

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

by

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PREVIEW

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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.

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PREVIEW

Contents

List of Figures	ix
List of Tables	xiii
1 Introduction	1
1.1 Motivation	1
1.2 Contributions of This Work	6
1.3 Organization of The Dissertation	7
2 Atomic Force Microscopy	9
2.1 Introduction	9
2.1.1 Probe	11
2.1.2 Scanning Mechanism	16
2.1.3 Detection Mechanism	17
2.1.4 Vibration Isolation	17
2.2 Tip-sample Interaction Forces	18
2.2.1 Van der Waals Forces	20
2.2.2 Electrostatic Force	22
2.2.3 Capillary Forces	22
2.2.4 Adhesive Forces	23
2.3 Imaging Modes	23
2.3.1 Contact Mode Operation	23
2.3.2 Noncontact-Mode Operation	26
2.4 Summary	28
3 Cell Membrane Model	29
3.1 Continuum Mechanics Review	29
3.1.1 Kinematics	30
3.1.2 Theorem of Polar Decomposition	32
3.1.3 Stress	35
3.2 Nonlinear Cell Membrane Model	38
3.3 Summary	42

4	AFM Cantilever Calibration Methods	48
4.1	Theoretical Methods	49
4.2	Static Response Techniques	49
4.3	Dynamic Response Methods	54
4.3.1	Flexural Vibration of Cantilever Beam	55
4.4	Calibration using a Piezolever	62
4.4.1	Piezolever Calibration	66
4.4.2	Calibration of the AFM Stage	70
4.4.3	Calibration of AFM Cantilevers	71
4.5	Experimental Results	76
4.6	Summary	78
5	Dual-Probe Atomic Force Microscopy	82
5.1	Experimental Techniques	83
5.2	Elasticity Measurements with AFM	90
5.3	Experimental Details	94
5.3.1	Design Steps and Challenges	94
5.4	Summary	103
6	Results and Discussion	107
6.1	Theoretical Assessment	117
6.2	Summary	122
7	Summary and Conclusions	123
7.1	Calibration of AFM Cantilevers Using Piezolevers	124
7.2	Dual-Probe AFM	124
7.3	Future Work	125
	Bibliography	128

List of Figures

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 piezo-electric 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.	10
2.2	The AFM probe. The cantilever and the tip is etched from a chip for easy handling.	12
2.3	Tip based AFM resolution. Opening angle of the tip (a) is bigger than the opening angle of the tip (b).	13
2.4	Schematic representation of different aspect ratio AFM tips and some tip effects. 1. Broadening 2. Compression 3. Not proper tip for the sample 4. Low aspect ratio tip (high opening angle) for proper sample. 5. High aspect ratio (low opening angle) tip 6.Modified	14
2.5	An AFM cantilever with rectangular cross section.	15
2.6	An AFM cantilever with triangular cross section.	15
2.7	The reflected laser beam location depends on the cantilever.	18
2.8	Interatomic force-distance curve for atomic force microscopy.	19
2.9	The deflection of the AFM cantilever in different distances from the sample.	21
2.10	The detection of adhesive forces between the AFM tip and the sample is possible. Case 1 illustrates the presence of adhesive forces whereas case 2 illustrates the ideal case with no or negligible adhesive force.	24
2.11	AFM image acquisition at contact mode with constant force.	25
2.12	Working point selection during "tapping" mode. The cantilever is brought close to the sample such that at the lower semioscillation, the tip gets in contact with the sample surface.	27
3.1	The mapping between the reference configuration and the current configuration.	30
3.2	The deformation gradient can be decomposed into rotation tensor and a stretch tensor.	33
3.3	a) A traction force vector is applied arbitrarily on each of the three faces of the cube. b) Each traction vector in 3D will have three components.	36

3.4	The geometry of deformation under the force F	39
3.5	The nondimensional deformed shape of the cell membrane under the point load.	43
3.6	The deformation of the membrane due to different loads.	44
3.7	The deformed shape of the membrane due to different values of bending moduli $-k$	45
3.8	The deformed shape of the membrane due to different values of stretching moduli $-\lambda$	46
4.1	Schematic of static response to pendulum force method.	50
4.2	Schematic of cantilever on reference cantilever method. 1) AFM cantilever is positioned above the reference cantilever. 2) The contact between the cantilevers is obtained. 3) Lowering the AFM cantilever with a known displacement results in deflection in both the reference and AFM cantilevers.	52
4.3	Schematic of added mass method. 1) A known mass is positioned under the AFM cantilever. 2) The mass is attached to the AFM cantilever by lowering the AFM cantilever and establishing a contact between the mass and the cantilever. 3) The cantilever is lifted up. The deflection of the cantilever due to the added mass is measured.	53
4.4	Uniform cantilever beam.	56
4.5	First three vibrational modes of a rectangular cantilever.	60
4.6	The commercially available Si- self sensing piezoresistive cantilever. (PRC400:SiII Nanotechnology Inc.)	63
4.7	The piezolever resistors are placed in a Wheatstone bridge.	65
4.8	Piezolever calibration using nanoindentation. a) The piezolever is located with the optical microscope. b) The indenter tip is brought within microns of the piezolever. c) The indenter tip is engaged with the piezolever. d) The stiffness of the piezolever is measured with averaging multiple indents at the very end of the piezolever.	67
4.9	A typical force-distance curve when nanoindentation is performed on an AFM cantilever.	70
4.10	The designed device is used to calibrate the AFM stage. The AFM cantilever chip is brought in contact with the piezolever. Here there is no need to calibrate the AFM laser.	72
4.11	Experimental setup for the calibration using the piezolever method. An apparatus is designed to fix the piezolever under the AFM cantilever. The piezolever is then placed in contact with the AFM cantilever.	74
4.12	Calibration methodology using piezolever. a) The piezolever is located using the optical microscope of the AFM. b) The uncalibrated AFM cantilever is brought brought within microns of the piezolever with the z control of the AFM. c) The AFM cantilever is aligned directly above the piezolever and using the AFM software, the cantilevers are brought into contact.	75

4.13	Schematic of the calibration procedure of the AFM cantilevers using a piezolever. 1) The AFM cantilever is placed into contact with the piezolever. 2) The AFM stage is moved in +z direction while the AFM cantilever is kept in its original location. The deflection of the stage δ_T is controlled by the AFM software. The deflection of the piezolever δ_P is extracted by the voltage change of the piezolever. The displacement of the AFM cantilever δ_C is calculated using eq 4.35.	75
5.1	Schematic showing the micropipette aspiration technique. A micropipette is used to partially or wholly suck the cell and the known pressure is then used to calculate the mechanical properties of the cell. a) The micropipette is brought within microns to the cell. b) A suction pressure is applied to the micropipette.	84
5.2	Optical tweezers method. a) Two silica beads are attached to opposite quadrants of a cell. One of the beads is trapped by a laser beam. b) One of the beads is trapped with a laser array and the other particle is moved with the microscope slide causing the cell to deform.	86
5.3	Microindentation or so called "cell poker" technique. a) A calibrated horizontal glass beam is used as the cantilever which applies the force. b) A long glass stylus is attached to this cantilever serving as the tip. c) An advantage of this technique over AFM is the glass stylus used as the tip is long enough that the horizontal beam is completely out of focus therefore it does not prevent microscopic observation of the deformed cellular region.	87
5.4	The AFM is tip applying a known force on a soft sample(cell). The deformation of the sample is also seen.	91
5.5	Typical force-distance curves obtained on stiff and soft samples. a) The probe is not in contact. b) Indentation on a stiff sample – the sample does not deform. c) Indentation on soft sample – the difference of the force curves represent the deformation of the sample.	91
5.6	Schematic of the proposed new technique. The topography of the deformed shape of the sample is imaged using an AFM.	95
5.7	The tip is "hidden" beneath the cantilever from the optics located above.	97
5.8	The modified tip of the piezolever.	98
5.9	The designed setup for piezolever calibration using nanoindentation technique.	99
5.10	The optical view of the calibration setup. The location of the piezolever tip is not easy with this setup.	101
5.11	The modified setup for piezolever calibration using nanoindentation technique.	102
5.12	The design process. 1. The micropositioner is selected. 2. The part that attaches the PCB to the micropositioner in the demanded orientation is designed. 3. The PCB is designed. 4. The spring that pushes the piezolever with a sufficient force is designed. This pushing force establishes a continuous connection between the piezolever and the Wheatstone bridge.	104
5.13	The designed stand alone system used to apply precise loads on the sample under the commercially available AFM – Autoprobe M5.	105

6.1	The data collected from calibration of the piezolever using nanoindentation technique.	109
6.2	The voltage output of the piezolever is measured during the trapezoidal indents to characterize the piezolever behaviour.	110
6.3	The undeformed topography image of the PDMS sample taken by tapping mode AFM.	111
6.4	Force application on the sample.	112
6.5	The deformed shape of the polymer sample obtained by the AFM.	113
6.6	The 2D plots of the deformed shape along the y axis.	114
6.7	The 2D curve that is just before the piezolever tip (data 1) is selected and considered to be the 2D deformed shape of the polymer sample. The piezolever tip is clearly seen in the other curves.	115
6.8	The piezolever tip is removed from the plot data.	116
6.9	The distance between the piezolever apex and the 2D deformed shape of the polymer is predicted to be approximately $3 \mu\text{m}$ by the SEM image of the piezolever at 15 degrees (the scaling bar is total of $10 \mu\text{m}$).	117
6.10	The built-in least squares function in Matlab7 is used to calculate the nondimensional coefficients of the RHS of the experimental data which in turn give Φ_{RHS} and λ_{RHS}	119
6.11	The built-in least squares function in Matlab7 is used to calculate the nondimensional coefficients of the RHS of the experimental data which in turn give Φ_{RHS} and λ_{RHS}	120
7.1	Dual probe AFM technique may be used to quantify the mechanical properties of thin films. AN AFM cantilever may be coated with thin film and using the setup. Then the second probe may be used to bend the precoated cantilever. Finally imaging the topography is possible with the AFM. . . .	126
7.2	AFM cantilever is imaged as a sample. The second probe is used to bend the sample AFM cantilever. Considering the 10 nm resolution of a typical AFM, images of the deformed cantilever with thin film coating has a significant potential in investigation of the mechanical properties of the thin film. . . .	127

List of Tables

1.1	Characteristics of experimental approaches.	4
4.1	Wave numbers of the characteristic equation.	59
4.2	Comparison of the calibration methods for AFM cantilevers.	80
4.3	Nominal specifications of the piezolever.	81
4.4	Experimentally measured stiffness values of two types of cantilevers are compared to the nominal values supplied by the manufacturer. Nanoindentation and the proposed piezolever method are used for the measurements.	81
4.5	Instrumentation constants used in nanoindentation measurements.	81
6.1	The parameters obtained by the curve fitting procedure.	118
6.2	The calculated values of the parameters for shifted datasets.	121

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 $\sim 0.2 - 5.5 \mu\text{m}$
Magnitude of force	$\sim 0.01 - 1 \text{ kPa}$ (P) and $\sim 0.01 - 2 \text{ kPa}$ (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 piezo-lever 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 Binnig 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 Binnig, 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

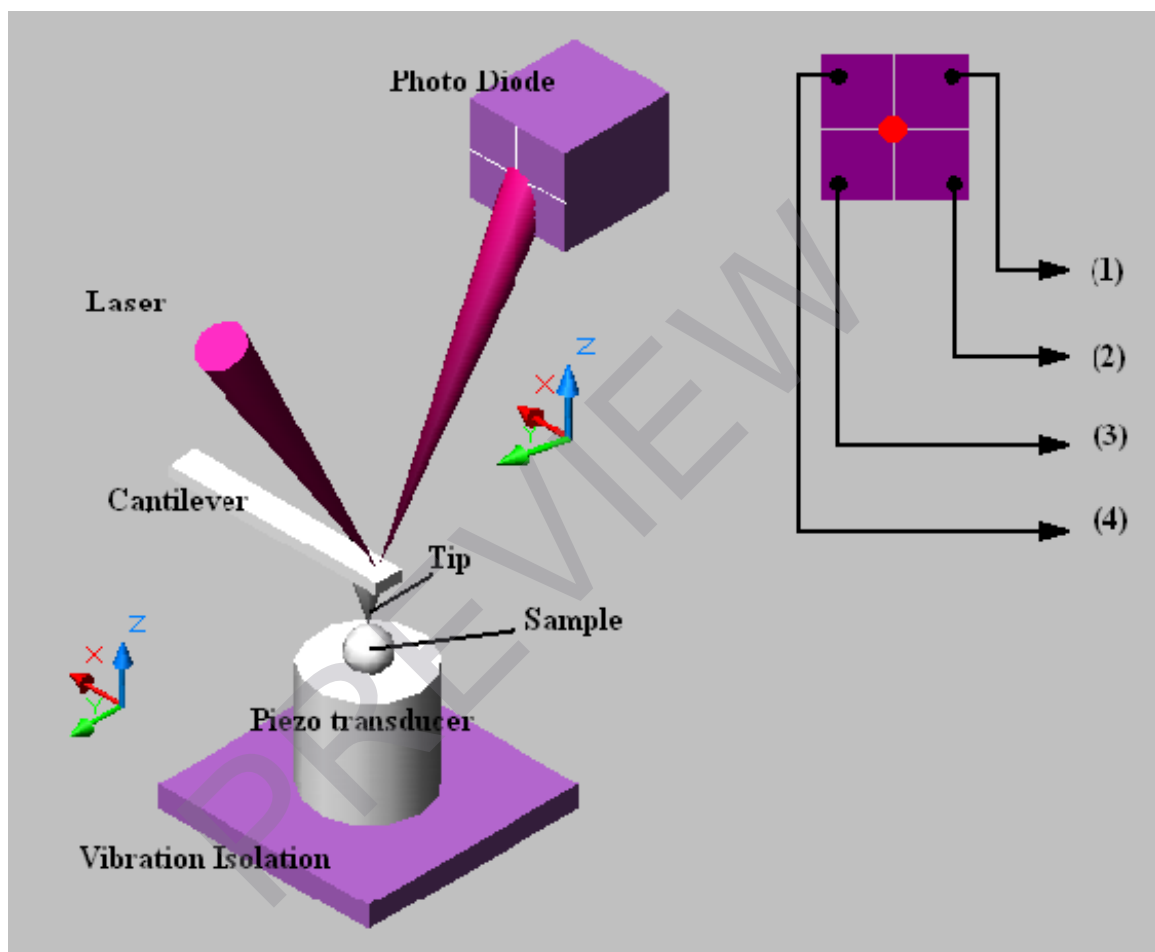


Figure 2.1: Schematic representation of the atomic force microscope. All AFMs have five essential components: a cantilever with sharp tip mounted at the end, a mechanism of sensing the cantilever deflections, a feedback system, a piezoelectric scanning system which provides the relative motion between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