An Apparatus for High Throughput Muscle Cell Experimentation

BY

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Abstract

The cardiac ventricular muscle cell (myocyte) is a key experimental system for exploring the mechanical properties of the diseased and healthy heart. The myocyte experimental model provides a higher level of physiological relevance than molecular or myofibril studies while avoiding problems inherent to multicellular preparations including heterogeneity of cell types and diffusion limited extracellular spaces. Millions of primary myocytes that remain viable for four to six hours can be readily isolated from animal models. However, the mechanical properties of only a few physically loaded myocytes can be explored in this time period using current, bulky and expensive instrumentation.

In this thesis, a prototype instrument is described that is modular and inexpensive and could form the basis of an array of devices for probing the mechanical properties of single mammalian myocytes in parallel. This would greatly increase the throughput of scientific experimentation and could be applied as a high content screening instrument in the pharmaceutical industry providing information at the level of a critical cellular phenotype, myocyte mechanical properties, for drug development and toxicology studies.

The design, development and experimental verification of the modular instrument are presented here. The mathematical, mechanical and electrical characteristics of the novel force sensor and actuator system, $H_{\infty}$ control implementation and data processing methodology are discussed. Finally, the functionality of the instrument is demonstrated by implementing novel methodologies for loading and attaching healthy, single mammalian ventricular myocytes to the force sensor and actuator and measuring their isometric twitch force and passive dynamic stiffness at varied sarcomere lengths.

Thesis Supervisor: Ian W. Hunter
Title: Hatsopoulos Professor of Mechanical Engineering
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1 Introduction

A recent study performed by the American Heart Association found that cardiovascular diseases including coronary heart disease, stroke, congestive heart failure, high blood pressure and others were the leading cause of death in all males and females in the United States in 2001. These diseases accounted for 38.5% of all deaths and were estimated to cost approximately $368 billion including both direct costs for health care and indirect costs in lost productivity [1]. To put this in perspective, this was approximately 3% of the $11.252 billion, 2003 annual gross domestic product of the United States or twice the estimated cost of all cancers. These statistics demonstrate the clear socioeconomic cost of cardiovascular diseases and motivate research that can help understand and alleviate them.

The cardiovascular system has been explored at many levels from population based studies through to the structure and interaction of individual proteins [2]. In general moving away from the study of in vivo human hearts downward through isolated hearts, muscle tissue and individual cells toward isolated proteins increases the quantity and quality (repeatability) of the data gathered while reducing the clinical relevance. More invasive studies often require nonhuman experimental systems such as large animals (bovine, dog and pig) and small animals (mouse, rat, rabbit and guinea pig) that further reduce clinical relevance.

To consider the function or dysfunction of the heart as an organ, researchers must rely on its experimentally accessible properties from the molecular to the gross physiological level. These include cardiac morphology, homodynamic indices of pump function, rate, rhythm and electrical activity, biochemistry and biology of cellular metabolism, excitation-contraction coupling and ion regulation and the level and activity of intra and extracellular proteins and other macromolecules controlled via the regulation and expression of the genetic profile of individual cells. As the primary function of the heart is to pump blood, mechanical properties are crucial experimental endpoints that are accessible at the level of the whole organ through to the interaction of individual proteins.
In this thesis, modular instrumentation was developed to study the mechanical properties of individual heart muscle cells (cardiac myocytes) from female Guinea pigs. More specifically, system identification techniques were rigorously applied to provide quantitative linear models of the passive mechanics of the cell including the first measurement of the dynamic stiffness of a myocyte. This project represents a first step toward an instrument that is capable of thoroughly characterizing the mechanics of living heart muscle cells in parallel. This could be applied to high throughput studies of muscle physiology and for drug development and toxicology testing in the pharmaceutical industry.

The introduction of this thesis will discuss the key aspects of using mammalian myocytes as an experimental system including their isolation, stimulation and attachment to force sensors and actuators. It will present some common mechanical measurements used to explore the physiology of muscle cells (and multicellular tissue) and describe the attributes and limitations of current instrumentation used in these experiments. This discussion will provide a framework for presenting the goals of the project; to design a compact, inexpensive and modular instrument (one element of an array) capable of characterizing the mechanical properties of myocytes under varied loading conditions and to demonstrate its functionality by providing the world’s first measurement of the dynamic stiffness of a single cell.
1.1 The myocyte as an experimental system

We used isolated cardiac myocytes as an experimental system to explore the mechanics of the heart to exploit the following advantages:

- A myocyte has only a few myofibrils in a physiologically relevant orientation (an advantage over studies on isolated proteins).
- A myocyte provides excellent optical access to sarcomere length, a critical physiological parameter (far better than that provided by larger tissue samples).
- Studies on myocytes avoid complications seen in multicellular preparations (tissue) including heterogeneity of cell types [3], diffusion limited extracellular spaces [4,5], non-uniform shortening of sarcomeres during isometric contraction and the mechanical influence of the extracellular matrix (ECM) [6].
- The myocyte represents a clear division on which to base modeling efforts such as the physiome project [7].

Mammalian myocytes are approximately 110 μm long, $10^{-10}$ m$^2$ in cross section, have sarcomere lengths between 1.8 and 2.4 μm and can potentially produce up to 10 μN of force or 100 kPa of stress (see reviews [8, 9, 10]). An intact Guinea pig ventricular myocyte’s modulus of elasticity at 0 Hz varies between 40 and 400 kPa depending on cell length and activation state. The full physiological range of muscle length corresponds to approximately 30 % strain [11] and spans the nonlinear ascending limb of the classic force length relationship. As a result, muscle tissue exhibits significant sarcomere length and force load dependent nonlinearities.

Measuring the mechanical properties of myocytes is a significant instrumentation challenge. It is complicated by small forces (< 10 μN) and necessarily small displacements (1 nm to 30 μm) that typically require expensive force transducers and position actuators. Furthermore, it is very difficult to attach a living muscle cell with intact cell membrane to a force transducer and actuator with sufficient strength to support contractions and sufficient care to avoid damaging the cell or introducing compliance at the attachment point that distorts measurements (see review by [12]). Finally, as there are
no cell lines that maintain the rod shaped morphology and proteins required for excitation contraction coupling, it is typically necessary to obtain primary cells from animal models on the day of measurement rather than relying on cell culture [13]. The following sections introduce these and other issues relevant to cellular muscle physiology experiments.

1.1.1 Myocyte Isolation

The adult myocardium is composed of individual muscle cells separated laterally by the sarcolemma and the extracellular matrix and transversely by intercalated discs. Contractile myofilaments, a series of repeat units termed sarcomeres terminate on these discs and do not pass from one cell to another. The combination of the independence of the contractile apparatus and the multilaminated nature of the intercalated disc allows cells to be separated by various means while still maintaining their functional and morphological integrity (see reviews [9,10]).

MECHANICAL DISRUPTION

Mechanically disrupting muscle tissue was one of the earliest techniques used to isolate myocytes [14]. Muscle tissue was ground in solutions such as Tris maleate at pH 7.4 that were known to disrupt connective tissue. Ca$^{2+}$ chelators such as EDTA (ethylenediamine tetra-acetic acid) were often included to minimize cell damage through contraction during isolation. However, while these techniques could isolate myocytes they were far too aggressive and resulted in significant damage to cell membranes producing very few healthy, viable myocytes.

ENZYMIC DISPERSION

The disappointing performance of isolation via mechanical disruption led to the development of enzymatic digestion [15] a technique that is still in use today. The heart was perfused through the aorta with a series of solutions culminating in a digestive mix containing proteolytic enzymes capable of hydrolyzing connective tissues. The cells could then be separated by very mild mechanical perturbation and enriched with a gentle centrifugation for a few minutes at low speeds (200 rpm).
An extended process of trial and error was used to find quasi optimal solutions for enzymatic dispersion (see review [10]). The use of heterogeneous dissociating enzymes at their lowest effective concentration was found to be critical as was the need to provide enough extracellular Ca$^{2+}$ to preserve structural integrity of the membrane and glycoproteins but not so much as to inhibit separation of cells at the intercalated discs. Ca$^{2+}$ and K$^+$ concentrations were dependent upon the type and concentration of enzymes used to separate the cells and the species from which the tissue was taken. Typical collagenase solutions had low levels of Ca$^{2+}$ and K$^+$, glucose at around 5-11 mM, bicarbonate or HEPES as a buffer and a net osmolarity of approximately 280 mOsm/kg. Finally, the addition of Mg$^{2+}$ATP and Albumin at 1 – 20 g/L also seemed to stabilize the cell membrane and avoid disruption of the glycocalyx.

In this work we applied a proven protocol kindly provided by Professor Peter Kohl of the University of Oxford that used a blend of bacterial collagenases and proteases. The approach was sufficiently optimized in our laboratory to routinely provide high yields of healthy myocytes that remained viable for up to 6 hours.

1.1.2 Stimulating myocytes

The ability of an isolated muscle cell to contract is a key indication of cell viability. Muscle physiologists have explored the mechanical properties of myocytes with an intact cell membrane and skinned cells with perforated membranes (first introduced by Natori in 1954 [16]). Intact cells are technically alive, maintain physiological separation of actin and myosin, have a complete excitation contraction coupling apparatus and intact signaling pathways [13]. Skinned cells provide precise control of the chemical environment surrounding the myofibrils allowing biochemical studies of the interaction of proteins in the cross bridge cycle to be explored in a more physiologically relevant setting. Furthermore, they permit the exchange or partial replacement of some regulatory proteins within the cell, a technique pioneered by Moss et al in 1985 [17]. The excitation contraction coupling process is clearly different in intact and skinned cells necessitating different methodologies to induce contractions.
Skinned cells are stimulated by rapidly changing the solution in the myocyte bath from relaxing, \([\text{Ca}^{2+}] < 10 \mu\text{M}\), to contracting, \([\text{Ca}^{2+}] < 100 \mu\text{M}\). Several appropriate solutions can be found in the literature \([18,19]\). If necessary, free ionic concentrations can be predicted according to Fabiato \([20]\). The timescale for diffusion into a cell with radius 10 \(\mu\text{m}\) at 20 \(^{\circ}\text{C}\) is approximately 10 ms (assuming \(D_{\text{ion}} \approx 10^{-10} \text{m}^2/\text{s}\)) and is inversely proportional to temperature \([21]\). It is typical to ensure that diffusion of chemical species is the rate limiting step. This requires that solutions be changed rapidly using, for example, a split flow technique \([22]\).

Alternatively, an electric field can be used to stimulate twitches in intact cells. Typically, platinum electrodes parallel to the long axis of the myocyte are used to apply step changes in electric field of up to 10 kV/m at frequencies between 0.1 and 10 Hz \([23,24]\). It is worth noting that although it is not necessary to be able to rapidly change the solution surrounding intact cells to induce a contraction, this capability is still very useful for exploring the response of a cell to a step change in its environment. The fluidic system for intact cell experiments often incorporates rapid switching and minimal dead volume and could, therefore, be easily adjusted to support skinned cells.

### 1.1.3 Measuring sarcomere length

The regular banded structure visible under an optical microscope gave striated muscle its name and inspired optical interrogation of sarcomere length. Uniform sarcomere spacing along the length of the cell is often considered an indicator of cell health after isolation. Furthermore, it is important to consider sarcomere length when making physiological measurements on muscle tissue as it is a critical determinant of force \([25,26,27]\) and muscle dynamics \([28]\). It is also desirable to ensure that the sarcomere spacing remains uniform across the length of the cell if inferences are to be made between contraction of whole muscle cells and the underlying regulation of contraction or protein architecture. Optical measurements of sarcomere length can be broadly classified as either diffraction or direct imaging based.
LIGHT DIFFRACTION

The diffraction of light caused by the crystalline structure of the A-I band has been observed since the nineteenth century [29]. It was applied to cardiac papillary muscle in 1974 using a laser beam and a video camera, but the technique suffered from broad diffraction lines due to structural heterogeneity of multiple cellular preparations [30]. Single cells provide much clearer diffraction patterns and multiple theoretical and experimental approaches have been used to explore interpretations of the separation, intensity, fine structure and dynamics of these patterns (see review in [31]). The spacing of the diffraction pattern gives a direct measure of average sarcomere length with sarcomeres modeled as a plane grating [31]. This relationship is only approximate and does not account for structural heterogeneity [32], Bragg effects due to the crystalline structure of the myocyte [33] and spatially asynchronous behavior [28]. Despite these drawbacks laser diffraction is still being used as a measure of sarcomere length as it is simple to implement and has high bandwidth (2 to 28 kHz) so it can easily be used online for control [24,27].

DIRECT IMAGING

Directly imaging sarcomeres with bright field, interference or polarizing microscopes using high quality, high numerical aperture optics and high bit rate CCD cameras provides considerably more information than laser diffraction at the expense of complexity, physical size and money (see review [34]). While frame rates of 200 to 250 Hz [35,36] have been achieved, off-line processing, typically using spatial Fourier transforms, was required to find sarcomeric periodicity [25]. This technique is particularly useful in myofibril preparations where A-I band overlap contrast can be high enough to monitor the length of individual sarcomeres [28]. More recently, Ion Optix [37] began providing a commercial system that combines fluorescent monitoring of internal Ca$^{2+}$ concentration with sarcomere length measurement at up to 1 kHz.

1.1.4 Measuring myocyte mechanical properties

After the concept of the cross bridge cycle was introduced by A.F. Huxley, physiological experiments on the mechanics of muscle tissue focused on the magnitude and rate of
force generation and relaxation in cardiac, skeletal and smooth muscle, exploring the mechanisms behind the generation and regulation of force. The work identified the importance of Ca$^{2+}$ as a trigger for contraction and motivated its inclusion in ionic models of action potentials (see review from 1968 [38]). In 1954, Natori conducted experiments on skinned muscle tissue (in which the cell membrane had been dissolved) allowing direct control of the intracellular environment [16]. This facilitated comparisons of physiological and biochemical experiments, exploring how the mechanical constraints provided by the arrangement of the contractile apparatus in muscle tissue affected reaction rates between isolated proteins in the cross bridge cycle.

The mechanical effects of many different perturbations (chemical, thermal, genetic, electromagnetic and mechanical) on isolated muscle tissue have been explored in depth over the last century. Almost all of these tests can be replicated on individual cells that avoid the problems associated with multicellular preparations as discussed in the beginning of Section 1.1. Table 1.1-1 summarizes a collection of some of common mechanical tests used by muscle physiologists to explore the regulation of contraction and the excitation contraction coupling process (see review [39]).

<table>
<thead>
<tr>
<th>Perturbation</th>
<th>Parameters Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant [Ca$^{2+}$]</td>
<td>Fit steady state force as a function of [Ca$^{2+}$] to the Hill equation. Measures cooperative activation.</td>
</tr>
<tr>
<td>Shorten under constant load then step muscle length</td>
<td>$k_{TR}$: rate of tension redevelopment [40].</td>
</tr>
<tr>
<td>[Ca$^{2+}$] step using optically triggered chelators</td>
<td>$k_{Ca}$: rate of tension development</td>
</tr>
<tr>
<td>Phosphate concentration, [P$_i$], step using optically triggered chelators</td>
<td>$k_{P}$: rate of force relaxation after step change in [P$_i$]</td>
</tr>
<tr>
<td>Release from isometric contraction under constant load.</td>
<td>V$_{u}$: velocity of shortening at a given load. Measures muscle power output [41].</td>
</tr>
<tr>
<td>Release from isometric contraction via removal of stimulus (absorption of Ca$^{2+}$)</td>
<td>Rate of relaxation (complicated by many factors)</td>
</tr>
<tr>
<td>Electric field stimulation rate</td>
<td>Peak force produced</td>
</tr>
<tr>
<td>Step, sinusoidal or stochastic length changes</td>
<td>Complex mechanical impedance. Gives a measure of the number of intact cross bridges and insight into both the active and passive mechanical properties.</td>
</tr>
</tbody>
</table>

Table 1.1-1. Some of the common mechanical physiological tests used to characterize muscle tissue and explore the regulation of contraction (see review [39]). These tests have been applied to skinned and intact muscle preparations under varied conditions including altered [Ca$^{2+}$], pH, [Mg$^{2+}$], sarcomere length, actin myosin lattice spacing, external and internal ionic strength, [MgATP], [MgADP] and temperature (where [X] is the concentration of X).
Muscle is clearly a nonlinear system as is evidenced by the classic Frank Starling force length relationship. As can be seen in Table 1.1-1, step inputs have been the stimulus of choice for exploring the mechanics and regulation of muscle tissue in the presence of these nonlinearities. For example, step displacements of greater than 1 % of the sample length were the basis of a technique to measure rate of force development introduced by Brenner [40] and the direct measurement of the unloaded velocity of shortening as proposed by Edman [41]. Other examples include the use of photolysis to provide step inputs of Ca$^{2+}$, MgATP or phosphate (see review [39]).

The techniques of Brenner and Edman attempt to force the muscle tissue into a particular state to simplify the interpretation of results (no strong cross bridges [40] or no load during contraction [41]). More recently, the development of techniques such as optical or magnetic tweezers that are capable of manipulating the contractile apparatus isolated from single cells have provided a more direct means for exploring the regulation of contraction. Despite the advent of these molecular techniques, whole cell / tissue mechanical studies are still very important and are clearly more physiologically relevant than experiments at the level of individual proteins.

The complexity of the living cell introduces several experimental challenges. The mechanical properties of a cell depend on the history of perturbations it has been subjected to including the process by which it was isolated and attached to a force sensor and actuator. Cells are typically used within four to six hours of isolation allowing the possibility of significant changes in the molecular state of the cell. Finally, once mechanical testing begins each perturbation can potentially induce both short term and long term changes in protein expression and signaling pathways within the cell. While these problems cannot be avoided, their effects can be minimized by tightly controlling experimental conditions. Furthermore, these problems are indicative of the tradeoff between clinical relevance and precise experimental control that occurs as the experimental model moves from simple (the interaction of individual proteins) to complex (whole organisms or populations).
In this thesis we focus on the development of modular instrumentation capable of exploring the mechanical properties of single cells. To verify the operation of the instrument, we chose to measure the dynamic stiffness or complex mechanical impedance of a muscle cell as this requires the application and measurement of broadband displacements and forces and hence provides a thorough demonstration of the capabilities of the instrument. In addition, to the author's knowledge this measurement would be a world first.

1.1.5 Dynamic Stiffness

The dynamic stiffness is a complex number representing the ratio of stress to strain as a function of frequency. Early research characterized this frequency response function using sinusoidal perturbations to explore oscillatory work in insect muscle [42]. In 1980, Kawai and Brandt extended the use of dynamic stiffness to striated vertebrate muscle and introduced a model that decomposed the frequency response data of activated muscle into the sum of three, 1st order, high pass filter elements and a constant [43]. The Laplace transform equivalent of this model is

\[
Y(s) = \frac{F(s)}{X(s)} = H + A \frac{s}{s+a} - B \frac{s}{s+b} + C \frac{s}{s+c},
\]

where \(Y(s)\) is the dynamic stiffness (force / displacement), \(H\) is the stiffness at 0 Hz and \(H+A-B+C\) is the instantaneous stiffness (at infinite frequency) typically interpreted as a measure of the number of cross bridges attached. Kawai linked the exponential time constants of the step response of this system (1/a, 1/b and 1/c) to previously identified phases in the force transient produced by the application of a length step to muscle tissue observed by A.F. Huxley [44] and hence to the underlying physiology of muscle. As the features captured by this model are present when the muscle tissue is actively contracting and absent when the tissue is relaxed or in rigor, Kawai asserted the time constants represent fundamental properties of cycling cross bridges [43]. Since its inception in 1980 this decomposition, with slight variations, has been extensively used by Kawai and widely adopted by various other research groups [45,46,47,48,49].
In subsequent work on papillary, psoas and soleus muscle from ferrets and rabbits [50,51,52,53], Kawai et al developed a 10 parameter, 7 state model of the cross bridge cycle similar to that based on biochemical studies [54]. In this work Kawai measured the dynamic stiffness at varied concentrations of MgATP, MgADP, P_i (phosphate) and temperature and explored changes in the rates of Equation 1.1-1. These data were used to estimate rates in the cross bridge cycle model based on the assumptions that any reaction significantly faster than the frequency of mechanical perturbation would be at equilibrium while any reaction slower than the frequency of oscillation would appear not to occur. Further, Kawai asserted that sinusoidal length perturbations affected the rates of transition between states in the cross bridge cycle only at or around the applied sinusoidal frequency (or rate of disturbance) and these altered rates were captured by changes in the time constants of Equation 1.1-1.

Another interpretation of dynamic stiffness data was presented by Campbell et al [55,56] and is based on an 18 parameter nonlinear model of the cross bridge cycle that captured the length dependence of transition rates between states, the cooperative interaction of neighboring regulatory units and the effect of sliding filament overlap. In Campbell’s early work [55] this model was linearized and fit to complex dynamic stiffness data [46, 47] using nonlinear least-squares techniques. Given that the dynamic stiffness data could be adequately represented using Equation 1.1-1 (with 7 parameters), Campbell’s fit was unsurprisingly over-parameterized. In a more recent paper Campbell et al [56] corrected this problem with a simplified 4 parameter model representing the dynamic stiffness as the sum of a low pass element (due to mechanisms of recruitment) and high pass element (due to distortion of attached cross bridges). Expressed in the Laplace domain the model was

\[
\sigma(s) = \frac{\Delta F(s)}{\Delta L(s)} = E_0 \frac{b}{b + s} + E_\infty \frac{s}{c + s},
\]

(1.1-2)

where E_0 and E_\infty are the 0 and infinite frequency stiffness while b and c are characteristic frequencies that are complicated functions of underlying rates in the cross bridge model (with b < c).
Campbell et al provided three links between the model of Equation 1.1-2 and the underlying physiology. $E_\infty$ was correlated with force and gave a measure of the number of strongly attached cross bridges. The rate constant $c$ was correlated with the ratio of the ATPase rate (a measure of cross bridge detachment) and the force produced. Finally, the rate of force redevelopment, $k_{TR}$, was correlated with $b$ (a measure of recruitment) [56].

In comparison, Kawai et al [43,51] used the following modified version of Equation 1.1-1 to give physiological significance to the parameters

$$\frac{Y(s)}{s} = \frac{H - B}{s + b} + \frac{C}{s + c} + \frac{D}{s + d}.$$  \hspace{1cm} (1.1-3)

Kawai related the fastest rate, $d$, to step 1b in their proposed cross bridge cycle model, representing the transition between two isomerization states of the actin myosin ATP complex. Rate $b$ and $c$ were considered together and related to both step 2, the transition between the actin myosin MgATP complex and the detached states, and step 4, the transition between the detached states and the force generating actin myosin ADP complex (hydrolysis having occurred during weak attachment in the detached states). Kawai hypothesized that rate $b$ was influenced more by step 4 (force generation) in agreement with a study on rabbit psoas muscle [50] while rate $c$ was primarily determined by step 2 (detachment).

Although not normally the case, if $H$ was approximately equal to $B$ then Equation 1.1-3 would reduce to

$$\frac{Y(s)}{s} = H - B \frac{s}{s + b} + C \frac{s}{s + c} + D \frac{s}{s + d},$$  \hspace{1cm} (1.1-4)

that can be compared to Campbell's model (Equation 1.1-2) by ignoring the high frequency component associated with rate $d$ and setting $E_0 = H$ and $E_\infty = C$. However, Campbell associated rate $b$ ($b < c$) indirectly with the rate of detachment and rate $c$ with the rate of force development. Kawai found the reverse, associating rate $b$ with force development and rate $c$ with detachment.
In summary, Campbell, Kawai and other researchers have found that the features of the dynamic stiffness can be captured by a model with 2 to 4 poles and zeros [50,51,56]. However, current models of the cross bridge cycle used by these authors require 10 [50] and 18 [55] parameters. Clearly the dynamic stiffness spectrum alone contains insufficient information to predict all the parameters in these underlying models. Kawai et al approached this problem by measuring changes in the dynamic stiffness due to perturbations in the concentration of MgATP, MgADP, Pi and temperature. Based on these results, Kawai claimed that he had developed a unique model of the cross bridge cycle with the qualification that no other model with the same degree of simplicity would fit his data [51]. While Campbell’s model has more parameters (complexity), it is also supported by experimental evidence [55] and yet comes to vastly different conclusions about the physiological processes underlying the features of the dynamic stiffness. This suggests that satisfying the dynamic stiffness is a necessary but not sufficient condition for validation of models of the regulation of contraction.

Furthermore, a fundamental problem underlying the association of kinetic parameters to features of the dynamic stiffness is the assumption that these rates are influenced by length perturbations in a linear manner at frequencies at or around the reaction rate. Kawai addressed the linearity of the system using the amplitude of harmonics in measured force data [43] and found that for length changes less than 0.23% of the initial length the total power in the first four harmonics was less than 3% that of the fundamental frequency. However, harmonics are not the only potential end effect of nonlinearity. A frequency domain, transfer function representation of a system assumes that a sinusoid (or complex exponential) is an eigenfunction of that system. Nonlinearities could distribute the input power arbitrarily over many frequencies (other than the fundamental and its harmonics). Conceptually, it is entirely feasible that a sinusoidal length perturbation applied to a highly nonlinear system like muscle tissue could affect rates that are significantly faster or slower than the applied perturbation.

In this thesis we are not using the dynamic stiffness as a means to infer details of the underlying regulation of contraction within muscle. That work is perhaps better
performed by direct measurement using molecular techniques that can provide sufficient information. Instead we treat the dynamic stiffness as a simple linear model of the mechanical properties of muscle and a means to validate the capabilities of the modular instrument under development. Furthermore, rigorously applying stochastic system identification techniques to measure the dynamic stiffness is the first step toward estimating parameters in nonlinear or time varying models [57,58] that are more able to reproduce the actual response of heart muscle cells to mechanical stimuli. These models could be used to strike an efficient balance between computational complexity and predictive accuracy in efforts to model the response of the heart as an organ [59].
1.2 Current instrumentation

A wide variety of transducers both commercial and developmental have been used to measure the mechanical properties of single cardiac myocytes. Complete systems require a position actuator and force transducer. Capacitive force transducers from Cambridge Technology (Watertown MA) [60] and precision actuators from Physik Instrumente (PI) [61] and Queensgate Nanopositioning [62] are commonly used commercial systems. However their high cost and size make them inappropriate for use in a high throughput device. Developmental devices predominantly use some form of cantilever as the force transducer including optical fibers, suction pipettes, glass needles, microfabricated polysilicon beams [63,28], 12 μm wire [64] and steel foil [65]. Displacement of the cantilever is detected via strain gauges [63], video analysis or optoelectronic methods [65]. In these devices the cantilever acts as a mechanical filter with properties that are adjusted using the trade off between compliance and resonant frequency. The former sets the minimum resolvable force and the latter the bandwidth of the measurement system. Several of the recent instruments developed for exploring cardiac myocyte mechanics will now be considered and their deficiencies highlighted. For a summary of older devices see Table I in [66].

Kenneth Roos’ group at UC Berkeley developed a disposable MEMS transducer for measuring the mechanics of single myocytes [63] (see Table 1.2-1) with the original intent of measuring the dynamic stiffness of cardiac myocytes at frequencies greater than 1 kHz [67]. The device was fully submersible and used polysilicon beams in a cantilever arrangement with a piezoelectric strain gauge to measure displacement. The length of the cell and the average sarcomere length were optically monitored directly. The system lacked a position actuator and was severely limited by position noise (the quoted force accuracy was over a bandwidth of only 7.2Hz).
Researchers at the Hanover Medical School in Germany have developed a device based on a simple atomic force microscope (AFM) design [65] (see Table 1.2-2). The apparatus used a 75 μm thick steel cantilever with a 1 mm tungsten needle extension to hold the myocyte in solution. Displacement of the beam was measured by reflecting a laser off the cantilever onto a split photodiode. The other end of the myocyte was manipulated by an open loop piezoelectric transducer (P-844.10, PI, Germany). The system had excellent force resolution (18 nN over 16 kHz) however it was band limited to 200 Hz by the low resonant frequency of the transducer, limited by stability to < 20 % strain (of the muscle) and sensitive to evaporation from the recording chamber.
More recently another German group headed by Stehle [28] has extended the use of the AFM system by coupling it with video microscopy to directly image individual sarcomeres (see Table 1.2-3). While the device specifications have not yet been published in detail [69], it appears to have excellent force resolution and high bandwidth. The device was designed to explore myofibrils (not myocytes) and hence has relatively high compliance and uses a very small cantilever [70].

<table>
<thead>
<tr>
<th>Force Transducer</th>
<th>Etched Si-Probe type AFM cantilever (Nanosensors, Germany [70])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution</td>
<td>Unspecified (inspection of graphs suggest ≈ 10-20 nN over bandwidth)</td>
</tr>
<tr>
<td>Resonance</td>
<td>25 kHz in air, unspecified in preparation</td>
</tr>
<tr>
<td>Compliance</td>
<td>≈ 0.25 to 0.5 mN ⇒ &lt;5% strain for 10 μN force</td>
</tr>
<tr>
<td>Calibration</td>
<td>Unspecified</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell Length Transducer</th>
<th>PI-P-821.20 piezoelectric transducer</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW</td>
<td>Sub millisecond measurement time [61]</td>
</tr>
<tr>
<td>Accuracy</td>
<td>±0.9 nm</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sarcomere Length Transducer</th>
<th>Off line image processing from video microscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW</td>
<td>25 fps</td>
</tr>
<tr>
<td>Accuracy</td>
<td>Unspecified (quoted results accuracy implies &lt; 40 nm over bandwidth)</td>
</tr>
</tbody>
</table>

| Attachment                  | 1:3 mixture silicon (3140 RTV coating, Dow Corning): 2% nitrocellulose in amylacetate |

**Table 1.2-4.** Specifications of the instrument developed by Stehle group [28].

The inherent nonlinear relationship between force and length in muscle has led to the use of isometric measurements of force and places an upper limit on the compliance of the cantilever. This problem has been addressed by utilizing feedback and an actuator to limit the deflection of the cantilever [64, 66]. Luo et al [66] placed a motor in series with a cantilever greatly increasing the moving mass of the system and hence lowering the bandwidth (27 Hz). Iwazumi avoided the latter problem with an elegant design that combined the cantilever and actuator into one structure [64] (see Table 1.2-4). By attaching a myocyte to two compliant wire loops in a magnetic field and independently controlling the current in each, Iwazumi was able to fix the position of one end of the muscle and measure force at the other. The resulting system had sufficient bandwidth and resolution to perform dynamic stiffness measurements on myocytes. However, it was designed to measure forces up to 1 μN (individual myofibrils) and displacements of at most a few μm. The high bandwidth, very low active compliance and compact design of Iwazumi's device [64] made it an attractive choice as the basis for our muscle testing apparatus.
<table>
<thead>
<tr>
<th>Force Transducer</th>
<th>Current through platinum wire coated in polystyrene (13 mA/μN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution</td>
<td>5 pN/Hz over 6 kHz BW</td>
</tr>
<tr>
<td></td>
<td>3 pN/Hz with no servo control and 2 kHz BW</td>
</tr>
<tr>
<td>Resonance</td>
<td>2.33 kHz with attached tungsten wire submerged in solution.</td>
</tr>
<tr>
<td>Compliance</td>
<td>≈ 2.8 m/N without servo control</td>
</tr>
<tr>
<td></td>
<td>≈ 0.5×10⁻³ m/N with control ⇒ 5×10⁻³ % change for 10 μN</td>
</tr>
<tr>
<td>Calibration</td>
<td>A 70 mm long quartz fiber (compliance 10000 m/N)</td>
</tr>
<tr>
<td>Length Transducer</td>
<td>Platinum wire coated in polystyrene (maximum range 1-3 μm) under servo control from split photodiode detection of wire position</td>
</tr>
<tr>
<td>PD Resolution</td>
<td>≈ 2 nm over 50 kHz ⇒ ≈ 9 pm/Hz on average</td>
</tr>
<tr>
<td>Wire BW</td>
<td>Wire position could be adjusted at 0.12 m/s</td>
</tr>
<tr>
<td>Sarcomere Length Transducer</td>
<td>Diffraction of light from 20W halogen light incident on linear PD array</td>
</tr>
<tr>
<td>BW</td>
<td>Unspecified</td>
</tr>
<tr>
<td>Accuracy</td>
<td>Read from oscilloscope at 2 μm/division</td>
</tr>
<tr>
<td>Myocyte Attachment</td>
<td>Dow Corning 3140 Silicon adhesive compliance of ≈3×10⁻³ m/N</td>
</tr>
</tbody>
</table>

Table 1.2-5. Specifications of the instrument developed by Iwazumi [64].

The instrumentation described in this section directly measured muscle or myofibril dynamics by attaching a force sensor and actuator to either end of the cell. Other approaches have been presented in the literature. For example, Ziles et al surrounded many cells with agarose gel [71] and applied length perturbations indirectly by stretching the agarose. This distributed forces across the entire surface of the cell perhaps mimicking natural connections to the extracellular matrix. However, it was very difficult to accurately determine the force applied to or produced by the cell.

Another recent novel approach involved depositing cells onto an array of micro-fabricated rods [72]. (Note the paper discusses the use of this technology with fibroblasts and smooth muscle cells however it would be equally useful with cardiac myocytes if sufficient attachment strength could be provided.) This technique has the potential to provide parallel measurements of many cells simultaneously. However, as currently described the rods are not actuated and the cells cannot be imaged with transmission microscopy (the substrate is opaque).

Finally, Yin et al attached a laminin coated magnetic bead to one end of the cell and calculated forces produced by the cell from the motion of the bead [73]. The cell was purportedly attached to the laminin coated slide in its central region to create a
mechanical base. This paper provided very little detail on the strength of attachment or how they estimated the reported 5 µN force of contraction. The idea of using electromagnetic fields rather than mechanical springs (cantilevers) to constrain the motion of a cell is discussed in Section 2.3.2.

In this thesis, the mechanical properties of single cells were measured directly using a novel actuator and force sensor design based conceptually on the work of Iwazumi [64]. As with all instruments designed to directly measure the mechanical properties of living myocytes, the attachment of the sensing apparatus to the cell was a key issue.
1.3 Myocyte Attachment

1.3.1 Myocyte attachment strategies

Attachment has long been a significant problem in studying the mechanical properties of single muscle cells (see review by Garnier et al [74] and introduction of [75]). While tetanic isometric tension was measured in 1940 in intact skeletal muscle cells [76], methodologies to explore intact mammalian ventricular cells only began appearing in the literature in 1979 [77] (see review by Brady [8]). Mammalian cardiac muscle cells are difficult to work with because of their relatively small size (compared with amphibian cardiac muscle cells) and, in the case of intact cells, the extreme sensitivity of the sarcolemma to applied stress.

Several attachment techniques for mammalian ventricular cells have been used including suction micropipettes [77, 75], immobilization against microscope slides via pressure [78] and various natural and artificial adhesives such as fibronectin [79], laminin [80, 73], poly-L-lysine [81, 82] and silicon adhesive [83]. Another technique developed by Le Guennec et al [84] and subsequently applied by Cazorla et al [85, 86] and others used an etched carbon fiber which was reported to provide successful attachment 80% of the time. The hypothesized mechanism of attachment was electrostatic attraction. It was thought that etching the fiber increased the charge density. Nishimura et al replicated this approach with custom carbon fibers that provided increased surface area [87].

None of these techniques were able to sustain electrically stimulated contractions of intact mammalian muscle cells at optimal sarcomere lengths of approximately 2.2 μm (the length at which maximal force is produced by the cell). Typically the cell would detach from the force sensor or actuator at sarcomere lengths on the order of 2 μm. In this project we develop a novel attachment strategy that is capable of supporting large contractions and is limited by contraction induced damage leading to cell death. Natural mechanisms of attachment could guide improvements in the current strategy.
1.3.2 Natural myocyte adhesion

An ideal methodology for connecting a force sensor and actuator to a myocyte would mimic natural attachment mechanisms. There are several key protein structures that cross the cell membrane and play a role in coupling forces between the extracellular matrix and the actin-myosin contractile apparatus in the adult myocyte. Two will be considered in more detail here; intercalated discs and costameres.

INTERCALATED DISCS

Intercalated discs are an important region of cell to cell coupling between cardiac myocytes and consist of three major junctional complexes (see review [88]):

- Fascia adherens connecting the actin cytoskeleton and contractile apparatus to the cell membrane.
- Desmosomes that attach intermediate filaments to the muscle termini.
- Gap junctions which serve as the means of electrical conduction.

Of these structures, the fascia adherens represent the most likely target for an attachment strategy. Cadherins are a major component of both fascia adherens and desmosomes. A recent paper by Chu et al [89] used a micropipette aspiration assay to assess the force of attachment between S180 cells stably expressing E-Cadherin and N-Cadherin. The two cells were gently pressed together and left for up to 60 minutes before being pulled apart. The minimum aspiration force required to hold the cells in both pipettes (separating the pair) was termed the separation force (SF). They found that attachment proceeded in phases, initially forming in approximately 30 seconds then linearly increasing in strength over 30 minutes before gradually increasing to a peak of 200 nN at 60 minutes. The authors noted that the cells were dissociated using a combination of trypsin and EDTA which disrupted the cadherins on the cell membrane (the population of cadherins on the surface recovered after 4 to 12 hours).

This study highlights three key issues relevant to the development of a myocyte attachment strategy. Firstly, the attachment strength observed in this and other studies
was less than 1 \( \mu \text{N} \) (too small to support myocyte contractions, perhaps suggesting that surface coatings alone would not provide sufficient attachment strength). Secondly, the time course of the attachment development would clearly be very important when attempting to bind myocytes to force sensors. A generally accepted model for the time course of cell attachment via integrins was proposed by McClay and Erickson who found that attachment strength increased rapidly in the first 15 minutes then gradually slowed to reach a maximum after 60 minutes [90]. Finally, the effect of the cell isolation protocol (more specifically the collagenase) on the cell membrane protein structures could critically affect the attachment. It would not be possible to wait 4 to 12 hours for these structures to regenerate as the quality of the myocytes would decline.

Despite their obvious role in attachment, intercalated discs would be a difficult target for an artificial attachment mechanism. This is primarily due to their location at the end of the cell that provides limited surface area for attachment (the ends of isolated myocytes are often jagged). As a result, most attachment strategies used in the muscle research literature bind to the sides of the myocyte making costameres a more likely target.

**COSTAMERES**

Costameres are membrane associated plaques that link the contractile apparatus at the Z and M lines (features of the sarcomere) to basement membrane extra cellular matrix (ECM) proteins such as collagen, laminin and fibronectin. The costameres are thought to provide mechanical stability and transmit contractile force from the Z line to the basement membrane. They consist of three primary junctional complexes (see review [88]):

- Integrins / focal adhesions that play a well studied role in both signaling (transduction of mechanical signals to cellular response) and cell adhesion to the ECM.
- Dystroglycan complex (DGC) which is composed of dystrophin, sarcoglycans and other proteins and is responsible for linking costameric actin to the muscle cell membrane.
- Spectrin based cytoskeleton elements whose roles are not yet well understood.
In an interesting study, Dalen et al explored the effect of an unspecified collagenase on the surface expression of immunoreactive fibronectin and laminin [91]. They found that fibronectin was thoroughly disrupted by the isolation process while laminin maintained its distribution in bands wrapped around the cell that conceivably could line up with the costameres on the Z and M lines [92, 93]. Dalen et al also found that neither laminin nor fibronectin were present at the ends of the cell in the intercalated disc regions.

As discussed earlier in this section, costameres, and more specifically integrins, are the most likely target for attachment using artificial methodologies that press coated glass or other materials against the surface of cells. Several researchers have attempted to use fibronectin [79] or laminin [80, 73] to bind myocytes with limited success presumably acting through integrins within costameres.

AREA, MECHANICAL DISTURBANCES AND ATTACHMENT STRENGTH
A recent paper by Gallant et al [94] used an elegant hydrodynamic force assay based on a spinning disk with microfabricated circular fibronectin coated patches varying from 5 μm to 100 μm in diameter to provide a very quantitative analysis of cell attachment as a function of surface area (using NIH3T3 fibroblasts). They found that cell attachment strength increased linearly with the area available up to about 75 x 10^{-12} m^2. Surprisingly, above this area attachment strength appeared to depend more on the formation of an adhesive patch (focal adhesion) at the edge of a cell that countered the torque of hydrodynamic drag with a peak force of approximately 200 nN. The authors demonstrated that the apparent saturation limit in adhesion was not set by the amount of fibronectin, integrin receptors, vinculin or talin. Instead, the authors hypothesized it could be an internal set point controlled by cellular processes.

The importance of cellular regulation of attachment strength is further demonstrated by earlier work by Riveline et al [95] which found that the application of force via a fibronectin coated substrate induced the formation of focal contacts at the edges of NIH3T3 fibroblasts. Interestingly if the cells were plated on poly-L-lysine coated
substrate mechanical stimulation did not increase focal contact formation suggesting that specific interactions with the ECM might be required to trigger this effect. Taken together, these studies suggest that significant contact area, appropriate surface coatings and gentle mechanical stimuli could improve the strength of myocyte attachment.
1.4 Thesis synopsis

The primary goal of this project was to develop instrumentation that was capable of parallel measurements of the loaded mechanical properties of individual cardiac myocytes. As is demonstrated in this introduction, leading research groups in this area consider it difficult to measure the mechanical properties of a single cell in slow, bulky and expensive custom instrumentation. Furthermore, attaching the cells to the force sensor and actuator in these systems remains a significant problem. The design of a modular, inexpensive apparatus capable of parallel measurement of the mechanical properties of muscle cells represented a significant instrumentation challenge that required the integration of mechanical and electrical engineering, optics, physiology, biology and mathematics. Such a device would have applications in high throughput studies of muscle physiology and for drug development and toxicology testing in the pharmaceutical industry.

This thesis describes the design and development of a novel modular system that is capable of performing mechanical tests on intact guinea pig ventricular muscle cells and is suitable for integration into an array for high throughput measurements. Chapter 3 and Chapter 4 are formatted as Journal papers. Chapter 3 describes details of the instrument design and presents the proof of principle measurement, the world's first characterization (to the author's knowledge) of the passive dynamic stiffness of a single cell. Chapter 4 outlines the design and development of an \( H_\infty \) control of muscle length and its application to the measurement of myocyte twitch force. Chapter 2 provides details of the design and development of the instrument beyond those presented in Chapters 3 and 4. It covers the position sensor, novel motor structure, signal processing strategy used to interpret measurements and digital control of cantilever position and force applied to the cell. It also describes the isolation, loading, attachment and electrical stimulation of the intact cardiac myocytes used to demonstrate the functionality of the device. Chapter 5 presents some preliminary measurements of the active dynamic stiffness of intact myocytes while Chapter 6 concludes the thesis and suggests possibilities for future work.
2 Details of the Design

Before beginning a detailed description of the design and testing that represent the body of this thesis it is worthwhile noting that a complete list of specifications for the ideal device did not exist at the beginning of the project. Tradeoffs and specifications were uncovered as the work progressed and, where practical, integrated into subsequent iterations. A collection of proof of principle measurements of myocyte mechanics were performed at the end of this project. These experiments gave critical insights into the desired operation of the instrument. The integration of these final considerations into the device should be the subject of future work (see Section 6.2.1). This section describes the development of the current design and subsequent experimentation roughly in chronological order covering the position sensor, current source, motor system, signal processing strategy, $H_\infty$ controller and myocyte experimentation.
2.1 Position Sensor

2.1.1 Broad design requirements

The position sensor was the first part of the system to be designed and is a critical element of the system as its resolution limits both the minimum detectable force and position. The original design goals for the sensor required a bandwidth greater than 10 kHz and position resolution below a nanometer and a dynamic range of at least $10^5$ (peak signals greater than 100 μm). To be suitable for an array, the sensor also needed to be inexpensive, easy to align and be capable of simple integration with a digital data acquisition system.

Many different position transducer mechanisms were considered. Strain gauges are compact, relatively easy to align, inexpensive and require simple analog signal processing. However, they are highly temperature-sensitive and the relatively high currents through the cantilevers (see Section 2.3) would introduce significant thermal noise. Capacitive position sensors can have very high resolution, are inexpensive and provide lower temperature coefficients than strain gauges. However, they would be difficult to integrate into this application as they require significant surface area, would be highly nonlinear (non parallel plate configuration), are not well suited to displacements as large as ± 100 μm and would suffer from variations in the dielectric constant of the physiological saline when fully immersed.

In this design, optical position sensors had the advantage of separating the sensing element from the difficult environment around the cantilevers. Interferometers have excellent bandwidth, resolution and dynamic range and would be an ideal solution for a stand alone system to measure the mechanical properties of a single cell. They are less suitable for an array application as they are bulky, relatively expensive and difficult to align. A more appropriate choice might be the combination of a laser and split photodiode (similar to that used by Stehle et al [28]). The laser is incident at an angle to the surface to be monitored and reflected onto the center of the split photodiode.
Displacement is sensed by comparing the amplitude of the two photodiode currents. However, this system would be reasonably difficult to align and the components would need to be packaged together (light source and photodiode).

After considering the available sensor options a position sensor system using a quasi-confocal optical detection system similar to that of Brennan et al [96] was selected. The sensor, a HEDS-1300 bar code scanner, was commercially available from Agilent (now sold as the HBCS-1100 by Avago Technologies) and could provide sub nanometer resolution after suitable amplification.

2.1.2 The HEDS-1300 sensor

The HEDS-1300 sensor combines a light emitting diode (LED), photodiode and bifurcated aspheric lens in a compact TO5 package. Light from the sensor was reflected from a target and generated a current in the photodiode. A schematic of the sensor and the current profile as a function of distance from the reference plane of the sensor are provided in Figure 2.1-1. To maximize the sensitivity of the position sensor, the distance from the sensor to the reflective surface was set to ensure the steepest gradient between displacement and photodiode current.

![Figure 2.1-1](image-url)
The LED could sink a maximum of 50 mA continuous current and, according to the data sheet, the reflected light from a piece of white tape generated a maximum 650 nA in the photodiode. In our experience currents in excess of 1.3 µA could be generated if the sensor was reflected from a mirror. Under typical operating conditions when the light was reflected from a stainless steel cantilever immersed in physiological saline the device produced on the order of 1 pA/nm requiring a sensitive transimpedance amplifier to generate a useable position signal. The transimpedance amplifier was designed with a gain of $21 \times 10^6$ V/A and provided approximately 20 mV/µm at its output.

Both the photodiode and the LED were quite sensitive to temperature fluctuations. The data sheet describes the relationship between temperature, $T$, and photodiode current, $I_p$, as

$$I_p(T) = I_p(T_0) e^{K(T - T_0)}, \quad (2.1-1)$$

where $T_0$ is the initial temperature and $K$ is -0.01 1/°C for a forward current in the LED of 35 mA ($I_F$). For small changes in photodiode temperature ($\Delta T_{PD}$), the photodiode current decreased by approximately $I_p(T_0) \times 0.01 \times \Delta T_{PD}$. Given that the operating current was typically 300 nA this corresponds to -3 nA/°C or -63 mV/°C at the output of the position sensor. Thus, thermal fluctuations on the order of mK will degrade system performance (as discussed in Section 2.1.3).

The LED optical output also changes with temperature. Both the saturation current, $I_s$, and the forward voltage, $V_F$, are functions of temperature. They can be related to the current through the LED and hence its optical output by

$$I_{LED} = I_s \left( e^{\frac{V_F V}{kT}} - 1 \right), \quad (2.1-2)$$

where $k$ is Boltzmann's constant, $q$ is the charge on an electron and $n$ is constant that depends on the material properties of the diode. The data sheet suggests that, for small changes in temperature, the optical output of the LED, $I_{out}$, and hence the current through the photodiode, $I_p$, decreases by approximately $I_p(T_0) \times 0.008 \times \Delta T_{LED}$ (assuming $I_{out}$ is proportional to $I_p$). For an operating photodiode current of 300 nA this corresponds to
-50 mV/°C. The combined effect of thermal interference on the photodiode and LED is considered in more detail in Section 2.1.4.

As the two position sensors necessarily face each other in the design, the profile of the beam incident on the reflective surface determines the amount of cross talk between the two position sensors (see Section 2.3.2). The beam profile at varied distances from the sensor was measured using a firewire CCD camera that provided approximately 10 μm/pixel (Orange IBOT). It was found that the profile at the focal point had a primary central peak 400 μm in diameter and two ancillary peaks above and below this (see Figure 2.1-2). The beam profile in a typical operating position 500 μm from the focal point was also provided in this Figure. At the operating position the central spot had expanded to an ellipse with major and minor diameters of approximately 500 μm and 800 μm respectively.

The position sensor was reflected from the tip of the cantilevers that had dimensions 1 mm × 0.4 mm. To minimize cross talk between the sensors they should be aligned with the orientation tab vertical. This ensured the majority of light would be incident on the back surface of the cantilever. The need to have the entire spot captured by the sensor to minimize cross talk and maximize signal strength was balanced by the requirement to minimize the moving mass of the force sensor (see Section 2.3.3).
2.1.3 Circuit design

Having chosen the appropriate position sensor for our system a compact low noise circuit was required to amplify the position signal and offset it such that it filled the dynamic range of an analog to digital converter (ADC). The electronics can be divided into two parts, a current source for the LED within the HEDS-1300 and a transimpedance amplifier for the photodiode current.

LED CURRENT SOURCE

The LED required a current source that could provide approximately 35 mA of continuous current. Noise in the current flowing through the LED would translate directly into intensity fluctuations of its optical output and hence amplitude noise in the position signal. According to the data sheet the photodiode current increases linearly with $I_{LED}$ such that

$$I_{PD} = I_{PD35}(1 + 0.03(I_{LED} - 35)), \quad (2.1-3)$$

where $I_{LED}$ is the current through the LED (in mA) and $I_{PD35}$ is the current in the photodiode when $I_{LED} = 35$ mA. Again assuming an operating photodiode current of 300 nA this corresponds to a position sensor output voltage error of 189 V/A change in LED current.

To minimize current fluctuations, the final current source design (see Figure 2.1-3) used a low noise buffer amplifier, the LT1806, capable of providing 80 mA of output current. The op-amp set the combined voltage across a 22 $\Omega$ resistor and the LED equal to a reference voltage provided by a precision bandgap integrated circuit (the LT1019).

![Figure 2.1-3 A simplified circuit diagram of the LED current source. $R_{SENSE}$ was chosen to set the current through the HEDS-1300 LED at 35 mA and $V_{REF}$ was provided by an LT1019 precision voltage reference.](image-url)
Noise Analysis

The voltage across the resistor is approximately \(2.45 - V_F = 0.75\) V where \(V_F\) is the forward voltage of the LED (1.7 V while on). Fluctuations in the output voltage of the op-amp will fall mostly across the resistor as the minor changes in current will be absorbed with very little alteration of \(V_F\). The LT1806 has 800 nV peak to peak (p-p) of voltage noise, \(e_n\), (565 nV\(\text{RMS}\) if sinusoidal) between 0.1 Hz and 10 Hz and 3 nV/\(\sqrt{\text{Hz}}\) at 10 kHz. Thus in a 10 kHz bandwidth the standard deviation, \(\sigma\), of the voltage noise of the LT1806 referred to the output would be approximately 0.86 \(\mu\)V. This would create current noise in the LED with \(\sigma = 39\) nA equivalent to voltage noise at the output of the position sensor with \(\sigma = 7.4\) \(\mu\)V. In this and subsequent calculations, it was assumed that the amplitude of peak to peak noise, \(V_{\text{p-p}}\), was related to the RMS amplitude, \(V_{\text{RMS}}\) according to \(V_{\text{p-p}} = \sqrt{2} \times V_{\text{RMS}}\) (a weak assumption) and that the noise was zero mean so that its standard deviation was equal to \(V_{\text{RMS}}\). The current noise of the LT1806 is 1.5 pA/\(\sqrt{\text{Hz}}\) which would flow through the very low output impedance of the LT1019 (0.02 \(\Omega\)) and create negligible current noise in the LED.

The LT1019 voltage reference has 6.1 \(\mu\)V p-p noise between 0.1 and 10 Hz (4.3 \(\mu\)V\(\text{RMS}\)) and additional voltage noise between 10 Hz and 10 kHz with standard deviation of 6.1 \(\mu\)V. This would create current noise in the LED with \(\sigma = 470\) nA or voltage noise at the output of the sensor with \(\sigma = 89\) \(\mu\)V.

Thermal noise or drift will also affect all the components including the LED. In this analysis we will assume the thermal noise is 1/\(f\) distributed with \(\sigma = 0.1\) \(^\circ\)C between 0.1 Hz and 10 Hz. The LED, LT1019 and 22 \(\Omega\) resistor are dissipating significant power and reach a steady state temperature approximately 5 to 10 \(^\circ\)C above ambient during operation. This increases their sensitivity to wind and ambient thermal fluctuations. The 22 \(\Omega\) surface mount resistor has a temperature coefficient (tempco) of 100 ppm/\(^\circ\)C creating negligible fluctuation in the LED current. The LT1806 offset voltage drifts by 1.5 \(\mu\)V/\(^\circ\)C which is transferred to its output with unity gain and creates position sensor output voltage noise with \(\sigma = 1.3\) \(\mu\)V. The LT1019 output drifts by 12.5 \(\mu\)V/\(^\circ\)C producing
output voltage noise with $\sigma = 11 \mu V$. In comparison, using the analysis from the previous section, this level of thermal noise will create noise at the output of the position sensor with $\sigma = 5 mV$ when the operating current is 300 nA.

This analysis suggests that displacement noise introduced by the current source circuit is dominated by thermal fluctuations of the LED that alter $V_F$ and hence $I_{LED}$ and the intensity of light produced. This issue could be addressed by using a differential amplifier such as the AD620 to monitor the voltage across (and current through) the 22 $\Omega$ resistor directly. The LT1806 could then be used to regulate this voltage (rather than the potential across both the resistor and LED). This would ensure that $I_{LED}$ was insensitive to the influence of the temperature of the LED on $V_F$ and potentially eliminate the dominant noise source in the current source design.

Another possible improvement of this design would be modulation of the LED drive current. By modulating the drive, higher peak currents could be used (up to 75 mA) which would create larger photodiode currents. Furthermore, independent modulation of each position sensor could limit cross talk between the two (see Section 2.3.2). However, this would significantly increase the complexity of the drive circuit, potentially degrade low frequency performance and introduce high frequency noise near the very sensitive transimpedance amplifier.

**TRANSIMPEDANCE AMPLIFIER**

The transimpedance amplifier (see Figure 2.1-4) served to amplify the photodiode current in the HEDS device to provide the resolution and dynamic range necessary to measure the mechanical properties of myocytes. The amplifier (with net gain of $21 \times 10^6 V/A$) consisted of three stages, an AD795 configured as a transimpedance amplifier with gain $2 \times 10^6 V/A$, a second stage OP1177 summing amplifier with gain of 10.5 V/V which set the operating position by removing an offset set by a precision LT1019 voltage reference and an LT1881 output buffer. The operating point was defined as the current through the photodiode that would produce 0 V at the output of the amplifier.
Noise Analysis

The goal of the circuit design was to ensure the position signal was shot noise limited for the majority of the measurement bandwidth (0.1 Hz to 100 Hz). Shot noise is a white noise source that is a result of the discrete nature of electrical current and can be estimated using

\[ I_{SN} = \sqrt{2qI_{DC}} \cdot A/\sqrt{Hz} \tag{2.1-4} \]

where \( q \) is the charge on an electron and \( I_{DC} \) is the current level. The 300 nA operating point of this system would create approximately 6.5 \( \mu V/\sqrt{Hz} \) voltage noise at the output of the position sensor (\( \sigma = 650 \mu V \) over a 10 kHz bandwidth). As is discussed in Section 2.1.4 it was difficult to limit the output noise at this level for frequencies below 50 to 100 Hz.

The choice of op-amp for the first stage of the amplifier was influenced by the need to limit output voltage noise. The primary motivation for selecting the AD795 was its very low input current noise, \( i_n \), of 13 fA p-p (9.2 fA RMS) between 0.1 and 10 Hz and 0.6 fA/\( \sqrt{Hz} \) at 1 kHz. Over a 10 kHz bandwidth this would produce voltage noise on the position sensor output with \( \sigma = 1.4 \mu V \). Input voltage noise of the AD795 would be amplified by the 10.5 voltage gain of the circuit to produce output noise with standard
deviation 18.6 \mu V. Another source of noise in this stage is Johnson noise in the 1 M\Omega feedback resistors governed by
\[ V_j = \sqrt{4kTR} \, V/\sqrt{Hz} \, . \] (2.1-5)
where k is Boltzmann's constant, T is the temperature in Kelvin and R is the resistance. This will produce approximately 2.5 \mu V/\sqrt{Hz} of white voltage noise at the output of the position sensor (\sigma = 250 \mu V over a 10 kHz bandwidth).

The OP1177 summing amplifier has relatively low input voltage noise (0.4 \mu V p-p between 0.1 Hz and 10 Hz and 7.9 nV/\sqrt{Hz} at 1 kHz) which after accounting for the gain of the amplifier produces approximately \sigma = 10.7 \mu V of output noise. The effect of its current noise (0.2 pA/\sqrt{Hz}) is negligible due to the low impedance seen by the amplifier inputs (approximately 3.4 k\Omega). The same precision reference, the LT1019, used in the current source provides the offset voltage and introduces voltage noise with a standard deviation of 10.4 \mu V at the summing input or 35 \mu V at the position sensor output.

Thermal noise or drift can be considered by again assuming it is 1/f distributed with \sigma = 0.1 \degree C between 0.1 Hz and 10 Hz (although the majority of the components in the transimpedance amplifier dissipate less power than those of the current source and hence would probably suffer less from thermal fluctuations). Drifts in the offset voltages of the two op-amps produce negligible output noise. The LT1019 output drifts by 12.5 \mu V/\degree C producing output voltage noise with \sigma = 4.2 \mu V after accounting for gain. The 100 ppm tempco of the resistors in the circuit are reasonably well matched so the summing amplifier gain remains approximately constant. In comparison, drift in 1 M\Omega resistors used in the first stage amplifier will produce output voltage noise with standard deviation of approximately 63 \mu V (assuming a 300 nA operating current). However, the dominant noise source is again expected to be thermal fluctuations within the HEDS device. Using the analysis from Section 2.1.2 thermal drift of the photodiode current around a 300 nA operating point will create noise at the output of the position sensor with a standard deviation of roughly 6.3 mV.
CIRCUIT LAYOUT
The following measures were taken when laying out the double sided printed circuit boards (PCBs) to minimize interference:

- Ground planes on both sides of the PCB split to separately surround the current source and photodiode amplifier.
- 0.1 μF ceramic capacitors at the power pins of all integrated circuit components.
- Very short traces between the photodiode pins and the first stage transimpedance amplifier.
- As much separation between the photodiode pins and the current source as physically possible.
- Star configurations on power and ground traces to minimize voltage drops on common paths.

2.1.4 System characterization

CALIBRATION
The calibration strategy for the cantilevers and position sensors are described in Sections 3 and 4 and the reference [97]. The system was calibrated with all forces and displacements referred to the tip of the cantilever where the myocyte would attach.

LINEARITY
The relationship between photodiode current within the HEDS-1300 and distance between the front of the device and a reflective surface is inherently nonlinear. To quantify this relationship, an aluminum surface mounted on a micrometer was placed in front of the device. The output of the first stage of the transimpedance amplifier was measured as a function of displacement of this surface. The normalized results are presented in Figure 2.1-5A. Applying a linear fit to a small operating region of ± 100 μm on the rising edge of this profile yields an excellent R^2 of 0.9988 (see Figure 2.1-5B). The accuracy of this measurement was limited by the manually operated micrometer which was moved in steps of approximately 20 μm.
RESOLUTION

The resolution of the sensor could be determined by estimating the power spectral density (PSD) of the position signal when light from the HEDS-1300 device was reflected from a stationary surface. Ideally, the position signal would be constant and (after the removal of the mean) the PSD would be zero at all frequencies. The resolution of the device over a given bandwidth \( f \in [f_{\text{min}}, f_{\text{max}}] \) was defined as the standard deviation, \( \sigma \), of the position signal after it had been bandpass filtered with cutoffs at \( f_{\text{min}} \) and \( f_{\text{max}} \). This could be related to the PSD (in units of V\(^2\)/Hz) using

\[
\sigma = \frac{\sqrt{\int_{f_{\text{min}}}^{f_{\text{max}}} \text{PSD}(f) df}}{G_{\text{PS}}} \ m, \tag{2.1-6}
\]

where \( G_{\text{PS}} \) is the sensitivity of the device in V/m or the change in the output of the amplifier for a given displacement of the reflective sensor (proportional to the gradient of Figure 2.1-5A). As is evident in this figure, the optimal operating region (with high linearity and steep gradient) extends from approximately 0.6 to 0.9 of \( I_{\text{PD MAX}} \). The sensitivity of the position sensor in this region was approximately 60 kV/m when the sensor was reflected from a mirror and 5 to 20 kV/m when the sensor was reflected from a cantilever in solution.
The position signal was sampled at 20 kHz with a 16 bit National Instruments data acquisition card (NI6052E). The PSD was estimated from a $4 \times 10^6$ point position signal using the \textit{pwelch} function in Matlab with 50 \% overlap of 131 072 point sections and a Hamming window. To gain further understanding of the sources of noise in the position signal multiple measurements were made at varied operating points (currents through the photodiode) by adjusting the distance between the sensor and a mirror. A plot of the PSD at four currents between 250 nA and 1342 nA is presented in Figure 2.1-6.

![Figure 2.1-6](image)

\textbf{Figure 2.1-6.} The PSD of the position signal at four operating points (currents through the photodiode). The low frequency noise is roughly proportional to $I_{PD}$ while the noise above 300 Hz is proportional to $\sqrt{I_{PD}}$.

A detailed noise analysis of position sensor amplifier was presented in the previous section and concluded that the dominant sources of output noise in this circuit were shot noise in the photodiode (proportional to $\sqrt{I_{PD}}$), noise due to thermal fluctuations of the photodiode (proportional to $I_{PD}$) and noise due to thermal fluctuations of the LED (also proportional to $I_{PD}$). As can be seen in Figure 2.1-6, the PSD is not shot noise limited in
the measurement bandwidth between 0.1 Hz and 50 to 200 Hz (depending on the current level). The average PSD at 1 Hz and 1 kHz are plotted as a function of \( I_{PD} \) in Figure 2.1-7A and B. This Figure demonstrates that the low frequency noise was proportional to \( I_{PD} \) (supporting the hypothesis that it was a result of thermal fluctuations in the LED and photodiode) while the high frequency noise was proportional to \( \sqrt{I_{PD}} \) and very close to theoretical shot noise limit. As the current through the photodiode increases a larger frequency range is dominated by the noise proportional to \( I_{PD} \). The noise begins rolling off above 3 kHz due to a single pole anti-aliasing filter.

![Figure 2.1-7A](image)

**Figure 2.1-7A** The average PSD at 1 kHz compared with the theoretical shot noise. The measured noise is a function of \( \sqrt{I_{PD}} \) and close to the theoretical shot noise limit. **B.** The average PSD at 1 Hz. There is a linear relationship between \( I_{PD} \) and the noise floor in this region.

The noise proportional to \( I_{PD} \) rises above the shot noise limit at frequencies up to 200 Hz (depending on the current level). The temperature of the outside of the HEDS-1300 case was monitored using a thermistor and it was found that the time constant in response to step changes in temperature (observed at switch on) was approximately 10 seconds which is far slower than the observed noise. Assuming the shot noise and hypothesized thermal noise are uncorrelated the total variance of the position signal is the sum of the variance of each noise source. When \( I_{PD} \) was 1340 nA, the excess noise above the shot noise limit had standard deviation of 5.3 mV (the total noise had \( \sigma = 5.5 \) mV and the theoretical shot noise had \( \sigma = 1.3 \) mV).

Using the results of Section 2.1.2 at this operating point the thermal fluctuations in the photodiode and LED would introduce noise with \( \sigma = 280 \) mV/°C and 225 mV/°C.
respectively. Therefore, making the simplistic assumption that the two thermal noise sources are uncorrelated, thermal fluctuations with a standard deviation of only 15 mK would be required to introduce the observed noise variance ($\sigma = 5.3 \text{ mV}$). The LED and photodiode are very small (< $25 \times 10^{-12} \text{ m}^3$) with equivalently small thermal mass (< 50 $\mu$J/K, assuming they are constructed primarily from Si). They are attached to a small gold coated disc at the base of the HEDS-1300 device with thermal mass of approximately 60 mJ/K (see Figure 2.1-8). Depending on the quality of thermal connection it is plausible that mK fluctuations in temperature could occur rapidly enough to account for the observed noise at 100 Hz.

As a final note, the results presented in Figure 2.1-6 and Figure 2.1-7 were measured while the sensor was reflected from an aluminum surface. At the typical operating point in the device, when the reflective surface was a parylene coated cantilever immersed in physiological saline, the value of $I_{PD}$ was typically between 250 nA and 300 nA. The PSD of the position noise of the experimental system is presented in Section 3.

**The effect of quantization**

The input range of the 16 bit analog to digital converters (ADCs) of the NI6052E were set to ± 1V which introduced a minimum step size of $\Delta = 3 \mu$V (between 0.3 nm and
0.05 nm depending on the reflective surface). The quantization would limit the precision of a single measurement of position to ± 1.5 µV. A 16 bit ADC only provides a dynamic range of approximately $0.65 \times 10^5$. Ideally, an 18 bit ADC would be used as a minimum to ensure displacements as large as ± 100 µm could be accommodated.

Quantization errors have less effect on stochastic system identification techniques or the estimation of power spectra where averaging is employed. To consider this, first define the error sequence as $e[n] = x[n] - y[n]$ where $x[n]$ is the actual position signal and $y[n]$ is the output of the ADC. Assume that $e[n]$ can be modeled as an additive noise source that is a sample of an ergodic, white, stationary random process uncorrelated with $x[n]$ and uniformly distributed between ± $\Delta/2$. The variance of each sample of $e[n]$ would then be $\sigma = (\Delta^2/12)^{1/2} = 0.9$ µV [98]. Furthermore, when the signal is sampled at 20 kHz, the PSD of $e[n]$ will have an amplitude 9 nV/√Hz which is well below the estimated PSD of the position signal. While these assumptions are unlikely to be completely satisfied the results presented in Figure 2.1-6 suggest that the quantization noise did not dominate the estimate of the position signal PSD (as it achieved the shot noise limit albeit for frequencies greater than 100 Hz). Thus it was concluded that the quantization error limited the accuracy of a single measurement of position but did not limit the resolution of the sensor as defined by Equ 2.1-6.

### 2.1.5 Possible improvements

In the current design the HEDS-1300 position sensor provided sub nanometer resolution for frequencies above 10 Hz and was robust, inexpensive and easy to align. However, there was room for improvement. The noise floor increased with increasing current through the photodiode (as a function of $I_{PD}$ or $\sqrt{I_{PD}}$) while the sensitivity of the sensor increased linearly with the current through the photodiode. Provided the noise floor remains shot noise limited for a region of the measurement bandwidth increasing the current will improve the position resolution as defined by Equation 2.1-6. Therefore it is desirable to improve the quality of the reflective surface seen by the position sensor in the device. Furthermore, the spot size of the device could possibly be reduced by integrating an external lens with the HEDS-1300 device. Avago Technologies sells several possible
devices (HBCS-2999 and others). Reducing the spot size would ensure all light was reflected from the back surface of the cantilever. In addition, focusing the light would serve to increase the position sensitivity at the cost of sensor range.

Ideally, the sensor would be limited by the shot noise in the photodiode (a fundamental physical limit of photodiode based detectors). To achieve this, the thermal noise that is hypothesized to dominate at low frequency would need to be reduced. An approach to correct the thermal drift of $I_{LED}$ was discussed in Section 2.1.3. The forward voltage of the LED, $V_F$, varies by approximately -1.2 mV/°C. Given the close proximity of the LED to the photodiode, this voltage could be amplified and used as a measure of the temperature of the photodiode. It could then provide the basis of a digital compensation scheme designed to limit the effect of thermal noise on $I_{PD}$. However, the success of such a compensation strategy would probably be limited by the temperature resolution given that the estimated standard deviation of thermal noise causing this problem was only 15 mK. By combining these improvements it should be possible to increase the position sensor resolution by a factor of 3 to 10 which would be critical for future experimental work (see Section 6.2).
2.2 Current Source

2.2.1 Circuit design

Two identical, independent current sources were designed to drive the cantilever actuators. A simple design (see Figure 2.2-1) was used to provide the basis of the current source; a power op-amp in a unity gain negative feedback configuration setting the voltage across a series connection of the cantilever and a sense resistor. The principle concerns were:

- Sufficient dynamic range and adequate noise performance to produce forces as small as 10 nN and as large as $\pm 200 \mu$N.
- Sufficient slew rate and peak current to impose rapid step changes in length on the muscle cell. Preferably capable of stretching a cell through its full physiological range (30 $\mu$m) in under 100 $\mu$s (1.5 kHz bandwidth).

![Figure 2.2-1. A simplified circuit diagram of the current source. The LT1970 power amplifier set the voltage across and current through the cantilever and sense resistor. An AD620 measured the voltage across the sense resistor.](image)

The choice of amplifier to provide the current drive was set by the inherent tradeoff between amplifier output performance (slew rate and peak current) and noise performance. The slew rate ($V_{SLEW}$, V/s) provides one limit on the maximum achievable bandwidth ($\omega_{MAX}$) for a given maximum peak to peak output current ($I_{MAX}$) according to

$$\omega_{MAX} = \frac{V_{SLEW}}{R_{NET}I_{MAX}},$$  \hspace{1cm} (2.2-1)

where $R_{NET}$ is the net resistance of the series connection of the cantilever and sense resistor in this circuit.
The size of $R_{NET}$ sets the conversion between current and voltage which dictates the minimum detectable current and potentially limits the output current due to saturation at the voltage rails. In the unity gain configuration, input voltage noise ($e_n$) from the current source amplifier will appear directly at the output and translates to a current noise of $e_n/R_{NET}$ in the cantilevers. Increasing $R_{NET}$ improves the current (force) resolution but decreases the slew rate bandwidth limit and increases power dissipation.

The peak output current sets the maximum steady state displacement of the cantilevers that could be achieved. The motor structure could produce approximately $1 \times 10^{-3}$ N/A of current drive (see Section 2.3.3) so for a cantilever with stiffness of 2 N/m the actuator could provide $0.5 \times 10^{-3}$ m/A. The combination of peak applied force and desired step size ($\Delta x$) also limited the maximum bandwidth by limiting the peak stiffness or magnitude of the dynamic stiffness, $|H(j\omega)|_{\text{MAX}}$, at a given frequency, $\omega_0$ according to

$$|H(j\omega_0)|_{\text{MAX}} = \frac{I_{\text{MAX}} L\mathbf{B}}{\Delta x},$$

(2.2-2)

where $L\mathbf{B}$ is the coupling between current and force in N/A.

Based on these design equations the LT1970 was selected as the current source amplifier. It could provide a peak current of $\pm 0.4$ A ($\pm 200$ μm peak displacement) and had an output slew rate of $1.6 \times 10^6$ V/s. The slew rate limited the bandwidth to 22.7 kHz for full signal swing. The amplifier was able to provide $\pm 30$ μm steps provided $|H(j\omega)| < 13.3$ N/m. For our cantilevers with stiffness at 0 Hz of 2 N/m, damping coefficient of 0.1 and first resonance at 330 Hz (see Section 2.3.3) this limits $\omega$ to less than $2\pi \times 2.5$ kHz (approximately 65 μs rise times).

The LT1970 has an input noise voltage of $3$ μV p-p ($2.1$ μV RMS) between 0.1 Hz and 10 Hz and 15 nV/√Hz at 1 kHz. Over a bandwidth of 1 kHz this corresponds to voltage noise with a standard deviation of 2.6 μV (current noise beyond resonance will be attenuated by the mechanical transfer function of the cantilever and can be ignored). For
\( R_{\text{NET}} = 14 \ \Omega \) this is equivalent to force noise with standard deviation of 0.19 nN more than satisfying the design criteria.

Thermal noise or drift can be considered by again assuming it is 1/f distributed with \( \sigma = 0.1 \ ^\circ \text{C} \) between 0.1 Hz and 10 Hz. Further assuming the combination of the sense resistor and cantilever has tempco of 100 ppm/°C (the sense resistor alone has a tempco of 50 ppm/°C) this will introduce force noise with standard deviation of approximately 40 nN at the peak operating current of 0.4 A. At typical operating currents of 25 mA this would produce noise with a standard deviation of 2.5 nN. Other sources of noise such as Johnson noise in the sense resistor and cantilevers and amplifier current noise and offset voltage thermal drift and had negligible effect on current output.

It should be noted that the position sensor was still the dominant source of noise limiting the resolution of force a fact easily verified experimentally. Further improvements in the current source noise performance would only be necessary if the position sensor resolution increases significantly.

2.2.2 Other considerations

The layout of the printed circuit board used the methodologies discussed in Section 2.1.3. In addition, completely independent power sources were used to separate the high current supply to the cantilevers from the sensitive electronics used to measure current levels and a large plane of copper was connected to the power amplifier base to ensure adequate thermal dissipation. Furthermore, care was taken to ensure no high current traces were in close proximity to the position sensor to limit interference coupling observed in early designs.

The final circuit design also used an AD620 differential amplifier with gain of 0.5 to measure the voltage across the sense resistor. The output of the AD620 was passed through a buffer to a National Instruments 6052E (NI6052E) analog to digital converter (ADC) to record the current through the cantilevers. The input range of the 16 bit ADC of
the NI6052E was set to ±5V which introduced a minimum step size of \( \Delta = 15 \mu V \). The quantization would limit the precision of a single measurement of force to approximately ±1 nN. Earlier designs attempted to close an analog control loop using the output of the AD620 in the feedback path however this introduced instability due to significant phase lag.

Finally, it should be noted that by using the power amplifier in a negative feedback configuration to regulate the voltage across the sense resistor and cantilever minimized effects such as electromagnetic coupling of current between the cantilevers and back electromotive force (see Section 2.3.2). The feedback would act to cancel any changes in voltage across the sense resistor created by currents induced in the cantilevers.
2.3 Motor structure

2.3.1 Design requirements

The motor structure is the heart of the instrument. At the beginning of the project, the basic design objectives for the motor structure were to attach a compact, inexpensive force sensor and actuator to both sides of a myocyte. Actuating both ends of the cell conveyed several advantages. Firstly, the entire length of the cell could be scanned under a stationary imaging system. Secondly, a point on the cell could be held stationary with respect to an external imaging system or patch clamp even while applying length perturbations potentially allowing simultaneous mechanical and electrophysiological measurements. Thirdly, the force sensor signals could be averaged to provide common mode noise rejection. Finally, by actuating the force transducer, feedback could be used to adjust the sensors effective stiffness as discussed in Section 4.

Based on the properties of a myocyte (see Section 1.1), it was decided that the actuators should have at least ± 100 µm of travel and a bandwidth of at least 300 Hz while the force sensors should have sub nano-Newton resolution. Appropriate force sensor and actuator technologies were required to achieve these objectives.

FORCE SENSORS

While typical force transducers quantify force by measuring the deflection of a mechanical bending element other approaches were considered. Conceptually, the force sensor required a small mass trapped in a three dimensional potential energy well. The gradient of the well in the direction of motion sets the restoring force when the mass is displaced by Δx. The energy well could be provided by a simple one dimensional spring with stiffness k and potential energy $E = \frac{1}{2} \times k(\Delta x)^2$ (which has very high stiffness or steep potential energy gradients in all but one dimensions). Alternatively it could be provided by a magnetic field (magnetic traps [99, 80,73]) or electromagnetic field (optical trap, see review [100]). To provide actuation in the later two cases, the field could be adjusted to displace the energy minimum. The “spring” could then consist of a
small particle that was somehow integrated into a fluidics system for loading and inspecting the cells. Another potential advantage of electromagnetic fields is that they could create a nonlinear relationship between displacement and corrective force that would allow a greater dynamic range of forces to be detected for a given position sensor (at the cost of increased design and calibration complexity). However, current optical and magnetic traps are only suitable for forces between 100 pN and 10 nN and would not be able to constrain the 10 μN a cardiac myocyte can produce during contraction (see [101] and review by [100]). As a result of this limitation, we decided to use a mechanical bending element to measure myocyte force.

ACTUATORS
Many actuator design concepts were considered early in the project. Of these, micro-electromechanical systems (MEMS) initially appeared quite attractive. Assuming force was to be sensed with some form of bending element, the small feature size of microfabrication techniques would lower moving mass of the element and increase its resonant frequency (and hence measurement bandwidth) for a given stiffness (or force sensitivity). Furthermore, the possibility for combining the actuator and force sensor with a microfluidics system that could perhaps load cells and apply solutions was appealing. The integration of function for muscle cell experimentation on a single chip (the “lab on a chip” concept) could be taken further. For example, the cell could be lysed and gene expression profiles could be measured after performing mechanical tests. Finally, the inherent potential for parallel manufacture of MEMS devices would be ideal for an array instrument.

There were two key disadvantages to a MEMS approach. Firstly, the required ± 100 μm range of travel would push the capabilities of MEMS actuators [102]. Secondly, due to the exploratory nature of the project and large number of unknown specifications the slow iteration cycle of MEMS design would have limited what could be achieved in the project. Instead, the rapid three dimensional micro-fabrication facilities available in the Bioinstrumentation Laboratory including the electrical discharge machine (EDM) were used to build a prototype that could be rapidly iterated as critical design requirements
were exposed. The possibility of transitioning to a MEMS design once the system requirements are well understood is raised in Section 6.

A second actuator technology considered was a piezoelectric stack. Piezoelectric actuators are very stiff which meant they could not be used directly as a combined force transducer and position actuator. However, there are several examples in the literature of developmental devices for single cell muscle physiology that attach a compliant cantilever force sensor to a piezoelectric actuator [103, 28]. The measurement bandwidth in this case is normally limited by the resonant frequency of the cantilever and these systems can be very effective given the high quality of commercially available piezoelectric actuators (that are also highly expensive). A piezoelectric actuator was not used in this design due to the large range of displacements required (necessitating a large piezoelectric stack) and the bulky electronics required to drive the actuator and provide position feedback to correct for hysteresis and other nonlinearities.

DESIGN CONCEPT
Based on these considerations, we chose a design concept similar to that of Iwazumi [64] utilizing two Lorentz force actuators that were compliant enough to be simultaneously used as force sensors. Lorentz force actuators are advantageous as they are highly linear, require only a simple current source drive and, with appropriate design, can be made small and compact. Once the actuator technology was selected, the challenge became how best to implement the motor system to achieve maximum coupling between current and force, sufficient force resolution and bandwidth and the necessary biological compatibility.

2.3.2 Design considerations

CHOICE OF ACTUATING STRUCTURAL ELEMENT
After deciding to combine a Lorentz force actuator with a bending element force sensor an appropriate structure for the actuator was needed. The design constraints introduced by the physiology of single muscle cells were not well understood in the early stages of the project. From an engineering perspective, the fundamental design tradeoff was the
relationship between resonant frequency (measurement bandwidth) and stiffness (force sensitivity). Three simple structures were considered; wires and beams fixed at both ends and cantilevers. Analytical equations for stiffness and resonant frequency provided a means of comparison and are summarized in Table 2.3-1 [104].

As can be seen in Table 2.3-1, the cantilever has a first resonant frequency that is approximately 7.5 times higher than that of a beam or wire fixed at both ends for a given stiffness. In other words, the cantilever achieves better measurement bandwidth for a given force resolution. This comparison ignores the difference in the moment of inertia for a wire (with circular cross section) and a beam (with rectangular cross section). These equations also assume that no tension is applied to the wire or beams fixed at both ends. Early design iterations empirically explored different actuator structures and found that a mild tension, T, was often required with beam (or ribbon) structures to avoid sagging. As stiffness increases with T while resonant frequency increases with $\sqrt{T}$ the addition of tension reduces the resonant frequency achievable for a given stiffness.

<table>
<thead>
<tr>
<th>Structure</th>
<th>$k$ (N/m)</th>
<th>$\omega_1$ (Hz)</th>
<th>$k/\omega_1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wire / beam fixed at both ends</td>
<td>$\frac{384EI}{l^3}$</td>
<td>$\frac{22.4EIg}{2\pi wl^4}$</td>
<td>$7.5 \times \frac{16\pi}{3.52} \sqrt{\frac{EIw}{gl^2}}$</td>
</tr>
<tr>
<td>Cantilever</td>
<td>$\frac{24EI}{3l^3}$</td>
<td>$\frac{3.52EIg}{2\pi wl^4}$</td>
<td>$3.52 \sqrt{\frac{EIw}{gl^2}}$</td>
</tr>
</tbody>
</table>

Table 2.3-1. Analytical relationships for stiffness and resonant frequency for cantilevers and wires / beams fixed at both ends [104]. The critical ratio of stiffness to resonant frequency is also provided.

Optimizing the mechanical tradeoff between resonant frequency and stiffness was not the only engineering design objective. To be useful as a force transducer the structure had to be coupled with a suitably sensitive position sensor. Given the HEDS-1300 confocal sensor was the current best choice for a position sensor (see Section 2.1), the actuator structure needed a flat surface to reflect a 400 $\mu$m diameter spot. This was achieved in early iterations that used wires fixed at both ends by flattening a central region of the wire. However this was less than ideal, further supporting the use of a cantilever.
For actuation, the structure needed to be placed in an appropriate magnetic field. Both wires and beams fixed at both ends could be easily actuated by inserting them in an air gap with a constant magnetic field at right angles to the current flow. The creation of a cantilever Lorentz force actuator was not as straightforward. First of all, it was desirable to make the actuating element from a metal which could support a high current density (to maximize available actuation force). As there was no inherent current path in a simple cantilever, a central section was removed to create a current loop. If this cantilever loop was placed in a constant magnetic field at right angles to the current flow the force on the arms of the cantilever would be in opposite directions creating torque (see Figure 2.3-1). To get useful linear actuation for our application it was necessary to expose the two arms of the cantilevers to opposing magnetic fields.

![Figure 2.3-1](image)

**Figure 2.3-1** A cantilever current loop in a constant magnetic field. The force on either arm is in opposite directions creating torque.

The first design idea that showed promise for creating the required actuation involved placing the cantilevers in the diverging B field between two like poles of a magnet (see Figure 2.3-2). When the cantilever was appropriately placed its arms were exposed to fields pointing in opposite directions. However, the magnetic field would be highly non uniform and it was difficult to design an appropriate arrangement of field guides to create maximal flux.
Figure 2.3-2 A top view slice through the midsection of the cantilevers of Figure 2.3-1 placed in the center of a diverging magnetic field. The current (blue arrows) is directed out of the page in the right cantilever arm and into the page on the left arm. The resulting Lorentz force would bend the cantilever in one direction.

A more elegant design solution used a central magnetic field guide in the gap between the cantilever arms to create uniform magnetic fields at right angles to current flow in each arm (see Figure 2.3-3). The cantilevers in this design can be thought of as a single turn in a voice coil actuator, the motor used in the majority of cone speakers for its excellent linearity. This structure formed the basis of a US patent application [105] and the final motor system used to explore the mechanical properties of single cells. It is described in more detail in Section 3.

Figure 2.3-3 A cut away side view of the motor structure and a single cantilever. The magnetic field is concentrated in the air gap (approximately 1 T). The magnetic field direction is reversed on either side of the central field guide (connected to the South Pole) so that current through both arms of the cantilever loop generates force in the same direction (out of the page for the current and field directions in this figure).
CHOICE OF ACTUATOR MATERIAL

The original specifications for the cantilever actuator material included;

- High conductivity to minimize power dissipation
- High peak current density to maximize peak actuation
- High electromagnetic reflectivity at $\lambda = 655$ nm to ensure a strong position signal
- Biological compatibility and high corrosion resistance as it would be immersed in physiological saline (to avoid problems due to evaporation and surface tension)
- Low susceptibility to fatigue

To maximize conductivity and peak current density the cantilever was manufactured from metal rather than a metal coated semiconductor (which could be built using standard microfabrication techniques). The need for biological compatibility and corrosion resistance in physiological saline suggested a stainless steel alloy would be appropriate. The first cantilevers were cut from 25 $\mu$m stainless steel 304 foil using the wire EDM with the 100 $\mu$m wire. However, it was observed that after approximately one month in the device the cantilevers became magnetized by the strong magnetic field within the air gap (see Section 2.3.3). Some nonmagnetic austenitic stainless steels transform to ferromagnetic martensite when cold worked. To avoid this problem, subsequent cantilevers were made out of high quality 25 $\mu$m thick stainless steel 316 foil from Goodfellow, which had been annealed after being rolled and had very low magnetic susceptibility (see Table 2.3-2).

High strength Beryllium copper alloys were also considered and have excellent elasticity, approximately 100 fold greater electrical conductivity, equivalent density to stainless steel (same moving mass) and are paramagnetic with very low magnetic susceptibility. However, the risk of cytotoxicity when the beryllium copper was placed in solution ruled the material out. The final motor structure was coated in 3 to 5 $\mu$m of parylene rendering it biologically inert. Nonetheless, the cantilevers continued to be made from stainless steel to avoid the possibility of subtle damage to the parylene coating exposing the underlying material and leading to cell death. If, in future designs, the cantilevers are suitably separated from the cells, beryllium copper should be reconsidered.
### Table 2.3-2. Material properties of the alloys selected for use as the actuator element. Aside from its toxicity, Beryllium Copper was the best choice. Stainless steel alloy 304 was slightly more appropriate than the 316 alloy however it was slightly ferromagnetic.

<table>
<thead>
<tr>
<th>Property</th>
<th>Stainless Steel 304</th>
<th>Stainless Steel 316</th>
<th>Beryllium Copper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conductivity (1/Ωm)</td>
<td>$1.39 \times 10^6$</td>
<td>$1.35 \times 10^6$</td>
<td>$118 \times 10^6$</td>
</tr>
<tr>
<td>Peak current density (A/m²)</td>
<td>good</td>
<td>good</td>
<td>excellent</td>
</tr>
<tr>
<td>Density (kg/m³)</td>
<td>7930</td>
<td>7960</td>
<td>8250</td>
</tr>
<tr>
<td>Young’s modulus (GPa)</td>
<td>190 - 210</td>
<td>190-210</td>
<td>120-160</td>
</tr>
<tr>
<td>Yield stress (MPa)</td>
<td>205</td>
<td>205</td>
<td>140</td>
</tr>
<tr>
<td>Fatigue strength, 10⁸ cycles (MPa)</td>
<td>240</td>
<td>170</td>
<td>330</td>
</tr>
<tr>
<td>Corrosion resistance</td>
<td>good</td>
<td>excellent</td>
<td>reasonable</td>
</tr>
<tr>
<td>Toxic</td>
<td>no</td>
<td>no</td>
<td>yes</td>
</tr>
</tbody>
</table>

### DAMPING DUE TO BACK EMF

A charge, $q$, within a conductor moving at velocity, $v$, in a magnetic field, $B$, will experience a force, $F_B = q \times B$. The resulting separation of charges creates an electrical field. The back EMF is a steady state potential that develops when the force on a charge, $q$, due to the electric field, $E$, ($F_E = qE$) balances $F_B$. In this application the cantilevers with length, $L$, moved at right angles through the magnetic field and the potential, $V$, that developed across them was

$$V = L|B||v|,$$

(2.3-1)

This potential created a current in the cantilevers that opposed motion of the cantilevers. This is illustrated in the system model of Figure 2.3-4A and B. In these figures the voltage, $r$, applied to a cantilever with resistance, $R$, is converted into a force which displaces the cantilevers. An opposing voltage equal to the gradient of the displacement multiplied by $B \times L$ is generated across the cantilevers.

**Figure 2.3-4A** The plant without back EMF. The current source applies a reference input voltage, $r$, across the sense resistor, $R$. The resulting current is converted to force $F = LI \times B$ which displaces the cantilevers.

**B.** The plant including back EMF. The motion of the cantilevers creates a voltage that provides damping.
The cantilevers actuators are highly under-damped in air ($\zeta = 0.005$, see section 2.3.3). Highly resonant poles would be problematic for feedback position control. As a result it might be desirable to use back EMF to provide additional damping. If the cantilever is modeled as simple 2nd order system with resonance, $\omega_n$ (rad/sec) damping coefficient, $\zeta$, and stiffness $k$ then when the back EMF is integrated as in Figure 2.3-4B the transfer function between $r$ and $y$ becomes

$$\frac{Y(s)}{R(s)} = \frac{|B|\omega_n^2}{s^2 + \left(2\zeta\omega_n + 2\omega_n \left(\frac{\omega_n|L|}{2kR}\right)\right)s + \omega_n^2}.$$  \hspace{1cm} (2.3-2)

The back EMF creates an additional damping coefficient with magnitude

$$\xi_{EMF} = \frac{|B|L\omega_n^2}{2kR}.$$  \hspace{1cm} (2.3-3)

For typical design values ($|B|L = 1 \times 10^{-3}$ N/A, $\omega_n = 2\pi \times 630$, $L = 7$ mm, $k = 2$ N/m and the sense resistor $R = 10\Omega$) this term will be approximately $1 \times 10^{-4}$ or 50 fold smaller than the damping of the cantilevers themselves.

It would be possible to adjust the system to improve the ability of back EMF to damp the cantilevers by increasing the coupling between current and force and the resonant frequency and decreasing the sense resistor and stiffness. However, it is worth noting that in the present design, the current source that drives the motor uses feedback to regulate the voltage across a sense resistor (see Section 2.2). The current induced by the back EMF across the cantilevers will change the voltage across this resistor and the feedback loop will act to reject this disturbance. Therefore, at frequencies below the bandwidth of the current source, back EMF will be greatly reduced by the very high gain of the operational amplifier feedback in the current source.

**COUPLING BETWEEN CANTILEVERS**

The potential for interference via coupling between the cantilevers was a major design consideration. Any measure of the mechanical properties of a sample connected between the cantilevers was effectively a measure of mechanical coupling between the cantilevers.
It was desired to limit the magnitude of coupling due to interference to less than 10% the mechanical coupling when a cell was attached. The original estimate of myocyte stiffness was approximately 0.6 N/m using a Young's modulus of 200 kPa, a length of 100 µm and a circular cross section with radius 10 µm (note that it was later empirically found to be as low as 1/10th of this initial estimate depending on myocyte length and activation state).

Assuming cantilever stiffness was 2 N/m, and using the lumped parameter system model presented in Section 4 it was originally expected that 23% of the displacement applied to one cantilever would couple to the second cantilever and a design goal of less than 1% coupling between the cantilevers due to interference was set. However, under certain conditions the stiffness of the myocyte was 0.05 N/m and only 2.5% of the displacement applied to one cantilever would couple through the cell. Under these conditions, coupling interference and position noise became a significant problem (see Section 3).

The design required two sensitive force transducers (cantilevers) facing each other separated by approximately 100 µm. As a result, there were many potential sources of coupling including:

- Mechanical coupling
  - Directly through debris or contact points between the cantilevers
  - Indirectly through the fluid between the cantilevers (air or water)
  - Through bubbles that were trapped or formed between the cantilevers
- Electrical coupling
  - Capacitive coupling between two cantilevers
  - Electrical shorts on the cantilever or the current source circuits
  - Capacitive coupling between the current source and high impedance of the position sensor transimpedance amplifier.
- Magnetic coupling
  - Magnetization of the cantilevers
- Optical coupling
  - The beam profile produced by a position sensor could spill over the target cantilever and be detected by the opposite sensor.
Significant coupling was observed in each design iteration and tests where devised to identify and reduce the major sources to satisfy the design objective. Direct mechanical coupling through contact could be detected via visual inspection under a microscope and was relatively easy to rule out. An example of this type of coupling occurred when the arms of the cantilever brushed the sides of the magnetic field guides that created the strong magnetic field in the air gap due to an assembly error. Another form of mechanical coupling, the creation of bubbles between the cantilevers, was a significant problem in all design iterations that immersed the cantilevers. However, bubbles could also be detected visually and avoided with appropriate measures (see Section 2.3.3). The last source of mechanical coupling considered, indirect coupling though the fluid connecting the cantilevers, was a serious concern. It was very difficult to model the magnitude of possible coupling via this mechanism and to separate it empirically from optical coupling of the position signal as discussed later in this Section.

Electrical coupling between cantilevers due to shorts could be detected by measuring the impedance between the cantilevers. The magnitude of the impedance between the cantilevers was approximately 78 kΩ at 0 Hz when the system was correctly assembled. Note this value is lower than would perhaps be expected and is a result of the finite impedance between the high and low power grounds on the LT1970 integrated circuit. This issue was observed when one of the cantilever edges was assembled in contact with the permendur magnetic field guides (which were grounded). It was corrected by insulating subsequent designs with 3 μm of parylene and ensuring the motor structure was not grounded.

Capacitive coupling between the cantilevers was a concern. Assuming the cantilevers could be treated as a parallel plate capacitor with area $A$ and separation $d$ and ignoring fringing (a weak set of assumptions but suitable for a simple worst case estimate) the capacitance between would given by

$$C = \frac{\varepsilon_0 \varepsilon_r A}{d}.$$  \hspace{1cm} (2.3-4)
When the two cantilevers were fully immersed in water with $\varepsilon_r = 80$, there would be approximately 15 pF of capacitance between them. This suggests that capacitive coupling could be ignored as it would only become significant at GHz frequencies when the magnitude of the electrical impedance between the cantilevers was reduced to the order of the resistance of the cantilevers and sense resistor (14 $\Omega$). Furthermore, the feedback loop of the current source would act to reject any coupled currents as disturbances from the desired set point so, for frequencies below the bandwidth of the current source control loop, capacitive coupling would be further reduced.

Another form of interference was observed between the current source and position sensor. In early designs the elements of the current source drive circuit were in close proximity to the critical first stage of the high gain transimpedance amplifier of the position sensor (sensitive to pA of current). It was found that current signals were directly coupling into the position signal even when the light from the position sensor was blocked. This problem was addressed in subsequent iterations by replacing the current source components near the position sensor with a ground plane.

Another interesting form of coupling that was a challenge to diagnose occurred when the cantilevers themselves became magnetized. The issue was first observed approximately one month after a new motor structure had been constructed. It was found that the cantilevers moved when mildly magnetized tweezers were brought close to them. This deflection did not occur when ceramic tweezers were used. Furthermore, it was observed that if one cantilever was pushed toward the opposite cantilever using the ceramic tweezers the second cantilever was repulsed. The solution to this problem was to replace the cantilevers with a different stainless steel alloy that did not become magnetized over time (see discussion earlier in this Section).

Optical coupling between the position sensors was routinely observed in all iterations. Secondary spots in the profile of the position sensor beam (see Section 2.1.2) would spill over the edges of the cantilevers and be detected by the opposite position sensor. The position sensors could be aligned to minimize the static component of this effect by
adjusting the spot location of position sensor one (PS$_1$) on cantilever one (C$_1$) to reduce the offset introduced in the photodiode current of PS$_2$. The alignment was optimal if the position signal from PS$_2$ did not change when the light from PS$_1$ was blocked at the output of the device. A second form of optical coupling that was referred to as indirect optical coupling occurred when light produced by PS$_1$ was detected by the photodiode in PS$_1$ after reflecting from C$_2$. This effect could be observed in the PS$_1$ signal when PS$_2$ was blocked or off and C$_2$ was displaced.

Of the many possible sources of coupling, most could be ruled out either through visual inspection or modeling that suggested they would be negligible. However, it was difficult to empirically or theoretically separate indirect optical coupling from indirect mechanical coupling through the fluid separating the cantilevers. Both forms of coupling are potentially highly nonlinear; they could have different responses when the cantilevers move toward or away from each other and any fluidic effects would be strongly dependent on the velocity of displacement. In an attempt to identify the dominant source of coupling, the following test was performed. With PS$_2$ off and the cantilevers in air, the position signal of PS$_1$ was monitored as either a sinusoidal or stochastic current was applied to C$_2$. This test was repeated with the cantilevers in water. The mechanical coupling was expected to increase in water. If there was no significant increase in the position sensor coupling between the two tests this would suggest that optical coupling was dominant. However the test was inconclusive as the position sensors had to be realigned when the cantilevers were immersed.

To conclude, the best way to limit interference coupling would be to separate the cantilevers and ideally remove them from solution leaving only the two attachment mechanisms in close proximity at either ends of the cell. While this will introduce other problems such as surface tension effects on the sensitive force sensors, it is still suggested as a possible improvement in future iterations (see Section 6.2.1).
2.3.3 Final design

The initial design goals of the mechanical structure of the motor had an engineering focus. The device needed to support the cantilevers and to optimize the coupling between current and force by maximizing the magnetic flux in the air gaps though which the arms of the cantilevers passed. Once muscle cell experimentation began in earnest, it became apparent that for loading and testing it was also desirable that the structure be fully submersible in physiological saline to avoid the effects of surface tension and evaporation on the cantilevers. The materials exposed to the solution also needed to be biologically inert and electrically insulated to facilitate stimulation of the cells. Finally it was critical to provide a means for transmission microscopy of the cell while it was attached to the cantilevers to ensure the condition of the cell could be monitored in real time.

The design illustrated in Figure 2.3-5 met these objectives. The core of the device was the cantilever assembly consisting of the two cantilevers each attached to 100 µm thick glass rectangles with a 130 µm diameter, multimode optical fiber between them. The cantilever assembly was glued onto one half of the motor structure and the remaining elements of the motor were held together using magnetic attraction. Small aluminum spacers ensured the structure was stable and supported two stainless steel tubes that were inserted into fluidic inlets at the base of the structure. Key details of the final design were the subject of a paper (see Section 3). The following report will focus on additional details.
Figure 2.3-5. The modular system used to perform the proof of principle tests on cardiac myocytes. The inset provides more detail of the motor structure mounted on the current source circuit board and inserted into a glass tube. Fluidic inlets (not shown) allow this tube to be filled with physiological saline.

CANTILEVER ASSEMBLY

The cantilever assembly is illustrated in Figure 2.3-6. The 100 μm wire in the EDM was used to cut the cantilevers from 316 stainless steel foil sheets, (50 mm × 50 mm × 25 μm) that were annealed after being rolled. To support the foil in the EDM it was clamped onto a 50 mm × 50 mm × 2 mm stainless steel sheet. Originally the foil was sandwiched between two sheets of stainless steel however it was found to be difficult to separate the pieces without damaging the cantilevers. By using an appropriate cutting path the cantilevers were left attached to the sheet at the base of one arm and could be cut free for use.

The cantilever apparatus was assembled by hand and secured using UV curable glue. The cantilevers were attached to glass rectangles that provided electrical insulation and support. The glass pieces were approximately 0.2 mm × 1.2 mm × 15 mm (T × W × L) and cut from borosilicate glass cover slips using a diamond scribe. To ensure both
cantilevers had the same unsupported length, the width of the cantilever arms increased from 200 \( \mu m \) to 400 \( \mu m \), 5 mm from the tip. This change was aligned with the top of the glass rectangle during assembly. An optical fiber and two 0.2 mm \( \times \) 0.3 mm \( \times \) 15 mm glass sections were glued between the cantilevers to separate them and provide the means for transmission microscopy.

![Figure 2.3-6A](image)

**Figure 2.3-6A.** The cantilever assembly. B An exploded view of the assembly showing the optical fiber and glass pieces used to electrically isolate the cantilevers from each other and the surrounding motor structure.

**MAGNETIC DESIGN**

The magnetic field guides provided the bulk of the motor structure. The primary function of the guides was to create a high magnetic flux density in the air gap through which the cantilevers passed. They also needed to mechanically support the cantilevers, simplify assembly and facilitate the fluidic system by providing access for inlets/outlets and a shape suitable for insertion into a glass tube.

Permandur was used as the base material taking advantage of its excellent saturation flux density. Nickel plated, sintered Neodymium Iron Boron magnets were used for their high residual magnetic flux density. The main structure of the motor was designed in Solid Edge and manufactured using the wire EDM and 5 axis machining center (HAAS). Three dimensional magnetic finite element analysis (Ansys) and iterative redesign were used to optimize the motor structure by identifying regions of saturation or peak flux density in the permandur field guides and flux leakage outside of the air gap. The simulation used a
three dimensional model that was imported from Solid Edge, taking advantage of quarter symmetry in order to minimize computation time. The finite element model required approximately 106000, 8 node elements (Solid96, Ansys). The magnets were modeled with isotropic relative magnetic permeability \( \mu_r = 1.044 \) and \( H_c = 0.6 \times 10^6 \) A/m. The permandur was modeled with a B-H curve that saturated at 2.4 T (see Figure 2.3-7). Care was taken meshing the model to ensure the solution would converge. The Ansys script used to run the simulation is provided in Appendix 8.1. The FEM mesh and results of the simulation illustrating the magnetic flux density in the air gap are provided in Figure 2.3-8A and B. Thermal effects and the magnetic fields produced by passing current through the cantilevers were ignored in this study.

![Figure 2.3-7](image)

**Figure 2.3-7.** The relationship between magnetic flux density \( B \) and magnetic field intensity \( H \) used to model the permandur material in the FEM.
Figure 2.3-8A. The FEM mesh used to simulate the magnetic flux within the motor structure (quarter symmetry was used to minimize the number of elements required). The entire structure was surrounded by another air mesh which was omitted for clarity. B The FEM solution showing the magnitude of the magnetic flux density. The magnetic flux density saturates in the central keeper (red region) due to its limited cross section. The magnetic field in the air gap was uniform with magnitude 0.7 T.

The simulation was not used to predict the actual flux density in the air gap. Instead, it provided insight into the behavior of magnetic flux in the permandur and highlighted several key elements of the design. Firstly, it revealed regions on the base of the motor structure that were good candidates for a fluidic inlet (i.e. areas of permandur that, if removed, would provide minimal interruption of the magnetic circuit). Secondly, it identified that the weakest point in the design was the central magnetic field guide (between the arms of the cantilever). Due to the necessarily narrow cross sectional area of this region it was prone to saturation (see Figure 2.3-8B). The curved structure illustrated in Figure 2.3-9B increased this cross section as much as physically possible. The critical nature of the center region meant that the inclusion of an optical fiber between the cantilevers (an effective magnetic open circuit) would slightly reduce the magnetic field strength. Finally, the simulation served as a means to ensure that flux leakage was minimized. That is, that the air gap that housed the cantilevers was not bypassed with a
magnetic short circuit elsewhere in the design. This required sufficient separation of the magnetic field guides by air or the aluminum spacers (see Figure 2.3-9).

![Figure 2.3-9A](image)

**Figure 2.3-9A.** A cutaway view of the motor structure showing the magnets, permandur field guides and cantilever assemblies. The aluminum spacers hold the permandur sections apart to maintain the air gap. Stainless steel tubes (800 µm outer diameter) were inserted into the fluidic inlet to apply solutions. B The cantilever assembly attached to one piece of the bottom magnetic field guide. The center region of this field guide was the critical component of the magnetic circuit. The top surface of this guide was angled to avoid obstructing the light from the position sensor.

**PERFORMANCE AND SPECIFICATIONS**

**Calibration**

The calibration strategy for the cantilevers and position sensors are described in Section 3 and [97]. The system was calibrated with all forces and displacements referred to the tip of the cantilever where the myocyte would attach.

**Actuation**

The motor structure was mounted directly on a circuit board and the ends of the cantilevers were soldered to two independent, low noise current sources each capable of providing up to ±0.4 A (see Section 2.2). The resistance of the cantilevers was approximately 4 Ω and 1 µW of power was dissipated into the solution during a typical measurement of the dynamic stiffness of a myocyte (σ_{force} approximately 1 µN). The relationship between current and force at the tip of each cantilever was approximately the same, typically between 1 ×10⁻³ and 1.1 ×10⁻³ N/A. This could be used to estimate the
magnetic flux density in the air gap. Each 5 mm long cantilever had two 3.5 mm sections at the base of each arm in the magnetic field. All forces and displacements were referred to the tip of the cantilever where the muscle cell would be attached. The ratio of the displacement of the tip of a cantilever of length, L, when a force F is applied to the tip (\(Y_{\text{tip}}\)) to the displacement when a uniform force is distributed along the lower x % of its length (\(Y_{\text{dist}}\)) is [104]

\[
\frac{Y_{\text{tip}}}{Y_{\text{dist}}} = \frac{8}{x^3 (4 - x)} .
\]  

(2.3-5)

In this case, \(x = \frac{3.5}{5} = 0.7\) so the coupling between current and force distributed across the arms of the cantilever was roughly \(7 \times 10^{-3}\) N/A (to produce an effective \(1 \times 10^{-3}\) N/A at the tip). This suggests the flux density in the air gap was approximately 1 T.

**Mechanical response**

The dynamic compliance of the cantilevers in air and fully immersed in physiological saline were found using stochastic system identification (see Section 2.4.4). A \(10^6\) point band limited between 0 and 2 kHz force (current) signal with Gaussian distribution sampled at 10 kHz was applied to a cantilever and the resulting displacement measured. The estimated Markov parameters were fit to a 2nd order mechanical impulse response using nonlinear LSE. The magnitude and phase of the dynamic compliance of the cantilevers in air and physiological saline are presented in Figure 2.3-10. The resonant frequency decreased from 640 to 395 Hz and the damping factor, \(\zeta\), increased from 0.004 to 0.105 when then cantilevers were immersed. The stiffness remained the same at 2.04 N/m. One of the significant advantages of this design approach is its ability to tune the trade off between force resolution and measurement bandwidth by adjusting the cantilever dimensions. Based on thorough analysis of the proof of principle results, subsequent designs will probably have slightly lower stiffness while maintaining the current resonant frequency by reducing the thickness of the cantilevers (see Section 6.2.1).
Figure 2.3-10. The magnitude and phase of the dynamic compliance of a cantilever in air and water characterized using stochastic system identification. The $\text{coh}^2(j\omega)$ of the system identification is also provided. Note the applied stochastic signal was band limited at 2 kHz hence the $\text{coh}^2(j\omega)$ rolls off above this frequency.
2.4 Signal processing strategy

Key details of the signal processing strategy are presented in Sections 3 and 4. The following serves to complement the information provided there.

2.4.1 Graphical user interface (GUI)

The experimental system was controlled using a computer and a 6052E National Instruments data acquisition card (NI6052E). VB.Net code was used to coordinate experiments and perform initial signal processing. Early versions included a custom signal processing class that had methods for implementing the FFT, power spectral estimation and toeplitz matrix inversion. In the current version of the code much of this processing was performed in Matlab after the experiment had been completed. In a final parallel instrument it is expected that some processing will be performed locally at each module using an embedded processor while the rest will be performed by a central computer. The code can be separated into two sections, forms that provided the GUI and classes that performed signal processing and interfaced with the NI6052E.

FORMS

The frmMain form was the primary GUI for controlling the software. It allowed the user to set input and output channels and the sample rate. Typical experiments were $20 \times 10^3$ to $2 \times 10^6$ points long, sampled at frequencies between 2 kHz and 20 kHz and involved applying a known input and logging four channels of output data (two position sensor signals, PS$_1$ and PS$_2$ and two current source monitors CS$_1$ and CS$_2$). Input sequences were created in Matlab and saved as text files that could be loaded by the GUI before each test. For system identification the variance of the input file was typically set at unity in Matlab and appropriately scaled when the input was loaded by the GUI. Similarly, reference inputs for the controller were amplitude normalized in Matlab then scaled.

The frmLog form was used to display and log data arbitrary length sections of data from two input channels simultaneously. The user could set the sample rate, signal length, input channels and input channel gains then save the data. If desired, sequential
sequences could be acquired, displayed and saved in separate files. This was useful for calibration, power spectral estimation and recording the performance of the system. The final form, $\text{frmOpt}$, was used to define the location of files for control filter coefficients, system identification outputs and other parameters.

**CLASSES**

The $\text{CNIDAQ\_Control}$ class provided an interface for all the relevant functions from the dynamic link library, nidaq32.dll. It had methods for:

- Initializing the data acquisition card.
- Initializing input and output channels (by setting sampling rates and gains).
- Generating output waveforms.
- Applying an output to one or both channels while logging four inputs at arbitrary sampling rates (used for system identification).
- Applying an output on one channel while running closed loop control and logging four inputs.

An instance of the $\text{CNIDAQ\_Control}$ class was created when the program started and used to control the NI6052E. Details of the implementation of the system identification functions and digital control will be discussed in Section 2.4.4 and 2.5.1 respectively.

The primary function of the $\text{CFilter}$ class was to implement the digital control by providing a direct form II, $n^{th}$ order digital filter [98]. It had methods to:

- Update the numerator and denominator coefficients or impulse response of a filter.
- Reset the delay banks (memory) of a filter.
- Calculate the output of a filter using the direct form II algorithm in response to a single point or an array of data.
- Calculate the output of a system that convolved a known impulse response with a single point or array of data.
- Find the magnitude response of an infinite impulse response digital filter with known numerator and denominator coefficients.
• Perform the bilinear transform to convert poles in the s-domain to poles in the z-domain

Of these functions, the first three were put to significant use in the final version of the software to calculate the next value of the controller output. Details of the implementation will be discussed in Section 2.5.1.

The `CComplex` class was written to perform complex number math necessary for the bilinear transform and magnitude response calculations in the `CFilter` class. It was also used in many of the functions in the signal processing class that was left out of the final software version. The `CComplex` class had methods to add, subtract, multiply and divide any combination of real and complex numbers. It also provided methods to conjugate an instance of the class and calculate its magnitude.

### 2.4.2 Sarcomere length estimation

The algorithm for sarcomere length detection was implemented in Matlab. A simple script specified the bitmap or .avi file location (and the number of frames to process within the .avi file). This script then called a separate function that displayed the image or first frame of the movie. The user could define an appropriate region of the image using the mouse from which the sarcomere length (SL) was to be estimated.

Several methodologies were considered to estimate the SL within the selected region. The first approach found the peak of the average of the spatial discrete Fourier transform of the rows within the region. The cell was aligned to ensure the myofibrils ran along the rows of the image (so the striations due to the sarcomere protein structure were vertically aligned). The spatial frequencies, $\Omega_k \,(\text{m}^{-1})$, of the Fourier transform of an N point sequence of pixels are given by

$$\Omega_k = \frac{k}{N\Delta x} \quad k \in [0, N-1], \quad (2.4-1)$$

where $\Delta x$ is the conversion factor between pixels and distance (0.09 $\mu$m/pixel when the 50 times Mitutoyo M-plan objective was used). The bin spacing, $\Delta\Omega \,(\text{m}^{-1})$ (separation between spatial frequencies) was
\[
\Delta \Omega = \frac{1}{N \Delta x}.
\] (2.4-2)

However, simply averaging the Fourier transform of the rows was not an accurate means to estimate the SL. In the majority of cases, only small regions of the cell image were used to estimate the SL due to poor image quality and other issues discussed below. If, for example, only 5 sarcomere lengths were used (approximately a 10 \( \mu \text{m} \) long section) then \( N = 111 \) pixels and the bin spacing was \( 0.1 \times 10^6 \text{ m}^{-1} \). In this case, the only spatial periods represented near physiological sarcomere lengths would be 1.67 \( \mu \text{m} \), 2 \( \mu \text{m} \) and 2.5 \( \mu \text{m} \) (for \( k = 4, 5 \) and 6 in Equation 2.4-1). Even in the best case where 50 \( \mu \text{m} \) of a cell could used the bin spacing would only drop to \( 0.02 \times 10^6 \text{ m}^{-1} \) and the spatial periods would be separated by approximately 0.08 \( \mu \text{m} \) around 2 \( \mu \text{m} \).

The problem of poor spatial frequency resolution due to short sequence length could be addressed by interpolating between the bins using an appropriate function (an approach often used in identifying tones). Alternatively, each row could be padded with \( M \) zeros before taking the Fourier transform. It was found that by setting \( M \in [5N, 10N] \) (where \( N \) was the original signal length) and averaging the Fourier transform of each row the SL resolution was significantly improved.

Another attempted technique involved averaging each column of a section (with sarcomeres running along the rows) and then fitting sinusoids of varied frequencies to the averaged row data. The range of frequencies used spanned the expected physiological SL range and the final estimate was calculated from the frequency that provided the lowest mean squared error between the fit and the data. This process was typically run twice, first using a broad range of frequencies then using a narrow range of spatial frequencies around the SL estimate from the first run. A simple brute-force algorithm was used for this minimization as the processing was typically done after the experiment. While there are many algorithms (for example the bisection algorithm) that could be used to reduce this computation time, the zero padded Fourier transform estimate was selected as the method for SL estimation for its simplicity and speed.
To calibrate the SL estimation an image of a 100 μm graticule with 2 μm spacing was captured with the different objectives used to view the myocytes. The value of Δx was set to ensure the peak of the average of the zero padded Fourier transform was at $0.5 \times 10^6$ m$^{-1}$. Each row of the image was typically bandpass filtered with cutoffs 2.9 μm and 0.6 μm ($0.35 \times 10^6$ m$^{-1}$ and $1.7 \times 10^6$ m$^{-1}$). An image of the graticule taken with the 50 × Mitutoyo M-plan objective and the averaged Fourier transform of a selected region of the image are provided in Figure 2.4-1A and B.

![Image of graticule](image)

**Figure 2.4-1A** The image of the graticule with 2 μm spacing used to calibrate the 50 × Mitutoyo M-Plan Apo objective. The graticule was on a slight angle and hence was slightly out of the focal plane on the right hand side of the image. The inset was the region of the image selected for processing. **B** The spatial FFT of the selected region. When Δx was set to approximately 0.09 μm/pixel, the clear peak was at a spatial wavelength of 2.008 μm and the spatial frequency bin spacing was 0.0012 $\times 10^6$ m$^{-1}$.

To demonstrate the use of this algorithm, a representative cell image and the associated average Fourier transform are presented in Figure 2.4-2A and B respectively. The SL in this case was estimated to be 2.28 μm with a bin spacing of 0.0038 $\times 10^3$ m$^{-1}$. Comparing Figure 2.4-1B and Figure 2.4-2B it is clear that the Fourier transform of the cell data has a lower signal to noise ratio and a broader peak. This is possibly due to the reduced image contrast and slight variation in SL across the region of the image selected. As can be seen in Figure 2.4-2A, only small sections of the muscle cell were selected to ensure a clean estimate of the SL. The other regions produced SL estimates of 2.24 μm and 2.30 μm.
Figure 2.4-2. A representative image of a cell and different regions that could be used to estimate the SL. B The FFT of one of the selected regions. The SL estimate in this case was 2.28 μm (this was expected as the cell had been significantly stretched from resting SL or 1.85 μm).

Ideally sarcomere length would be resolved to within at most ± 0.02 μm. Due to physical limitations in the current design a long working distance objective is required which has a relatively low numerical aperture of 0.42. For broad band illumination of an slit aperture (a reasonable model of a sarcomere), the diffraction limit for resolution is approximately

\[ Y = 0.5 \frac{\lambda}{NA}. \]  

(2.4-3)

Assuming an average wavelength of 550 nm the resolution limit is approximately 650 nm. Although this is sufficient to observe the striation pattern of single cells, we require the ability to detect changes in SL on a much finer scale. As discussed by Roos et al [106] the ability to resolve SL is limited primarily by contrast between the light and dark regions of the cell. By using standard image processing algorithms for enhancing contrast, the Fourier transform to detect the peak frequency in the image and averaging many rows to further improve the signal to noise ratio it is possible to resolve very fine changes in SL. The accuracy of this process could be further improved by using higher numerical aperture optics and phase contrast (instead of bright field illumination) to increase image contrast. In addition, the point spread function of the optical system could be characterized (perhaps using an impulse or point light source) and then used to correct the image via deconvolution. Finally, interpolation could be used between the points of the Fourier transform to more accurately predict the maximum.
However, in practice it was found that the process of SL estimation was limited by other experimental problems. Bubbles and debris accumulated on the glass slip above the cantilevers and distorted the image of the cell (as is evident in the left side of Figure 2.4-2A). Sometimes as little as 20 \( \mu m \) of the cell was exposed between the two glass elements of the attachment mechanism providing an undistorted image of at most 10 sarcomeres for inspection. In earlier iterations of the device the cell would sometimes be slightly angled due to differences in height between the two attachment mechanisms. In the worst case this affected image focus along the cell length. The cell could also be on slight angle in the horizontal focal plane so the myofibrils did not run directly along the rows of pixels. Even if the boundaries of the cell were well aligned horizontally, it was observed that myofibrils within the cell could still be slightly angled themselves. Finally, cells often had non uniform SL distribution along their length. This could have been an indication of uneven distribution of stress within the cell or a sign of cellular damage. Note that if this problem was significant, the cell was discarded.

Given the importance of sarcomere length to muscle mechanical properties, problems affecting the accuracy of SL estimation are of significant concern. However it was often possible to get at least one or two good measurements of SL from a given cell during an experiment and any remaining values could be extrapolated or interpolated from known length changes that were applied to the cell. In the case where image quality remained high throughout the experiment it was observed that there was good correlation between the cell length and SL.

2.4.3 Edge detection and system calibration

The position sensor was calibrated by comparing the position signal to displacement of the tip of the cantilevers measured optically using the digital microscope. The inward edge of the attachment mechanism on a cantilever (approximately where a cell would attach) was illuminated from below with the optical fiber and imaged using the Sony DFW-SX900 digital camera and 50 times Mitutoyo M-Plan Apo objective. The brightness, contrast and focus of the image were adjusted to ensure a sharp transition between dark and light at the edge of the attachment system. The Sony camera was
rotated such that the image of the cantilever edge was vertical and the cantilever was driven with a 0.5 Hz sinusoidal force. Six seconds of video were captured at seven frames per second and the position sensor signal was simultaneously recorded.

Frame by frame edge detection was implemented in Matlab. The frame was converted to a grayscale image and its intensity scaled to fill the 8 bit dynamic range using the `imadjust` function. The image was cropped to select the region with the edge reducing the computation time. The edge of the attachment mechanism was detected by applying the `edge` function in Matlab. The Canny method was used to search for local maxima of the gradient of image intensity found using the derivative of a Gaussian filter. The user provided two thresholds that defined the cutoffs for strong and weak edges. The intensity scaling and thresholds were manually adjusted using the first frame of the movie. Once these parameters were set, the script ran through all the frames finding the position of the edge in each row of the image as a function of time. The data from each row were offset to remove the mean and then averaged. The quality of the edge detection was assessed by considering the standard deviation of distribution of displacements measured in each row.

A 0.5 Hz sinusoid was fit to the averaged edge data and its amplitude scaled (using the calibration between pixels and µm described in Section 2.4.2) to estimate the displacement. The position sensor signal was also fit to a sinusoid and the ratio of amplitudes provided calibration between position and displacement in V/m (typically between 10 and 20 kV/m when the cantilevers were fully immersed).

### 2.4.4 System identification implementation

We chose to use stochastic system identification [107] to estimate the mechanical impulse response and dynamic stiffness / compliance of the muscle cell and cantilevers. The signal processing strategy used to estimate the dynamic stiffness of a single cell is presented in Section 4.

The deconvolution of the output and input data to find the impulse response was implemented in the time domain using biased auto and cross correlation estimates (as
discussed in Section 4). It was found that the best results were achieved when the full input and output sequences were used to estimate the correlation and typically only the central 2048 time lags were kept. It was also possible to deconvolve the output and input data to find the frequency response (magnitude and phase) using power spectral estimates. These estimates were made in Matlab using periodogram averaging with 50% overlap and a Hamming window (the function \textit{pwelch}).

It is worth noting that a typical stimulus to probe the mechanical response of a cell was band limited between 0 and 100 Hz. The signals were sampled at 2 kHz. The variance accounted for or coherence$^2$ ($\text{coh}^2$) dropped rapidly above the stimulus bandwidth and the estimate of the mechanical transfer function in this region was ignored in the frequency domain. To properly estimate the impulse response in the time domain the sampling rate could be lowered to 200 Hz and the analog antialiasing filter appropriately adjusted.

LUMPED PARAMETER MODEL AND DYNAMIC COMPLIANCE

The lumped parameter model is described in Section 4. Briefly, it is a MIMO linear transfer function model that uses the dynamic stiffness of the muscle actuator and force sensor cantilevers ($H_M(j\omega)$, $H_1(j\omega)$ an $H_2(j\omega)$ respectively) to relate forces and displacements at the tip of the cantilevers where the muscle cell was attached (see Figure 2.4-3).

\[
\begin{bmatrix}
X_1 \\
X_2
\end{bmatrix} = H(s) \begin{bmatrix}
F_1 \\
F_2
\end{bmatrix}
\]  

\[H(s) = \begin{bmatrix}
\frac{H_1 + H_m}{H_1H_2 + (H_1 + H_2)H_m} & \frac{H_m}{H_1H_2 + (H_1 + H_2)H_m} \\
\frac{H_m}{H_1H_2 + (H_1 + H_2)H_m} & \frac{H_1 + H_m}{H_1H_2 + (H_1 + H_2)H_m}
\end{bmatrix}
\]  

\[
\begin{bmatrix}
F_1 \\
F_2
\end{bmatrix} = H^{-1}(s) \begin{bmatrix}
X_1 \\
X_2
\end{bmatrix}
\]  

\[H^{-1}(s) = \begin{bmatrix}
\frac{H_1 + H_m}{H_1H_2 + (H_1 + H_2)H_m} & \frac{-H_m}{H_1H_2 + (H_1 + H_2)H_m} \\
\frac{-H_m}{H_1H_2 + (H_1 + H_2)H_m} & \frac{H_1 + H_m}{H_1H_2 + (H_1 + H_2)H_m}
\end{bmatrix}
\]
The dynamic stiffness of a muscle cell was found by rearranging the second row of Equation 2.4-5A and letting $s = j\omega$ to give

$$H_M(j\omega) = \frac{X_2(j\omega)}{X_1} \frac{1}{1 - \frac{X_2(j\omega)}{X_1}} H_2(j\omega), \quad (2.4-6)$$

where $X_2/X_1(j\omega)$ is an estimate of the transfer function relationship between the measured displacements $x_1(t)$ and $x_2(t)$. However $H_M(j\omega)$ can be estimated using three other formulations derived from Equation 2.4-4A and 2.4-5A.

$$H_M(j\omega) = \frac{F_1(j\omega) - H_1(j\omega)}{X_1} \quad (2.4-7)$$

$$H_M(j\omega) = \frac{1}{1 - \frac{F_1(j\omega)}{X_1}} \frac{1}{1 - H_1(j\omega)} - \frac{1}{H_2(j\omega)} \quad (2.4-8)$$

$$H_M(j\omega) = \frac{H_1(j\omega)H_2(j\omega)}{F_1(j\omega) - (H_1(j\omega) + H_2(j\omega))} \quad (2.4-9)$$

Originally it was planned to use the average of all four estimates. However, using simulated data with realistic parameter values in the presence of additive Gaussian white noise it was found that the mean squared error between the estimate of Equation 2.4-6 and the simulated values of $H_M(j\omega)$ was lower than that of any of the other equations or any combination of averages.
These equations are difficult to implement in the time domain where multiplication and division become convolution and deconvolution. However, rearranging Equation 2.4-6 gives

\[
H_M(j \omega) = \frac{X_2(j \omega)}{X_1 - X_2(j \omega)} H_2(j \omega),
\]  

(2.4-10)

where \(X_2(X_1 - X_2(j \omega))\) is an estimate of the transfer function between \(X_2(j \omega)\) and \(X_1 - X_2(j \omega)\). This equation could be evaluated using estimates of the impulse response of the system \(X_2(X_1 - X_2)\) and \(H_2\). In practice the compliance impulse response of the muscle tissue would be estimated as the dynamic stiffness of a cantilever was an improper system (more zeros than poles in the frequency range of interest implying the magnitude of the dynamic stiffness of the cantilever would increase without bound at high frequencies).
2.5 Control of the system

The details of the final implementation of the controller are provided in Section 4. This chapter will present implementation issues not discussed in that paper and provide some additional details of other attempted approaches.

2.5.1 Implementation of the digital controller

DATA ACQUISITION SYSTEM AND DELAY

The final modular device used the 6052E PCI (peripheral component interconnect) National Instruments data acquisition card (NI6052E) in a Dell personal computer (PC) to implement a 10th to 16th order digital controller running at 2 kHz. The major issues with using the NI6052E system for digital control were delay and timing. The input data had to pass from the card across the PCI to the processor of the PC that performed the control computations then back across the PCI to be output by the NI6052E digital to analog converters. This transfer was relatively slow and limited the closed loop sample rate. Furthermore, there were no simple functions or instructions on how to perform this task in the nidaq32.dll library provided by National Instruments.

Of the many approaches trialed the best performance was provided by the following sequence of functions. The same framework was used for both system identification and control with minor modifications.

DAQ_Rate()  'Set the clock rate such that four inputs would be read in at 2 kHz
SCAN_Setup()  'Set the number of input channels and gain for each channel
DAQ_DB_Config()  'Enable the use of double buffering for continuous data acquisition
SCAN_Start()  'Started the scan
while ( i < max)
    DAQ_Monitor()  'Capture the four inputs (forcing sequential acquisition)
    Control calculations
    AO_VWrite()  'Write single points on each channel (update immediately)
    i++
end while
DAQ_Clear()  'Cancels the scan
The critical factor in the performance of this system was the delay. The NIDAQ 6052E multiplexes data acquisition so each channel is acquired sequentially. When running at 2 kHz and acquiring 4 channels there is an automatic delay of 125 µs between each acquisition and up to 375 µs between the first and the last channel. There are two common approaches for compensating for delay in digital control systems. One is to attempt to output the new data “instantaneously” so that the delay is an insignificant fraction of the sample period. In the other approach, the output can be updated on the next sample of the input and the delay can be included in the controller design (through an additional $z^{-1}$ term). Unfortunately, the NI6052E cannot implement either of these approaches. The “instantaneous” output was clearly not possible due to the multiplexed input and there was no way to synchronize data output with the NIDAQ internal clock at the same time as acquiring clocked inputs. Instead, a suboptimal approach was used, ensuring the control system was tolerant to a variable delay of up to one sample period. In the pseudo code fragment above, the implementation used a single point output function was used which creates the output immediately after being called.

The effect of the arbitrary delay, $T$, was modeled using two approaches. Firstly, as a second order expansion of $e^{-sT}$

$$e^{-sT} = \frac{1 - \frac{Ts}{2} + \frac{(Ts)^2}{8}}{1 + \frac{Ts}{2} + \frac{(Ts)^2}{8}}. \quad (2.5-1)$$

When this model was included in an earlier design that used plant inversion it explained an unexpected resonance that was observed in the magnitude response of the controller. The second approach included the delay directly in a Matlab Simulink model of the system. Matlab Simulink was the modeling methodology used in the final design as it could include the effects of additive shot noise from the photodiode, digital quantization at the DAC, saturation of the position sensors and current source and variable delay. A simplified schematic of the Simulink model used to design the final controller is provided in Figure 2.5-1. A limited version of the Matlab script used to design the $H_\infty$ controller is provided in Appendix 8.2.
Figure 2.5-1. A simplified version of the Simulink model used to design the isometric $H_\infty$ control system. The objective was to minimize the displacement of the cantilevers in response to a muscle disturbance. The model allowed the effects of quantization, sensor and output saturation, additive position noise and delay to be considered.
DELAY AND STOCHASTIC SYSTEM IDENTIFICATION

The delay could be ignored in the controller design provided it did not significantly affect control performance or stability. However, it also affected the stochastic system identification results. The four inputs in the scan were position sensor one and two, (PS₁, PS₂) and current source one and two (CS₁, CS₂). The CS inputs monitored the voltage across the sense resistor in the current source (see Section 2.2). To identify the transfer function of a cantilever, for example cantilever one, the stochastic output was applied using the pseudo code illustrated above (with unsynchronized outputs). The transfer function was estimated between data gathered from CS₁ and PS₁. To estimate the delay between these inputs the low frequency phase response was considered. At frequencies sufficiently below resonance (330 Hz in physiological saline) the cantilever transfer function should have very little phase change. Given the damping coefficient of ζ = 0.1, the phase of the cantilever system should be 0.018 rad at 30 Hz. The estimated phase in this region is plotted in Figure 2.5-2 as a function of frequency. A linear fit to this data had a gradient of -345 μs and provides an estimate of the delay.
Figure 2.5-2. The change in phase as a function of frequency between 0 and 190 rad/s (30 Hz). The equivalent delay between the input and output data was approximately 345 µs.

At 60 Hz this delay would have introduced a phase change of -7.4 degrees. A similar delay between the PS₁ and PS₂ would impact the estimation of the mechanical transfer function of the myocyte. While this could have been characterized and compensated for it was considered small enough to ignore. The delay between the inputs was included naturally in the controller design by fitting 4th order models to the raw cantilever characterization data (see Section 4).

CONTROL ALGORITHM IMPLEMENTATION

The state-space $H_\infty$ optimal controller produced by the Matlab function $hinf\text{syn}$ was implemented using digital filters in direct form II [98]. For the SISO isometric controller discussed in Section 4 there were two 16th order filters (one for each cantilever). All loops in the direct form II algorithm were rolled out to minimize computation time (by
avoiding branches and pipeline flushes due to conditional statements). The algorithm for a 2\textsuperscript{nd} order filter is given below.

\[
\begin{align*}
\text{dOA} &= -\text{dA}(1) \ast \text{dA}(1) - \text{dA}(2) \ast \text{dA}(2) & \text{The pole coefficient calculation} \\
\text{dOB} &= \text{dA}(1) \ast \text{dB}(1) + \text{dA}(2) \ast \text{dB}(2) & \text{The zero coefficient calculation} \\
\text{dZ}(0) &= \text{dDataIn} + \text{dOA} & \text{The new zero\textsuperscript{th} delay value} \\
\text{dY} &= \text{dZ}(0) \ast \text{dB}(0) + \text{dOB} & \text{The output} \\
\text{dZ}(2) &= \text{dZ}(1) & \text{Update the delay array} \\
\text{dZ}(1) &= \text{dZ}(0)
\end{align*}
\]

Where \text{dA} is the filter memory (a vector of length 2 in this case) and \text{dB}, and \text{dB} are the denominator and numerator coefficients.

In implementing the controller it was necessary to carefully account for offsets to avoid applying damaging step displacements to the muscle cell when the controller was switched on. Zero mean reference inputs were typically generated independently in Matlab and saved as ASCII files. When the reference input was loaded into the VB.Net program it was scaled and the current offset applied to the cantilever was added to the signal to create the true reference input \( V_{\text{REF}}[n] \). The position signal, \( V_{\text{PS}}[n] \), was also corrected for any offsets by subtracting the first measured value, \( V_{\text{PS}}[0] \). The control implementation is illustrated by the following pseudo code.

\[
\begin{align*}
V_{\text{OUT}}[n+1] &= \text{control\_filter}(V_{\text{REF}}[n]-V_{\text{REF}}[0]-V_{\text{PS}}[n]-V_{\text{PS}}[0]) \\
V_{\text{OUT}}[n+1] &= V_{\text{OUT}}[n+1] + V_{\text{REF}}[0]
\end{align*}
\]

Where \text{control\_filter} evaluates next output of the control filter using direct form II (it is implemented in the CFilter class see Section 2.4.1). \( V_{\text{OUT}}[n+1] \) is then applied to the current source. This implementation ensures that control filter responds to any changes in the reference input and position signal from their initial values.
EMBEDDED SYSTEM DESIGN
To be true to the modular concept, it was desirable to design an embedded system to provide data acquisition and control. Furthermore, the embedded system would be able to run at significantly greater sampling rates allowing digital feedback control of cantilevers with higher resonant frequencies. Sections of the required digital electronic design and programming for this project were assigned to another student in the Bioinstrumentation Laboratory. Unfortunately, this project had to be terminated before it could be completed.

Several design possibilities were considered. The first significant design iteration used a TMS320C6711 Texas Instruments (TI) digital signal processor (DSP) in combination with a field programmable gate array (FPGA), 128 Mbytes of random access memory (RAM) and four 100 ksps, 18 bit analog to digital converters (ADC) and two 100 ksps, digital to analog converters (DAC). The system was to have a 32 bit bus to load data from the ADC and DAC in parallel (thereby minimizing delay). The 32 bit floating point processor on the TI DSP was to be used for the controller implementation while the FPGA coordinated the system managing experiments and controlling the data bus to shuttle data from the ADCs to the TI DSP then back to the DACs (simultaneously logging inputs in the RAM). I was responsible for implementing the control algorithm on the TI DSP.

A TI DSP starter kit (DSK) in combination with an integrated development environment, Code Composer v2 was used to develop the code. There was a significant learning curve in which several problems relating to processor initialization were overcome. For example, the use of the correct memory map at initialization in the dsk6xinit.gel file that was called at startup. However, as with the NIDAQ implementation, the primary issue was how to efficiently transfer data between the TI DSP and ADCs/DACs via the FPGA without introducing significant delay. The TI DSP provided an enhanced direct memory access (EDMA) for transferring data between level two cache and external peripherals via a 32 bit external memory interface EMIF. We chose to use a combination of interrupts and a subset of the EDMA called quick direct memory access (or QDMA) to provide the fastest transfer of data.
The initialization of the TI DSP and the implementation of the digital filter using the direct form II algorithm were programmed in C for simplicity and the code was compiled to run on the TI DSP using the Code Composer. However, it was not possible to achieve tight timing and control of interrupts via the interrupt vector table when programming in C. As a result, a function was written in assembly to coordinate the core of the control implementation. This function ran on the TI DSP in an infinite loop until it was forced to exit by a pulse on external interrupt 4 (manually generated using a signal generator). The control cycle began in a loop waiting for interrupt 5 that was to be set high by the FPGA when the input data was ready. The assembly function would then trigger a QDMA transfer to read the 32 bit bus and then monitor interrupt 8 that was set by the EDMA when the transfer was complete and the data stored in a known location in level two cache. The assembly function then called an external function written in C that performed the necessary control calculations (for a 4\textsuperscript{th} order filter in this case) and output the data again using a QDMA transfer. The process is illustrated in the following pseudo code.

Loop1: If interrupt 4 triggered drop out of function
If interrupt 5 triggered continue 'Data ready on bus
Else go to Loop1
Trigger a QDMA to read data
Loop2: If interrupt 4 triggered drop out of function
If interrupt 8 triggered continue 'Data read into L2 cache
Call control filter function 'Implement filter, output data
Go to Loop1

The actual assembly code is provided in Appendix 8.3. The TI DSP has dual arithmetic, load and store units and the assembly code was manually optimized to take advantage of this by executing operations in parallel. A balance was struck between optimization and the ease with which the code could be read by a human (for debugging purposes).

To test the operation of this code external interrupt 5 was triggered with an 8 µs pulse repeated at 50 kHz and pin 0 of the EMIF was monitored using a TDS3102B oscilloscope.
from Tektronix. Both the read and write events were evident as spikes on the EMIF pin as illustrated in Figure 2.5-3. The total delay between interrupt 5 going high and the control output being set on the EMIF was 1.38 μs (at total of approximately 207 clock cycles in the TI DSP). According to the data sheet, function calls and interrupts took approximately 15 cycles to execute and the control implementation in C took 110 cycles (independently measured). This left approximately 35 cycles for each of the QDMA transfers.

![Graph](image)

**Figure 2.5-3.** The delay between when interrupt 5 was set (blue trace, indicating the input data was ready on the bus) and data being read/written on EMIF pin 0. The second pulse on pin 0 of the EMIF represents the write operation (the output of the digital controller). The delay introduced by the TI DSP was only 1.38 μs.

It is worth noting that the ADC and DAC introduced an additional 1.5 μs and 2.8 μs of delay. In the ADC this occurred after the conversion process had finished before the data could be read while in the DAC this was for data transfer and minimizing glitches. The total delay, ignoring the negligible processing time in the FPGA to coordinate activities,
would have been 5.7 µs. This would have been insignificant even if the controller was running in closed loop at 20 kHz (the original specification for the project).

As the embedded system project progressed the design was simplified by using an Analog Devices fixed point Blackfin processor to both control the experiments and run the control and utilizing SPI (a serial data transfer protocol) to communicate with the ADCs and DACs. A Cirrus processor was also considered, but discarded due to the very poor documentation and support and unreliable behavior of the processor. Unfortunately, the digital design remains incomplete.

2.5.2 Isotonic control

As discussed in Section 4 muscle mechanical properties depend significantly on muscle length and the load under which it is operating. It was therefore desirable to design an isotonic $H_c$ controller to complement the isometric controller and allow the muscle to contract against a constant load. For example if the load is set to zero and the myocyte produces a force, $F$, while contracting it should shorten by approximately $F/k_m$ where $k_m$ is the stiffness of the muscle cell. Of course, the cell stiffness will change with muscle length and the state of contraction as will the amount of force produced so this is only a weak approximation.

In typical experimental systems with an actuator on one side of the muscle tissue and a cantilever force sensor on the other, isotonic control is implemented by moving the actuator to hold the force sensor at a constant displacement (or force) [108]. Other researchers have attached one end of the cell to a fixed surface and the other to a cantilever force sensor. By actuating the base of the force sensor cantilever, the location of the cantilever tip relative to the base and hence the force applied to the muscle cell can be regulated [103].

In our case, the controller design is somewhat counterintuitive. Isotonic control cannot be implemented by detecting the displacement of one cantilever and actuating it directly to correct for this displacement. The Lorentz force applied to the cantilever via the motor
system would displace the tip not the base of the cantilever and hence add to the force applied to the muscle cell. Instead, the displacement of the tip of one cantilever must be countered by moving the opposite cantilever and having the force couple through the cell. To clarify this point, consider the estimate of the load on a cell using cantilever one, \( F_{\text{EST1}} \)

\[
F_{\text{EST1}} = k_1(\Delta x_{11} + \Delta x_M + \Delta x_{21}) - F_{11} = k_1(\Delta x_M + \Delta x_{21}),
\]

(2.5-2)

where \( k_1 \) is the stiffness of the cantilever, \( \Delta x_{11} \) is the displacement introduced by Lorentz force of current source one, \( F_{11}, \Delta x_{21} \) is the displacement of cantilever one due to forces coupling through the cell from current source two and \( \Delta x_M \) is the displacement of the cantilever due to the muscle contraction. If \( \Delta x_{21} = 0 \) then \( F_{\text{EST1}} = F_M \) (the twitch force). To set the force load to a given value, for example 0 N, the displacement \( \Delta x_{21} \) must cancel the displacement due to the muscle force.

To conceptually design an \( H_{\infty} \) isotonic controller it was necessary to account for the coupling of forces and displacements between the two cantilevers (through the cell). This required the full MIMO system model of Equation 2.4-4. The control objective was to design the controller \( K \) to minimize the error between the output \( y \) of Figure 2.5-4A and a desired set point, \( r \) in the presence of force disturbances produced by the muscle. Note that the output \( y \) is

\[
y = \begin{bmatrix} \Delta x_M + \Delta x_{21} \\ \Delta x_M + \Delta x_{12} \end{bmatrix}.
\]

(2.5-3)

The elements of \( y \) are scaled estimates of the force load on a cell (see Equation 2.5-2). In a similar manner to the isometric controller design described in Section 4, the control objective can be quantified using the ratio of the closed loop transfer function between disturbance input and plant output, \( G_{YD} \), and the open loop transfer function \( G_p \)

\[
S = \frac{G_{YD}}{G_p} = \frac{I}{I + K(G_p - H)} = \begin{bmatrix} S_{11}(s) & S_{12}(s) \\ S_{21}(s) & S_{22}(s) \end{bmatrix}.
\]

(2.5-4)

This ratio is the mathematical equivalent of the sensitivity of the system illustrated in Figure 2.5-4B where the isotonic plant is now modeled using only the cross coupling. From this point on the development of the isotonic controller, \( K \), paralleled that of the isometric controller with the objective being to minimize the sensitivity of the system illustrated in Figure 2.5-4B over the bandwidth of the muscle twitch.
The closed loop system with reference input, $r$, scaled muscle disturbance, $d$, photodiode shot noise (the dominant noise source), $n$, controller, $K$, current source, $CS$ (scaled to account for the conversion between current and force) and position sensor, $PS$. The coupled cantilevers are represented using the full MIMO model of Equation 2.4-4. The inputs and outputs are two element vectors and the block elements are $2 \times 2$ transfer function matrices. The output, $y$, is a scaled estimate of the force load on the muscle cell. B The simplified closed loop system used to design the isotonic controller. The design objective required the sensitivity of this system to be minimized over the bandwidth from 0 to 10 Hz.

To design the isotonic controller the myocyte was modeled as a spring with stiffness $H_{M\text{Est}}(j\omega) = k_{M\text{Est}} \text{ N/m}$. The performance of the isotonic controller was strongly dependent on how $k_{M\text{Est}}$ compared to the actual cell stiffness ($H_M(j\omega)$). The effect of using a controller designed with $H_{M\text{Est}}(j\omega) = k_{M\text{Est}}$ on a plant modeled using stiffness $H_M(j\omega) = k_M$ was explored mathematically. It was found that for $k_M > k_{M\text{Est}}$, stability became a problem as displacements introduced by the controller coupled more effectively through the cell to the opposite cantilever (analogous to too much gain). For $k_{M\text{Est}} > k_M$, the performance of the controller was reduced as it was not applying enough force to displace the opposite cantilever. Given the stiffness of muscle tissue can increase by a factor of 20 during a twitch [57] some form of adaptive controller might be more appropriate.

However, it would be certainly be beneficial to implement the $H_o$ isotonic control and explore its performance before investing the time necessary to design an adaptive controller.
2.6 Cell Experimentation

The previous sections of this document have focused primarily on the design and implementation of aspects of the instrument. In this section, the key issues that arose while performing experiments on single muscle cells are discussed including cell isolation, imaging, fluidics, loading, attachment and electrical stimulation.

2.6.1 Myocyte Isolation

The myocyte isolation methodology is described in Section 3. Professor Peter Kohl, Rebecca Rowland and Dr. Gentaro Iribe of the University of Oxford kindly provided the protocol, two weeks of training at Oxford and some glassware and other materials. The procedure was performed in laboratory space provided by Professor W.G. Thilly of the Bioengineering Department at Massachusetts Institute of Technology. When properly implemented the protocol repeatedly produced high yields of healthy, viable myocytes. An image of several healthy cells captured using the Sony DFW-SX900 digital camera and the Olympus microscope is provided in Figure 2.6-1.

Figure 2.6-1. An image of several healthy cells isolated at MIT using the protocol provided by Professor Peter Kohl of the University of Oxford.
The collagenase blend used in the isolation, C8176 from Sigma Aldrich, was a key determinant of both cell health and attachment properties. The use of a blend of different collagenases and proteases was advantageous as it minimized batch to batch variability and provided a high level of repeatability. Unfortunately, for a critical period of six months while the attachment protocol was being developed the C8176 collagenase was unavailable in the United States. During this period an alternate collagenase blend was used (C8051, Sigma Aldrich).

Minor modifications to the original protocol were required in order to obtain viable cells using the C8051 collagenase. Interestingly, as discussed in Section 2.6.5, when healthy cells were successfully isolated using this collagenase they adhered strongly to glass without the need for a clamp. However, soon after this was property was established the C8051 protocol stopped reliably producing healthy cells. The following possible sources of the considerable variablitiy in cell viability and yield from one experiment to the next were explored:

- Fluidic system contamination. The entire fluidic system was carefully washed repeatedly with 95 % ethanol then flushed with deionized water and all tubing replaced.
- Deionized water purity. The Millipore system was inspected and all glassware used in solution preparation was carefully cleaned.
- Changes in solution osmolarity and pH due to poorly calibrated meters. The pH probe was replaced and the osmolarity meter calibration checked.
- Collagenase storage and use. If the collagenase was not warmed to room temperature before being opened condensation would cause the powder to aggregate over time. However, the effect of temperature cycling on collagenase activity was a concern. The final protocol distributed the collagenase into several smaller bottles that were opened while cold and used very quickly.
- Bottle to bottle variation in collagenase. Several bottles of C8051 collagenase were used (they were all from the same batch).
- Variation in the time to achieve digestion (collagenase perfusion time) with animal weight and heart size.
• Correlation of cell health with many different parameters including:
  o Heart rate immediately after perfusion started.
  o Time elapsed during tissue dissection and mounting.

Ultimately, Sigma Aldrich, agreed to import 500 mg of C8176 collagenase from Germany for our consumption. When the C8176 was substituted for the C8051, we were once again able to isolate healthy, viable myocytes (with minor adjustments in the digestion time). It was concluded that, on the whole, the C8051 collagenase was unreliable and all subsequent isolations were performed using the C8176 collagenase blend.

2.6.2 Imaging

The ability to image the cells was critical for several reasons. Firstly, it was necessary to visually inspect the myocytes to judge their health as indicated by a clean cell membrane (no early signs of cell lysis) and a uniform, clear sarcomere structure. Cell health was also assessed by observing if a cell would contract (greater than 5% strain) when stimulated with an electric field (see Section 2.6.6). Secondly, the imaging system was critical for manually loading the cells. Early attempts to load cells onto the cantilevers without a clear, real-time image of the loading area universally failed. The final major application of the imaging system was sarcomere length estimation which was discussed in Section 2.4.2.

In the majority of experimental systems designed to explore the mechanics of myocytes the cells are imaged on a slide in a conventional microscope. Once a healthy cell is selected the actuator, force sensor and attachment mechanism are brought to the cell using some form of micromanipulation system. In our design the motor structure was not easily adapted for integration with a bench top microscope and we adopted the strategy of bringing the cell to the cantilevers. As a result, the imaging system needed to be capable of tracking the cell from the inspection region to the loading area.
Early iterations of the imaging systems attempted to rely on reflective top down illumination given the difficulty of bringing light into the area around the cantilevers. However, it was not possible to clearly see the cells using this approach suggesting that, at a minimum, transmission microscopy with bright field illumination was necessary. This problem was solved by threading an optical fiber through the center of the motor structure (at the expense of a small amount of motor performance, see Section 2.3.3). The 130 μm diameter optical fiber acted as a point light source located approximately 500 μm below the cell when it was loaded on the cantilevers.

The contrast of images could potentially be improved by using phase contrast rather than bright-field illumination. Furthermore, the system would benefit from a redesign which allowed a high quality condenser and high numerical aperture objective to be brought to bear on the cell. As discussed in Section 2.4.2, the quality of the imaging system was also degraded by issues such as cellular debris in the fluid between the cell and the objective and bubbles produced by the electrodes or trapped on the cantilevers when the bath was filled with solution. These elements could also be minimized by appropriate redesign.

2.6.3 Fluidics

The fluidic system integrated with the motor structure was critical for repeatable physiological experiments. To perform the proof of principle measurements the fluidic system used three gravity driven wells, an oxygen supply (95 % O₂/5 % CO₂) that could be bubbled through the solutions and tubing connections to the motor system bath (R-3603 Tygon Lab tubing from Saint-Gobain with low O₂ permeability). In the latest iteration the solutions were suspended about 0.4 m above the optical table in hollow walled 200 to 400 mL glass wells. Water from a thermally regulated bath was passed through the hollow walls of the wells to heat the physiological solutions to 36 °C. Solution flow from the wells to the inlets in the base of the motor structure bath was controlled using manual valves.

In early iterations, the solution in the motor bath had a negative meniscus. This left the surface relatively flat and permitted undistorted imaging of the cantilevers in solution.
with a top down microscope. However, the negative meniscus affected the mechanical response of the cantilevers (measured using system identification techniques) possibly through a combination of surface tension and the close proximity of the surface to the tips of the cantilevers. If the motor bath was filled to create a positive meniscus above the top of the glass tube, the mechanical response of the cantilevers became the expected damped second order system with smaller higher order modes (see Section 2.3.3). Unfortunately, the surface of the positive meniscus was curved which distorted images captured using the microscope.

To overcome this problem, a small glass slip was suspended about 3 mm above the top surface of the motor bath using a plastic structure. A positive meniscus formed between the glass tube of the bath and the suspended glass slip. The plastic structure also supported a 600 \( \mu \text{m} \) diameter nozzle that was used to suck solution out of the bath. If the nozzle was properly adjusted it was found that the fluidic system was self regulating. Solution would be removed from the motor bath faster than it flowed in at the base until the nozzle’s connection with the solution surface was broken. The bath would then refill until the connection was restored (the complete cycle typically had a period of 5 to 10 seconds). It was found that when the nozzle was set at an appropriate location, this process would minimally disturb the cantilevers allowing continuous flow through the bath during measurement.

The most significant problem with the fluidic system was the formation of bubbles between the cantilevers or in the optical path between the position sensor and the back surface of the cantilevers. Given the close proximity of the cantilevers (approximately 250 \( \mu \text{m} \) in the final design) and surrounding permandur motor structure it was difficult to avoid trapping air when filling the bath. It was found that the best approach involved pushing the cantilevers apart (by about 1 mm at the tip) using the cell loading capillary (see Section 2.6.4) before filling the bath. Trapped air bubbles could be detected via visual inspection or indirectly through their effect on the mechanical response of the cantilevers (see Section 2.3.2).
In earlier iterations, bubble formation around the cantilevers was also observed during experimentation. As discussed in Section 2.6.6 the bubbles that formed around the cantilevers were sometimes a result of an electrochemical reaction when the parylene insulation of the cantilevers was damaged. However, it was found that the primary source of these bubbles was a change in the solubility of O₂ and CO₂ as the temperature of the solution around the cantilevers increased due to resistive power dissipation in the cantilevers. To overcome this problem the solutions were degassed by using the temperature controlled wells to heat them to 36 °C (above the steady state temperature of the fluid in the bath so no gas was forced out of solution around the cantilevers).

The bath volume was approximately 0.5 mL. Simply considering the heat capacity of the water, the bath temperature would change by approximately 0.5 K/J. In a typical experiment, approximately 25 mA of current was applied to offset one cantilever tip to adjust for the length of the cell. The offset current was significantly larger than that used to apply small length changes to the muscle to characterize its mechanical properties. For a 25 mA offset the cantilevers would dissipate 2.5 mW of power producing a temperature change of 1.2 mK/s. In practice, it was found that with solution flowing continuously through the bath, the temperature reached a steady state of approximately 28 °C in most experiments. The temperature of the bath was monitored with a very small platinum resistive temperature detector, RTD, (HEL-700, Honeywell) and custom Wheatstone bridge amplifier that used an LT1019 voltage reference, precision matched resistors and an AD620 instrumentation amplifier to detect changes in the RTD resistance. The RTD was electrically insulated with parylene and inserted into the bath a small distance from the cantilevers. The relationship between temperature and output voltage, V\text{OUT}, of the temperature sensor could be found using

\[ T = \frac{1}{\alpha} \left( \frac{R_3}{R_0} \cdot \frac{1 - \gamma}{\gamma} - 1 \right), \quad \gamma = \frac{V_{\text{OUT}}}{GV_{\text{REF}}} + \frac{R_1}{R_1 + R_2}, \tag{2.6-1} \]

where \( \alpha = 0.00385 \), \( R_1 = R_2 = 1.18 \, \text{kΩ} \), \( V_{\text{REF}} = 2.45 \, \text{V} \), \( G \) is the gain of the differential amplifier (sensing the voltage difference between the arms of the Wheatstone bridge) and \( R_0 \) is the RTD resistance at 0 °C (\( R_T = R_0(1 + \alpha T) \, \Omega \)). The theoretical relationship between temperature and RTD voltage was checked using an independent thermometer and is
displayed in Figure 2.6-2. A simplified version of the Wheatstone bridge amplifier is also included.

![Diagram of Wheatstone bridge amplifier](image)

**Figure 2.6-2.** The relationship between temperature and output voltage of the Wheatstone bridge amplifier. A linear fit is also provided. The inset is a simplified version of the Wheatstone bridge circuit (R_T represents the RTD).

One additional problem with the fluidic system is worth noting. It took a significant amount of time for the solution in the baths to change. Due to the many interstices of the design, the bath could not be simply purged via convection instead the solution change relied on diffusion which was far slower. This was obviously an issue for experimental protocols that required solution changes and was also a problem when cleaning the bath. It was found that to avoid salt build up in the bath the system needed to be flushed by at least 30 mL of deionized water after experimentation (typically driven through the system at reasonable pressure with a syringe).

The fluidic system used in the final iteration was sufficient to provide proof of principle measurements. However, there is considerable room for improvement. Ideally it should provide rapid switching between the solutions (minimum dead volume and an inlet right
by the cell). Furthermore, the temperature of the solutions should be regulated as close as possible to the bath perhaps using a thermoelectric cooler and temperature sensor. Also, it would be ideal if the entire system could be sealed to remove the need for the pump to extract solution from the motor bath and avoid any mechanical perturbations this introduces. As was discussed in Section 2.4.2 scattering from cellular debris in the solution and on the glass slip above the motor bath affected the image quality. It would be preferable if the solution volume above the cell was minimized and the system adjusted so that direction of solution flow did not wash cell debris from the inspection region across the optical path of the microscope. Finally, while immersing the cantilevers prevents surface tension effects from distorting cell measurements it also introduces cleaning problems. It might be preferable to remove the cantilevers from the bath.

### 2.6.4 Myocyte loading

As discussed in the Section 2.6.2 we adopted the slightly unusual approach of bringing the myocyte to the force sensor and actuator instead of the reverse and several methodologies to achieve this goal were attempted. The first serious iteration attempted to suck cells into a 200 μm internal diameter glass capillary for transport (see Figure 2.6-3). Fluid was moved through the capillary using a quasi-microfluidic system composed of a 50 μL syringe driven by a micrometer. Initially, it was intended that the cell be selected under an independent microscope, sucked into the capillary then transported to the device and gently laid out on top of the cantilever. It quickly became apparent that this method did not have sufficient accuracy to place a cell on top of the cantilever.
Figure 2.6-3. A cell just before it was sucked into a hollow capillary. It was found that while the cells could be transported within the capillary they could not be loaded onto the cantilevers with sufficient accuracy.

To avoid having to move a cell from one bath to another, it was necessary to integrate cell inspection into the existing motor bath. The need to maintain access to the cantilevers both optically (for the position sensors and imaging system) and physically (to load the cells and manipulate the cell attachment mechanism, see Section 2.6.5) made the design of the inspection system a challenge. The problem was solved with an inspection mount that could slide along the top of the motor system bath (see Figure 2.6-4). The mount was constructed from stainless steel 304 using the wire EDM. A small section of plastic cover slip from VWR Scientific Products was placed over a circular hole in the mount and secured using Dow Corning silicone sealant compound 111. It had previously been found that myocytes adhered less readily to the plastic cover slip (compared with glass).

To load a myocyte, the motor bath was partially emptied of solution, and a dry inspection mount placed above the bath (see Figure 2.6-4). A 10 μL drop of dilute cell suspension (approximately 1:8 by volume of cell pellet to BSA Tyrode solution) was applied to the slide and allowed to settle for five minutes. The suction pump was switched on and the motor bath was filled with Ca^{2+} Tyrode solution. Fluid was flushed through the bath for
about one minute to minimize the chance that cellular debris would settle onto the cantilevers. Once the system had stabilized the fluidic system was switched off and the inspection mount pushed over the cantilevers such that it was illuminated from beneath by the optical fiber in the motor system. A healthy cell could then be selected via inspection using the 10 times Mitutoyo M-plan apo lens and bright field illumination.

Figure 2.6-4A. The cell inspection mount on top of the motor bath. The dilute cell suspension was placed on top of the mount while it was in this position B. The inspection mount positioned above the cantilevers so the cells could be imaged using brightfield illumination and top down microscopy. A healthy cell was selected here and picked up using the loading capillary (see text). The inspection mount was then slid back to its position in image A.

Once the inspection system was designed an alternative to the failed hollow capillary and quasi microfluidics system for loading the cell was required. While testing this system, it was observed that cells stuck readily to the tip of the hollow capillary. This was the basis of the final approach that used a capillary that had been pulled to a fine tip (1 μm outer diameter) to pick up the cells. It was found that bringing the tip under a cell on the plastic cover-slip and letting it sit for 10 seconds was sufficient to attach to the cell. The myocyte was then lifted up and the inspection mount gently slid out of the way (see Figure 2.6-4). The cell could then be lowered down to the cantilevers for attachment. An image of the cell attached to the loading capillary is shown in Figure 2.6-5.
Several additional issues are worth mentioning. Firstly, a small drop of Ca\(^{2+}\) Tyrode was placed on the under side of the plastic cover slip of the inspection mount before it was placed in the well. This avoided condensation when the room temperature cell suspension was applied to the slide and prevented air from being trapped under the slide when the bath was filled. Secondly, the adhesion of the cells to the loading capillary was greatly improved if the BSA in the original cell suspension was washed out of the system for a minute or two (with Ca\(^{2+}\) Tyrode). Finally, the design of the cell inspection mount was adjusted to minimize rotation and sticking while sliding across the motor bath (by moving the center of mass). This minimized mechanical disruption of the solution which, in earlier designs, was sufficient to wash cells off the loading capillary when the inspection mount was pushed back to the position of Figure 2.6-4A.

The final iteration of the loading system could bring a cell to the cantilevers in an average of five minutes after the cells had settled on the inspection mount. However, the system was less than ideal. Many features of the design were limited by mechanical and optical constraints created by preexisting features of the motor structure. The problems associated with loading the cells were not well understood when the motor system was designed. Given that efficient and preferably automated loading would be critical to the
operation of an array, ease of loading should be a significant consideration in the design of subsequent iterations.

2.6.5 Myocyte Attachment

Attachment has long been a significant problem in studying the mechanical properties of intact single muscle cells (see review by Garnier et al [74]). It became one of the most challenging problems of this thesis. It was complicated by many factors including the difficult and time consuming isolation process, the limited life time of cells post isolation (four to six hours), the challenge of loading cells, the inherent fragility of the cell membrane and the large forces produced when a cell contracts (up to 4 μN in our hands). As a result, it was typically only possible to test between one and five cells per experiment. Furthermore, the extensive preparation required meant that only one experiment could be performed every second day.

Several different strategies and materials were explored to promote cell attachment. As described in Section 3 we achieved the best results with a novel clamp structure on each capillary (see Figure 2.6-6) and the collagenase blend C8176 from Sigma-Aldrich (St Louis, Missouri). The cell was clamped between a square cross section borosilicate glass capillary and an etched carbon fiber [84] (kindly provided by Professor Peter Kohl, University of Oxford). The carbon fiber was glued at one end to the top surface of the glass capillary. To attach a cell, the carbon fiber was bent upward and the cell was gently placed between it and the glass capillary. The carbon fiber was then slowly lowered onto the cell. The process was repeated with the opposite end of the cell (setting the separation between the cantilever actuators according to the length of the myocyte). The clamp force could be adjusted by varying the distance between the cell and the point where the carbon fiber was glued to the glass. We found that very good attachment could be achieved with mild deformation of the cell. Using this methodology it was possible to test several cells in a single day of experiments.
OTHER ATTACHMENT MECHANISMS

It is worth discussing some of the other attachment mechanisms that were attempted. Originally we intended to use the etched carbon fiber [84] on its own to attach to the cells to the cantilever. The fibers had been reported to provide successful attachment 80% of the time and an alternate carbon fiber with very high surface area had been used by Yasuda et al [87] to bind single cells. Based on a literature review and discussions with Professor Peter Kohl who used Le Guennec's fibers in his laboratory, the carbon fiber approach appeared the clear choice for cell attachment.

To test carbon fiber attachment, the experimental system used by Professor Peter Kohl’s group in Oxford was replicated in our laboratory using an agarose incubation well from Warner Instruments that was integrated into an Olympus microscope and two carbon fibers attached to micro manipulators. Cell health was tested visually and via electric field stimuli (see Section 2.6.1) and then the carbon fibers were lightly pressed against the cell for two to five minutes. The microscope slide was coated with poly HEMA to limit competitive attachment (although the cells still adhered mildly to the coated glass after pressure was applied). If attachment was successful cells were lifted from the glass.

Figure 2.6-6. The cell attachment clamp. To load a cell the carbon fiber was bent upward at its free end and a myocyte gently placed between it and the glass capillary. The carbon fiber was then lowered onto the cell. The upper inset is an image of a myocyte attached between the cantilever while the lower inset is a scanning electron microscopy image of the carbon fiber.
and the strength of attachment could be tested by stimulating the cells to contract using an electric field (see Figure 2.6-7A).

In our hands the carbon fiber did not successfully attach to the cells more than 5% of the time. Very strong attachment at both ends of the cell was even less likely. Many experiments were performed to try to identify the cause of this problem focusing on potential differences between our experimental system and that of Oxford. The low success rate could be tolerated when the attachment mechanism was brought to the cells under a microscope as it was quite possible to attempt to attach more than 30 cells in one four to six hour experimental period. However, for an array application the time required to load and attach cells is likely to be a critical limitation and a far higher success rate is required.

In early experiments using carbon fibers glued to the tips of the cantilevers not even a 5% success rate of attachment was achieved (see Figure 2.6-7B). It was hypothesized that the poor performance was due to the lack of applied pressure. As discussed in Section 1.3 cell attachment strengthens over time in many cell types and pressing the cells against the carbon fiber would presumably increase the contact surface area.

Figure 2.6-7A. Two cells connected end to end and attached relatively strongly to two carbon fibers as viewed using the Olympus microscope experimental system. B The first picture of a weakly attached cell on a carbon fiber within the device and the loading capillary used to place it there (see Section 2.6.4).
It was difficult to apply pressure to cells placed across the carbon fiber in the modular device. A custom jig was designed to present two surfaces that could be brought gently down on the cell on either cantilever using a three axis micromanipulator. The space above the motor structure bath was becoming quite crowded necessitating the somewhat convoluted design structure illustrated in Figure 2.6-8.

The pressure jig did not significantly increase the attachment success rate. It was very difficult to avoid killing the cell while applying pressure using the manual micromanipulator. This problem was addressed by using a piezoelectric actuator to apply the final displacement. In addition, the cells often adhered with more strength to the surface applying the pressure rather than the carbon fiber. Both poly HEMA coated square cross section glass capillaries and small sections of 200 µm thick plastic cover slips were used to squash the cells.

The low success rate of the carbon fiber on its own prompted the search for alternate attachment mechanisms. Based on a literature review it appeared that none of the natural or artificial adhesives that had been used were able to provide sufficiently strong attachment by themselves (see Section 1.3). This suggested that some form of mechanical enhancement was required and led to the use of clamp structures. The first clamp used

**Figure 2.6-8A.** The cell pressure jig, motor structure and inspection mount. The cell pressure jig was bolted onto a three dimensional micromanipulator via the slot in the upper right of this image. **B.** A close up view of the cell pressure jig showing the square glass capillaries originally used to apply pressure to a myocyte resting on carbon fibers. In more recent design these capillaries were used to bend the carbon fiber clamp.
two square cross section borosilicate glass capillaries. The lower capillary was glued to the cantilever as in Figure 2.6-6 and the second capillary replaced the carbon fiber in this Figure (again glued only at one end). The principle of operation was identical, the cell was to be inserted between the two glass capillaries and clamped.

The primary problem with this glass clamp structure was the difficulty inserting the cell between the two glass capillaries (due to the size of the upper arm of the clamp). However, the attachment mechanism was tested during the period in which the alternate collagenase blend, C8051, was used in the isolation protocol (see Section 2.6.1). It was observed that cells isolated with this collagenase adhered directly to glass with significantly more strength than cells isolated with collagenase blend C8176 presumably due to differences in cell surface composition after digestion. This was evident in their interaction with the glass loading capillary.

It was further found that bending the glass in the clamp increased the attachment strength (as assessed by stretching the cells using the cantilevers). The cells would adhere immediately on being brought in contact with the top surface of the bent clamping capillary. This unusual result was observed on at least five separate days of experimentation. There was a clear difference in attachment strength between cells that were lightly pressed onto straight capillaries compared with those pressed onto bent capillaries. Two hypotheses were put forward to explain this effect. The first was that the surface tomography of the glass was altered as a result of the bending stress. The second was that the bending stress positively biased the dissociation reaction of the silanol groups on the surface of the glass thereby increasing the surface charge density and cell attachment.

Regardless of the underlying mechanism, the increased attachment allowed the first successful measure of forces coupling between the cantilevers through the cell. Unfortunately, the quality of the cell isolation with the C8051 collagenase degraded rapidly soon after these initial experiments (as discussed Section 2.6.1). When the original collagenase, C8176, became available in the United States again, the isolated
cells did not attach to glass with equivalent strength. This motivated the design of the carbon fiber clamp structure which provided rapid, strong attachment.

### 2.6.6 Cell Stimulus

The generation of a twitch contraction is an important indicator of cell health, demonstrating that the excitation contraction coupling mechanism within the cell is functioning. An electric field was required to stimulate twitches in intact cells via net depolarization of the membrane, influx of Ca\(^{2+}\) through L-type channels and release of Ca\(^{2+}\) from the sarcoplasmic reticulum (see review [109]).

We chose to use platinum electrodes to apply the electric field to the cell and adopted standard stimulus parameters (2 to 10 ms pulses at an electric field strength 20 to 40 % above the level required to induce contractions). The ability of the cells to contract was first tested in the agarose bath under the Olympus microscope. Platinum wires 1 mm in diameter were arranged on opposite edges of the bath. The pulses were generated by a Techron 8803 power amplifier driven by an Agilent 33220A signal generator. It was found that the fraction of cells with healthy appearance that responded to the electric field depended on the strength of the stimulus, the quality of the isolation and the length of time since isolation. About 1 hour after a good isolation approximately 80% of the cells that appeared healthy responded to an estimated 600 V/m electric field stimulus. The majority of the unresponsive cells with healthy appearance could be made to contract by increasing the electric field strength up to approximately 2000 V/m. No attempt was made to consider the effect of cell orientation with respect to the electric field in this simple experiment.

The placement of the electrodes in our system was complicated by the compact environment around the cell. It was necessary to find an orientation that did not affect the ability to load cells into the device or occlude the position sensor light incident on the back surface of the cantilever. The final design used Teflon insulated, 100 \(\mu\)m platinum wire attached to either side of the jig that was used to manipulate the cell clamp (see Figure 2.6-8). The tips of the wire used to stimulate the cell (where the insulation was
removed) were separated by approximately 2 mm. Once the cell was attached the three axis micromanipulator attached to the pressure jig could be used to move the electrodes to a suitable stimulus position near the cell.

Several problems became apparent when electrical stimulation was attempted in the device. Firstly, it was found that the potential difference between the electrodes needed to be as large as 20 V to generate a contraction. Bubbles of oxygen and hydrogen produced at the electrodes accumulated on the glass slip above the cantilevers and often affected the image quality of the microscope.

The voltage required to stimulate the cells was considerably higher than expected given experiments under the microscope and simple predictions of the electric field between the electrodes. However, the two stainless steel cantilever actuators were connected to ground through a very low impedance path (a 10 $\Omega$ resistor current sense resistor) and acted to shield the myocyte. A two dimensional electro-quasi-static finite element model of the space around the cell was used to explore this issue. The cantilevers were modeled as infinite sheets with a uniform potential (0V) and were surrounded by 3 $\mu$m of parylene. The electrodes were modeled as infinite rods and set to the stimulus potential. It was found that the electric field strength was greatly reduced in the space between the cantilevers. However this effect decreased rapidly with distance above the tip of the cantilevers. Based on the findings of this simulation, subsequent iterations of the attachment mechanism used an extra piece of glass capillary to ensure the cell was positioned a further 100 $\mu$m above the cantilever tip.

Finally, it was observed that repeated adjustment of the myocyte attachment mechanism could damage the parylene insulation coating the cantilevers. This resulted in bubble formation on the cantilevers when the electric field was applied by the platinum electrodes which affected the image quality of the top down microscope (see Section 2.6.2). If the bubbles were large enough they could cause significant coupling between the cantilevers and interfere with mechanical measurements (see Section 2.3.2).
3 Instrumentation Paper

The experimental objective of this thesis was to verify that the modular system designed could, in fact, measure the mechanical properties of single cells. The proof of principle test selected was a measurement of the dynamic stiffness. The following draft paper, entitled "A modular instrument for exploring the mechanics of cardiac myocytes", describes details of the instrument design and the world’s first measurement (to the author’s knowledge) of the passive dynamic stiffness of an intact mammalian ventricular myocyte. It should be noted that the draft papers in Sections 3 and 4 are intended to stand alone. As a result, there is some overlap of information content, equations and figures in these papers and the remainder of the thesis.
3.1 Introduction

The cardiac myocyte is a key experimental system for exploring the mechanical properties of the diseased and healthy heart. Compared with molecular or myofibril studies, the contractile protein apparatus is arrayed in a physiologically relevant orientation within a living cell. Furthermore, myocytes avoid problems inherent to multicellular preparations including heterogeneity of cell types [3], diffusion limited extracellular spaces [5,4], non-uniform shortening of sarcomeres during isometric contraction, the mechanical influence of the extracellular matrix [6] and allow clear optical interrogation of sarcomere length.

Protocols for isolating millions of functional, primary cardiac muscle cells from ventricular tissue are readily available [8,10,110]. Myocytes are typically used for experimentation within four to six hours of isolation. However, using current instrumentation it is difficult for a single researcher to successfully explore the mechanical characteristics of more than one or two myocytes under loaded conditions within this time. An instrument array that could perform these measurements on 10 to 100 cells in parallel would be advantageous from both ethical and scientific perspectives.

To provide a suitable basis for an instrument array, the mechanical testing device upon which it is based should be modular, compact and inexpensive. Many different actuators and force transducers have been used to explore the loaded mechanical properties of cardiac muscle cells. However, the majority of current instruments were not designed for parallel measurements. They use commercial displacement actuators that are both expensive and bulky (when associated electronics are considered) and some form of cantilever as the force transducer. Researchers have used optical fibers, suction pipettes, glass needles, steel foil [65], microfabricated polysilicon beams [63,28] and 12 μm wire [64] for the cantilever and detected its displacement with strain gauges [63,66], video analysis or optoelectronic techniques [65]. The position sensor is the key element of this style of force transducer and the tradeoff between resolution and cost is critical for an array application.
While advances in instrumentation have provided actuators and force transducers with sufficient resolution to perform mechanical experiments on individual cardiac myocytes, the attachment of these cells to the instrument remains a significant challenge (see review by Garnier et al [74]). Many attachment methodologies have been used including suction micropipettes [77], immobilization against microscope slides via pressure [78] and various natural and artificial adhesives such as fibrinogen [79], poly-L-lysine [81], silicon sealants [111] and adhesive foams [83]. Another technique developed by Le Guennec et al [84] used an etched carbon fiber which was reported to provide successful attachment 80% of the time. The hypothesized mechanism of attachment was electrostatic attraction and etching increased surface area and hence net charge. More recently, Yasuda et al [87] used carbon fibers with even greater surface area and they have reported success in stretching single cells using this technique.

Here we describe the design and development of a modular instrument and novel attachment mechanism that is capable of exploring the mechanics of single muscle cells and is suitable for use in an instrument array for high throughput science. The mechanical and electrical characteristics of the device are presented and its functionality demonstrated by providing the first measure of the passive dynamic stiffness (a complex number representing the ratio of stress to strain as a function of frequency) of a single intact ventricular myocyte at varied sarcomere lengths.
3.2 Design Considerations

The core of the instrument illustrated in Figure 3.2-1 is conceptually similar to the design of Iwazumi [64] utilizing two Lorentz force actuators that can simultaneously be used as force sensors. A permandur motor structure concentrates a magnetic field in two air gaps and was manufactured using a wire electrical discharge machine, EDM (Charmilles, Lincolnshire, IL) and a 5 axis machining center (HAAS Automation Inc., Oxnard, CA). Stainless steel cantilevers with a rectangular section removed from the center to form a loop serve as the actuator and force sensor. The long arms of the cantilever loop pass through the air gap magnetic field providing the basis for actuation. The motor structure is coated in 3 μm of parylene (to ensure biological compatibility and provide electrical insulation) using a bench top deposition system (Para Tech Coating Inc, Aliso Viejo, CA). Optical position sensors reflect from the back surface of each cantilever, their photodiode current is amplified and the resulting position signal is then used to control the current source driving the actuator (see Section 4). All electronics necessary to amplify position signals and drive currents through the cantilevers are built into the module illustrated in Figure 3.2-1. Circuit design and board layout utilized PSpice and Allegro packaged within Cadence Design Systems software (Cadence Design Systems, San Jose, CA) and double sided circuit boards were manufactured using a board plotter (LPKF, LPKF Laser & Electronics AG, Garbsen).
3.2.1 Data Acquisition Signal Processing and Control

Currently, data acquisition, signal processing and $H_\infty$ digital control are implemented using a PCI card (6052E National Instruments, Austin, TX) and custom Visual Basic.Net (VB.Net) code. Four analog inputs, two position signals and two current signals, are each sampled at up to 10 kHz and two analog outputs set the current through the cantilevers. Signal processing algorithms have been implemented in VB.Net and Matlab to perform digital control (see Section 4), power spectral estimation and stochastic system identification. Stochastic system identification was used to estimate samples of the impulse response or Markov parameters of a linear system model from measured input and output signals (typically a force input and position output).

CHARACTERIZING THE CANTILEVERS

A typical experiment to probe the impulse response of a cantilever involved the application of a 100 second current (force) to the cantilever, generated and sampled at a rate of 10 kHz. The signal was produced by filtering zero mean, unit variance, Gaussian
white noise. The filter was chosen to scale and distribute the power applied to the system over the appropriate measurement bandwidth (typically between 0.1 Hz and 2.5 kHz when characterizing the cantilevers). The standard deviation of the input (force) and output (position) were typically < 10 μm and < 20 μN respectively. To find the impulse response, 2048 point auto and crosscorrelation estimates were made of the force (X) and position (Y) data and used to populate the following [107]

\[
R_{XX} h_{XY} = R_{XY}
\]

where \( R_{XX} \) is a Toeplitz matrix of autocorrelation coefficients, \( R_{XY} \) is a vector of crosscorrelation estimates, \( h_{XY} \) is a vector of samples of the impulse response and \( n = 2048 \) in this case. Equation 3.2-1 was solved for \( h_{XY} \) by inverting the Toeplitz matrix using a Levinson recursion algorithm implemented in VB.Net and Matlab. The dynamic compliance is the Fourier transform of \( h_{XY} \) (normally expressed as a magnitude and phase).

The quality of the system identification was assessed by calculating the variance accounted for or coherence squared, \( \text{coh}^2(\omega) \), using [107]

\[
\text{coh}^2(\omega) = \frac{|S_{xy}(\omega)|^2}{S_{xx}(\omega)S_{yy}(\omega)} \quad 0 \leq \text{coh}^2(\omega) \leq 1,
\]

where \( S_{xy}(\omega), S_{xx}(\omega) \) and \( S_{yy}(\omega) \) are the cross, input and output power spectral densities respectively. Conceptually, the \( \text{coh}^2(\omega) \) will be less than unity at a particular frequency if there is noise or interference in the data at that frequency (e.g., sensor noise or extraneous inputs such as mechanical interference) or nonlinearities in the system under test (e.g., harmonics).

**CHARACTERIZING A SAMPLE**

The modeling strategy used to characterize a sample was described elsewhere (see Section 4). Briefly, static, modal and harmonic finite element analyses in Ansys (Ansys, Canonsburg, PA) were used to assess the effect of loading the cantilevers with a sample
with material properties similar to a myocyte. It was found that for frequencies up to the first resonance, an invertible lumped parameter model (see Figure 3.2-2 and Equation 3.2-3) of the two cantilevers and the sample was adequate to describe the dynamics of the system. The multi-input multi-output (MIMO) lumped parameter model uses the force applied to the cantilevers as inputs and the displacements of the tips of the cantilevers as outputs.

\[
\begin{bmatrix}
X_1 \\
X_2
\end{bmatrix} = H(s) \begin{bmatrix}
F_1 \\
F_2
\end{bmatrix}
\]  
(3.2-3a)

\[
H(s) = \begin{bmatrix}
\frac{H_2 + H_m}{H, H_2 + (H, + H_2)H_m} & \frac{H_m}{H, H_2 + (H, + H_2)H_m} \\
\frac{H_m}{H, H_2 + (H, + H_2)H_m} & \frac{H_m}{H, H_2 + (H, + H_2)H_m}
\end{bmatrix}
\]  
(3.2-3b)

\[
\begin{bmatrix}
F_1 \\
F_2
\end{bmatrix} = H^{-1}(s) \begin{bmatrix}
X_1 \\
X_2
\end{bmatrix}
\]  
(3.2-4a)

\[
H^{-1}(s) = \begin{bmatrix}
H_1 + H_m & -H_m \\
-H_m & H_2 + H_m
\end{bmatrix}
\]  
(3.2-4b)

**Figure 3.2-2.** Lumped parameter system model \(H_m(j\omega), H_1(j\omega)\) and \(H_2(j\omega)\) are the dynamic stiffness of the sample, and two cantilevers respectively. Forces and displacements can be applied on either side of the sample.

To explore the properties of a myocyte suspended between the cantilevers, a 100 second force signal, \(F_1\), was applied to cantilever one (see Figure 3.2-2) sampled at 2 kHz. The applied current was typically band limited between 0.1 Hz and between 40 and 100 Hz and scaled to produce a standard deviation of position and force of < 1 \(\mu m\) and < 2 \(\mu N\) respectively. The positions of the two cantilever tips, \(x_1\) and \(x_2\), were recorded and the
transfer function \( X_1/X_2(j\omega) \) estimated using Equation 3.2-1. The dynamic stiffness of the sample could then be estimated by rearranging the second row of Equation 3.2-4a to give

\[
H_M(j\omega) = \frac{X_2(j\omega)}{1 - \frac{X_2(j\omega)}{X_1}} H_2(j\omega),
\]

where \( H_M(j\omega) \) and \( H_2(j\omega) \) are the dynamic stiffness of the sample and cantilever two.

**SARCOMERE LENGTH ESTIMATION**

A custom digital microscope was built using a 10, 20 or 50 times Mitutoyo M-Plan Apo lens (Mitutoyo America Corporation, Aurora, IL), an infinity-correct tube lens (Edmund Optics Inc, Barrington, New Jersey) and a Sony DFW-SX900 CMOS digital video camera (Sony, Tokyo). This was used to capture \( 1280 \times 960 \) pixel images of the myocyte at 7 frames / second. The sensitivity of the digital microscope (4.6 pixels/\( \mu \)m) was found using the spatial Fourier transform of an image of a 2 \( \mu \)m graticule. An initial estimate of the sarcomere length (SL) was made using the average spatial Fourier transform of each row of a user defined region of the myocyte image that had been band pass filtered and zero padded to reduce the bin spacing.

### 3.2.2 Position Sensor

The position sensor was a critical element of the design as its resolution limited both the minimum detectable force and position. A commercially available confocal sensor consisting of an LED, split lens and photodiode (HEDS-1300, Agilent, Palo Alto, CA) was selected for its simplicity, robustness, low cost and ease of alignment [96]. These characteristics made it ideal for use in an instrument array.

A low noise transimpedance amplifier was designed to ensure that the position resolution was limited by the shot noise of the photodiode within the HEDS-1300 device. Circuit noise and interference were quantified at several operating points (current levels through the photodiode) by estimating the power spectra of the position signal output. In the current device iteration with the cantilevers fully immersed in physiological saline it was
found that the circuit was shot noise limited between 10 Hz and 10 kHz with a typical noise floor of 0.75 nm/√Hz and sensitivity of approximately $15 \times 10^3$ V/m (see the Section 3.3)

### 3.2.3 Force Sensor

The cantilevers are typically 5 mm × 1 mm × 0.025 mm (L × W × T) and are cut from 25 μm annealed stainless steel 316 foil using the wire EDM. Stainless steel 316 was used for its high conductivity, low cost, reasonable reflectivity and low magnetic susceptibility (provided it is annealed after being cold worked). Originally stainless steel 304 was used however it was found that it became magnetized over time by the strong magnetic field within the motor structure.

Early designs used straight sections of wire or ribbon fixed at both ends which simplified the motor structure as only one air gap with a uniform magnetic field was required. However, the ratio of stiffness to first resonant frequency for a uniform beam fixed at both ends is approximately 8 times that of a beam fixed only at one end [104]. Therefore, a cantilever provides better force resolution (lower stiffness) for a given measurement bandwidth (set by the resonant frequency). This motivated the design of a novel cantilever actuator with a rectangular section removed from its center to create a current loop (see Fig 3.2-1).

It is desirable for the force sensor to be significantly stiffer than the myocyte so that the loading effects of the myocyte can be ignored (see Section 4). However, due primarily to limitations in position sensor resolution, this is difficult to achieve. To appreciate this problem, consider the position resolution required to measure the stiffness of muscle tissue. Rearranging Equation 3.2-4 and treating the muscle and cantilevers as simple springs the relationship between the displacement applied to one side of the cell, $Δx_1$, (the actuator) and the displacement of the other cantilever $Δx_2$ (the force sensor) is

$$Δx_2 = \frac{k_m}{k_2 + k_m} Δx_1,$$

(3.2-6)
where $k_m$ and $k_2$ are magnitude of the stiffness of the muscle and force sensor respectively. The displacement, $\Delta x_1$, is often limited to avoid nonlinear effects in the muscle tissue and position sensor resolution provides a lower bound on $\Delta x_2$. The combination of these constraints sets the maximum stiffness, $k_2$, of the force sensor. We have found that female guinea pig ventricular myocytes have stiffness between approximately 0.04 and 0.4 N/m at 0 Hz depending on cell length and contraction state. The cantilevers were designed with a stiffness of 2 N/m. In the worst case when $H_m = 0.04$ N/m, only 2% of the displacement applied by the actuator will couple through the cell to the force sensor.

### 3.2.4 Motor Design and Actuation

The primary goals of the mechanical structure of the motor were to support the cantilevers and to maximize the coupling between current and force by concentrating the magnetic flux in the air gaps through which the arms of the cantilevers passed. However, it was also desirable that the structure be fully submersible in physiological saline (to avoid the effects of surface tension and evaporation on the cantilevers), biologically inert, electrically insulated and provide the means for transmission microscopy of the cell while it was attached to the cantilevers (necessary for direct optical measurement of sarcomere length). The design illustrated in Figure 3.2-3 met these objectives. The core of the device is the cantilever assembly consisting of the two cantilevers each attached to 100 $\mu$m thick glass rectangles with a 130 $\mu$m diameter, multimode optical fiber between them. The cantilever assembly was glued onto one half of the motor structure and the remaining elements of the motor are held together using magnetic attraction. Small aluminum spacers ensure the structure is stable and support two stainless steel tubes that are inserted into fluidic inlets at the base of the structure.
Figure 3.2-3A A cutaway CAD diagram of the motor structure illustrating the arrangement of Permandur magnetic field guides and NdFeB magnets that creates a magnetic flux density of approximately 1.0 T in the air gap surrounding the arms of the cantilevers. B The cantilever assembly (two cantilevers glued to glass plates with an optical fiber between them) and one side of the base of the motor structure.

MAGNETIC DESIGN

Permandur was used as the base material for the motor structure taking advantage of its high saturation flux density. Nickel plated, sintered Neodymium Iron Boron magnets (Dexter, Elk Grove Village, IL) were used for their high residual magnetic flux density. The main structure of the motor was designed in Solid Edge (UGS, Plano, TX) and manufactured using the wire EDM and 5 axis machining center. Three dimensional magnetic finite element analysis (ANSYS Inc., Canonsburg, PA) and iterative redesign were used to optimize the motor structure by identifying regions of saturation or peak flux density in the permandur field guides and flux leakage outside of the air gap. The simulation used approximately 106000, 8 node elements (Solid96, Ansys). The magnets were modeled with isotropic relative magnetic permeability \( \mu_r = 1.044 \) and \( H_c = 0.6 \times 10^6 \) m. The permandur was modeled with a B-H curve that saturated at 2.4 T. Thermal effects and the magnetic fields produced by passing current through the cantilevers were ignored in this study.

FLUIDICS

The motor structure was designed to be inserted in a glass tube (15 mm × 6 mm × 12 mm, \( W \times L \times H \)) which could then be sealed at the base using Dow Corning compound 111.
(Dow Coming, Midland, Michigan). This approach allowed the entire motor to be submerged during tests. Solutions were applied through two inlets in the base of the motor structure or through the open top of the glass tube. A small section of a coverslip was suspended over the bath to ensure a flat surface for imaging and to allow the stable formation of a positive meniscus above the glass tube. A small nozzle inserted into the meniscus was used to pump fluid from the bath and was adjusted to minimize mechanical disruption of the surface. The temperature of the fluid in the bath was recorded during experiments using a resistive temperature detector.

**ACTUATION**

The motor structure was mounted directly on a circuit board and the ends of the cantilevers were soldered to two independent, low noise current sources each capable of providing up to ± 0.4 A. The resistance of the cantilevers was approximately 4 Ω and 1 μW of power was dissipated into the solution during a typical measurement of the dynamic stiffness of a myocyte (σ\text{force} approximately 1 μN). The relationship between current and force applied to each cantilever (referred to the tip) was approximately the same, typically between 1 \times 10^{-3} and 1.1 \times 10^{-3} N/A corresponding to an air gap magnetic flux density of approximately 1 T. The motor could provide peak forces of ± 400 μN and peak displacements of approximately ± 200 μm. The Lorentz force driving the actuation was distributed along the cantilever arms starting about 1.5 mm below their top edge. The estimation of cantilever and myocyte stiffness both assume force is applied to the tip of the cantilevers at the point of cell attachment. The system is calibrated such that all forces are expressed as an equivalent force at this point.

### 3.2.5 System Calibration

The position sensors were calibrated in the apparatus before each test by focusing the digital microscope on the top edge of each cantilever as it was being driven by a 0.5 Hz sinusoidal current. Custom edge detection code written in Matlab was used to track the displacement. The position sensor sensitivity was typically around 20 \times 10^3 V/m when the cantilevers where immersed in physiological saline.
The relationship between force and current was calibrated using a combination of modeling and stochastic system identification described elsewhere (see Section 4). Briefly, a finite element model (FEM) of the cantilever structure in a vacuum was generated using ANSYS. Eleven simulations were run over a range of lengths from 3 to 6 mm. The results of the simulation were used to express the change in the first resonant frequency and the stiffness at 0 Hz (estimated by applying a 1 mN force distributed across the top edge) as functions of cantilever length.

Stochastic system identification was then used to measure the mechanical transfer function of each cantilever between 0.1 Hz and 10 kHz. The estimated Markov parameters were fit to a 2nd order mechanical impulse response using nonlinear LSE. Comparing the empirical resonant frequency to the finite element model results provided an estimate of the stiffness of the cantilever. Then, by measuring the displacement resulting from a known current using the digital microscope, the relationship between current and force could be determined.

3.2.6 Myocyte Isolation, Loading and Attachment

ISOLATION

Ventricular myocytes were isolated from Dunkin-Hartley female guinea pigs approximately 2 to 3 months of age using standard enzymatic techniques [112,113]. Briefly, animals were anaesthetized with 50 mg/kg telazol (equal parts by weight tiltemamine-HCL and zolazepam-HCL) then decapitated and the heart quickly excised and placed in heparin-Tyrode solution (500 IU/L heparin added to Normal Tyrode solution, mM: NaCl 140; KCl 5.4; MgCl₂ 1; CaCl₂ 1.8; HEPES 5; Glucose 11). The heart was mounted on a Langendorff apparatus and subjected to perfusion via the aorta of heparin-Tyrode, EGTA solution (mM: NaCl 140; KCl 5.4; MgCl₂ 1; HEPES 5; Glucose 11; EGTA 1) and High K⁺ solution (mM: NaCl 4; KCl 10; MgCl₂ 1; CaCl₂ 0.025; K- Glutamate 130; HEPES 5; Glucose 11) followed by collagenase solution (1g/L collagenase blend added to High K⁺ solution). The ventricles were then cut from the heart and myocytes mechanically dissociated in High K⁺ solution through a series of steps. Cell
suspensions were spun in a centrifuge at 300 rpm for 1 minute and the pellet resuspended in BSA-Tyrode (normal Tyrode with 1 g/L bovine serum albumin and 620 IU/L trypsin inhibitor). Myocytes were used for experimentation with 4 to 6 hours of isolation.

LOADING
The motor structure was immersed in oxygenated Tyrode and 10 μL of cell suspension was placed on a custom stage directly above the cantilevers. The cells were illuminated using the optical fiber passing through the center of the motor (see Figure 3.2-3B) and imaged using the digital microscope. The myocytes were stimulated with 100 μm diameter platinum electrodes using 3 to 6 V, 0.2 Hz, 10 ms pulses. Given the configuration of the electrodes and the presence of the metallic cantilevers it was difficult to accurately estimate the electric field strength applied to the cells. A cell was selected for experimentation if it contracted more than 5 % under electric field stimulation and its sarcomere pattern was ordered (with average resting sarcomere length between 1.7 μm and 1.9 μm). A pulled borosilicate glass capillary with a tip diameter of approximately 1 μm was gently touched against the cell and allowed to attach for approximately 20 seconds. The cell was then lifted from the inspection stage and lowered onto the cantilevers.

ATTACHMENT
Several different strategies and materials were explored to promote cell attachment. It was found that the attachment characteristics of isolated cells were highly dependent on the collagenase / protease blend used in their isolation. We achieved the best results with a novel clamp structure on each capillary (see Figure 3.2-5) and the collagenase blend C8176 from Sigma-Aldrich (St Louis, Missouri). The cell was clamped between a square cross section borosilicate glass capillary and an etched carbon fiber [84] (kindly provided by Professor Peter Kohl, University of Oxford). The carbon fiber was glued at one end to the top surface of the glass capillary. To attach a cell, the carbon fiber was bent upward and the cell was gently placed between it and the glass capillary. The carbon fiber was then slowly lowered onto the cell. The clamp force could be adjusted by varying the
distance between the cell and the point where the carbon fiber was glued to the glass. We found that very good attachment could be achieved with mild deformation of the cell.

Figure 3.2-5. The cell attachment clamp. To load a cell the carbon fiber was bent upward at its free end and a myocyte gently placed between it and the glass capillary. The carbon fiber was then lowered onto the cell. The upper inset is an image of a myocyte attached between the cantilever while the lower inset is a scanning electron microscopy image of the carbon fiber.
3.3 Experimental Validation

To demonstrate the functionality of the instrument the passive dynamic stiffness of a single ventricular myocyte was measured. A cell was selected and attached to the top of the cantilevers then allowed to stabilize for five minutes in 28 °C, oxygenated Tyrode solution with 1.8 mM calcium. The resting sarcomere length (1.81 μm) was measured and then mild tension was applied (producing approximately 5 % strain). After pretensioning, the cell had a sarcomere length of 1.9 μm and was 21 μm wide and 86 μm in length (measured between the attachment points).

To measure the dynamic stiffness, a 10^6 point displacement signal with Gaussian distribution and 1 μm standard deviation (± 4 μm peak) was applied to the actuator cantilever. The signal was sampled at 10 kHz and shaped using a custom digital filter with a cut off frequency between 40 and 60 Hz. The displacement of both the actuator and force sensor cantilevers were recorded. The cell was then stretched to sarcomere lengths of 2.14 μm and 2.37 μm and the measurement repeated. There was good agreement between the relative increase in sarcomere length and the increase in cell length (96 μm and 105 μm) for these stretches. This result demonstrates the attachment mechanism was successfully transmitting motion of the cantilevers to displacement of the contractile apparatus within the cell.

The dynamic stiffness of the myocyte and $\text{coh}^2(j\omega)$ of the measurement were estimated at 215 frequencies linearly separated by 0.31 Hz using Equation 3.2-5 and Equation 3.2-2 and are illustrated in Figure 3.3-1. The dynamic stiffness of the force sensor cantilever ($H_2(j\omega)$ in Equation 3.2-5) was measured before the cell was attached and after it was removed using the same input sequence that was applied to the muscle scaled to produce a standard deviation of 10 μm. The stiffness of the cell was converted to a modulus by optically measuring cell width and assuming that the cell had elliptical cross section and that the minor axis of cross section was a third of the major axis (approximately 21 μm and 7 μm respectively) [114, 115]. To demonstrate the increase in dynamic modulus with
sarcomere length, the magnitude of the dynamic stiffness at 10 frequency points between 9 and 12 Hz was averaged and the results are plotted in Figure 3.3-2.

Fig 3.3-1. The dynamic stiffness of a myocyte at 28 °C in Normal Tyrode solution with 1.8 mM Ca\textsuperscript{2+}. Three separate measurements were made at increasing sarcomere lengths (1.9 μm, 2.14 μm and 2.37 μm). Data points are linearly spaced by 0.3 Hz.
The average dynamic modulus (at 10 frequencies between 9 and 12 Hz) of the myocyte at 28 °C in Normal Tyrode solution with 1.8 mM Ca²⁺ plotted as a function of sarcomere length.

The 1 μm standard deviation (± 4 μm peak) displacements applied to the actuator cantilever would be large enough to disrupt any strongly bound cross bridges. The peak displacements are considerably above the noise floor of the position sensor. However, a band limited, stochastically varying signal distributes power across many frequencies. Furthermore, at short sarcomere lengths when \(|H_m(j\omega)|\) at 0 Hz was approximately 0.04 N/m only 2% of the applied displacement would couple through the cell to the force sensor cantilever. The power spectra of the measured displacement of the actuator and force sensor cantilevers at sarcomere lengths of 1.9 μm and 2.37 μm are presented Figure 3.3-3A and B respectively. The noise floor or power spectrum of the position signal when the force sensor cantilever was stationary is also given in this Figure. As can been seen in Figure 3.3-3A, the signal to noise ratio (SNR) of the force sensor displacement is approximately 2.4 at 30 Hz. In comparison, at the longer sarcomere length of Figure 3.3-3B the SNR was 4.4 at 30 Hz as the muscle cell was considerably stiffer and the displacement of the force sensor was larger (as suggested by Equation 3.2-6).

It is also worth noting that the displacement power applied to the actuator cantilever was deliberately increased for frequencies below 10 Hz. The increase in displacement was required to compensate for the low frequency position sensor noise that rose above the shot noise limit at approximately 10 to 20 Hz as is evident in Figure 3.3-3A and B.
Figure 3.3-3A The power spectral density of the measured displacement of the actuator and force sensor cantilevers when the SL was 1.9 μm. The position sensor noise floor is provided for comparison. The SNR of the force sensor displacement was approximately 2.4 at 30 Hz. B The same power spectra measured when the SL was 2.37 μm. Significantly more displacement coupled through the cell to the force sensor cantilever producing an improved signal to noise ratio of 4.4.
3.4 Discussion

We have described the design and development of a modular instrument for exploring the mechanics of intact mammalian myocytes. The instrument utilized a novel motor design and attachment strategy that allowed cells to be stretched to sarcomere lengths in excess of 2.4 μm. As a proof of principle, stochastic system identification techniques were used to measure the dynamic stiffness of a myocyte in 1.8 mM Ca^{2+} Tyrode solution between 1 and 60 Hz at three sarcomere lengths. To the author’s knowledge, this represents the first measurement of the passive dynamic stiffness of a single, intact mammalian cardiac myocyte.

A frequency-shaped, 100 second noise sequence with a Gaussian amplitude distribution was used to perform the stochastic system identification and generate estimates of the dynamic stiffness of a single cell at 215 distinct frequencies. As discussed by Rossmanith [116], the use of broad band signals such as shaped white noise instead of sinusoids is advantageous because it can greatly reduce the time required to measure the complex modulus at low frequencies and simplify nonlinear analysis. The standard deviation of the stimuli used in this study was approximately 1 μm (1 % strain) and the peak to peak variation in displacement was ± 4 μm. These displacements were large enough to disrupt any strongly bound crossbridges (see review [44]).

As was illustrated in Figure 3.3-3, the larger displacements were necessary to ensure the displacement of the force sensor was above the noise floor. The coh^2(jω) given in Figure 3.3-2 is less than unity indicating the presence of noise in the data or nonlinearities in the system. The SNR improves as the cell is stretched (see Figure 3.3-2) and it is likely that this is in part responsible for the parallel increase in coh^2(jω). This hypothesis is further supported by the concomitant reduction in SNR and coh^2(jω) at frequencies below 3 Hz as the position sensor noise rises faster than the applied displacement (see Fig 3.3-3A and B). The position sensor in this instrument was chosen as it is ideal for an array application due to its low cost, robustness and ease of alignment. We are considering
design modifications to boost the resolution of the sensor by an order of magnitude which would allow an equivalent reduction in the applied displacement.

Activated muscle is highly nonlinear as demonstrated by the classic step experiments of Huxley & Simmons [117]). Several researchers have observed nonlinearities in the form of harmonics when sinusoids with amplitude greater than between 0.02 and 0.2 % of muscle length were applied to activated rabbit psoas muscle or glycerinated flight muscle [43, 118]. Although we measured passive mechanical properties in this proof of principle experiment, it is possible that a nonlinear response of the muscle was also responsible for the reduction in $\text{coh}^2(j\omega)$ especially given the relatively large displacements applied. The passive dynamic stiffness measured in this study can be compared to that measured by other researchers using intact cardiac muscle tissue. Shibata et al [119] measured the passive dynamic stiffness of intact papillary muscle from rabbits at 24°C with 2.5 mM $[\text{Ca}^{2+}]_o$ (external calcium concentration) at 8 frequencies between 0.05 Hz and 30 Hz. Their results show the same gradual increase in stiffness and phase that we observed and the magnitude of the dynamic modulus was of similar order (approximately 80 kPa at the muscle length that produced peak force). Pinto and Fung [120,121] measured the passive dynamic stiffness of rabbit papillary muscle between 0.01 Hz and 100 Hz using sinusoids with amplitude equal to 4.06 % of muscle length over a range of temperatures between 5 °C and 37 °C.

Finally, Kirton et al [122] conducted a detailed analysis of the effect of BDM and $[\text{Ca}^{2+}]_o$ on the dynamic stiffness of intact passive rat trabecula at 20 °C. They also measured the same gradual increase in magnitude and phase when 20 mM BDM was applied. However at $[\text{Ca}^{2+}]_o = 1.25$ mM without BDM they observed significant changes in phase as a function of frequency possibly suggesting the muscle tissue was mildly activated. Although we had higher $[\text{Ca}^{2+}]_o$ than used by Kirton et al we did not observe any significant changes in phase in this study. The phase response observed by Kirton et al has been associated with the cycling of cross bridges and the generation of force. It is
likely that the large displacements used in our proof of principle experiment would have disrupted both of these processes.

The novel motor structure described here has several important advantages. Firstly, the unique means of actuation of the cantilever, analogous to a single loop of a conductor in a voice coil motor ensures the actuator / force sensor has very low moving mass. Secondly, this design conveys flexibility as the dimensions of the cantilevers can be adjusted to tune the trade off between bandwidth (resonant frequency) and force sensitivity (stiffness). Thirdly, by designing the motor system such that it could be inserted into a glass tube and fully immersed we avoid interference due to surface tension and evaporation. Finally, the motor structure was well suited to feedback control of cantilever position. We successfully implemented H∞ optimal control of muscle cell length increasing the effective stiffness of the cantilevers 30 fold while measuring isometric contractions of a myocyte at varied sarcomere lengths (see Section 4).

Another novel aspect of this work was the attachment methodology. The approach combined a mild clamping force with the inherent attachment of intact myocytes to both borosilicate glass and etched carbon fiber. This technique does not replicate natural attachment mechanisms between intact myocytes and the extracellular matrix that couple forces through protein structures such as the intercalated discs or costameres (see review by [88]). However, the strategy was repeatable, gentle enough to avoid damaging the cells, strong enough to support significant forces and successfully coupled external displacements to the contractile apparatus. Furthermore, the cells were firmly attached immediately after clamping, an important property for rapid parallel testing.

The primary goal of this work was to design a system that was capable of measuring the mechanical properties of individual cells and was also appropriate for use in an instrument array. To be suitable for an array, the major components of the design including the actuators, position sensors, fluidics and data acquisition system must be compact and inexpensive. The majority of these requirements are satisfied by the current design and we are currently working on integrating the fluidic system with the module.
and designing an embedded digital system to localize data acquisition and control. It is also necessary to simplify as much as possible the challenge of working with single myocytes. It currently takes an average of five minutes to select and load a cell onto the cantilevers. The loading time is limited by the manual manipulation of the cells and we are considering means to automate this process.

In conclusion, we have described the design and development of a modular instrument that has successfully been used to make the first measurement of the passive dynamic stiffness of intact ventricular myocytes. The system utilized a novel motor structure and attachment mechanism and represents a successful first step toward an instrument array for high throughput single cell muscle physiology.
4 $H_{\infty}$ Control and Signal Processing Paper

Given the importance of muscle length on muscle mechanical properties, it was desirable to implement closed loop control of cantilever position to increase the ability of the system to provide support for isometric contractions. The following draft paper entitled “$H_{\infty}$ control of isolated ventricular myocyte length” describes the development and application of a MIMO model and $H_{\infty}$ controller. To reiterate, the draft papers in Sections 3 and 4 are intended to stand alone. As a result there is some overlap of information content, equations and figures in the papers and the remainder of this thesis.
4.1 Introduction

It is very well established that sarcomere length and muscle load have significant impact on muscle mechanical properties. It is therefore desirable to maintain precise control of these factors during experiments to simplify the interpretation of results. This has motivated the use of feedback control in muscle physiology. For example, to measure the rate constant of force redevelopment, \( k_{TR} \), Brenner and Eisenberg [40] used laser diffraction monitoring and feedback to regulate sarcomere length as force developed. The velocity of shortening during muscle contraction, \( V_u \), is strongly dependent on the force load as described by Hill’s classic equation [123]. Isotonic feedback control has been applied to measure \( V_u \) in single cells under varied loads [124]. More recently, an adaptive control strategy has been used to subject muscle tissue and cells to changes in length and force that are close to the conditions seen in vivo [103, 125]. In all of these examples, loading conditions are controlled to regulate both passive and active muscle properties including the distribution of actin-myosin interactions among the different states of the cross bridge cycle and the rate of transitions between these states.

Mechanical experiments on myocytes require extremely sensitive force sensors. Force is typically measured with some form of bending element that functions as a mechanical filter whose properties can be adjusted through a tradeoff between force sensitivity (stiffness) and bandwidth (resonant frequency). Decreasing stiffness to improve force resolution increases the change in muscle cell length during contraction. Given the effect of altering muscle length on the distribution of cross bridges it is often desirable to perform isometric contractions (allowing less than 5 nm/half sarcomere length change or approximately 0.5 % strain [44]). Feedback control of force sensor displacement has been used to increase the effective stiffness of the force sensor while maintaining its sensitivity.

Feedback gain and hence control performance are limited by the need to maintain stability. This is especially important in isotonic control where significant variations in muscle stiffness during contraction [57] affect controller stability. Early isotonic and
isometric control of muscle tissue was implemented using analog compensators and classical control theory [40, 64, 66, 124, 108]. This approach requires significant empirical tuning and often produces sub-optimal controllers due to its inability to directly influence the location of all system poles. More recently, a feed forward adaptive control scheme was proposed which adjusted the actuator drive signal to achieve desired length and force changes during muscle contraction [126]. This approach has since been successfully applied to intact single cells using a carbon fiber attachment mechanism [103]. As the authors acknowledge, the disadvantage of this approach is that control can only be applied for a single twitch presumably because loading the cell during the twitch changes the mechanical response of the cell in subsequent twitches requiring further adaptation.

Modern control techniques provide a mathematical framework based on state space representations of systems that allow the “optimal” adjustment of all pole locations to achieve a desired level of stability and performance. $H_\infty$ optimal control allows the importance of criteria such as stability, sensor noise feedback and disturbance rejection (critical for isometric and isotonic control during muscle contractions) to be simply integrated into the cost function to be optimized [127]. For example, an $H_\infty$ controller was recently described that provided high bandwidth robust control of the tip of a micro-fabricated cantilever using a piezoelectric stack with significantly better performance than a classical controller [128].

Here we describe the design and development of an $H_\infty$ controller that is one element of a modular instrument and signal processing strategy designed to explore the mechanics of single muscle cells (see Section 3). We first present system identification techniques and a lumped parameter model that were used for controller design and stability analysis. Then, static, modal and harmonic mechanical finite element models (FEMs) of the system are described that support the lumped parameter model and assist in system calibration. Finally the design and implementation of the $H_\infty$ controller are illustrated and its functionality is demonstrated by measuring the twitch of an intact mammalian myocyte under open loop and isometric conditions at varied sarcomere lengths.
4.2 Modeling Considerations

4.2.1 Device Description

A detailed description of the device is provided elsewhere (see Section 3). Briefly, the core of the instrument illustrated in Figure 4.2-1 and 4.2-2 is conceptually similar to the design of Iwazumi [64] utilizing two Lorentz force actuators that can simultaneously be used as force sensors. The actuators are stainless steel cantilevers with a rectangular section removed from the center to form a single loop. A permandur motor structure guides the magnetic field and concentrates it in two air gaps. The motor structure is manufactured using a wire electrical discharge machine, EDM (Charmilles, Lincolnshire, IL) and a 5 axis machining center (HAAS Automation Inc., Oxnard, CA). The long arms of the cantilever loop pass through the magnetic field within the air gap providing the basis for actuation (see Figure 4.2-2). The motor structure is coated in 3 μm of parylene (to ensure biological compatibility and provide electrical insulation) using a bench top deposition system (Para Tech Coating Inc, Aliso Viejo, CA). Confocal optical position sensors reflect from the back surface of each cantilever. The position sensor photodiode current is amplified and the resulting position signal (with < 1nm/√Hz noise above 10 Hz) can be used to control the current source driving the actuator. A block diagram description of the system is illustrated in Figure 4.2-3. All electronics except those for data acquisition and control are built into the module. Circuit design and board layout utilized PSpice and Allegro packaged within Cadence Design Systems software (Cadence Design Systems, San Jose, CA) and double sided circuit boards were manufactured using a board plotter (LPKF, LPKF Laser & Electronics AG, Garbsen, Germany).
Figure 4.2-1 The central motor structure. Permandur magnetic field guides concentrated the magnetic field in two air gaps to provide the basis of actuation. The entire motor structure was inserted into a glass tube (not shown) and could then be fully immersed in physiological saline. Cells were attached to the top surface of the cantilevers.

Figure 4.2-2 A cut away side view of the motor structure and a single cantilever. The magnetic field was concentrated in the air gap (approximately 1 T). The magnetic field direction was reversed on either side of the central field guide (connected to the South pole) so that current through both arms of the cantilever loop generated force in the same direction (out of the page for the current and field directions in this figure).
The two position sensors produced approximately 1 to 5 pA/nm which was amplified by low noise transimpedance amplifiers (Amp) then filtered by simple analog low pass filters (LPF) to minimize aliasing. A National Instruments data acquisition card (NI 6052E) provided four 16 bit analog to digital converters (ADCs) and two 16 bit digital to analog converters (DACs) and ran at 2 kHz in closed loop. Control algorithms were implemented in Visual Basic.NET.

### 4.2.2 System Identification

A combination of stochastic and parametric system identification was used to generate a model of the device. Custom Visual Basic.Net (VB.Net) code was written to control a National Instruments 6052E data acquisition card (National Instruments, Austin, TX). A $2 \times 10^5$ point white noise force (input) sequence sampled at 2 kHz was applied to the cantilever and the resulting displacement (output) was recorded. The mechanical impulse response was estimated using stochastic system identification techniques as described in Section 4. Briefly, 2048 point biased auto and cross-correlation estimates were made of the force ($X$) and position data ($Y$) and used to populate the following [107]
\[
R_{XX} h_{XY} = R_{XY}
\]

\[
\begin{bmatrix}
R_{XX}[0] & R_{XX}[1] & \ldots & R_{XX}[n] \\
R_{XX}[1] & R_{XX}[0] & \ddots & \\
\vdots & \ddots & \ddots & \ddots \\
R_{XX}[n] & R_{XX}[0] & \ldots & R_{XX}[n]
\end{bmatrix}
\begin{bmatrix}
h_{XY}[0] \\
h_{XY}[1] \\
\vdots \\
h_{XY}[n]
\end{bmatrix}
= \begin{bmatrix}
R_{XY}[0] \\
R_{XY}[1] \\
\vdots \\
R_{XY}[n]
\end{bmatrix}.
\]

\(R_{XX}\) is a Toeplitz matrix of autocorrelation estimates, \(R_{XY}\) is a vector of crosscorrelation estimates, \(h_{XY}\) are samples of the impulse response and \(n = 2048\) in this case. Equation 4.2-1 was solved for \(h_{XY}\) by inverting the Toeplitz matrix using a Levinson recursion algorithm implemented in VB.Net and Matlab.

The quality of the system identification was assessed by calculating the variance accounted for or coherence squared (\(\text{coh}^2(j\omega)\)) using [107]

\[
\text{coh}^2(j\omega) = \frac{|S_{xy}(j\omega)|^2}{|S_{xx}(j\omega)||S_{yy}(j\omega)|},
\]

\(0 \leq \text{coh}^2(j\omega) \leq 1\),

(4.2-2)

where \(S_{xy}(j\omega), S_{xx}(j\omega)\) and \(S_{yy}(j\omega)\) are the cross, input and output power spectral densities respectively.

Although the estimated compliance impulse response (between force input and displacement output) could be fit to a classic second order response, it was found that a fourth order, discrete, infinite impulse response (IIR) model provided an excellent fit and captured the high-frequency system dynamics. The magnitude and phase of the Fourier transform of \(h_{XY}\) and the auto regressive moving average (ARMA) fit are presented in Figure 4.2-4. This characterization was performed with the cantilevers fully immersed in physiological saline (Tyrode solution). Under these conditions the dominant resonant poles were at approximately 330 Hz with damping ratio, \(\zeta\), of 0.1 (when the cantilevers were in air the primary resonance was at 640 Hz with \(\zeta = 0.006\) results not shown).
4.2.3 Lumped parameter modeling

To facilitate control design, a three component, MIMO lumped parameter model was used to describe the dynamics of the system (see Figure 4.2-5). It is a series connection of three complex mechanical impedances. The two cantilevers and the muscle cell are treated as independent systems with transfer functions relating input displacement to output force \( (H_1(s), H_2(s) \text{ and } H_M(s) \text{ for cantilever one, cantilever two and the muscle respectively}) \). The model assumes that the two forces are applied at the tip of the cantilevers where the muscle cell is attached and the displacements are measured at this point (see Figure 4.2-5). The system can be represented as:

\[
\begin{bmatrix}
X_1 \\
X_2
\end{bmatrix} = H(s) \begin{bmatrix}
F_1 \\
F_2
\end{bmatrix}
\]

(4.2-2a)

\[
H(s) = \begin{bmatrix}
\frac{H_z + H_m}{H_1H_z + (H_1 + H_z)H_m} & \frac{H_m}{H_1H_z + (H_1 + H_z)H_m} \\
\frac{H_m}{H_1H_z + (H_1 + H_z)H_m} & \frac{H_z}{H_1H_z + (H_1 + H_z)H_m}
\end{bmatrix}
\]

(4.2-2b)
\[ \begin{bmatrix} F_1 \\ F_2 \end{bmatrix} = H^{-1}(s) \begin{bmatrix} X_1 \\ X_2 \end{bmatrix} \]  
(4.2-3a)

\[ H^{-1}(s) = \begin{bmatrix} H_1 + H_m & -H_m \\ -H_m & H_2 + H_m \end{bmatrix} \]  
(4.2-3b)

**Figure 4.2-5.** Lumped parameter system model $H_m(j\omega)$, $H_1(j\omega)$ and $H_2(j\omega)$ are the dynamic stiffness of the sample, and two cantilevers respectively. Forces and displacements can be applied on either side of the sample either by the actuators or the muscle.

The dynamic stiffness of a muscle cell can found by rearranging the second row of Equation 4.2-3a and letting $s = j\omega$ to give

\[ H_m(j\omega) = \frac{X_2(j\omega)}{X_1(j\omega)} H_2(j\omega), \]  
(4.2-4)

where $X_2/X_1(j\omega)$ is an estimate of the transfer function relationship between the measured displacements $x_1(t)$ and $x_2(t)$. Equation 4.2-4 can also be evaluated at a single frequency, $\omega_0$, using a sinusoidal analysis and measuring the change in magnitude and phase between $x_1(t)$ and $x_2(t)$ to give $X_2/X_1(j\omega_0)$.

It is of interest to consider a simplification of Equation 4.2-4. If the cantilevers had sufficient mass and stiffness then the effect of loading them with a myocyte would be negligible. Equivalently $|H_m(j\omega)| << |H_1(j\omega)|$, $|H_2(j\omega)| << |H_2(j\omega)|$ and $|X_2/X_1(j\omega)| << 1$. The cantilevers could then be treated as independent SISO systems and Equation 4.2-4 would reduce to

\[ H_m(j\omega) = \frac{X_2(j\omega)}{X_1(j\omega)} H_2(j\omega). \]  
(4.2-5)

In the muscle physiology literature the dynamics of the force sensor are typically ignored and it is treated as a simple spring with stiffness $k_2$. This assumption is valid if the
applied displacement $x_1(t)$ is band limited to frequencies significantly below the first bending resonance, $\omega_n$, such that magnitude of $H_2(j\omega)$ is approximately constant and no phase lag is introduced. The frequency at which this assumption fails depends on $\omega_n$ and the damping factor of the force sensor.

If $x_1(t_0)$ is a low pass filtered step with magnitude $\Delta x_1$ and $\Delta x_2$ is measured once the system has settled Equation 4.2-5 reduces to

$$\hat{H}_M (j0) = \frac{\Delta x_2 \times k_2}{\Delta x_1} = \frac{\Delta F}{\Delta x_1}.$$  \hspace{1cm} (4.2-6)

Equation 4.2-6 provides the most straight forward means of estimating the stiffness of a sample using an actuator and force sensor. However, according to the lumped parameter model, this equation will underestimate the stiffness of the muscle by a factor $\alpha$

$$H_M (j0) = \alpha \hat{H}_M (j0) \hspace{1cm} \alpha = \frac{1}{1 - \frac{\Delta x_2}{\Delta x_1}} = 1 + \frac{k_m}{k_2}.$$  \hspace{1cm} (4.2-7)

Given that force sensors used in single cell experiments are often less than an order of magnitude stiffer than the myocyte being explored, this error can be quite significant.

4.2.4 FEM Modeling

Finite element modeling of the cantilevers served two primary purposes. Firstly, it gave a theoretical measure of stiffness and first resonant frequency as a function of cantilever length for use in calibration. Secondly, it allowed static, modal and harmonic analyses of the effect of a muscle cell load on the cantilevers for exploring the validity of the lumped parameter model. Standard material properties were used and geometries were imported into Ansys (ANSYS Inc., Canonsburg, PA) from three dimensional computer aided design models (Solid Edge, UGS, Plano, TX). To ensure all FEMs were identically meshed for comparative purposes, the full system including both cantilevers and the muscle cell was meshed and then unwanted elements in a particular simulation were cleared. Despite the inherent symmetry of the problem a full model of both cantilevers and the cell was used to ensure all rotational modes could be captured.
CANTILEVERS

Each annealed stainless steel 316 cantilever was modeled as a 25 μm thick, 1 mm wide beam that was between 3 and 6 mm in length and had a rectangular cross section removed (see Figure 4.2-6). It was meshed using approximately 10000, 10 node tetrahedral elements (solid92, Ansys) with three translational degrees of freedom at each node. The stainless steel was modeled as an isotropic elastic material with a Young’s modulus of 193 GPa, density of 7960 kg/m³ and Poisson ratio of 0.29. A constant damping ratio of 0.1 was used for the harmonic simulation of the cantilevers based on empirical measurements of the dynamics of the cantilevers immersed in physiological saline solution (see the Figure 4.2-4).

![Figure 4.2-6](image)

Figure 4.2-6. The FEM of the two cantilevers and muscle cell. The 10 μN force used in the static and harmonic simulations was applied to the nodes connecting the muscle cell to the tip of the cantilevers. The bases of the cantilever arms were immobilized in all simulations.

MUSCLE

Detailed constitutive models of muscle tissue have been used as the basis of finite element analysis of muscle tissue and the whole heart [121]. As our objective was to validate the lumped parameter model of Figure 4.2-5, an isotropic elastic model of the cell was used for simplicity. The muscle cell was modeled as an 80 element, 150 μm long
rectangle with cross sectional area $0.4 \times 10^{-9}$ m$^2$. Its Young’s modulus and Poisson ratio were 150 kPa (see Section 3) and 0.5 $[71]$ respectively.

CALIBRATION SIMULATION

The stiffness of the cantilever at 0 Hz was measured by applying a 10 μN force distributed across the 5 elements on the top surface of the cantilever where the muscle was attached in the complete model (see Figure 4.2-6). As a boundary condition, the bottom surfaces of both cantilever arms were immobilized. A static analysis was run for 11 lengths evenly spaced between 3 and 6 mm and a plot of stiffness versus length is provided in Figure 4.2-7B. An inverse cubic function of length was fit to the data using least squares estimation (based on the analytical relationship between stiffness and length [104]). A modal analysis of the cantilever in a vacuum with the base of both arms immobilized was conducted for the same range of lengths and the first resonant frequency, $\omega_n$, is plotted as a function of length in Figure 4.2-7A. Due to the slightly unusual geometry of the cantilevers, the fit of the first resonant frequency was a function of $(\text{length})^{-1.83}$ rather than the standard analytical relationship of $(\text{length})^{-1.5}$ for a beam fixed at one end [104].

![Figure 4.2-7A](image1)

![Figure 4.2-7B](image2)

**Figure 4.2-7A** The results of the modal FEM simulation demonstrating the relationship between first resonant frequency and length. **B** The results of the static FEM simulation demonstrating the inverse cubic relationship between cantilever stiffness and length.
The simulation results in Figure 4.2-7A and B provided the basis of a methodology to calibrate the position sensor and the relationship between current through the cantilevers and force at the cantilever’s tip. A $10^6$ point random force input (current) sampled at 10 kHz was applied to the cantilever (in air) and the resulting displacement (output) was measured. Equation 4.2-1 was used to estimate the impulse response relating current input to displacement output. The resonant frequency of the cantilever was then found by fitting a second order impulse response to the empirical data. The relationship between resonant frequency and length in Figure 4.2-7A could then be used to predict the cantilever length which, using the fit in Figure 4.2-7B, provided the stiffness of the cantilever.

To complete the calibration, a known sinusoidal current was applied and the displacement of the tip of the cantilever measured using both the position sensor and a digital microscope. The later had been independently calibrated in pixels/μm using a 2 μm graticule. The position sensor sensitivity (m/V) was calibrated directly using the optical measure of displacement. The force at the cantilever tip was estimated using the simulated cantilever stiffness and the observed displacement. This provided the relationship between current and force (N/A). The current to force calibration was performed only once immediately after the motor had been built before the cell attachment structure was glued to the tip of the cantilevers.

**VERIFYING THE LUMPED PARAMETER MODEL**

A harmonic analysis was conducted to explore the validity of the lumped parameter model described by Equation 4.2-2 and 4.2-3. The objective was to determine if Equation 4.2-4 could be used to estimate the dynamic stiffness of the simulated muscle cell from the displacement of appropriate nodes in the FEM. Three different models were used, a single cantilever, two cantilevers separated by 100 μm with a muscle cell attached between them and the muscle alone. The cantilever length was set to 5.15 mm (cantilever stiffness of 2.42 N/m) and a 10 μN sinusoidal force at 100 frequencies between 10 and 1000 Hz was applied to each model. In each case the force was distributed across the 5
nodes where the muscle attached to the tip of the actuating cantilever and the
displacement of one of those nodes recorded as a function of time. For the dual cantilever
simulation, the displacement of a node in the same location on the second cantilever (the
force sensor) was also recorded. For the muscle only simulation, the set of 5 nodes where
the muscle would attach to the second cantilever were immobilized (on the opposite end
of the cell to the applied force) and both cantilever meshes cleared. The dynamic
compliances of the systems were found using the change in magnitude and phase
between the input (force) and output (displacement) sinusoids.

The dynamic compliances of the loaded and unloaded cantilevers are illustrated in
Figure 4.2-8. The effect of loading the system with a muscle is strongly dependent on the
relative stiffness of the cantilevers (2.42 N/m at 0 Hz) and the muscle cell (0.43 N/m at 0
Hz). As can been seen in Figure 4.2-8, the apparent stiffness of the loaded cantilever
increased (due to the presence of the muscle cell and second cantilever) while the
resonant frequency was not significantly affected.

Figure 4.2-8. The dynamic compliance at 100 frequencies between 10 and 1000 Hz found using Ansys
harmonic simulation of the single cantilever model (unloaded) and the actuated cantilever in the dual
cantilever model with muscle attached (loaded).
The ability of Equation 4.2-4 to predict the dynamic stiffness of the simulated muscle cell using position data from the loaded cantilever simulation was then explored. The dynamic stiffness of the force sensor cantilever, \( H_2(j\omega) \), was found using the single, unloaded cantilever simulation (see Figure 4.2-8). The transfer function \( X_2/X_1(j\omega) \) between the simulated displacement of the actuating cantilever (input) and the force sensor cantilever (output) was calculated from the loaded cantilever simulation. \( H_{\text{M Est}}(j\omega) \) was then estimated using Equation 4.2-4. For comparison, the dynamic stiffness of the myocyte, \( H_M(j\omega) \) was also found directly using the results of the harmonic simulation of the muscle alone. The magnitude and phase of \( H_{\text{M Est}}(j\omega) \) and \( H_M(j\omega) \) are plotted in Figure 4.2-9. The excellent agreement between the direct finite element simulation result, \( H_M(j\omega) \), and the calculated result, \( H_{\text{M Est}}(j\omega) \), serves to validate the use of the lumped parameter model to describe the cantilever system for small displacements and forces and supports the use of Equation 4.2-4 to interpret experimental results.

Substituting \( H_2(j\omega) = k_2 \) into Equation 4.2-5 (modeling the force sensor as a spring) gives

\[
H_M(j\omega) = \frac{X_2}{X_1}(j\omega) \times k_2 = \frac{F}{X_1}(j\omega),
\]

(4.2-8)

Equation 4.2-8 was used to provide a second estimate the dynamic stiffness of the muscle, \( H_{\text{M Est2}}(j\omega) \), and the magnitude and phase of this estimate are compared with
$H_{\text{Mest}}(j\omega)$ and $H_M(j\omega)$ in Figure 4.2-10. Equation 4.2-8 overestimated the stiffness of the muscle cell at low frequencies by a factor of 1.18 as predicted by Equation 4.2-7 and introduced significant phase and magnitude error as the cantilevers approach resonance.

![Graph showing stiffness and phase versus frequency](image)

**Figure 4.2-10.** The dynamic stiffness of the muscle cell estimated using Equation 4.2-4 ($H_{\text{Mest}}(j\omega)$), Equation 4.2-8 ($H_{\text{Mest2}}(j\omega)$) and directly simulated in Ansys ($H_M(j\omega)$) at frequencies between 10 Hz and 500 Hz (approximately 80% of resonance). Equation 4.2-8 provides a poor estimate of the dynamic stiffness of the muscle as the cantilevers approached resonance.
4.3 Controller Implementation

4.3.1 Control Design

The primary objective of the control system was to improve the ability of the cantilevers to support isometric muscle contractions by minimizing their displacement. The plant to be controlled, \( G_p \), is a MIMO system with two inputs that set the current through each cantilever and two outputs from the independent position sensors. The dynamics of the system were described using Equation 4.2-2 (the current source and position sensor are modeled as constants within the bandwidth of the controller). The control objective could be achieved by improving the ability of the closed loop system illustrated Figure 4.3-1A to reject muscle force disturbances that lie between 0 and 10 Hz. This objective can be quantified using the ratio of the closed loop transfer function between disturbance input and plant output, \( G_{YD} \), and the plant transfer function, \( G_{VU} = G_p \) (the open loop disturbance rejection).

\[
S = \frac{G_{YD}}{G_p} = \frac{I}{I + KG_p} = \begin{bmatrix} S_{11}(s) & S_{12}(s) \\ S_{21}(s) & S_{22}(s) \end{bmatrix}, \tag{4.3-1}
\]

Where \( I \) is a 2×2 identity matrix and \( K \) is the transfer function matrix of the controller (see Figure 4.3-1A). \( S \) is also known as the sensitivity of the closed loop system. The effective stiffness of the cantilevers is inversely related to the magnitude of the elements of \( S \).

Initially, simple analog proportional control was considered (treating the cantilevers as independent SISO systems). However, a root locus analysis (see Figure 4.3-2) found the maximum stable loop gain of a proportional control scheme to be a mere 0.8. This would only increase the effective stiffness of the cantilevers at 0 Hz by at most a factor of 1.8 (significantly less if reasonable gain and phase margins were required). More complicated classical analog compensators such as lead, lag or lead-lag (PID) were considered however their performance suffered due to the inherent tradeoff between closed loop gain and stability. Plant inversion to cancel the dominant second order poles
was also explored but dismissed as it was insufficiently robust to accommodate the variable myocyte load.

![Diagram of closed loop system with reference input, muscle disturbance, photodiode shot noise, controller, current source, and position sensor.](image)

**Figure 4.3-1A.** The closed loop system with reference input, muscle disturbance, photodiode shot noise (the dominant noise source), controller, current source, and position sensor. The inputs and outputs are two element vectors and the block elements are $2 \times 2$ transfer function matrices. B. The augmented plant used to ensure optimizing the $H_\infty$ norm produced the desired closed loop response. $W_1$, $W_2$, and $W_3$ are the digital filters that scale the outputs of the augmented plant.

![Root locus of fourth order IIR model of one cantilever.](image)

**Figure 4.3-2.** A root locus of the fourth order IIR model of one cantilever. The maximum stable loop gain was 0.8 which only increased the effective stiffness at 0 Hz a factor of 1.8.

Controller design using digital state space solutions has the advantage that the location of all of the plant poles can be specified using algorithms that can be applied with equal ease to SISO and MIMO systems. Direct pole placement using the Matlab function *place* was not suitable for this application as it is difficult to relate control objectives to pole
locations for systems that are either greater than second order or MIMO. Linear Quadratic Gaussian (LQG) control was considered however the Kalman filter state estimator is optimal only for white noise disturbances and while LQG control be modified to treat non white disturbances [127], the $H_\infty$ approach provides an intuitive means to relate the disturbance rejection design objective to the optimization norm.

The $H_\infty$ norm is the maximum gain between all input-output combinations of a generic system $G$ over all frequencies [127]. It is defined as

$$\|G\|_\infty = \sup_{\omega} \sigma[G(j\omega)].$$  \hspace{1cm} (4.3-2)

To effectively use the $H_\infty$ norm to design an “optimal” controller it is necessary to augment the plant by introducing additional outputs that quantify the control objectives. By weighting these outputs at each frequency using filters, their impact on the $H_\infty$ norm can be adjusted. Through this process the relative importance of each control objective can be set at each frequency. The plant augmentation used for this design is shown in Figure 4.3-1B. The filter matrices $W_1$, $W_2$ and $W_3$ produce scaled outputs that capture disturbance rejection (the sensitivity function, $S$), controller effort, and a measure of noise feedback and stability margin (the complementary sensitivity function, $T$). In the augmented plant of Figure 4.3-1B, $u_1$, $u_2$, $y_1$ and $y_2$ are vectors comprising a total of 4 inputs and 8 outputs. The Matlab function $hinf$ was used to design $K$ such that the $H_\infty$ norm of the transfer function matrix relating $u_1$ (2 inputs) and $y_1$ (6 outputs) was minimized

$$\|G_{y_1u_1}\|_\infty = \begin{bmatrix} W_1S \\ W_2KS \\ W_3T \end{bmatrix} < \gamma,$$ \hspace{1cm} (4.3-3)

where $T$, the complimentary sensitivity function, is defined as

$$T = \frac{KG_p}{1 + KG_p} = \begin{bmatrix} T_{11}(s) & T_{12}(s) \\ T_{21}(s) & T_{22}(s) \end{bmatrix},$$ \hspace{1cm} (4.3-4)

and $W_1$, $W_2$ and $W_3$ are diagonal transfer function matrices that scaled both inputs identically using a filter described by $W_{xa}(s)$ (for $x = 1$, 2 or 3)
\[
W_x = \begin{bmatrix}
W_{xa}(s) & 0 \\
0 & W_{xa}(s)
\end{bmatrix}.
\] (4.3-5)

Initially, the controller was designed while ignoring the effects of the muscle implying each cantilever could be treated as an independent SISO system or combined in the following simple MIMO system

\[
\begin{bmatrix}
X_1 \\
X_2
\end{bmatrix} = \begin{bmatrix}
\frac{1}{H_1} & 0 \\
0 & \frac{1}{H_2}
\end{bmatrix} \begin{bmatrix}
F_1 \\
F_2
\end{bmatrix}.
\] (4.3-6)

Increasing the gain of the controller increases the ability of the closed loop system to reject a twitch force disturbance. The inherent tradeoff between the gain of a stable controller and bandwidth of the closed loop system can be managed by adjusting the amplitude and cutoff of the filter \(W_{1a}(s)\). The majority of the power in Guinea pig ventricular muscle cell twitches lies between 0 and 2 Hz (unpublished results). A low pass Butterworth filter with a cutoff of 5 Hz was chosen for \(W_{1a}\) to stress the importance of low frequency gain. The filter roll off was cancelled with two zeros at 30 Hz to avoid significant overshoot in the step response. The complimentary sensitivity, \(T\), represents the transfer function matrix \(G_{VN}\) between the shot noise from the photodiode in the position sensor, \(n\), and the displacement of the cantilever, \(v\) (see Figure 4.3-1B). \(W_{3a}(s)\) was used to ensure the magnitude of \(T\) rolled off for frequencies above 100 Hz. When the system \(T\) was driven by noise sampled from the position sensors at 2 kHz the standard deviation of the output was approximately 90 nm. Given that the design objective was to minimize physical displacement of the muscle cell, the feedback of position sensor noise modeled by \(T_n\) limits the improvement that can be achieved with position control (see Section 4.4). \(W_{2a}(s)\), which bounds the control effort, was a constant 0.001 as the current source was more than capable of providing the force required to correct the small displacements introduced by the muscle. The magnitude and phase of \(W_{1a}(j\omega)\) and \(W_{3a}(j\omega)\) are presented in Figure 4.3-3.
Fig 4.3-3. The magnitude and phase of \( W_{1a}(j\omega) \) and \( W_{3a}(j\omega) \). \( W_{1a}(j\omega) \) sets the bandwidth of disturbance rejection in the closed loop system. \( W_{3a}(j\omega) \) influences the coupling of shot noise from the position sensor to the cantilevers and the stability of the system.

The controller, \( K \), with a gain at 0 Hz of approximately 110 was designed using the Matlab function `hinfsyn` and Equation 4.3-6 to model the plant, \( G_p \), ignoring mechanical coupling between cantilevers due to the myocyte. A simple robustness analysis of the controller was performed by replacing the plant with the MIMO cantilever model, \( G_{PM} \), of Equation 4.2-2. The myocyte was modeled as a spring with stiffness, \( k_m = 0.4 \text{ N/m} \) (a worst case error). The performance and stability of the closed loop system was analyzed using the sensitivity and complementary sensitivity functions and the gain and phase margins.

The critical design objective was to maximize disturbance rejection by ensuring the sensitivity of the system was significantly less than one in the frequency band between 0 and 10 Hz. The effective stiffness of the cantilever \( x \) is increased by a factor of \( 1/|S_{xx}(j\omega)| \). The Bode diagrams of the first element of the sensitivity and complementary sensitivity matrices calculated using \( G_p (S_{11}(j\omega) \text{ and } T_{11}(j\omega)) \) and \( G_{PM} (S_{M11}(j\omega) \text{ and } T_{M11}(j\omega)) \) are compared in Figure 4.3-4. As can be seen in this figure, \( 1/|S_{M11}(j0)| = 37 \) was smaller than \( 1/|S_{11}(j\omega)| = 51 \) indicating a minor loss in disturbance rejection due to
the inclusion of myocyte coupling in the plant model. The magnitude of the sensitivity $S_{M12}(j\omega)$ at 0 Hz was 1/289 and was ignored (results not shown). The bandwidth of the controller, estimated by the first time $|S_{11}(j\omega)|$ crosses -3 dB, was approximately 55 Hz.

The magnitude and phase of the loop transfer functions with and without the myocyte ($L = K \times G_P$ and $L_M = K \times G_{PM}$) are illustrated in Figure 4.3-5. The gain and phase margins of the two transfer functions were almost identical at approximately 18.8 dB and 76.5 deg respectively implying the controller could tolerate expected variation in the plant. It was possible to trade the large gain and phase margins for increased disturbance rejection. Alternate designs produced up to a 280 fold increase in stiffness with a 4.8 dB gain margin and 47 degree phase margin. However these were not optimal designs due to sensor noise feedback (see Section 4.4).

![Figure 4.3-4](image)

**Fig 4.3-4.** The magnitude and phase of the sensitivity and complimentary sensitivity functions ($S_{11}(j\omega)$ and $T_{11}(j\omega)$ respectively) between input force, $F_1$ and output displacement, $x_1$. The bandwidth of the controller was approximately 55 Hz (as judged by the point where the S crosses -3 dB for the first time). $S_{M11}(j\omega)$ and $T_{M11}(j\omega)$ are the sensitivity and complementary sensitivity function when the closed loop controller, $K$, was applied to the MIMO system of Equation 4.2-2 with $H_M(j\omega) = k_m = 0.4$ N/m.
A controller $K_{\text{MIMO}}$ was designed using the MIMO plant model, $G_{\text{PM}}$, using $k_m$ between 0.04 N/m and 0.1 N/m. It was found (results not shown) that while the MIMO controller mitigated some of the performance loss when $k_m$ was set to 0.4 N/m the improvement was not sufficient to justify the dramatic increase in the order of the controller (from 16 to 44).

### 4.3.2 Experimental Verification

**CONTROLLER IMPLEMENTATION**

The block diagram description of the control system is given in Figure 4.2-3 and 4.3-1A. The transfer function matrix $K$ (designed using the SISO plant model of Equation 4.3-6) was converted to two discrete IIR filters, $K_{11}(z)$ and $K_{22}(z)$, that were implemented using a direct form II algorithm in VB.Net [98]. The position sensor signal for each cantilever was sampled by the NI 6052E data acquisition card at 2 kHz. This signal was adjusted for offsets, subtracted from a reference input and then passed through the control filter for that cantilever. The output of the filters was converted to an analog voltage by the NI.
6052E and set the current through each cantilever. The step response of the open loop and closed loop response of a single cantilever were recorded and are illustrated in Figure 4.3-6. The rise times are 0.48 ms and 2.9 ms for open and closed loop respectively. While features of the step response were not deliberate design objectives for the isometric controller the closed loop system was able to apply smooth, rapid length changes to the muscle cell.

![Figure 4.3-6. The open and closed loop step response of one cantilever sampled at 2 kHz (0.48 ms and 2.9 ms rise time respectively).](image)

**CELL ISOLATION AND LOADING**

Ventricular myocytes were isolated from Dunkin-Hartley female guinea pigs approximately 2 to 3 months of age using standard enzymatic techniques as described elsewhere (see Section 3.2.6). A myocyte was selected for experimentation if it contracted more than 5% under electric field stimulation and its sarcomere pattern was ordered (with average resting sarcomere length between 1.7 μm and 1.9 μm). Sarcomere lengths were estimated using averaged spatial Fourier transforms of images of the cell captured with a digital microscope (see Section 2.4.2). The selected cell was moved to the cantilevers from the inspection mount using a borosilicate glass capillary with a tip diameter of 1 μm. The myocytes were then clamped between a square cross section borosilicate glass capillary and an etched carbon fiber [84] (kindly provided by Professor
Details of the cell loading and attachment procedure are provided in Section 3.2.6.

**TWITCH RESPONSE**

To verify the design and implementation of the isometric $H_\infty$ controller its ability to reject the displacement disturbance introduced by a muscle cell twitch was explored. A healthy cell was selected and attached to the cantilever in Tyrode solution (mM: NaCl 140; KCl 5.4; MgCl$_2$ 1; CaCl$_2$ 2.0; HEPES 5; Glucose 11) at approximately 28 °C. After attachment the cell was allowed to equilibrate for two minutes and then it was electrically stimulated with 10 ms pulses at 0.2 Hz approximately 20 % above threshold using two 100 μm diameter platinum electrodes separated by 2 mm. The twitch force was allowed to stabilize for an additional 5 minutes before beginning the experiment and the electrical stimulation was maintained until the end of the test.

After the twitch force had stabilized, the cell was stretched in approximately 4 μm increments that were each applied over approximately 3 seconds. After each stretch the twitch force was allowed to reach a steady state over 30 to 60 seconds then the displacements of both cantilevers were logged for 5 to 10 twitches. The open loop twitch force could be roughly estimated by multiplying the measured displacement by the known stiffness of the cantilevers. The $H_\infty$ controller was then switched on for an additional period of 30 to 60 seconds to hold the cantilevers in their current position (generating isometric contractions). The displacement of and current through both cantilevers were recorded. The closed loop twitch force was estimated from the sum of the displacement multiplied by cantilever stiffness and the force applied via the current source. The control was then switched off and another stretch applied. This process was repeated until the cell began to spontaneously contract which invariably led to cell death after a period of 5 to 20 minutes. Spontaneous contractions typically began when the cell was producing approximately 2 μN of force at sarcomere lengths between 1.95 μm and 2.1 μm. The peak twitch force recorded across all experiments was approximately 4.3 μN (46 kPa, $0.94 \times 10^{-11}$ m$^2$ cross sectional area) at a diastolic sarcomere length of approximately 2.23 μm and an electrical stimulus frequency 0.5 Hz.
The average peak twitch force (mean ± standard deviation, n ∈ [5, 10]) of a single representative cell at three sarcomere lengths between 1.84 μm and 2.04 μm (diastolic) was measured under closed and open loop conditions and the results displayed in Figure 4.3-7. As can be seen in this figure, the peak force under closed loop control was larger than that in open loop at the two longer sarcomere lengths.

![Graph](https://via.placeholder.com/150)

**Figure 4.3-7.** The average peak twitch force produced by a characteristic cell at three sarcomere lengths with and without H∞ control (closed and open loop respectively). Results are an average of 5 to 10 twitches ± standard deviation (error bars).

To demonstrate the operation of the H∞ controller, the average twitch induced displacement of one cantilever (mean ± standard deviation, n ∈ [5, 10]) under open and closed loop for the three sarcomere lengths are displayed in Figure 4.3-8. The closed loop displacement was measured directly using a position sensor. In this figure, the equivalent open loop displacement was calculated from the closed loop twitch force and cantilever stiffness. The directly measured open loop displacement was not used as the twitch force produced under closed loop was larger than that in open loop (see Figure 4.3-7). It was found that the H∞ controller reduced the peak displacement of the cantilevers by a factor of 29.6 ± 2.3 at the three sarcomere lengths (mean ± standard deviation, n = 3). At the longest sarcomere length this reduced the net change in muscle length (considering both
cantilevers) from approximately 960 nm in open loop to 30 nm in closed loop or approximately 0.96 % strain to 0.03 % strain.

Figure 4.3-8. Average displacement of one cantilever (mean ± standard deviation, n ∈ [5, 10]) during a twitch under open (OL) and closed (CL) loop conditions at three sarcomere lengths (SL). The open loop displacement was predicted from the twitch force measured when the $H_o$ controller was switched on. The $H_o$ isometric control increased the effective stiffness of the cantilevers by a factor of 29.6 ± 2.3 (mean ± standard deviation, n = 3).
4.4 Discussion

4.4.1 Control of Cell Length

We have presented the design and implementation of a robust $H_\infty$ controller that was based on a comprehensive understanding of system dynamics developed through modeling and system identification. The controller is one part of a modular instrument designed to measure the mechanical properties of living muscle cells.

By minimizing the $H_\infty$ norm defined in Equation 4.3-3 the optimal controller manipulates the locations of system poles balancing the tradeoff between disturbance rejection (reflected in $S$) and stability and sensor noise feedback (governed by $T$). The magnitude of the elements of $S$ represent the ratio of the open and closed loop coupling between a muscle force disturbance and displacement output (see Figure 4.3-1A). The primary objective of this design was to ensure $|S_{11}(j\omega)|$ and $|S_{22}(j\omega)|$ were sufficiently low for frequencies less than 5 to 10 Hz (several times the bandwidth of a muscle twitch). A healthy myocyte can produce up to 10 μN during contraction (approximately 50 to 70 kPa). This would bend the tip of the cantilevers at either end of the cell by approximately 5 μm in open loop (5 % strain at each end).

The $H_\infty$ controller achieved approximately a 30 times increase in the effective cantilever stiffness (see Figure 4.3-8). This would be sufficient to reduce net strain produced by a 10 μN contraction from 10 % to 0.3 % achieving the design objective (less than 0.5 % strain). The control model predicted a 51 fold increase in cantilever stiffness which was reduced to 37 fold in the simple robustness analysis that modeled the myocyte as a spring with variable stiffness (see Figure 4.3-4). The discrepancy between modeled and actual controller performance is presumably a result of system properties that were not captured in the model such as the time varying, nonlinear characteristics of the muscle cell and the delays introduced in the control loop by the NI 6052E data acquisition card.
The significant gain and phase margins (see Figure 4.3-5) could be traded for higher loop gain (producing up to a 280 fold increase in stiffness with a 4.8 dB gain margin and 47 degree phase margin). However, under closed loop, shot noise from the photodiodes in the position sensors is fed back to the output of the cantilevers. A 280 fold increase in apparent stiffness would reduce the peak displacement due to a 10 μN twitch to 18 nm. However, the model predicts the standard deviation of position noise fed back would be 115 nm in this case. As the goal of the feedback was to avoid disrupting the cross bridges due to length changes, the optimal feedback gain should reduce the twitch amplitude to match the standard deviation of the cantilever tip displacement produced by sensor noise feedback. As designed, the $H_\infty$ controller used in this paper would theoretically reduce a 10 μN twitch displacement to 98 nm and create position noise via feedback with a 90 nm standard deviation ($< 0.1 \%$ strain). The plant augmentation of Figure 4.3-1B provides an intuitive means to include this tradeoff in the optimization norm by adjusting the weights $W_1$ and $W_3$.

The bandwidth of the controller, also limited by the need to minimize sensor noise feedback, is approximately 55 Hz (see Figure 4.3-4) corresponding to a rise time on the order of 2.9 ms in the step response between reference input $r$ and displacement $y$ (see Figure 4.3-1A). It must be stressed this is the bandwidth of the closed loop system not the limit of system response which is bound loosely by the first resonance of the cantilevers in physiological saline (approximately 330 Hz). To apply a known signal (a step for example) with faster dynamics a pre-filter or a separate controller could be used to generate a suitable reference input that would drive the closed loop system faster. This approach would be limited practically by the current source driving the actuator or the maximum current density of the cantilevers.

Our design can be favorably compared with others in the literature. The adaptive approach of Nishimura et al [103] provided an approximate 20 fold increase in stiffness under isometric control however their controller could only function for one twitch. Luo and Tung [66] used a bimorph actuator to move a glass force probe with a resonant frequency of 361 Hz and designed an elegant controller that achieved a 96 fold increase
in stiffness with a controller gain of 198. This instrument was let down by the poor performance of the bimorph actuator rather than the controller design. An instrument designed by Iwazumi [64] to measure myofibril mechanics achieved excellent closed loop bandwidth of approximately 15 kHz and increased open loop stiffness by a factor of 5600. However, the compensator had a gain of 5000 and was designed to cancel dominant system poles (at 1.6 kHz). Combining pole cancellation with large feedback gains can create instability from minor fluctuations in the plant (due to changes in the myofibril stiffness during contraction for example).

Both Luo et al [66] and Iwazumi [64] acknowledge the problem of sensor noise feedback affecting the performance of the system. Luo et al had to roll off the low frequency gain of their controller (using a high pass filter) to limit the coupling of 1/f noise. The adaptive controller of Nishimura et al [103] does not suffer from this problem as it can take advantage of averaging during the training period. This benefit can be weighed against the inability of their adaptive controller to function for more than one contraction.

Optimal control using the $H_\infty$ norm will not provide dramatic improvements in closed loop performance when compared with carefully tuned classical controllers applied to relatively simple SISO plants. However, the design effort is spent in appropriately augmenting the plant to highlight design objectives (see Figure 4.3-1B). Once that is achieved, an optimal controller can be generated for many similar plants without the often painstaking process of empirical tuning. Furthermore, modern state space control techniques are easily expanded to MIMO systems. Using the MIMO plant model to design an isometric controller did not convey sufficient performance improvement in this case to justify the increase in controller order. However, the coupling between the cantilevers (using the full MIMO model) must be included to design an $H_\infty$ isotonic controller, a subject being actively pursued in our laboratory.

### 4.4.2 Modeling Strategies

The lumped parameter model provided a linear framework that formed the basis of control design. The harmonic FEM data demonstrated that the lumped parameter model
could also separate the cantilever dynamics from those of the sample. This creates the possibility of extending the measurement bandwidth of the device to include the first resonance of the force sensor. This could be applied constructively to the tradeoff between resonant frequency and stiffness, increasing the force sensitivity without loosing measurement bandwidth.

The modeling strategies presented here have several limitations. Firstly, muscle cells are not simple springs. Even passive cells demonstrate viscoelastic properties at frequencies less than 50 Hz (see Section 3) and there are significant length and load dependent nonlinearities ([40, 123] and review in Huxley [44]). In fact, using stochastic system identification to generate improved linear and nonlinear models of muscle cell dynamics motivates other experiments with this instrument. Secondly, the simulations are all done in a vacuum, the slight increase in effective moving mass for cantilever measurements in air would affect the resonant frequency leading to minor errors in calibration. Finally, the finite element model does not capture many factors like the nature of the attachment between then cells and the cantilevers. Despite these limitations the models allowed system dynamics to be explored in silico increasing understanding and aiding in design.

4.4.3 Physiological Significance

Isometric contraction of muscle tissue requires that myosin heads bound to actin filaments (cross bridges) not be physically disrupted due to shortening during the contraction process. This limits the net strain of a myocyte to less than 0.5 % [44]. The $H_\infty$ controller discussed in this paper was able to support isometric contractions of a single myocyte, reducing the displacement of the cantilever tips in response to a muscle force disturbance by a factor of approximately 30. The controller would limit the net myocyte strain to < 0.3 % for a 10 $\mu$N contraction. Imposing isometric conditions on the muscle cell during contraction had the effect of increasing the net force produced by the cell by as much as 23 % (see Figure 4.3-7) in agreement with studies on trabecula and papillary muscle [129, 126]
The peak twitch force measured in this study was 4.3 μN (46 kPa, 0.94 × 10⁻¹¹ m² cross sectional area) at a diastolic sarcomere length of approximately 2.23 μm and an electrical stimulus frequency 0.5 Hz. This can be compared with twitches observed by other researchers using intact ventricular myocytes (see table in [82]). Cazorla et al have published a series of papers using the etched carbon fibers of Le Guennec [84] to stretch ventricular myocytes from Guinea pigs [85,86]. In these studies the cells were stimulated at 0.25 Hz and the peak active stress measured was approximately 1 kPa (between 0.1 and 0.2 μN of force) at sarcomere lengths between 2.0 and 2.1 μm. The authors state that the tension was limited by the myocyte separating from the carbon fiber.

Bluhm et al [82] used glass plates coated in poly-L-lysine to attach a force sensor and actuator to myocytes from New Zealand white rabbits. They varied the stimulus rate between 0.1 Hz and 1 Hz and measured a peak active tension of 2.7 μN at a sarcomere length of approximately 1.97 μm. Shepherd et al [81] used glass rods coated with poly-L-lysine to measure a peak force of 1.23 ± 0.44 μN using Guinea pig ventricular myocytes at passive sarcomere lengths (between 1.81 and 1.88 μm).

Currently, the largest twitch force produced by a single intact cardiac myocyte reported in the literature is 9.52 μN at a sarcomere length of 1.83 μm corresponding to a peak stress of 69 kPa [103] (the average twitch force for n = 17 was 5.72 μN). In this study the authors used custom carbon fibers to attach to the myocytes from male Wistar rats and electrical stimulation at 0.5 Hz using 10 ms pulses. It is worth noting that in calculating peak force the authors used an unusual equation for the bending stiffness of the carbon fiber that increased the effective stiffness of the beam and hence the estimate of force by a factor of four (compared with a previous study by the group) [87].

The critical limitation when measuring the twitch force produced by an intact cardiac myocyte is the attachment strategy. The attachment methodology used in this study provided rapid, repeatable attachment that was strong enough to support significant cellular contractions without releasing the cell (up to 46 kPa) and stretch cells to sarcomere lengths beyond 2.4 μm (see Section 3). However, as myocytes were stretched
toward optimal sarcomere lengths the action of the twitch appeared to damage the cells
inducing spontaneous contractions. This typically occurred when the cell began
producing approximately 2 μN force (approximately 20 kPa of stress) at sarcomere
lengths between 2.0 and 2.1 μm and a stimulus frequency of 0.2 Hz. The improvement of
the attachment strategy to support healthy contractions at longer sarcomere lengths is the
subject of ongoing research.

In conclusion, we have presented the design and implementation of an $H_\infty$ controller that
was applied to a novel actuator and force sensor combination as part of a modular
instrument designed to explore the mechanical properties of living muscle cells. This
work represents the first step toward an array instrument capable of $H_\infty$ isometric and
isotonic control of muscle contractions that would allow the effects of load on the
mechanical properties of 10 to 100 mammalian cardiac myocytes to be explored in
parallel across the full physiological range of sarcomere lengths.
5 Additional Experimentation

Sections 3 and 4 described the proof of principle experiments that were performed to demonstrate the ability of the system to make mechanical measurements on intact mammalian ventricular muscle cells. The results presented were the culmination of over 100 independent myocyte experiments performed over the course of 1.5 years to optimize the isolation protocol, develop the loading and attachment strategy, refine the instrument and develop the experimental protocols for the proof of principle tests. In this section, the preliminary results of a final series of experiments aimed at measuring the dynamic stiffness of an activated (continuously contracting) myocyte are presented. The difficulties encountered in this work highlighted the need for improvements in the attachment mechanism and the combined requirement to increase the sensitivity of the position sensor and reduce the stiffness of the cantilevers. These improvements will be implemented in future iterations of the device.
5.1 Active Dynamic Stiffness Measurement

Unlike skeletal muscle, cardiac muscle cannot be put into tetanus by a series of closely spaced stimulating pulses due to the long refractory period in its action potential. It was therefore necessary to artificially stimulate cardiac cells to force them into a steady state contraction. This was achieved by substituting Ba$^{2+}$ for Ca$^{2+}$ in the bathing solution.

The simple environment in the agarose bath under the Olympus microscope was used to begin exploring the effect of Ba$^{2+}$. Myocytes were isolated using the standard protocol and several drops of dilute cell suspension in BSA Tyrode were placed on an uncoated glass slide in the bath. Oxygenated Ca$^{2+}$ Tyrode solution at room temperature was then run through the chamber for five minutes to remove the BSA. The cells were stimulated at 0.5 Hz with 10 ms electric field pulses at approximately 600 V/m for a further five minutes. During this period, pictures of the relaxed and contracting cells were captured using the Sony DFW-SX900 camera (see Figure 5.1-1A and B).

After the contraction of the cells had stabilized, the solution being washed over the cells was switched to 2 mM Ba$^{2+}$ Tyrode (while maintaining the electrical stimulus). The condition of the cells was monitored over the next five minutes. As the Ca$^{2+}$ was removed from the bath the cells stopped twitching in response to the electric field stimulus and some cells became visually contracted (see Figure 5.1-1C). A small fraction of the cells either died during the transition or did not appear to have contracted at all in response to the Ba$^{2+}$. 
After observing that the cells would respond to Ba\(^{2+}\) a series of experiments were conducted within the modular instrument to attempt to make the first measurement of the activated dynamic stiffness of a living muscle cell. The standard isolation and loading and attachment protocols were used (as described in Sections 2.6 and 3). Once the solution in the bath had been switched to Ba\(^{2+}\) Tyrode the dynamic stiffness of the muscle tissue was measured. However, the typical features of the magnitude and phase of the dynamic stiffness of cardiac tissue (papillary and trabecula) described in the literature were not observed. Instead, the dynamic stiffness was very similar to that measured in 1.8 mM Ca\(^{2+}\) Tyrode. Furthermore, none of the cells visually contracted after the Ba\(^{2+}\) was applied and spontaneous contractions were often seen when cells died in the device while immersed in Ba\(^{2+}\) Tyrode. If a Ba\(^{2+}\) contracture was properly induced then further vigorous contraction should not have been possible even during cell death. Two hypotheses were put forward to explain these observations:

- The stimulus amplitude applied during stochastic system identification (typically ± 4 μm peak to peak) was large enough to break any strongly bound cross bridges implying that the measured response was dominated by the passive dynamic stiffness of the myocyte regardless of the contractile state of the cell.
• The solution in the bath and specifically in the vicinity of the cell was not being replaced within the timeframe of the experiment (30 minutes).

The first hypothesis was tested by measuring the dynamic stiffness using a swept sinusoidal analysis with much smaller amplitude. As was discussed in Section 3, the minimum amplitude of the stochastic signal used to probe the muscle cell was limited by the noise floor of the position sensor. It is easy to demonstrate that, for a given peak amplitude, a broad band stochastic signal will provide lower power at a given frequency within the signal bandwidth than a sinusoid (at its dominant frequency). As a result, lower amplitude peak to peak sinusoidal signals could be applied while maintaining the same signal to noise ratio. Ideally, the resolution of the position sensor would be improved and / or the cantilevers stiffness decreased so that the benefits of stochastic system identification techniques could be realized in activated muscle (see Section 6).

To test the second hypothesis, a fluidic inlet was mounted on a micromanipulator such that it could be brought close to the cell. When 2 mM Ba\(^{2+}\) Tyrode was applied directly in this way it was found that many cells died. As a result, the concentration of Ba\(^{2+}\) was reduced to between 0.2 mM and 0.5 mM. Interestingly, the cells did not visibly contract even when the solution was applied directly. It is possible that the contraction was too slow to be visually obvious. If this was the case, it also would be very difficult to differentiate the expected mild contraction from gradual low frequency drift in the position sensor signals. It is also possible that 0.2 mM Ba\(^{2+}\) was insufficient to elicit a contraction and the concentration of Ba\(^{2+}\) should be gradually increased from 0.2 mM to 2 mM (generating a contraction while avoiding cell death).

Despite these issues the dynamic stiffness of a single cell in approximately 25 °C, 0.2 mM Ba\(^{2+}\) Tyrode solution was measured at four different sarcomere lengths between 2.04 \(\mu\)m and 2.41 \(\mu\)m. A swept sinusoidal analysis was used with 32 logarithmically spaced frequencies between 1 Hz and 40 Hz and peak to peak amplitude of 0.3 % \(L_0\) (approximately ± 150nm). The magnitude and phase of the measured dynamic stiffness are given in Figure 5.1-2.
Fig 5.1-2A. The magnitude of the dynamic stiffness of a muscle cell in 0.2 mM Ba\(^{2+}\) measured using swept sinusoidal analysis at 4 different sarcomere lengths. B The phase of the dynamic stiffness. Note the necessarily small signal to noise ratio affected the phase estimation more than the magnitude estimation.
As can be seen in Figure 5.1-2 the general shape of the magnitude of the dynamic stiffness in 0.2 mM Ba\textsuperscript{2+} Tyrode is similar to that measured in Ca\textsuperscript{2+} Tyrode (see Section 3). Interestingly, the magnitude of the dynamic stiffness in Ba\textsuperscript{2+} Tyrode was approximately four times larger at a given sarcomere length. However, the features often observed in the active dynamic stiffness of cardiac muscle tissue were still noticeably absent. It is possible that these features are a result of other factors in multi-cellular tissue preparations such as heterogeneous cell types, the presence of significant extracellular matrix or cellular damage at the attachment points.

However, there were several problems with this experiment. Firstly, the muscle cell spent over an hour in Ba\textsuperscript{2+} Tyrode. Other studies have demonstrated that after 25 minutes in Ba\textsuperscript{2+}, ATP and phosphocreatin levels within the cardiac muscle tissue decrease and the cell enters a quasi rigor state [130]. Shepherd et al found that when papillary muscle from New Zealand white rabbit was bathed in 0.5 mM Ba\textsuperscript{2+} for 45 minutes the features in the magnitude of the dynamic stiffness had become less obvious. They also found that after 117 minutes in Ba\textsuperscript{2+} the dynamic stiffness had increased in magnitude by a factor of approximately 3.5 (as the tissue entered a rigor like state). In our experiment, the dynamic stiffness was measured sequentially from shortest to longest sarcomere lengths over a period of approximately 120 minutes.

Secondly, it was not possible to determine if the increase in measured stiffness was a result of the Ba\textsuperscript{2+} or simply due to the use of small amplitude perturbations. It has been shown that large displacements that break strongly bound cross bridges will reduce the stiffness of muscle tissue [40]. This question could be addressed by measuring the dynamic stiffness of a muscle cell in 1.8 mM Ca\textsuperscript{2+} Tyrode using swept sinusoidal analysis with equally small stimulus amplitude.

Finally, the majority of protocols for measuring the dynamic stiffness of Ba\textsuperscript{2+} activated muscle tissue begin by electrically stimulating the muscle tissue for up to 60 minutes at a sarcomere length that produced close to peak twitch force, L\textsubscript{MAX} [119, 122, 131, 132]. The muscle would then be transferred to Ca\textsuperscript{2+} free solution while maintaining electrical
stimulus until the twitch response disappeared. This ensured that Ca\(^{2+}\) stores within the cell were depleted before the Ba\(^{2+}\) was applied. As was discussed in Section 4, electrically stimulating the cell for an extended period at optimal sarcomere lengths (L\(_{\text{MAX}}\)) was not possible in our system due to cell fragility. It is possible that the lack of stimulation affected the cells contractile processes and, while the myocyte remained morphologically healthy, it was unable to properly respond to Ba\(^{2+}\) activation. A second possibility is that Ca\(^{2+}\) was not properly flushed from the cytoplasm and sarcoplasmic reticulum, limiting the Ba\(^{2+}\) activation via competitive binding.

After conducting this series of preliminary experiments it was concluded that the measurement of the active dynamic stiffness of an intact mammalian myocyte (a world first to the author’s knowledge) would require additional improvements in the position sensor sensitivity and attachment methodology and a reduction in cantilever stiffness. Implementing these and other improvements discussed throughout this thesis would greatly improve the quality and repeatability of experiments performed with this instrument (see Section 6.2.1).
6 Conclusions and Future Work

6.1 Conclusions

In this thesis, modular instrumentation was developed to study the loaded mechanical properties of individual cardiac myocytes from female Guinea pigs. More specifically, the myocyte twitch force was measured and system identification techniques were rigorously applied to provide quantitative linear models of the passive mechanics of the cell including the first measurement of the dynamic stiffness of a myocyte. Several key milestones were achieved during the design, development and verification of this instrument.

Firstly, a robust, easy to align and inexpensive position sensor was designed to sense force by measuring the displacement of small cantilevers that were placed on either side of a cell. The low noise transimpedance amplifier for the sensor provided $< 1 \text{ nm}/\sqrt{\text{Hz}}$ of position noise at frequencies above 10 Hz and was shot noise limited at frequencies above 10 to 100 Hz depending on the operating conditions. The position sensor proved sufficiently sensitive to measure the passive dynamic stiffness and twitch force of a single myocyte.

Secondly, a unique motor structure (US utility patent pending) was designed and built that provided two cantilever actuators with $\pm 100 \mu\text{m}$ of travel and a force resolution of $< 2 \text{ nN}/\sqrt{\text{Hz}}$ above 10 Hz. The motor structure was combined with a fluidic system to allow the entire system to be fully submerged in physiological saline creating a local environment suitable for the cells. The design also integrated an optical fiber for bright field transmission microscopy of myocytes in the device and provided sufficient space for the position sensor to illuminate the back surface of the cantilevers.

Thirdly, a loading and inspection system was integrated into the motor well, allowing a healthy cell to be selected for experimentation just above the cantilevers. The cells were manipulated using a simple pulled capillary that provided an average loading time from
cell inspection to attachment of five minutes. A novel attachment mechanism was also
designed that rapidly and robustly bound the 100 μm long, 20 μm wide cells to the tips of
the cantilevers, facilitating mechanical measurement. The attachment mechanism was
capable of stretching cells beyond the physiological range of sarcomere lengths and
supporting contractions up to approximately 2 μN without damaging the cells.
Furthermore, the very high success rate of attachment made this technique ideal for use in
an array.

Finally, the operation of the modular instrument was verified by running two sets of
proof of principle experiments that were world firsts (to the author’s knowledge):
- The measurement of the passive dynamic stiffness of an intact mammalian
cardiac myocyte.
- The application of H∞ optimal control of cantilever position to make isometric
measurements of muscle cell twitch force.
These initial experiments served to satisfy the original goals of the project, laid the
ground work for more complicated studies and highlighted necessary improvements in
the modular system to be addressed in the future before integrating the module into an
array instrument.

To conclude, this thesis described the design, development and application of a first step
toward an instrument that is capable of thoroughly characterizing the mechanics of living
heart muscle cells in parallel. The future instrument could be applied to high throughput
studies of muscle physiology or to drug development and toxicology testing in the
pharmaceutical industry.
6.2 Future work

There are two significant thrusts that could serve to focus future work on this project. Namely, continued iteration of the instrument design and extension of the experimental work.

6.2.1 The next instrument iteration

The next iteration of the instrument should focus on modularity and improving the quality of experimental results by implementing the improvements discussed in the body of this thesis. The combined lessons of the instrument characterization and muscle cell experiments are summarized in the following list of specifications and suggested improvements.

Electrodes and cell stimulation

The electrodes must be capable of applying an electric field of 0.5 kV/m to 5 kV/m across the cell. Currently the electrical stimulation is limited by bubble formation and the shielding effect of the grounded stainless steel cantilevers in close proximity to the cell. To alleviate these problems consider:

- Removing the cantilevers from solution or using a non conductive attachment mechanism that separates the cells from the cantilevers.

- Avoiding bubble formation by:
  - Limiting applied potential to < 2 V by placing the electrodes closer together.
  - Using solution flow to wash bubbles away from cantilevers.

Fluidics

The fluidic system used in the final iteration was sufficient to provide proof of principle measurements. It could be improved by providing:

- Rapid switching between at least three solutions (minimal dead volume).
- Thermal regulation as close as possible to the myocyte to control immediate environment.
• Improved modularity using small wells and automated micro valves.
• The ability to flush the system with deionized water (or mild detergents) for cleaning purposes.

The core of the system (force sensor / actuator and position sensors)
The following combination of improvements to the force and position sensors could provide the required order of magnitude increase in sensitivity to make the first measurement of the active dynamic stiffness of single cells using stochastic system identification:
• Use smaller, lighter cantilevers to simultaneously increase resonance (toward 1 kHz) and force sensitivity (stiffness < 0.2 N/m). The higher force sensitivity would lessen the significance of interference coupling between the cantilevers.
• If possible, construct the cantilevers from a Beryllium Copper alloy.
• Take the cantilevers out of solution to increase the primary resonant frequency and improve the quality of reflected position signal (increasing position sensor sensitivity).
• Apply temperature compensation to the position sensor LED current source and photodiode to limit low frequency noise (improving position sensor resolution).
• In the longer term consider moving to a MEMS approach once the design requirements are thoroughly understood.

Cell attachment, loading and imaging
The attachment, loading and cell imaging systems used in this project were, like the fluidic system, adequate to provide proof of principle measurements. However, they could be improved by:
• A strategy that maintains strength of attachment while not damaging cells during contractions that produce greater than approximately 2 μN of force.
• A large inspection region for selecting cells with independent electrodes to stimulate contractions.
• A methodology for loading and attachment that could be completed in approximately one minute with the potential for automation.
- Elements of the system being either disposable or cleanable (using solvents to remove cell debris)
- Using a high numerical aperture objective and matched condenser to image the cells and potentially other forms of illumination such as phase contrast to improve the quality of sarcomere length detection
- Providing the possibility for fluorescent monitoring of sub-cellular features such as internal Ca\(^{2+}\) concentration.

As a final note, subsequent module iterations should also consider issues related to the design of an array of testing devices. Of primary importance would be the balance between the complexity of an individual module and its level of independence from the array. That is, which elements of the system (fluidics, power supply, imaging etc.) should be implemented globally at the array level and which should be implemented at the modular level to provide the “best” instrument as judged by size, cost and flexibility.

### 6.2.2 Future experimentation

The quality of future experimental work would benefit from a significant redesign of the instrument incorporating the objectives described in Section 6.2.1. Once that is complete there are several lines of experimentation that would be interesting to pursue.

The relationship between twitch force and the full physiological range of sarcomere length of an intact cardiac myocyte could be measured (characterizing the Frank Starling relationship for a single cell). The \(H_\infty\) isometric control could be combined with isotonic control to measure the myocyte mechanical properties while the cell is loaded in a manner similar to that seen in the heart (extending the work of Nishimura et al [103]). In addition, with an improved attachment system and higher position sensitivity the dynamic stiffness of an intact cardiac muscle cell activated by Ba\(^{2+}\) could be measured. Stochastic system identification techniques could then be used to create nonlinear and time varying models of the muscle cell (extending the work of Nielson et al [57]).
Finally, the effect of a collection of drugs from the pharmaceutical industry on the mechanical properties of intact mammalian cardiac myocytes could be measured using an appropriate mix of novel stochastic system identification techniques and “classical” physiological experiments. The characterization of myocyte state could be further extended by adding functionality to the instrument including fluorescent monitoring of sub-cellular features such as the internal concentration of Ca$^{2+}$ and patch clamp monitoring of electrophysiological properties. Changes in observed state could then be correlated with desired or adverse physiological phenotypes leading to the application of the array instrument for drug development and toxicology screening in the pharmaceutical industry.
7 References


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8 Appendix

Over the course of this project thousands of lines of code were written in Matlab scripts, Ansys scripts, VB.Net, C and assembly. The following sections contain examples of some of this code for illustrative purposes.

8.1 Ansys magnetic FEM for motor design

Ansys was used for many different finite element modeling tasks within this project including magnetic, mechanical and electrical simulations. Each of these projects were implemented using scripts that executed sequential text commands in Ansys and allowed the simulation parameters to be quickly adjusted. To illustrate this concept, the script used to optimize the magnetic flux within the motor structure is presented here (see Section 2.3.3 for more details).

```
finish
/CLEAR, all
/prep7
/title 3D Motor Design

!Define Element Types
ET,1,96       !For volume meshing
ET,2,200      !For area meshing
KEYOPT,2,1,4

!Define the material properties
!**********************************************************************
MAT,1          !NdFeB permanent magnet magnetized in Z+ direction
MP,MURX,3,1.044
MP,MURY,3,1.044
MP,MURZ,3,1.044
MP,MGZZ,3,600000 !A/m
MAT,2          !Vandium Permandur
TB,BH,4,,12
TBPT,,136.77,1.412
,,237.68,1.786
,,641.21,2.066
,,2000,2.192
,,5000,2.247
,,10000,2.283
,,20000,2.331
,,40000,2.368
,,80000,2.412
,,160000,2.475
,,320000,2.537
,,640000,2.59
MAT,3          !Air
MP,MURX,5,1
```
!Load geometry file (from a 3D CAD model)

!display the volume numbers

!shrink from meters to millimeters (then delete old volumes)

VLSCALE,all,,0.001,0.001,0.001

VDELE,1,8,1
ADELE,1,61,1
LDELE,1,134,1
vsel,all
vplot,all

!Manipulate volumes to removing overlapping elements

bopt,keep,1 !divide center keeper using first air gap volume
vinv,14,16
vsbv,14,1
vdele,14 !divide center keeper using second air gap volume
vinv,15,2
vsbv,2,3
vdele,2
vsel,all

!set the BTOL to small enough size to avoid volume errors

BTOL,4.88095E-7

!subtract the structure volumes from the air boundary

vsel,u,,13 !ignore the volume used to subdivide the central keeper
vsbv,10,all,SEPO,DELETE,KEEP

!subtract the structure volumes from the air gaps

vsbv,15,all,SEPO,DELETE,KEEP
vsbv,16,all,SEPO,DELETE,KEEP

!split the central keeper volume

vsel,all
BOPT,KEEP,1
vinv,13,4
vsbv,4,7
vsel,s,,4
vsel,a,,13
vdele,all

!glue the remaining volumes together

vsel,all
BOPT,KEEP,0
vglue,all

!Assign materials

!Volume list after vglue

!14 Bottom keeper
!4 Magnets
!!1,10,13,15,18 Top and center keepers
!!16,17,19 Air
vsel,s,,4
vatt,1
vsel,s,,13,15,1

!NdFeB magnets
vsel,a,,1,10,9
vsel,a,,18
Vatt,2 !Permandur
vsel,s,,16,17,1
vsel,a,,19
vatt,3 !Air
vsel,all

!Mesh the design
************************************************************************************
!The critical volumes where meshed using an area mesh sweep to ensure sufficient quality

!Mesh the areas then refine them

!Now sweep the volumes using the area meshes

!Mesh the remaining volumes

!Remove the mesh elements (the area mesh)

!Solve for magnetic potentials
************************************************************************************

/SOLU
!Dont need any BC for parallel flux as this is the natural condition
!for magnetic scalar solutions
!Ideally force the far field solution to be normal to the surface
!However this would require a spherical air shell (rather than the rectangular one used)
!Instead, set magnetic field to zero in far field

!Select all features of the simulation

!Solve
8.2 $H_\infty$ controller design in Matlab

Here we present a simplified version of the $H_\infty$ isometric controller design. In this case, the plant is treated as a simple SISO system with 4th order ARMA models for each cantilever (that were previously fit to experimental data, see Section 2.5.1). The full script covered MIMO controller design, sensitivity analyses, Simulink model details and isotonic controller design.

clear all
close all

%Sampling rate
fs = 2000;

%Load the ARMA models of the cantilevers
aC1 = load('C:\aC1.mat');
bC1 = load(' C:\bC1.mat');
aC2 = load('C:\aC2.mat');
bC2 = load(' C:\bC2.mat');
C1 = tf(bC1,aC1,1/fs);
C2 = tf(bC2,aC2,1/fs);

%Define the SISO plant model
%***********************************************************************
H11 = C1;
H12 = 0;
H21 = 0;
H22 = C2;
%***********************************************************************

%Generate the state space representation of the cantilevers
%***********************************************************************
HT = [H11 H12; H21 H22]; %The plant transfer function matrix
HTS = ss(HT);
Hmin = minreal(HTS); %Removes pole zero cancelations within a fixed tolerance
SysD = canon(SysD,'modal');
HTSD = SysD;
%***********************************************************************

%Set up the weighting transfer function matrices, W1DM, W2DM, W3DM
%***********************************************************************
%Good disturbance rejection: epsw1 = 10, epsw2 = 0.01 epsw3 = 0.1
%Good step response: epsw1 = 1, epsw2 = 0.01 epsw3 = 0.1
scale = 1;
epsw1 = 5*scale;
epsw2 = 0.001*scale;
epsw3 = 0.1*scale;
w1 = 2*pi*5; %emphasis on disturbance rejection
w12 = 2*pi*30; %flattening out of W1
w3 = 2*pi*800; %control effort to follow input
w32 = 2*pi*500;
W1 = tf(1,[1/wl 1])*tf([1 w12],w12)*epsw1;
W2 = [1 0; 0 1]*epsw2;
W3 = tf([1,[1/w3 1])*tf([1 w32],w32)*epsw3;

W1D = c2d(W1,1/fS,'zoh');
W1DM = [W1D 0;0 W1D];
W1DM = W1DM^2;

W3D = c2d(W3,1/fS,'zoh');
W3DM = [W3D 0;0 W3D];
W3DM = W3DM^2;

W2 = c2d(tf(eye(2,2))*epsw2,1/fS,'zoh');
W2ss = ss(W2);
W2DM = W2;

%Implement the controller
%Augment the plant with the weighted transfer functions
HTSDW = augw(HTSD,W1DM,W2DM,W3DM);
%Find the optimal hInfinity controller
[Kh,CL,GAM,INFO] = hinfsyn(HTSDW,2,2);

%******************************************************************************

%**********
8.3 Assembly control loop for TI DSP

The core of the control loop for the Texas Instruments digital signal processor was implemented in assembly to improve the timing of the interrupt handling. This code was one part of a larger program written in C to read data from a 32 bit bus, perform control calculations then send the data back out over the bus (see Section 2.5.1).

;A function to loop continuously while monitoring interrupts
;void intLoop(void)
;REGISTER USE
;A0,A1,B1,B2
;++must preserve A/B 10-15, A3 and B3

.reff_intControl
.asg B15, SP

.sect ".text"
.global __intLoop
.def L0,L1,L2,B1

__intLoop:

STW B3,*SP--(8)

L0:

mvkl E1,A0 ;store the exit function line

MVC ISTP,B2

mvkh E1,A0

EXTUB2,22,27,B2

CMPEQB5h,B2,B1 ;check for interrupt 5

CMPEQB4h,B2,B2 ;check for interrupt 4 to drop out of function

L1:

[B1] B L1 ;loop to L1 if no interrupt pending

[B2] B A0 ;exit function if B2=1 then B1=0 so B L1 doesn’t occur

[B1] MVC ISTP,B2 ;check for new interrupts

[B1] EXTUB2,22,27,B2

[B1] CMPEQB5h,B2,B1 ;check for interrupt 5
CMPEQ 4h, B2, B2 ; check for interrupt 4 to drop out of function
nop 1
; [[B1] branch executes
mvkl 0000FFFFh, B1
mvkl 02000024h, A1 ; clear all flags in the IFR
mvkl 02000024h, A1
mvkl B1, ICR
mvkl 80000000h, A0
mvkh 80000000h, A0 ; the start address of the input data from ADC
MVC ISTP, B2 ; look for EDMA interrupt when data ready
stw A0, *A1 ; triggers the QDMA transfer
EXTU B2, 22, 27, B2
CMPEQ 8h, B2, B1 ; check for interrupt 8
mvkl E1, A0 ; store the exit function line
CMPEQ 4h, B2, B2 ; check for interrupt 4 to drop out of function
mvkh E1, A0

L2:

; [[B1] B L2 ; loop to L1 if no interrupt pending
; [B2] B A0 ; exit function if B2=1 then B1=0 so L1 doesn't occur

; [[B1] MVC ISTP, B2 ; check for new interrupts
; [[B1] EXTU B2, 22, 27, B2
; [[B1] CMPEQ 8h, B2, B1
; CMPEQ 4h, B2, B2 ; check for interrupt 8
nop 1
; [[B1] branch executes
B intControl
mvkl 0000FFFFh, A0 ; prepare to clear the IFR (using ICR)
mvkl L0, B3 ; store start of this loop as return address
mvkh L0, B3
MVC A0, ICR ; clear the flag in the IFR
nop 2
; interrupt handler function executes

E1:

LDW **SP(8), B3 ; reload the SP and exit function
nop 4
B B3
nop 5