Instrumentation to Characterize Needle Insertion into Biological Tissue

by

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Abstract

The Transdermal Drug Delivery Project in the BioInstrumentation Laboratory involves the design of a device to deliver drugs through the human skin using micro needles. It is crucial to characterize the insertion of micro needles into biological tissues. Hence, instrumentation will be designed and fabricated for the characterization of micro needle insertion. This thesis focuses on the design and fabrication of such instrumentation. The instrument is multi-modal, multi-axis, mobile and compact. It is capable of precise insertion positioning and acquiring accurate insertion force data. Characterization of micro needle insertion into biological tissues is done successfully using the data acquired by this instrument and an existing physical force model.

Thesis Supervisor: Ian W. Hunter

Title: Hatsopoulos Professor of Mechanical Engineering and Professor of BioEngineering

Acknowledgements

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I specially thank my roommate, Gene Yeo, for his wonderful friendship and advice. I also thank all the friends I have known outside the lab. They have been kind and helpful in providing other sources of entertainment outside MIT. Finally, I thank my family for their constant support and understanding.

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Chapter 1
Introduction and Background

1.1 Introduction

The objective of the Transdermal Drug Delivery Project in the BioInstrumentation Laboratory is to build a device that will delivery drugs through the human skin using micro needles [11]. These stainless steel needles are approximately 100 μm in outer diameter and approximately 60 μm in inner diameter. It is crucial to know the characteristics of micro-needle insertion into skin. It is also important to measure the maximum insertion force required for a micro-needle to puncture the skin. With the knowledge of a micro-needle's penetration force and behavior during an insertion, an appropriate injecting device can then be designed.

In view of this, instrumentation needs to be designed and fabricated so that micro-needle insertion into biological tissues can be characterized. The design and fabrication of the appropriate instrumentation will be described and discussed in Chapter 2 of this thesis.

There is also a need to investigate whether or not different insertion angles and velocities affect the required penetration force into skin. The experimental procedure will be described in Chapter 3. Results and discussion will be in Chapter 4. Lastly, conclusion and future work will be in Chapter 5.

1.2 Background

1.2.1 Anatomy of Human Skin

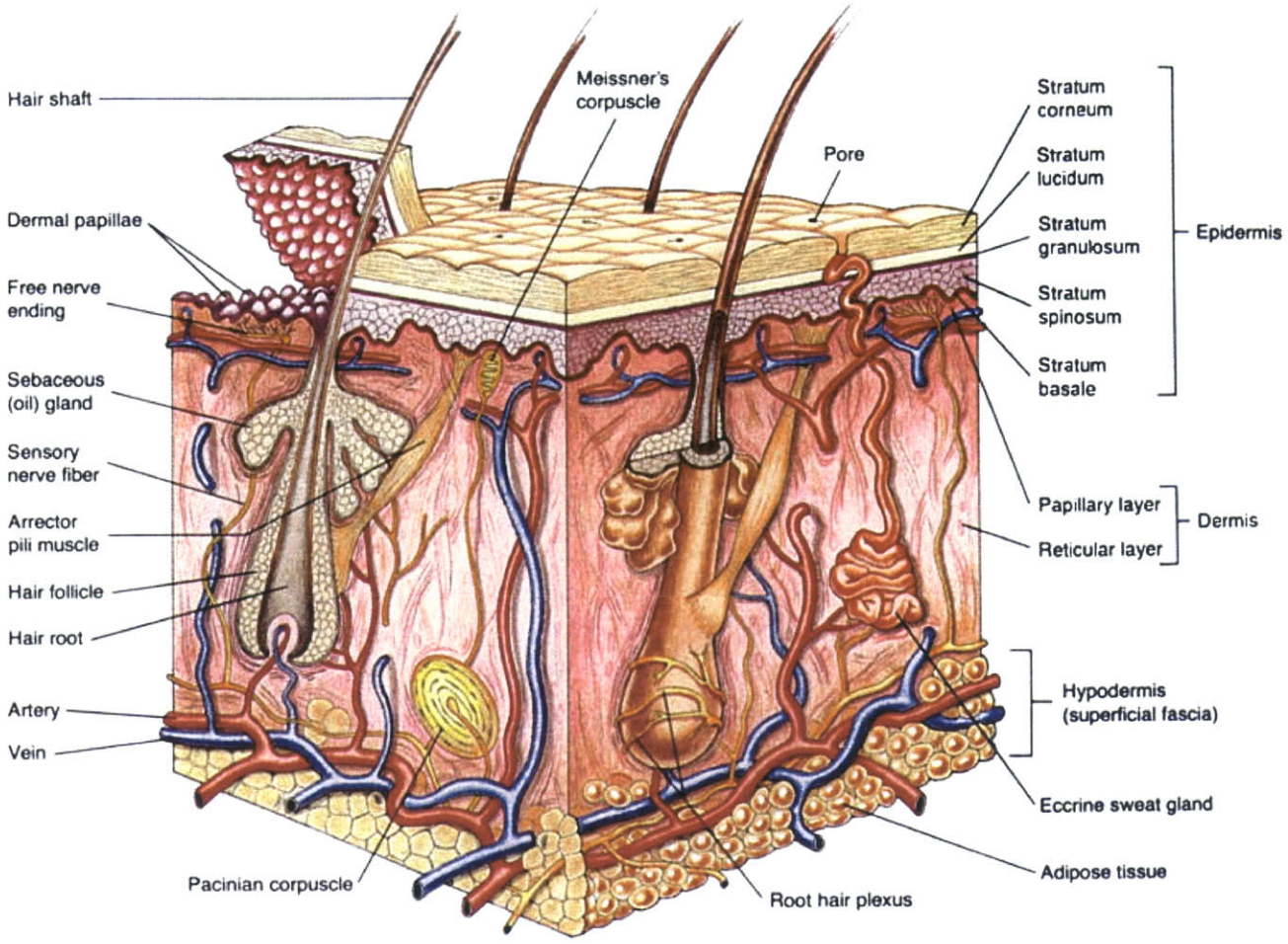

Figure 1-1. Structure of human skin (image taken from [6]).

It is important to understand the structure of the human skin prior to characterizing micro needle insertion into skin. Figure 1-1 shows the structure of the human skin.

The skin is made up of 2 main parts. The epidermis is the outer, thinner portion of the skin. The epidermis is connected to the inner, thicker part called the dermis [20]. The

thickness of epidermis varies from about 0.06 mm to 0.09 mm in the eyelid to about 0.5 mm to 0.8 mm, in the palm and sole [10].

The epidermis consists of 5 layers. From the deepest to the outer most layer, they are the stratum basale, stratum spinosum, stratum granulosum, stratum lucidum and stratum corneum. It is useful to know that the stratum corneum is a layer of about 30 rows of flat, dead cells. The stratum corneum of the skin of the forehead and cheeks averages about 0.02 to 0.04 mm in thickness, whereas on the palm and sole it is about 0.4 to 0.7 mm [10].

The dermis is made up of connective tissue containing collagen and elastic fibers. The combination of collagen and elastic fibers gives the skin its strength, extensibility (ability to stretch), and elasticity (the ability to return to original form after deformation) [20]. This knowledge is useful for characterizing the behavior of skin during a needle insertion.

1.2.2 Existing Instrumentation

858 Mini Bionix II testing system

The 858 Mini Bionix® II testing system (MTS Corporation, Eden Prarie, MN) is being used commercially to conduct biomechanical testing (Figure 1-2) such as needle insertion tests [9].

Figure 1-2. 858 Mini Bionix® II testing system (image taken from [9]).

The apparatus has force transducers of 15 kN and 25 kN capacity. The force range is too large for the kind of expected insertion force for the micro needles used in this project (50-300 mN). The device functions only on a single axis in the z-direction and therefore is limited in terms of degrees of freedom. The instrumentation is bulky, takes up a lot of space and is very costly. However, its flexibility and versatility make it easy to be modified to conduct different kinds of tests such as soft tissue strain measurement and spine loading experiments.

JHU Steady Hand Robot

The Steady Hand Robot designed in John Hopkins University (JHU) is used for microsurgical tasks such as needle insertions into organs [17].

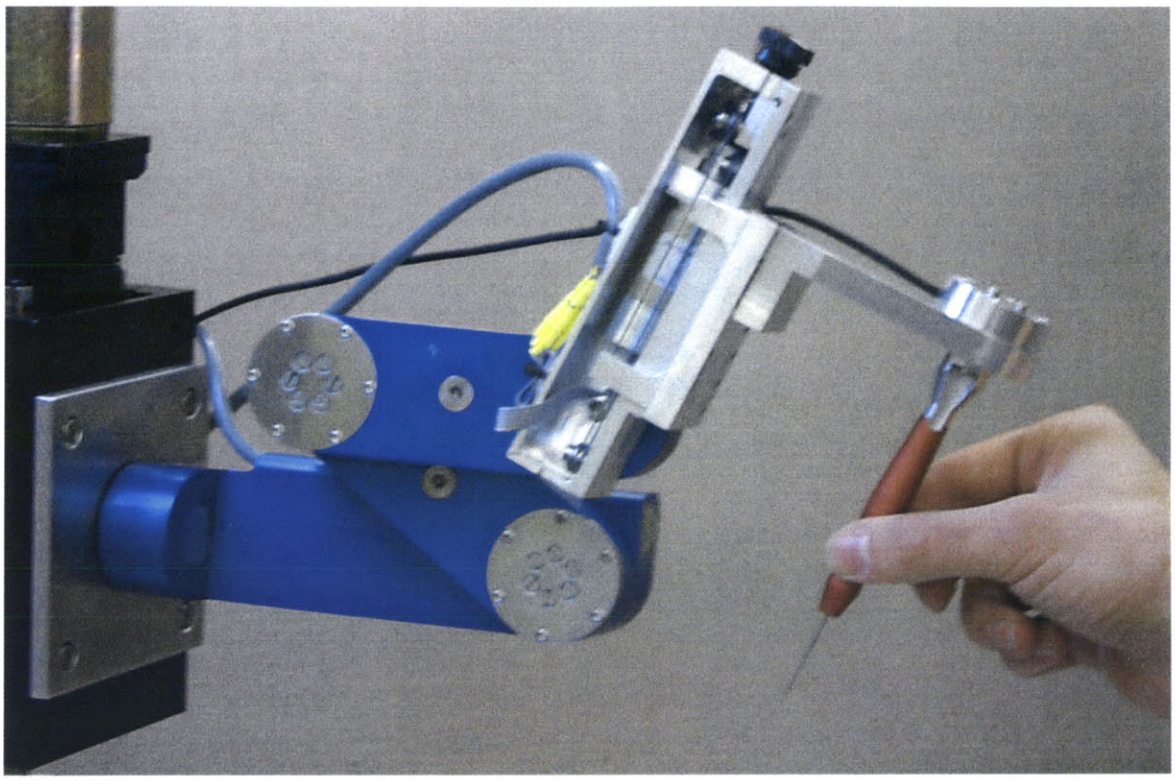

Figure 1-3. Steady hand robot from JHU (image taken from [17]).

This robot is designed for "steady hand" microsurgery so as to extend human ability to perform micromanipulation. It is being used in the Department of Mechanical Engineering at JHU to conduct needle insertion into organs for haptic modeling [19]. It is integrated with a needle holder and load cell to carry out needle insertion experiments. The load cell has a range of 10 N so it is appropriate for accurately measuring micro needle insertion force. The robot has 7 degrees-of-freedom, capable of performing needle insertion in various axes and directions. The disadvantage of this robot is that there is

human intervention during needle insertion experiments and hence velocity control is very limited.

Summary

In view of existing instrumentation, there is a need to design and build a highly versatile, and compact apparatus that is capable of performing precision micro needle insertion experiments, acquiring accurate results, having multi degrees of freedom, desired velocities control and having no human manipulation of the needle during insertion.

Chapter 2
Design and Fabrication of Instrumentation

To characterize needle insertion into biological tissue, such as skin, instrumentation with a high degree of precision and accuracy is needed. In this chapter, the design and fabrication of such a measurement system is discussed in detail. The design of this single semi-automated multi-modal instrument is divided into three main systems, namely the precision motion system, data acquisition and measurement system and the optical system. Figure 2-1 shows the assembled version of the multi-modal apparatus.

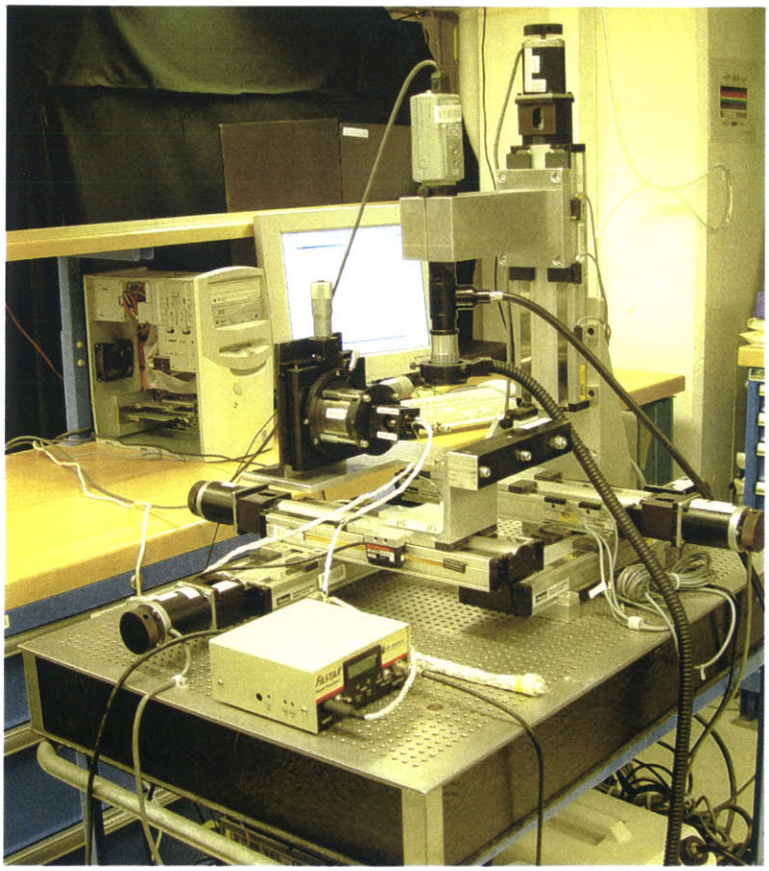

Figure 2-1. Multi-modal instrument.

2.1 Precision Motion System

In this section, details of the precision motion system of the instrumentation are discussed. The precision motion system consists of micro stepping linear stages, controllers, and stepper motors. The use of computer control for this system is presented as well.

2.1.1 Micro Stepping Linear Stages, Micro Stepping Motors and Controllers

The precision motion system is made up of five micro stepping linear stages (Parker Daedal Division Model no. 404150ZRMP, Hudson, NH) to provide five axes of motion: one Z-axis, two X-axes (X1 and X2 axis) and two Y-axes (Y1 and Y2 axis). The optical system is mounted on the Z-axis. Figure 2-2 shows a single linear stage. Each linear stage is attached to a micro stepper motor (Parker Compumotor ZETA57-83-MO, Hudson, NH) and provides a resolution of motion of 50 nm. One controller (Parker Compumotor ZETA 6104, Hudson, NH) is used to control each micro stepper motor. Figure 2-3 shows the ZETA 6104 controllers.

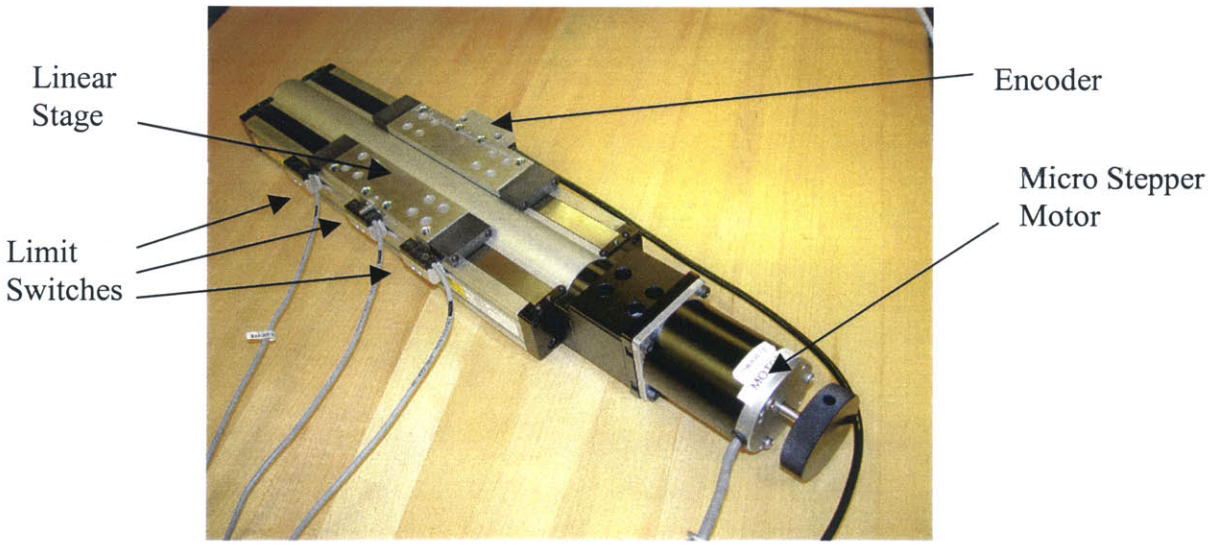

Figure 2-2. A single micro stepping linear stage with micro stepping motor.

Figure 2-3. ZETA 6104 controllers.

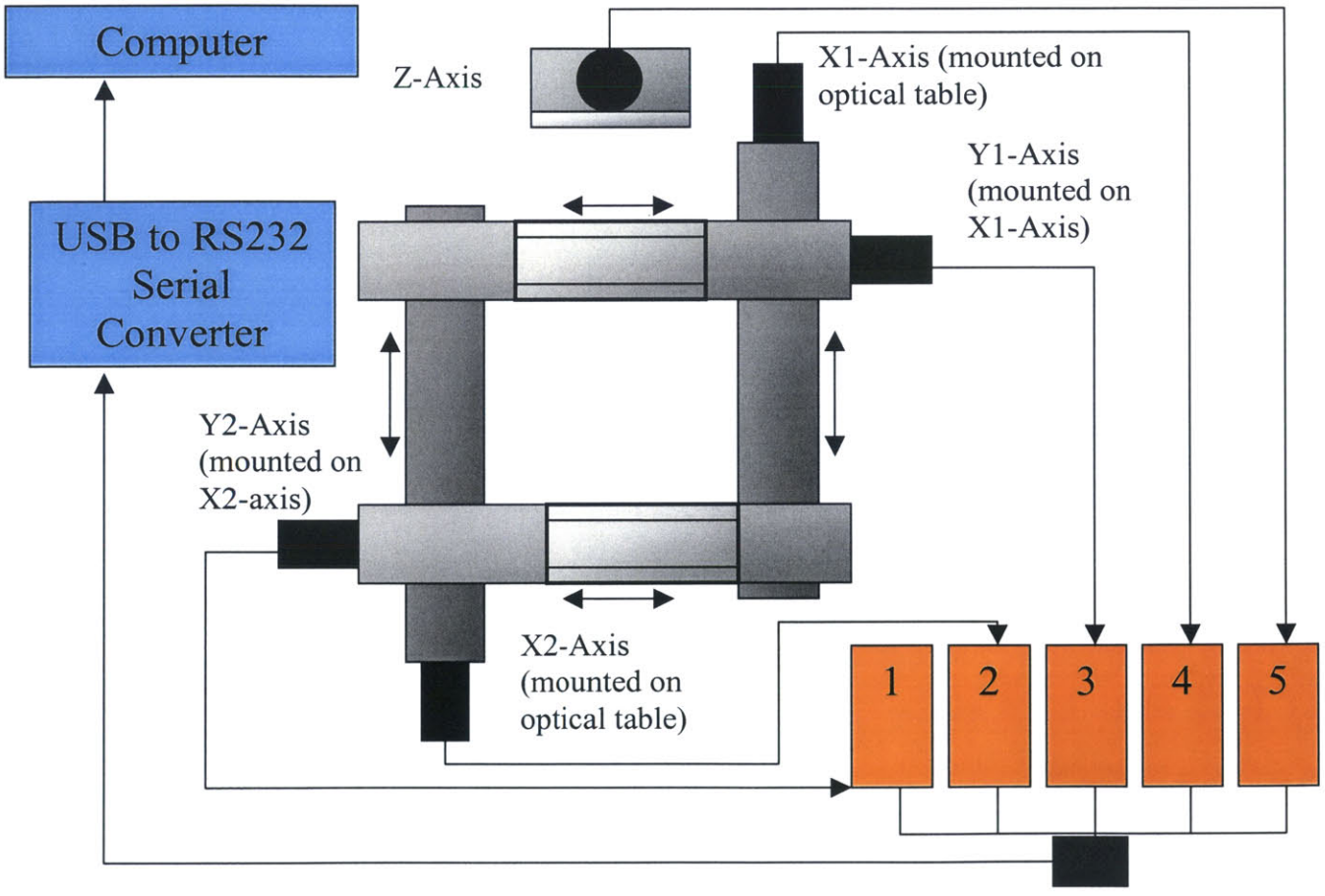

Figure 2-4. Integration of precision motion system.

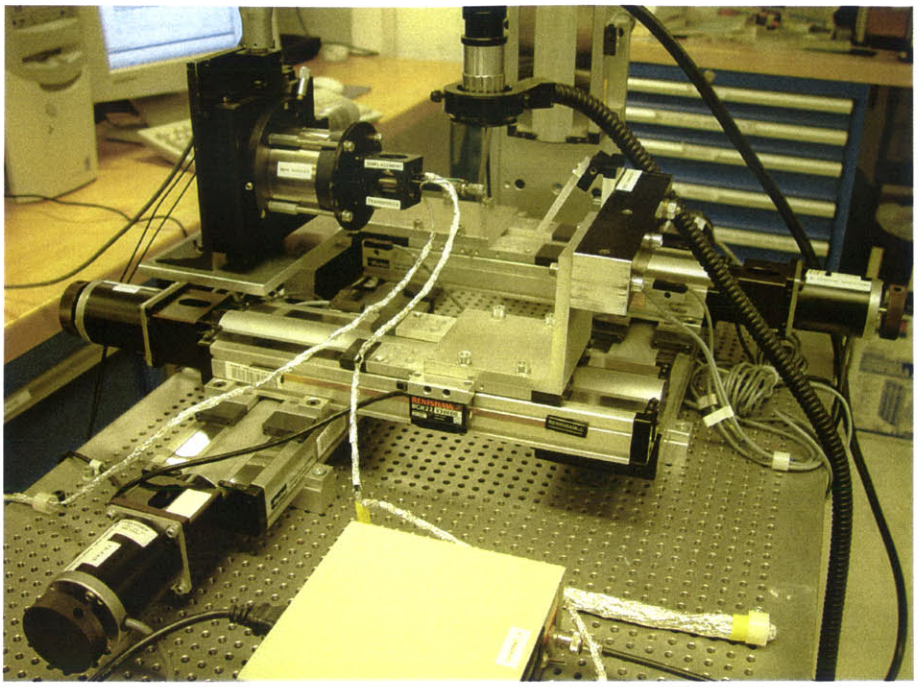

Figure 2-5. Actual precision motion system mounted on optical table.

Figure 2-4 shows the schematic layout of how the linear stages, micro stepper motors, controllers, and the computer are integrated. The computer communicates with the controllers via RS232 serial port connections. The actual precision motion system set up is shown in Figure 2-5. Each controller is connected to a single RS232 cable, which is in turn connected to an RS232-to-USB (Universal Serial Bus) connector (Edgeport, Austin, Texas) linked to the computer via USB [8].

The five controllers can communicate in another way with the computer by connecting a single RS232 cable to the first controller and linking the rest of the controllers using a Daisy-chain technique. Figure 2-6 shows the schematic of the Daisy-chain connections among the five controllers.

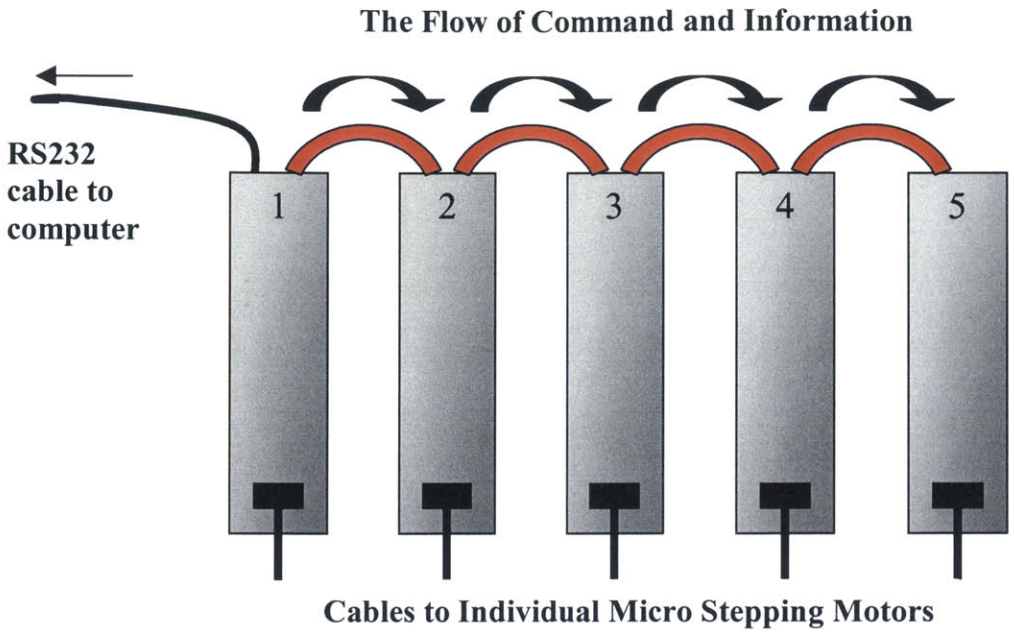

Figure 2-6. Daisy-chain connection schematic of the five controllers.

In this Daisy-chain connection, communication speed is slower because the information sent back and forth from the last controller has to pass through the first few controllers. For example, commands from the computer sent to controller number 5 have to pass through controller number 1, 2, 3 and 4 and vice versa. The Daisy-chain technique is undesirable because of the time delays during communication.

2.1.2 Encoders and Limit Switches

Three of the micro stepping linear stages (Z-axis, X2-axis and Y2-axis) are mounted with displacement encoders (Renishaw Model No.RGH22 X30F00) and limit switches. Figure 2-2 shows the encoders and limit switches mounted on the linear stage.

The displacement encoders are installed to determine the actual displacements made by the linear stages. The readings of these encoders are also used to ensure accurate analysis of needle insertions into biological tissue. The encoders are connected to the

same controllers used to control the micro stepping motors and the readings can be retrieved from the controllers. 1 mm of travel corresponds to 250 counts on the encoder reading:

$$Displacement = \frac{x}{250} mm, \tag{2.1}$$

where x is number of counts from the start point.

The limit switches are used to ensure the lead screws in the micro stepping motors are not damaged by jamming at the end of travel. The limit switches consist of: forward limit switch, home limit switch and backward limit switch. The limit switches are also connected to the controllers. Both the forward and backward switches (end-of-travel limit switches) are activated when a metal probe from the linear stage moves over the proximity sensors on the switches. Figure 2-7 shows the metal probe and a limit switch. Once the end-of –travel limit switches are activated, the controller will stop the linear stage from moving in the intended direction. However, the opposite direction of travel is valid. In this way, the lead screws in the micro stepping motors will not be damaged.

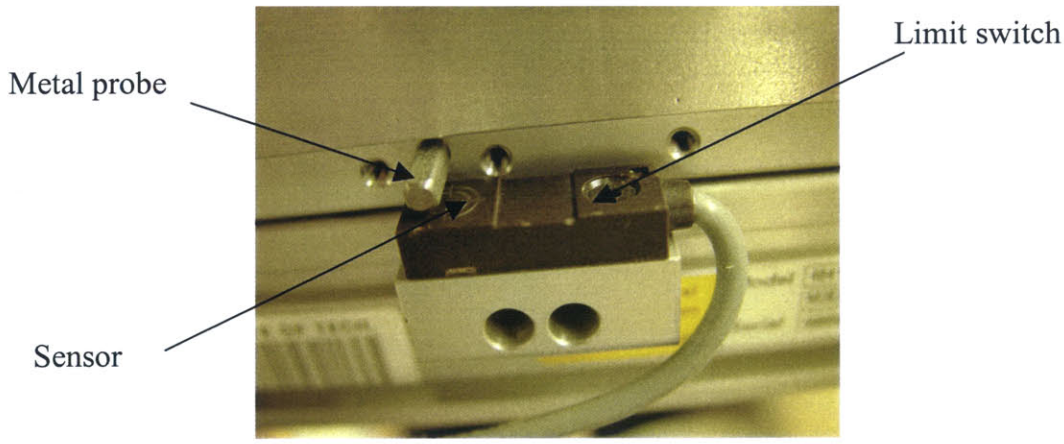

Figure 2-7. Limit switch and metal probe.

2.1.3 Computer Controlled Motion System

The precision motion system is computer controlled using the Visual Basic Program. Figure 2-8 shows the Graphics User Interface (GUI) of the program to control the linear stages. The same GUI is also used for the data acquisition and measurement system. The Microsoft Visual Basic 6.0 [13] code for the GUI is shown in the Appendix.

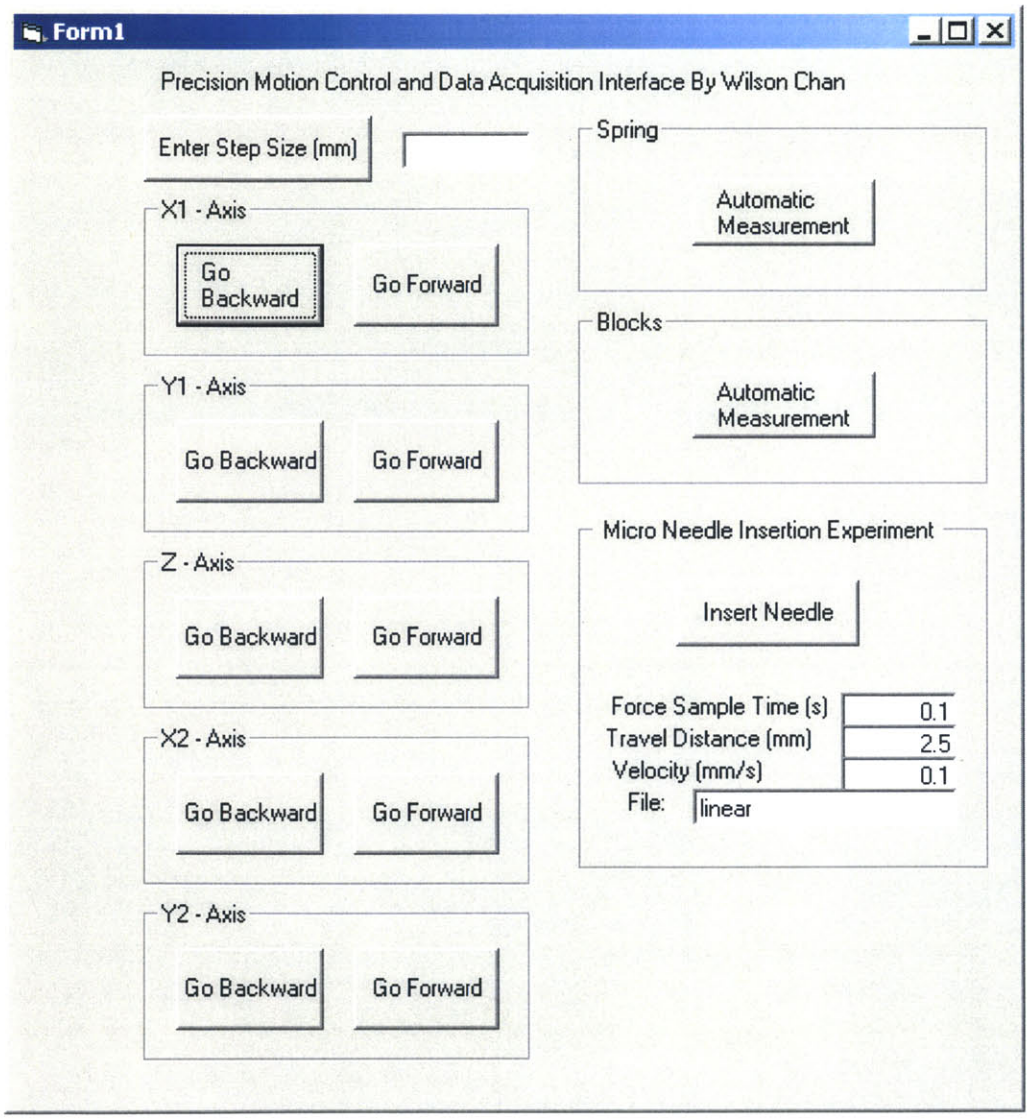

Figure 2-8. Graphics user interface used in precision motion control and data acquisition.

2.2 Data Acquisition and Measurement System

In this section, the details of the data acquisition and measurement are discussed. The integration of the data acquisition and measurement system with the precision motion system is also shown. The data acquisition and measurement system consists of force transducers, a displacement transducer, a voice-coil actuator, data acquisition unit, signal conditioning amplifier, power amplifier, function generator, low pass filters, and power supply.

2.2.1 Force Transducers

In this experiment, two types of force transducers, Entran and Omega load cells, are used to make needle insertion forces measurements. The Entran load cell (ELFS-T3M-10N) is shown in Figure 2-9. It can measure both tensile and compressive forces within the full-scale of 10 N (between –5 N and +5 N). It activates at an excitation voltage of 15 V DC and operates well at temperatures between –40 °C and 120 °C. It also has M5 thread shafts from both sides for easy mounting. It has been calibrated to 1 N/V.

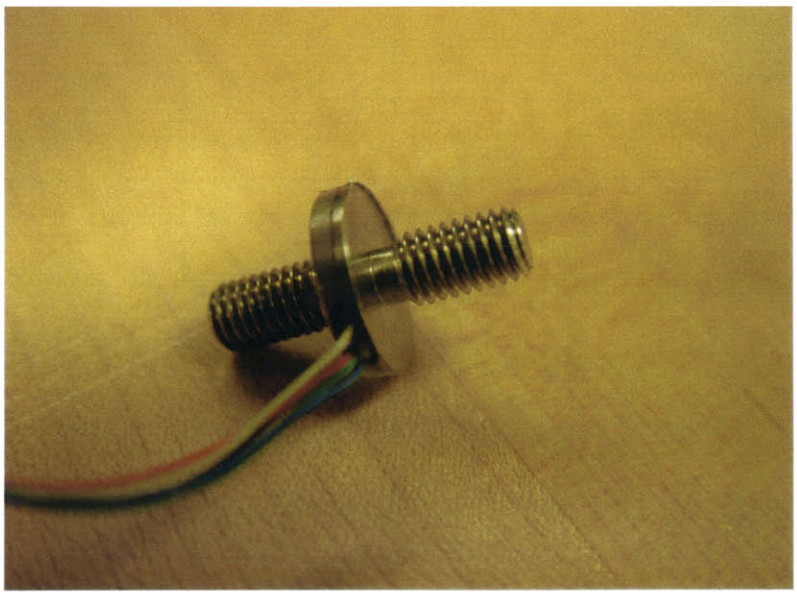

Figure 2-9. Entran ELFS-T3M-10N load cell.

The Omega load cell (Model LCCA-200) is shown in Figure 2-10. This is a more robust force transducer and has a full-scale range of 900 N (between –450 N to +450 N). Although the range is high, the Omega load cell is capable of accurately detecting very small forces (resolution of 0.1 mN) such as needle insertion forces. The excitation voltage is 15 V DC and operates well at a temperature between 0 °C to 53 °C. The Omega load cell is calibrated to 10 N/V.

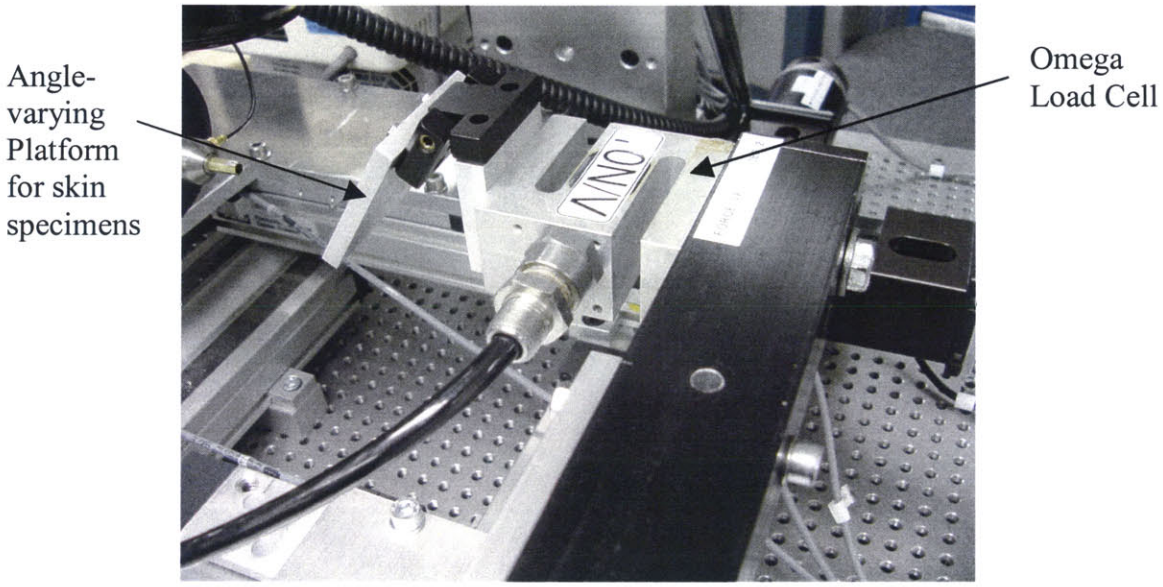

Figure 2-10. Omega model LCCA-200 load cell.

2.2.2 Voice-Coil Actuator

The Voice-Coil Actuator is also known as the Type 4810 mini-shaker from Brüel & Kjaer [2]. Figure 2-11 shows the mini-shaker. The mini-shaker is used for the dynamic excitation of lighter objects. It is of the electrodynamic type with a permanent field magnet. A coil, which is an integral part of the table structure, is flexibly suspended in one plane in the field of the permanent magnet. A sinusoidal current signal, provided by an external oscillator such as a function generator, is passed through the coil to produce a

vibratory motion at the table. This will be used to vibrate the needle in the axis of insertion for one of the experiments.

An object to be vibrated can be attached to the mini-shaker's table by means of a 10-32 UNF screw. The frequency range is DC to 18 kHz and maximum peak-to-peak displacement of table is 6 mm. The dynamic stiffness of flexures holding the table is 2 kN/m.

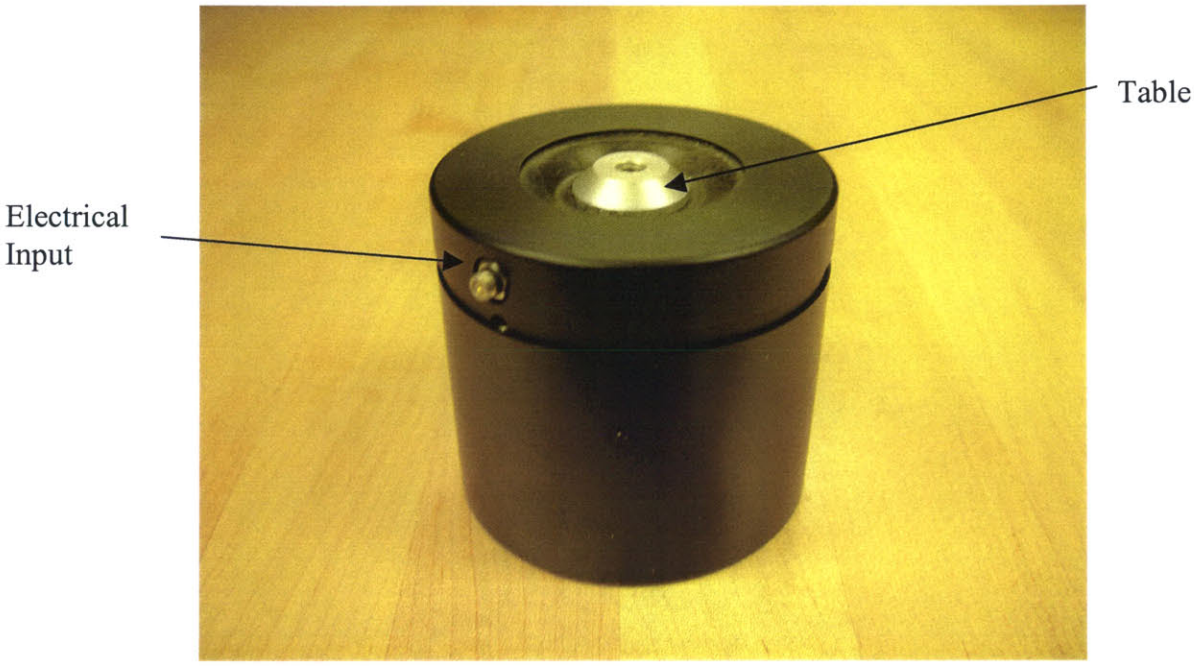

Figure 2-11. Brüel & Kjaer Type 4810 mini-shaker.

2.2.3 Displacement Transducer

The Fastar LD100-20 displacement transducer (from Omega) is shown in Figure 2-12. It is mounted on the table of the mini-shaker to determine the distance of the flexures being pushed back when a force is exerted during needle insertion. It is crucial to know this distance because the actual displacement reading from the encoder of the micro

stepping linear stage needs to be compensated with the distance recorded by the Fastar displacement transducer.

The Fastar displacement transducer operates and measures displacement with an aluminum core moving inside a polyimide encased coil. It is ideal for measuring oscillating linear displacement with frequencies as high as 15 kHz. It measures displacement up to 19 mm and has a resolution of 0.19 μm. It operates well at temperatures ranging from –50 °C to 125 °C.

The output of the displacement transducer is recorded in the high-speed signal conditioner (Fastar SP200A from Omega) shown in Figure 2-12.

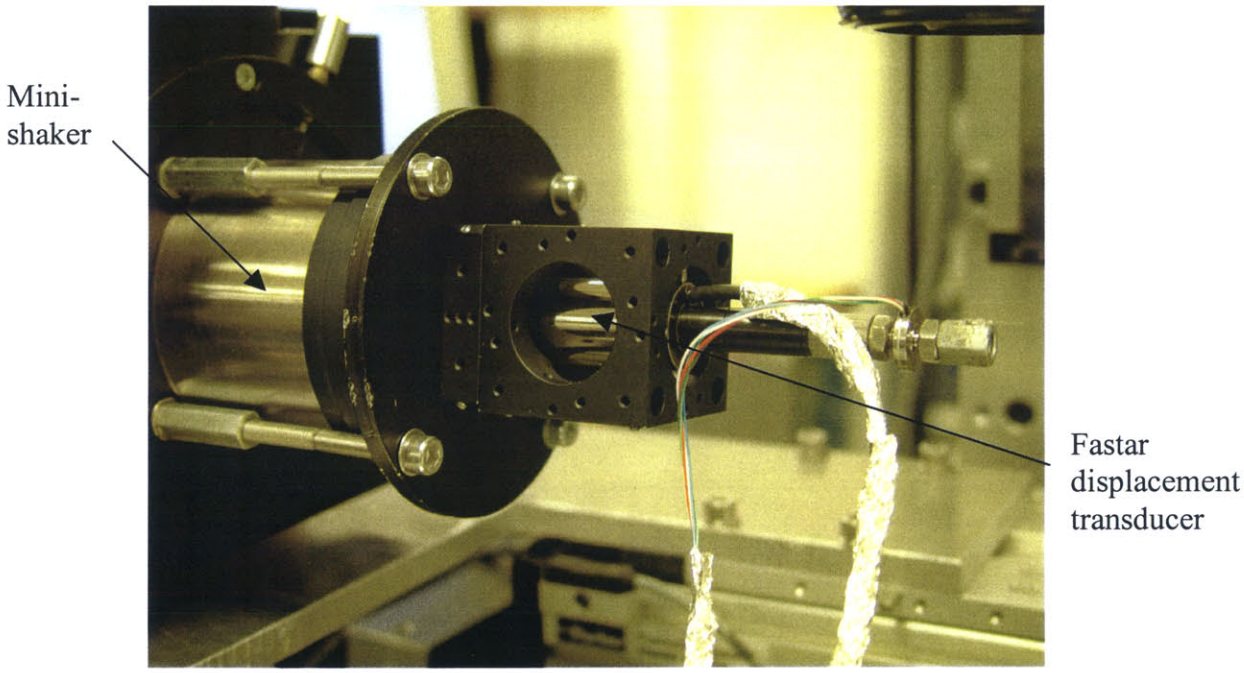

Figure 2-12. Fastar displacement transducer mounted on mini-shaker.

2.2.4 Data Acquisition

Outputs from the Entran and Omega load cells are fed into signal conditioning amplifiers (2300 System from Measurements Group, Inc.) [1]. These signal conditioning

amplifiers help calibrate the load cells to a desirable value. The Entran load cell is calibrated to 1 N/V while the Omega load cell is calibrated to 10 N/V.

The outputs of these signal conditioning amplifiers are in turn fed into the data acquisition unit (Agilent 34970A, Hewlett-Packard Company) through a 1 kHz first-order low pass filter to minimize noise. The readings from the Fastar displacement transducer are fed directly into the data acquisition unit. The data acquisition unit is set to scan and sample readings from these transducers during experiments. Readings from the output buffer will be retrieved using a Visual Basic program.

A function generator (HP 3314A, Hewlett-Packard Company) is used to supply a sinusoidal current signal into the mini-shaker to create oscillatory motion. A power supply (HP E3631A, Hewlett-Packard Company) and a power amplifier are used to power up the mini-shaker and the low pass filter.

Figure 2-13 shows the signal conditioning amplifiers, data acquisition unit, function generator, power supply, power amplifier and low pass filter.

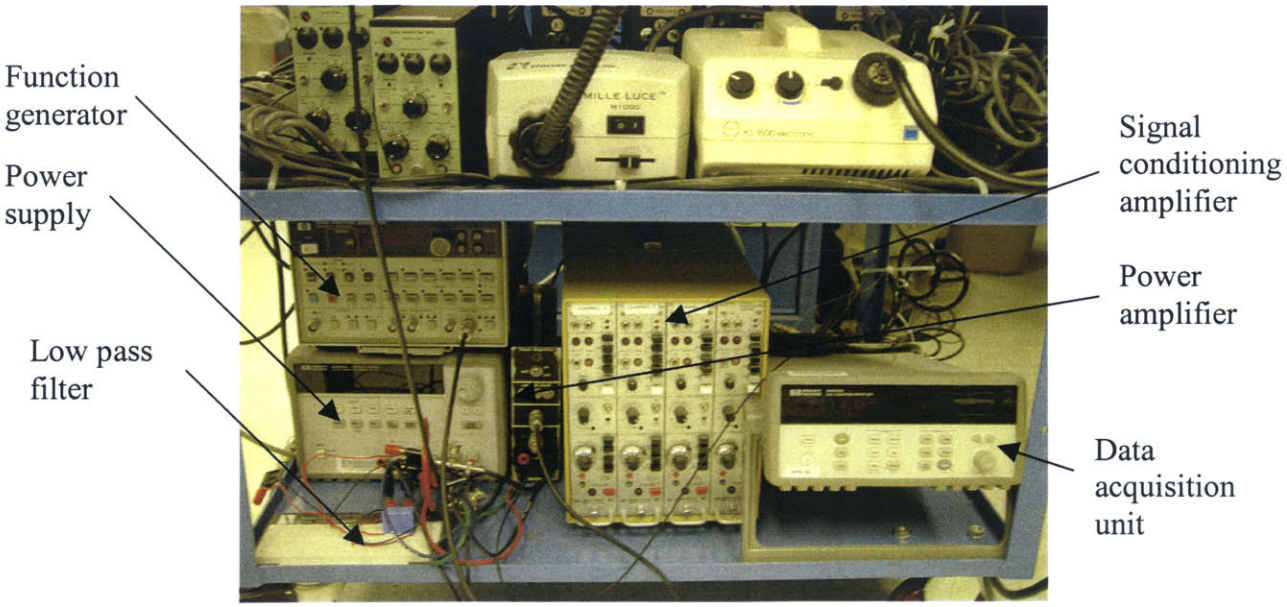

Figure 2-13. Data acquisition system.

Figure 2-14 shows the schematic of the Integration of Data Acquisition System.

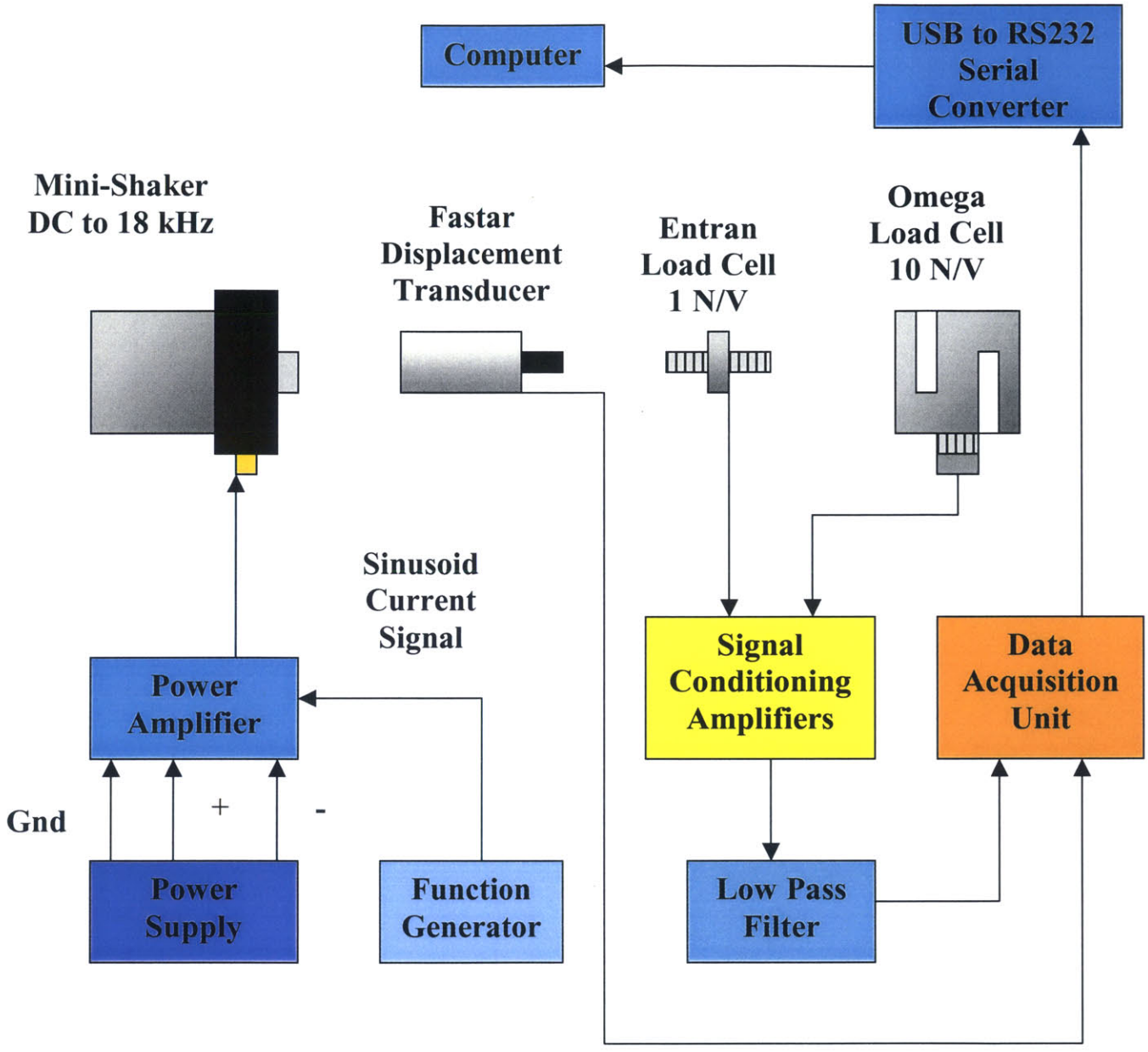

Figure 2-14. Schematic of data acquisition system.

2.2.5 Integration of Data Acquisition System with Precision Motion System

The micro testing instrumentation is a multi-modal one. It can be readily modified to conduct different experiments on needle insertions. Figure 2-15 shows how the mini-shaker, Fastar displacement transducer and the Entran load cell are integrated with the precision linear stages.

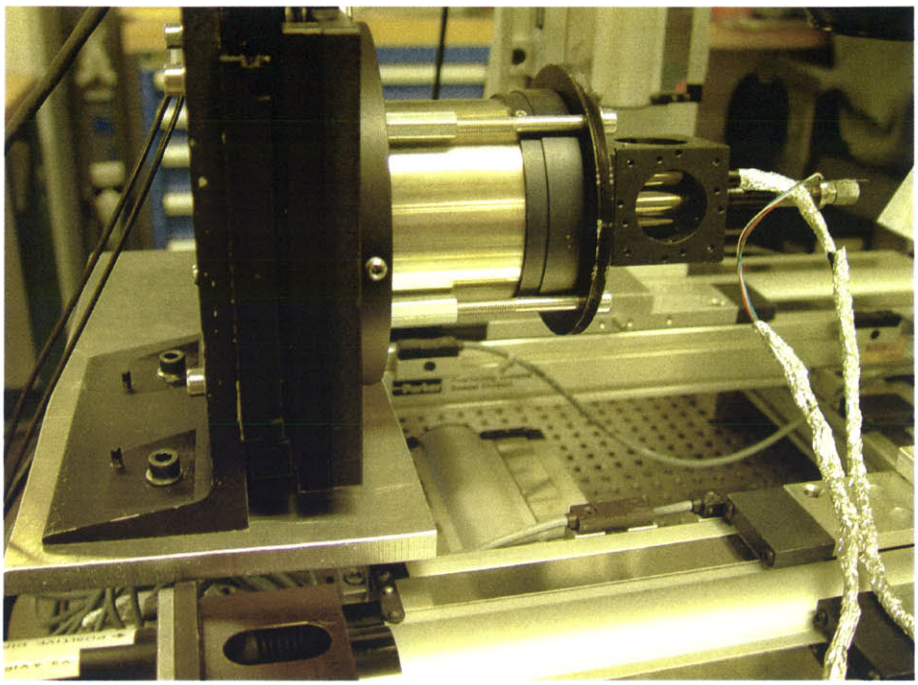

Figure 2-15. Integration of voice-coil, displacement transducer and load cell with linear stages.

The above module was used to conduct experiments on both the linear needle insertions and linear needle insertions with vibratory motion in the axis of insertion.

2.2.6 Simple Experiments to Test Instrumentation

Two simple experiments were conducted to test the capabilities of the multi-modal instrumentation before performing any needle insertion experiments. The first experiment involved compressing rubber blocks of different stiffness as shown in Figure 2-16 and obtaining the force versus deformation curves using the data acquisition system.

These blocks were compressed in 10 μm steps until a maximum force of 50 N, then returned to the uncompressed state. The Omega load cell measured the forces at every step. The results of this experiment are shown in Figure 2-17. Hysteresis occurs for all blocks [18].

The second experiment involved stretching a spring as shown in Figure 2-18. The force versus extension curves are shown in Figure 2-19. The spring was stretched in steps of 1 mm to a total extension of 10 mm. Results show a linear relationship between spring force and extension of spring with spring constant k of 1.27 kN/m, thus obeying Hooke's law.

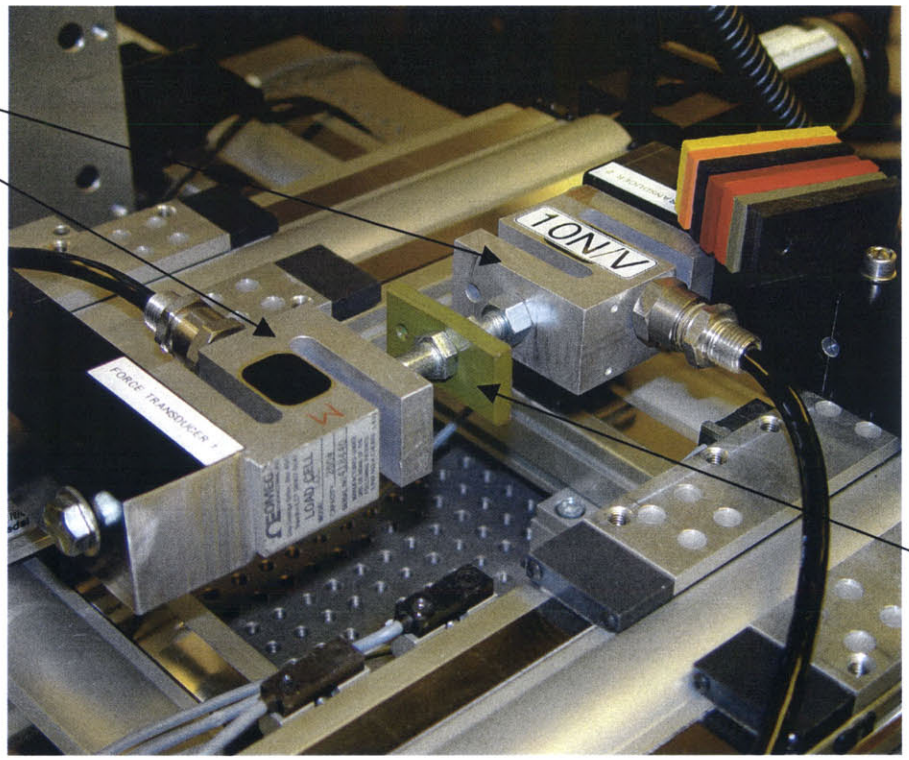

Figure 2-16. Rubber blocks compression experimental rig.

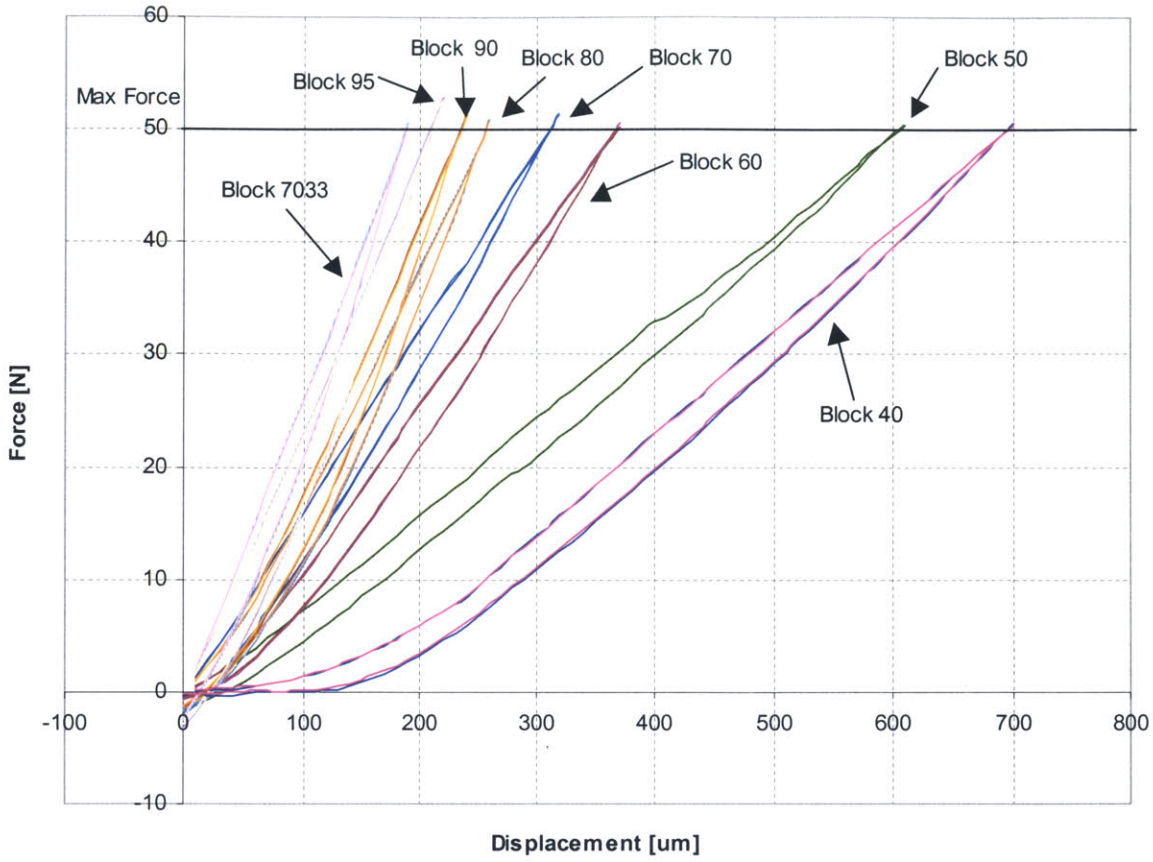

Figure 2-17. Force versus deformation results for rubber blocks (increasing numbers corresponds to increasing stiffness).

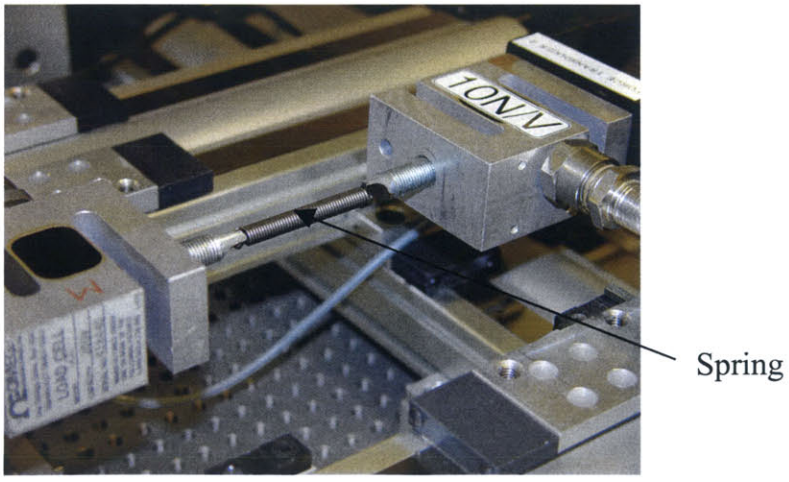

Figure 2-18. Spring extension experimental rig.

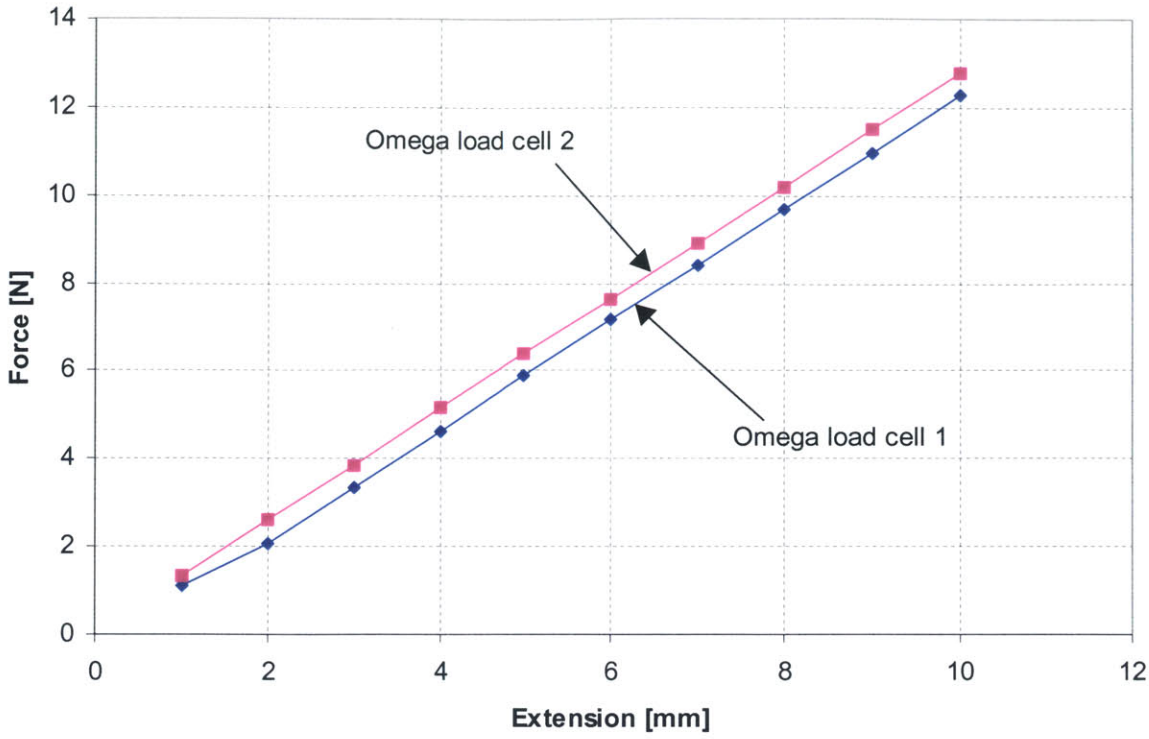

Figure 2-19. Force versus extension results for a spring (k = 1.27 kN/m).

2.3 Optical System

Part of the instrumentation includes an optical system. The optical system was integrated to image needle insertions into biological tissue. In this section, the details of the optical system are discussed. The optical system includes a CCD camera, microscope objectives, beam splitter, light sources, and imaging software.

2.3.1 Integration of the Optical System

The schematic of the optical system is shown in Figure 2-20. A CCD camera (1280 × 960 resolution Sony DFW-SX900) was used to capture images of needle insertions, using an IEEE 1394 Digital Interface [7] at a frame rate of 7.5 frames/s.

Mitutoyo [4] infinity-corrected microscope objectives (M Plan Apo series with magnification of 2X, 5X and 20X) were used to magnify the images of needle insertion.

The InfiniTube™ in-line assembly unit [3] allowed the Mitutoyo objectives to be coupled with the Sony CCD camera. It had an effective secondary lens system of focal length of 200 mm, providing 1X magnification onto the CCD image plane for the Mitutoyo objectives. It utilized an in-path beam splitter and a side port illumination tube to allow light from light sources to be directed to the Mitutoyo objectives and the Sony CCD camera.

Two light sources were used in the optical system. The Zeiss KL1500 electronic light source is used to provide illumination in the InfiniTube™ in-line assembly unit. The Mille Luce fiber optic illuminator M1000 light source is used to provide illumination for the ring guide around the Mitutoyo objectives. The ring guide supplied a radial illumination on the objects to be imaged and created 'shadow' effects to produce distinct features.

Microsoft Direct X8A [5] provided a video capturing and imaging software to record needle insertion videos and images captured by the Sony CCD camera.

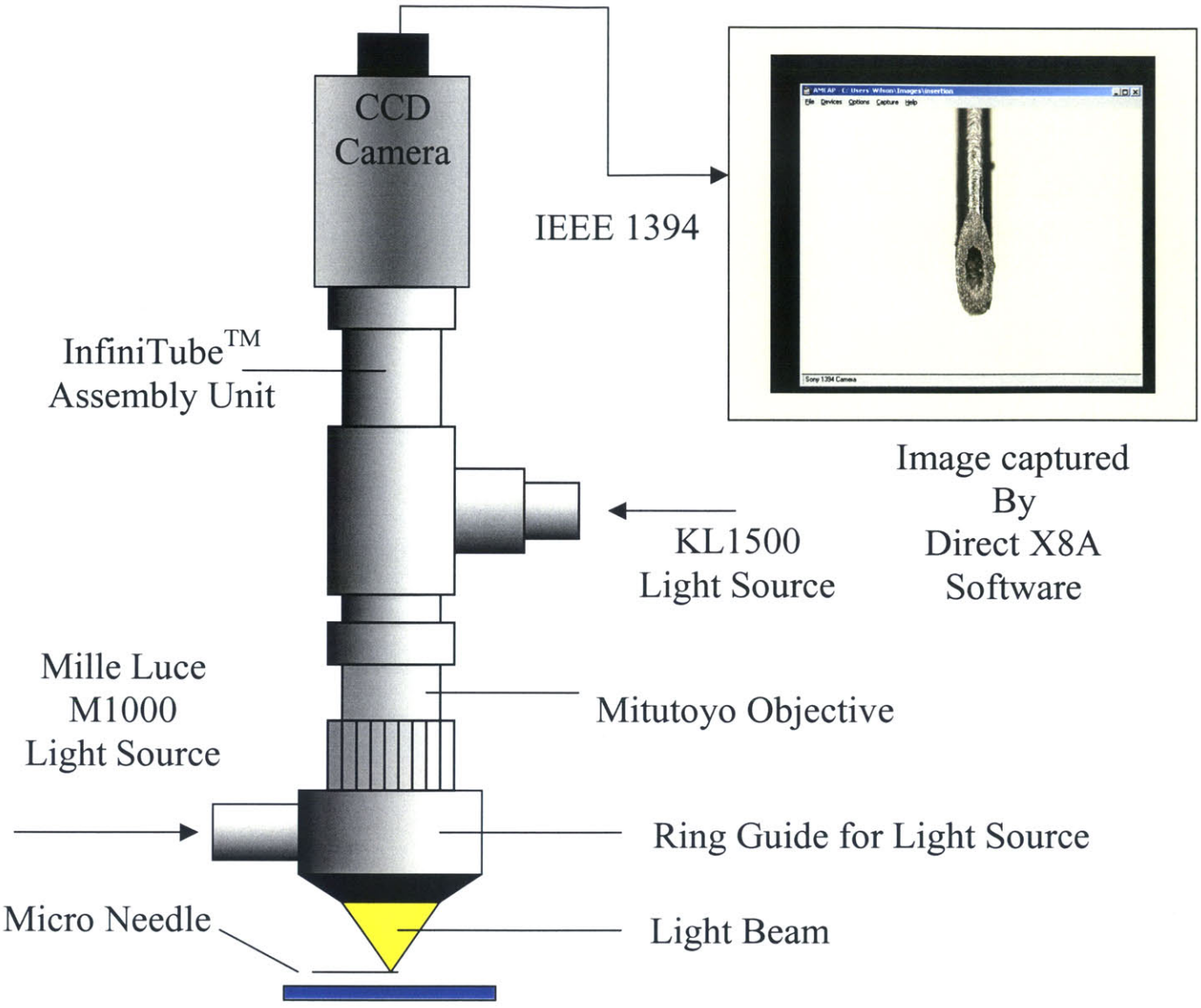

Figure 2-20. Schematic of optical system. The image on the right is of a stainless steel needle with 100 μm OD and 60 μm ID.

Figure 2-21 shows the optical system mounted on the Z-axis of the precision motion system. Figure 2-22 shows an image of a micro needle.

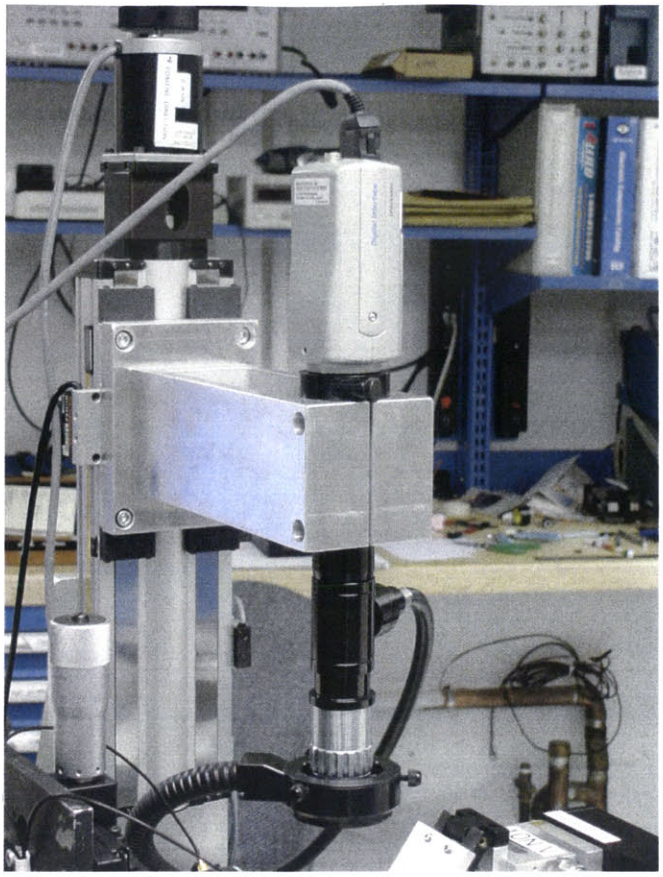

Figure 2-21. Optical system mounted on Z-axis of precision motion system.

Figure 2-22. Image of micro needle taken by optical system.

Chapter 3
Experimental Procedure

The objective of the instrumentation was to characterize micro needle insertions into biological tissue. The relationship of insertion forces and insertion displacements of micro needles will be investigated. In this chapter, the procedure for this experiment is described.

3.1 Specimen

The biological tissue used for the needle insertion experiment is skin from the shoulder of a pig. It is selected based on its similar characteristics to human skin and is readily available in supermarkets.

3.2 Experiment to Characterize Micro Needle Insertions

In this experiment, there were 2 methods of insertion: 1) the linear insertion and 2) the linear insertion with oscillatory motion (frequency of 5 kHz and amplitude of 20 mV) in the axis of insertion. This was done to investigate whether the oscillatory motion has a significant effect on lowering insertion forces or ease of puncture into skin. Illustrations of methods of insertion are shown in Figure 3-1 and Figure 3-2.

Intuitively, higher insertion speeds should generate higher insertion forces. Therefore, in this experiment, two different speeds of linear insertion: 1) 0.1 mm/s and 2) 1 mm/s were used to investigate the effect of insertion velocity on insertion force.

Investigation of whether a smaller angle of insertion would generate a lower insertion force was also done. There were two different angles of insertion into the skin: 1) 90° and 2) 15° in this experiment (angles measured from the surface of the skin to the shaft of the needle).

Similar experiments were conducted on a 24-Gauge (OD = 570 μm) surgical needle to compare results with the micro needle.

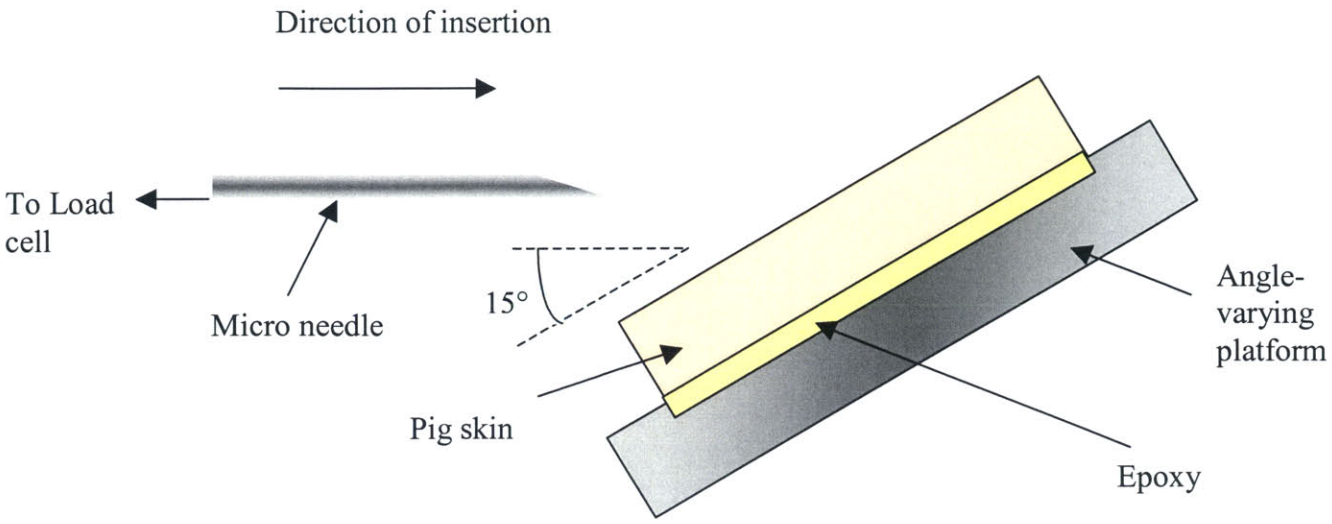

Figure 3-1. Linear insertion method with insertion angle of 15°.

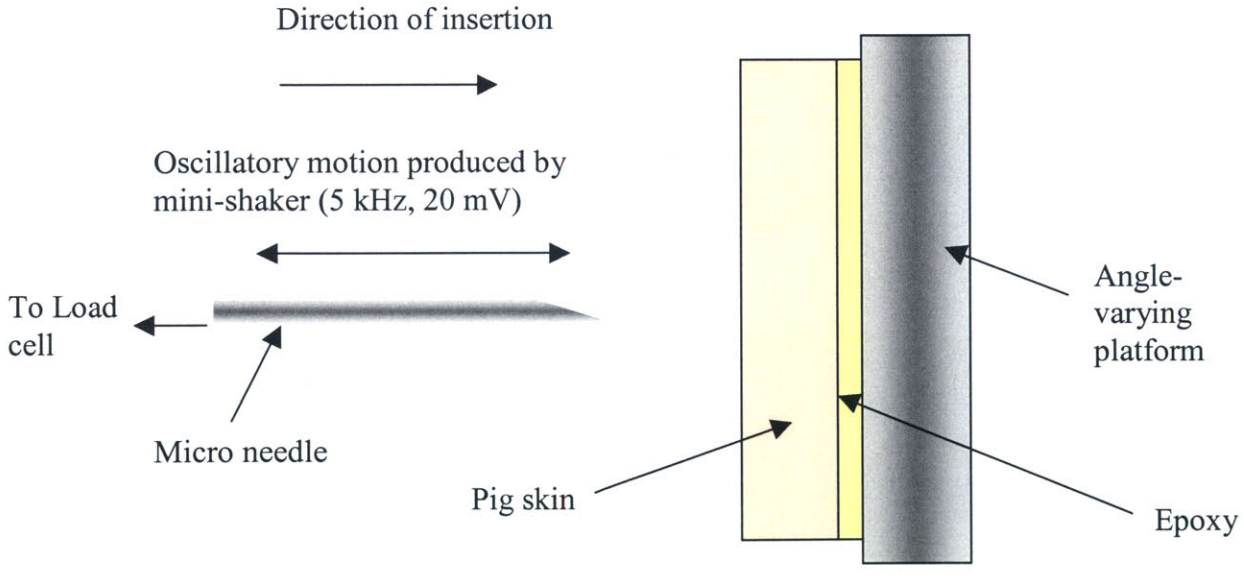

Figure 3-2. Linear insertion with oscillatory motion with insertion angle of 90°.

3.3 Experimental Procedure

A piece of 40 mm×40 mm pig skin was cut using a surgical scalpel. The skin was attached with epoxy onto a platform mounted on the instrumentation, which allows the user to vary the angle of insertion.

The micro needle was soldered onto a M5 stainless steel nut. The nut was then screwed directly onto the Entran load cell so that the micro needle is parallel to the axis of insertion. The 24-Gauge needle was threaded with M5 threads, and was screwed directly onto the Entran load cell.

At the beginning of each test, each needle was located approximately 0.5 mm away from the skin. During each experiment, each needle was advanced to a total displacement of 2.5 mm, penetrating the skin to an approximate depth of 2.0 mm. The needle was held in the punctured skin for about 5 seconds, and then pulled out from the skin to its original position. Insertion force readings were registered by the load cell during each test and recorded by the computer via the data acquisition unit. Insertion force data were collected corresponding the displacement readings from the encoders from the linear stages. A plot of insertion force against displacement could be obtained for each needle insertion test.

Figure 3-3 and Figure 3-4 show the micro needle inserting into pig skin at angles of 90° and 15°, respectively. Figure 3-5 and Figure 3-6 show the 24-Gauge surgical needle inserting into pig skin at angles of 90° and 15°, respectively.

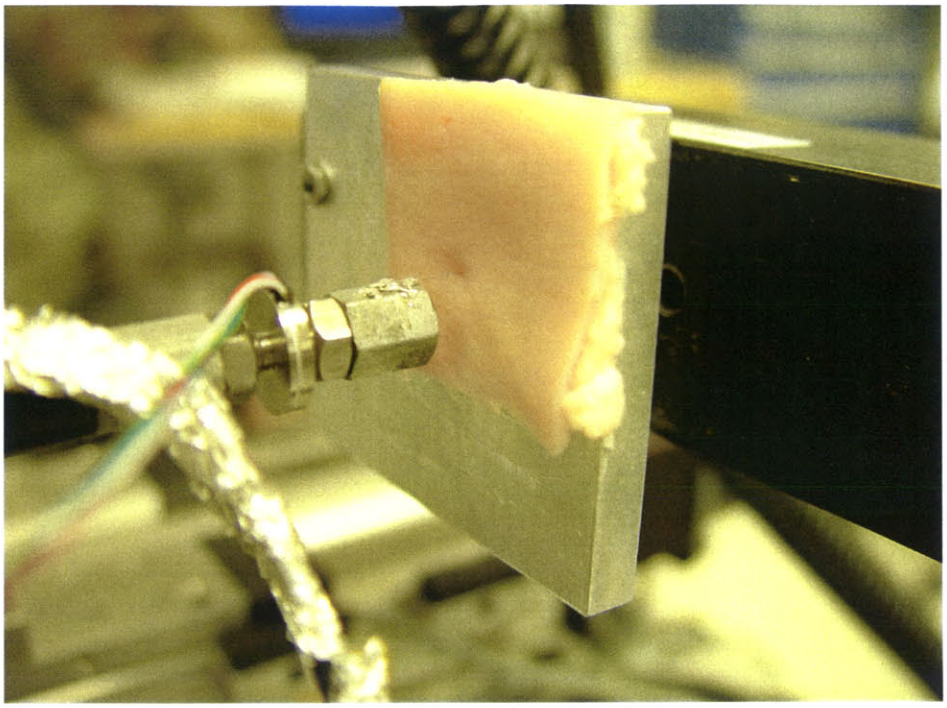

Figure 3-3. Micro needle inserting into pig skin at 90°.

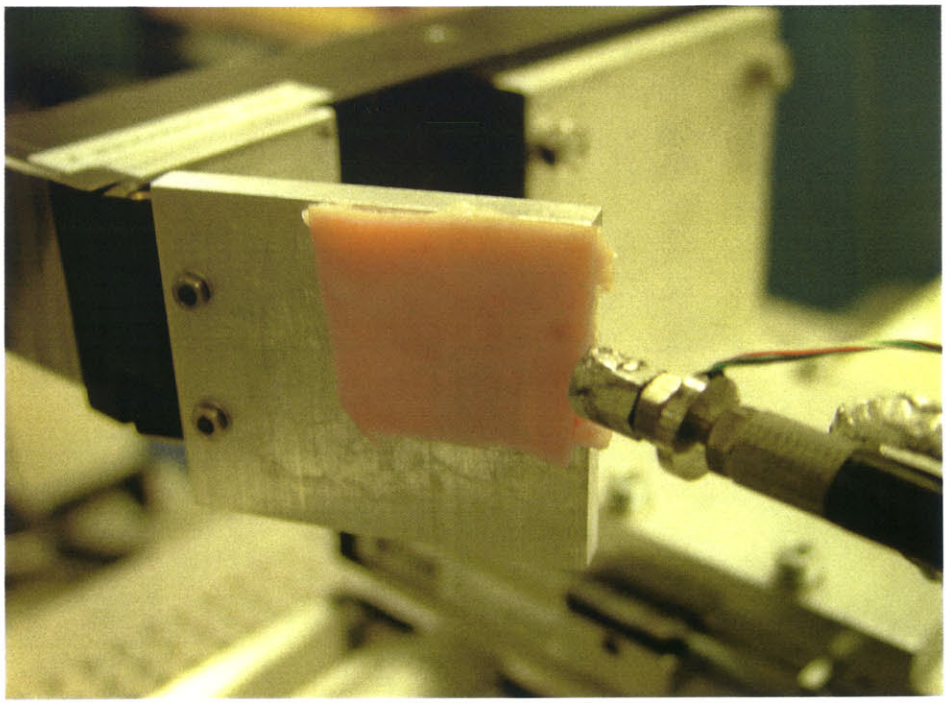

Figure 3-4. Micro needle inserting into pig skin at 15°.

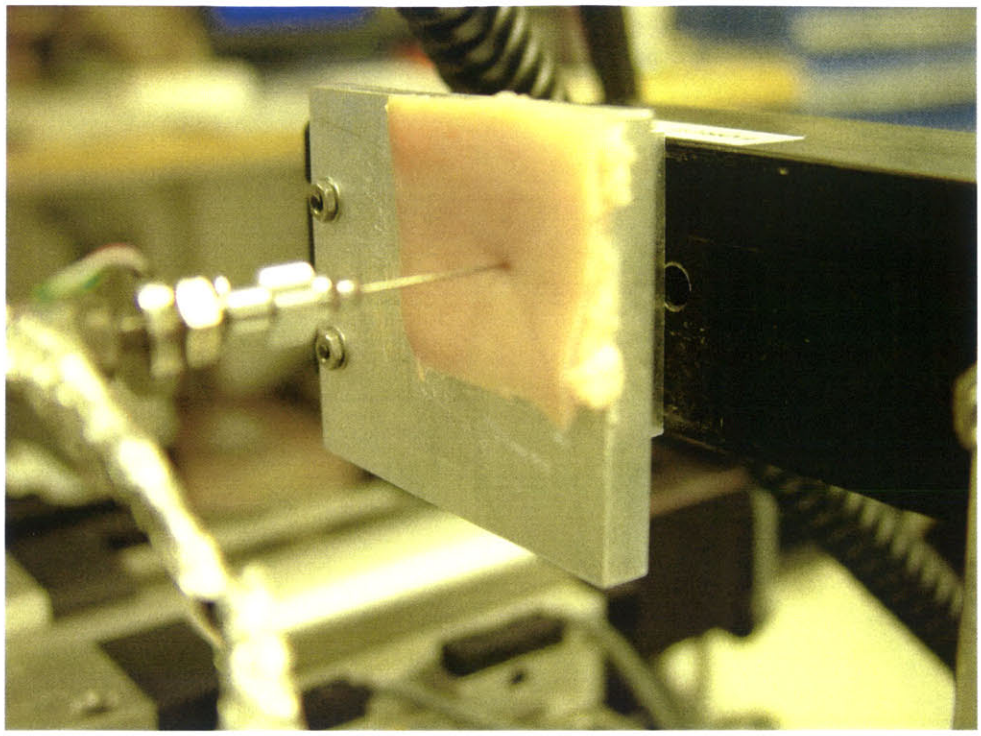

Figure 3-5. 24-Gauge surgical needle inserting into pig skin at 90°.

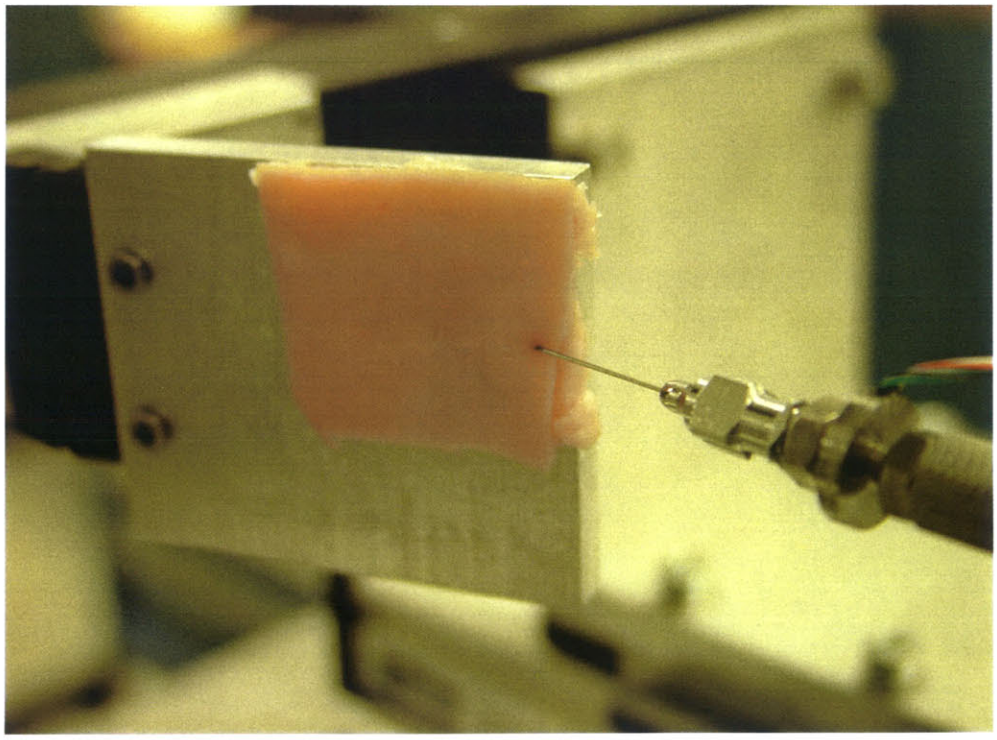

Figure 3-6. 24-Gauge surgical needle inserting into pig skin at 15°.

There were a total of 16 tests in each experiment. Table 3-1 shows the 16 different tests in each experiment. Tests were conducted in the order listed.

Table 3-1. 16 different tests in each experiment.

No.	Method of Insertion	Angle	Speed	Needle type
1	Linear	15 degrees	0.1 mm/s	100 μm
2	Linear	15 degrees	1 mm/s	100 μm
3	Linear	15 degrees	0.1mm/s	570 μm
4	Linear	15 degrees	1 mm/s	570 μm
5	Linear	90 degrees	0.1mm/s	100 μm
6	Linear	90 degrees	1 mm/s	100 μm
7	Linear	90 degrees	0.1 mm/s	570 μm
8	Linear	90 degrees	1 mm/s	570 μm
9	Linear with Oscillatory Motion	15 degrees	0.1 mm/s	100 μm
10	Linear with Oscillatory Motion	15 degrees	1 mm/s	100 μm
11	Linear with Oscillatory Motion	15 degrees	0.1mm/s	570 μm
12	Linear with Oscillatory Motion	15 degrees	1 mm/s	570 μm
13	Linear with Oscillatory Motion	90 degrees	0.1mm/s	100 μm
14	Linear with Oscillatory Motion	90 degrees	1 mm/s	100 μm
15	Linear with Oscillatory Motion	90 degrees	0.1 mm/s	570 μm
16	Linear with Oscillatory Motion	90 degrees	1 mm/s	570 μm

Each set of experiments is conducted on the same piece of specimen pig skin to avoid skin variation. The tests in each experiment were conducted in a rapid succession in order to obtain consistent results, since the texture of the pig skin changes after prolonged exposure to the air. Each experiment took about 30 minutes to conduct. Ten sets of experiments were performed to verify repeatability of this experiment. Each experiment uses a different pig skin sample.

Chapter 4
Results and Discussion

In this chapter, the various needle insertion force results are presented and discussed. The micro needle insertion is also characterized using existing models.

4.1 Results and Discussion

Ten experiments were conducted and data were collected. A typical set of needle insertion force results are shown in Figure 4-1 and Figure 4-2. All experiments demonstrate a general trend and therefore verify the repeatability of the experiments.

The results show that the micro needle requires lower insertion force (peak force from plots) than the 24-Gauge (570 μm) surgical needle. Since the 24-Gauge needle is larger in diameter, it makes sense that more force would be required to push the larger needle into skin.

Results also show that the insertion force of a micro needle into skin at 15° angle of insertion is lower than that of a 90° one.

A higher speed of needle insertion does not appear to have a significant effect on insertion force. There is no significant difference in the shape of the plots. The mechanical behavior of the skin is not affected by higher insertion speed.

The results also show that the method of linear insertion with oscillatory motion (frequency of 5 kHz and amplitude of 20 mV) has no significant effect on lowering insertion forces into skin.

The following pages show the plots and an analysis of the results follows.

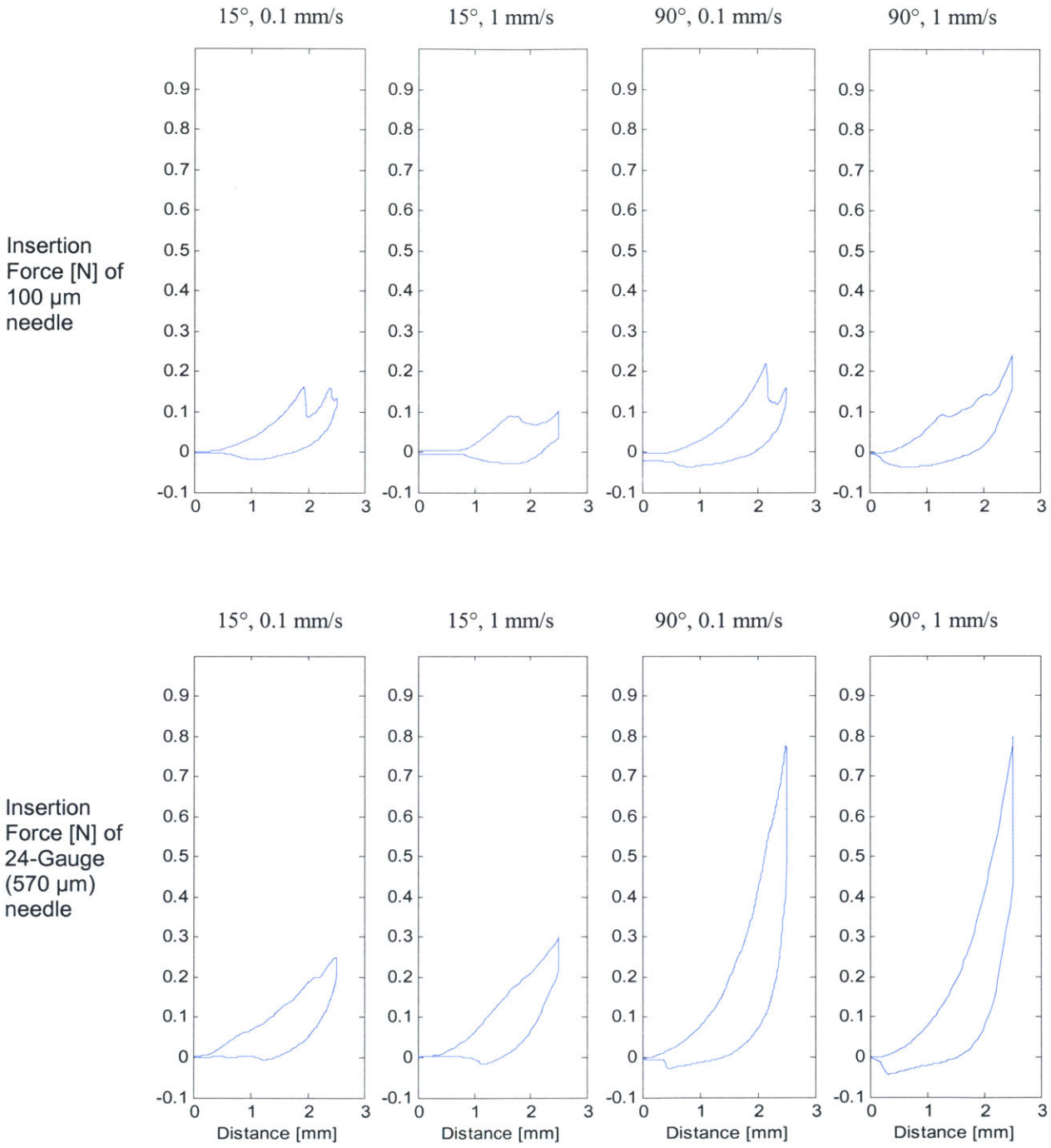

Figure 4-1. Insertion force versus displacement results using linear insertion method.

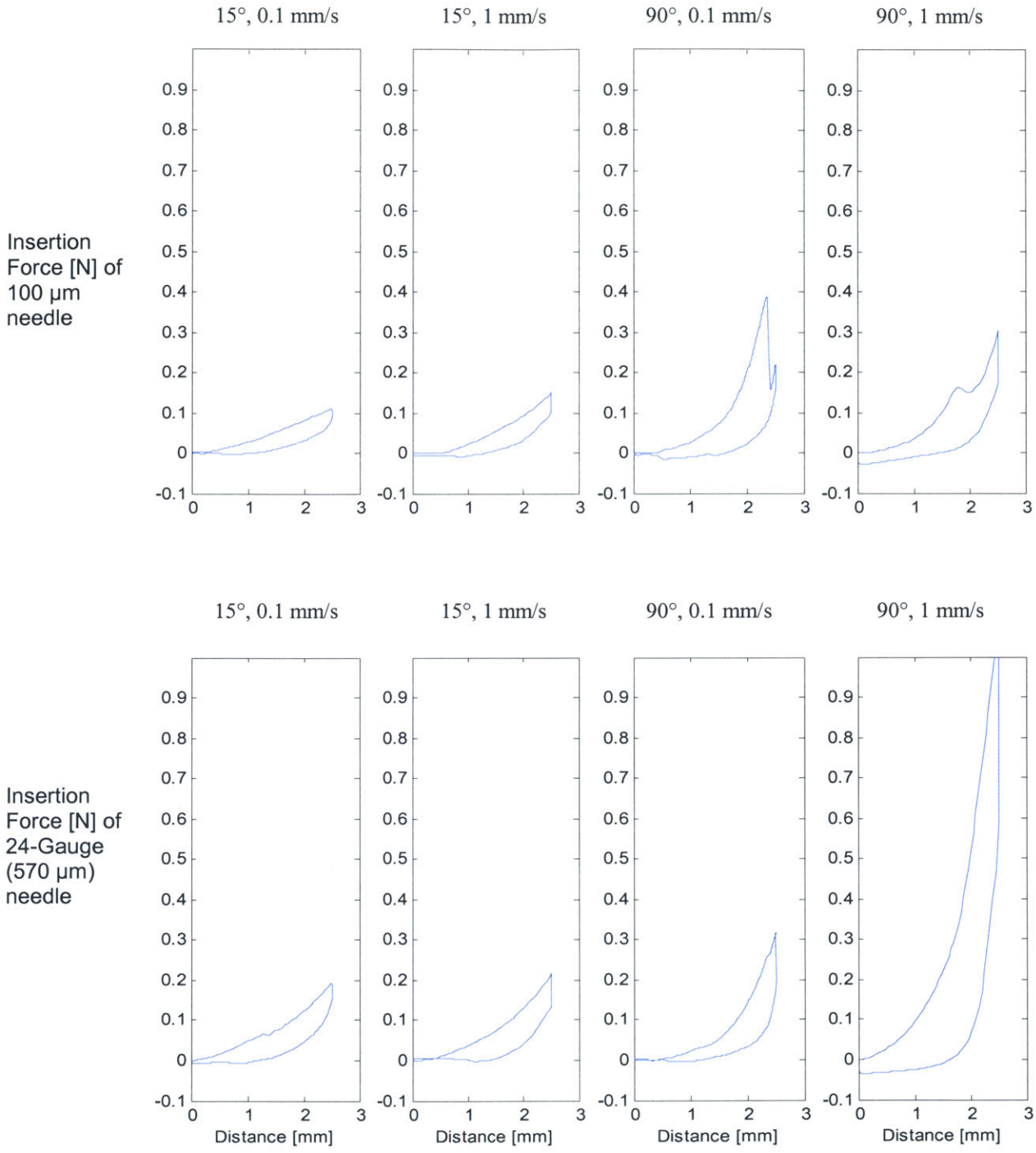

Figure 4-2. Insertion force versus displacement results using linear insertion with oscillatory motion method.

4.2 Characterization of Micro Needle Insertion into Skin

In this section, a detailed data analysis of one of the needle insertion tests is made. Data are also fitted to an existing physical model.

4.2.1 Data Analysis

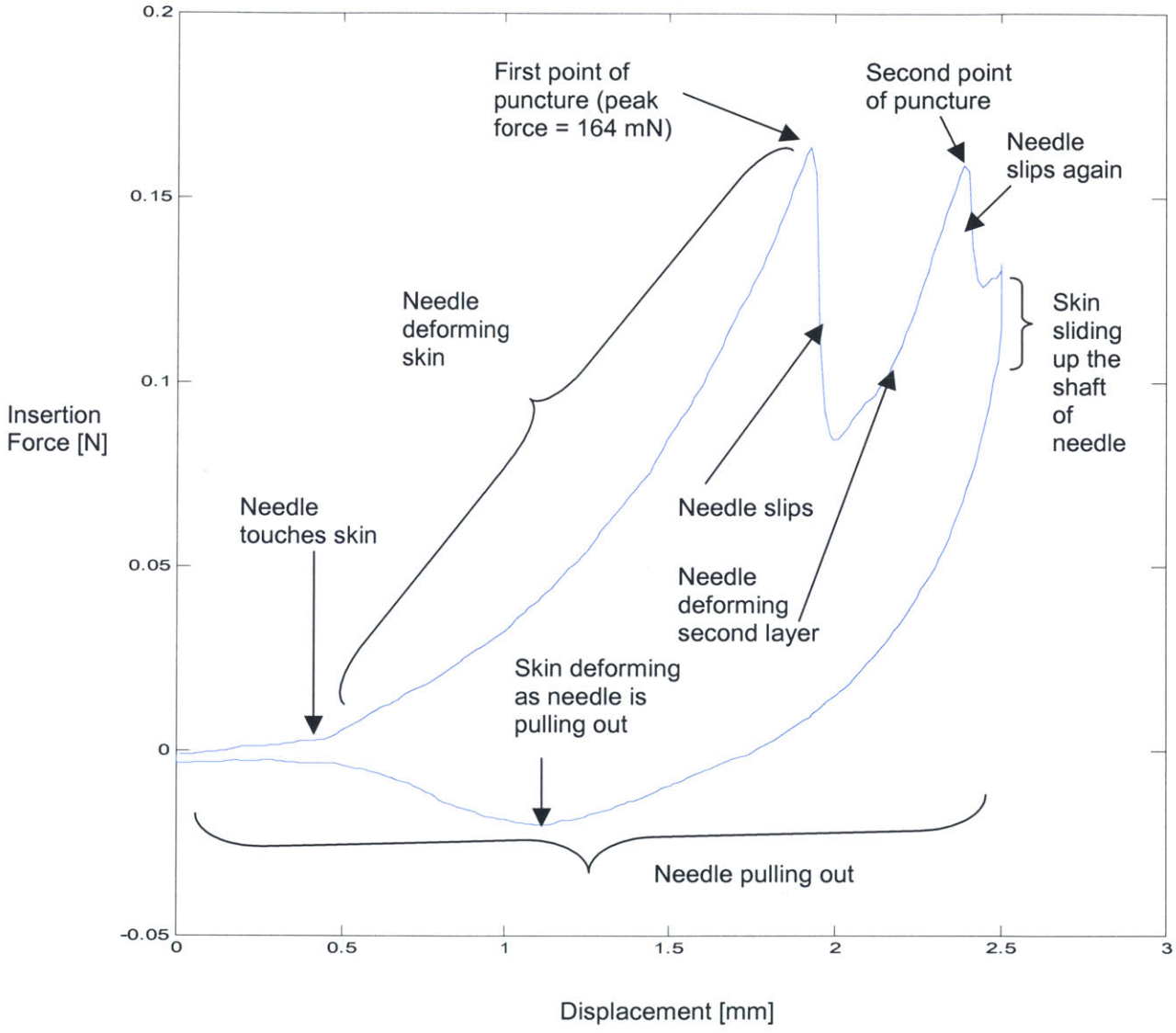

Figure 4-3. Typical insertion force versus displacement curve for linear insertion of micro needle into skin for insertion angle of 15° and insertion speed of 0.1 mm/s.

A typical insertion versus displacement plot is shown in Figure 4-3. Figure 4-4 illustrates how the skin behaves when a micro needle penetrates it. As the micro needle approaches the skin, it touches the tissue and deforms the elastic portion (due to collagen and elastic fibers of dermis) of the skin (see Figure 4-4(A)). The initial nonlinear portion of Figure 4-3 shows the deformation of skin. The skin deforms until a point where the needle first punctures the skin. This is shown at the first peak (point of puncture) in the Figure 4-3. After the needle punctures the first layer (epidermis), it slips, and this is evident in the sudden drop in insertion force after the first peak. As the needle slips, it comes in contact with an internal layer (possibly the dermis) and continues to deform the internal layer (see Figure 4-4(B)). The deformation continues until the needle punctures the internal layer of skin. This is shown in the sudden drop of insertion force after the second point of puncture.

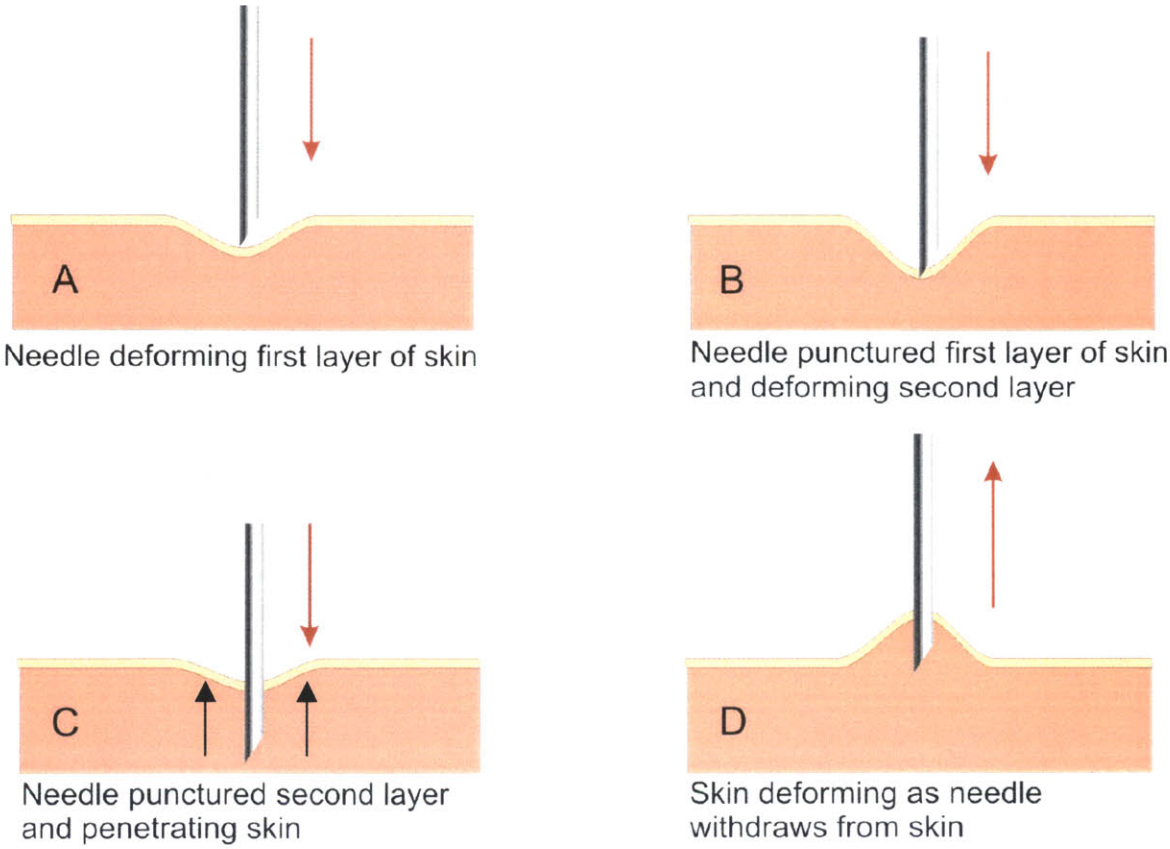

Figure 4-4. Illustration showing the mechanics of micro needle insertion into skin.

After penetrating the skin to a certain depth, the micro needle is held in place for 5 seconds before it is withdrawn from the skin. At this point, the skin slides up and this is shown in the drop in insertion force after the second peak in Figure 4-3 (see illustration in Figure 4-4(C)). From the negative force results in the removal portion of the plot in Figure 4-3, its can be shown that the skin deforms in the opposite direction as the needle is withdrawing out of the skin (see illustration in Figure 4-4(D)). This is due to elastic nature of skin and friction between the skin and the shaft of needle. When the needle punctures and penetrates the skin, the skin springs back radially and exerts a radial force on the needle [16]. This increases the friction force experienced by the needle and thus when the needle is pulling out of the skin, the skin deforms in the direction of withdrawal

until the friction force acting on the walls of the needle is insufficient to stretch and hold the skin any longer. At this point, the skin slips from the needle and returns to the unstretched position. The analysis of the skin mechanics during needle insertions is based on results and verified by physical observations of behavior of the skin.

4.2.2 Physical Model

From Figure 4-3, it can be shown that force data collected is a summation of stiffness, friction, and cutting forces, as shown in Equation 4.1 [19]. The stiffness force is pre-puncture, and the friction and cutting forces (penetration force) are post-puncture.

$$f_{needle}(x) = f_{stiffness}(x_1) + f_{friction}(x_2) + f_{cutting}(x_2), \tag{4.1}$$

where x is the displacement of needle, x_1 is the pre-puncture displacement of needle and x_2 is the post-puncture displacement of needle.

The stiffness force is due to the elastic properties of the skin. The elasticity of the skin can be identified from the pre-puncture force data in Figure 4-3. Biological tissue is linearly elastic for small deformations [14]. However, it is clear from Figure 4-3 that the deformation is significant, so the force must be modeled by a nonlinear method. Assuming a quasi-static stiffness response, a nonlinear spring model demonstrated by d'Aulignac in modeling deformation of a human thigh is used [12]. The graphical representation of this nonlinear model is shown in Figure 4-5.

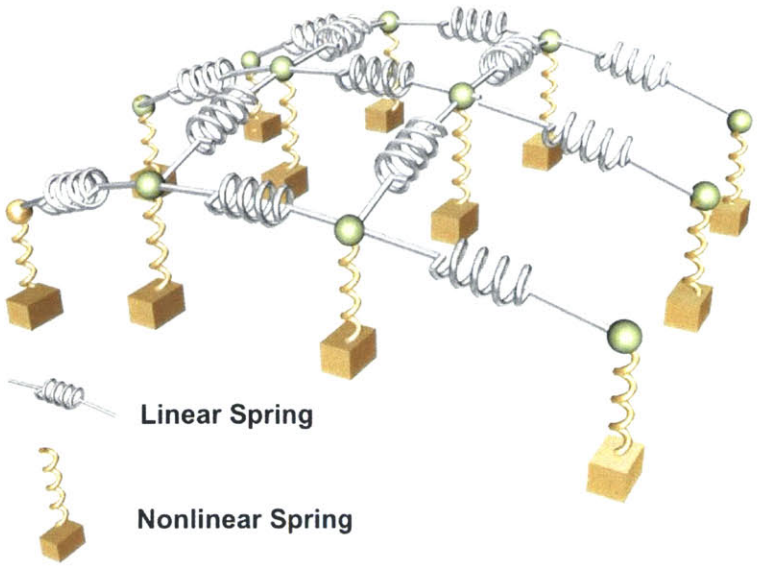

Figure 4-5. Nonlinear model of human thigh.

The nonlinear force model is given by

$$f_{stiffness}(x) = \frac{x}{ax+b}, \tag{4.2}$$

where x is the difference in the length of the nonlinear springs with respect to their original, resting length. The nonlinear stiffness parameters a and b are fitted to match the deformation measured on skin.

Based on deformation of skin from five tests on the linear insertion of micro needles into skin at an angle of 90° and a speed of 0.1 mm/s, the average values of stiffness parameters a and b were found and are shown in Table 4-1.

Table 4-1. Average Values of Nonlinear Stiffness Parameters a and b.

Parameter a [1/N]	**Parameter b [m/N]**
-152.67	0.15035

Figure 4-6 shows the fit of the physical model to a typical deformation result during a linear insertion of micro needle into skin at an angle of 90° and a speed of 0.1 mm/s.

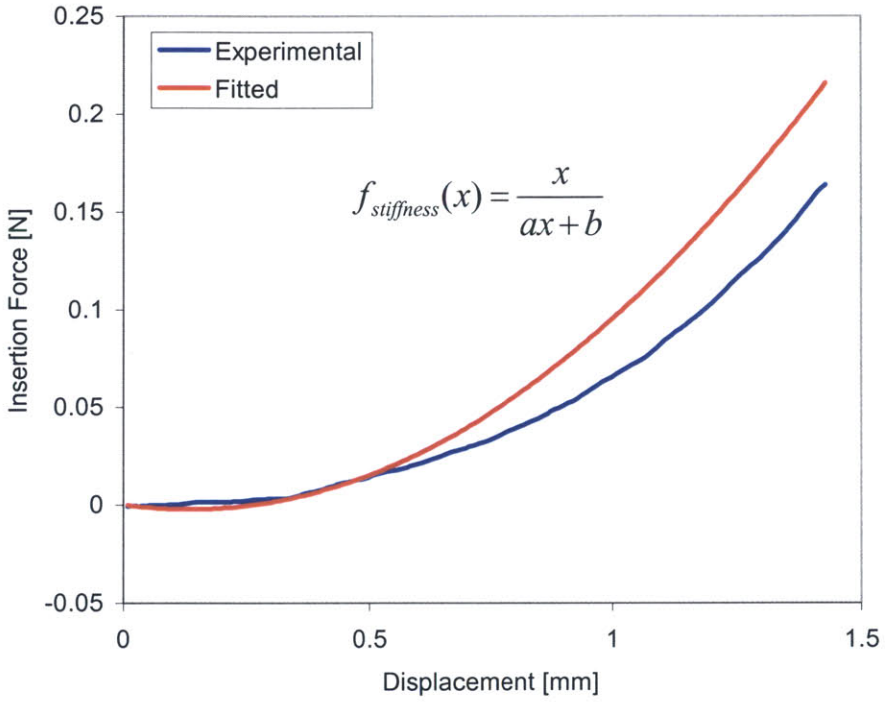

Figure 4-6. The fit of physical model to a typical experimental deformation result.

The penetration force is made up of the friction and cutting forces. The friction force occurs along the length of the needle, and is due to tissue adhesion and damping. The friction force can be modeled as

$$f_{friction} = b_p l v_{needle}, \tag{4.3}$$

where b_p is the damping coefficient per unit length, l is the length of needle in the skin and v_{needle} is the velocity of the needle tip.

Within the limits of this experiment, only v_{needle} can be determined accurately. The damping coefficient per unit length, b_p, is not known accurately, and varies from specimen to specimen. This is especially true when the properties of skin specimen change over time due to exposure to the atmosphere. The length of needle in skin, l, also cannot be accurately determined within the limits of this experiment because the position of the skin surface is not measured. Hence, the friction force cannot be accurately modeled.

The cutting force is that which is necessary to slice through the skin. This force exists as a combination of cutting forces and tissue stiffness at the tip of the needle, since the needle encounters stiffness as it cuts through new tissue. The cutting force can be calculated by subtracting the friction force from the total penetration force. However, in this experiment, the friction force cannot be accurately modeled. Thus, it is difficult to model cutting force of a needle through a skin, although ideally, cutting forces will be constant and unrelated to the needle depth.

4.3 Limitations

One of the limitations of this experiment is the instrumentation's inability to detect the actual length of needle into skin during an insertion. This limits the ability to

model the friction force and cutting force in skin. Another limitation is that the properties of skin specimen change over time after it is exposed to the atmosphere, probably due to the loss of moisture. There was no test chamber where humidity could be controlled and thus the change in properties may have affected the insertion force results. It is also difficult to compare results from one insertion to another because skin properties are different at different spots of insertions.

Chapter 5
Conclusion

5.1 Summary

Instrumentation was designed and fabricated to characterize micro needle insertion into biological tissues. Maximum force of micro needles puncturing skin at various insertion angles and velocities were also measured and quantified. Higher insertion speed does not have a significant effect insertion force. A smaller angle of insertion requires less force for the micro needle to puncture skin. The micro needle (100 μm OD) requires less insertion force than a 24-Gauge (570 μm OD) surgical needle. The linear insertion method with an oscillatory motion, when compared to a regular linear insertion method, does not have a significant effect on lowering insertion force. The lowest force required to puncture skin is when insertion angle is 15° and insertion speed is 0.1 mm/s. This force ranges from 120 to 200 mN. For physical model representation, only the stiffness force can be modeled accurately. The friction and cutting force of micro needle cannot be modeled due to the limitation of the instrumentation. All these pieces of information are crucial for a micro needle injection transdermal drug delivery project.

5.2 Future Work

Although the instrumentation is able to measure and quantify micro needle insertion forces successfully, there are certain aspects of it that need improvement.

5.2.1 Temperature and Humidity Control Chamber

One of problems faced when conducting the experiment was that the properties of the skin specimen changed over time during exposure to the atmosphere. If the test is

conducted in an enclosed chamber where temperature and humidity can be controlled, the skin specimen properties may be maintained for a longer time.

5.2.2 Rotational Insertion Module

A linear insertion with rotational motion about the axis of insertion method may reduce micro needle insertion force [15]. A module may be included in the instrumentation to performed rotational needle insertion into skin.

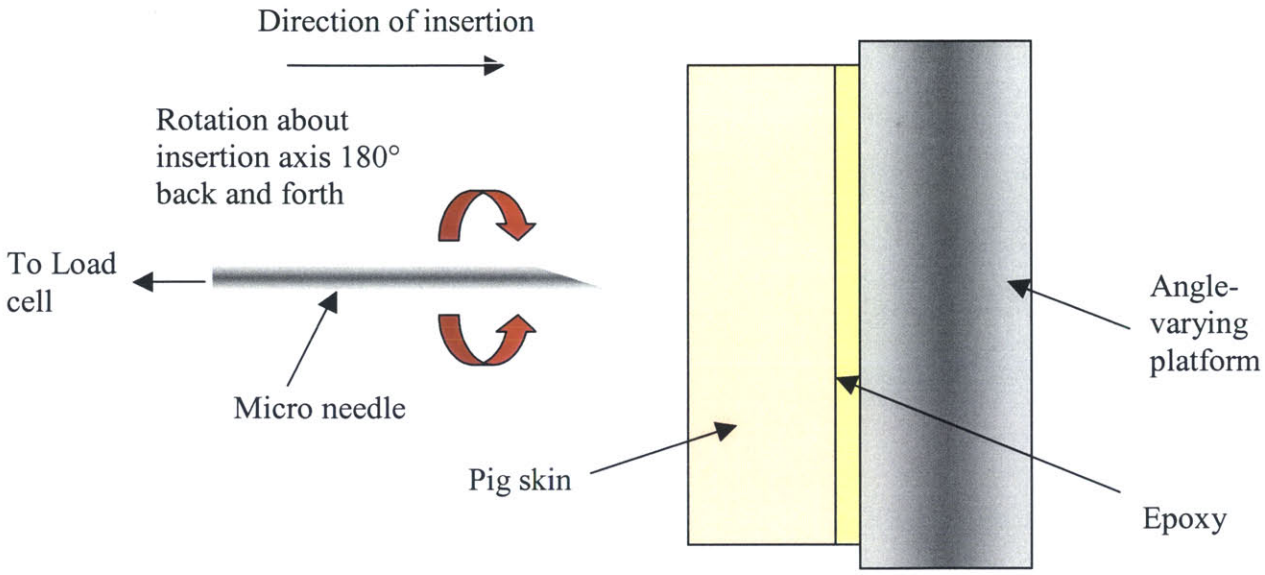

Figure 5-1. Rotational insertion method.

Similar analysis can be made with this new method of insertion, and then compared with the experiments presented here.

5.2.3 X-ray Imaging System

Because the exact penetration length of micro needle in the skin is not known during insertion, an x-ray imaging system may be integrated with the present

instrumentation to generate radiographs to reveal actual length of needles in skin. With the x-ray imaging, deflection of needles in skin can also be studied [15].

5.2.4 Synthetic Skin Specimen

Synthetic "skin" specimen of known stiffness and damping properties can be used to replace real skin samples. The problem of skin variation will be reduced if the needle insertions are made on synthetic "skin" specimens. In this way, it can be known for sure whether or not the variations in insertion forces are due to different methods, angles, and velocities of needle insertions.

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Appendix
Visual Basic 6.0 Code for Precision Motion Control and Data Acquisition

```
Option Explicit

'Precision Motion Control and Data Acquisition Interface by Wilson Chan

Dim i As Double        'Loop Iteration
Dim data1 As String    'Voltage Text from Channel 1
Dim data2 As String    'Voltage Text from Channel 2
Dim data3 As String    'Voltage Text from Channel 3
Dim data4 As String    'Voltage Text from Channel 4
Dim Vdata1 As Double   'Voltage data in numeric form from Channel 1
Dim Vdata2 As Double   'Voltage data in numeric form from Channel 2
Dim Vdata3 As Double   'Voltage data in numeric form from Channel 3
Dim Vdata4 As Double   'Voltage data in numeric form from Channel 4
Dim m1 As New sleep
Dim MotorSteps As Long  'steps made by stage
Dim MotorSteps1 As Long  'steps made by rotation stage
Dim Distance As Double  'distance in mm
Dim velocity1 As Double  'velocity in rev/s
Dim Speed As Double     'velocity in mm/s
Dim angle1 As Double    'angle in degrees
Dim Forward2 As Boolean 'Represent Postive Direction if True
Dim StepsPerRev As Long 'Steps Per Revolution
Dim DistPerTurn As Double ' Distance in mm per Revolution

Private Sub Form_Load()

  'Initial Values

  StepsPerRev = 25000
  DistPerTurn = 5
  Speed = 1
  Distance = 0.001
  Forward2 = True

  'Initialize RS-232 Serial Ports when Form Loads

  PortA.PortOpen = True
  PortB.PortOpen = True
  PortC.PortOpen = True
  PortD.PortOpen = True
  PortE.PortOpen = True
  PortF.PortOpen = True
  PortG.PortOpen = True
  PortH.PortOpen = True

  'Disable travel limits
  PortH.Output = "1_LH0" + vbCr

    ' Turn the timer off
  Timer1.Enabled = False
```

```
End Sub

Private Sub cmddist_Click()
   Distance = CDbl(txtDist.Text)
End Sub

Private Sub velocity_Click()
   Speed = CDbl(txtvel.Text)
End Sub

Private Sub GOForwardX1_Click() 'Move Stage in the Positive X1 Direction

   velocity1 = Round(Speed / DistPerTurn, 5)
   PortA.Output = "1_V" + CStr(velocity1) + vbCr
   MotorSteps = Round(Distance * StepsPerRev / DistPerTurn, 0)
   PortA.Output = "1_D" + CStr(MotorSteps) + vbCr
   PortA.Output = "1_GO" + vbCr

End Sub

Private Sub GOBackwardX1_Click() 'Move Stage in the Negative X1 Direction

   velocity1 = Round(Speed / DistPerTurn, 5)
   PortA.Output = "1_V" + CStr(velocity1) + vbCr
   MotorSteps = Round(Distance * StepsPerRev / DistPerTurn, 0)

   Forward2 = False
   If Forward2 = False Then
      MotorSteps = -1 * MotorSteps
   End If

   PortA.Output = "1_D" + CStr(MotorSteps) + vbCr
   PortA.Output = "1_GO" + vbCr


End Sub

Private Sub GOForwardY1_Click() 'Move Stage in the Positive Y1 Direction

   velocity1 = Round(Speed / DistPerTurn, 5)
   PortB.Output = "2_V" + CStr(velocity1) + vbCr
   MotorSteps = Round(Distance * StepsPerRev / DistPerTurn, 0)
   PortB.Output = "2_D" + CStr(MotorSteps) + vbCr
   PortB.Output = "2_GO" + vbCr

End Sub

Private Sub GOBackwardY1_Click() 'Move Stage in the Negative Y1 Direction

   velocity1 = Round(Speed / DistPerTurn, 5)
   PortB.Output = "2_V" + CStr(velocity1) + vbCr
   MotorSteps = Round(Distance * StepsPerRev / DistPerTurn, 0)

   Forward2 = False
   If Forward2 = False Then
```

```
    MotorSteps = -1 * MotorSteps
  End If

  PortB.Output = "2_D" + CStr(MotorSteps) + vbCr
  PortB.Output = "2_GO" + vbCr

End Sub

Private Sub GOForwardZ_Click() 'Move Stage in the Positive Z Direction

  velocity1 = Round(Speed / DistPerTurn, 5)
  PortC.Output = "3_V" + CStr(velocity1) + vbCr
  MotorSteps = Round(Distance * StepsPerRev / DistPerTurn, 0)
  PortC.Output = "3_D" + CStr(MotorSteps) + vbCr
  PortC.Output = "3_GO" + vbCr

End Sub

Private Sub GOBackwardZ_Click() 'Move Stage in the Negative Z Direction

  velocity1 = Round(Speed / DistPerTurn, 5)
  PortC.Output = "3_V" + CStr(velocity1) + vbCr
  MotorSteps = Round(Distance * StepsPerRev / DistPerTurn, 0)

  Forward2 = False
  If Forward2 = False Then
    MotorSteps = -1 * MotorSteps
  End If

  PortC.Output = "3_D" + CStr(MotorSteps) + vbCr
  PortC.Output = "3_GO" + vbCr

End Sub

Private Sub GOForwardX2_Click() 'Move Stage in the Positive X2 Direction

  velocity1 = Round(Speed / DistPerTurn, 5)
  PortD.Output = "4_V" + CStr(velocity1) + vbCr
  MotorSteps = Round(Distance * StepsPerRev / DistPerTurn, 0)
  PortD.Output = "4_D" + CStr(MotorSteps) + vbCr
  PortD.Output = "4_GO" + vbCr

End Sub

Private Sub GOBackwardX2_Click() 'Move Stage in the Negative X2 Direction

  velocity1 = Round(Speed / DistPerTurn, 5)
  PortD.Output = "4_V" + CStr(velocity1) + vbCr
  MotorSteps = Round(Distance * StepsPerRev / DistPerTurn, 0)

  Forward2 = False
  If Forward2 = False Then
    MotorSteps = -1 * MotorSteps
  End If

  PortD.Output = "4_D" + CStr(MotorSteps) + vbCr
```

```
   PortD.Output = "4_GO" + vbCr

End Sub

Private Sub GOForwardY2_Click() 'Move Stage in the Positive Y2 Direction

   velocity1 = Round(Speed / DistPerTurn, 5)
   PortE.Output = "5_V" + CStr(velocity1) + vbCr
   MotorSteps = Round(Distance * StepsPerRev / DistPerTurn, 0)
   PortE.Output = "5_D" + CStr(MotorSteps) + vbCr
   PortE.Output = "5_GO" + vbCr

End Sub

Private Sub GOBackwardY2_Click() "Move Stage in the Negative Y2 Direction

   velocity1 = Round(Speed / DistPerTurn, 5)
   PortE.Output = "5_V" + CStr(velocity1) + vbCr
   MotorSteps = Round(Distance * StepsPerRev / DistPerTurn, 0)

   Forward2 = False
   If Forward2 = False Then
      MotorSteps = -1 * MotorSteps
   End If

   PortE.Output = "5_D" + CStr(MotorSteps) + vbCr
   PortE.Output = "5_GO" + vbCr
End Sub


Private Sub insert105_Click()

    'Micro Needle Insertion Experiment

    'channel 105 scan for Entran Force Transducer


   Dim aStr As String
   Dim cnt As Long
   Dim TotalScans As Long
   Dim TimerIntSeconds As Double
   Dim Nstored As Long
   Dim firstcomma As Integer
   Dim secondcomma As Integer


   Const Chan = 105
   Dim aStr2 As String


   Dim vel As Double              'velocity in rev/second
   Dim SpeedLocal As Double       'speed in mm/s
   Dim DistanceLocal As Double    'total travel distance in mm


   Dim Data() As Double  'col 1: time, col 2:chan 105
```

```
'Variables for the needle insertion:


   TimerIntSeconds = CDbl(txttime.Text)    'seconds
   DistanceLocal = CDbl(txtdisp.Text)      'mm          'Stage moves by a total of 2.5 mm'
   SpeedLocal = CDbl(txtspeed.Text)        'mm/s

   Open "c:\Users\Wilson\data\pigshoulder2\sine\ch" & CStr(Chan) & "_" & txtfile.Text & ".txt" For
Output As #1



   TotalScans = (DistanceLocal / SpeedLocal) / TimerIntSeconds 'Number of data points taken per
direction

   ReDim Data(1 To TotalScans, 1 To 2) As Double


'Open file for data storage:

'Insertion

   vel = Round(SpeedLocal / DistPerTurn, 5)          'Convert to rev/second
   PortB.Output = "2_V" + CStr(vel) + vbCr
   MotorSteps = Round(DistanceLocal * StepsPerRev / DistPerTurn, 0)
   Forward2 = False
   If Forward2 = False Then
   MotorSteps = -1 * MotorSteps
   End If
   PortB.Output = "2_D" + CStr(MotorSteps) + vbCr  'Do not start stepper motors yet.

   'Before starting motion, we need to initialize the force measurement.

   PortF.Output = "*RST" + vbCrLf
   Call m1.SleepMS(100)

   PortF.Output = "CONF:VOLT:DC 10,0.00003,(@105)" + vbCrLf
   Call m1.SleepMS(100)
   PortF.Output = "ROUT:CHAN:DELAY " & CStr(TimerIntSeconds) & ",(@105)" & vbCrLf
   Call m1.SleepMS(100)

   PortF.Output = "ROUT:SCAN (@" & CStr(Chan) & ")" + vbCrLf
   Call m1.SleepMS(100)

   PortF.Output = "TRIG:COUN " & CStr(TotalScans) + vbCrLf       'Number of scans for the Data
Acquisition scanning
   Call m1.SleepMS(100)

   PortB.Output = "2_GO" + vbCr                  'Start the motors
```

```
PortF.Output = "INIT" + vbCrLf                'Start Scan
Call m1.SleepMS(100)

Call m1.SleepMS(CLng(TimerIntSeconds * TotalScans * 1000 + 1000))


'Retrieve data
PortF.Output = "FORM:READ:ALAR OFF" + vbCrLf      'Output Alarm Status Off
Call m1.SleepMS(50)
PortF.Output = "FORM:READ:CHAN ON" + vbCrLf       'Output Channel Number On
Call m1.SleepMS(50)
PortF.Output = "FORM:READ:TIME ON" + vbCrLf       'Output Time On
Call m1.SleepMS(50)
PortF.Output = "FORM:READ:UNIT OFF"               'Output Units Off
Call m1.SleepMS(50)

Nstored = TotalScans 'Number of data points that should have been taken


For i = 1 To Nstored
   PortF.Output = "R? 1" + vbCrLf
   Call m1.SleepMS(100)
   aStr = PortF.Input                     'Read Output Buffer as data
   cnt = 0
   Do While cnt < 100 And Left(aStr, 1) <> "#"
      cnt = cnt + 1
      PortF.Output = "R? 1" + vbCrLf
      Call m1.SleepMS(100)
      aStr = PortF.Input                  'Read Output Buffer as data
   Loop

   aStr = Right(aStr, Len(aStr) - 4)
   firstcomma = InStr(1, aStr, ",")
   secondcomma = InStr(firstcomma + 1, aStr, ",")

   aStr2 = Left(aStr, firstcomma - 1)
   Data(i, 2) = CDbl(aStr2)               'Read channel
   aStr2 = Mid(aStr, firstcomma + 1, secondcomma - firstcomma - 1)
   Data(i, 1) = CDbl(aStr2)               'time data
Next i



Print #1, "Channel 105"
Print #1, "Force_Sample_Time  " + CStr(TimerIntSeconds)
Print #1, "Travel_Distance    " + CStr(DistanceLocal)
Print #1, "Velocity           " + CStr(SpeedLocal)

Print #1, "Time(s)" + "   " + "Force(N)"

For i = 1 To Nstored
   Print #1, CStr(Data(i, 1)) + "              " + CStr(Data(i, 2))
Next i

Call m1.SleepMS(5000)
```

```
'Removal

vel = Round(SpeedLocal / DistPerTurn, 5)
PortB.Output = "2_V" + CStr(vel) + vbCr
MotorSteps = Round(DistanceLocal * StepsPerRev / DistPerTurn, 0)

PortB.Output = "2_D" + CStr(MotorSteps) + vbCr  'Don't start yet.

'Before starting motion, we need to initialize the force measurement.

PortF.Output = "*RST" + vbCrLf
Call m1.SleepMS(1000)

PortF.Output = "CONF:VOLT:DC 10,0.00003,(@105)" + vbCrLf
Call m1.SleepMS(100)
PortF.Output = "ROUT:CHAN:DELAY " & CStr(TimerIntSeconds) & ",(@105)" & vbCrLf
Call m1.SleepMS(100)


PortF.Output = "ROUT:SCAN (@" & CStr(Chan) & ")" + vbCrLf


PortF.Output = "TRIG:TIM " & CStr(TimerIntSeconds) + vbCrLf     'Use the internal timer to trigger
scans
Call m1.SleepMS(100)

PortF.Output = "TRIG:COUN " & CStr(TotalScans) + vbCrLf      'Number of scans for the Data
Acquisition scanning
Call m1.SleepMS(100)

PortB.Output = "2_GO" + vbCr                'Start the motors
PortF.Output = "INIT" + vbCrLf              'Start the Scan


Call m1.SleepMS(CLng(TimerIntSeconds * TotalScans * 1000 + 1000))

Nstored = TotalScans

'Retrieve Data
PortF.Output = "FORM:READ:ALAR OFF" + vbCrLf      'Output alarm status Off
Call m1.SleepMS(50)
PortF.Output = "FORM:READ:CHAN ON" + vbCrLf       'Output channel number On
Call m1.SleepMS(50)
PortF.Output = "FORM:READ:TIME ON" + vbCrLf       'Output time On
Call m1.SleepMS(50)
PortF.Output = "FORM:READ:UNIT OFF"               'Units output Off
Call m1.SleepMS(50)



For i = 1 To Nstored
   PortF.Output = "R? 1" + vbCrLf
   Call m1.SleepMS(75)
   aStr = PortF.Input        'Read Output Buffer as data
```

```
    cnt = 0                 'if read is wrong, try again...
    Do While cnt < 100 And Left(aStr, 1) <> "#"
      cnt = cnt + 1
      PortF.Output = "R? 1" + vbCrLf
      Call m1.SleepMS(100)
      aStr = PortF.Input      'Read Output Buffer as data
    Loop

    aStr = Right(aStr, Len(aStr) - 4)
    firstcomma = InStr(1, aStr, ",")
    secondcomma = InStr(firstcomma + 1, aStr, ",")

    aStr2 = Left(aStr, firstcomma - 1)
    Data(i, 2) = CDbl(aStr2)    'channel
    Data(i, 1) = CDbl(Mid(aStr, firstcomma + 1, secondcomma - firstcomma + 1)) 'time data
  Next i



  'Save the removal data:
  For i = 1 To Nstored
    Print #1, CStr(Data(i, 1)) + "              " + CStr(Data(i, 2))
  Next i


  Close #1

End Sub

Private Sub measure_Click()

  Open "c:\Users\Wilson\data\spring.txt" For Output As #1

  Print #1, "Displacement (mm)" + "   " + "Force 1 (N)" + "          " + "Force 2 (N)"

  For i = 1 To 10

    'Moves Stage by 1 Step for each loop

    velocity1 = Round(Speed / DistPerTurn, 5)
    PortB.Output = "2_V" + CStr(velocity1) + vbCr
    MotorSteps = Round(Distance * StepsPerRev / DistPerTurn, 0)
    PortB.Output = "2_D" + CStr(MotorSteps) + vbCr
    PortB.Output = "2_GO" + vbCr

    'Measure Output Voltage from HP 34970A Data Acquisition Unit Channel 1

    PortF.Output = "CONF:VOLT:DC 10,0.00003,(@101)" + vbCrLf
    Call m1.SleepMS(100)
    PortF.Output = "INIT" + vbCrLf
    Call m1.SleepMS(100)
    PortF.Output = "FETC?" + vbCrLf
    Call m1.SleepMS(50)

    data1 = PortF.Input 'Read Output Buffer as data
```

```
    Vdata1 = CDbl(data1) 'Change data from string to double
    Vdata1 = Vdata1 * 10 'Converts voltage value to Force Using 10N/V Calibration

    'Measure Output Voltage from HP 34970A Data Acquisition Unit Channel 2

    PortF.Output = "CONF:VOLT:DC 10,0.00003,(@102)" + vbCrLf
    Call m1.SleepMS(100)
    PortF.Output = "INIT" + vbCrLf
    Call m1.SleepMS(100)
    PortF.Output = "FETC?" + vbCrLf
    Call m1.SleepMS(50)

    data2 = PortF.Input 'Read Output Buffer as data
    Vdata2 = CDbl(data2) 'Change data from string to double
    Vdata2 = Vdata2 * 10 'Converts voltage value to Force Using 10N/V Calibration

    Print #1, CStr(i) + "              " + CStr(Vdata1) + "              " + CStr(Vdata2)

    Call m1.SleepMS(100)

  Next i

End Sub

Private Sub measureblock_Click()

'Compression

  Open "c:\Users\Wilson\data\block80a.txt" For Output As #1

  Print #1, "Displacement (um)" + "   " + "Force 1 (N)" + "              " + "Force 2 (N)"

  Distance = 0.01 'Stage moves by 10 um'
  i = 0

  Do
  'For i = 1 To 100

    'Moves Stage by 1 Step for each loop

    i = i + 1

    velocity1 = Round(Speed / DistPerTurn, 5)
    PortB.Output = "2_V" + CStr(velocity1) + vbCr
    MotorSteps = Round(Distance * StepsPerRev / DistPerTurn, 0)

    Forward2 = False
    If Forward2 = False Then
    MotorSteps = -1 * MotorSteps
    End If

    PortB.Output = "2_D" + CStr(MotorSteps) + vbCr
    PortB.Output = "2_GO" + vbCr

    'Measure Output Voltage from HP 34970A Data Acquisition Unit Channel 1
```

```
  PortF.Output = "CONF:VOLT:DC 10,0.00003,(@101)" + vbCrLf
  Call m1.SleepMS(100)
  PortF.Output = "INIT" + vbCrLf
  Call m1.SleepMS(100)
  PortF.Output = "FETC?" + vbCrLf
  Call m1.SleepMS(50)

  data1 = PortF.Input 'Read Output Buffer as data
  Vdata1 = CDbl(data1) 'Change data from string to double
  Vdata1 = Vdata1 * 10 'Converts voltage value to Force Using 10N/V Calibration

  'Measure Output Voltage from HP 34970A Data Acquisition Unit Channel 2

  PortF.Output = "CONF:VOLT:DC 10,0.00003,(@102)" + vbCrLf
  Call m1.SleepMS(100)
  PortF.Output = "INIT" + vbCrLf
  Call m1.SleepMS(100)
  PortF.Output = "FETC?" + vbCrLf
  Call m1.SleepMS(50)

  data2 = PortF.Input 'Read Output Buffer as data
  Vdata2 = CDbl(data2) 'Change data from string to double
  Vdata2 = Vdata2 * 10 'Converts voltage value to Force Using 10N/V Calibration

  Print #1, CStr(i * 10) + "              " + CStr(Vdata1) + "              " + CStr(Vdata2)

  Call m1.SleepMS(50)

Loop Until Vdata1 >= 50 And Vdata2 >= 50 'Loop stops when Max force of 50 N is reached

'Extension

Do
  'Moves Stage by 1 Step for each loop

  i = i - 1

  velocity1 = Round(Speed / DistPerTurn, 5)
  PortB.Output = "2_V" + CStr(velocity1) + vbCr
  MotorSteps = Round(Distance * StepsPerRev / DistPerTurn, 0)
  PortB.Output = "2_D" + CStr(MotorSteps) + vbCr
  PortB.Output = "2_GO" + vbCr

  'Measure Output Voltage from HP 34970A Data Acquisition Unit Channel 1

  PortF.Output = "CONF:VOLT:DC 10,0.00003,(@101)" + vbCrLf
  Call m1.SleepMS(100)
  PortF.Output = "INIT" + vbCrLf
  Call m1.SleepMS(100)
  PortF.Output = "FETC?" + vbCrLf
  Call m1.SleepMS(50)

  data1 = PortF.Input 'Read Output Buffer as data
  Vdata1 = CDbl(data1) 'Change data from string to double
  Vdata1 = Vdata1 * 10 'Converts voltage value to Force Using 10N/V Calibration
```

```
    'Measure Output Voltage from HP 34970A Data Acquisition Unit Channel 2

    PortF.Output = "CONF:VOLT:DC 10,0.00003,(@102)" + vbCrLf
    Call m1.SleepMS(100)
    PortF.Output = "INIT" + vbCrLf
    Call m1.SleepMS(100)
    PortF.Output = "FETC?" + vbCrLf
    Call m1.SleepMS(50)

    data2 = PortF.Input 'Read Output Buffer as data
    Vdata2 = CDbl(data2) 'Change data from string to double
    Vdata2 = Vdata2 * 10 'Converts voltage value to Force Using 10N/V Calibration

    Print #1, CStr(i * 10) + "              " + CStr(Vdata1) + "              " + CStr(Vdata2)

    Call m1.SleepMS(50)

  Loop Until i = 0

End Sub
```