summary, Stamenovic[18] showed that the contribution of microtubules to deformability of adherent cells depends on the degree of cell spreading. It is minor in highly spread cells, whereas it is substantial in less spread cells. Since in their natural habitat cells seldom exhibit highly spread forms, the above results suggest that the contribution of microtubules to cell deformability cannot be overlooked.

C. Intermediate filaments

Intermediate filaments (IF) are much more varied in sequence and structure than AF or MT. Indeed, there are more than fifty different types of IF, such as vimentin, keratin, desmin, neurofilaments, or lamins. They have a common structure formed by coiled-coil dimers. The dimmers then assemble into a staggered array to form tetramers which connect end to end to each other and form protofilaments. About 8 protofilaments bundle together to form a single rope-like filament. The diameter of IF

is ~ 10 nm and their persistence length is ~ 1 -3 μm . The Young's modulus of IF dimers has been estimated to be of ~ 1 -5 GPa [3]. Intermediate filaments play an important reinforcing role in cells, especially in cells like epithelial cells, which have to withstand high elastic stresses. They are not polar and do not exhibit any direct active behavior. They are not highly dynamic like actin filaments and microtubules and are not easily disrupted by toxins.

As they are believed to play only a passive reinforcement role, they have not generated the same interest as in the case of the other two filament types. The structure, organization, and composition of IF vary from one cell type to another. IF are usually bonded to other parts of the cytoskeleton via linking proteins, such as desmoplakins, filaggrin, or plectins, forming an integrated system. Their mechanical properties suggest that IF may contribute to the elasticity of the cells via tensile loading. Intermediate filaments have small contribution in low to moderate strain deformation but they maintain their resistance to shear deformation at large local strains to provide structural integrity to the cell. They also strain harden to bear the mechanical stress at strain levels where actin networks do not retain their structural integrity. It has been also suggested that IF may prevent from excessive buckling of MT, stabilizing the CSK and having a protective role [16].

D. Motor proteins

Motor proteins, together with actin filaments or microtubules, have the ability to produce forces or execute directed motion along the filaments [8]. These motor molecules consume chemical energy derived from the hydrolysis of ATP to ADP and perform mechanical work efficiently. They act as transporters, moving vesicles and other organelles within the cell, and as mechanical force generators controlling cell shape, cell division and other mechanical functions. There are three major classes of motor proteins: myosin, kinesin, and dynein. Myosin proteins work together with actin filaments. Kinesin and dynein proteins collaborate with microtubules.

The *myosin* family can generate contractile stresses in actin networks. Among myosins, *Myosin-II*, which causes muscle contraction and can generate contractile stresses in non-muscle cells, plays an important mechanical role. The microtubule

associated proteins, *kinesin* and *dynein* can generate plus-end and minus end directed motion, respectively. They are responsible for cellular transport and they act in the organization of microtubules and other cellular substructures.

E. Crosslinking proteins

Crosslinking proteins are involved in the crosslinking of the cytoskeletal filaments [8]. The most common crosslinkers of actin filaments are alpha-actinin and filamin. Even at relatively low concentrations, these proteins can have a dramatic effect in enhancing the elasticity of in-vitro actin filament networks. Such gels, while able to resist fast deformations, are able to flow under a very slow deforming force suggesting that the crosslinkers are dynamic in nature. There are multiple other actin associated proteins which can crosslink actin filaments. Some of these also take part in anchoring actin filaments to the plasma membrane. Microtubules can also be crosslinked by protein crosslinkers. In axons, for example, the microtubules form a bundled core due to the crosslinking of these filaments by microtubule. Cell spreading and movement occur through the process of binding of cell surface [integrin](#) receptors to [extracellular matrix](#) adhesion molecules.

Some authors have tried to explain cellular mechanics in terms of measuring the mechanical properties of actin gels [13]. It seems difficult to find a complete and satisfactory explanation to cell mechanical behavior by means of individual CSK components. The complexity of cellular mechanics comprises its structural organization, cross-linking between filaments, attachment to other cells and to the extracellular matrix, and cooperativeness with other cellular organelles. It seems thus the *in situ* measurement of cellular mechanics under physiological conditions is important.

2.1.2 Extracellular matrix

The extracellular matrix (ECM) can be seen as an external extension of the CSK [3-5,8]. The ECM is a dense mesh formed by several kinds of protein fibers, such as fibronectin, collagen, or laminin secreted by cells themselves. The diameter of the components of this network range from tens to hundreds of nm. Cells attach to the ECM via focal adhesion contacts, which are clusters of membrane receptors (integrins) and cytoplasmic molecules. An important component of ECM is the

aminoacid sequence Arg-Gly-Asp (RGD), which is the binding site for several integrin receptors to ECM fibers.

The composition of the ECM depends primarily on the tissue type. Its stiffness is mainly determined by collagen fibers and it is of the same order of magnitude of that of living cells, 100-1000 Pa. The ECM serves as a supporting scaffold for cells when forming living tissues. It has been shown that the density of ECM proteins strongly determines movement, shape and stiffness of cultured cells as well as it causes strengthening of cell-ECM adhesion sites. Indeed, the ECM supports part of the mechanical forces within the CSK. The balance of forces between cells and the ECM regulates cellular shape, stiffness, and structural stability. Thus, the ECM appears crucial to the developing and function of living tissues, and therefore to the way cells sense and response to mechanical stimulus.

Cells form tissues by adhering to neighboring cells and to the ECM. There is a wide amount of adhesion receptors and related structures. Integrins, cadherins, immunoglobulin cell adhesion molecules, and selectins are among the most important adhesion molecules. The adhesion molecules most related to the support and application of mechanical forces, critical for motility, or contraction, and related to the maintenance of the tissue integrity

2.1.3 Cytoplasm

The cytoplasm of vertebrate cells is composed by water with a 20-40% (w/v) of proteins. This high concentration of molecules lead to a crowded cell interior, in contrast with the traditional view of the cell as a water baloon with freely moving molecules. Recent developed microscopy techniques enabled us to visualize the cytoplasm with high resolution and with few changes from its native structure and composition [63]. Cryoelectron tomography images of *Dictyostelium discoideum* cells revealed a dense cell interior which has lead to a new term to described the cell cytoplasm *molecular crowding* [64]. This dense organization implies that between 5% and 40% of total cell volume is occupied by macromolecules, which may compete between each other to occupy the available volume. Although the implications of macromolecular crowding have not been already studied in detail, it seems likely an important contribution to the overall cellular viscoelasticity.

2.1.4 Nucleus

The nucleus of vertebrate cells is formed by a lipid membrane and encloses the genetic material densely packed in the form of chromosomes. AF, MT, and IF surrounds the nucleus at higher concentration than in other cellular regions. Caille et al. applied uniaxial force to cells and isolated nuclei and found a bulk modulus of 5 kPa for nuclei in cells and 8 kPa for isolated nuclei [67]. These studies reveal that the nucleus is relatively stiff compared to that of cytoplasm [67-68]. Due to the importance of the nucleus in the organization of the CSK it seems difficult to isolate its contribution to cellular mechanics. Indeed, it has been shown that cells nucleus is directly linked to the CSK and indirectly to the cell membrane via integrins [65,69]. Thus, forces applied to the cell contribute to the deformation of the CSK and to the indirect deformation of the cell nucleus, contributing to the mechanical response of living cells.

2.1.5 Cell membrane

The cell membrane is formed by a lipid bilayer with integrated protein molecules of different type and function. The area of the lipid layer forms the 50% of the total membrane surface. The lipid bilayer serves as a semipermeable barrier between cells and their microenvironment. Water and small molecules (O_2 , CO_2) are able to pass through the cell membrane. Another important task of the cell membrane is the regulation of the osmotic equilibrium. The embedded molecules serve as receptors, enzymes, or transport proteins. Both lipid and protein molecules can diffuse within the membrane, which can be seen as a two-dimensional fluid. The tension supported by a lipid bilayer of red blood cell has been calculated to be ~ 10 nN/ μm using the micropipette aspiration technique[9]. Similar measurements on neutrophils revealed a softer tension for the cellular cortex of ~ 30 pN/ μm [9]. The surface tension contributes to the whole mechanical properties of living cells and may control cellular functions, such as endocytosis and exocytosis. It was found there is a close binding between the lipid bilayer and the actin cortex and other attached molecules. Because of this, it is difficult to isolate the purely elastic behavior of the cell membrane. Poking measurements on living cells reflected forces from hundreds of pN to tens of nN needed to lyse cell membranes using various AFM tips such as cone [49],

pyramidal [51], and needle (44-45) tips has been done. Similar result is also found using cell poker technique (70).

2.2 Viscoelastic Materials

Viscoelastic materials have viscous and elastic properties [6]. An elastic material has a capacity to store mechanical energy without dissipation of energy. Viscous material in nonhydrostatic stress state implies a capacity of energy dissipation but not storing it. Viscoelastic materials have a capacity to both store and dissipate mechanical energy. Examples of viscoelastic materials are plastic, synthetic rubber, biomaterials (cells and tissues), and some of the modern composite material.

As viscoelastic material, cell can be represented as a densely crosslinked material [5]. When the deformation time is long enough, a major displacement can happen. The long time allows the cross links to be unraveled and created a new stable structure (There is no changing of phase). If the deformation time is short, only local arrangement happens. Between these extreme conditions, the material acts rubber-like with a constant modulus regardless of the frequency/time of deformation.

2.2.1 Complex modulus of Viscoelastic Material

As it is mentioned before viscoelastic material has viscous and elastic properties. This caused the modulus of viscoelastic material to be a complex function. The strain (ε) lagged behind the stress (σ) with a phase of δ .

$$\sigma = \sigma_0 \sin(\omega t) \dots\dots\dots (2.1)$$

$$\varepsilon = \varepsilon_0 \sin(\omega t - \delta) \dots\dots\dots (2.2)$$

$$\sigma = \varepsilon E^* \dots\dots\dots (2.3)$$

$$E^* = E' + iE'' \dots\dots\dots (2.4)$$

E^* is the complex Young's modulus which is divided into two parts: E' is the storage modulus and E'' is the loss modulus. The tangent of the angle called loss factor can be defined as in equation 2.5.

$$\tan \delta = \frac{E''}{E'} \dots\dots\dots (2.5)$$

Storage modulus is the measure of energy saved by the elastic part of material to push back. Loss modulus is the measure of loss of heat energy by viscous part during

dissipation. That means loss factor is the measure of loss energy by the viscous part relative to stored energy by the elastic part.

2.2.2 Cell microrheology

Rheology or viscoelasticity deals with the elastic and viscous properties of materials[8,13]. Due to the micrometer size of the common used probes to study cellular mechanics, the use of the term microrheology is widely accepted. Cells behave not only elastically but also in a viscous manner, *i.e.* cells are viscoelastic.

The viscoelasticity of living cells can be measured by different procedures. One of the most common is the application of a step of force or deformation and the measurement of the corresponding response over time. Using this simple approach, it has been observed that cells respond initially as an elastic body and then either force and deformation relax in time. Elastic and viscous moduli, damping factors, and relaxation times of living cells have been estimated using this technique[22,50,78].

A more robust approach to determine the viscoelasticity of materials is the application of controlled low amplitude oscillations. This method resulted in stress-strain loops with hysteresis reflecting the viscoelastic behavior of living cells. Both elastic and viscous response showed also to depend on the rate at which the deformation was applied, cells behaved stiffer when probed at higher rates. In particular, both shear complex modulus real and imaginary elements, G' and G'' respectively, followed a weak power law at low frequencies, with G'' approaching to a purely Newtonian fluid at frequencies above 100 Hz [2,19,21,34-37,40]. Figure 2.2 shows the experimental techniques that have been used to calculate the microrheology of cells (For further details on the techniques see section 2.5).

Table 2.1 Microrheology properties of some cells done by oscillatory AFM and MTC (in bracket). [19,21,34-37,40]

Cell Type	Storage Modulus (G')	Loss Modulus (G'')	Frequency range (Hz)
Human alveolar epithelial cells(A549)	~438 -1500Pa	~150 -1500 Pa	0.1-100
A549 (MTC)	~ 0.55-1.5kPa	~0.2-1.5kPa	0.1-100

Human bronchial epithelial cells	~400 -1500Pa	~130 -1700 Pa	0.1-100
Airway Smooth Muscle cells	~ 0.9-1 Kpa	~0.15-0.5kPa	0.5-100
(MTC)	~2-6kPa	~0.7-8KPa	0.1-1000
Fibroblast NIH 3T3	0.35-0.7 kPa	0.2-0.4 kPa	80-300
Fibroblast BALB 3T3	0. 2-0.5kPa	0.15-0.2 kPa	50-300
Fibroblast SV-T23T3	0.15-0.2 kPa	0.05-0.07 kPa	50-300

Figure 2.2 Examples of experimental technique used to find the dynamic response of cells. Figure cited from [8]

The stress-strain relation of living cells has been shown to be fairly linear for low deformations or applied forces. However, when high strain is applied, cells show a higher noticeable stiffness [34,40,47]. This effect may be due to the underlying hard substrate where adherent cells grow and to an inherent cellular characteristic. Some authors have taken the observed effect of apparent higher stiffness of living cells probed at high strain as an evidence of strain hardening [16]. However, recent AFM measurements by Mahaffy et al. [35] taking into account cells thickness have shown a linear behavior, even when cells were at indentation depths $>50\%$ of their thickness.

2.3 Mechanical Models of Single Cell

As physical entities, living cells have structural and physical properties that enable them to withstand the physiological environment as well as mechanical stimuli

occurring within and outside body. To further understand these mechanical properties, cell models are developed.

Cell models are derived using either micro/ nanostructural approach or continuum approach. Structural approach deems cytoskeleton as the main structural component with other part such as cell membrane and spectrin network give an effect also. Continuum approach treats cell as comprising materials with continuum material properties. Although continuum models give less insight to detailed molecular event, it is easier and more straightforward to be used in computation. Lim et al in [52] review some continuum mechanical models that various researchers have developed until now. Below are the models presented by Lim [52].

Figure 2.3 Overview of mechanical models for living cells. Figure was cited from [52]

2.3.1 The Single Phase Models

Single phase methods model the cell cytoplasm as one phase material. As it was known, the cell is actually not a single phase material as it has several different parts such as liquid and organelles. Single phase model simplify the model and assume it has

only one phase. There are three types: Cortical shell liquid core model, solid model, and power-law damping model.

A. Cortical-Shell Liquid Core Model

This model show the cell consist of a membrane (shell) filled with liquid. This model can be divided into 4 types: Newtonian, Compound, Shear Thinning and Maxwell.

Newtonian model can be used to model Leukocytes which is a suspension type cell. It behaves like a liquid drop and adopts spherical shape when suspended. In this model, the cell interior is assumed to be a homogenous Newtonian viscous liquid and the cell cortex is taken as an anisotropic viscous fluid layer with static tension but without bending tension. Figure 2.4 shows the Newtonian model.

Figure 2.4 Newtonian model. Figure was cited from [52]

Figure 2.5 Compound Newtonian Model. Figure was cited from [52]

Compound Newtonian model was developed to model a more complex cell such as eukaryotes. The cell comprised of three-layered structure. The plasma membrane and the ectoplasm make up outer layer. The middle layer is the endoplasm which is fluid

like and soft. The core layer is composed of the condensed region of nucleus and surrounding cytoskeleton. Figure 2.5 shows the compound Newtonian model.

The shear thinning liquid drop model presents the cortex as a layer with constant tension and the cytoplasm as shear thinning (power law) liquid droplet. The instantaneous apparent viscosity of the cytoplasm takes the form of power law function for the instantaneous mean shear rate. Figure 2.6 shows the shear-thinning liquid drop model.

Figure 2.6 Shear-Thinning Liquid Drop Model. Figure was cited from [52]

Maxwell Liquid Drop Model can be applied to study Leukocytes behavior in micropipette aspiration [8]. The cell consisted of a pre-stressed cortical shell containing Maxwell liquid inside. Maxwell liquid consists of elastic and viscous element. So the cell was modeled as viscoelastic material. Figure 2.7 shows the Maxwell liquid drop model.

Figure 2.7 Maxwell Liquid Drop Model. Figure was cited from [52]

B. Solid model

This method assumed the whole cell as homogenous without considering cortical layer. By assuming homogeneity, the mechanical parameters needed are reduced so

simplify the data analysis. It can be divided into two types: Linear elastic and Viscoelastic model.

Linear elastic model assumes the cell as linear elastic material. However, it is generally inadequate for describing the mechanics of cells because the apparent elasticity of a cell depends upon loading rate and history. This is actually a behavior of viscoelastic and viscous material.

Linear Viscoelastic Model that is usually used is Kelvin model (Standard Linear Solid). The model is consist of spring (k_1) in series with a viscous damper (μ_1). Both of them parallels with a second spring (k_2). Figure 2.8 shows the Kelvin model.

Figure 2.8 Kelvin or Standard Linear Solid model. Figure was cited from [52]

C. Power Law Structural Damping Model

As cells are frequently subjected to dynamic forces in their physiological environment, the dynamic behavior of cells is of particular interest. The model is developed by including the effect of sinusoidal excitation which introduce frequency variable in the formula developed in previous models.

Oscillatory MTC [2,19,21] and AFM [34-37,40] are popular techniques used for conducting dynamic tests on adherent cells. For both dynamic tests, low amplitude sinusoidal force signal resulted in a sinusoidal displacement at the same frequency but exhibited a phase lag in the steady state.

Importantly, in both the oscillatory MTC and AFM experiments, the storage modulus G_0 of the cells (including human bronchial and alveolar epithelial cell lines, human airway smooth muscle cells, human lung epithelial cells [2,40,52] was found to

depend on the frequency (ranging from 10^{-2} to 10^3 Hz) according to a weak power law with a constant exponent between 0.1 and 0.4. The storage modulus G' exhibited similar power-law frequency dependence at low frequency ($< \sim 10$ Hz) but a Newtonian viscous component became significant at high frequency [2,40]. Such weak power-law dynamic behavior cannot readily be explained by the spring-dashpot models (e.g. Newtonian, Maxwell, standard linear solid models), because the spring-dashpot models will always overestimate the power of the frequency dependence. To model the observed rheological behavior of the adherent cells, the power-law structural damping model is proposed:

$$G^*(\omega) = G'(\omega) + G''(\omega) \\ = G_0 \left(\frac{\omega}{\omega_0} \right)^\alpha (1 + i\eta) \Gamma(1 - \alpha) \cos \frac{\pi\alpha}{2} + i\omega\mu$$

where α is the exponent of the power law ($0 < \alpha < 1$); $\eta = \tan(\pi\alpha/2)$ is the structural damping coefficient, μ is the Newtonian viscous term, ω is the angular frequency, $\Gamma(\cdot)$ denotes the gamma function and G_0 and ω_0 are scaling factors for stiffness and frequency, respectively (note that $G' = G_0 \cos(\pi\alpha/2) \approx G_0$ when $\omega = \omega_0$ and $\alpha \rightarrow 0$). In the experiment for a single-cell type, the plot of G' against angular frequency curves for different drug treatments have been found to pass a common point ($G_0; \omega_0$), which indicates G_0 and ω_0 are approximately invariant with drug treatments. This power-law structural damping model was later shown to be explained by the fractional derivative viscoelasticity [52]. More interestingly, the dynamic rheological properties of adherent cells were found to be related to the contractile stress—the larger the contractile stress, the higher the dynamic moduli [16].

2.3.2 Biphasic Model

Biphasic model treats the cytoplasm as two phases separately: solid polymeric content and interstitial fluid [4,52]. This model is widely used to model musculoskeletal cell mechanics. Even though this model give a more accurate representation than single phase continuum model, the complexity of biphasic theory and the irregular geometry often render analytical solution too challenging or even impossible. The newest development of this model is called multiphasic model where cells not only modeled as solid and fluid but also as ion [4].

2.3.3 Tensegrity Model

Cellular tensegrity model is one of the theory on cell modeling based upon micro/nanostructural approach [4,52]. Micro/ nanostructural model depicted cells as being composed of a large number of discrete elements that do not fill the space. This discrete element is a stress bearing element and finite in size. Each discrete element's behavior is subject to the conditions of mechanical equilibrium and geometrical compatibility of each node. In the case of tensegrity model, the structure of cells is modeled as stress-supported or prestressed structure. This structure requires tensile prestress in its structural members even before external loading application to maintain structural integrity. In the early 1980's, Donald Ingber is the first person who introduced a novel model of cytoskeletal mechanics as tensegrity structure and the model is termed as cellular tensegrity model from then on [4].

Tensegrity model take each elements of cytoskeleton into account for a discrete formulation of the model. It can range from very simple to very complex, multicomparmental, and multimodular structure. Compared to continuum models, this approach require a more complex modeling of the cell. Tensegrity model could account for static elastic behavior of cells but it still can not explain the frequency response of the cell which conforms to power law.

2.4 Experimental Techniques for Single Cell Mechanics

There are several techniques appropriate to used in the study of cell mechanics such as Optical Tweezer (OT), Magnetic Tweezer (MT), Micropipette Aspiration (MA), Magnetic Twisting Cytometric (MTC), and Atomic Force Microscopy (AFM). The selection of the techniques is usually dependent on the size of the samples and force needed [1]. For example, Atomic Force Microscopy and Optical tweezer would be better used on nanoscale biostructures. Micropipette Aspiration would be appropriately used for microstructure.

Figure 2.9 Force range of some experimental techniques. Figure cited from [3]

Figure 2.10 Size sample range of some experimental techniques. Figure cited from [3]

2.4.1 Optical Trap /Laser Tweezer (OT)

This method uses a laser to trap, control or manipulate dielectric particles attached to the cell in a medium. A laser light is made to shine on a dielectric particle (microbead) whose refractive index is higher than the medium. When the laser is transmitted through this particle, the photons of this laser are subjected to change in momentum due to refraction. This momentum change will exert a restoring force on the particle in the direction of greater photon flux (the focal point of the laser beam). The reflection of the laser will cause a repulsive force to the particle and cause it to go away from the focal point. The restoring force however is usually greater than the repulsive force. Because of that, the net force will move the particle towards the focal

point rather than moving the particle away from it. This net force will eventually exert a pulling force to the cell under testing.

An advantage of optical laser tweezer is the use of laser so there is no physical contact with the sample. It is also able to sense the force less than 1 pN which is very hard to achieve using other techniques. The disadvantage of this method is the maximum force is limited to a few hundred pN. Besides that, there is a possible photo damage effect on biological sample at high laser power limits.

The pioneer of this method is Arthur Ashkin who was first conducted a range of laser trap experiment to viruses and *E. coli* bacteria [1]. After that, many kind of studies has used this method to mechanically probe the membrane properties of cell and DNA. Lim et al [1] conducted an experiment to human red blood cells infected by malaria. It was found that there is significant stiffening in the infected red blood cell. This result agrees with previous work using micropipettes technique.

Figure 2.11 Optical Tweezer experiment on red blood cell. Figure was cited from [1]

2.4.2 Magnetic Tweezer

Magnetic Trap or Tweezer is a further modification of Optical Trap. Instead of laser, magnetic beads serve as the grips. The electromagnetic field will impose a magnetic force on these beads and compel displacement. Alenghat et al [71] constructed a magnetic tweezer that can apply controlled tensional forces to transmembrane receptors via bound ligand-coated microbeads while optically measuring lateral bead displacements within individual cells. The magnitude of the force is controlled directly and calibrated by electromagnetic field intensity.

Magnetic tweezer has an advantage to laser tweezer that the possible damage by radiation is not a problem anymore. However, the magnetic susceptibility of each bead is difficult to determine which make it difficult to calibrate and to control it precisely.

Figure 2.12 Magnetic Tweezer Experiment. Figure was cited from [71]

2.4.3 Micropipette Aspiration

Micropipette is a simple method to probe mechanical properties of single cells. This technique applies a suction pressure to partially or completely suck a single cell into a micropipette whose diameter may range from less than 1 to 10 micrometer and uses video microscopy to record the shape and change of the cell [1]. By measuring the cell elongation into pipette, the mechanical properties of the cell can be evaluated. Micropipette is compatible for probing suspension cell who constituted primarily by liquid.

Mitchison and Swann was the first to use MA to measure the elastic properties of sea urchin eggs in 1954 [1]. Since then, it has been used to characterize the properties of cells such as leukocytes, fibroblasts, neutrophils, chondrocytes, and red blood cells [1,7-11]. Trickey et al. [12]., for example, use this method to find the Poisson's ratio of chondrocytes cell

The main advantage of micropipette aspiration is its simplicity. However, the stress concentration at the pipette edge and the friction happen between micropipette surface and cell membrane can be a main disadvantage. These might affect the force calibration process and cause error in mechanical response calculation. Haider and Guilak [11] proposed an axisymmetric boundary integral model for incompressible linear viscoelasticity to solve micropipette aspiration contact problem.

Figure 2.13 Micropipette aspiration experiment to chondrocytes. Figure was cited from [7]

2.4.4 Atomic Force Microscopy

This technique involves a very sharp tiny tip mounted at the end of a flexible cantilever which directly senses the sample surface. A very precise relative vertical and lateral displacement between the sample and AFM tip is achieved via a computer controlled piezoelectric stage or cantilever holder. The interaction force between the tip and the sample surface induce deflection and this is recorded by a laser beam reflected off the back of cantilever. A sensitive photodiode is then used to sense the change of the movement and direction of the reflected laser beam.

Fig. 15. Schematic of an AFM set up.

Figure 2.14 Atomic Force Microscopy Experiment Setting. Figure was cited from [1]

AFM can act as both a powerful imaging tool and force sensor with pN resolution. The most important characteristic of AFM is its ability to image samples in fluids so it allows imaging of the samples in its natural physiological environment. The disadvantage of AFM is its limitation by Hertz' Contact Theory. Because of this, if the tip probes at different place, the elastic modulus would also vary. Besides that, the complexity of the molecular structure which make up cell membrane also a problem. Lastly, some error due to the laser and photodiode sensor can affect measurement especially in liquid. As the laser will travel from open air into liquid environment and vice versa, refraction happens during measurement.

2.4.5 Magnetic Twisting Cytometry (MTC)

This technique is done by partially embedding magnetic beads into the cells. Large numbers of beads are coated with ligands to bind them to cell surface. After that, several electromagnetic fields are introduced to the system and cause the beads to rotate. The force and displacement by the beads to the cell are measured by magnetometer. This method was introduced by Crick [1] and has been used to study cytoplasm and cell wall properties until now [7]. For example, Fabry [2] used MTC to dynamically characterizing a single cell.

Figure 2.15 Magnetic Twisting Cytometry (MTC) experiment. Figure was cited from [9]

The advantage of MTC is that it can give a much bigger force compared to Optical Trap or AFM (from 0.1 pN to 1 μ N). Not only that, cells may be mechanically probed continuously for hours or even days without potential heating problems and forces can be applied inside cells by allowing cells to engulf the beads [14]. However, it has a

disadvantage that there are difficulties in controlling the beads. Because of this, to get the average bead displacement, tens of thousands cells experiment had to be done. Not only that, there is a concern that the embedded bead will alter the cell mechanical response.

2.4.6 Micromanipulation Techniques

Micromanipulation is done by using a probe to apply force to the cell and using sensors measures the force and deformation of the sample. Micromanipulation has two types of test, compression and indentation. The difference between these two is just the shape and size of the probe.

Zhang et al. [72] developed a micromanipulator device to squeeze a single mammalian cells. The two flat surfaces were made by grinding the ends of optic fibre probes with diameters about 50 micrometers. Cells were captured from a drop of culture medium on a slide. The slide was put on a copper stage held by third micromanipulator. While a cell was squeezed, the force transducer will measure the force. This micromanipulation technique can be used to measure the bursting membrane tension, bursting pressure and compressibility modulus.

Figure 2.16 Micromanipulator device developed by Zhang et. al. Figure was cited from[72]

Ju et.al. [73] use a video enhanced depth-sensing indentation technique for characterizing biomaterials. Using a dc motor incorporated with a PZT actuator was fixed on a manual position adjustor. It could generate a long traveling distance of up to 12 mm with up to 0.1 μm resolution. A force transducer is attached to the termination face of the PZT to sense the force. A visual system of the inverted optical microscope captured the variation of samples.

Figure 2.17 Micromanipulation technique developed by Ju et.al. Figure was cited from [73]

Peters et al [74-76] is also develop a device based upon compression technique which is rather similar as Zhang's [72] and Bing's [73] device. However, it has a different force and deflection resolution. Peters tried to use this device to find the viscoelastic properties of single cell.

Figure 2.18 Compression technique device developed by Peters et.al. : (a) picture; (b) real device. Figure was cited from [74]

There are other research developed using a microplate manipulation methods. Thoumine et al [77] used this technique to determine the viscoelastic properties of a fibroblast for a certain range of frequency.

The micromanipulation technique is very simple. It only needs a probe, force and displacement transducers. Compression manipulation eliminates micropipette aspiration contact problem. However, it was found that there is some inaccuracy due to transducers error.

2.5 AFM Fundamentals

AFM appeared in 1986 and it was originally designed to obtain topographical images of surfaces [23-28]. AFM was rapidly applied to measure the mechanical properties of different samples. The possibility of working under liquid conditions made AFM straightforwardly applied to the study of structural properties of. The application of AFM to probe the mechanics of biological samples arrived in 1992. After these works, a great amount of studies appeared, and continue to appear, to study the morphology and the mechanical properties of living cells under different conditions, as well as various novel methods and techniques to improve measurements on biological samples, and specially on living cells [23-51]. Even though, a series of limitations arise in practical use, especially when using commercial systems. In this section, we briefly review the most common AFM methods and techniques to obtain both topographical images and mechanical measurements on living cells, emphasizing on the limitations that arise in the day to day use.

2.5.1 Principles of operation in imaging living cells

As mentioned above, AFM and its precursor scanning tunnelling microscopy, was firstly applied to obtain topographical information of hard surfaces in air. However, its capability of imaging non conducting surfaces in liquid conditions induced its immediate application to biological specimens. AFM is indeed mostly used as an imaging technique, especially in the field of material sciences.

AFM images are obtained by scanning the sample surface with the cantilever tip at a fixed force (fig. 2.14). The force is fixed by maintaining the cantilever to be always in constant deflection value using feedback electronics (usually digital proportional integral circuits) continuously correcting the vertical position of the cantilever (relative to the sample) as the input signal varies due to topographic obstacles. The sample topography is reconstructed from the vertical movements used to maintain the fixed force set point. The different imaging modes available depend on the input signal used in the feedback circuit to control the vertical position. The two main modes used in biology are *contact* and *intermittent contact* (IC or *tapping*) modes, which will be briefly discussed in the following. Other techniques and improvements

have been introduced such as magnetic modulation, frequency modulation, or ultrasonic force microscopy.[53]

Contact mode as the name implied requires the cantilever tip to be always in contact with the sample. Contact mode can provide high lateral resolution. Imaging mode, tip sharpness, sample stiffness and applied force determine the maximum lateral resolution that can be reached. In contact mode, soft cantilevers ($k \sim 0.01$ N/m) carrying sharp pyramidal tips (with an apex radius < 50 nm), low tip velocities (< 100 $\mu\text{m/s}$) and applied forces of few hundreds of pN are desired to obtain lateral resolutions of tens of nm on living cells. However, to obtain good contrast of the underlying CSK, higher forces might be applied. Another disadvantage found in this mode is the possibility of damaging the sample. In experiments, there will be an adhesion happen between the tip and the sample. A high contact force between them may damage the sample. Not only that, to make sure the cantilever can deflect upon encountering soft biological samples, the cantilever has to have a low stiffness as mentioned before. A soft cantilever would of course easier to be broken during experiments.

Figure 2.19 Illustration of two modes in AFM : contact and tapping modes [53]

Tapping or Intermittent Contact (IC) mode consists in making oscillate the cantilever at its resonant frequency (few kHz) so that it will only have an intermittent contact with the sample. This solves the adhesion problem that can happen in contact mode. Under liquid conditions, the driving oscillation is usually applied by the same piezoelement that controls the vertical position. When imaging living cells with soft cantilevers (~ 10 pN/nm) the driving oscillation leads to a cantilever deflection

amplitude of few tens of nanometers. The driving amplitude of oscillation is adjusted depending on the characteristics of the sample. The scanning is carried out by using the amplitude signal for the feedback. IC-mode is applied when low shear and lateral forces are required. In this mode the tip taps the sample only intermittently leading to lower perturbation and reduced shear forces as the tip scans the sample. In the case of living cells, IC-mode takes advantage of the mechanical properties of living cells. At the resonant frequencies of the cantilevers, cells behave almost as a viscous fluid. Therefore, the force exerted by the cell is proportional to the velocity at which it is probed leading to apparently higher forces when imaging in IC-mode. Even with its suitability for acquiring images of living cells without damaging them, IC-mode images have low lateral resolution and it is difficult to resolve cellular structural components, such as the CSK. However, IC-mode may be useful when only morphological parameters (perimeter, area, volume) are intended to be imaged.

AFM image can bring quantitative information such as height, perimeter or volume of living cells together with structural details of the surface and the inside of the cells, *e.g.*, cytoskeleton. The possibility of changing the liquid environment while imaging makes AFM an excellent tool to track structural and dimensional responses of living cells under drug treatment.

As mentioned above, AFM image is obtained by scanning the sample surface with the cantilever tip continuously correcting the vertical position (height) as the deflection (in contact mode) or amplitude (in IC-mode) vary as a result of sample topography. If the feedback circuiting was ideal, the error signal might be constant during the scanning. However, the responses of actual PID control circuits depend on the good tuning of the proportional and integral values and on the properties of the cantilever and the sample. Thus error signal are not constant but provide images that can reflect properties of the sample. Depending on the imaging mode, error images provide information about minute corrugations of the sample surface (deflection in contact mode) or viscoelastic properties of the sample (amplitude in tapping mode).

The type of the cantilever tip also affects the result of the image. For the purpose of high resolution imaging, a sharp cantilever tip is needed. A conical or pyramidal tip can give a higher resolution image compared to spherical tip. The drawback of a sharp

tip though is that it can damage the cell. A small contact area would create a higher pressure value to the part under investigation.

One of the main limitations of AFM imaging is its low acquisition rate, especially in the case of large samples (tens of microns), such as living cells. High resolution images of living cells can last several minutes, and are thus restricted to low cellular changes. Drift artifacts due to thermal deformations of both AFM system and samples are commonly observed in AFM imaging. These effects may be minimized by thermal stabilization of the whole system, even if it is usually difficult on living organisms. Piezoelectric elements also introduce creep and hysteresis effects when imaging large samples.

2.5.2 Mechanical measurements

Apart from imaging purposes, AFM is widely used as a force sensor, detecting forces ranging from tens of pN to hundreds of nN, which is the range involved in cell mechanics. Static mechanical measurements can be obtained by applying triangular oscillations in the vertical direction and measuring the resulting deflection as the tip contacts the sample. The deflection is translated into force using the spring constant of the cantilever which ranges from few mN/m to hundreds of N/m. The obtained curves are commonly known as force-distance ($F \cdot z$) curves, or simply force curves. The analysis of the approaching or loading and the retracting or unloading curves provides information about the elastic and adhesion properties of the sample, respectively, although different information can be extracted from them.

Commercially available tips used to be pyramidal or conical shaped (Olympus, Tokyo, Japan; Veeco, Santa Barbara, USA; MikroMasch, Tallinn, Estonia). Modified cantilevers with spherical bead and cylindrical needle attached at their ends had also been used in measuring cell mechanics. All these geometries lead to nonlinear force-indentation ($F \cdot \delta$) relationships, the force increases as the tip indents the sample as a result of the increasing area of contact between the tip and the sample as shown in figure 2.20.

Figure 2.20 Force-displacement curve [29]

The $F(\delta)$ relationships obtained with the various tip geometries is described by a contact elastic model of a rigid punch indenting an elastic half space. Heinrich Hertz was the first who described and solved the problem of two elastic bodies in contact [96], and Sneddon solved the problem for different tip geometries [97]. Table 2.2 summarizes the most relevant contact elastic models described in the literature. As it was mentioned by Butt et al. [27], a smooth spherical particle of defined radius is the preferable tip for characterization purpose. It is because (1) the force can be analyzed more quantitatively; (2) the total force is higher as the contact area is higher thus measurement can be more sensitive; (3) it is possible to make a variety of probe by attaching particles of different chemical properties; (4) hydrodynamic force measurement become possible.

Table 2.2. Contact models for common tip geometries

Tip geometry	Force-indentation relationship, $F(\delta)$
Sphere of radius R	$F = \frac{4E}{3(1-\nu^2)} R^{1/2} \delta^{3/2} \quad [96]$
Cone of semi-included angle θ	$F = \frac{2E \tan \theta}{\pi(1-\nu^2)} \delta^2 \quad [97]$
Flat ended cylinder of radius a	$F = \frac{2E}{(1-\nu^2)} a \delta \quad [97]$
Four-sided regular pyramid of semi-included angle θ	$F = \frac{0.7453 E \tan \theta}{(1-\nu^2)} \delta^2 \quad [98]$

.Note: E and ν are Young's modulus and Poisson ratio of the sample, respectively.

The most common approach to obtain the elastic modulus or Young's modulus (E) of living cells consists of several steps. First, the F - z curves are transformed into F . δ curves by computing the indentation of the tip into the sample, determining the point

at which the tip contacts the sample (point of contact). Second, the suitable contact elastic model to the geometry of the tip is fitted to the loading part of the curve. Choosing the approaching part prevents from possible artifacts related to adhesion observed in the retracting region. Due to the nonlinearity of the force response, two issues are crucial for a reliable estimation of E : the determination of the point of contact and the suitability of the contact model to the actual tip geometry [29, 34,37,40]. The point of contact is defined by the force (or deflection) offset (F_{off} or d_{off}) and the vertical point at which the cantilever starts to deflect (z_c). In the case of soft samples, the point of contact is difficult to determine visually due to the smooth deflection of the cantilever at low indentations. Thus, an objective estimation of these parameters should be established. Table 2.3 shows the current research founding about the Young's Modulus of various types of cells.

Table 2.3 Young's Modulus of living cells. Cited from [43]

Young's modulus of mammalian cells		
Cell type	E (kPa)	Commentary
Endothelial cells		
HUVEC	10–11	
–	1.3–7.2	Spatial heterogeneity
–	0.9–1.7; 12.0–18	Shear stress, different models
BPAEC	0.2–2.0	Spatial heterogeneity
Leukocytes		
Leukemia myeloid cells (HL60)	0.2–1.4	
Leukemia lymphoid (Jurkat) cells	0.02–0.08	
Neutrophils	0.2–0.07	
Corti organ's cells		
Outer hair cells	300–400	Cortical lattice
Guinea pig's outer hair cells	2–4	Different levels of cochlea
Mouse outer hair cells	2–4	
Guinea pig's inner hair cells	0.1–0.5	
Hensen's cells	0.3–1.1	
Osteoblasts	0.3–20.0	Changes at adhesion
Astrocytes	2–20	Spatial heterogeneity
Fibroblasts	4–5	
Migrating 3T3 cells	3–12	Spatial heterogeneity
–	0.6–1.6	Changes at adhesion
L 929	4–5	
Epidermal keratocytes	10–55	Spatial heterogeneity
Platelets	1–50	Spatial heterogeneity at activation
Skeletal muscle cells		
Murine C ₂ C ₁₂ myoblasts	11–45	Change at differentiation
Murine C ₂ C ₁₂ myotubes	8–14	Changes at differentiation, Treating with L-arginine
–	10–17	
–	28–21	
Myofibrils	40–45	
Cardiocytes		
Rat	90–110	Aging changes
Chicken	32–42	Spatial heterogeneity
Erythrocytes		
	5–200	
	14–18	Normal
	19–33	
	22–64	Hereditary spherocytosis, thalassemia, G6PD deficiency
	16–64	
	70–110	

Kuznetsova et al.[43] mentioned that there are several factors that affect the elasticity measurement using AFM. The first factor is a question of AFM sample handling such as the buffer and immobilization method. The second factor is related to the heterogeneity of mechanical properties of cells. There are significant variations of the values of elastic modulus at different cell regions. The third factor influencing elastic modulus measurement is connected with cell thickness. Substrate contributions can be neglected if AFM tip never indented more than 10% of the cell thickness. If the cell compartment under the study is very thin (<1000 nm), e.g. lamellipodium, it is necessary to impart special challenges for accurate measurements of its viscoelastic behavior instead of the usual Hertz contact model. Mahaffy et al.[35] reported the method for AFM-based microrheology that allowed to estimate the viscoelastic constants of thin parts of cell (<1000 nm) as well as those of thick areas, applying two different models—a model for well-adhered regions and a model for nonadhered regions.

Figure 2.21 Effect of heterogeneity and indentation depth to AFM result [24]

Even if the Young's modulus is a reliable estimate of cell stiffness, cells are not elastic bodies but viscoelastic. Several authors have measured the viscoelasticity of living cells by applying force or deformation steps and measuring the corresponding response [22,50,78]. The main limitation of these approach is that the obtained mechanical parameters are limited by the width of the time window used during the measurements.

A more robust method to probe cell viscoelasticity is the application of low amplitude oscillations at a wide frequency range to estimate the complex shear modulus G^* . The complex shear modulus is divided into a real or in-phase component, which stands for the elasticity or the stored energy, and an imaginary or out-of-phase component, which stands for the viscosity or lost energy. The real part is known as the elastic modulus (G'), while the imaginary part is called the loss modulus (G''). The oscillatory technique has been successfully applied to a wide variety of living cells including lung epithelial cells, airway smooth muscle cells, macrophages, and neutrophils in basal conditions and under mechanical or pharmacological treatment [2,19,21,34-37,40].

The oscillatory technique used in AFM measurements on living cells consists in applying low amplitude oscillations (50-100 nm) at an operating indentation (δ_0). Under these conditions, the contact elastic model described by $F(\delta)$ can be approximated taking the first two terms of its Taylor expansion [34]

$$F = F(\delta_0) + [(\partial F / \partial \delta)(\delta_0) * (\delta - \delta_0)] + \dots \quad (2.1)$$

For example, in the case of a flat ended cylindrical tip of radius a , the above expansion leads to

$$F = F_0 + 2E a (\delta - \delta_0) / (1 - \nu^2). \quad (2.2)$$

Expressing Eq. 2.2 in terms of the complex shear modulus $G^* = E/2(1+\nu)$ and solving for G^* we obtain

$$G^*(\omega) = \frac{(1 - \nu)}{4a} \frac{(F - F_0)}{(\delta - \delta_0)} \quad (2.3)$$

by invoking the Parseval theorem, Eq. 2.3 can be transformed into the frequency domain to obtain an expression for the complex shear modulus

$$G^*(\omega) = \frac{(1 - \nu)}{4a} \frac{F(\omega)}{\delta(\omega)} \quad (2.4)$$

where ω is the angular frequency ($\omega = 2\pi f$), and $F(\omega)$ and $\delta(\omega)$ are the Fourier transforms of force and indentation, respectively.

Table 2.4. Complex Shear Modulus for common tip geometries

Tip geometry	Complex Modulus $G^*(\omega)$
Sphere of radius R	$G^*(\omega) = \frac{(1 - \nu)}{4R^{1/2} \delta_0^{1/2}} \frac{F(\omega)}{\delta(\omega)}$

Cone of semi-included angle θ
$$G^*(\omega) = \frac{\pi(1-\nu)}{8(\tan\theta)\delta_0} \frac{F(\omega)}{\delta(\omega)}$$

Flat ended cylinder of radius a
$$G^*(\omega) = \frac{(1-\nu)}{4a} \frac{F(\omega)}{\delta(\omega)}$$

Four-sided regular pyramid of semi-included angle θ
$$G^*(\omega) = \frac{(1-\nu)}{2.9812(\tan\theta)\delta_0} \frac{F(\omega)}{\delta(\omega)}$$

Note: δ_0 and ν are initial indentation and Poisson ratio of the sample, respectively.

When probing biological samples under liquid conditions the hydrodynamic drag force exerted on the cantilever should be corrected at frequencies above ~ 0.3 Hz [39]. The drag force depends on the geometry of the cantilever, by the drag factor $b(0)$, and on the velocity of the cantilever free end $v = d\delta/dt$. It can be expressed by $Fd = b(0)v$. As described in the work by Alcaraz and co-workers, the drag factor increases as the tip approaches the surface. The factor $b(0)$ is usually obtained by applying low amplitude oscillations at different heights above the sample surface and extrapolating to zero separation [37,39,40]. The hydrodynamic drag contribution in the frequency domain has the form $Fd(\omega)/\delta(\omega) = i\omega b(0)$ (where $i = \sqrt{-1}$), and may be included into Eq. 2.4 to correct for the drag force, obtaining

$$G^*(\omega) = \frac{1-\nu}{4a} \left[\frac{F(\omega)}{\delta(\omega)} - i\omega b(0) \right] \quad . (2.5)$$

Due to the softness of living cells, mechanical measurements must be carried out using soft cantilevers (tens of mN/m) to apply low forces usually below 1 nN. Another important factor to take into account is the thickness of the cells. Living epithelial cells plated on glass coverslips have a thickness of 5-10 μm . Thus, the indentation should also be controlled in order not to sense the underlying hard substrate. The maximum indentation reachable when measuring soft samples without sensing the hard substrate can be estimated to be 10-20% of sample thickness [24].

With a fast development of MEMS technology, an idea to create a self-sensing-actuating cantilever was born [54-60]. This new cantilever can be made by using a piezoelectric effect of piezoelectric material. Previously, for tapping mode, an external piezoactuator has to drive the cantilever. This new cantilever however would not need this as it has its own piezoelectric material layer on top of it. This piezo layer

also can detect the displacement of the cantilever tip by measuring the sensing impedance. To get this sensing impedance, a bridge electric circuit (figure 2.22) was needed. The sensing impedance will be proportional to the tip deflection. This method has been used for imaging. According to Rogers et al [58], this new cantilever has an advantage of higher sensitivity compared to optical sensing system. Not only that, the driving voltage amplitude is smaller compared to the external piezoactuator driving voltage. This is because the piezoelectric layer only actuates the cantilever instead of the whole substrate package. This makes this new cantilever more efficient. This new cantilever has been proven to be working to image gold layer and E. coli bacteria [57-60].

Figure 2.22 Bridge Circuit used in the self-sensing actuating cantilever [58]

2.6 Piezoelectricity

Piezoelectric material has been popularly developed as transducers because of its electromechanical properties. Because of this, piezoelectric material can be developed as actuators and sensors. As an actuator, piezoelectric behaved based upon indirect piezoelectric effect where the actuating electrical voltage is converted into mechanical energy. On the other hand as a sensor, piezoelectric behaved based upon direct piezoelectric effect where mechanical force will generate electrical charge.

In linear piezoelectricity the equations of elasticity are coupled to the charge equation of electrostatics by means of piezoelectric constants. Below are the constitutive equations that couple the piezoelectric mechanical and electrical response.

$$\{T\} = \{c^E\} \{S\} - \{e\} \{E\} \dots \dots \dots (2.6)$$

$$\{D\} = \{e^T\} \{S\} + \{\epsilon^S\} \{E\} \dots \dots \dots (2.7)$$

or equivalently

$$\begin{Bmatrix} \{T\} \\ \{D\} \end{Bmatrix} = \begin{bmatrix} \{c^E\} & -\{e\}^t \\ \{e\} & \{\epsilon^S\} \end{bmatrix} \begin{Bmatrix} \{S\} \\ \{E\} \end{Bmatrix} \dots\dots\dots (2.8)$$

where:

$\{T\}$ = stress vector or $\{\sigma\}$ (N/m²)

$\{D\}$ = electric charge density vector (C/m²)

$\{S\}$ = strain vector (or $\{\epsilon\}$)

$\{E\}$ = electric field intensity vector (N/C)

$[c^E]$ = elasticity matrix (evaluated at constant electric field)

$[e]$ = piezoelectric stress coupling matrix

$[\epsilon^S]$ = dielectric matrix (evaluated at constant mechanical strain)

Due to this unique property, piezoelectric material has been used for manifolds of application as actuator and sensor in structural health monitoring, vibration control, modal testing, and mechanical design [79-91]. Piezoelectric has high resolution, zero friction, no wear and tear, and fast response. Not only that, it is rugged, cheap, light, and space efficient.

PZT can be modeled as a pure capacitor (C_p) and a voltage source (V_p) caused by self-sensing actuation. The impedance sensing circuit consists of either a sensing resistor (R), a capacitor (C), or parallel connection of the two, as shown in the figure 2.20. Any appropriate combinations can be used considering the balance of the impedance mismatch of the circuit.

Figure 2.23 Representation of piezoelectric (PZT) circuit. [84]

With the direct and inverse effect that exists in PZT materials, they can be used for both actuation and sensing simultaneously, referred to as the self-sensing actuator, which has been a recurrent research topic in the area of vibration control.

Traditionally, to achieve the self-sensing actuation, the sensing signal from the PZT is separated from the applied input signal by a special electric circuit referred to as a bridge circuit, as shown in Figure 2.21 under assumption that the PZT acts like a pure capacitor in the electric circuit analysis. When an actuation signal is applied, the PZT and the matched capacitance will receive an equal voltage. At the same time, vibration is induced in the structure and a sensing voltage will be added to the PZT patch. Each branch of the circuit is then feed through an op-amp, which cancels the actuation signal and leaves only the sensor signal out. However, because the magnitude of the applied voltage is usually much larger than that of the sensor signal, and the capacitance of the PZT is significantly influenced by temperature changes, the signal is easily mixed with input voltage, making physical realization of self-sensing actuation extremely difficult.

Figure 2.24 Bridge Circuit Model for self-sensing actuating mode. [85]

2.7 Simultaneous Sensing Cum Actuating (SSA) Method

Pseudo mechanical impedance is the ratio between the force applied to and the velocity along the force direction measured at the same given point on the structure. It is one of the frequency response functions used in modal analysis that is important to monitoring structural integrity of a model. It also has been utilized as means of

characterizing viscoelastic materials. One of the problems found to measure mechanical impedance is the bulky nature of equipments. The equipments are consists of a shaker, a force sensor, and an accelerometer. This will cause problem if the samples are small. Besides that, there is inconvenience found during installation and calibration of the equipments on site.

In 1994, Liang et al. developed an impedance method for dynamic analysis of active material systems [84-85, 88]. Liang shows that the electrical admittance, which is an inverse of the electrical impedance, of the PZT actuator is a combined function of the mechanical impedance of the PZT actuator and that of the host structure. This indicates that the electrical impedance of the PZT bonded onto the structure is directly related to the mechanical impedance of a host structure. Structural damage causes changes in structural parameters, and hence, changes in electrical impedances. The variation in the PZT electrical impedance over a range of frequencies is analogous to that of the frequency response functions (FRF) of a structure, which contains vital information regarding the health of the structure. In 1996, Rogers and Liang further studied on the coupling of the electrical impedance of piezoceramic materials and mechanical impedance of the structures. In their work, PZT patches were embedded into a structure. Then, a one-DOF model governing the electromechanical coupling was developed. This impedance technique later further developed for multidimensional model such as beam, shell, plate, and ring [84-85, 89]. The dynamic interaction between the PZT patch and the host structure has to be predetermined by an analytical solution. Because of this, this method is difficult to be applied in complex structure.

Ling and Xie [90] then further develop this technique by using a PZT inertial actuator(PIA) affixed to a structure as a collocated sensor cum actuator for monitoring structural integrity. Later on, Ling and Xie [91] managed to model the relationship between electrical impedance and mechanical impedance through a set of transduction matrix.

Ling and Xiel [90] developed a PIA as shown in the figure 2.25. An aluminum plate with a piezoceramic patch bonded to its inner surface is clamped as the cap onto the base of PIA. Then, the proof/inertial mass with appropriate weight is fixed at the

centre of the outer surface of the plate. When excited by electrical power, expansion and contraction of the PZT patch generated caused bending of the plate (introducing stress and strain to it) which also accelerates the mass. This is called inverse piezoelectric effect. A reaction force due to this accelerated mass will be exerted to the structure under testing through the base. The piezoelectric effect will cause a variation on the electrical impedance. This input electrical impedance is the variable that sensing cum actuating device wanted.

Figure 2.25 PZT Inertial Actuator (PIA). Figure was cited from [91]

Since the PZT inertial actuator (PIA) is a linear power transducer without internal energy generation, the relationships between the electrical voltage and current in the input port and the mechanical force and velocity at the output port can be described as 2x2 transduction matrix. This transduction matrix is consist of four frequency-responses transduction functions.

The transfer matrix can be written as the formula below.

$$\begin{Bmatrix} E \\ I \end{Bmatrix} = \begin{bmatrix} T_{11} & T_{12} \\ T_{21} & T_{22} \end{bmatrix} \begin{Bmatrix} F \\ v \end{Bmatrix} \dots\dots\dots(2.9)$$

This technique calculates the mechanical impedance using the electrical impedance without usage of force or motion sensor. Because of that, it makes sensor-less and in situ dynamic measurement of mechanical systems possible and avoid difficulties of sensor installation. Wang [93] had successfully used SSA method to create a miniature PZT coated cantilever (21mm x 6 mm x 3 mm dimension) for dynamic testing of structures. The experimental results measured by this method were found quite closed to the numerical results calculated by finite element method.

Figure 2.26 Experimental diagram of SSA indentation on soft viscoelastic material.

Figure was cited from [94]

Yin and Ling [94-95] used this sensing cum actuating method to characterize viscoelastic material. Yin used an electromagnetic shaker as a dynamic exciter in this SSA method. This device then used to dynamically indent some set of viscoelastic samples: EPDM280C rubber, NE264C rubber, and commercial eraser. It was found that the complex modulus found from this technique has similar values as Dynamic Measurement Analysis (DMA) result. Some discrepancies found when NE264C, the hardest sample, was done. This might be caused by the deformation in the suspension mechanism when it tried to indent a hard material.

It is proposed that this method is particularly suitable to characterize soft incompressible viscoelastic material. Thus, there might be a high possibility to use this method to characterize biomaterials such as cells.

Chapter 3

Transduction Functions Determination of the Cantilever Transducer by ANSYS Simulation

In the subject of cell mechanics, Atomic Force Microscopy has been one of the popular devices utilized. With the rapid development of MEMS fabrication, a new type of AFM cantilever has been developed. This new cantilever is created by adding a piezoelectric layer on top of the cantilever. This adds improvement over the conventional cantilever as it will be able to perform self-sensing and actuating due to the piezoelectric layer.

Ling et al [90-95] introduced a concept called transduction functions that can define an electromechanical transducer. This concept was based upon the determination of the system dynamics that relates the input and output port of the transducer. As actuator is a device that imposes a state on the system (input) whereas sensor is a device that monitors a parameter of the system (output), transduction functions enable an actuator to act simultaneously as a sensor. Ling et al [90-95] has developed this theory for years to measure system integrity and characterize viscoelastic material.

Wang [93] fabricated piezoelectric cantilevers transducers and modeled them in ANSYS program. Using Ling's technique, the transduction functions were determined through a simulation in ANSYS. The transduction functions found were then tested in laboratory experiment and found to be quite closed with the transduction functions found through laboratory calibration. The ANSYS transduction functions can also determine the mechanical impedance response that was given to each transducer during tests. These mean that ANSYS can help as transduction functions determination tool in Ling's technique especially if the size or shape of the transducer is a major obstacle in doing calibration experiment.

This chapter will cover all details about the process to calibrate the piezoelectric coated cantilever using transduction matrix theory. Firstly, the details about the

piezoelectric coated cantilever will be explained. After that, a detailed explanation about the transduction matrix theory would be stated. Then, the process would be done using ANSYS simulation to determine the transduction functions. Lastly, experimental measurement result will be used to verify the transduction functions.

3.1 Piezoelectric-coated cantilever

3.1.1 Piezoelectric Constitutive Equations

In linear piezoelectricity the equations of elasticity are coupled to the charge equation of electrostatics by means of piezoelectric constants. Below are the constitutive equations that couple the piezoelectric mechanical and electrical response.

$$\{T\} = \{c^E\} \{S\} - \{e\} \{E\} \dots\dots\dots (3.1)$$

$$\{D\} = \{e^T\} \{S\} + \{\epsilon^S\} \{E\} \dots\dots\dots (3.2)$$

or equivalently

$$\begin{Bmatrix} \{T\} \\ \{D\} \end{Bmatrix} = \begin{bmatrix} \{c^E\} & -\{e^T\} \\ \{e\} & \{\epsilon^S\} \end{bmatrix} \begin{Bmatrix} \{S\} \\ \{E\} \end{Bmatrix} \dots\dots\dots (3.3)$$

where:

$\{T\}$ = stress vector or $\{\sigma\}$ (N/m²)

$\{D\}$ = electric charge density vector (C/m²)

$\{S\}$ = strain vector (or $\{\epsilon\}$)

$\{E\}$ = electric field intensity vector (N/C)

$[c^E]$ = elasticity matrix (evaluated at constant electric field)

$[e]$ = piezoelectric stress coupling matrix

$[\epsilon^S]$ = dielectric matrix (evaluated at constant mechanical strain)

This constitutive equations can also be rewritten in strain- charge form

$$\begin{Bmatrix} \{S\} \\ \{D\} \end{Bmatrix} = \begin{bmatrix} [s^E] & -\{d^T\} \\ \{d\} & [\epsilon^T] \end{bmatrix} \begin{Bmatrix} \{T\} \\ \{E\} \end{Bmatrix} \dots\dots\dots (3.4)$$

$[s^E]$ = compliance matrix (evaluated at constant electric field) (m² /N)

$[e]$ = piezoelectric strain coupling matrix

$[\epsilon^T]$ = dielectric matrix (evaluated at constant mechanical stress)

To change the strain charge equations to stress charge equations, a set of equations in Eq.3.5 can be done.

$$\begin{aligned} \mathbf{c}_E &= \mathbf{s}_E^{-1} \\ \mathbf{e} &= \mathbf{d} \cdot \mathbf{s}_E^{-1} \\ \boldsymbol{\varepsilon}_S &= \boldsymbol{\varepsilon}_T - \mathbf{d} \cdot \mathbf{s}_E^{-1} \cdot \mathbf{d}^t \end{aligned} \dots\dots\dots(3.5)$$

3.1.2 Piezoelectric-coated cantilever under testing

With the rapid development in MEMS technology, a new cantilever with a piezoelectric layer coat has been developed. One of this cantilever has been commercialized and sold by Veeco.co as DMAPS Active Probe cantilever. The cantilever substrate can be mounted and wire bonded to a PCB board as shown in figure 3.1.

Figure 3.1 Mounted DMAPS

The probe is a MEMS device, micro-machined from bulk silicon with a piezoelectric film (Zinc oxide) patterned along a portion of the micro-cantilever. At the free end of the cantilever, there is a pyramidal tip with nanometer scale sharpness optimally shaped for high-resolution imaging. The micro-cantilever is capable of bending by way of bimorph actuation resulting in a controlled vertical displacement of the tip.

The Active Probe contains a ZnO stack (consisting of 0.25μm Ti/Au, 3.5μm ZnO, 0.25μm Ti/Au) at the base of each probe. This stack, along with the silicon cantilever, acts as a bimorph to control the vertical displacement of the tip. The ZnO bimorph can be used to 1) statically deflect the tip (for high speed imaging), 2) oscillate the cantilever (for tapping and active control), and 3) in reverse for self-sensing. The actuation sensitivity is nominally 20nm/V. The breakdown voltage is nominally 10V. The fundamental resonance is nominally 50kHz (range 40-60 kHz), the TappingMode imaging is best preformed on the second resonance, which is nominally 200kHz.

Sensor length	A	125 μm
Sensor width	B	55 μm
Actuator Length	C	375 μm
Actuator width	D	250 μm
Cantilever thickness		4 μm
Piezoelectric thickness	ZnO	3.5 μm
Ti/Au electrodes (2 electrodes)		0.25 μm each
ZnO width		160 μm
ZnO length		330 μm
Fundamental Res. Freq.		50kHz
Operating Res. Freq.		200 kHz

Figure 3.2 Active Probe cantilever with its specifications

Tip Height:	15μm - 20μm
Front Angle:	25°
Back Angle:	
Side Angle:	17.5°
Tip Radius (Nom.):	<10nm
Tip Radius (max.):	15nm
Tip Set Back (Nom.):	15μm
Tip Set Back (Rng.):	5μm - 25μm

Figure 3.3 The tip specifications

Figure 3.4 Cantilever SEM Pictures: from top left to bottom right: cantilever + die substrates, cantilever transducer, cantilever transducer, cantilever tip.

The cantilever is mounted on a die substrate. The piezoelectric layer and the electrodes were deposited along the substrate also to make two electrode areas. These areas are wire bonded to connect this cantilever to an electrical source.

3.2 Transduction Matrix Theory

Ling et al introduce the possibility to use a set of transduction matrix to define a PZT inertial actuator (PIA) [90-91]. With the aid of this matrix, mechanical impedance of a structure can be measured without force and motion sensor. Instead, by measuring the electrical impedance, mechanical impedance can be calculated. The PZT actuator is not only functionalized as actuator but also as a sensor that's why this method is called simultaneous sensing cum actuating (SSA).

Transduction matrix theory originated from four pole theory which was applied in the electrical system [90-95]. According to four pole theory, a complex system can be separated as many subsystem. Every subsystem is a 'black box' which can be viewed as a multi port device. The relationship of variables at different ports can be described with a transfer matrix. Among various multi port theories, two ports or four poles

theory, that has only one input port and one output port, is commonly used due to its simple form.

Figure 3.5 Two-port and four-pole system. Figure was cited from [94]

The four pole device exchanges energy with others at only two ports. At each port we define two variables, one of which is selected as being of primary practical interest to use. The product of the two variables gives instantaneous power flowing through the port. Each of the input, output, and transfer functions is Frequency Response Function (FRF).

$$\begin{Bmatrix} \varepsilon_{in} \\ f_{in} \end{Bmatrix} = \begin{bmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{bmatrix} \begin{Bmatrix} \varepsilon_{out} \\ f_{out} \end{Bmatrix} \dots\dots\dots (3.6)$$

Several subsystems can be connected. Each subsystem has a transfer matrix that defines its relation ship with the next subsystem. For many subsystems, the relation ship of the first subsystem to the n subsystem can be described as formula 3.7.

$$\begin{Bmatrix} \varepsilon_{01} \\ f_{01} \end{Bmatrix} = M_{01} M_{12} \dots \begin{bmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{bmatrix}_{(n-1)n} \begin{Bmatrix} \varepsilon_n \\ f_n \end{Bmatrix} \dots\dots\dots (3.7)$$

Making use of that theory, figure 3.6 shows the four pole diagram of electromechanical transducer. As the four pole theory states, an assumption that there is only one input port and one output port for the transducer is taken. The mechanical structure is assumed as single DOF system with a single input and output point. In the case of an electromechanical system, the input port is electrical and the output port of this diagram is mechanical. Electrical excitation can only be applied from a single input port. Force and velocity can only happen at the designated ouput point.

Figure 3.6 The four pole diagram of electromechanical transducer. Cited from [94]

The transfer matrix of the diagram in figure 3.6 can be written as the formula below:

$$\begin{Bmatrix} E \\ I \end{Bmatrix} = \begin{bmatrix} T_{11} & T_{12} \\ T_{21} & T_{22} \end{bmatrix} \begin{Bmatrix} F \\ v \end{Bmatrix} \dots\dots\dots(3.8)$$

All of the terms in Eq.3.8 are frequency response functions. The symbol E on the equation 3.3 representing the voltage given to the system, I is the current given to the system, F is force output of the system or actuating/transmitted force and v is the velocity output of the system. The four functions inside the 2x2 matrix define the relationship between the electrical input and the mechanical output. These functions are called transduction functions.

$$T_{11} = \left. \frac{E}{F} \right|_{v=0} \quad \text{transfer function of voltage to force if the output point is clamped}$$

$$T_{21} = \left. \frac{I}{F} \right|_{v=0} \quad \text{transfer function of current to force if the output point is clamped}$$

$$T_{12} = \left. \frac{E}{v} \right|_{F=0} \quad \text{transfer function of voltage to velocity if the output point is free}$$

$$T_{22} = \left. \frac{I}{v} \right|_{F=0} \quad \text{transfer function of current to velocity if the output point is free}$$

The transfer matrix is called transduction matrix as this matrix not only define the actuating functions but also the sensing functions of the cantilever transducer. The four elements of the transduction matrix could be determined by clamped or free boundary condition. This determination method is called direct method.

Assuming that the transducer is a linear system then the determinant of the transduction matrix should be unity according to reciprocity theorem.

Using transduction matrix method, a bridge circuit is not needed. Only electrical voltage and current was measured. This would decrease the complexity in the set up process.

3.3 Modelling of the Centilever Transducer in ANSYS

To determine the transduction matrix of the cantilever transducer, ANSYS software was used to simulate the response of the transducer. The cantilever and electrode were modeled as element solid45. The ZnO layer was modeled as solid5 element. The damping ratio was put as 0.005. Below are the mechanical properties of the transducers.

Table 3.1 Material Properties

Silicon Cantilever		
Density	Kg/m ³	2330
Young's modulus	GPa	100
Poisson's ratio		0.22
Gold Electrode		
Density	Kg/m ³	19300
Young's Modulus	GPa	120
Poisson's ratio		0.44
ZnO		
Density	Kg/m ³	5680

Compliance Matrix (s^E)	$\times 10^{-12} \text{ m}^2/\text{N}$	$\begin{bmatrix} 7.86 & -3.43 & -2.21 & 0 & 0 & 0 \\ -3.43 & 7.86 & -2.21 & 0 & 0 & 0 \\ -2.21 & -2.21 & 6.94 & 0 & 0 & 0 \\ 0 & 0 & 0 & 23.6 & 0 & 0 \\ 0 & 0 & 0 & 0 & 23.6 & 0 \\ 0 & 0 & 0 & 0 & 0 & 22.6 \end{bmatrix}$
Piezoelectric Strain Coupling (d)	$\times 10^{-12} \text{ C/N}$	$\begin{bmatrix} 0 & 0 & 0 & 0 & -11.34 & 0 \\ 0 & 0 & 0 & -11.34 & 0 & 0 \\ -5.43 & -5.43 & 11.67 & 0 & 0 & 0 \end{bmatrix}$
Dielectric ϵ^T	$\text{z}8.854\text{pF/m}$	$\begin{bmatrix} 9.16 & 0 & 0 \\ 0 & 9.16 & 0 \\ 0 & 0 & 12.64 \end{bmatrix}$

Figure 3.7 Top and side view of the cantilever

To check whether the simulation correctly model the cantilever, modal analysis was conducted to find the resonant frequencies of the transducer model. As shown in table 3.2, the resonant frequencies found were still within the range of the expected natural frequencies which are 50 and 200 kHz for first and third resonant frequency. Figure

3.8-3.10 shows the modal shape of the cantilever transducer at each resonant frequency. A twisting resonant frequency was also found between the first and third resonant frequency. This twisting frequency can emerge due to the inertia effect.

Table 3.2 Modal resonant frequencies of the cantilever

SET	Frequency (kHz)
1	50.912
2	189.36
3	201.9

Figure 3.8 First resonant frequency deformed shape

Figure 3.9 Second resonant frequency deformed shape

Figure 3.10 Third resonant frequency deformed shape

Figure 3.11 shows the velocity response of the transducer model in free output boundary condition. Figure 3.12 shows the electrical current response simulated during this condition. As this transducer is an electromechanical system, both figures should show the resonances happening on this transducer model. The first and third resonant frequencies can be easily identified in figure 3.11, and 3.12. The twisting resonant frequency can not be identified from the graphs. That is because the velocity is only measured at the tip in the z direction and electrical current is measured primarily due to strain at z direction. At twisting motion, there is no change in the z displacement of the tip. From figure 3.11, it can also be seen that an anti-resonance happens at 135 kHz as there was a change of phase by π radians but the velocity magnitude was very low around that frequency. As the current response can only detect the resonance condition, the anti-resonance at 135 kHz remains undetected in the current response of the piezoelectric layer as shown in figure 3.12.

Figure 3.11 Velocity response during free output boundary condition

Figure 3.12 Electrical Current response during free output boundary condition

3.4 Determination of Transduction Functions of cantilever transducer by simulation (ANSYS)

The most important step in this technique is to determine the transduction functions of the transducer. There are four transduction functions that define an electromechanical system which has electrical inputs (Voltage E and current I) and mechanical outputs (Force F and velocity v). Each transduction function identifies the relationship between one the electrical input to one of the mechanical output.

$$\begin{Bmatrix} E \\ I \end{Bmatrix} = \begin{bmatrix} T_{11} & T_{12} \\ T_{21} & T_{22} \end{bmatrix} \begin{Bmatrix} F \\ v \end{Bmatrix} \dots\dots\dots(3.8)$$

$$T_{11} = \left. \frac{E}{F} \right|_{v=0} \quad \text{transfer function of voltage to force if the output point is clamped}$$

$$T_{21} = \left. \frac{I}{F} \right|_{v=0} \quad \text{transfer function of current to force if the output point is clamped}$$

$$T_{12} = \left. \frac{E}{v} \right|_{F=0} \quad \text{transfer function of voltage to velocity if the output point is free}$$

$$T_{22} = \left. \frac{I}{v} \right|_{F=0} \quad \text{transfer function of current to velocity if the output point is free}$$

In the case of cantilever transducer, the electrical input should be where the excitation was given to the transducer. The mechanical output should be the cantilever tip because this tip will be used to indent future test sample. So this technique assumes

that there is only one output point which is the tip. Any force happens at any parts of transducer beside the output point is impossible to be calculated using this method.

Two methods were done to find the transduction functions: direct method and spring method.

3.4.1 Direct Method in Determining the Transduction Functions

Direct method determines the four transduction functions based on their own definitions (see eq.3.8). This method can be done by applying two extreme boundary conditions to the output of cantilever transducer model. In this case, the output of this transducer is the tip. The first boundary condition is free boundary condition. In this boundary condition, the tip will vibrate freely without any restriction. The second boundary condition is clamped boundary condition. The mechanical output point is clamped to prevent any motion in the z direction. In this case, the tip will be constrained in z direction so as not to move.

Figure 3.13 Top and Side View of the cantilever under clamped condition

In the free boundary condition, the electrical signals (current I and voltage E) will be taken from ANSYS result. Not only that, the velocity (v) at the output point will also be taken. Based upon the definition of T_{12} and T_{22} , then the value of T_{12} is E/v and T_{22} is I/v . In the clamped boundary condition, the electrical signals (current I and voltage E) will also be taken from ANSYS result. Not only that, the force (F) at the output point in the Z direction will also be taken. Based upon the definition of T_{11} and T_{21} , then the value of T_{11} is E/F and T_{21} is I/F .

Figure 3.14 and 3.15 shows the current magnitude and phase under free and clamped boundary conditions. The current magnitude was so closed to each other that they look like they overlapped. From the phase curves, resonances can be seen. At free boundary condition, there would be resonance near 50 and 200 kHz. At clamped boundary condition, there would be resonance at 80 kHz.

Figure 3.14 Current Magnitude under free and clamped condition

Figure 3.15 Current Phase under free and clamped condition

3.4.2 Spring Method

The second method, spring method, was done by attaching a spring with a defined stiffness and no mass to the tip of the cantilever. When a spring is attached to the transducer, the electromechanical system can be represented as fig 3.16. This figure shows transducer and spring as the whole system. The electrical inputs are the electrical signals given to the transducer. The mechanical responses are the mechanical responses at the output of the transducer and spring.

Figure 3.16 Electromechanical system of transducer and spring

This system can be separated into two subsystems: an electromechanical system for transducer and a mechanical system for the spring (see fig. 3.17). F_{o1} and v_{o1} are the force and velocity response induced by the transducer to the spring. This output force from the transducer is the same as the force and velocity input to the spring (F_{i1} and v_{i1}). F_2 and v_2 is the same force and velocity response as fig. They are the force and velocity response by the electromechanical system and spring as a whole system. As the spring and transducer vibrates freely, so there is nothing constraint the spring output. This means that F_2 is zero. As the tip of the transducer and the spring vibrate together, it can be said that $v_{i1}=v_2$.

Figure 3.17 The electromechanical transducer subsystem and spring subsystem

The transducer subsystems' four pole model equation is:

$$\begin{Bmatrix} E_1 \\ I_1 \end{Bmatrix} = \begin{bmatrix} T_{11} & T_{12} \\ T_{21} & T_{22} \end{bmatrix} \begin{Bmatrix} F_{o1} \\ v_{o1} \end{Bmatrix} \dots\dots\dots(3.9)$$

To actuate the spring itself, the force needed will be : $F_{i1}=-k/x$ where x is the displacement of the tip and spring. The displacement is a frequency response function and it's an integration of velocity.

$$x(j\omega) = v(j\omega)/j\omega \dots\dots\dots(3.10)$$

$$F_{i1}(j\omega) = -k v(j\omega)/j\omega \dots\dots\dots(3.11)$$

The spring subsystems' four pole model equation is:

$$\begin{Bmatrix} F_{i1} \\ v_{i1} \end{Bmatrix} = \begin{bmatrix} 1 & -k/j\omega \\ 0 & 1 \end{bmatrix} \begin{Bmatrix} F_2 \\ v_2 \end{Bmatrix} \dots\dots\dots(3.12)$$

With $F_2=0$, $F_{i1}=F_{o1}$ and $v_{i1}=v_{o1}$, Eq 3.12 can be substituted into Eq.3.9 and the ensuing equation is E.g 3.13.

$$\begin{Bmatrix} E_1 \\ I_1 \end{Bmatrix} = \begin{bmatrix} T_{11} & T_{12} \\ T_{21} & T_{22} \end{bmatrix} \begin{Bmatrix} -jkv_{i1}/\omega \\ v_{i1} \end{Bmatrix} \dots\dots\dots(3.13)$$

By using a set of springs with a known stiffness value, the transduction functions can be approximated using E.q. 3.14.

$$\begin{bmatrix} E_1 & I_1 \\ E_2 & I_2 \\ \cdot & \cdot \\ \cdot & \cdot \\ E_n & I_n \end{bmatrix} = \begin{bmatrix} -jk_1v_1/\omega & v_1 \\ -jk_2v_2/\omega & v_2 \\ \cdot & \cdot \\ \cdot & \cdot \\ -jk_nv_n/\omega & v_n \end{bmatrix} \begin{bmatrix} T_{11} & T_{21} \\ T_{12} & T_{22} \end{bmatrix} \dots\dots\dots(3.14)$$

Where n = number of equations.

In ANSYS, the spring was modeled as COMBIN14 element in ANSYS simulation as shown in figure 3.16. The calculation of transduction functions was performed using two stiffness values: $k_1=1\text{N/m}$ and $k_2=5\text{ N/m}$.

Figure 3.18 Spring Combin14 Type connected to the tip

Figure 3.19 Current Magnitude with different springs attached

Figure 3.20 Current Phase with different springs attached

3.4.3 Transduction Functions

Figures 3.21 to 3.24 show the transduction functions found using direct method and spring method in ANSYS simulation. T11 which is shown in Fig 3.21 identifies the relationship between Electrical Voltage and Force. T12 which is shown in Fig 3.22 identifies the relationship between Electrical Voltage and Velocity. T21 which is shown in Fig 3.23 identifies the relationship between Electrical Current and Force. T22 which is shown in Fig 3.24 identifies the relationship between Electrical Current and Velocity.

Figure 3.21 T11 Magnitude (spring and direct method comparison)

Figure 3.22 T12 Magnitude (spring and direct method comparison)

Figure 3.23 T21 Magnitude (spring and direct method comparison)

Figure 3.24 T22 Magnitude (spring and direct method comparison)

From 0-50 kHz, the T21 (Fig.3.23) increases from 10^{-1} to 10 A/N. This means that the current sensitivity towards force increases by 100 times of its initial value along the frequency 0-50 kHz. Whereas for T11 (Fig.3.21), the value is about $1-2 \times 10^6$ V/N. This would mean that changes in the force response magnitude along the frequency range of 0-50 kHz will cause more obvious change in the current signal than in the voltage signal. It would be easier to detect any change of force in current signal than in voltage signal. This might be due to the indirect effect of piezoelectric material.

From 0-50 kHz, T22 (Fig.3.24) decreases from 10^{-3} to 10^{-5} As/m. This means that the current sensitivity towards velocity decreases by 100 times of its initial value along the frequency 0-50 kHz. Whereas for T12 (Fig.3.22), the value is approximately 10^4 Vs/m near 0 kHz and closed to 1 Vs/m near 50 kHz. This means that the voltage sensitivity towards velocity decrease by 10000 times of its initial value along the frequency 0-50 kHz. This might be because the direct effect of piezoelectricity where change in voltage will cause a direct change in velocity. The effect of a voltage change will be more profound to the velocity magnitude of the transducer than a current change.

From Fig 3.21 and 3.23, it can be seen that at clamped boundary condition, the resonance happen at around 80 kHz. This is shown as a deep valley at around 80 kHz for both graphs. This means that at that resonance point, the force need to maintain a clamped condition is very high even with a small excitation voltage and current.

From Fig 3.22 and 3.24, it can be seen that at free boundary condition, the resonance happen at around 50 kHz. This is shown as a deep valley at around 50 kHz for both graphs. This means that at that resonance point, the velocity sensed is very high even with a small excitation voltage and current.

From figures 3.21-3.24, it can be concluded that in ANSYS simulation both method generated the same transduction functions. Figure 3.25 shows that the determinant found was unity. It can be concluded that the transducer model as an electromechanical system follows the reciprocity theorem. A unity determinant means that the electrical energy given was transformed completely as mechanical energy. A determinant that is closed to one will be assumed as a system with very low loss of energy.

Figure 3.25 Determinant Magnitude

The transduction functions determined from both methods were then tested by attaching a spring with a different stiffness to the transducer FEM model in ANSYS. The cantilever was connected to a spring with a stiffness of 20 N/m. The test was done in the range of 0 to 60 kHz. The transduction functions found from direct and spring method were then used to calculate the expected motion by only inputting the

electrical signals measured by ANSYS. Figure 3.22 shows the mechanical impedance calculated by transduction matrix and its theoretical value (k_{20}/ω). The result calculated gives quite an accurate estimation. The results are actually overlapping each other.

Figure 3.26 Comparison of mechanical impedance calculated by transduction matrix and its theoretical value of a spring with 20N/m stiffness

3.4 Verification of Transduction Functions in Experiment

To verify whether the results of ANSYS simulation can be applied in the real laboratory experiment, the electrical impedance (E/I) of the cantilever transducer in free boundary condition was measured by impedance analyzer (Solartron SI 1260A) in laboratory environment.

Figure 3.27 Schematic Diagram of the Electrical Impedance by Impedance Analyzer

Figure 3.28 below show the current magnitude of the transducer experimentally measured and predicted using ANSYS. As shown in figure 3.28 the experimental

current and ANSYS current magnitude is very closed in value. Figure 3.29, though show that the phase of the impedance measured differed from the calculated impedance phase by ANSYS. This shows that the transducer may have a high dielectric loss factor. Using the phase data at 100 Hz, the dielectric loss factor of this transducer is about 14%.

Figure 3.28 Comparison of Current Magnitude measured in experiment and simulated by ANSYS

Figure 3.29 Comparison of Current Phase measured in experiment and simulated by ANSYS

Using the values measured by impedance analyzer, the velocity was calculated using the transduction matrix. Figure 3.30 compared the velocity magnitude calculated from

experimental result and ANSYS simulation. As it can be seen, after 80 kHz, the velocity error increases. However, even at range 0-70 kHz, the calculated velocity still show a high error.

Figure 3.30 Comparison of velocity calculated by transduction functions from experimental data source and velocity simulated by ANSYS: from 0-120 kHz(left); from 0-70 kHz(right)

The most possible cause of this error is of course the high dissipation loss factor that this transducer apparently has. The modeling in ANSYS could not put into account the dissipation effect to the overall response of the piezoelectric material. To make use ANSYS program to determine the transduction functions, the dissipation loss of the transducer has to be assumed very small. This means that the ANSYS model can not adequately represent the current transducer because of its high dissipation loss factor.

Another possible cause of error in the modeling process was the geometry of the piezoelectric layer on this transducer. This possible cause of error should be taken into account in any effort designing future transducer. The left figure of fig. 3.31 shows the whole top view of this transducer. The part acting as transducer is actually only the part in red circle. However, the area that is coated with ZnO is much larger than that (the part in the blue circle). The ZnO layer on top of the stable die was constrained not to move. The impedance happen in this area also affects the overall impedance measured.

Figure 3.31 Top views of the transducer

Figure 3.32 Piezoelectric transducer created by Wang [93]

Previous research by Wang [93] had successfully confirmed that FEM analysis program such as ANSYS can estimate the transduction functions of a piezoelectric cantilever transducer. Wang's transducer is a cantilever beam with a PZT patch glued on top of it as shown in Fig 3.32. All of the piezoelectric material acted as an actuator to the beam.

Figure 3.33 Current Magnitudes with a different geometry

Using ANSYS simulation, two models were made for comparison. The first model is the current model where the whole area where ZnO is coated is included. The second model only includes the cantilever area. The current magnitudes were then taken and shown in figure 3.33. The current magnitude from the first model was about ten times bigger than the second model. Besides that, the peak due to resonance of the first model also was not as visible as the resonant peak of the second model. It can be said that for the first model the change in current magnitude due to the mechanical movement would not be as significant as for the second model. The area of ZnO layer on the die affect the overall current signal significantly.

Another cause of error can also come from instrumental error. ANSYS simulation was done under ideal condition and assumption of zero processing time. However, in reality, to measure the signals, the instrument need some processing time which can increase the delay and affect the results.

3.4 Conclusion

A piezoelectric coated cantilever can act like a transducer. This new type of transducer has been developed for AFM application. With piezoelectric layer, the cantilever can perform self-sensing and actuating.

This cantilever transducer was then calibrated using transduction matrix theory. The transduction matrix should define the system's dynamic properties. Based upon the detail of the cantilever dimension and material properties, the cantilever was then modeled in ANSYS software. A solid5 element was used to model the piezoelectric layer and solid45 was used for other material. The damping ratio was assumed to be 0.005.

Based upon the definitions of each transduction functions, the functions were determined using direct method. Direct method was done by applying two extreme boundary conditions to the system: free boundary condition and clamped boundary condition. Another type of calibration was also done to get the transduction functions. In this calibration, a spring was attached to the tip of the cantilever. The force was

then can be measured by Hooke's law ($F=-kx$). By setting several set of equation (varying the value of stiffness k) to create an over-determined problem, the transduction matrix can be found. The determinant found from both methods is very closed to unity along the frequency from 0-220 kHz.

A test was done by attaching a new spring to the cantilever tip. The result of the mechanical response was then calculated using the transduction functions found from spring method and direct method. It was found that the transduction functions was quite accurate as the result was closed to the theoretical value.

Verification of the transduction matrix with experimental result shows a discrepancy. The velocity calculated by transduction functions was very different from the predicted velocity. The most possible cause of this error is of course the high dissipation loss factor that this transducer apparently has. The modeling in ANSYS could not put into account the dissipation effect to the overall response of the piezoelectric material. To make use ANSYS program to determine the transduction functions, the dissipation loss of the transducer has to be assumed very small. This means that the ANSYS model can not adequately represent the current transducer because of its high dissipation loss factor. Another cause of error also can be due to instrumental error. Instrument will cause delay due to its own processing time which will affect the measured signal.

Another possible cause of error can also come from the design of the cantilever itself. The large unmoving area of ZnO on top of the die will affect the total electrical signals measured. Considering how much wider area on the die than on the cantilever, even a slight variation of this area will cause a significant change to the electrical signals. Optimization would be difficult to achieve due to the large variation.

Chapter 4

Experimental Determination of Transduction Functions

In this chapter, the determination of transduction functions through experimental calibration will be done. From previous chapter, the transduction functions had already been found through finite element method using ANSYS software. A simulation model representing the transducer was made in ANSYS and a calibration process was simulated. The transduction functions were tested in the simulation and they calculated the mechanical force and velocity quite accurately. However, when the transduction functions were tested with electrical impedance measured by impedance analyzer during a free vibration experiment in laboratory, a high error in predicting the mechanical impedance happened. It was concluded that due to the design of the transducer, it is difficult to model the transducer to give an electrical signals that is as closed as reality. Besides that, error due to instruments depend on how small it is, may affect the measurement. Because of this problem, an experimental calibration was done to find the transduction functions of the transducer. The calibration would be done by contacting the output tip with beam of known mechanical properties. A set of beams will be used so that the transduction functions can be predicted.

4.1 The theoretical foundation of calibration process

The transfer matrix of the transducer can be written as the formula below.

$$\begin{Bmatrix} E \\ I \end{Bmatrix} = \begin{bmatrix} T_{11} & T_{12} \\ T_{21} & T_{22} \end{bmatrix} \begin{Bmatrix} F \\ v \end{Bmatrix} \dots\dots\dots(4.1)$$

The symbol E on the equation 4.1 representing the voltage given to the system, I is the current given to the system, F is force output of the system or actuating/transmitted force and v is the velocity output of the system. The four functions inside the 2x2 matrix defines the relationship between the electrical input and the mechanical output. As mentioned before in the previous chapter, to calibrate the transducer and get the functions, the electrical and mechanical signals under two extreme boundary

conditions (free and clamped) must be measured. There are some disadvantages arise using this method. First, it is difficult to achieve a perfectly clamped boundary condition without affecting the transducer. Second, for some of the transducers either due to the geometry or size, it would be impossible to measure their mechanical force and velocity. Because of that, a new method of calibration was done [93-95]. This new method would measure only electrical impedance (E/I) without measuring any mechanical signals. This was done by introducing a set of known mechanical impedance value. Wang [93] and Yin [95] did this by adding a set of masses at the output point of their transducer.

Equation 4.1 can be rewritten into Equation 4.2 to calculate the mechanical impedance Z_m which is actually Force over Velocity.

$$Z_m = \frac{t_{22}Z_e - t_{12}}{t_{11} - t_{21}Z_e} = Z_{mo} \frac{Z_e - Z_{ef}}{Z_e - Z_{ec}} \dots\dots\dots(4.2)$$

$$Z_{mo} = \left. \frac{F}{v} \right|_{i=0} = -\frac{t_{22}}{t_{21}} \text{ is the mechanical impedance when it is open circuited}$$

$$Z_{ec} = \left. \frac{E}{i} \right|_{v=0} = \frac{t_{11}}{t_{21}} \text{ is the electrical impedance when it is clamped}$$

$$Z_{ef} = \left. \frac{E}{i} \right|_{F=0} = \frac{t_{12}}{t_{22}} \text{ is the electrical impedance when it is free.}$$

Equation 4.2 introduces 3 different transduction functions. These new functions are actually originated from the original 4 transduction functions (see Chapter 3) and the relationship between them can be seen from their definition above. In this new calibration method there are two functions needed to be found Z_{mo} and Z_{ec} . Z_{ef} can be found simply by measuring the electrical impedance when the output is free. The process to find these two functions is explained below.

Equation 4.2 can be rewritten into equation 4.3. Putting this equation into a matrix form, Eq. 4.3 can be rewritten as Eq.4.4. Equation 4.5 to 4.7 further defined the matrices.

$$Z_{mo} \frac{Z_e - Z_{ef}}{Z_m} + Z_{ec} = Z_e \dots\dots\dots(4.3)$$

$$AZ = C \dots\dots\dots(4.4)$$

$$Z = \begin{bmatrix} Z_{mo} \\ Z_{ec} \end{bmatrix} \dots\dots\dots(4.5)$$

$$A = \begin{bmatrix} a_1 & b_1 \\ a_2 & b_2 \\ \cdot & \cdot \\ a_n & b_n \end{bmatrix} \text{ where } \rightarrow a_n = \frac{Z_e^n - Z_{ef}}{Z_m^n} \text{ and } b_n = 1 \dots\dots\dots(4.6)$$

$$C = \begin{bmatrix} c_1 \\ c_2 \\ \cdot \\ c_n \end{bmatrix} \text{ where } \rightarrow c_n = Z_e^n \dots\dots\dots(4.7)$$

$$Z = A^{-1}C \dots\dots\dots(4.8)$$

Matrix A is an nx2 matrix and matrix C is an nx1 matrix. The variable n represents the number of sets. By introducing an n set of equations into Eq. 4.3, this problem will become an over-determined problem and the transduction functions can be found using Eq. 4.8.

A calibration using a set of cantilever beams was done for this test. This calibration method was done instead of adding mass such as what Yin [95] and Wang [93] done because of the difficulties in attaching mass to the cantilever transducer which size is in the micrometer range. Not only the size problem, another problem emerged is how to attach and reattach the mass without deforming the transducer. Because of that, this calibration method using a set of beams with known mechanical properties was proposed.

Figure 4.1 Schematic picture of the calibration process (left), cantilever beam modeled as 2 finite element beam(right)

The cantilever beam will be represented as a clamped beam at one end. It will be modeled as two-finite-element beam. There is only one point force assumedly happen at the tip of the beam. This model can safely representing the beam as long as the frequency of interest below the lowest second natural of frequency of any beam under test. The vector of nodal displacements for this model is $a_j = [Y_1 \ h\theta_1 \ Y_2 \ h\theta_2]^T$

The stiffness and mass matrix of this 2-element beam is stated in Eq. 4.9 and 4.10.

$$k = \frac{EI n^3}{L^3} \begin{bmatrix} 24 & 0 & -12 & 6 \\ 0 & 8 & -6 & 2 \\ -12 & -6 & 12 & -6 \\ 6 & 2 & -6 & 4 \end{bmatrix} \dots\dots\dots(4.9)$$

where E =elastic modulus, I =moment of inertia, and L =length of beam, and n = numbers of element (in this case $n=2$)

$$M = \frac{\rho AL}{420n} \begin{bmatrix} 312 & 0 & 54 & -13 \\ 0 & 8 & 13 & -3 \\ 54 & 13 & 156 & -22 \\ -13 & -3 & -22 & 4 \end{bmatrix} \dots\dots\dots(4.10)$$

Where ρ is the density of the beam material and A is the area of the beam

The eigenvalues ω_r^2 and eigenvectors U_r (where $r=1,2,\dots,n$) of the beam can be found.

The beam was given a harmonic force at the end of the beam. So the Force Matrix is:

$$F = \begin{bmatrix} 0 \\ 0 \\ F_{given}(j\omega) \\ 0 \end{bmatrix} \dots\dots\dots(4.11)$$

The nodal displacement vector can be calculated using Eq.4.12 below:

$$a = \begin{bmatrix} Y_1 \\ h\theta_1 \\ Y_2 \\ h\theta_2 \end{bmatrix} = \sum_{r=1}^n \frac{U_r^T [F]}{\omega_r^2 - \omega^2} U_r \dots\dots\dots(4.12)$$

As the value needed is only the displacement at the end of the cantilever, only Y_2 is extracted. The variable Y_2 is a function of $F_{given}(j\omega)$. Velocity can be calculated by differentiating the displacement. So velocity of the tip :

$$v(j\omega) = j\omega Y_2(j\omega) \dots\dots\dots(4.13)$$

The mechanical impedance can be calculated by substituting Eq. 4.13 to 4.14.

$$Z_m(j\omega) = F_{given}(j\omega) / v(j\omega) \dots\dots\dots(4.14)$$

Then by substituting Eq.4.14 into Eq.4.6, Eq.4.8 can be solved and transduction functions can be calculated.

4.2 Methodology

4.2.1 The Cantilever Beam Set for Calibration

Figure 4.2 The picture of the cantilever beams

Figure 4.2 shows the cantilever beams that would be used for calibration. They are CLFC-NOBO calibrated cantilever beams bought from Veeco. These cantilever beams were already calibrated to confirm their mechanical properties such as their stiffness and resonant frequency. Table 4.1 lists the properties of each cantilever beams.

Table 4.1 Mechanical properties of the cantilever beams

	Length (μm)	Width (μm)	Thickness (μm)	Frequency (kHz)	Spring Constant (N/m)
Long	400	29	2	17.366	0.1658
Medium	197	29	2	68.99	1.336
Short	97	29	2	272	10.35

4.2.2 Experimental Set Up

Figure 4.3 shows the schematic diagram of the experimental set up. The cantilever transducer was set on a Z Positioner so as to move in Z direction. The cantilever beams for testing was set on the X and Y Positioner so it can move in the X and Y direction. The X and Y positioners will be controlled to place each cantilever beam under test at required position. The X,Y, and Z positioners used are M111. DC Controlled Positioners from Physik Instrumente. Each positioners were driven by C-862 Mercury DC Motor and can be easily controlled through the computer. It has a resolution of 0.007 μm per step and maximal movement of 15 mm.

Figure 4.3 Schematic diagram of the experimental set up

The transducer was monitored through a frame grabber (GAIA-Blue) which has 100 x magnification. Figure 4.2 and 4.5 was taken by this instrument.

Figure 4.4 Pictures of the experimental set up

The transducer was given an electrical voltage from the voltage source. To measure the electrical signals there would be two kinds of instrument sets can be used.

The first set, the current probe (Keithley 428) and dynamic signal analyzer (HP35670) would be used to measure the current and voltage separately. The dynamic signal analyzer can give the actuating voltage and current probe measure the current. The voltage and current were then sent back to dynamic signal analyzer to be recorded. Some of the advantages of this measurement set are its portability (compared to the second measurement set) and its ability to give a high voltage source. The disadvantage of this set is that the accuracy of the current probe in measurement of a very low current or high frequency current signal is not as good as the second measurement set. Besides that, DSA can only analyze signals with a frequency below 51.2 kHz.

The second set was impedance analyzer (Solartron SI 1260A). Impedance analyzer measure and record the electrical impedance only. Impedance analyzer frequency range is up to 32 MHz and it can measure impedance higher than 10^{14} ohm. However, it can only give voltage up to $3V_{RMS}$ and it is bulky and difficult to move.

4.2.3 Experimental Calibration Technique

Figure 4.5 shows the picture of the transducer contacted with one of the cantilever beams. To position the beams in the x and y direction, a monitoring through frame grabber image was performed. The positioning was executed by observation so it would be subjected to human error. The tip of the cantilevers was positioned to be as closed as possible to the end of the cantilever beams. However, as the beams become shorter, this objective was more difficult to achieve.

Figure 4.5 Picture of the calibration process

Positioning in the Z direction would be difficult to achieve accurately through only visual monitoring. To make sure that the transducer tip touches the cantilever surface, the electrical impedance signal in the first resonant region was observed. The first resonance will happen under free vibration condition. When the tip contacts another surface, the boundary condition will change. Now, the tip will be constrained by the beam surface. This would cause the resonant frequency to change and at the first resonant frequency region, the resonant peak would be gone. This way, the Z position where the tip contacts the surface can be predicted.

Figure 4.6 Electrical Impedance Magnitude as the tip approach a surface

Figure 4.6 shows the electrical impedance magnitude at the first resonant frequency region which was around 51 kHz for this transducer. As the tip approaching the beam surface and in contact with it, the impedance signals near resonance would change and vanish from that region when it was fully in contact with the beam surface. Besides observing the electrical impedance magnitude, electrical impedance phase can also give the same information.

An electrical actuation voltage of 3 V_{RMS} was given by a dynamic signal analyzer and a frequency range of 12-51 KHz was observed.

4.3 Results and Discussion

4.3.1 Results and discussion on calibration performed by the Current Amplifier and DSA

Figure 4.7 Electrical Impedance Magnitude with different cantilever beams attached

Figure 4.8 Electrical Impedance Phase with different cantilever beams attached

Figure 4.7 shows the electrical impedance magnitude of the transducer when it's free or attached by cantilever with different stiffness at frequency range of 11-51 kHz. As it can be seen, the magnitude of each curve is closed to each other. This shows that the change in force given to the transducer only produced a small amount of change in its electrical impedance magnitude. This phenomenon can be caused by the low value

of the ZnO piezoelectric strain coupling and its capacitance value. Because of these, the current to force ratio of this transducer will be small.

Figure 4.8 shows the electrical impedance phase of transducer when it was free or attached with cantilever beams. From this figure, an error caused by the current amplifier can actually be shown clearly. It can be seen that the phase continually increased as frequency increased. This was due to delay introduced by current amplifier. This current amplifier will add larger delay as frequency of the current signal increases.

Figures 4.9 to 4.11 shows the transduction functions found from calibration experiment done by the first measurement set. As it can be seen from Fig 4.9-4.11, the transduction functions found from FEM and the current experimental calibration differed largely. This was indeed expected as the functions calculated by FEM did not consider the effect of dielectric loss and instrumental error in their calculation. The value of Z_{ef} and Z_{ec} differed slightly due to the difficulty in modeling the ZnO area on top of the die. A very large difference was actually seen in Z_{mo} graph. Z_{mo} is the mechanical impedance when it's electrically opened. This means that the electrical current was considered to be zero. This way, the effect of dielectric loss should be less to the value of Z_{mo} . The experimental Z_{mo} magnitude can actually be lower than the FEM value by 1000 times. Besides that, their patterns are also different. Z_{mo} found by FEM has a high gradient along the frequency. This low value of experimental Z_{mo} might be caused by the addition of instrument noise into the current signals. As the charge to force properties of ZnO is quite low, the addition of this error will cause the Z_{mo} to decrease.

Figure 4.9 Comparison of Z_{ef} magnitude of FEM calculation and experimental calibration

Figure 4.10 Comparison of Z_{ec} magnitude of FEM calculation and experimental calibration

Figure 4.11 Comparison of Z_{mo} magnitude of FEM calculation and experimental calibration

Besides that, the problem is also enhanced by the large area of ZnO on top of the die instead of cantilever. Because of this, the sensed signal or signal due to motion and force on the cantilever only change the current signal at smaller proportion compared to if the ZnO layer only on the cantilever. Figure 3.33(see Ch.3) actually shows that due to the large ZnO area on the die, the current increased by 10 times of the current of the model which only has ZnO layer on the cantilever.

Usually the current signal due to the mechanical response sensed is small compared to the actuated signal. With an addition of current from the die, the sensed current proportion to the total current would become even smaller. Because of this, the current measured could be hugely affected by the instrument error itself.

After finding the transduction functions, these functions were used to calculate the mechanical impedance of each cantilever beams and then compared with the theoretical values of them (theoretical values were calculated using eq. 4.9-4.14). Figure 4.11 to 4.13 show the comparison of each cantilever mechanical impedance result from transduction functions and from theory.

Figure 4.12 Comparison of Z_m magnitude of long cantilever calculated using transduction functions and theory

Figure 4.13 Comparison of Z_m magnitude of medium cantilever calculated using transduction functions and theory

Figure 4.14 Comparison of Z_m magnitude of short cantilever calculated using transduction functions and theory

From these three figures, fig. 4.12-4.14, it can be seen that the transduction functions result curve of long and medium cantilevers quite resemble their theoretical curve. For the short cantilever though, the transduction result curve seems to be much lower than the expected value. In fig.4.12, both curves show the same pattern at lower frequency range but the transduction result curve starts to show fluctuation closed to 40 kHz. In fig 4.13, both curves become very closely resemble each other around 25 kHz onward. Whereas in fig.4.14, both curve differs in pattern and value but there is a possibility that both curves may meet at higher range of frequencies.

As mentioned before, the instrument error actually change the value of Z_{m0} found through this calibration. It can be said that the value of transduction function found in this experiment also include an addition of error values from the instrument. This would mean that the transduction function found will only be able to be used with the same set of instruments with same setting condition. This is of course under assumption that the instrumental error will always remain constant any time these instruments used.

A source of error can also come from the positioning of the transducer tip either on the length of the cantilever beam or in the z direction during calibration. As mentioned before, the tip was positioned to be as closed as possible to the edge of the beam but the shorter the cantilever, the less easy it is to ascertain the position. In the z direction, the transducer was positioned based upon the electrical impedance curve in the resonance region. If the resonance signal was gone from the impedance curve, then the transducer touches the beam's surface. However, as this was also done through subjective observation, a case where the transducer's position was too low and deflected the beams can happen.

4.3.2 Results and discussion on calibration performed by the Impedance Analyzer

Another experiment was done using an impedance analyzer (Solartron SI 1260A) to find the functions at wider range of frequencies (5-105 kHz). The cantilever transducer used in this experiment is different with previous transducer. It has a resonant frequency in 60 kHz. The difference of the resonance frequency was expected as this transducer model has a 40-60 kHz resonant frequency range due to the thickness range of 3-5 μm . This transducer was calibrated again with the cantilever beams set.

Figure 4.15 Comparison of Z_e magnitude : at 5-105kHz (left), at 50-90 kHz (right)

Figure 4.16 Comparison of Z_e phase

Figure 4.15 shows the electrical impedance magnitude of the cantilever transducer when it vibrated freely and when it touched the cantilever beams. It shows that the electrical impedance magnitude of each of them was quite closed to each other until it looked like they overlapped. Figure 4.15 (right) is a zoom in figure of figure 4.15(left) at frequency range of 50-90 kHz. From this figure, it can be seen that the resonance frequency is indeed shifted when it touched a cantilever beam. Figure 4.15(right) shows that the magnitude of each curves was very closed to each other even after the figure was magnified. This shows that the current change due to force given to the transducer by the beam was very small.

This small current change was mainly due to the low piezoelectric strain coupling and low capacitance of ZnO material. With the low value of its impedance change, the impedance magnitude measured would be prone to noise introduced by the measuring instrument itself.

Figure 4.16 is showing the electrical impedance phase curves as the transducer contacted with the cantilever beams. It can be seen that the resonant frequency shifts when the transducer contacted with the beams. The resonance shifts to the left when the transducer contacted with the long cantilever because, the mass portion of the long cantilever is larger than the stiffness portion. For the other cantilevers, it shifts to the right as stiffness of portion of the beam is larger than the mass portion. It can also be

observed that the impedance analyzer had lower instrumental error compared to current amplifier. The gradient of impedance phase measured by impedance analyzer was not as high as phase measured by current amplifier (see fig.4.8). Fig. 4.8 also shows that dielectric loss was happening to the transducer.

As mentioned before, the value of transduction functions will change with the instruments used. This in fact might be because each instrument added its own characteristic such as its own resolution and accuracy to the transduction functions. Figure 4.17 shows the Z_{mo} magnitude of this transducer compared to the Z_{mo} found previously from ANSYS. The Z_{mo} found from the current experiment calibration is different from the Z_{mo} found from previous calibration in Fig.4.11. This in fact shows how much the effect of different instrument change the functions. Considering that the Z_{mo} value of this is more closely resemble the ANSYS calibration, it can be said that the current instrument, impedance analyzer, may have been more accurate than previous instruments used, the current probe and DSA. However, the difference between them is still quite significant.

Figure 4.17 Comparison of Z_{mo} magnitude of found using ANSYS and experiment calibration

Figure 4.18 Comparison of Z_m magnitude of long cantilever calculated using transduction functions and theory

Figure 4.19 Comparison of Z_m magnitude of medium cantilever calculated using transduction functions and theory

Figure 4.20 Comparison of Z_m magnitude of short cantilever calculated using transduction functions and theory

Figure 4.18 till 4.20 show the comparison between the mechanical impedance calculated from transduction functions and from theoretical values at range 5-105 kHz. As it can be seen, these figures show the same trend as fig 4.12-4.14. The long cantilever's mechanical impedance calculated from transduction method resembles the theoretical values at range below 30 kHz. The medium cantilever curves closely resemble each other in between 40-90 kHz. The short cantilever curves resemble each other at high frequency range 40 kHz onward.

Based upon these findings, it can be said that the cantilever transducer have a certain range of mechanical impedance where it will work properly. Based on fig. 4.18-4.20, it might be around or less than 5×10^{-6} Ns/m for frequency range between 20-60 kHz.

4.3.3 Verification on Source of Error and Improvement recommendation for future research

This cantilever transducer has many things to be improved on to be a better sensor in dynamic mechanical properties measurement. Rogers [57-60] successfully used the same transducer model for imaging the surface topology of some samples. This can be done because Rogers [57-60] only used the electrical signals at the resonant frequency. The electrical current at the resonance will be the most sensitive so upon contact with a surface the electrical current will experience an immediate change. Based upon the changes of the current, the control circuit can control the height of the transducer and image the surface.

The major cause of the transducer failure to measure mechanical impedance accurately seems to be the low piezoelectric strain coupling of ZnO. Fig. 4.21 shows the change of current from being free to being clamped as it was calculated by ANSYS. This means fig. 4.21 shows the ideal current change for this transducer excluding loss and instrumental error. As it can be observed, the current difference range from 10^{-13} to 10^{-7} A. Assuming the cantilever transducer didn't have any dielectric loss, to measure this transducer properly, a measurement instrument with a high accuracy, wide current range, and wide frequency range would be needed.

Figure 4.21 Current change from free to clamped condition (FEM calculation from 0 to 220 KHz at 1Volt excitation)

Figure 4.22 Current change from free to clamped condition (calculated using transduction functions from signals measured from 11-51kHz by current amplifier at 1Volt excitation)

Fig. 4.22 shows the change of current from being free to being clamped as it was calculated using transduction functions from signals measured by current amplifier. As it can be observed, the current difference ranges from 10^{-8} to 10^{-7} A from 11-51 kHz. This means the current measured by the current amplifier whether it's clamped or free had a steady change throughout frequency increase. This can be caused by the inability of the instrument to measure current when its value was too low. As the value of the current is low, it would be more prone to noise.

Fig. 4.23 shows the change of current from being free to being clamped as it was calculated using transduction functions from signals measured by impedance analyzer. As it can be observed, the current difference ranges from 10^{-8} to 10^{-7} A from 5-105 kHz. This means the current measured by the current amplifier whether it's clamped or free had a steady change throughout frequency increase. This can be caused by the inability of the instrument to measure current when its value was too low. As the value of the current is low, it would be more prone to noise. Fig. 4.23 seems similar to fig 4.21 at 40-80kHz frequency range.

Figure 4.23 Current change from free to clamped condition (calculated using transduction functions from signals measured from 5-105 kHz by impedance analyzer at 1Volt excitation)

For its future purpose as dynamic measurement transducer, some improvements on the design and material of the transducer may need to be made. ZnO proves to be a low sensing indicator. It has a low piezoelectric strain coupling value, a charge to force ratio, compared to some other piezoelectric material such as PZT. It might improve the sensing capability of the transducer if PZT was used. Wang [93] successfully created and calibrated a miniature PZT cantilever transducer with a 21mm x 6mm x 3mm dimension. The experimental calibration was performed using a set of masses and impedance analyzer. The transduction functions were found to be similar to its FEM calculated-functions. Besides that, it also could sense the mechanical properties of a test beam. Lee et.al [54] also chose PZT for the actuator layer in his micro cantilever transducer because PZT (50 pF) has higher capacitance than ZnO (1 pF). These properties would make PZT a better sensor than ZnO.

Piezoelectric Strain Coupling of PZT (x 10⁻¹² C/N) =

$$\begin{bmatrix} 0 & 0 & 0 & 0 & 440 & 0 \\ 0 & 0 & 0 & 440 & 0 & 0 \\ -60 & -60 & 152 & 0 & 0 & 0 \end{bmatrix} \dots\dots\dots(4.15)$$

Piezoelectric Strain Coupling of ZnO (x 10⁻¹² C/N) =

$$\begin{bmatrix} 0 & 0 & 0 & 0 & -11.34 & 0 \\ 0 & 0 & 0 & -11.34 & 0 & 0 \\ -5.43 & -5.43 & 11.67 & 0 & 0 & 0 \end{bmatrix} \dots\dots\dots(4.16)$$

Besides that, the dielectric loss factor of the piezoelectric material needs to be considered. FEM analysis of transducer can be done only if the dielectric loss was small. FEM analysis should be able to remove some of the experimental difficulties that can happen during laboratory experiment such as instrumental error and size constraint.

Piezoelectric material area on top of the die should also be minimized. The larger area of piezoelectric material on top of the die than on the cantilever will affect the electrical current measured. Considering how much larger the area on the die compared to the area on the cantilever, this can be a problem in the modeling. The larger the area, the more geometrical variation can happen. The determination of the transduction functions by FEM, which was implemented by Wang[93] and the current study, was done by creating a transducer model whose natural resonant frequencies resemble the transducer's real natural frequencies as closed as possible. However, the stable area on the die would not contribute anything to the resonance but the total current magnitude would be largely affected by how large the area of the ZnO on the die. Considering how large the area on the die itself, the varying range of the electrical current or impedance would be wider and difficult to optimize. In other word, a slight variation in the area of ZnO on the die can cause large variation in the electrical signals measured.

A method introduced by Lee [54] might be a good solution for this problem. Lee [54] created a reference pattern of piezoelectric layer with same dimension as the piezoelectric layer that actuates his cantilever transducer (see fig. 4.24). He did this so that he can get the sensing current signal by subtracting the reference pattern current from the actuating pattern current.

This method can be modified for our purpose. For transduction method the total current happen at the actuating piezoelectric layer was needed. To remove the effect of the current of piezoelectric material on top of the die we can create a reference pattern to be the same size as the piezoelectric layer on the die. This way, we will only take into account the current signal flows on the piezoelectric layer on top of the

cantilever. This reference layer should be added during MEMS fabrication to ensure it to be identical as the original layer.

Figure 4.24 Self-sensing Actuating Cantilever Transducer made by Lee [54]

Third, larger numbers of beam used for cantilever calibration would be needed to improve the determination of the transduction functions. Besides that, a high focus laser displacement sensor would also be needed to incorporated during experiment for verification purpose.

4.4 Conclusion

Calibration to determine the transduction functions was done experimentally. The calibration was done using a set of beams with known mechanical properties. The transduction functions need to be determined were Z_{ef} , Z_{mo} , and Z_{ec} . A calibration first was done in the range of 12-51 kHz using a current probe and Dynamic Signal Analyzer to measure the electrical signals. It was found that the values of the transduction functions found were shows much different than the functions found using ANSYS simulation. The value of Z_{mo} magnitude especially was about 1000 times lower than the ANSYS' Z_{mo} . This might be due to several reasons.

The first reason is that ZnO has a low piezoelectric strain coupling (charge to force ratio) which cause a very small change in current magnitude even with a change of force. This added with the second reason can cause the Z_{mo} magnitude to be low. Compared to PZT for example, the piezoelectric strain coupling of ZnO is more than 10 times smaller. This would mean the charge response of ZnO would be 10 times smaller compared to PZT charge response with the same level of force given to both of them. The second reason is that due to the smallness of the sensing current

magnitude, an instrument error will have a larger effect on the overall signals measured. These instruments added their own error to the determination of the functions. It was indeed observed that the current amplifier will add delay and magnitude error for a very low current signal or signals with high frequency. What happen is the electrical impedance measured will be affected by the instrument error itself and this will change the value of Z_{mo} . The fact is the ANSYS' Z_{mo} would only be true under ideal condition. The transduction functions found on the calibration then will only be true if the same set of measurement instrument is used for any subsequent measurement.

The third reason is the large area of ZnO on top of the die compared to the one on the cantilever. This will increase the current signal measured and because of that the signals due to force or velocity sensed portion will seem smaller compared to the total current signal.

The transduction functions found were then used to calculate the mechanical impedance of cantilever beams under testing. The results were then compared to their theoretical mechanical impedance. The long cantilever beam impedance result shows a small discrepancy in the low region of frequency or below 40 kHz. The medium cantilever beam impedance result shows a small discrepancy at region higher than the 40 kHz. It was found that even though the functions can predict the mechanical impedance of long and medium cantilever beams but for the short cantilever beam, it shows a high discrepancy.

This might be caused the error in positioning of the transducer tip along the length of the beam and in the z direction. For the shortest cantilever beam, positioning the tip to be as closed as possible to the edge of the beam is rather difficult. In the z direction, positioning can also be difficult and a case where the transducer is too low and actually deflect the beam can happen. This error can happen because the positioning was done under subjective observation.

Calibration was done again in the range of 5-105 kHz using an impedance analyzer. Impedance analyzer has larger range of working frequency and better accuracy. It was found that the Z_{mo} magnitude was much closer to the ANSYS' Z_{mo} . This might be

because the impedance analyzer has higher accuracy than current probe used in the first calibration. This proves that instrument will add its own error in the calibration process and change the transduction functions accordingly.

In the range of 5-105 kHz, the long and medium cantilever shows the same patterns as previous calibration founding. The long cantilever beam shows small discrepancy in the a range below 30 kHz whereas the medium cantilever shows small discrepancy between 40-90 kHz. The short cantilever beam curves show that both curves are quite closed at frequency higher than 40 kHz. From this information, it can also be seen that the transducer can only predict the mechanical impedance in the range of 5×10^{-6} Ns/m or lower than that.

This transducer is not a good sensor for measuring force happen at its tip. Rogers [57-60] successfully used the same transducer model for imaging the surface topology of some samples. This can be done because Rogers only used the electrical signals at resonant frequency. The electrical current at the resonance will be the most sensitive so upon contact with a surface the electrical current will experience an immediate change. Based upon the changes of the current, the control circuit can control the height of the transducer and image the surface.

This cantilever transducer has many things to be improved on to be a better sensor in dynamic mechanical properties measurement. For future purpose on using it for biological specimen, some improvements on the design and materials of the transducer may need to be made. First, piezoelectric material area on top of the die should be minimized. Another solution is to remove the effect of the current of piezoelectric material on top of the die by creating a reference pattern the same size as the piezoelectric layer on the die. By subtracting the current of the transducer with the reference pattern current, only the current signal flows on the piezoelectric layer on top of the cantilever will be taken into account. This reference layer should be added during MEMS fabrication to ensure the likeness of it with the original layer.

Second, ZnO prove to be a low sensing indicator. It has a low charge to force ratio compared to some other piezoelectric material such as PZT. For a better transducer, PZT could be the material that can be considered. Third, larger numbers of beam used

for cantilever calibration would be needed to improve the determination of the transduction functions. Besides that, a high focus laser displacement sensor can be useful during experiment for verification purpose.

Chapter 5

Cantilever Transducer in Liquid Environment

To be used in the measurement of mechanical properties of biological specimens such as cells, the transducer will need to be used in liquid environment such as medium. This transducer, DMAPS, was not insulated or capable to perform in liquid environment. Because of that, it had to be coated with an insulating layer to be used. A coating procedure using electropolymerization method was done. The transducer was then tested in liquid environment such as water. Besides that, a FEM analysis was done to check the capability of transduction method to predict a distributed force to the cantilever transducer as liquid drag force is this type of force. This study was done in parallel with the study at chapter 4.

5.1 Insulation layer coating method

Rogers et al [57] or Lee et al [54] used a cantilever transducer to be used in liquid environment. They added insulating later such as Si₂O or Si₃N₄ on top of the cantilever. Rogers found Si₃N₄ addition using PECVD process showed a good insulating condition. However, PECVD method will be best used during wafer fabrication or transducer fabrication. However, it will not be efficient to be used in the current case where the transducer was already made and packaged.

A coating method using an electropolymerization method was done with the help of Pr. Li Chang Ming and Guo Chun Xian from Chemical and Bioengineering School of NTU. In electropolymerization, monomer is electrochemically oxidised at a polymerization potential giving rise to free radicals. These radicals are adsorbed onto the working electrode (positive electrode) surface and undergo subsequently a wide variety of reactions leading to the polymer network (polymerization). The working electrode will be coated with the polymer.

Insulating layer made of Poly(oxyphenylene) to coat the gold layer of the transducer was done using electropolymerization method. Poly(oxyphenylene) is a copolymer of phenol and 2-Allyl-phenol. It is often used as a protective coating to prevent corrosion

of metals. The experimental procedure followed the procedure done by Strein and Ewing [99] who successfully insulated a carbon fiber wire. Solutions for electropolymerization was prepared by dissolving 60mM phenol and 90mM 2-allylphenol in a 2% by weight solution of butyl-cellusolve in 25 mL of 1:1 methanol:water mixture. Ammonium hydroxide was used to adjust the pH to 9.0. The transducer was then insulated with a copolymer of phenol and 2-allyl phenol by holding the potential at +4 V (vs a platinum wire) for 12 minutes to dispose a 250 nm thickness of insulating layer.

Figure 5.1 Electropolymerization process

Figure 5.2 Cross section view of a gold sample insulated by electropolymerization process. (Photo was taken by Mr. Guo Chunxian using SEM)

This process has a major advantage compared to other disposition method such as sputtering or PECVD. It is that the coating layer will only be disposed on the electrically actuated layer. This means that only gold and piezoelectric layer is coated which makes the coating disposition to be efficient.

5.2 Testing of the insulating capability of the coating layer

The coated transducer was immersed in water while an electrical voltage was given by an impedance analyzer to make the transducer freely vibrate. The electrical impedance was then recorded. Figure 5.3-5.4 show the electrical impedance magnitude and phase when the transducer is immersed in water compared to when it is vibrating normally in air.

Figure 5.3 Electrical Impedance Magnitude when transducer is in air and in water

The effect of water damping can be seen clearly in the figure 5.4. The resonant frequency in air happen at around 60 kHz as a sharp peak can be seen in that region in the phase curve. From figure 5.4, a smaller and damped peak can be seen around 25 kHz in the phase curve of transducer immersed in water.

Figure 5.4 Electrical Impedance Phase when transducer is in air and in water

To check whether the coating layer work well, the after immersing in water, the transducer was then taken out back into air and the electrical impedance was measured immediately. Figure 5.5 and 5.6 show the electrical impedance magnitude and phase in air, before and after immersion.

Figure 5.5 Electrical Impedance Magnitude in air before and after immersion

Figure 5.6 Electrical Impedance Phase in air before and after immersion

From fig. 5.5-5.6, it can be seen that after immersion the electrical impedance curve start to resemble the original curve before immersion. The slight difference between the curves can be caused by the water molecule still adhered to the transducer. Upon drying, the electrical impedance should return to the original impedance. These prove that the coating layer can insulate the conductive layer on the transducer well.

5.3 Mechanical Impedance of transducer in liquid environment

It is known that hydrodynamic drag force will affect AFM measurement of mechanical properties of a sample in liquid environment [34-35, 39-40]. The hydrodynamic drag can be found as a function of velocity: $F_d = b(0)v$. The function b is a hydrodynamic coefficient changing according to the distance between sample and cantilever. The hydrodynamic coefficient when the cantilever touches the sample is called $b(0)$. The value of $b(0)$ was approximated by taking several measurement of oscillating cantilever at different distances above the sample by fitting it into spherical drag function. Mahaffy also mention that this drag force can be eliminated if the oscillation frequency is lower than 0.3 Hz.

Now the most simplified representation of drag force is a point force due to 1-DOF system consist of mass and damping.

$$F_d(t) = -m_d \frac{d^2 w(t)}{dt^2} - c_d \frac{dw(t)}{dt} \dots\dots\dots(5.1)$$

where F_d is the hydrodynamic drag force given to the transducer, m_d is the mass of water that is displaced and c_d is the damping constant due to water and w is the vertical displacement. Eq. 5.1 can be rewritten as Eq.5.2 as function of velocity with j as imaginary number.

$$F_d(j\omega) = -j\omega m_d v(j\omega) - c_d v(j\omega) \dots\dots\dots(5.2)$$

Eq.5.2 can then be rewritten to get the mechanical impedance of water drag.

$$Z_{m_d}(j\omega) = -j\omega m_d - c_d \dots\dots\dots(5.3)$$

From Eq. 5.3, it can be said that the effect of mass will be low in low frequency region but can be very high as frequency increases.

$$Z_{m_d}(j\omega) = \left| \sqrt{(\omega m_d)^2 + (c_d)^2} \right| \angle \left(a \tan \left(\frac{-\omega m_d}{-c_d} \right) \right) \dots\dots\dots(5.4)$$

From Eq. 5.4, it can be concluded that the mechanical impedance phase should always be within -3.14 to -1.57 rad or in the third quadrant. When frequency is low, the phase will be closed to -3.14 and as frequency increase will be increase to be closed to -1.57 rad.

However, the hydrodynamic drag force is actually a distributed force instead of a point force. A 1-DOF assumption will not be able to predict the drag force correctly. A hydrodynamic drag distributed force to a uniform cantilever should be written as

$$f_d(x,t) = -\rho_d A \frac{d^2 w(x,t)}{dt^2} - c'_d \frac{dw(x,t)}{dt} \dots\dots\dots(5.5)$$

where $f_d(x,t)$ is the hydrodynamic drag force per unit length due to liquid around the cantilever. ρ_d is the additional mass density. A is the cross section area of the cantilever. c'_d is the damping coefficient per unit length. Eq. 5.5

Some researchers have approximated the value of ρ_d and c'_d [100-101]. Hosaka et al. for example represent the cantilever as string of beads and express ρ_d and c'_d as

$$\rho_d = \frac{1}{12} \pi \rho_{liq} b^2 + \frac{3}{4} b \sqrt{\frac{2 \rho_{liq} \eta}{\omega}} \dots\dots\dots(5.6)$$

where η is viscosity of liquid, ρ_{liq} is liquid density, and b = width of cantilever.

Note: Eq. 5.6 and 5.7 was written for a cantilever with uniform width and thickness.

$$c'_d = c_\infty + c_s = \left(3\pi\eta + \frac{3}{4} \pi b \sqrt{2 \rho_{liq} \eta \omega} \right) + \left(\frac{\eta b^3}{h(x,t)^3} \right) \dots\dots\dots(5.7)$$

Note: Eq. 5.6 and 5.7 was written for a cantilever with uniform width and thickness.

As mentioned before, the value of damping coefficient change depend upon the distance of the tip from the surface ($h(x,t)$). That's why as the tip is near a surface an additional damping coefficient, c_s , is added to the original damping coefficient c_∞ .

Now, using the electrical impedance of the transducer immersed in water, the mechanical impedance was calculated after calibrating it with the method shown in chapter 4. Figure 5.7 and 5.8 show the mechanical impedance magnitude and phase calculated. If a point impedance assumption was taken, the electrical impedance phase should be within -3.14 to -1.57 rad with a curve pattern start from -3.14 rad at lower frequency and increase to -1.57 rad as frequency increases.

Figure 5.7 Mechanical Impedance Magnitude of water drag calculated with transduction functions

Figure 5.8 Mechanical Impedance Phase of water drag calculated with transduction functions

As it can be seen, the simplified assumption of water drag as one-DOF system with point impedance may not be able to explain the result. The drag force given to the transducer is actually a distributed force instead of a point force. Considering that SSA/transduction method can only predict point mechanical impedance, it will not give the true value of mechanical impedance due to the liquid. Instead, based upon the distributed force experienced by the transducer, it will predict point mechanical impedance. Because the transducer is calibrated only in the output point of the transducer which is the tip, the distributed force happen at any non output location will cause a miss estimation of mechanical impedance. This means that the mechanical impedance results shown in fig. 5.7-5.8 is not accurate because this method can't predict mechanical impedance due to a distributed force.

5.4 Transduction Method with Distributed Force

As transduction functions was found by calibration with a single output point where force and velocity happen as a point vector, distributed force happen along any area besides output point can't be predicted accurately. Mechanical impedance result calculated by transduction function is only an approximation which value is not the real mechanical impedance happen to the transducer. The next question that emerged

is if the transducer was experiencing a distributed force, whether it can still measure any additional point mechanical impedance due to the sample accurately.

A FEM model was made where an additional volume (330umx160umx4um) was adhered on top of the original transducer model. This added volume of course will give a distributed force in the form of distributed mass and stiffness to the original transducer.

Figure 5.9 Transducer with added volume V4

Figure 5.10 Distributed force due to the added volume

Now if the transducer was modeled in transduction matrix, then it can be written as:

$$\begin{bmatrix} E_w \\ i_w \end{bmatrix} = \begin{bmatrix} t_{11} & t_{12} \\ t_{21} & t_{22} \end{bmatrix} \begin{bmatrix} F_w \\ v_w \end{bmatrix} \dots\dots\dots(5.8)$$

$$\begin{bmatrix} F_w \\ v_w \end{bmatrix} = \begin{bmatrix} Z_w v_w \\ v_w \end{bmatrix}$$

where F_w and v_w is the approximated force and velocity at the output point (not the true force and velocity) and Z_w is the approximated mechanical impedance.

Now, even if the transduction matrix can't predict the mechanical impedance accurately of distributed force, assuming it will give constant value Z_w under same

condition, it should still be able to be used to predict additional point force and velocity happen at output point such as the case in Fig. 5.11.

Figure 5.11 Distributed force due to the added volume plus a spring ($k=5\text{N/m}$) at output point/tip

Below are the transduction matrix for the model shown in Fig. 5.11.

$$\begin{bmatrix} E_{w+k} \\ i_{w+k} \end{bmatrix} = \begin{bmatrix} t_{11} & t_{12} \\ t_{21} & t_{22} \end{bmatrix} \begin{bmatrix} F_{w+k} \\ v_{w+k} \end{bmatrix} \quad \dots\dots\dots (5.9)$$

$$\begin{bmatrix} F_{w+k} \\ v_{w+k} \end{bmatrix} = \begin{bmatrix} Z_w v_{w+k} + (k v_{w+k} / j\omega) \\ v_{w+k} \end{bmatrix}$$

Under assumption that Z_w is constant, then the mechanical impedance of the spring should be able to be calculated.

$$Z_{w+k} = Z_w + (k / j\omega) \quad \dots\dots\dots (5.10)$$

$$Z_k = (k / j\omega) = Z_{w+k} - Z_w$$

The model of the transducer with added volume and spring on tip was made. Then the mechanical impedance of each model (with spring and without spring) was inputted to Eq. 5.10. Figure 5.12 shows the mechanical impedance result calculated from Eq. 5.10 of the added volume has a density of 1000 kg/m^3 and modulus of 2.2 GPa . This is actually the density and bulk modulus of water. Figure 5.13 shows the mechanical impedance result calculated from Eq. 5.10 of the added volume is silicon and has a density of 2330 kg/m^3 and modulus of 170 GPa .

Figure 5.12 Comparison of mechanical impedance of spring ($k=5\text{N/m}$) found from Eq. 5.10 and its theoretical value if added volume has water mechanical properties

Figure 5.13 Comparison of mechanical impedance of spring ($k=5\text{N/m}$) found from Eq. 5.10 and its theoretical value if added volume has silicon mechanical properties.

It can be seen from Fig. 5.12 and 5.13 that Eq. 5.10 can't predict the expected value of mechanical impedance of spring with 5N/m stiffness. Instead, if the distributed force

was large (as in the case of silicon added volume), the predicted value would be much smaller than expected.

These proved that a distributed force on transducer would cause error in the SSA/transduction measurement method. The assumption that even under an additional point force excitation on the tip, the mechanical impedance of the distributed force calculated would always remain the same was proven wrong. And because of that, even if there is a force sensed at the output point/tip, the mechanical impedance calculated using transduction method would give a wrong mechanical impedance approximation if there was any distributed force happens at the body of transducer.

5.5 Comparison with other researches in piezoelectric transducer

As it was mentioned before, it seems that transduction method will not be able to be used in liquid environment with a piezoelectric transducer to measure dynamic mechanical properties of a sample. This is because this method can't accurately approximate the drag force given by the liquid and separated it from the total force measured.

Even though this transducer can't be used for dynamic measurement, this did not mean this transducer can be used to draw an image of the surface of a sample. Rogers et al [57] had successfully used this transducer to image the surface of a sample (gold sample) in the air and liquid environment. This can be done, because to do this test, only electrical signal at resonance would be needed. In liquid environment, the resonance still can be detected even though it will have a lower resonant frequency and a reduced magnitude due to damping. If this resonance peaks could be detected, the imaging process can be done. Imaging process was done by lowering the transducer vibrating in resonant frequency until its tip contacted the sample surface. Upon contacting the surface, the current signal magnitude will change. A control circuit will detect this and note the z position when this happened. This z position will be noted as the position of the surface at that particular point. The transducer was then moved in x and y direction, and the data collected will be mapped to represent the surface topology of the sample.

Lee [54] also created a piezoelectric transducer similar to Rogers' [57]. However, Lee decided to use PZT because of its high capacitance compared to ZnO. This will increase the sensitivity of the transducer. Lee also only used his transducer to draw the surface topology in air and liquid environment. Lee found the vertical resolution of his transducer to be better than the ZnO transducer used by Rogers [57]. Same as Rogers', the transducer was excited at its resonant frequency which was around 188 kHz in air.

To be able to be utilized in dynamic measurement of a biological sample, the transducer has to have the same accuracy as the current laser sensor which was used in current AFM. Mahaffy [34-35] and Smith [37-38] for example used conventional AFM to measure the dynamic mechanical properties of biological cells. The measurement was done in a frequency below 1000 kHz.

To be able to match this capability then piezoelectric transducer has to be able to work in the low range of frequency also. The current transducer though has low sensitivity at low frequencies. The resonance usually happens at high frequency (higher than 10 kHz) so the transducer is usually operated at high frequency. As piezoelectric transducer is the most sensitive in its resonance region, the next thing can be done either, lower the resonant frequency or use a piezoelectric material with a high sensitivity at low frequencies.

Transduction method can not determine the drag force given by the liquid. This will be a major obstacle for measurement in liquid environment. What can be done so that the transduction method can be used in liquid environment would be if the calibration was done in liquid environment also. Assuming the z position of the transducer remain the same, the transduction functions can be found by calibrating it in the liquid. The transduction functions found will be the unique transduction functions of this transducer in that liquid only. This method though will only work if the transducer has a high sensitivity as the force given by the sample might be considerably small compared to the drag force by the liquid surrounding. Besides that, the properties of liquid which changes due time (evaporation rate) or location (far or near surface) will cause difficulty in determination of transduction functions.

5.6 Conclusion

Insulator coating layer was deposited on top of the transducer's conductive layer using electropolymerization method. The coated transducer was then tested in water to check the insulation's quality. The insulation seems to work well as the electrical impedance measured return to normal after immersion in water.

In liquid environment, the cantilever transducer will experience a hydrodynamic drag force due to the liquid surrounding it. Modeling the liquid environment as one-DOF system with point mechanical impedance is not sufficient. Hydrodynamic drag force is actually distributed force given along the length of cantilever and not only at the output point/ tip of transducer. This means that the mechanical impedance calculated by SSA/transduction method is not the true mechanical impedance experienced by the transducer. The transduction method can't measure mechanical impedance due to distributed force such as hydrodynamic drag force.

A model of the transducer with an added volume on top of it was then made using ANSYS simulation. This added volume will simulate a distributed force given along most length of the transducer. Another similar model was then made with not only additional volume but also a spring connected at the output tip. Using these two models, it was then tested whether the SSA/transduction method can still predict the point mechanical impedance due to the spring even with distributed force happening in another location. The results showed that the transduction method failed in determining the spring mechanical impedance if there is distributed force happening. Besides that, it is also seen that the larger the distributed force is, the larger the error in the result.

This means that the transduction functions found while calibrating the transducer in air can't predict the hydrodynamic drag force of the liquid. If that's the case, for liquid usage, calibration may need to be done also in liquid environment. However, with the current transducer, it is difficult to do the calibration in liquid environment. This is because the current transducer has a low sensitivity and it might be difficult to

sense the force given to the tip compared to the much bigger drag force due to liquid. Besides that, the high damping due to liquid will create a low damped resonant peak. This will make the alignment of transducer in z direction difficult. These problems might be able to be resolved with a different design of the transducer so as to make the electrical impedance curves sharper and more sensitive to mechanical signals for example by using a material with a higher piezoelectric strain coupling value (charge to force ratio) such as PZT and minimizing the piezoelectric layer area on top of stable/unmoving parts such as the die.

Besides that, the hydrodynamic drag force changes as it moves closer to a surface. This means that the distributed force happening to the cantilever while in calibration will be different to the distributed force if it is moving closer to sample.

Chapter 6

Conclusion and Recommendation

A cantilever transducer, DMAPS (bought from Veeco) was used in this project to check the possibility of using a piezoelectric transducer with transduction method to be a sensing cum actuating transducer in measurement of mechanical properties of biological samples such as cells. A finite element analysis had been executed to find the transduction functions of the micro piezocantilever. This method can be done easily and remove the calibration problem due to its small size. This numerical analysis can be done using software such as ANSYS by performing harmonic analysis to predict the transduction matrix. This numerical analysis can be done accurately if all the information such as material properties and system properties (damping ratio, stiffness) are known. However, this analysis can only give an approximation result.

The transduction matrix result of this FEM analysis was then verified in an experimental procedure. A free vibration experiment was done. The electrical current was measured and compared with ANSYS' results. The velocity calculated using experiment's electrical signals show big discrepancy compared to the predicted velocity. This was found due to the high dielectric loss factor of this piezoelectric transducer. At 1000 Hz, the value of the loss factor is about 14 %. This finding proved that the ANSYS model can't represent the transducer. The model created in ANSYS was created under assumption that there is no or very small dielectric loss happening. ANSYS can not take the dielectric loss into consideration when it simulate the piezoelectric material responses. Besides that, the design of this transducer can also cause problem in refining the transduction functions found from ANSYS. The large area of piezoelectric on top of the unmoving/stable part of the die contributes very little to the movement of the cantilever transducer but affect the electrical signals greatly. As the change in the area of the piezoelectric layer on the die has large effect to the electrical signals, the refining of the transduction functions is difficult.

Calibration was done in real life experiment condition. The cantilever transducer was calibrated with a set consisting of three cantilever beams with known mechanical

properties to find the transduction functions which Z_{mo} , Z_{ec} , and Z_{ef} . The transduction functions found were different from the functions found from ANSYS simulation. The value of Z_{mo} especially is much smaller than the ANSYS' Z_{mo} . This might be caused by the low charge to force ratio of the ZnO material which caused the low current value even with a change of force. Besides that, instrument error also affects in the transduction functions. This would mean that the transduction functions found were not only unique to the transducer but also unique to the measuring instruments. This was proven when the values of Z_{mo} were found different with different measuring instrument sets.

The transduction functions were then used to calculate the mechanical impedance of the cantilever beams. The results were then compared to their theoretical mechanical impedance. It was found that at low frequency range or below 40 kHz, the transduction functions can calculate the mechanical impedance of the long cantilever well. It can calculate the mechanical impedance of the medium cantilever at around 40-80 kHz range and for the short cantilever at high frequency range or higher than 80 kHz. It is also shown that the transducer can approximate the mechanical impedance value if it's lower than or around $5e-6$ Ns/m.

The transducer conductive area was coated with an insulating layer so that it can work in liquid environment. An electropolymerization method was used and a test was done by giving an actuation voltage while it's immersed in water. This test proved that the insulating layer worked well.

In liquid environment, the cantilever transducer will experience a hydrodynamic drag force due to the liquid surrounding it. Hydrodynamic drag force is actually distributed force given along the length of cantilever and not only at the output point/ tip of transducer. Considering that the transducer was calibrated to measure only point mechanical impedance, the transduction method can't measure mechanical impedance due to distributed force such as hydrodynamic drag force.

A model of the transducer with an added volume on top of it was then made using ANSYS simulation. This added volume will simulate a distributed force given along most length of the transducer. Another similar model was then made with not only

additional volume but also a spring connected at the output tip. Using these two models, it was then tested whether the transduction method can still predict the point mechanical impedance due to the spring even with distributed force happening in another location. The results showed that the transduction method failed in determining the spring mechanical impedance if there is distributed force happening. Besides that, it is also seen that the larger the distributed force is, the larger the error in the result.

This means that the transduction functions found while calibrating the transducer in air can't predict the hydrodynamic drag force of the liquid. If that's the case, for liquid usage, calibration may need to be done also in liquid environment. However, with the current transducer, it is difficult to do the calibration in liquid environment. One of the cause is the high damping due to liquid will create a low damped resonant peak. This will make the alignment of transducer in z direction difficult. This might be able to be resolved with a different design of the cantilever so as to make the electrical impedance curves sharper and more sensitive to mechanical signals for example by using a material with a higher charge to force ratio such as PZT and minimizing the piezoelectric layer area on top of stable/unmoving parts such as the die.

The objective of this research is to use a cantilever transducer to measure dynamic mechanical properties of biological cells using SSA method. Using ANSYS and experimental approach, the transduction functions of this cantilever transducer had been found. A modification by adding a coating of insulating layer has also been done. However, it was found that this cantilever transducer and SSA/ transduction method can't work properly in liquid environment where distributed forces in the form of hydrodynamic drag happen to the whole body of transducer. Because of this, the last objective would not be able to be achieved.

Based upon the results measured and calculated in this project, it can be said that the DMASP transducer is not compatible to be used to measure mechanical properties of biological cells. The first cause will be the low piezoelectric strain coupling (charge to force ratio) of ZnO material. Because of this, the transducer has low sensitivity. ZnO has low piezoelectric strain coupling (charge to force ratio) value compared to other

piezoelectric material such as PZT. PZT can have a piezoelectric strain coupling as high as 20 times of the ZnO's. Using PZT might improve the sensing capability of this type of cantilever transducer.

Besides that, the design of the transducer also posed a problem. The piezoelectric layer was deposited not only on the cantilever but also on the unmoving/ stable die. This area was large compared to the area on top of the cantilever. This would cause a higher total current measured. The signals due to mechanical stimulation will be much smaller compared to this total current and difficult to measure. Besides that, due to this large area, a refinement of transduction functions in ANSYS is difficult as change of area can cause big difference in the electrical signals measured.

This cantilever transducer has major shortcomings in measurement in liquid environment. This is because the liquid surrounding cantilever will give a distributed hydrodynamic drag force instead of a point force. A calibration inside the liquid environment may be able to solve this problem. However, the parameters of liquid which change due time (evaporation rate) or location (far or near surface) will cause difficulty in determination of transduction functions.

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