Design and Construction of an NMR Force Microscope to Image Single Biological Cells \textit{in vivo}.

\begin{center}
\textit{by}
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Chapter 1

Introduction

1.1 Theory

The phenomenon of magnetic resonance has been used, with ingenious implementation, to image objects in three dimensions. This technique can be used to look at not only the electron structure of an object, as in visible imaging, but also nuclear structure in nuclear magnetic resonance (NMR). The essence of this phenomenon is that any particle with a magnetic moment will have its energy states split when placed in a static magnetic field. The energy between these two states is given by $-\mu B_z$ where $\mu$ is the magnetic moment of the particle, also given by $\gamma m \hbar / 2\pi$: the gyromagnetic ratio times the spin quantum number multiplied by Planck’s constant over $2\pi$. $B_z$ is the magnitude of the static field and $m$ is the spin state of the particle. The particle usually observed is the proton, i.e. hydrogen, since it has the strongest signal. Alternatively a more complex nucleus may be used provided that the total moment of the nucleus is not zero. In the case of the proton, with spin $\frac{1}{2}$, the energies of the states are $\pm \mu B_z$ for alignments with and anti-parallel to the field respectively. Once aligned anti parallel the spin will decay into the ground state in a time $T_1$.

The spin of the proton has a total magnitude of $\frac{1}{2}(\frac{1}{2}+1)$ though the eigenvalue for the $z$ component of the spin $s_z$ may only take on a value of $\pm \frac{1}{2}$. This results in an $x$-$y$ component to the spin which precesses around the static magnetic field $B_z$ due to the Lorentz force. If immersed in a radio frequency (RF) field of frequency equal to the precession frequency, the spins will be encouraged to precess in phase with one another. Fittingly, this phenomenon has been dubbed magnetic resonance. Depending on the strength and duration of the RF pulse the amplitude of the spinor wavefunction may be made equal in the $+z$ and $-z$ directions. This results in only the $x$-$y$ component of the spin being apparent. Since this moves the moment from, on average, the $z$-direction to the $x$-$y$ plane, it is called a $\pi/2$ pulse as it rotates the direction of the moment $\pi/2$ radians. Similarly, $\pi$ pulses are also possible.

Indeed, using a sequence of $\pi$ and $\pi/2$ pulses one may determine information about the motion of particles in the sample as well as their location. This is useful in analyzing biological systems by allowing one to study diffusion rates within a cell. To accomplish this, the spins are rotated into the $x$-$y$ plane using a $\pi/2$ pulse. Because the spins are not in a uniform field, but rather a gradient, different spins will precess at slightly different rates. They are also not stationary and will move, thereby encountering different field strengths as they wander through the gradient. As different particles precess at different speeds some will run ahead of others in their phase relation to the other spins. If after some short period a $\pi$ pulse is applied those that are precessing faster will now be flipped in their position relative to the other spins. Now the fastest spins are behind the slower ones. In time they will catch up and begin to overtake their slower cousins. When all of the spins are, momentarily, at the same phase again a signal may once again be detected. This is known as a spin echo. If, of course, the spins have deviated from their original positions in the field gradient too far then the speed of precession of a given spin will not be constant before and
after the \( \pi \) pulse. This destroys the possibility of rephrasing after a time \( T_2 \). Since the time one may wait before an echo may be detected is proportional to the movement of the spins, i.e. diffusion of the molecules, spin echo may be used as a tool in diffusion studies as well as spectroscopy and imaging.

Magnetic resonance is used as a diagnostic device to analyze the composition of materials. Since each nuclei has a different gyromagnetic ratio, each resonates at a different frequency for a given \( B_z \). This makes possible nuclear magnetic resonance (NMR) spectroscopy. Further fine scale splitting of energy levels due to dipole-dipole interactions of adjacent nuclei allow the interconnectedness of atoms in a molecule to be determined from such a spectrum. Further, frequency shifts due to varying degrees of electron shielding allow the relative electro-negativity of nuclei to be inferred from the local spin polarization. To take such a spectrum, an RF photon generated by a coil, with an energy equal to the energy difference between the two states \( \Delta E = 2\mu B_z \) is used to flip the state of the proton from parallel to anti-parallel. This absorption can then be detected as a load across the coil. If a field gradient, show as the shaded regions in Fig. 1, is introduced and a sufficiently narrow frequency spectrum used in the RF pulse then only certain nuclei at a particular field will be under conditions needed for resonance. The condition for resonance is met along lines of constant field, labeled 1-5 in the figure. Each of these areas of constant field constitutes a resonance slice. With known field gradients a specific group of nuclei may be excited to resonance at a particular point in the sample and recorded as a single voxel of data. By moving the sample, varying the field gradient, or changing the RF frequency, a signal may be elicited from small regions throughout the sample. The data from each region may then be assembled into a 3-D image of the sample. This is known an magnetic resonance imaging (MRI).

![Figure 2](image)

Because water has two hydrogen atoms per molecule it is an excellent molecule to analyze. Additionally, since biological systems have a great deal of water, and hydrogen in other molecules, such as fats, proteins and carbohydrates, NMR has become an invaluable tool in the medical community. Alas, for exceedingly small samples on the order of tens of microns, i.e. a single cell, the load induced by the signal is below current electronic detection methods.
1.1.1 Microscopy Setup

Although the sensitivity of electronic circuits is insufficient to detect exceedingly small signals, mechanical oscillators can be constructed using silicon lithography techniques that are sensitive enough to be used in the detection of the spin flips in the sample. John Sidles fathered this technique, called nuclear magnetic resonance force microscopy (NMRFM) [Sidles]. NMRFM utilizes a high quality oscillator to detect small forces with a frequency equal to the resonance frequency of the oscillator. Since the amplitude of the oscillator is the amplitude generated per cycle multiplied by the Q, signals used to drive the oscillator may be amplified to Q times their typical magnitude.

Despite the fact that mechanical oscillators can be constructed with much higher Q than electronic circuits, the amplitude of oscillation excited is still vanishingly small and must be detected by means of optical interferometry. Additionally, mechanical oscillators are limited, for practical reasons, from having a resonant frequency in the high RF. This means that a degree of ingenuity is required to couple the nuclear moments and the oscillator at the appropriate frequency. These problems along with the practical issues of alignment and detection are what separate the theory and experimentation of NMRFM.

The general setup for NMRFM consists of the use of an interferometer to detect the position of the oscillator head, and hence its amplitude of oscillation. In this setup an optical fiber is used to route coherent light to the oscillator and to accept the reflection to form an interference fringe. This interferometry setup is discussed in more depth in section 1.2.2. A magnet, placed on the oscillator head, is then brought near the sample. When driven by an appropriate RF signal the spins will couple to the magnet on the oscillator and drive it.

![Figure 3](image)

Unfortunately the natural precession frequencies of nuclei is much higher than the resonant frequencies of our oscillators. In order to couple the magnetic moment of the magnet on the oscillator to the spins, the RF signal used to synchronize the spins is frequency modulated at the characteristic frequency of the mechanical oscillator. This is accomplished by using a high frequency HP8656B Signal generator with a modulation signal provided by the lock-in Amplifier. Since each amplitude peak in the signal is equivalent to a $\pi$ pulse, the spins are made to flip at a frequency equal to that of the amplitude modulation, i.e. the resonant frequency of the mechanical oscillator.

By using this magnet on oscillator setup, there is only one field gradient available. This means that only one dimension may be scanned at a time. With a bit of ingenuity, however, it is unnecessary to
rotate the sample or oscillator. To construct a 3D image the sample may be scanned across the oscillator using the piezo-electric tube (PZT). At each point in the sample a measurement is taken for each resonance slice. Since the first slice to intersect the sample is only one voxel, as seen in region 1 of the resonance slice of Fig. 1, a 2D construction of the surface of the sample may be acquired. The next slice, region 2, may then be read. Since the signal generated by the surface is known from the previous scan, one may subtract the contributions from the surface layer when measuring the second layer of the sample. This procedure may be repeated as far into the sample as the field gradient can be maintained.

1.1.2 Oscillators

The Q of a mechanical oscillator is limited by several factors including viscous air drag, defects in the material that limit resiliency, and mechanical coupling to the environment. Since it is the Q of the oscillator that is one of the major limiting factors to the sensitivity (i.e. resolution) of the microscope it is imperative that it be maximized. The limitation of viscous drag by air is overcome with relative ease by placing the oscillator in vacuum. Vacuums of 1 torr or better are easily attainable by any run-of-the-mill oil roughing pump. Below this pressure the viscous effects of air become negligible in comparison to other sources of energy loss [Graf]. The second problem, that of resiliency, is mostly due to defects in the crystal lattice of the material and interactions at the grain boundaries within the material. In order to overcome this problem a single crystal is used for the entire oscillator. Single crystal silicon is the material of choice as advanced lithography techniques have been developed for shaping silicon by the electronics industry, and silicon wafers are mass produced and available at a reasonable price. The use of a single crystal does not eliminate phonon-phonon interactions or thermoelastic gradients, but these factors are far less significant than the phonon scattering off defects and grain boundaries [Graf]. Despite all of this high-tech tomfoolery the oscillator will be inextricably coupled to the outside environment by whatever is holding it in place. By using a rigid base energy dissipated can be decreased. In order to accomplish this the mount holding it should be made of diamond and clamped down onto the base of the oscillator as tightly as possible, though even this is would not be terribly effective. Diamond prices being what they are, and silicon cracking easily under pressure this does not seem a useful solution.

To solve the problem of coupling between the oscillator and environment an elegant bandpass filter design is incorporated into the structure of the oscillator itself. Enter the high-Q oscillator. The oscillator consists of a head and body attached by a neck with the body attached, via a waist, to the base. The head is constructed with a much smaller mass than the body, and the neck and waist are made thin to act as torsion springs as shown below.

![Figure 4](image_url)

This oscillator has four main normal modes of oscillation; in order of frequency they are:

- **Lower Cantilever**: The whole oscillator sways as a unit like a reed would sway in the wind. Here the neck and waist act as flexural springs rather that torsion springs.
-Lower Torsional: The entire oscillator moves as a unit, but this time in a twisting fashion around its axis of symmetry.

-Upper Cantilever: The oscillator returns to its swaying motion, though, with the head and body now swaying in an anti-symmetric manner.

-Upper Torsional: The head and body move counter to one another in a twisting fashion around the axis of symmetry.

The upper torsional mode has the highest resonant frequency and the highest Q. It is therefore of greatest interest. Because the body and head move in an anti-symmetric fashion they must have the same period, but with the head being less massive than the body it must move faster to balance the angular momenta of the body. This results in most of the energy of oscillation being stored in the head with the body acting as a bandpass filter to the base. Any energy loss to the base must be extracted from the motion of the body, as there is no direct coupling of the head to the base. As only a small fraction of the energy is stored in the body to begin with the total energy loss from the oscillator is minimized.

Modern lithography techniques available at the Pickle Research Campus are used by Michelle Chabot to micro-machine these oscillators with dimensions on the order of microns. Indeed, the smaller the oscillator, generally, the higher the resonant frequency and the smaller the spring constant of the neck and waist both of which result in better sensitivity as will be discussed in the next section.

A 1µm dot of cobalt is vapor deposited on the oscillator head along with a thin 80 nm coating of gold to prevent oxidation. This acts as a magnet when placed in a static field and serves two purposes. It generates the field gradient in the sample by introducing a perturbation in the permittivity of the space around it, as well as allowing the oscillator to couple to the magnetic moments excited to resonance within the sample by an RF coil. Troy Messina has constructed a vapor deposition chamber to characterize the behavior of YHx films for optical switching applications and has kindly applied his expertise to the deposition of such magnets on both the high-Q oscillators as well as commercial AFM tips and UltraLevers.

1.1.3 Detection Limits

The signal to noise ratio, and thereby the resolution of the microscope, is limited by several factors including the resonant frequency of the oscillator, the temperature, the static field strength and perhaps most importantly the Q of the mechanical oscillator. The static field cannot be increased without breakdown of the external superconducting solenoid and the temperature is set by the desire to image a single cell in vivo. Other factors, such as the Q of the oscillator, may be improved only at the limitation of creativity. To determine the resolution possible in such a microscope we must analyze the force generated by a given volume of the sample at resonance and the minimum force detectable by the oscillator setup.

To calculate the force generated by an ensemble of spins we now turn to the classical force of a magnetic moment in a field gradient. The magnetization of an ensemble of spins is given by the Curie formula [Reif] which multiplied by the field gradient yields the force generated by N spins:

\[
F_{\text{gen}} = \frac{N \gamma^2 h^2 B}{3(2\pi)^2} k_B T (I(I+1)) \frac{\partial B}{\partial z} \tag{1}
\]

where \(\gamma h/2\pi\) is the gyromagnetic ratio times Planck’s Constant over \(2\pi\). B is the magnitude of the static magnetic field, T is the temperature, \(k_B\) is Boltzmann’s constant, I is the spin of the particle and \(\delta B/\delta z\) is the local field gradient. To determine N we must determine the number of spins within an acceptable field strength to resonate. An approximation for the thickness of the resonance slice \(\Delta z\) is given by [Barrett]:

\[
\Delta z = \frac{10^{-3}}{\gamma B h} \tag{2}
\]
where \(\frac{\partial \mathbf{B}}{\partial z}\) is given in tesla/meter, or equivalently centigauss/micrometer and \(10^{-3}\) is the range of acceptable field strength in tesla. A brief dimensional analysis confirms the units. Upon first glance the total force generated seems to be independent of gradient, as the gradients in the expressions for \(F_{\text{gen}}\) and \(\Delta z\) cancel. Indeed, for a given slice of constant area the force is constant regardless of the thickness, but if one desires a smaller slice, which as the reader will recall from section 1.1.1 is essential for the deconvolution into a 3D image, then a larger gradient is desirable.

Since 3D imaging is the ultimate goal it will be assumed that a volume element (voxel) of equal size in each dimension is desired as the smallest image element. Because the \(z\) direction is limited in resolution by the gradient and equal resolution is assumed in every dimension voxels of volume \(\Delta z^3\) will be used in future calculations for resolution. With these assumptions we may now calculate \(N\).

Given the mass density, in grams/cm, of the substance to be imaged \(\rho\), its molecular weight \(m\), and the number of measurable atoms per molecule \(n\) we may calculate \(N\):

\[
N = \frac{n N_a \rho}{\left(10^{-3}\right) m} \tag{3}
\]

where \(N_a\) is Avogadro’s number and the factor of 10 comes from the \(10^{-3}\) factor in equation (2) and from the conversion from CGS to MKS units. The force generated by a single voxel of the sample as a function of resolution may now be calculated.

Equation (3) assumes a pure concentration of the sample molecule as is the case in most superconducting studies done with NMRFM in the lab. This statement also holds true to good approximation for water in biological samples. For this reason the variable for the concentration of the sample substance has been omitted for simplicity. If, however, the concentration is not unity equation (3) may be multiplied by the mass fraction of the target substance to correct the result.

Of course the force generated is a meaningless number unless the detection threshold is known. The minimum force detectable by a mechanical oscillator is given by [Wago et al.]:

\[
F_{\text{det}} = \sqrt{\frac{4 k_b k T \cdot \Delta \nu}{\omega_o Q}} \tag{4}
\]

where \(k_b\) is Boltzmann’s constant, \(k\) is the spring constant of the oscillator, \(T\) is the temperature, and \(\omega_o\) and \(Q\) are the resonant frequency and quality factor of the oscillator, respectively. \(\Delta \nu\) is the spectral frequency of the measurement governed by the averaging time. The longer the oscillator is observed the more random fluctuations will cancel and the better the steady amplitude of the oscillator may be known.

Diffusion studies provide for the most useful and interesting data, but are limited to measuring pulse echoes. Because the echo signal is of limited duration the measurement time is similarly limited. One may take multiple measurements of the same point, but this is increasingly more troublesome as the number of pulses that must be measured increases.

Despite this limitation, many of these factors may be altered, such as \(\omega_o\), simply by changing the size of the oscillator. Generally the smaller the oscillator the higher its resonant frequency and the smaller its \(k\) [Barrett]. Others such as \(\Delta \nu\) and \(T\) are limited by experimental reasons of using spin echo in liquid water. Clearly the size and \(Q\) of the oscillators are the best bet to increasing resolution. Since the relation of size to \(Q\) is uncertain in any rigorous mathematical sense I will concentrate primarily on the requirements of \(Q\) for a given resolution.

To determine the conditions necessary to image a sample we first set the minimum detectable force, \(F_{\text{det}}\) equal to the generated force \(F_{\text{gen}}\). Substituting equation (3) in for \(N\) in equation (1) and assuming hydrogen is being imaged, i.e. \(I = \frac{1}{2}\) and \(\gamma = 42.577\ \text{MHz/tesla}\) we get:
\[
\frac{nN_x \rho \gamma^2 h^2 B_z}{3(2\pi)^2 10^3 k_B} m \left( \frac{\partial B}{\partial z} \right) (= \{1 + 1\}) \frac{\partial B}{\partial z} = \sqrt{\frac{4k_B T k \cdot \Delta v}{\omega \cdot Q}}
\]

Solving this equation for Q and plotting it as a function of the gradient, \( \frac{\partial B}{\partial z} \) and temperature T results in:

\[\text{Figure 5}\]

From this plot and corresponding equations we may determine that in order to achieve a resolution of 0.1 \( \mu \)m, i.e. using a gradient of 10,000 T/m, and signal to noise ratio of 10 either a Q of 2\times10^9 must be achieved at room temperature, assuming a resonant frequency of 60 kHz and a spring constant of 5\times10^{-3} N/m. Alternatively, a temperature of approximately 0.3 K a Q of merely 2 is sufficient. To date, the Q required for a room temperature scan are not practical we must either lower the temperature and suffer imaging a cell in cryostasis or settle for a lower resolution. With current technology oscillators the upper left portion of the plot is feasible. With Q of 10,000 at room temperature and frequencies in the 60 kHz regime resolutions of 1 \( \mu \)m are attainable. In the future stronger field gradients, by use of thicker magnets on the oscillators, and use of higher power RF signals will allow resolutions of 0.1 \( \mu \)m. At room temperature the Q required to for a given field gradient under current conditions may be represented as a slice of the plot above at \( T = 300 \) K.
Work is currently underway by Yong Lee to construct a similar probe for use with He³. This would allow temperatures approaching 0.3 K to be realized. Using such cryogenic temperatures the detection of single spins has been forecast to occur in the next two decades or so. In order to image at a linear resolution of \(10^{-10}\) m, that of a single atom, a Q of \(2 \times 10^{12}\) is necessary, though again with improvements in field gradient technology this may be brought to a more realistic number.

The detection limit of this setup does not vary linearly or identically with each of the variables. The sensitivity scales as \(Q^{1/2}\), for example. This means that only by quadrupling the Q is sensitivity doubled. Similarly the other variables scale as follows:

<table>
<thead>
<tr>
<th>Sensitivity Dependence</th>
<th>Change of 0.5x</th>
<th>Change of 2x</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Q^{1/2}) : Quality</td>
<td>0.707</td>
<td>1.414</td>
</tr>
<tr>
<td>(\omega_0^{1/2}) : Resonant Freq.</td>
<td>0.707</td>
<td>1.414</td>
</tr>
<tr>
<td>(\Delta f^{-1/2}) : Freq. Spread</td>
<td>1.414</td>
<td>0.707</td>
</tr>
<tr>
<td>(k^{-1/2}) : Spring Constant</td>
<td>1.414</td>
<td>0.707</td>
</tr>
<tr>
<td>B : Static Field</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>(T^{-3/2}) : Temperature</td>
<td>2.828</td>
<td>0.354</td>
</tr>
<tr>
<td>((\delta B/\delta z)^{-4}) : Field Grad.</td>
<td>16</td>
<td>0.0625</td>
</tr>
</tbody>
</table>

### 1.2 Original Probe Configuration

At the start of my project I was given responsibility of a probe created by a former graduate student, Tobias Graf, for the purpose of analyzing the characteristics of the large high-Q oscillators using a capacitive drive and a light interferometry pickup. The high-Q oscillators in question were first implemented by Dr. John Markert, under whose supervision I now work, during his graduate work at Cornell University. The high-Q oscillators have achieved quality factors as high as \(10^8\) at cryogenic temperatures under vacuum.

#### 1.2.1 Structure

The original probe components still in use include the overall body of the probe with vacuum feed-throughs and 1-D translation stage for the optical fiber. At the top of the probe, i.e. the side which will ultimately stick out of the magnet, there are two quick-flange (QF) vacuum ports opposite one another. One is for a thermocouple vacuum gauge and the other for attaching the pump itself. Perpendicular to the vacuum ports is a cluster of 7 BNC receptacles in the form of a hexagon with a single receptacle in the
A flange just below this assembly is used to mate the probe to magnet via a collar using a triclover flange. At the bottom of the probe, where the actual NMR takes place, there is a 1-D translation stage which holds the optical fiber. The stage is controlled by a micrometer screw for coarse approach and a piezo for fine control to allow fringe locking. Care should be taken when using the micrometer screw as the piezo is delicate and will shatter easily if compressed too severely. Additionally, a runway with an array of 2-56 clearance holes for mounting components is attached to the ventral side of the probe opposite the 1-D stage. A second flange is attached above the runway and fiber stage for the attachment of a vacuum can. The original dimensions of the end of the probe were insufficient to house all of the components necessary to complete the NMR Force Microscope. A longer runway with more mounting holes as well as a longer vacuum can were constructed. Ultimately even the longer can proved to confining. While using the probe to get oscillator data for an abstract Troy Messina submitted plans for a wider flange and vacuum can.

1.2.2 Optics

In order to detect the motion of the oscillator laser interferometry at 680 nm via an optical fiber is used. At the top of the probe a laser and detector assembly is connected to an optical fiber, which is routed to the tip of the probe and held in the 1-D fiber stage. Two directionally coupled optical fibers are used with the laser and detector fitted to one side and the free end and reference detector on the other pictured in figure 7a.

![Figure 7a](image)

Upon reaching the tip, as seen in figure 7b, of the cleaved fiber 4% of the light is reflected due to the impedance change from glass to air. The remainder of the light exits the tip and reflects off the oscillator. Some of this light is reflected back into the fiber and both the glare reflection and reflection off the oscillator traverse the fiber back to the detector where the intensity of the fringe is read.

By design the laser used has a coherence length of a few centimeters. This means that any stray reflections from areas of the probe or within the optical fibers not local to the fiber tip will not contribute definitively to the interference. Both the laser and the detectors used are powered by independent 15 volt power supplies.

1.3 Ultimate Goals

My goal in working on this probe is to modify it from its previous configuration into a device capable of NMR microscopy. I wish to generate a nuclear magnetic resonance image with a spatial resolution of 0.1 µm or better. To achieve this goal conversion of the probe to use the small oscillators is necessary. Because small oscillators will be used higher sensitivity of the nuclear resonance should be possible thereby allowing for higher resolution. If sensitivity of this magnitude is achieved, it will be used to image a single biological cell, *in vitro*.
Chapter 2

Design and Construction

2.1 Mechanics

Since the original probe was constructed to characterize the large high-Q oscillators several mechanical modifications had to be made to facilitate the use of the micro-oscillators and of a liquid sample. Basically, little more than the original shaft with interferometry setup survives. This includes the laser and diodes coupled to the optical fiber, as well as the 1-D translation stage that holds the fiber. All of the co-axial connections are used, but few retain their original wiring within the probe.

In keeping with the ideas used in the original construction, all components I have created are made of 304 stainless steel whenever possible. 304 stainless is strong, resistant to corrosion, and most importantly for our purposes, only weakly magnetic. Indeed, only when components were touched with a very strong neodymium magnet could I detect any magnetic response at all. Alnico and ceramic magnets had no discernable effect.

2.1.1 3-D Translation Stage

Since the probe was designed for the large oscillators in mind, is was not immediately suitable for the small oscillators. The fiber was designed at a height that was would place it exactly at the head height of a large oscillator placed in a small clamp constructed for use in the probe. The capacitive drive was likewise far too low for use with the small oscillators. In order to align the small oscillators with the fiber it was necessary to mount the oscillator on a 3-D translation stage. Simply shoving the oscillator in a clamp is far too inaccurate to align the oscillator properly, and tightening down the clamp would alter the alignment of the oscillator. The probe constructed by Tina Barret, now used by Michelle Chabot, had such a translation stage in it. Unfortunately, since my probe has no mechanical feedthroughs as well as less than one third the space available at the time, the design used in that probe was unsuitable for my purposes.

Perusing catalogs in an effort to find a stage small enough to fit within the confines of the probe’s vacuum can proved frustrating and ultimately unfruitful. All of the commercially available stages that could provide accurate translation in all three dimensions over a reasonable range of motion were both controlled with micrometer screws and far too large for the bore in the magnet, much less the vacuum can. Other stages, which were custom built to the specific application, were smaller, but were piezo driven and therefore had a very limited range of motion. Additionally they would require a constant high voltage input to keep them in one position and were rather costly. In the end, it was decided that a stage had to be designed and constructed.

Fine positioning and relatively large ranges of motion over all three dimensions are required for successful operation. The stage, in order to compensate for my butterfingers in epoxing the oscillator, had to provide for a few millimeters of movement. This would allow the crudely positioned oscillator to be brought into alignment with the fiber despite initially being off by up to a millimeter or more. In order to provide for the best alignment, the motion had to be controllable to a few thousandths of an inch. 0-80 screws are a simple and proven technique of controlling the motion of components. With 80 turns per inch and my hand able to turn accurately to perhaps 1/8 of a turn, motion accurate to within one 640th (0.0016) of an inch or better is theoretically possible.

Three independent moving stages of 304 stainless were constructed, with one orthogonally mounted atop the other. In this way each provided one dimension of movement isolated from the others. Each movement has two pieces, a stage and a base upon which it moves. Each base has two 1/16 inch pins pressure fit into it with a 0-80 clearance hole in between. The pins serve as tracks along which the stage portion rides. A 0-80 tapped hole was made in between the two clearance holes to provide purchase for the screw threads in the stage portion. The head design of the screws only allows the screw to be loaded under tension effectively, therefore compressive springs are placed on the pins in between two plates to keep them apart while a screw holds them together under tension.
In order to ensure that workability of this design a prototype model was first built out of brass because of its ease of machinability. By using brass the problem of drill bits frequently breaking when making long, narrow holes in harder materials, such as stainless steel, was avoided. Aluminum is yet more easily worked, but past prototypes of other failed stage designs showed that small pieces made of aluminum were too frail to withstand the compression needed to hold them fast within the vice. Despite brass’s relative softness, many drill bits were still called upon to make the ultimate sacrifice in order to see the prototype to completion.

Upon completion the stage behaved as desired, providing for an adequate range of motion and sensitivity along each axis independently. With the prototype proven, detailed plans were drawn up with the dimensions that had been finalized during the construction of the prototype. Note that the screw on the y-stage, shown in the plans below, is not in the center. This is to accommodate the optical fiber and allow it unrestricted access to the oscillator. Due to my difficulty with machining the prototype of brass it was ruled that submitting a drawing to the machine shop would be most efficient. Submitting the drawings then allowed me more time to concentrate on the construction of the other components. The plans submitted are found in the appendix of this paper.

With the 20/20 hindsight provided by the mounting and use of the finalized model in the probe several shortcomings were identified. First and foremost is that although parts can be machined to better than five thousands of an inch, pressure fit pins do not share this level of precision. Since the clearance holes for the pins were made as small as possible to eliminate play in the movement, the pins must be as true as possible to provide for smooth translation. Also the spacing of the two pieces of the primary base is does not align reliably with the clearance holes in the runway. This means that once they are both secured with all four screw holes the rods are inevitably bent and the stage portion will not ride smoothly. To avoid warping only one side of the primary base should be screwed down. In order to provide full range of motion 1½ inch long 0-80 screws are needed. These screws as well as the non-magnetic beryllium copper springs are hard to come by. Eventually the screws were located at Austin Bolt Co. and the springs from Century Springs Inc.

2.1.2 Coil Mount

The RF coil must be held in position over the sample perpendicular to the static field. Because the Larmor frequency of hydrogen is so high, 343 MHz at the current field of 8.073 tesla, any change in the position of exposed wire, including the coil, will significantly change the impedance of the coil. An RJ-45 co-axial cable is used to reduce the noise of the RF signal transmitted from the BNC assembly at the top of
the probe to the end. The coil is soldered to one end of a piece of rigid stainless coaxial line. The other end of the rigid co-ax is soldered to the RJ-45. This protects the RF signal from noise until it reached the exposed coil itself.

An L-shaped holder, houses the rigid piece of co-ax to which the coil is attached. A clearance hole for the rigid co-ax is located at the top of the L-shaped coil mount, and a 0-80 tapped hole is drilled perpendicular to it to allow a small screw to hold the co-ax securely in place. In the foot of the mount a linear hole has been drilled wide enough to provide clearance for a 2-56 screw. The hole is elongated to provide some range of motion when positioning the coil. Since the 3-D translation stage has 2-56 tapped holes drilled clear through its base, and is secured to the runway via 2-56 screws shorter than the thickness of the base, there are already 2-56 tapped holes near the oscillator’s location readily available.

In keeping with the rest of the probe design the mount was to be made of stainless, but lack of time and the typical frustration of drilling small holes through a quarter inch of stainless on a piece less than an inch in any dimension proved too trying. Instead an identical mount was constructed from aluminum. A replacement mount made of stainless will soon follow.

2.1.3 PZT mount

After the oscillator is aligned with the fiber, the sample must then be aligned to the oscillator. To this end, a 2-D translation stage built by Nathan Adair is used with slight modification to allow screw mounting of a tubular piezo element (PZT). The PZT is then fitted with a one-dimensional friction stage as a coarse approach mechanism.

The PZT used is a Stavely tubular piezo made of EBL 2 material with the dimensions 1.00x.375x.02 in³. It has a single inner electrode covering the entire inner surface and four outer electrodes, each covering one quadrant of the tube around the circumference. In order to secure the PZT to the 2-D stage it is first epoxied to a macor plate. Macor is used to prevent shorting the outer and inner electrodes across the base. Since the PZT itself blocks access to the screw holes in the 2-D stage a hole is simply drilled in the center of the plate inside the PZT. An adapter plate made of stainless is then used to mate the macor plate to the three 2-56 tapped holes in the 2-D stage. The adaptor plate has a central hole to mate with the PZT macor plate, as well as three 2-56 countersunk clearance holes to permit fastening to the 2-D stage.

With the PZT in place the electrodes are then connected. First a wire was silver-epoxied to the inner electrode. The epoxy is then cured in a 50°C oven for 5 minutes. The wire is then looped around the screw used to secure the macor to the adaptor plate. Since the adaptor screw, adaptor plate, and stage are all made of stainless steel the wire leading from the screw to the PZT provides a connection to probe ground.

Once the epoxy had set a collar was fitted to the free end of the piezo to allow mounting of the friction stage to the PZT. The collar is a cylindrical piece of stainless with one end designed to be mated to the PZT. The other end has eight 0-80 tapped holes drilled around the perimeter at even spacing to allow mounting of further components.

To secure the collar silver epoxy was swabbed around the inner circumference of the PZT to a depth of about a quarter-inch. The collar was then cleaned with acetone and gently pushed into the PZT and rotated back and forth a few times to evenly spread the epoxy. Careful attention must be paid in this procedure as the epoxy has a tendency to get squeezed out of the piezo and ooze out around the edge of the PZT. This spilled epoxy invariably caused a short in the PZT across the inner and outer electrodes. Still, with a steady hand and some patience the collar was fitted and cured in the oven.

Once the work on the inside of the PZT was completed and secure, a short segment of wire was then silver epoxied to each of the outer quadrants, one at a time. Once one wire was securely cured to an outer quadrant the next could then be placed without fear of disturbing the positioning of the priorly completed connections. These wires are then connected, via twisting and electrical tape, to long wires running the length of the probe to the BNC connector assembly at the top.

2.2.4 Friction Stage
Because the PZT has a longitudinal range of motion of only around 1 µm at best a coarse approach mechanism is required to get the sample within detection range of the oscillator. It was decided that a friction stepper stage was the best approach.

The friction stage consists of a base and a pair of moving platforms. The base is a cylindrical plug, fitted to the inner dimension of the PZT collar, terminating with a disk having two 0-80 clearance holes aligned with the tapped holes in the PZT collar. Two $\frac{1}{16}$ inch snug clearance holes are drilled parallel to the axis of symmetry and slightly off center, 180° apart. Two stainless steel $\frac{1}{16}$ inch diameter pins were then silver epoxied into the holes. These pins provide rails upon which the paired platforms ride. The platforms themselves are flat oblong pieces of stainless with spherical pits drilled into one side in which $\frac{1}{16}$ inch diameter ball bearings are nestled and subsequently silver epoxied. There are three pairs of such holes in each piece: two pairs to hug one pin and the third pair to provide contact along one side of the second pin. Since the platform is not committed to following the second pin absolutely, only to touching it, there is a degree of play in the motion of the platform around the second pin. This compensates for any deviations in the pins from parallel. Up to this point in construction each platform is identical, but a final coup de grace differentiates the two. A 0-80 tapped hole is made in the center of one platform while an untapped #53 hole is made in the center of the other. Here, #53 is specified because a typical 0-80 clearance hole provides too much play in the relative motion of the two stages. A 0-80 screw is then threaded through one of the beryllium copper springs used for the translation stages, then the #53 clearance hole of one platform, and finally to the threaded hole of the other. The platforms are then placed onto the rails of the base and the screw tightened until the spring pushes the ball bearings onto the rails. Simply tightening or loosening the screw will change the frictional force between the fixed ball bearings.

The premise of operation of the friction stage relies on the coefficient of static friction being greater than that of dynamic friction. Provided the degree of friction is appropriate, the platform should move with the base as it is moved by the PZT. If however the base is moved suddenly, the inertia of the platform will be great enough to overcome the static friction and the platform will remain at its original position, despite the fact that the base has moved. In this way, the platform may be moved to the desired position slowly, and the base then retracted quickly to its original position without disturbing the platform. By repeating this process the platform can be moved in increments of the distance the PZT can jerk. This provides for a range of motion equal to the length of the rails since the effect of each jerk is cumulative. Once the platform is positioned to the desired location, the PZT can then be used for fine scale manipulation.

![Figure 9](image_url)
A long period saw-tooth wave is a perfect input signal for such motion. As the voltage to the PZT gradually increases the base and platform move in unison. At the end of the wave, the sudden drop in voltage causes the PZT to quickly return to its rest length while leaving the platform where it was. The platform’s direction of the motion may be reversed by simply reversing the sign of the saw-tooth wave.

Ultimately the friction stage was mounted to the PZT collar diagonally to avoid hitting the coil mount over its range of motion. The flexibility of putting extra holes in the collar had paid off. This is a valid point of all design that should be followed in any prototyping situation: always look to build flexibility into your device in how it is put together or operated, especially if it requires negligible effort.

2.1.5 Sample Mount

With the oscillator properly aligned with the optical fiber and the piezo, complete with coarse approach mechanism in place, mounted on the 2-D stage the time has come to mount the sample. As stated, hydrogen is the element of choice when looking to find a signal. In order to minimize uncertainty and difficulty from attempting to image an unknown system, such as a cell, a glass capillary full of pure water will be used as the first test sample. The glass capillary must have an inner diameter of at least 20 um to allow the possibility of an entire eukareotic cell, be it human or otherwise, to fit comfortably within its confines. The wall thickness should be as small as possible to allow the oscillator to approach the sample more closely. The closer the magnet on the oscillator to the sample, the higher the field gradient, and thus the higher the resolution possible within the cell. The walls must be thick enough, however, to withstand normal handling without breakage and the vacuum they will be submitted to within the probe. It is necessary for the sample to be vacuum safe in order to raise the Q of the oscillator and achieve higher resolutions.

Such capillaries were very difficult to find in any catalog and ultimately they had to be made in house. Typically the wall thickness of commercial capillaries far exceeded 20 um and were routinely coated with a polymer on the outside for protection. This leads to further difficulties involved with stripping the polymer away. Soon the idea dawned that since most other parts had been created in our own backyard of UT then perhaps the capillaries could be too. Far from precise, the methods of the glassblowers do work. A glass tube with the appropriate inner and outer diameter was heated in a hydrogen flame and simply pulled into a long strand. This long hollow fiber of glass is thickest at the ends and narrowest at the middle portion. All that is necessary is for one to excise a short portion from the appropriate location to get a capillary of the desired diameter. With a segment of glass capillary in hand all one must do is dip the end in water and rely on capillary action to draw the water in.

The dimensions of the capillary were then checked to ensure that they matched the dimensions of a single cell. To do this a slurry of cells was produced scraping the end of a wooden swab against the inside of an undergraduate’s cheek. The swab was then used to stir a drop of water on a microscope slide. The result was a large drop of water with a suspension of cells. To test the dimension of a capillary the cells were simply drawn into one and the filled capillary was then observed under a light microscope at 400x. The thick end was used first and found to have far too large an inner diameter, as multiple cells could be seen clumped together within the capillary.

After a short bout of trial and error it was found that the smallest portion that was made is about the right size. In other words, the lower limit of capillary size possible is very near the desired dimensions. Provided the solution of cells is dilute enough the capillary should draw in only one cell at a time and one is able to observe a single cell nestled within the glass capillary. Under the microscope the walls of the capillary were observed to be approximately of the same thickness as the inner diameter. Since the inner diameter snugly fit the cell’s diameter it was deemed to be of the appropriate dimensions, that is: about 20 µm, the diameter of a typical eukareotic cell.

After a capillary of the appropriate size was garnered it was flame sealed. In order to prevent the water in the sample from evaporating in the vacuum of the probe the ends had to be sealed. Although small, and expensive, end caps for capillaries are available it was found that simply heating the ends of the capillary in the flame of a torch works well. If there is any water at the end of the capillary then a pop will be heard as the water evaporates after which the end will seal itself under the surface tension of the liquefied glass. For the purpose of sealing water this evaporation of part of the sample in of no concern, but there exists fear of cooking the cell. In order to prevent this the capillary may be only partially filled
and the sample then moved to the center of the tube, by either blowing in the end or by gently flicking the tube, prior to sealing. Once sealed the ends were inspected under the optical microscope again to confirm a seal. Once satisfied that the ends were sealed, the capillary containing water was then put into a vacuum bell which was roughed down to 1 torr. The capillary was left in vacuum for 20 minutes without any adverse effects.

To hold the capillary containing the sample a pair of nickel-chrome wires were attached to the friction stage. To provide a large surface to epoxy the wire to the friction stage on side of each wire was put through rollers to flatten it. The other side was then deformed into a V-shape using needle nose pliers to provide a potential well for the capillary to rest in. Since the friction stage is oriented at a slant the wires are of uneven length to keep the capillary horizontal. With a bit of good old-fashioned craftsmanship the wires were formed into a shape and size that placed the capillary holding the sample near the oscillator. A small drop of Duco cement was then used to secure the capillary in place. The capillary may be easily removed by immersing the end of the sample holder in acetone to dissolve the Duco cement.

Combining this and all of the aforementioned components yields the entirety of the mechanics of the probe as seen below.

![Mechanics of the probe](image)

**Figure 10**

### 2.2 Electronics

Of course all of this is useless without the control circuitry for the piezos and data acquisition. Several circuits had to be constructed for the purposes of fringe stabilization, voltage amplification, and
Much of how the electronics within the boxes functioned was a mystery to me during design and construction, as former students designed much of the circuitry. Now with a greater understanding of the inner workings of the boxes I will attempt to elucidate the inner workings of these boxes.

2.2.1 Fringe-Lock

At a resolution better than a wavelength of light (here 680 nm), the fiber interferometer is easily susceptible to noise. To remedy this, a feedback loop using operational amplifiers (op-amps) is used to modulate the piezo coupled to the 1-D translation fiber holder and hold the fiber on a fringe. Such a box had already been designed and built during the original probe design and construction. Unfortunately, the feedback box was broken. Since the previous design had used high-voltage op-amps (150 volts) which were suspected as the cause of breakage, a low-voltage design was decided upon as a replacement. Such a box had already been designed for the use with the other NMRFM probe.

A clone of the box was constructed with the aid of a schematic furnished by Michelle Chabot and using the preexisting box as an example.

![Figure 11](image)

Here the P+ and P- are connected to a linear piezo used to move the fiber along its axis. The P- output is used to provide an offset to the piezo’s motion, allowing manual fine control of the position of the optical fiber once the coarse approach has been used. If a voltmeter is attached across the signal input a fringe may be confirmed by looking for a sinusoidal response with the manipulation of the offset knob. The first op-amp encountered in the circuit is fed a voltage from set point potentiometer, which is added by the op-amp to the signal from the interferometer. The offset of this signal allows one to set the point on the fringe that the circuit will start locking from. The second op-amp acts as an integrator circuit to make the
corrections by the first op-amp cumulative. The correction frequency of the feedback is set around 1 kHz. This allows for correction of any slow drifts while still allowing high frequency vibrations, such as those from the oscillator, to be observed.

The construction of this circuit is straightforward except for the 25 K potentiometer used for offset and current limiting on the second op-amp. It must be set, roughly, to its middle point otherwise the op-amp will become overloaded in seconds. If this happens the op-amp will become hot for a few seconds during overload, but afterwards will remain cool leaving little evidence of the failure.

2.2.2 High Voltage Amplifier

The PZT can accept an electric field of approximately 15 volts per mil (0.001”) with the polarized direction and 5 volts against it. Since the wall of the PZT is 0.02 inches thick (i.e. 20 mil) it accepts –100 to +300 volts on its outer electrodes. Since most control circuitry functions on a ±10 volt scale a high voltage amplifier was needed to amplify the voltage to the PZT and allow for full range of motion.

The high voltage amplifier is constructed from four identical inverting amplifier circuits. Since most op-amps found on the shelf are only able to take ±15 to ±18 volts special high voltage op-amps had to be found.

The PA42 operational amplifier from Apex Microtechnology was used in the construction of the amplifier. They are able to accept up to ±175 volts, which is less than the maximum voltage the piezo is able to accept, but suitable to our purposes. Applying the maximum allowable voltage is avoided to prevent any hysteresis effects, and the operating voltage is kept at around 30% of maximum, i.e. +100 V to –30 V. The amplifier circuit is a typical inverting amplifier design of the following form:

![Figure 12](image)

This circuit was constructed in quadruplicate on a single board and mounted in an aluminum project box. Initially, since only DC signals will be used no capacitors or current limiting connections were made. This did not result in a working circuit, however, and several additions to the theoretical inverting amplifier had to be made. The capacitors shorted from the power leads to ground are to reduce noise from the power supplies to the op-amps. Additionally capacitor $C_C$ and resistor $R_C$ are placed across pins 8 and 9 for phase compensation purposes. For the gain of 10 used here the values are 10 pF and 2.2 kΩ respectively [Apex]. The resistor $R_{CL}$ is then put across the output and the current limiting pin 7. Of course all capacitors are rated to the supply voltage or better.
From the outside two BNC receptacles on the smallest face of the box accept the positive and negative voltage supply. ABC Regulated Power Supply and Harrison 6516A DC Power Supply were used to supply +100V and –30V respectively. By providing limited supply voltage the maximum output voltage is also limited to the maximum acceptable voltage of the PZT. This allows for complete range of amplification with a ±10V control signal while preventing possible damage to the PZT and avoids accidental histeresis effects. Formerly both supplies were 6516A models but one became a casualty of research and had to be replaced by the ABC supply.

On each of the two long side faces are a row of four BNC receptacles, one for each channel. One side of the box accepts the input signal of ±10V from a control source and the other side outputs the voltage to the PZT of ±100V. Each input and corresponding output is located opposite one another.

### 2.2.3 Impedance Matching

In order to have as strong a resonance as possible in the sample the RF coil must be allowed copious amounts of power. In order to provide the coil with the maximum amount of power from the HP8656B signal generator it must be impedance matched to the rest of the line. Since the coil is bound to have a different impedance from the transmission line it is best to create some kind of tuning device so that the signal sees something at the end of the cable that is of equivalent impedance. If a capacitor is put into the circuit along with the coil then an oscillating LC circuit can be constructed. If the resonant frequency can be made to match the signal frequency then the back emf from the coil will always be in sync with the signal emf. The resonant frequency of an LC circuit is given by $\omega^2 = 1/\text{LC}$.

The coil has a inner diameter of 0.021" and 20 turns. This results in an inductance of approximately 1μH. Given that the $B_z$ field in the superconducting magnet is currently at 8.073 Tesla and hydrogen resonates at 42.577 MHz/T we may calculate that the RF frequency the coil will need to carry is 343 MHz. Solving for C we get 330 pF as the capacitance necessary to impedance match our circuit. One should recall that even small displacements of the wires leading from the rigid co-ax to the coil and wires within the capacitor array may change these values significantly.

By matching the impedance of the coil in this way the signal treats the whole assembly as having the typical impedance of the transmission line. Because finding a capacitor of exactly the right capacitance is just as difficult as making a coil with the right impedance a tunable assembly of capacitors is used. With one capacitor in series with the coil and one in parallel a sufficient accuracy and range of capacitance may be achieved. Typically the capacitor assembly would be placed adjacent to the coil at the end of the probe but space limitations prevent this.

With the capacitors outside the probe and connected to the coil by several meters of cable a few things must be taken into account. Primarily is the issue of time delay over the cable. Since the speed of a signal in the co-ax is roughly 60% the speed of light in vacuum the signal will travel approximately 0.87 meters over one period. Because this is comparable to the distance of cable separating the coil and capacitors one must ensure that they are separated by an integer number of half wavelengths to keep the circuit elements in phase with each other. This is achieved by adding appropriate lengths of cable between the probe and capacitor assembly. The final tank circuit is:
Also, the $Q$ of the LC circuit will be lowered with the capacitors so far away from the coil due to losses and noise in the transmission line. This means that less energy will be stored in the LC circuit from the signal. Thus, we cannot rely on the $Q$ of the electronic circuit to amplify the signal much, and more power input is necessary for a given response of the coil. Since we have ample power available and are interested in reading the small signals generated by the resonance of the nuclei via a mechanical oscillator, and not the coil as in conventional NMR, this lack of gain is of negligible importance.

2.2.4 Complete Setup

Once all the custom built circuits were completed they are connected to the probe along with a Stanford Research Systems 830 lock-in amplifier and a 4 channel analog out (DAQ) board purchased from National Instruments for use with LabView via a GPIB interface card. Each channel of the DAQ is connected to each of the amplification channels of the high-voltage inverting amplifier. The output from the amplifier is connected to the BNC ports at the top of the probe leading down to each of the PZT quadrants. The fringe locking circuit is then connected to the probe to the BNC ports attached to the linear piezo controlling the fine motion of the fiber stage. The signal from the interferometer is then spliced and fed into the input ports of the fringe locking box and the lock-in amplifier. Last, the output from the lock-in internal signal generator is routed through the impedance matching circuit and into the BNC port on the probe connected to the RF coil.
2.3 Programs

Although it is theoretically possible to take data without the aid of computer control, the sheer volume of data desired as well and the number of pieces of equipment to be controlled makes automated control an essential luxury. Automated data taking greatly expedited checking that items, such as the high voltage amplifier and friction stage, were working properly. Coordinating the many pieces of equipment and various parts of the probe itself was also greatly simplified.

2.3.1 Op-Amp Diagnostics

In order to confirm that the high voltage op-amp circuit was working properly input voltages ranging over ±10V were input and the output from the amplifier measured. This is simple for a few points and a single channel, but to ensure that the behavior of the amplifier was linear across the entire range of operation several points had to be taken. To ensure proper operation of all components, testing needed to be performed as efficiently as possible. A simple LabView program was used to output and record the output and resultant amplified voltage into a tab delimited spreadsheet file.

The input voltage is stepped from –10 V to +10 V in a number of increments set by the user. The program determines the voltage difference between steps by dividing the total range, 20 V, by the number of steps and subtracting 10, the offset from 0 to 20 V. The recording procedure is then put into a For-Next loop and the input voltage calculated by the iteration number multiplied by the step value. An additive constant of one is inserted where necessary to compensate for the lack of a 0th loop.

Using this program the gain of the high voltage amplifier was tested. Below can be seen the results before and after the addition of current limiting resistors in the circuit and capacitors across the power leads.
The slope of the fit line gives the ratio of amplification: -9.44 with an offset from zero of 0.017V. This is slightly off from the design value of 10x amplification, but is sufficient for operation.

2.3.2 Friction Stage Control

As in the case of the amplifier circuit, many data points had to be taken to confirm the accurate operation of the friction stage. A large number of points are desirable to ensure that the motion can be detected if the motion of a single step is below detection threshold and that the motion of the stage is consistent. As stated in the mechanical description of the friction stage, a saw-tooth waveform is best suited to drive the PZT coupled to the friction stage. Although the Stanford Research Systems 345 Signal
Generator is capable of generating saw-tooth waves of most any period it is somewhat difficult to generate a single wave pulse or to generate a negative amplitude wave pulse. Additionally, this signal would have to be spliced out to each of the four input channels on the high voltage amplifier. Since all four inputs are already connected to the DAQ, and are indeed more versatile, it was decided to generate the waveform in the LabView software. This has the added benefit of allowing flexibility to the waveform generation should the need occur in the future.

To generate the pulse a For-Next loop similar to the one implemented in the Op-Amp Diagnostics program is used without a negative offset. Again, the number of steps desired during ramp up, as well as the final peak amplitude, are controlled by the user in the LabView front end. This signal is then inverted so that the inverting voltage amplifier will restore the original sign and prevent confusion to the user. Once inverted the signal is then sent to each of the four channels of the DAQ and ultimately to the PZT. Once the loop is complete the voltage is suddenly cut to zero. This sudden change should move the base of the friction stage while leaving the platform where it was.

With a reflective sample placed in the friction stage sample mount the interferometer can be aligned to take a reading of the position of the platform. Once the saw-tooth pulse is completed the computer then reads the DC interferometer signal from the Keithly 199 Multimeter into which it is fed. This value is then recorded in a file. This sequence is enclosed in a For-Next loop as well and may be completed once or a number of times as specified by the user. Continuous operation may be realized by requesting one iteration and running the VI continuously.

### 2.3.3 Frequency Sweep

Before the oscillator may be used in NMRFM its resonant frequency must be found. The Q of these oscillators is high by design and therefore the resonance peak should be narrow. Given that the oscillators have a resonant frequency of around 10,000 Hz and a Q of 1,000 or more the bandwidth should be about 10 Hz. Since lithography techniques are not precise enough to specify the resonant frequency in the design to any more than a few kilohertz the oscillator must be driven and the response measured over a frequency spectrum. Since the peak is narrow a high-resolution scan is necessary, making data taking time consuming without automated assistance.

By taking the resonance data in air, the Q is lowered by viscous damping and the resonance peak broadened. This reduces somewhat the frequency resolution necessary on the initial scans of the oscillator. Once in vacuum, however, the resonant frequency tends to rise. This necessitates a second scan in vacuum at higher resolution, though the approximate frequency is now known to within a few hundred hertz.

This program interfaces with the lock-in amplifier in order to take data rather than the 199 Multimeter. The reference signal generated by the lock-in is fed into the probe and to a driving coil or piezo depending on the preferred method. The AC signal from the interferometer is then fed into the input of the lock-in. Once the user has input the start and end frequencies as well as the resolution of the scan the computer outputs each frequency in turn to the lock-in and takes the measurement of the oscillator response. It then saves the frequency and response to a tab delimited spreadsheet file with the frequency in the left column and response to the right.

The amplitude of the output signal, which drives the oscillator is variable from 0 to 5V peak to peak. Furthermore, there is a panel to set the sensitivity and length of time averaging as well as the display and output of each channel. For the purposes of the scan, R and θ, the amplitude and phase, are used for channels one and two respectively. The time taken between measurements was made variable in response to the lock-in not recording random data points in the scan. It was thought that this was due to a lag in communication between computer and lock-in and the time delay was implemented to allow the system to ‘catch up’. This delay is modulated by means of a knob on the front end. Sadly, this method has met with limited success and to date, and data points are still lost during scans. If the frequency resolution is high enough these lost points can be taken as acceptable losses, the quantity of which vary from scan to scan though are typically less than 5%. Alternatively, multiple scans can be used to complete the data set if necessary.
2.3.4 PZT Controller

In order to control the PZT an algorithm was developed that allowed the user to input a voltage for the x and y directions of motion while ensuring that the voltage never exceeded −50 V or +100 V. To ensure that proper voltages would be sent to the PZT, a prototype algorithm was developed and tested. Once proven, it was used to test the PZT and will be further incorporated into future imaging and control software.

Since the algorithm governing the x and y control voltages are identical the program consists of two identical algorithms, one for each direction. A horizontal and vertical slide control on the front end, with ranges of ±10 V, control the x and y directions respectively. The program must then take each input and split it into two channels and invert them to compensate for the inverting amplifier. The sign of one signal is then reversed with respect to the other. This channel now has two inversions which cancel, both of which are left out of the end program for sake of efficiency. The channel that will ultimately be of positive voltage, the one with the negative value in the program, is kept at its raw input value of between 0 and 10 and fed into one side of the PZT. The other channel, the one with the positive value in the program, will ultimately be negative and is thus scaled down by 50% its original value. Because the PZT expands with positive voltage, selecting a positive input in the front end should move the PZT right or left depending on the axis. Since the PZT bends away from the side expanding the inverted signal, i.e. the one that is ultimately output from the amplifier with the same sign as the front end input value, is sent to the bottom and left sides of the PZT.

Because determining the sign of a non-binary number involved complex comparisons and several conditional loops it was decided that a simple mathematical algorithm would be used to condition the signals. The simple equation \( \frac{1}{2}s + \frac{1}{4} \) is used where \( s \) is the sign of \( x \), −1, 0, +1, computed by a Boolean operation in LabView. \( x \) itself is the value input from the front end. Thus \( x \) is scaled by \( \frac{1}{2}s + \frac{1}{4} \) with values of:

<table>
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<td>( \frac{1}{2} )</td>
</tr>
<tr>
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</tbody>
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When \( s \) negative \( x \) is then scaled by a factor of \( \frac{1}{2} \) and if positive by unity. In the case that \( x \) is 0 the scale factor is unimportant since it is ultimately multiplied by \( x \), i.e. zero, anyway. This algorithm is used for both the inverted and non-inverted inputs from the front end for each axis for a total of 4 times. I feel this is a much simpler and easier to read program that would be possible using conditional loops.
3.1 Characterization

3.1.1 Oscillator Resonance

Once Troy Messina succeeded in vapor depositing a magnet onto an AFM cantilever I was ready to take a resonance measurement. The AFM cantilevers do not have the high Q of the double torsional oscillators but as it was readily available from past work I made do with what was available. Each chip of AFM tips contains several cantilevers, each of a different size and thus resonant frequency. On this particular chip only the largest possessed an attached magnet.

To take a measurement the oscillator first had to be aligned with the optical fiber. To accomplish this the cantilever assembly, which had already been silver epoxied to an aluminum substrate, was silver epoxied to a small piece of piezo crystal attached to the stage. The stage was then used to bring the largest cantilever into alignment with the fiber. Alignment was confirmed visually using an oblique microscope mounted on a movable arm by observing the specular reflection of the laser light from the fiber off the oscillator. Once the oscillator was aligned properly the micrometer screw controlling the coarse approach of the fiber was used to bring the fiber to within a few microns of the oscillator.

Once in place the P- voltage from the fringe locking box was changed using the offset knob. The response from the interferometer was monitored using a handheld voltmeter. After a bit of tweaking the voltage oscillated with an increase in applied offset voltage, confirming the existence of a fringe. With the fringe in sight the middle was found and the fringe locking box was set to automatic. After the locking mechanism is engaged the fringe is stable to within a few hundredths of a volt and measurements can begin. The error of the interferometer is surprisingly good. Below are shown two plots, one is of the fringe intensity over time. The second plot shows the effect of disturbance.

![Interferometer Noise](image.png)

Figure 17
Since the oscillator will ultimately be driven by the spin flipping of the magnetic moments in the sample it would be prudent to show that the oscillator could be driven using a magnetic field gradient. To accomplish this a rare earth magnet was placed in line with the oscillator to provide a static field. This static field aligned the moments in the cobalt dot and allowed a coil mounted with its axis of symmetry along the z-axis to drive the oscillator.

As luck would have it, nothing happened. No resonance was detected. This is curious since Troy Messina and Michelle Chabot were successful in driving the very same cantilever magnetically not more than a month previous. This failing may be due to an insufficient static field, or an improperly aligned coil, but I suspect that the cobalt dot has most likely been oxidized or damaged in use to the extent that it can no longer be polarized sufficiently to drive the cantilever. Anticipating somewhat this turn of events, the piezo the cantilever array was attached to was used to shake the array. To do this the signal that was routed to the coil was simply attached aluminum substrate and grounded to the 3-D stage. With the piezo used as an, albeit crude, driving mechanism data was successfully taken.

Using the Frequency Sweep program multiple runs of data were taken in air to find the resonance peak of the cantilever and the Q. Later sweeps were taken of the second largest cantilever in air with two performed in vacuum. From these data the Q of both oscillators may be calculated as given by:

\[ Q = \frac{\Delta \omega}{\omega} \text{ for } Q \gg 1 \]
where $\Delta \omega$ is the full width at half maximum of the resonance peak and $\omega_o$ is the frequency at the peak.

Figure 19

The Q of the large cantilevers is approximately 15 as calculated from the data plotted to the left. The substrate resonance has been plotted along with the large cantilever to confirm that the signal is indeed from the oscillator. The substrate does show a gentle hump around the resonant frequency of the oscillator but this is most likely a sympathetic vibration or light scattered off of the oscillator when pointing to the substrate. The y-axis shows the amplitude of oscillation as read by the lock-in amplifier from the interferometer setup.
Unfortunately a signal was never realized from the large oscillator under vacuum. This is probably due to damage to the oscillator, having been accidentally rammed by the optical fiber. The medium sized cantilever was, however, still measurable. The above shows the resonance in air and vacuum. The amplitude of the oscillator in vacuum should, by all accounts, be higher. As many difficulties were encountered while attempting to seal the probe and pump it down to 1 torr or better the alignment of the oscillator was probably disturbed during pumping. The probe has a severe leak at the vacuum-can, flange interface. This should be resolved with further breaking in of the seal with lead wire. This results in the y-axis being useless for an absolute measurement of amplitude but it may still function as a relative measure of amplitude. In order to calculate the Q, only a frequency spread is required, and this information may still be garnered from the plot. Here the Q of the cantilever in air is comparable to the large cantilever, 15. The vacuum value, however, is found to be significantly better at 86. It should be noted that, as is typical, the absence of damping due to air raises the resonant frequency as well as the Q.

3.1.2 PZT Motion

In order to use the PZT for scanning purposes it is first necessary to characterize its motion as a function of input voltage. Since one-dimensional imaging is the first goal the z-dimension is most important to characterize, especially since the z motion is used to control the friction stage. To this end a flattened piece of nickel-chrome wire was placed in the sample holder of the friction stage to provide a point for the interferometer to look at. Once aligned the existence of a fringe was established using the fringe locking box. Since the locking box will track a fringe making detection of motion impossible the box was shut off once the existence of a fringe was established. Alternatively, the P+ feedback from the box could be measured to confirm the motion of the PZT, but those data are more difficult to convert into a calibrated scale of linear motion due to the non-linear response curve of the piezo controlling the fringe [Graf].

The friction stage control program was slightly modified to take a data point after every sample in a pulse rather than after the completion of the pulse. The program was run which ramped the voltage to the PZT from –50V to +100V in increments of 0.1V. In typical fashion the data are taken by reading the DC signal voltage from the detector of the interferometer setup. This constitutes the entire operating range of
the PZT. These data allow the motion of the PZT as a function of input voltage, uniform to every quadrant, to be measured. The voltage output to the PZT is plotted along with the response below.

From these data 5 fringe peaks appear to be distinguishable. The decrease in amplitude is presumed to be from a progressive misalignment of the target reflector on the PZT. This indicates that the PZT moved about 5 fringes over the entire operating voltage. This corresponds to 11.33 nm/V.

![Figure 21](image1.png)

During the first data runs, a buzzing was detected from the PZT. Since all oscillations in the amplifier circuit had, presumably, been eliminated the source of the noise was sought out. Op-amp oscillations typically cause large feedback effects, not a small sinusoidal overtone. To the extent of my ability to determine pitch, the noise did not sound like white noise, but rather a tone or combination of tones. Connecting the output of the amplifier to the oscilloscope confirmed that a dominant, relatively pure frequency did exist. As the use of music majors with perfect pitch was ruled ‘unscientific’ (and perhaps inhumane) in determining the Fourier transform the output of the amplifier was connected to the lock-in and a frequency spectrum taken. Several long runs over the entire audible spectrum turned up nothing. Ascertaining that the frequency must not be broadband the resolution of the scan was increased to 1 Hz and taken from 10 Hz to 20,000 Hz overnight. The noise seems to be the ubiquitous line noise from the power company, though at 40 Hz with an amplitude of 6.7 mV. 60 Hz noise was also detected, albeit at a lower amplitude of 1.3mV, as well as other frequencies all at integer ratios of 60. A small peak of 0.2mV was found at 120 Hz, as well as a very small, but consistent peak of 0.14 mV at 50,000 - 50,010 Hz.

![Figure 22](image2.png)

In an attempt to localize the source of the noise both power supplies were plugged into the oscilloscope and lock-in, and manual scans performed. The 6516A output had a white noise with constant amplitude of 15 mV regardless of the set output voltage up to 10 V. The ABC Regulated Power Supply had a clean sine wave apparent on the oscilloscope with an amplitude of 5 mV And a frequency of 437.5 kHz as determined by averaging the period of several waves. When fed into the lock-in, no such signal was apparent. Neither power supply showed a peak around 60 Hz, though the 6516A showed a 0.1mV response from 150-250 Hz.
The audible noise from the PZT increased in direct proportion to the output voltage from the amplifier. Direct measurements were not taken above 10 V direct output in fear of overloading the lock-in. Having placed capacitors across the power leads to the op-amps a means of eliminating this noise remains unclear, though increasing the capacitance across the power leads is a possibility, though solution is doubtful as the manufacturer places rather specific values on such capacitors. Assuming the signal to noise ratio is constant over all voltages, the noise should never exceed 0.1 V. This equates to a vibrational amplitude of 0.07 nm. It seems doubtful that such a small displacement should be audible, so a few more data runs were taken to confirm the noise. No noise could be detected on subsequent data runs. Boggled, but pleased, the problem of the former noise was abandoned.

3.1.3 Friction Stage

In order for the friction stage to operate correctly in the vertical position the static force holding the platforms must be greater than the force of gravity. At the same time the response of the PZT must be fast enough to generate an inertial force greater than the static force. That is: \( ma > kd > mg \) must be satisfied, where \( m \) is the total mass of the platforms, \( k \) is the spring constant, \( d \) is the distance the spring is compressed by the screw, and \( g \) is the acceleration due to gravity. Clearly the acceleration of the PZT must be greater than the acceleration due to gravity. With this in mind the screw compressing the spring was hand tightened until the platform cleaved to the rails with sufficient tenacity to oppose gravity. Again, an interferometer reading was taken using a reflective metal target placed in the sample holder. Upon successful alignment of the target to the fiber using a steady hand and the oblique microscope the fringe locking box was used to establish the existence of a fringe. To take data the locking mechanism was shut off to provide ease of characterization of the motion and to avoid the possibility of the stage moving farther than the piezo could compensate for. The friction stage control program was then run briefly to establish that the mechanism was working. Surprisingly, after a 60 step sample an almost complete sine wave was seen. To characterize the stage the program was run for 600 cycles, generating 10 V pulses with a period of one second. The pulses were generated at a sample frequency of one point per 10 ms, or 100 Hz. After each pulse the data from the interferometer was recorded and the data then plotted as seen in the figure below. As the fringes cycled with the motion of the platform the interferometer readings were recorded and then tabulated.

![Figure 23](image-url)
The sharp peak at step 379 is most likely due to someone sneezing, possibly slamming a door, or bumping the table. Fortunately the fringe stabilized after a few transients back to its original position. Considering the data run took about 15 minutes the existence of one anomaly is surprisingly good.

With knowledge of the wavelength of light used each cycle in the plot may be treated as the platform moving one fringe, 340 nm. By visual inspection the platform is seen to have moved roughly 6.25 fringes. This totals to 2,125 nm, and with 600 steps that amounts to roughly 4 nm per step. Attempts to vary the distance traversed per pulse by lessen the friction or increase the amplitude of the pulse have at present, met with no conclusive success. Reversing the polarity of the pulse did however move the stage successfully, though whether the direction was truly reversed is unknown.

Assuming that the platform is unaffected in its position upon the completion of the pulse, it may be inferred that the PZT moves 1.4 nm/V. Since the total range of motion of the PZT in the z direction should be 350 nm as calculated in section 3.1.2 for the full 150V operating range the platform must not be slipping completely.

It may also be further deduced that the sensitivity of the interferometer setup is $2,720 \text{ nm/V}$ when locked on a fringe in the region of maximum slope. The DC noise level as determined at the peaks is 0.05 V, which yields an error of 136 nm. This corresponds to the measurements taken of the interferometer noise in section 3.1.1. This puts an upper limit on the DC sensitivity of 136 nm. This sensitivity may be further increased, in theory, by a better reflected signal, but this is the best DC sensitivity realized to date. AC signals may be measured with much higher accuracy with the lock-in since a narrow bandwidth of 3 Hz is used. Here noise levels of 10 nV are typical. This corresponds to an AC sensitivity of around $10^{-3}$ nm.
Chapter 4

Summary

4.1 Future Work

Once everything is properly aligned and a reliable signal to noise ratio is realized, i.e. oscillators with high enough Q and appropriate field gradients are successfully used, a 3D image of the diffusion characteristics inside a living cell will be taken at a 1 µm resolution. This should be enough to measure the diffusion of water in and out of the nucleus of the cell, an important precursor to tracking genetic information within the cell in relation to the nucleus. Also a higher resolution scan in 1D would be possible. With future improvements in field gradient and to the RF coil power resolutions of 0.1 µm should be attainable. At this resolution fine scale structure of a cell, better than that of light microscopy, will be available for 3-D imaging.

Work to image a cell is underway as of this writing. Although the high Q oscillators with magnets were not available for initial studies they have been fabricated and will be in use shortly. After data is successfully taken of a cell \textit{in vivo}, it may be used to generate a force map of the cell defining the strength of pulse echoes from various areas of the cell. These data must then be deconvoluted by an, at present, unwritten program.

4.2 Conclusion

Adaptation of the probe to use the small oscillators was successful. The capacitive drive was replaced with piezo and magnetic driving systems. Resonance data of multiple oscillators were taken in both air and vacuum with an observed increase in Q and resonant frequency. A sample mount capable of securely holding a liquid sample in vacuum was also successfully devised in concert with a coarse approach friction stage to bring the sample within range of the oscillators detection for the purposes of magnetic resonance imaging. Although the probe has been successfully converted and modified certain idiosyncrasies in the probe’s operation, such as the small vacuum leaks and questionable field gradients of the magnets, remain to be fixed in order for accurate magnetic resonance data to be taken. These issues as well as the imaging of a liquid sample using magnetic resonance imaging will be tackled this summer when more time is available.
Bibliography