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A Few Nascent Methods for Measuring Mechanical Properties of the Biological Cell

Hartono Sumali, Steven J. Koch, Gayle E. Thayer, Alex D. Corwin, Maarten P. de Boer, Osvaldo H. Campanella, Steven Werely, David Nivens, Carlos Corvalan

Prepared by
Sandia National Laboratories
Albuquerque, New Mexico 87185 and Livermore, California 94550

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Hartono Sumali, Steven J. Koch, Gayle E. Thayer, Alex D. Corwin, Maarten P. de Boer
Sandia National Laboratories
P.O. Box 5800
Albuquerque, New Mexico 87185-1310

Osvaldo H. Campanella, Steven Werely, David Nivens, Carlos Corvalan
Purdue University
225 S. University Street
West Lafayette, IN 47907

Abstract

This report summarizes a survey of several new methods for obtaining mechanical and rheological properties of single biological cells, in particular:
1. The use of laser Doppler vibrometry (LDV) to measure the natural vibrations of certain cells.
2. The development of a novel micro-electro-mechanical system (MEMS) for obtaining high-resolution force-displacement curves.
3. The use of the atomic force microscope (AFM) for cell imaging
4. The adaptation of a novel squeezing-flow technique to micro-scale measurement.

The LDV technique was used to investigate the recent finding reported by others that the membranes of certain biological cells vibrate naturally, and that the vibration can be

detected clearly with recent instrumentation. The LDV has been reported to detect motions of certain biological cells indirectly through the motion of a probe. In this project, trials on *Saccharomyces cerevisiae* tested and rejected the hypothesis that the LDV could measure vibrations of the cell membranes directly.

The MEMS investigated in the second technique is a polysilicon surface-micromachined force sensor that is able to measure forces to a few pN in both air and water. The simple device consists of compliant springs with force constants as low as 0.3 milliN/m and Moiré patterns for nanometer-scale optical displacement measurement. Fields from an electromagnet created forces on magnetic micro beads glued to the force sensors. These forces were measured and agreed well with finite element prediction. It was demonstrated that the force sensor was fully functional when immersed in aqueous buffer. These results show the force sensors can be useful for calibrating magnetic forces on magnetic beads and also for direct measurement of biophysical forces on-chip.

The use of atomic force microscopy (AFM) for profiling the geometry of red blood cells was the third technique investigated here. An important finding was that the method commonly used for attaching the cells to a substrate actually modified the mechanical properties of the cell membrane. Thus, the use of the method for measuring the mechanical properties of the cell may not be completely appropriate without significant modifications.

The latest of the studies discussed in this report is intended to overcome the drawback of the AFM as a means of measuring mechanical and rheological properties. The squeezing-flow AFM technique utilizes two parallel plates, one stationary and the other attached to an AFM probe. Instead of using static force-displacement curves, the technique takes advantage of frequency response functions from force to velocity. The technique appears to be quite promising for obtaining dynamic properties. More research is required to develop this technique.

Acknowledgments

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The authors gratefully acknowledge the technical contribution from Phil Reu, Matt Banning, Gene Venturini, Bonnie McKenzie, Frank DelRio, Jim Martin, and George Bachand, and the programmatic support and guidance from Wahid L. Hermina.

Contents

Nomenclature

Variables

E	Young's modulus of elasticity
$\hat{F}$	Complex force in frequency domain [N]
j	$\sqrt{-1}$
k	Spring constant [N/m]
L_i	Inner-spring length [m]
L_o	Outer-spring length [m]
m	Tested material mass [kg]
R	Impedance real part [N/(m/s)]
s	Spring constant [N/m]
t	Time [s]
t	Spring thickness [m]
$\hat{u}$	Complex velocity in frequency domain [m/s]
w	Spring width [m]
$\hat{Z}$	Complex impedance in frequency domain [N]
Δw	Deviation of spring width from design [m]
ω	Radial frequency [1/s]

Acronyms

AFM	Atomic force microscope
BSA	Bovine serum albumin
LDV	Laser Doppler vibrometer, Laser Doppler velocimeter
LDRD	Laboratory-Directed Research and Development
MEMS	Micro electro-mechanical system(s)
RBC	Red blood cell
SNL	Sandia National Laboratories

1. Report Introduction

1.1. Motivation

Knowledge of the mechanical and rheological properties of biological cells is extremely important, partly because the mechanical properties are related to the functioning of the cells and to pathological conditions such as infection [1] and diabetes [2]. Consider for example the red blood cell (RBC). The role of red blood cells is to transport materials, notably oxygen, through the body. During the flow of red blood cells through capillaries, the cells are subjected to different types of deformations that are closely related to the mechanical properties of the cell membranes. Thus, the mechanics of red blood cells has been an intense subject of research over the years. Many publications in the literature (e.g. [3]) describe methods and results from testing red blood cells. Recent advances in microsystems science and technology can, and should, be leveraged to improve the quality and practicality of the existing methods.

Recent progress in microstructures and nanotechnology has spawned very promising static and dynamic methods for measuring biological cells' mechanical properties such as the moduli of elasticity and viscoelastic parameters. Some of those novel methods were explored in this project. The static methods involve the measurement of force-vs-displacement curves at near-zero velocity. The dynamic methods measure forces and/or velocities of oscillations in the cell membranes. The oscillations can be spontaneous or induced by external sources. The properties of the oscillations have been related to the cell membrane's elastic properties [2] and to nano-mechanical processes in the cell [4].

1.2. Scope of Work

The project explored the potential of a few new methods to obtain mechanical and rheological properties of single biological cells. It also attempted to develop a new method that has the potential to overcome the drawbacks of the existing methods. After a literature survey of the state of the art, the project investigated the following:

1. The use of laser Doppler vibrometry (LDV) to measure vibrations in certain cells.
2. The development of a novel micro-electro-mechanical system (MEMS) for obtaining high-resolution force-displacement curves.
3. The use of an atomic force microscope (AFM) probe for profiling the shape of the biological cells.
4. The development of a novel squeezing-flow technique using microsystems.

The LDV technique was used to investigate the recent finding reported in the literature that the membranes of certain biological cells vibrate spontaneously, and that the vibration can be detected clearly with recent instrumentation. A previous publication reported that the LDV was successful in measuring motions of certain biological cells indirectly through the motion of a probe. The project in this report tested the possibility

that the LDV could measure vibrations of the cell membranes of *Saccharomyces cerevisiae* without a probe.

The MEMS investigated in the second technique is a polysilicon surface-micromachined force sensor that is able to measure very small forces in both air and water. The simple device consists of extremely compliant springs and of Moiré patterns for nanometer-scale optical displacement measurement. Force fields from an electromagnet were used to create forces on magnetic micro beads glued to the force sensors. These forces were measured and compared with finite element prediction. Potential uses of the sensor will be discussed.

The use of atomic force microscopy (AFM) for profiling the geometry of red blood cells was investigated as the third technique. The project investigated the possibility that the method commonly used for attaching the cells to a substrate actually modified the mechanical properties of the cell membrane. If that is true, then the use of the method for measuring the mechanical properties of the cell may not be completely appropriate without significant modifications.

The last of the studies discussed in this report is intended to overcome some drawbacks of the AFM as a means of measuring mechanical and rheological properties. The squeezing-flow AFM technique utilizes two parallel plates, one stationary and the other attached to an AFM probe. Instead of using static force-displacement curves, the technique takes advantage of frequency response functions from force to displacement. A scaled-up version of this technique is shown to be successful in obtaining dynamic properties in millimeter-sized capsules made of biological materials. More development is required to adapt this technique to micro-scale measurements.

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2. Laser Doppler Vibrometry

2.1. Introduction

Pelling et al. [1] used the atomic force microscope (AFM) to detect spontaneous mechanical vibrations of the yeast cell. The cell was fixed to a filter pore. An AFM probe was placed on the cell. The motion of the probe tip was captured using photodiodes. The study showed the motions to be almost purely sinusoidal, with amplitudes ranging from 0.5 nm to about 5 nm. The frequency was a function of temperature, ranging from 873Hz at 22^{o}C to 1634Hz at 30^{o}C. Exposure of the cells to a metabolic inhibitor ceased the motion.

Alster et al. [2] showed that the human red blood cell (RBC) vibrates (flickers). They used a method based on point dark-field microscopy and recorded the image intensity from the RBC as a function of time. The amplitude of the displacement-induced intensity variation was 18.7% +/- 1.75% in non-diabetic patients and 13.9% +/- 1.7% in diabetic patients. From the frequency difference, they conclude that diabetes reduces the bending compliance of the RBC, thereby reducing the RBC's ability to pass through capillaries that are narrower than its own diameter.

The method used by Pelling et al. [1] required very precise and rigorous preparation. And although it was said to be "non-intrusive", it involves mechanical contact and interaction with the AFM probe tip. The method used by Alster et al. [2] did not show motion of the cell directly. Instead, it inferred the vibration based on the oscillation of the light intensity. An alternative to the two methods was a method that would not require mechanical contact, and had been shown to measure mechanical motion reliably. Below is the description of our attempt to detect the mechanical motion of biological cells using the Laser Doppler vibrometer (LDV).

The Structural Mechanics group at SNL has utilized the LDV to obtain various measurements of motions of micro electro-mechanical systems (MEMS). The LDV measures velocity by measuring the Doppler shift frequency between a laser beam reflected by the moving surface and a reference laser beam from the same source. The LDV has also been used to measure motions of biological tissues [3], by measuring an AFM probe attached to the tissues [4]. The purpose of this section is to present an attempt to explore the possibility of using the LDV for measuring vibrations of individual biological cells, directly, without the use of contacting probes. Brewer's yeast (*Saccharomyces cerevisiae*) cell was used as an example following Pelling et al. [1].

2.2. Test Procedure

A Polytec^{TM} MSV 600 Laser Doppler Vibrometer system was used with several laser transducers including a DD-300-HF displacement transducer module with a range of -75nm to +75nm and a bandwidth of 20MHz. The LDV was integrated into the optical train of a microscope. *S. cerevisiae* (Carolina Biologicals) was streaked on MV Media (Carolina Biologicals, 24mg/500ml) in a Petri dish at room temperature. The thickness of

the streaks was varied. After 24 hours, the grown colonies were examined under an optical microscope (Figures 2.1 and 2.2). Many of the colonies, especially large ones, were aggregates of several layers of cells. Figure 2.3 shows several small colonies with mostly single layers of individual cells. The

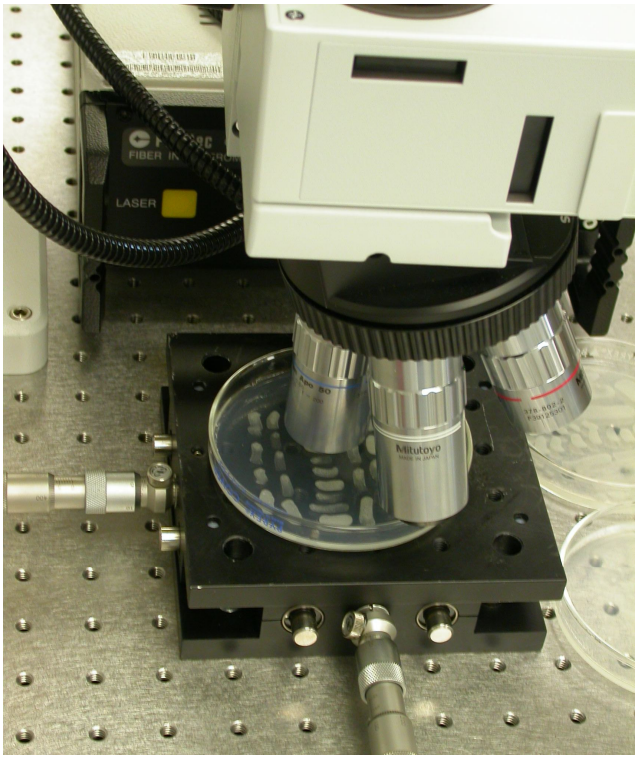

Figure 2.1: Petri Dish on X-Y Stages Under Microscope.

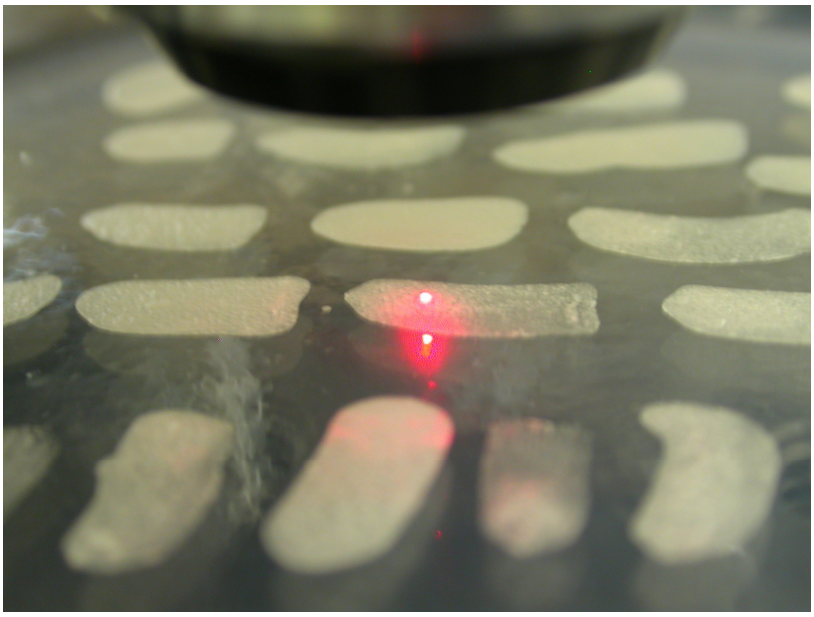

Figure 2.2: Laser Beam Focused on a Streak.

objective magnification was 20X. Figure 2.4, a 50X-objective image centered around the single cell at the center of Figure 2.3, indicated the presence of bud scars on the cells. Figure 2.5 shows that optical clarity started to deteriorate when the objective magnification was higher than 50X. A typical yeast cell has a diameter of about 5 μm. For vibration measurement with LDV, the laser beam was aimed at a single cell from a chosen colony. Colonies with a single layer of yeast cells were chosen to make sure that the laser beam did not measure the vibration of several cells clumped together. Lone cells without a colony were also found easily and targeted for LDV motion measurements.

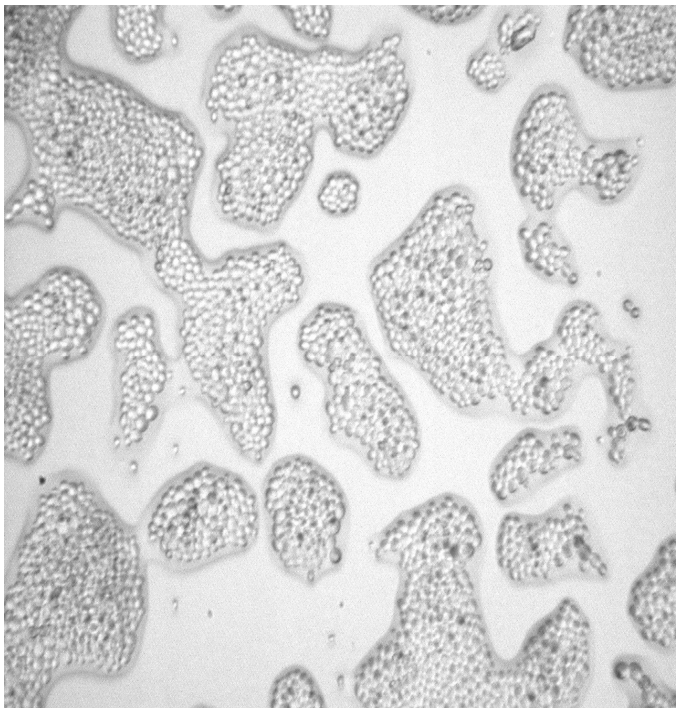

Figure 2.3: Small Colonies and a Single Cell Near the Center. Objective Magnification = 20x.

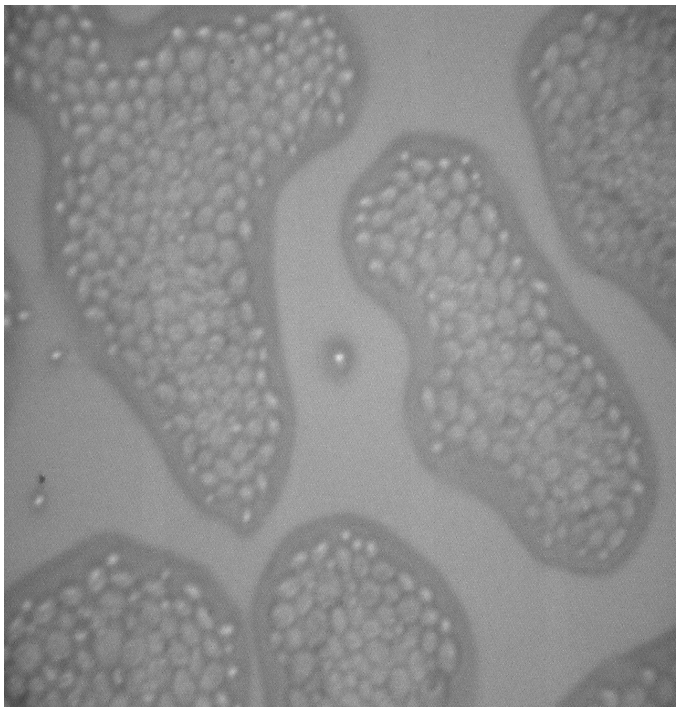

Figure 2.4: Small Colonies and a Single Cell Near the Center. Objective Magnification = 50x.

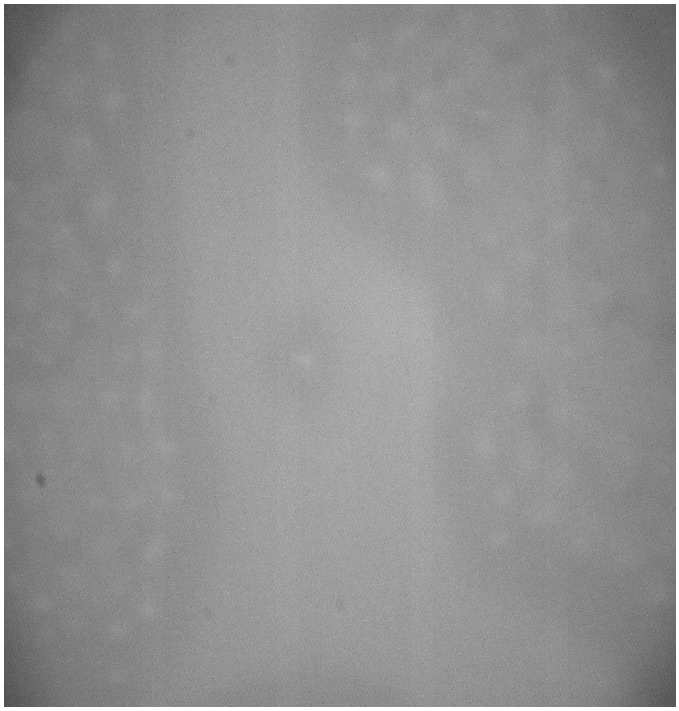

Figure 2.5: Laser Beam Focuse on a Single Cell Near the Center. Objective Magnification = 100x.

For each targeted cell, hundreds of time-domain measurements were averaged. Several averaging methods were used, including the amplitude-only averaging and peak averaging.

2.3. Result and Conclusion

A typical discrete Fourier transform of the LDV signal from a single cell is shown in Figure 2.6. That spectrum appeared to be the same as a spectrum obtained from the measurement on a part of the culture medium that had no cell (Figure 2.7). Both spectra appeared to show only the noise floor of the system. Thus, we did not observe any prominent periodic oscillation described in the literature [1]. At this time, we are still investigating the possible reasons why the LDV did not detect the cell oscillations.

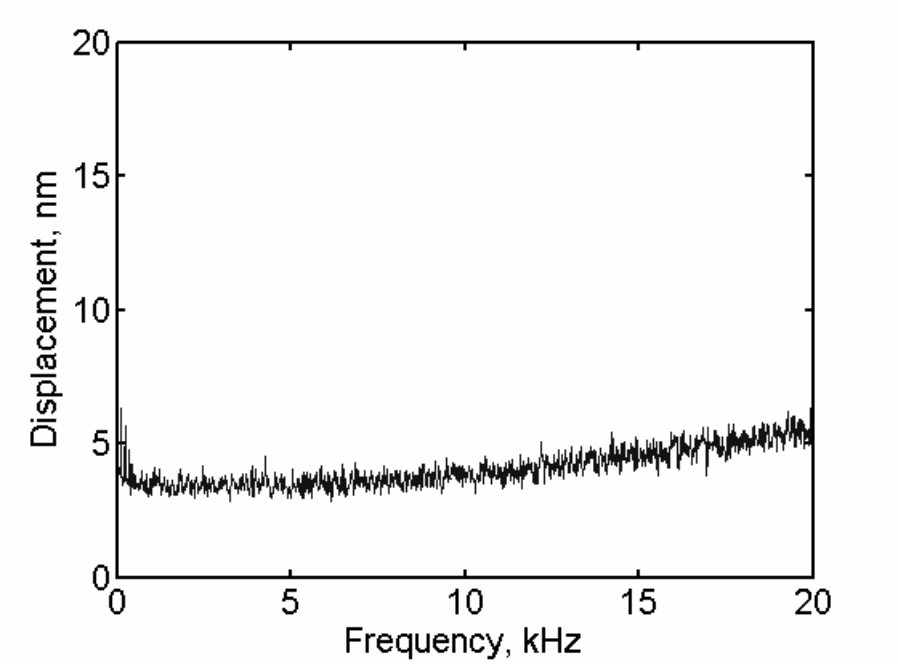

Figure 2.6: Discrete Fourier Transform of LDV Signal from Single Cell.

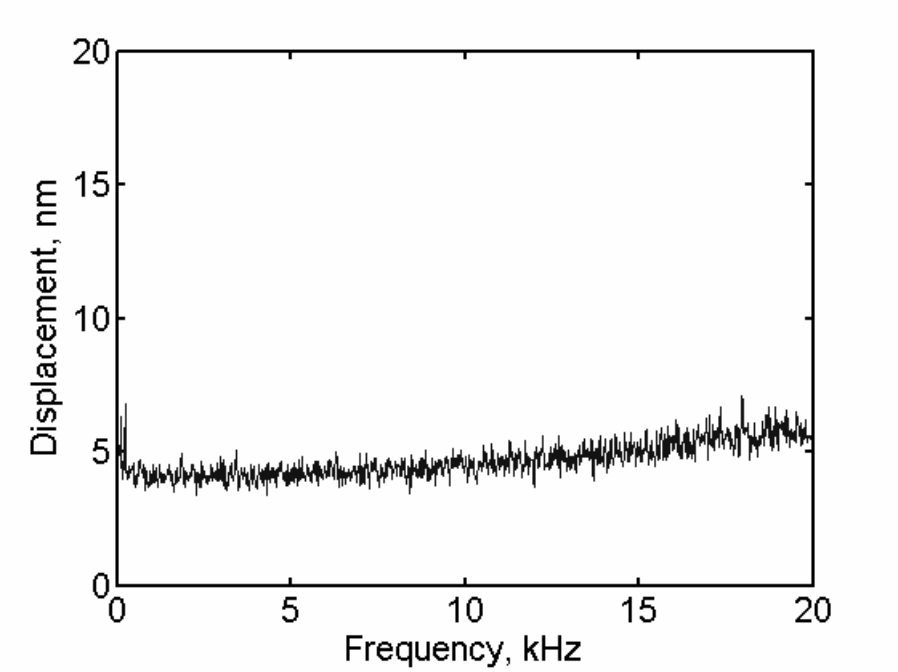

Figure 2.7: Discrete Fourier Transform of LDV Signal from Culture Medium.

2.4. References

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3. MEMS Force Sensor

3.1. Introduction

Magnetic microspheres have been used in a variety of intra-cellular and molecular biophysics applications [1-7]. However, their utility depends on the ability to apply well-controlled and calibrated forces. Popular calibration methods have relied on inference of force from Stokes drag [3,4,and 8] and Brownian dynamics [1], while other methods have used calibrated microneedles [6,9], gravity [10] or known properties of polymers [11]. While useful, these methods have drawbacks in some cases. In the case of Stokes drag calibration, the particle radius and solvent viscosity must be known accurately, and also the magnetic particle may travel a large distance compared with the particle diameter, leading to complications when the field gradient is large. When using Brownian dynamics, the length of the tether must be measured or deduced, and the temperature must be well controlled. To address these complications, we have designed, fabricated and tested a compliant surface-micromachined spring with which the lateral force field of an electromagnet on a single magnetic microparticle can be calibrated. This provides a simple force calibration method that does not depend on particle shape, polymer tethers of known length, or solvent conditions. Furthermore, the spring is sensitive enough to allow characterization of single bead, and therefore bead to bead variation.

3.2. Description of the Device

The principle of our device is similar to previous reports using long glass cantilevers [6,9], but with some important distinctions. While a long glass fiber is ideal for placing a microsphere into a very small gap, the fibers must also be calibrated for each fiber and may not have an easily controlled spring constant. In the device we describe here, the spring constant can be chosen by design, and furthermore, can be accurately verified by on-chip calibration. Furthermore, the device can operate both in air and water, leading to future possibilities of directly measuring biophysical forces on-chip with multiple adjustable sensors.

Figure 3.1 shows the device consists of a folded-beam suspension attached to a Moiré grating. It is fabricated by surface micromachining methods [12], and the structural material is polycrystalline silicon (polysilicon). The spring constant of the grating can be calculated from

$$k = 2E(w - \Delta w)^3 t\left(L_o^3 + L_i^3\right)^{-1} \tag{1}$$

where E = 164 GPa is Young's modulus, w = 1 μm is the nominal line width, Δw = 0.2 μm is the decrease in the nominal line width due to processing, t =2.25 μm is the spring thickness and L_o and L_i are the respective lengths of the outer and inner spring beams. For these designs, L_o - Li=19 μm, and L_o values were 186, 268, 388, 561 and 823 μm, resulting in nominal spring constant values of 34, 11, 3, 1 and 0.3 milliN/m.

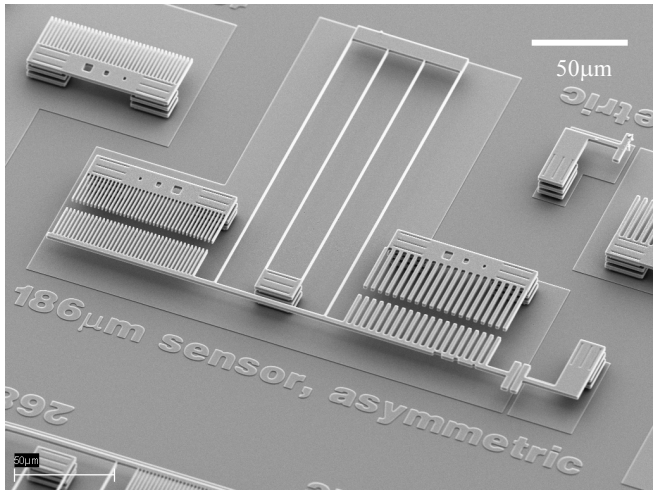

Figure 3.1: SEM image of a 186 µm force transducer. Shorter force sensor is shown here for ease of display.

The displacement sensor is comprised of an object grating attached to the end of the spring and a reference grating attached to the substrate. To make a displacement measurement, we compare the relative phase of the two gratings. Using a 50X objective, by applying sub-pixel interpolation and by virtue of averaging over the many pixels of the periodic grating, we can detect phase to one part in one thousand. Given the grating pitch of 2.5 μm, this translates to 2.5 nm in-plane measurement resolution. Force resolution is then the product of the displacement resolution and the spring constant.

3.3. Magnetic Force Measurements

Using two micromanipulators and pulled-glass fibers, we affixed with vacuum grease (Dow brand) individual beads (Dynal M270 streptavidin, product #653.05, lot# F72000) to a desired location on the MEMS force sensor with approximately 5 µm precision. Figure 3.2 shows a single bead affixed to a sensor and positioned about 200 microns away from the magnet face. (A long, 581 µm force sensor is used for data in this report.)

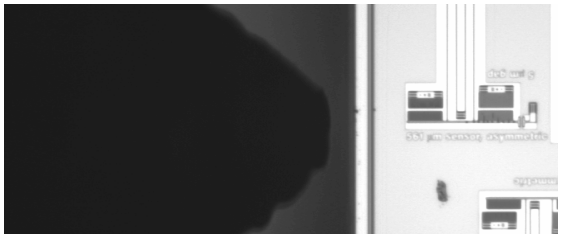

Figure 3.2: 5x picture of device with single bead [picture now is of 10 beads] positioned next to the electromagnet.

Figure 3.3 shows force versus magnet current for 12 different beads at an axial distance of 200 microns from the magnet pole piece. We assumed the saturated bead moment to be proportional to bead displacement at maximum applied current (20A, field = 560 mT), and constructed the histogram shown in the inset by averaging nine consecutive measurements (to reduce noise from stray air currents) after stepping current from 0 to 20 A. The magnet was degaussed prior to each sweep. For the 12 beads we measured a standard deviation of +/- 9%, and a possibly bimodal distribution.

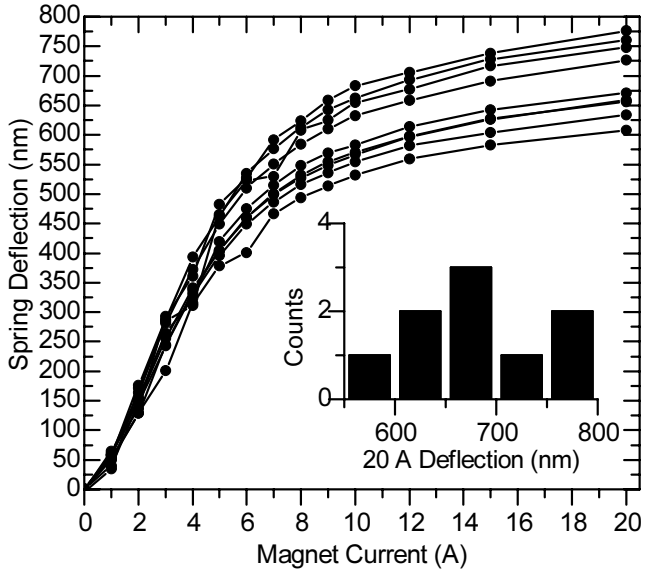

Figure 3.3: Measured spring deflection versus magnet current for nine different beads on three different sensors. Each trace represents an individual non-averaged sweep with current increasing from 0 to 20 amps. Inset: Histogram of spring deflection at 20 amps, bin size 50 nm.

We also used the micro-spring to calibrate the force field on a single bead for various currents and axial displacements from the pole, as shown by filled circles in Figure 3.4. The data are for axial displacements of (from top) 167, 207, 247, 307, 407, 607, 1007 μm.) For a given distance, the data represent a single current sweep (0 to 10 A), and the same bead and sensor were used for all data. Prior to each current sweep, the magnet was degaussed with a decaying 100 Hz current. As expected, due to remanence in the iron core, the return sweeps (not shown) showed significant hysteresis. The data compare well with values calculated with finite element modeling [16].

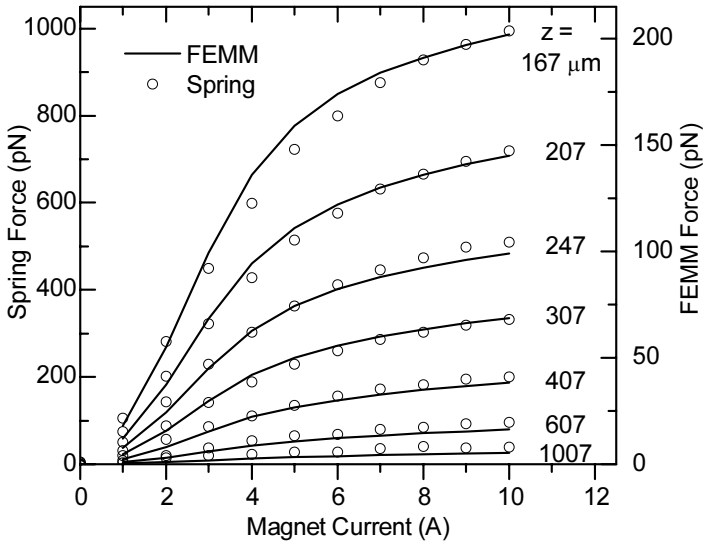

Figure 3.4: Measured (open circles) and calculated (lines) force versus applied magnet current for seven different axial displacements of a single bead.

Another feature of the spring force transducer is its relative insensitivity to environmental conditions such as temperature and solvent. Importantly, using a simple flow cell, we rendered the surface hydrophilic with an ozone treatment, then hydrated the device and demonstrated retention of full functionality. The hydrated force measurements were unchanged and noise was significantly damped compared with operation in air. We found that the major source of noise was stray air currents. Because results in buffer were the same as in air, it was not necessary to hydrate the device to characterize our magnet.

In addition to this example, there are other possible advantages to on-chip real-time force sensing. As seen in Figure 3.4 the force for this magnet can vary by as much as a factor of two across a distance of 40 microns, so one would need to accurately know the position of the magnet relative to the bead in order to know the force from magnet current alone. Real time force sensing would obviate the need to know the precise location of the bead and magnet properties.

More information on the new MEMS force sensor can be found in [16].

3.4. Conclusion

We have shown that a simple micromachined force sensor can be used to characterize individual micron-scale magnetic particles and also to map the magnetic force field of an electromagnet. In the current implementation, the spring constant is accurately determined via the fabricated line width and design parameters, but future designs will incorporate on-chip self calibration. Self-calibration combined with the insensitivity to temperature and buffer conditions make the force sensor an attractive alternative to standard calibration techniques. We anticipate using the device for characterization of

other commercial and custom microsphere preparations and other electromagnet designs. Furthermore, we anticipate incorporating the force sensor into future MEMS designs which will be used to measure biophysical forces in real time for a variety of biomolecular and sub-cellular processes.

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4. Atomic Force Microscopy

4.1. Introduction

Most of the previous work on red blood cells using AFM focused on imaging cells that had been fixed with cross linking agents such as glutaraldehyde [1]. These agents are excellent for fixing the cells and imaging them, however they significantly alter the properties of the cell membranes, complicating the study of the membranes' mechanical properties. During scanning, attached red blood cells are subjected to considerable forces applied by the AFM tip which detach the cells from the attachment surface (glass cover slip in our tests). That movement of the cells precludes the application of local forces that allow one to study the rheology of the cell membrane.

4.2. Attachment Protocol

Preliminary work was conducted to establish a protocol that allows attachment of the cells that could sustain the forces applied during scanning. The protocol is given below:

1. Coat glass with poly-l-lysine 0.02% for 15min. Rinse in double distilled water and allow it to dry at room temperature.
2. Pellet red blood cells (RBCs) by centrifugation at 1000g for 10 minutes. Remove plasma and buffy coat. Resuspend 2µL RBCs in 1mL PBS (137mM NaCl, 2.7mM KCl, 8.1mM Ka_2HPO_4, 1.5mM KH_2PO_4,) pH 7.4 containing 0.20% bovine serum albumin (BSA) and 5mM glucose. Allowed to attach in humidified chamber for 30 min.
3. Remove the excess of unattached cells by dipping glass in beaker with PBS. Repeat until no more cells come off. Fix with 0.5% acrolein* solution made in PBS (no glucose, no BSA) for 5 minutes at room temperature. Discard fixative and keep the fixed attached cells immersed in PBS (no glucose, no BSA) until analysis.
4. Mount the glass in the chamber with fresh PBS and observe.

Note: *Acrolein could be replaced by glutaraldehyde.

Figure 4.1 shows cells that were prepared and observed in an optical microscope, whereas Figures 4.2, 3 and 4 illustrates the different types of cells observed by optical microscope and that were identified with the AFM instrument.

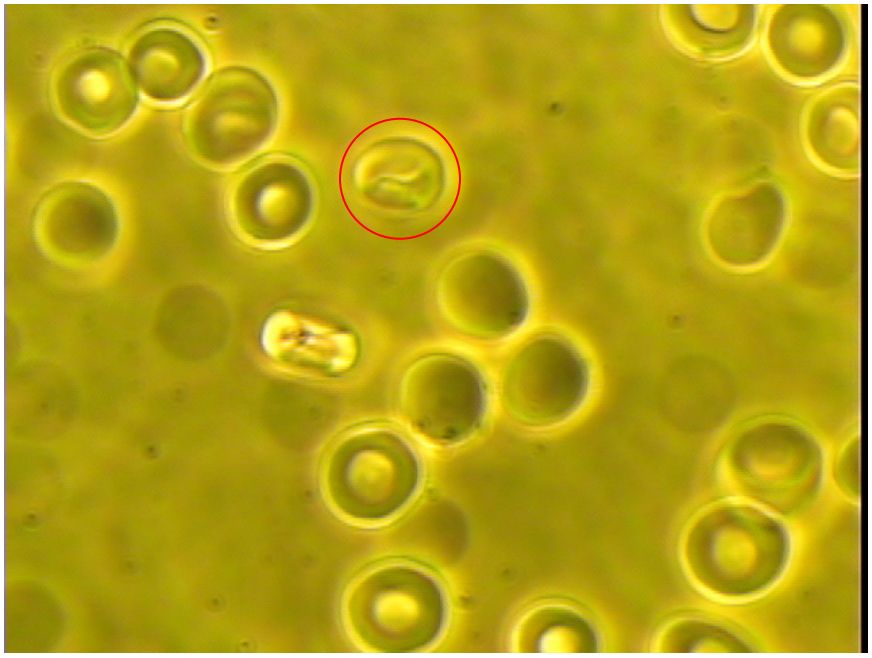

Figure 4.1: Optical microscope image of red blood cells that were attached to the glass and later fixed

As observed in the figure, three types of cells can be distinguished (1) red blood cells with the typical discoid shape, (2) red blood cells that have lost the dimple of the center, likely due to the attachment on the glass and the fixation procedure and (3) cells that appear to have a projection on the surface. We believe that these cells are produced by the fixation method in which more attachment on the sides produce the projection noted on the optical microscopy image. Images of these specific cells were obtained with the AFM instruments and they are illustrated in Figures 4.2, 3 and 4.

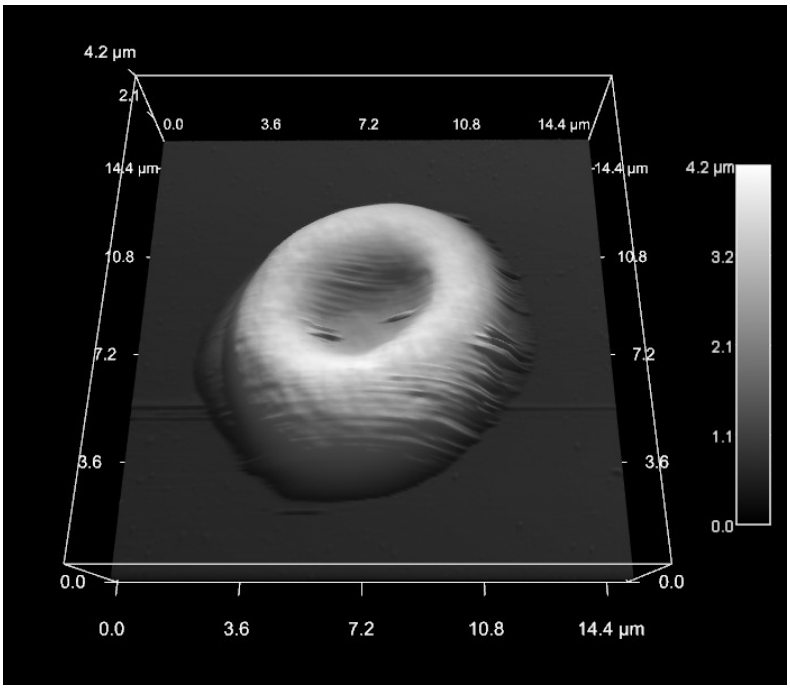

Figure 4.2: Red blood cell with the typical discoid shape. Dimensions are given by the grayscales for the z-direction and by the dimensions in the x-axis

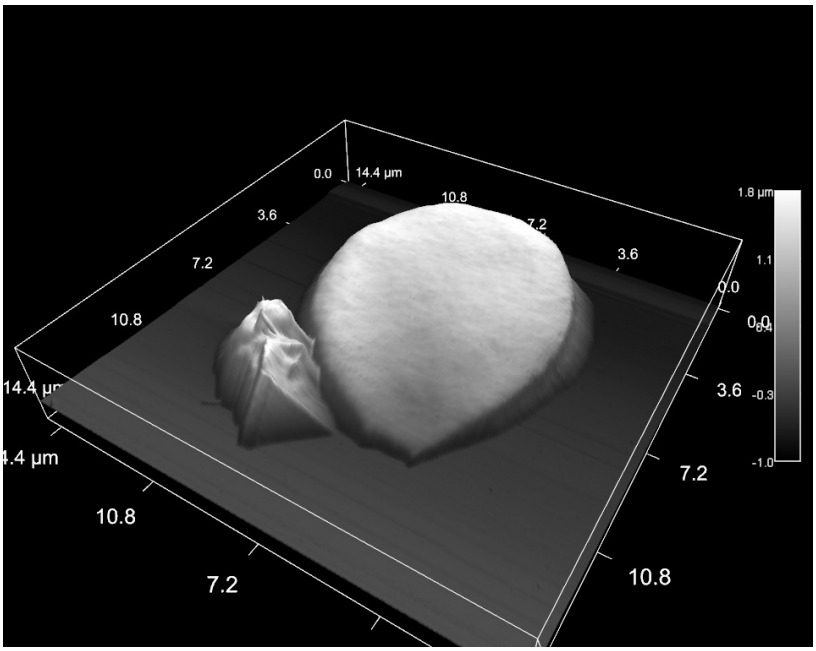

Figure 4.3: Red blood cells without the dimple. It can be noted that due to the attachment the thickness of the cells has reduced from approximately 4 μm to 1.8 μm. "Type-2" cell.

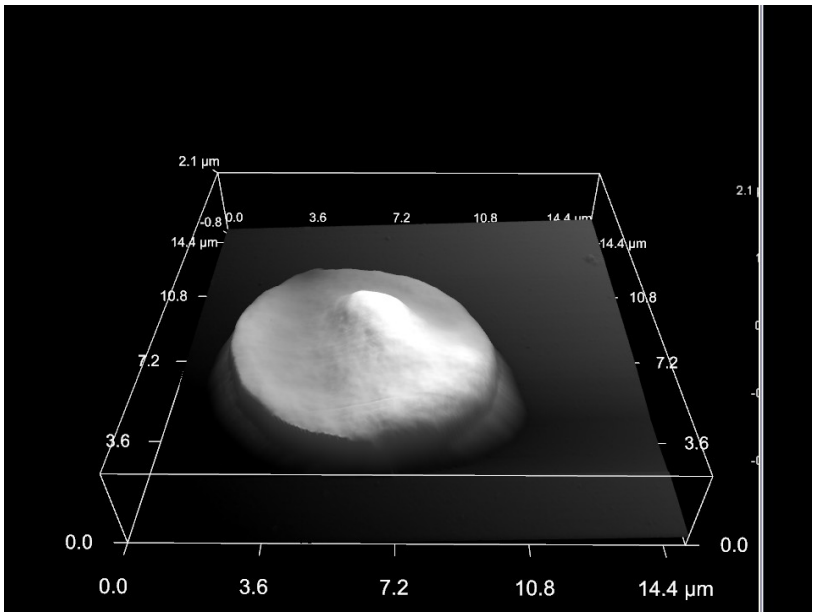

Figure 4.4: "Type-3" red blood cell

4.3. AFM Imaging without Cross-Linking Agent

Figure 4.5 shows AFM images of a group of red blood cells that have been attached to the glass cover. The cells were attached following the protocol given above, however, no cross-linking agent was used. The proposed attachment protocol provides a methodology to attach cells without affecting their properties. In order to measure the viscoelastic properties of the red blood cell membranes it is important that they be not modified. The image include dimensions of the cells about 6-7 μm in diameter and 3 μm height. One of the red blood cells was identified for further studies and the image plotted as an amplitude plot. Here the dimension in the z direction is given by the amplitude of the tip displacement in the z direction, which in this case (about 3.2 nm) is considerable lower than the thickness of the cell. That image is illustrated in Figure 4.5. Attempts were made to obtain force-vs-displacement curves for this individual cell, but they were not successful due to the lack of an appropriate calibration procedure. A proper calibration procedure is being evaluated at the moment. Figure 4.6 shows the position-vs-amplitude plot for the scan.

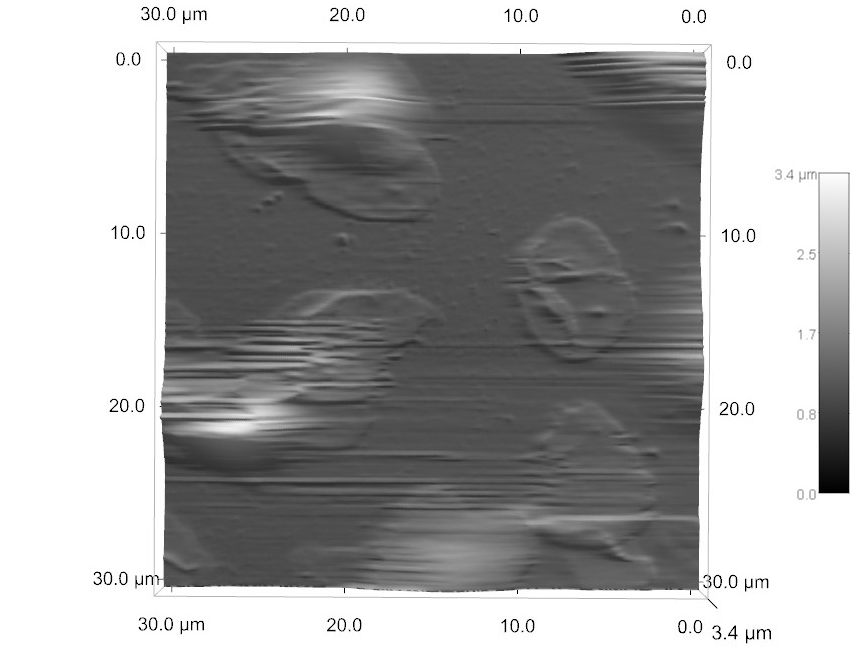

Figure 4.5: AFM Image of Red Blood Cells attached to the glass cover without a cross-linking agent.

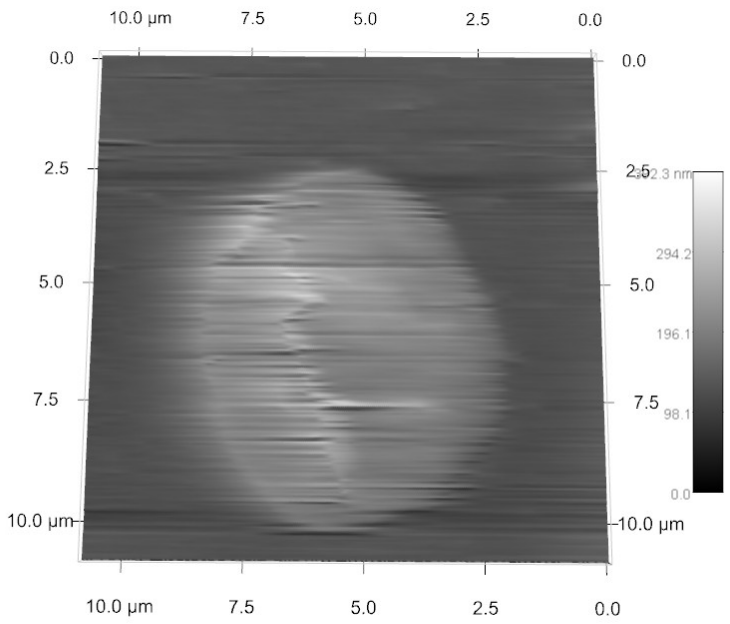

Figure 4.6: Amplitude plot of a red blood cell identified in Figure 4.5.

4.4. Conclusion

AFM imaging without a cross-linking agent did not reveal normal shapes. The cross-linking agent required in current procedures of attaching the cells to the substrate modifies the physical properties of the RBC. If the purpose of the AFM probing is related to the measurement of physical properties, then the current procedure for sample attachment is not likely to be appropriate. A new technique for using the AFM probe for measuring physical properties needs to be developed.

4.5. Reference

[1] Nowakowski, R., Luckam, P. and Winlove, P. 2001. Imaging erythrocytes under physiological conditions by atomic force microscopy. *Biochimica et Biophysica Acta*, 1514, 170-176.

5. Squeeze-Flow Technique

5.1. Introduction

The purpose of developing the squeeze-flow technique is to overcome the drawback of using AFM imaging as part of measuring mechanical properties of the biological cell, especially of the red blood cell. Biological cells have a structure that resembles capsules consisting of an internal deformable material enclosed by a semipermeable membrane. The primary role of the membrane is to confine and protect the encapsulated material as well as controlling the exchange of the capsule content and the environment [1]. Red blood cells are a typical example of these natural capsules. The role of the red blood cells is to transport materials, notably oxygen, through the body. During the flow of red blood cells through arteries, veins and capillaries the cells are subjected to different types of deformations that are closely related to the mechanical properties of the cell membranes. Thus, the area concerning the mechanics of red blood cells and the study of flow of blood in narrow tubes has been very active over the years. Secomb [2] describes methods and results from testing red blood cells. In general most of the methods described involve complex mechanical models, in fact a two phase system including complex geometries. However, with the aim of modeling these complex systems many assumptions have been relied on, in particular those concerning the application of axisymmetric geometry to the cells. Methods to test capsules have been also described by Barthes-Biesel [1]. Among many of the methods described, the squeezing of these capsules between two plates at a constant velocity appears to be the one that can provide better results and more fundamentals measurements due to the fact that strains and stresses can be better defined in this geometry. Results of the mechanical testing are generally reported as the squeezing force versus a parameter defined as the relative flattening of the capsule. Testing is generally carried out until the membrane bursts. This test can then be classified as a destructive test so it is not amenable to monitor changes in the mechanical properties of the cell membranes due to the action of different environments and conditions. The squeezing flow method has been used extensively for testing bio and food materials and its application has been described by Campanella and Peleg [3].

At Purdue University, we have developed a modified squeezing flow technique. Essentially the geometry is similar than that described by Barthes-Biesel but the type of deformation is different because it consists on the application of sinusoidally time-varying small strains to the sample. Details of the methodology are described by Mert [4]. The new methodology has the advantage of being non-destructive so it allows one the study of systems without affecting their structure, i.e. it is amenable to monitor changes of the mechanical properties due to environmental changes. It is envisaged that the developed system can be attached to an Atomic Force Microscope (AFM) which may serve the dual purpose of imaging the cells and determining their mechanical properties.

5.2. Adaptation to Micro-Scale Measurements

The main objective of this work is to develop models that can describe the rheology of biological cells using the squeezing flow geometry by building a micro-squeezing flow

device or an attachment for our AFM instrument. In order to test the feasibility of using the squeezing flow geometry and the newly developed method [4] for testing biological cells, we produced artificial cells (see Figure 5.1) of approximately 2mm diameter surrounded by membranes with different thickness using the gelation reaction Sodium Alginate-Calcium [5]. As depicted in the figure, the membrane thickness is a function of how long the reaction between the sodium alginate and the calcium ions takes place. Longer reaction times result in capsules with thicker membranes. The thicknesses of the membranes for different reaction times were measured using a digital caliper.

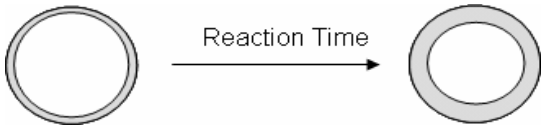

Figure 5.1: Formation of the artificial Calcium-Alginates Capsules

The test setup is illustrated in Figure 5.2. We used a function generator, a power amplifier and a piezoelectric actuator to generate a band-limited random frequency excitation. An impedance head was used to measure the resulting force and velocity of the top moving plate. A stepping motor is used to set the initial (DC offset) displacement of the capsule. A load cell measured the DC component of the squeezing force.

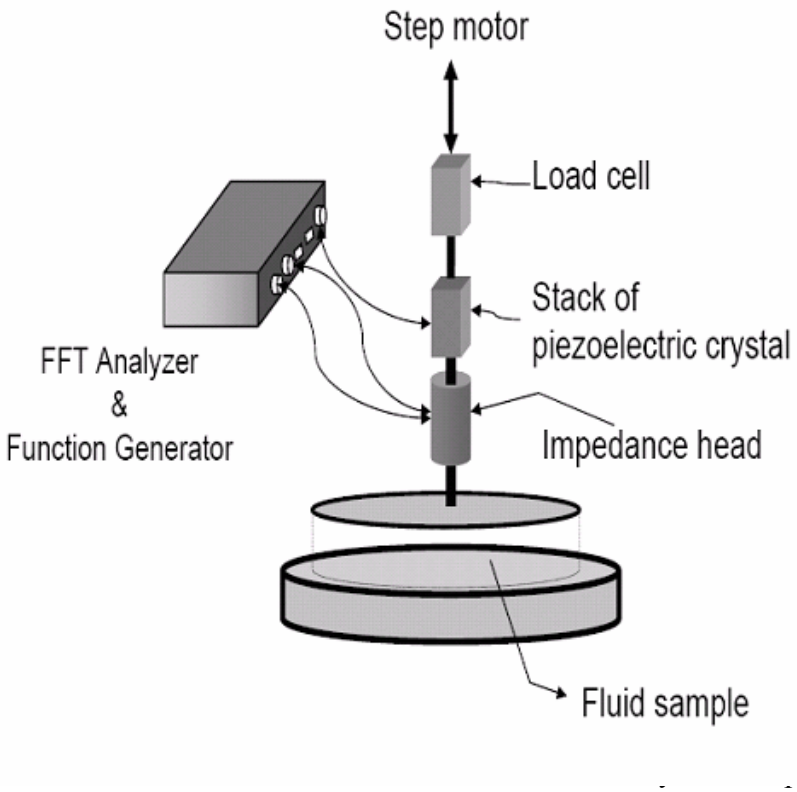

Figure 5.2: Squeezing flow test setup

The measured complex mechanical impedance of the tested material can be expressed in the frequency domain as:

$$\hat{Z} = \frac{\hat{F}}{\hat{u}} = R + i(\omega m - s/\omega) \quad (1)$$

In the above equation $\hat{F}$ and $\hat{u}$ are the complex force and velocity respectively obtained by the Fourier Transformation of the measured force and velocity. R is the resistance of the material due to damping, whereas m and s are the mass and spring stiffness of the tested material, and ω is the radian frequency of the harmonic oscillation.

For fluid samples we have developed appropriate equations that have in consideration the flow of fluids between the two plates. Currently we are developing equations that can be applied to capsule-like samples. In the mean time, we have tested artificial alginate-calcium capsules surrounded by membranes of different thickness using this modified squeezing flow apparatus. The results are illustrated in Figure 5.3. The curves indicate different reaction times between alginate and a calcium salt ($CaCl_2$). Longer reaction times result in capsules with thicker membranes.

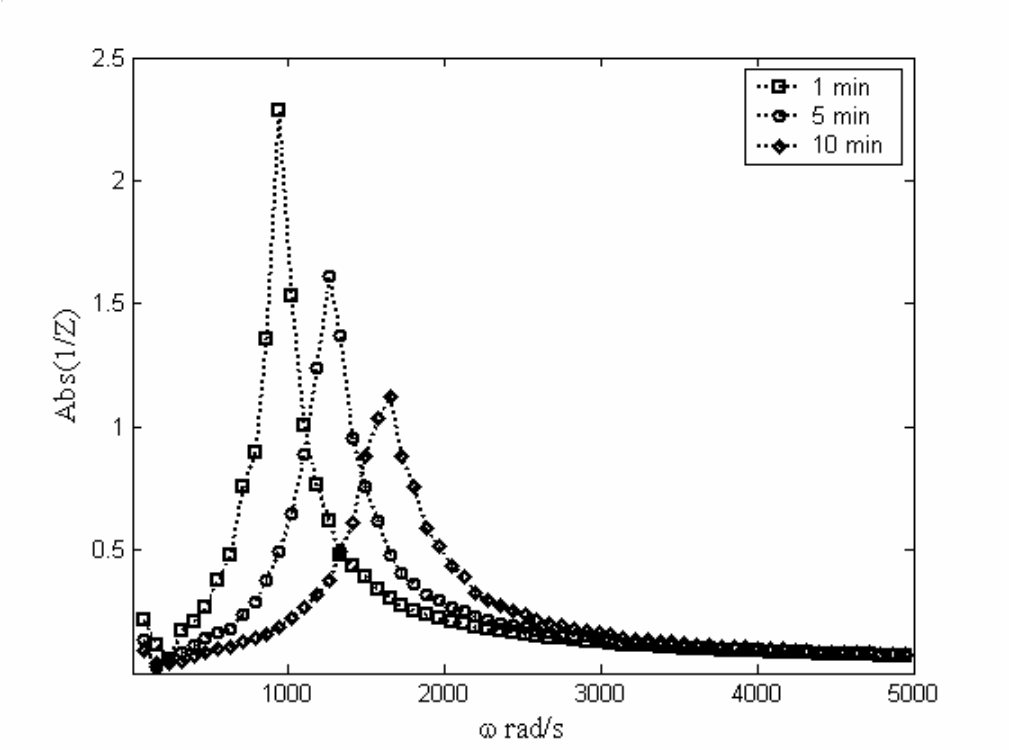

Figure 5.3: Mobility (velocity/force) magnitude of artificially fabricated alginate capsules with membranes of different thickness. The curves indicate different reaction times between alginate and a calcium salt ($CaCl_2$). Longer times result in capsules with thicker membranes.

As indicated in the figure distinctive resonant peaks are observed for each sample. Since samples with longer reaction times have thicker and stiffer membranes, they exhibit a higher elastic behavior which is represented by the shifting to the right of the resonance frequency. Appropriate equations (work in progress) will allow us to estimate the viscoelastic properties of the capsules from the mobilities depicted in Figure 5.3.

5.3. Future Work

Work is in progress at Purdue University for adapting the squeeze-flow technique to micro-scaled measurement for single cells. The design of a special attachment to an AFM probe is almost completed. Also in progress is the development of constitutive equations to describe the rheology of the red blood cells during squeezing and/or compression by the AFM probe fitted with the special attachment.

5.4. References

[1] Barthes-Biesel, D. 2003. Flow induced capsule deformation. *In "Modeling and simulation of capsules and biological cells"*, C. Pozrikidis Ed. Chapman and Hall/CRC Mathematical Biology and Medicine Series, London.

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[3] Campanella, O.H. and Peleg M. 2002**.** Squeezing flow viscometry for nonelastic semiliquid foods - Theory and applications. *Critical Reviews in Food Science & Nutrition*. 42(3), 241-264.

[4] Mert, B. 2004. Novel Acoustical and mechanical methods for rheological characterization of biological materials. PhD Thesis. Purdue University.

[5] Ak, M.M, Nussinovitch, A., Campanella, O.H. and Peleg, M. 1989. Crosslinking of thermally preset alginate gels. *Biotechnology Progress*, 5(2), 75-77.

6. Report Conclusions

A typical discrete Fourier transform of the LDV signal from a single cell did not show any prominent periodic oscillation described in the literature. Further investigation is necessary to find the reason why the LDV did not detect the cell oscillations.

The MEMS force-displacement sensor developed in the project can be used to characterize individual micron-scale magnetic particles and also to map the magnetic force field of an electromagnet. In the current implementation, the spring constant is accurately determined via the fabricated line width and design parameters, but future designs will incorporate on-chip self calibration. Self-calibration combined with the insensitivity to temperature and buffer conditions make the force sensor an attractive alternative to standard calibration techniques. We anticipate using the device for characterization of other commercial and custom microsphere preparations and other electromagnet designs.

AFM imaging of the red blood cell without a cross-linking agent did not reveal normal shapes. The cross-linking agent required in current procedures of attaching the cells to the substrate modifies the physical properties of the RBC. If the purpose of the AFM probing is related to the measurement of physical properties, then the current procedure for sample attachment is not likely to be appropriate. A new technique for using the AFM probe for measuring physical properties needs to be developed.

Work is in progress at Purdue University for adapting the squeeze-flow technique to micro-scaled measurement for single cells. The design of a special attachment to an AFM probe is almost completed. Also in progress is the development of constitutive equations to describe the rheology of the red blood cells during squeezing and/or compression by the AFM probe fitted with the special attachment.

The four techniques identified in this $100,000/one-year exploratory project have very good potential to develop into powerful experimental tools. Obviously, much more research is required.

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Prof. Osvaldo H. Campanella
Agricultural and Biological Engineering Department
Purdue University
225 S. University Street
West Lafayette, IN 47907

Prof. Bernard A. Engel, Head
Agricultural and Biological Engineering Department
Purdue University
225 S. University Street
West Lafayette, IN 47907