Quantification of the Properties of Nonlinear Media using Dual-Probe Atomic Force Microscopy

by

Saltuk Bugra Aksu

A DISSERTATION

Presented to the Faculty of
The Graduate College at the University of Nebraska
In Partial Fulfillment of Requirements
For the Degree of Doctor of Philosophy

Major: Engineering Mechanics

Under the Supervision of Professors Joseph A. Turner and Eveline Baesu

Lincoln, Nebraska

August, 2007

UMI Number: 3295233

UMI Microform 3295233

ProQuest Information and Learning Company
300 North Zeeb Road
P.O. Box 1346
Ann Arbor, MI 48106-1346

Quantification of the Properties of Nonlinear Media using Dual-Probe Atomic Force Microscopy

Saltuk Bugra Aksu, Ph.D

University of Nebraska, 2007

Advisers: Professors Joseph A. Turner and Eveline Baesu

The mechanical response of soft materials like certain polymers and biological media can not be described using simple linear elastic deformation theories. These materials have a response that is more complex and often nonlinear. Measurement of the mechanical response is important, because cells for example, have a strong correlation between mechanical response and internal changes and processes occurring within the cell. In other words the state of health of the cell is related to this mechanical response. Therefore, quantification of the mechanical properties of the live cell membranes is of great scientific interest. Despite the importance, until now a nondestructive experiment to achieve this goal was not available.

The properties of cell membranes have been investigated using a variety of methods. Chemical analyses of cells include a great risk of changing the very properties of the living system that are being probed during the time of investigation. Mechanical techniques used to quantify the physical parameters include the micropipette technique, optical tweezers, microindentation or so-called "cell poker" technique, magnetic bead rheometry and atomic force microscopy (AFM). Among these technologies for precise and controlled mechanical loading of cells, AFM with a calibrated cantilever offers the greatest potential

for gaining new insight into properties of biological samples at sub-micron scales, since AFM can provide quantitative results. However, current analyses of experimental data are often based on simple linear material models such that the results are qualitative at best.

In order to determine the mechanical properties of such nonlinear media, results from experiments must be accompanied with a consistent mathematical model. Therefore, a nonlinear constitutive model is used here to emulate the material response to a point load applied with an AFM tip. The main drawback of such models is that knowledge of the entire deformed shape of the sample under load is required. Currently it is not possible to acquire such information using AFM. AFM technology uses a single probe which performs only one task at a time – AFM can be used either to extract qualitatively local stiffness, to image surface topography, or to perform mechanical manipulation at the sub-micron scale. Integration of another probe to an already existing AFM would allow nanomanipulation and/or force application on the sample during topography imaging simultaneously. Thus, a new experimental technology is proposed – a dual-probe AFM.

In this dissertation, the technology for a dual-probe AFM is described in detail and is used on example materials. The nonlinear constitutive model is then used to analyze measured data for extracting nonlinear constitutive properties. Results show that the theoretical model used here gives reasonable and consistent results for the samples examined. The work presented here should expand the understanding of mechanical properties of nonlinear media.

Acknowledgements

I was never a man of words so it is not easy for me to simply thank to those who made this dissertation possible. I will try my best...

First and foremost I thank Dr. Joseph A. Turner, the best advisor, teacher and work partner I could ever have wished for. He provided a professional balance of guidance, support, friendship and encouragement to pursue my own ideas. Without his enthusiasm, inspiration, patience and great efforts, I would have been lost. Throughout my life he will always be a role model for me. It is difficult to overstate my gratitude to my co-advisor, Dr. Eveline Baesu. Throughout my study, not only she was readily available whenever I needed her but she always provided encouragement, exceptionally good advice, good teaching, good company, and vision that started this project. Her professional collaboration and her friendship meant a great deal to me. Thank you both for never doubting me and pushing me time after time. It has truly been an honor to work with you.

I am deeply indebted to my committee members Dr. Jiashi Yang, Dr. Mehmet Inan and Dr. Suzanne Rohde for their help, stimulating suggestions and encouragement. Dr. Inan deserves special recognition for providing me the biological samples I needed, in addition to his friendship, thoughtful criticism, time and attention during busy semesters.

I can't thank enough to Hiroshi Takahashi san for the help and support he provided. I am grateful to Hiroshi Takahashi san not only for providing me the piezolevers I used in this work, but also for his patience and unconditional help. Thank you for being kind enough to answer all the questions I had and also for the lunch we had in Tokyo.

A number of other people provided valuable discussions during the planning of

my course and the writing of this dissertation. My heartfelt thanks to: our department secretary Kathie Hiatt for helping the department to run smoothly, for being very kind and for assisting me in many different ways especially when I was teaching, Dr. Li Tan for providing me the PDMS samples that I used in this work, Dr. Lanping Yue for milling the piezolevers using focused ion beam (FIB) technique, Constantine Tarawneh for his wise recommendation to me about changing my major to Engineering Mechanics, James Dean McManis and the machine shop for manufacturing the tools I designed with great care and precision and Dr. George Schade who made it possible for me to start UNL in the first place. I will never forget his willingness to take a chance with me. In addition, I would like to thank the National Science Foundation for providing me the financial means to complete this project.

Additionally I would like to seize this opportunity to thank my colleagues for their help and support throughout this project: Alireza Tofangchi for sharing his enthusiasm, valuable discussions and comments on my work and Benjamin Jon Polly for proof reading my dissertation and his thoughtful suggestions.

I feel a deep sense of gratitude for my family who formed my vision and taught me the things that really matter in life. Their absolute confidence in me made me the man I am today. I always felt their unconditional love and support. I thank you all for being the source of my inspiration. My sincere thanks go to my grandfather Talip Aksu for making it possible for me to start my graduate study at UNL.

I can't express my appreciation to my son, Batuhan Aksu who changed the way I view the world. You filled our home with joy and happiness.

Finally, and most importantly, I wish to thank my wife, Gulin Aksu for having faith in me when nobody else did. I thank you for having the patience to put up with me, for providing a peaceful home, for all the emotional support, for simply being my best friend, my love and my life... You believed that I could do anything even when I could not quite believe in myself. You are the only reason that I never give up. How can I ever thank you enough... To you I dedicate this dissertation.

Contents

List of Figures

List of Tables

Chapter 1

Introduction

"*I could draw you a map of all the components in a cell and put all the proper arrows connecting them. But for even the simplest single-celled microorganism, I or anybody else would look at the map and have absolutely no ability to predict anything.*"

Nobel Prize winning biochemist Alfred G. Gilman

1.1 Motivation

All humans consist of a collection of cells. A complex heterogeneous structure forms a plasma membrane which surrounds the cell and therefore provides a barrier between the internal and the external environment. The equilibrium shape of a cell membrane is controlled by the elasticity of the lipid bilayer of the cell membrane [1]. The cell membrane reflects to a certain extent the functions of the cell and plays an essential role in cell to cell interactions. These interactions include cell signaling, division and motility. The membrane maintains molecular and potential gradients which have a role in the transport of substances

in and out of the cell [2], [3].

In order to create effective cures for diseases we need to have a good understanding of cell function. Therefore, the cell and all of its components have been studied for many years. The multi-scale hierarchy of the elements of tissue starts at the molecular level, then the intra- and extra- cellular elements, cells, connective fibers, and finally the scale of particular organs. It is clear that the macroscopic response of a tissue depends strongly on its microstructure, down to the level of the single cell. Changes in membrane properties can result in quick succession of changes in one or more of its functions, which in turn can induce a drastic change in behavior of the cell. This happens for example in catastrophic cellular change and degeneration leading to cancer, Alzheimer's, Parkinson's or multiple sclerosis [4], [5]. This implies that the mechanical response of the outer membrane of a cell is an indicator of internal changes and processes occurring within the cell. In other words, one can tell if a cell is healthy or not by looking at the mechanical response of that cell.

The processes occurring in the external and/or the internal environments of the cells trigger different mechanisms resulting cells to be subject to mechanical stimuli. For example, when motion is generated by muscular contractions within the musculoskeletal system, the external environment imposes mechanical stimuli on the cells within muscles, bones, and connective tissues. In [5], experiments on healthy and cancerous cells indicated a reduction of one order of magnitude in the force necessary to produce a certain deformation. Endothelial cells are exposed to shear stresses within the cardiovascular system whereas cardiac cells and vascular smooth muscle cells are subjected to stretch due to the beating heart. The mechanical stimuli of the external environment are directly transferred to the

cells, which are physically connected to the surrounding extracellular matrix. In addition, conversion of chemical energy into mechanical energy in the internal environment imposes mechanical stimuli on cells as well. The response of the cell to mechanical stimuli is not only the deformation of the cell membrane but also growth [6], differentiation [7], secretion [8], gene expression [9] and altered extracellular matrix production [10]. When the applied stimuli exceed the adaptive capacity of the cell, this results in cell damage or death.

However, measuring the mechanical response of the cell membrane is a difficult task. Performing a calibrated mechanical experiment on the cell membrane surface is a big challenge since it requires a calibrated force and a way of measuring the response simultaneously. In addition, the size of the equipment must be in micrometer scale. To date several techniques are utilized to study the properties of cell membranes, most of them chemical in nature. Methods of chemical analyses of cells include a great risk of changing the properties of the living system that is being probed during the time of investigation. Therefore mechanical methods are becoming more important. The techniques that make use of mechanical testing in order to investigate the health of the cells inside the body or in their natural medium are evolving due to the need of different parameters with more accurate results. Micropipette technique [11] [12], microneedles [13], optical tweezers [14] [15] [16] [17] [18] [19], microindentation technique [20] [21] [22] [23] [24] [25] [26], magnetic bead rheometry [27] [28], magnetic rotational microrheology [29] techniques are the most common ones used to probe mechanical properties of biological samples. Local loading of cells generally reveals the mechanical properties of certain structures or parts of cells (atomic force microscopy, cell poking). On the other hand, when it is more important to evaluate

the properties of the cell as a whole, global loading techniques are preferred (micropipette aspiration). The characteristics of these techniques are summarized in Table 1.1.

Table 1.1: Characteristics of experimental approaches.

Experimental Method	Partial (P) - Full (F) micropipette aspiration	micro-indentation	AFM	Bead micromanipulation
Physical principle	suction - part (P) or whole (F)	indentation	indentation	twisting or moving
Controlled parameter	pressure	axial deformation	force or axial deformation	torque or force
Size of load applicator	N/A	2 μm	$<$ 50 nm	bead diameter $\sim$ 0.2 - 5.5 μm
Magnitude of force	$\sim$ 0.01 - 1 kPa (P) and $\sim$ 0.01 - 2 kPa (F)	5-400 nN	pN-nN	20 pN - 10 nN
Deformation range	vary with the cell/pipette diameter	max 50 %	max 10 %	max 2 μm
Resolution	0.2 μm, 0.1 Pa	0.02 μm, 5 nm	nm, pN	0.1 μm - 1 pN
Visualisation	brightfield microscopy	brightfield microscopy	brightfield, confocal microscopy	brightfield, fluorescence, confocal microscopy
Operating temperature	Room temperature	Room temperature	Room temperature	Room temperature

Even for the same cell type, different techniques often report different range of results [30]. Because for a local loading technique such as microindentation, the indentation depth is in the nm range (in the cell membrane only), whereas for a global loading technique the response of the cell involves deformation in the membrane along with the underlying cytoskeleton, and the nucleus. Further details of these techniques are discussed in Chapter 5.

Among all the mechanical experimental techniques that are used, atomic force

microscopy (AFM) has the most potential, since a calibrated force needs to be applied in order to get quantitative results. The local interaction (as low as tenths of picoNewtons) between a sharp tip and the sample with high force resolution can be measured using AFM. In addition to probing local physical topography, AFM can probe the viscoelasticity, adhesion, charge density, and magnetic field.

In the last decade, the AFM has successfully evolved from a simple imaging tool to a multi purpose instrument that can be used for material characterization and nano manipulation. Essentially, AFM was developed to study solid materials in the first place but very few biological systems approximate that ideal. However, soon after its invention, its ability to explore samples in liquids was realized. Moreover, the intermittent contact mode is also successfully applied to liquid environments [31] [32]. These two developments captured the attention of many biologists and the experiments on biological samples started. The versatility of AFM along with the parameter to be measured play a significant role on the design of the experiments performed. Today, stretching single DNA strands, measuring single molecular bindings became routine applications of AFM. In addition, AFM renders elasticity measurements of different biological samples possible. Elasticity measurements of solid materials are successfully achieved by using AFM along with a Hertzian contact model. The use of the Hertzian model is also adopted for the elasticity measurements of biological samples. In this case, a significant irreproducibility problem occurs [33]. In [34], this problem is attributed to the mathematical model used to quantify the experimental results. The use of the linear Hertzian model is not suitable for soft materials because the assumptions made in the Hertzian theory are not valid for soft biological samples.

Hence, a new technique must be developed in order to overcome the inconsistency and irreproducibility problems.

1.2 Contributions of This Work

The objective of this research is the design of a new nondestructive purely mechanical experiment that will allow the cell membrane response to be measured under a calibrated load. In order to determine the mechanical properties of the cell membrane, results from experiments must be accompanied by a consistent mathematical model. Therefore, a nonlinear constitutive model is used to emulate the material response to a point load applied with an AFM tip. The main drawback of such models is that knowledge of the entire deformed shape of the sample under load is required. Currently it is not possible to acquire such information using AFM. AFM technology uses a single probe which performs only one task at a time – AFM can be used either to extract qualitatively local stiffness, to image surface topography, or to perform mechanical manipulation at the sub-micron scale. Integration of another probe to an already existing AFM would allow nanomanipulation and/or force application on the sample during topography imaging simultaneously. Thus, a new experimental technology is proposed – a dual-probe AFM. A commercially available piezolever is calibrated and modified. Then it is integrated to a commercially available AFM as the second probe.

This work contributes to the field of biomechanics by proposing and also describing the current state of a framework suitable for various experimental tasks in biomechanics. The key part of the framework is an experimental technique, followed by the related mathe-

matical model for the identification of material parameters. The new technology developed here will form the basis for a new generation of cantilever based sensor systems for nondestructive monitoring of changes in the nonlinear mechanical properties of materials. Note that although the primary purpose of our technique is to describe the mechanical properties of nonlinear media its applicability is much broader. It can be used for quantification of connective tissues, plant cells, thin films and nanofiber reinforced composites.

1.3 Organization of The Dissertation

This work is organized as follows:

- Chapter two provides an introduction to the subject of AFM. The scanning and detection mechanisms of AFM as well as some basic features of current operation modes are presented to understand the need for integration of a second probe.

- Chapter three gives a brief review of continuum mechanics and the nonlinear model that is compared to experimental results. In particular, nonlinear modeling of the cell membrane is discussed and the nondimensional deformation solution for the cell membrane is provided.

- Chapter four describes the AFM cantilever calibration methods in detail. Theoretical models and the experimental techniques are compared in terms of accuracy and applicability. In addition, a new tool which utilizes a commercially available piezolever to calibrate AFM cantilevers is presented. This chapter concludes with the results of the calibration experiments performed by using the developed tool.

- Chapter five presents and compares the experimental methods previously used to quantify the mechanical properties of biological samples. The procedure for elasticity measurements of biological samples with AFM is also given in this chapter. The proposed experimental method (dual-probe AFM) for mechanical characterization of cell membranes is explained. The details of the experimental design as well as the challenges are summarized. The characteristics, performance, and possible applications of this method are detailed.

- Chapter six discusses the acquired results. The nonlinear constitutive model is used to analyze the measured data and extract nonlinear constitutive properties. Results show that the theoretical model used in this work gives reasonable and consistent results for the samples examined. Therefore, it is shown that the proposed method is feasible.

- Finally in chapter seven the future prospects of the presented work are discussed. Suggestions for further modifications to the current experimental setup are provided in order to perform the experiment in aqueous environment.

Chapter 2

Atomic Force Microscopy

2.1 Introduction

The atomic force microscope is a member of the family of microscopes known as scanning probe microscopes (SPMs). The SPM was invented by Binning and Rohrer in the 1980s and the importance of this discovery is recognized through the Nobel prize in Physics (1986). SPM is the first of a large probe microscopy family which sense the structure of sample surface by means of a extremely sharp tip therefore measuring some form of interaction between the surface and the probe. The images are obtained by measuring the changes in these interactions. A few years later Binning, Quate and Gerber announced the birth of the second member of the SPM family - the AFM [35]. Commercial scanning tunneling microscopes (STMs) which are the first type of SPMs became available in the late 1980s. New types and refinements of SPMs have appeared and will undoubtedly continue to be developed in the future. In chapter 5 a new modification is presented for the AFM.

A simple schematic of a commercially available AFM is shown in Figure 2.1. The

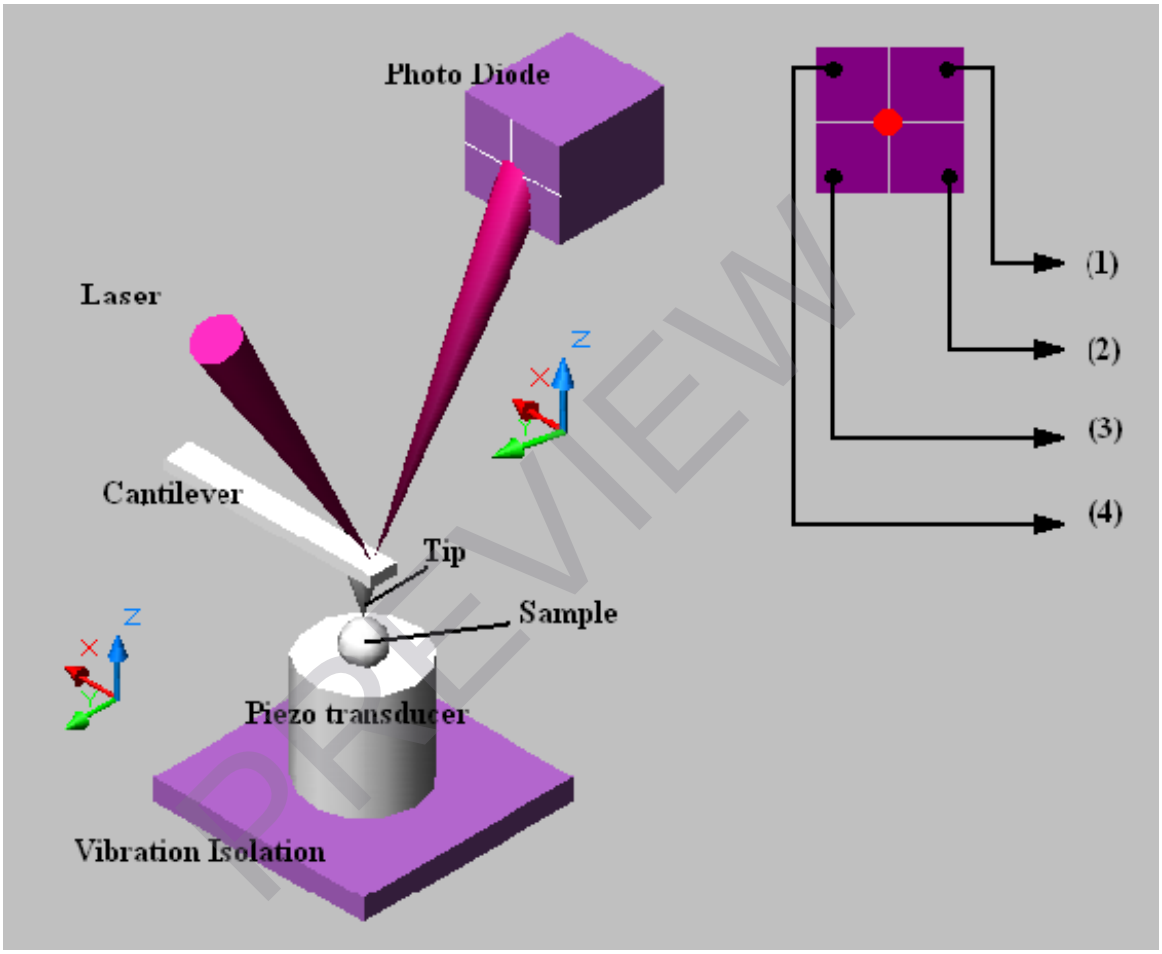

Figure 2.1: Schematic representation of the atomic force microscope. All AFMs have five essential components: a cantilever with sharp tip mounted at the end, a mechanism of sensing the cantilever deflections, a feedback system, a piezoelectric scanning system which provides the relative motion between the tip and the sample and finally a display system that converts the measured data into an image.