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UMI
TOWARD MRI MICROIMAGING OF SINGLE BIOLOGICAL CELLS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

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* * * * *

The Ohio State University
2001

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ABSTRACT

There is a great advantage in signal to noise ratio (SNR) that can be obtained in nuclear magnetic resonance (NMR) on very small samples (having spatial dimensions ~100 μm or less) if one employs NMR "microcoils" that are of similarly small dimensions. These gains in SNR could enable magnetic resonance imaging (MRI) microscopy with spatial resolutions of ~1-2 μm, much better than currently available.

We report the design and testing of a NMR microcoil receiver apparatus, employing solenoidal microcoils of dimensions of tens to hundreds of microns, using an applied field of 9 Tesla (proton frequency 383 MHz). For the smallest receiver coils we attain sensitivity sufficient to observe proton NMR with SNR one in a single scan applied to ~10 μm³ (10 fl) water sample, containing 7x10¹¹ total proton spins. In addition to the NMR applications, microcoils have been applied to MRI producing images with spatial resolutions as low as 2 μm x 3.5 μm x 14.8 μm on phantom images of rods and beads. This resolution can be further improved.

MRI imaging of small sample volumes requires significant hardware modifications and improvements, all of which are discussed. Specifically, MRI microscopy requires very strong (>10 T/m). rapidly switchable triaxial magnetic field
gradients. We report the design and construction of such a triaxial gradient system, producing gradient substantially greater than 15 T/m in all three directions, x, y, and z (as high as 50 T/m for the x direction). The gradients are powered by a custom-designed power supply capable of providing currents in excess of 200 amps and switching times of less than 5 μs corresponding to slew rates of greater than 10^7 T/m/s. The gradients are adequately uniform (within 5% over a volume of 600 μm^3) and sufficient for microcoil MRI of small samples.
ACKNOWLEDGMENTS

I wish to acknowledge and thank all of those who have aided and encouraged me during my graduate career. First, I must thank Dr. Charles Pennington, my advisor, for the idea for my research project, attempting to image a single biological cell. Though as of yet not accomplished, together we have made great strides in increasing the resolution of MRI microscopy imaging.

Next, I wish to thank Dr. John Hoftiezer for his help in designing the PIN diode duplexer and especially his help in designing the Gradient Power Supply. His help in fixing all of my "mistakes" and timely arrival to "put out fires" was greatly appreciated and will be missed in the future. Without John's help, I would still be several years from graduating and the "death drawer" would be overflowing. I also wish to thank Dr. H. Douglas Morris from the NIH for his timely advice and encouragement. His insight into the realm of microcoils and his sharing of his knowledge was treasured.

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begun. Robert Cooper, a summer undergraduate student, was instrumental in acquiring some further signal to noise measurements also in Chapter 4.

I wish to thank Bob Wells for help in designing the MRI probes and coil-winding machine. Without his help, we would still be winding coils by “hand”. His insights into materials and their interactions were a resource to me throughout my graduate career. I also wish to thank Kent for putting together the MRI probes. I would like to thank the people in the physics machine shop for putting up with my many modifications and versions throughout the development phase of my experiment. I am especially thankful that I was not thrown out of the machine shop for asking for just one more “tweak”.

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Tapering the slot holding the micropipette allows the shortest possible lead length to the microcoil. Minimizing lead length is important to reduce the effects of stray inductance.

The tapered slot on an rf board is shown. The microcoil, too small to be seen, is tuned with a 0402 capacitor. The 50 Ω stripline connects the microcoil to the SMA connector.

Three different orientations of the gradient boards are shown above. The gradient boards are shown for completeness in discussing the microcoil placement and are discussed in Chapter 5.

The rf board slides in-between the two planes of the gradient board. Both the gradient board and rf board are mounted on the same acrylic mounting piece. The mounting piece is machined to ensure that the rf board is vertically centered in-between the gradient planes.

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CHAPTER 1

PROSPECTS FOR IMAGING SINGLE BIOLOGICAL CELLS

1.1. INTRODUCTION

Magnetic Resonance Imaging (MRI) creates pictures based on molecular concentrations of certain nuclei with various contrasting mechanisms. The MRI commonly used in today’s hospitals provides resolution of a few cubic millimeters, adequate resolution when imaging the human body. The imaging of microscopic cells with MRI will require micron resolution, a level not yet attained, but being approached. Optical microscopy, X-ray imaging, and the electron microscope permit finer resolutions than any current MRI and finer than magnetic resonance imaging can ever attain.
However, MRI does have certain advantages over other forms of imaging. The MRI process is non-invasive; samples do not require dye, slicing, or radiation. This allows the imaging of living organisms. By improving the MRI resolution by a factor of $10^{10}$, as compared to current resolutions in hospital MRI's, the exploration of the inner workings of cellular biology is possible without damaging the cell. The resolution of the image depends on many factors: the signal to noise ratio, the diffusion rate of the $^1$H atoms, magnetic susceptibility inhomogeneities in the cell and others to be discussed later. Ultimately, resolution will depend on the strength of the applied gradient and the sensitivity of the rf coils. These problems must be surmounted to attain cellular MRI images.

1.2. NUCLEAR MAGNETIC RESONANCE

MRI uses the Nuclear Magnetic Resonance (NMR) technique; therefore to understand MRI, a brief review of Nuclear Magnetic Resonance is presented. An excellent account of the NMR process, including the apparatus, has been written by Hoult and Richards. NMR is possible because certain atoms possess intrinsic angular momentum, $J$, and magnetic moment, $\mu = \gamma J$. By placing the nuclei in a magnetic field, the nuclei acquire a net magnetization in the same direction as the applied field when at equilibrium. The net magnetization precesses about the external static magnetic field, $H_0$. 

2
at a fixed angle. Quantum mechanically, for a nuclei with spin $\frac{1}{2}$, like hydrogen, the number of nuclei with spin $\frac{1}{2}$ is greater than the number of nuclei with spins $-\frac{1}{2}$ resulting in the net magnetization.

The equation of motion of the magnetic moment is found by equating the torque on the magnetic moment to the change in the angular momentum.

$$\frac{d\vec{\mu}}{dt} = \vec{\mu} \times (\gamma \vec{H}_o)$$  \hspace{1cm} (1.1)

where $\gamma$ is the gyromagnetic ratio, see Figure 1.1. The gyromagnetic ratio is the magnetic moment divided by the angular momentum, expressed in units of hertz per field. Quantum mechanically, the energy spectrum of the nucleus is composed of discrete energy levels. Excitation at the Larmor frequency, $\omega_0 = \gamma H_o$, produces NMR transitions by exciting the nuclei between energy levels, since the energy levels are separated by $\hbar \omega_0$. Only specific nuclei possess a gyromagnetic ratio but every gyromagnetic ratio is unique. NMR is atomic specific, allowing information to be collected on certain atoms ($^1$H, $^7$Li, $^{13}$C, $^{14}$N, $^{19}$F, $^{23}$Na, $^{31}$P, and others) related to biological samples. The frequencies at nine Tesla are 10 to 400 MHz and are in the radio band of the electromagnetic spectrum. A radio frequency (rf) pulse must be on resonance to induce transitions.

To observe the net magnetization, an additional rf. at the resonance frequency, magnetic field is applied, $H_1$, that is typically 10 to 100 Gauss, much smaller than the static magnetic field. The $H_1$ field is applied perpendicular to the static field and causes the net magnetization to rotate away from the $H_0$ axis. The applied perpendicular field is
Figure 1.1. The magnetic moment precesses about the effective magnetic field much like a top precesses in the Earth's gravitational field.

\[ \vec{H}_1 = 2H_1 \cos(\omega_0 t) \]  

The mathematics of the net magnetization vector is better visualized in a rotating coordinate system at the Larmor frequency. The applied perpendicular magnetic field can be broken into two components, one rotating clockwise and the other counter-clockwise.
It can be shown that the clockwise component will rotate at twice the resonance frequency in the rotating frame of reference and can be ignored leaving only the counterclockwise component. A rotating coordinate system, at the Larmor frequency, then eliminates the precession of the net magnetization about the static field, leaving the magnetization vector aligned with the static field. The rotating frame quantum mechanical Hamiltonian is a Zeeman Hamiltonian. For spin \( \frac{1}{2} \) system and a pulse of strength \( H_1 \) along the \( x \) direction at angular frequency \( \omega \), the Zeeman Hamiltonian is

\[
H_{\text{net}} = -\gamma \left( H_0 - \frac{\omega}{\gamma} \right) I_z - \gamma H_1 I_x.
\]  

(1.3)

The \( H_1 \) pulse rotates the net magnetization toward the \( +y \) axis where \( I_x \) and \( I_z \) are quantum mechanical operators. At the resonance frequency, the first term in Equation 1.3 is zero, leaving a simplified Hamiltonian in the rotating reference frame. The net magnetization is rotated an angle

\[
\theta = \gamma H_1 \tau_p.
\]  

(1.4)

by the \( H_1 \) rf pulse, where \( \tau_p \) is the duration of the pulse. After applying a pulse of \( \theta \) equal to 90°, the resulting NMR signal is called a Free Induction Decay (FID) and is the simplest NMR experiment. When \( \tau_p \) gives a 90-degree pulse, for a constant \( H_1 \), it is commonly referred to \( T_{20} \) and it is found experimentally by varying the length of the pulse and recording the amplitude until a maximum appears, as the NMR signal amplitude is proportional to the sin \( \theta \).
1.2.1. BLOCH EQUATIONS OF MOTION

After the rf pulse, the net magnetization decays back to equilibrium due to relaxation effects. The components of the magnetization vector, x, y, and z, decay at different rates. The characteristic time for relaxation along the longitudinal $H_0$ axis, is $T_1$, also called the spin-lattice relaxation time. $T_2$, also called the spin-spin relaxation time, contributes to the decay of the transverse magnetization. In liquids the relaxation time $T_1$ is greater than or equal to $T_2$. Classical equations of motion have been derived for the net magnetization vector by Felix Bloch$^1$, and are

$$\frac{dM_x}{dt} = \gamma (\vec{M} \times \vec{H}_{\text{total}}) - \frac{M}{T_1} \tag{1.5}$$

$$\frac{dM_y}{dt} = \gamma (\vec{M} \times \vec{H}_{\text{total}}) - \frac{M}{T_2} \tag{1.6}$$

$$\frac{dM_z}{dt} = \gamma (\vec{M} \times \vec{H}_{\text{total}}) + \frac{M_0 - M}{T_2} \tag{1.7}$$

where $M_0$ is the equilibrium value of the magnetization and $H_{\text{total}}$ is the off resonance component of the static magnetic field plus the excitation pulse. Unfortunately, in NMR the effective transverse relaxation time, $T_2^*$ is not equal to $T_2$ and usually $T_2^* < T_2$ and are approximately related to each other by

$$\frac{1}{T_2^*} = \frac{1}{T_2} + \gamma \Delta H_0 \ . \tag{1.8}$$
where the inhomogeneity in the static field, $\Delta H_0$, increases the decay rate. In typical NMR experiments, the rf excitation pulse, Equation 1.2, is applied through a solenoid coil and the emf from the decaying spins is measured as an induced voltage using the same coil. This process will be fully explained below.

In NMR, the Fourier conjugate variables are frequency and time. Fourier transforming the time domain spectrum yields information on the spectrum of the NMR signal. The spectrum information obtained yields chemical shifts, structural information and many other factors.

1.2.2. SPIN ECHO PULSE SEQUENCE

A pulse sequence known as a spin echo can be used to illustrate the changing orientation of the net magnetization vector after rf pulses. In equilibrium, the magnetization vector is along the z-axis, or $H_0$. An rf pulse of sufficient duration to rotate the vector by $\pi/2$ about the x-axis is applied at time $t = 0$, see Figure 1.2. In the rotating frame, the magnetization after the pulse is in the x-y plane and dephases due to local inhomogeneities, typically by $T_1$ effects in the static field. This causes some components to rotate faster or slower than the rotating frame. The first signal produced is called Free Induction Decay or FID. After a time $t$, a $\pi$ pulse is applied, flipping the
Equilibrium after $t/2$. Before $t$.

Figure 1.2. The pulse sequence for a spin echo is shown at the top of the figure. First, a $\pi/2$ pulse is applied and after a time $t$, the second pulse, a $\pi$ pulse, is applied. The echo forms at a time $2t$ from the first pulse. The positions of the magnetization vectors are then shown as the spin echo pulse sequence progresses.

magnetization vectors $180^\circ$ about the x-axis. The magnetization components rephase: the faster vectors catch the slower vectors forming an "echo", eliminating the effects of field inhomogeneities. The field inhomogeneities only sum to zero at the center of the echo.
time progresses after the echo the spins dephase again due to field inhomogeneities. An emf is produced as the magnetization rephases and the vectors realign at a time $2t$ as the changing magnetic field produces a proportional voltage in the rf coil. The signal is called a spin echo.

1.3. MAGNETIC RESONANCE IMAGING

Magnetic Resonance Imaging involves the application of magnetic field gradients in addition to the static and rf fields to, usually, measure hydrogen density. The gradient fields can be applied in two different ways: along the static field, $H_0$, or along the rf field, $H_1$. The second method, $H_1$ gradients, results in poorer resolution compared to $H_0$ gradients and will not be discussed further. Applying static field gradients to the $z$ component of $H_0$ in three dimensions ($G_x = dB_z/dx$, $G_y = dB_z/dy$, $G_z = dB_z/dz$) cause local variations in the Larmor frequency.

$$\omega(\vec{r}) = \gamma H_0 + \gamma \vec{G} \cdot \vec{r}.$$  \hspace{1cm} (1.9)

The spatial variations in frequency provide the basis for the spatial resolution in MRI. The gradient is, of course, a tensor, but since only its $z$ components affect the magnetization, the gradient can be written as a vector.

In MRI, the Fourier conjugate variables are reciprocal space, commonly referred as "k-space" and real space. Defining the reciprocal space vector as.
\[ \tilde{k} = \frac{1}{2\pi} \gamma \tilde{G} t \]

where \( G \) is the gradient magnitude and \( t \) is the duration of the gradient; k-space can be traversed by changing the gradient strength or by allowing the duration of the gradient pulse to vary. Changing the amplitude of the gradient is referred to as "spin-warp imaging". While varying the gradient duration will traverse k-space, sufficient care must be used to not introduce any imaging artifacts related to \( T_2 \). The total time after an rf excitation pulse must remain constant as the duration of the gradients are changed to avoid \( T_2 \) artifacts in the image. The acquisition of any signal must occur at exactly the same instance, independent of the time the gradients are applied.

Assuming the dephasing due to the gradient is larger than the dephasing due to \( T_2 \) effects, the spin-spin relaxation time can be ignored. Fourier transforming the time domain signal with a linear applied gradient results in a spectrum corresponding to the location of the spins. The amplitude at each frequency is proportional to the number of spins experiencing that total field. At various points in k-space, a signal is acquired, the resulting signal \( S(k) \) is the conjugate variable of \( \rho(r) \), the spin density:

\[
S(\tilde{k}) = \iiint \rho(\tilde{r}) \exp(i2\pi \tilde{k} \cdot \tilde{r}) d\tilde{r}
\]

\[
\rho(\tilde{r}) = \iiint S(\tilde{k}) \exp(-i2\pi \tilde{k} \cdot \tilde{r}) d\tilde{k}
\]

By acquiring sufficient number of points in k-space, a map of the spin density in real space can be obtained by application of a Fourier transform. In MRI, a full three dimensional image requires significant data acquisition memory and time. To eliminate
this problem, successive two-dimensional images are usually recorded by selectively
exciting a plane of spins in the sample to be imaged.

1.3.1. SELECTIVE EXCITATION OR SLICE SELECTION

In FID and spin echo pulse sequences the spins are pulsed with a “hard” pulse.
This pulse is rectangular in nature and usually 5 to 10 μs in length. The reason for the
short hard pulses is that they excite a large frequency range of the spins, accounting for
the spins inadvertently off resonance due to inhomogeneous $H_1$ and $H_0$ magnetic fields.
This relationship is easily seen in Fourier space: the Fourier transform of a rectangular
function ($0$ everywhere except between $t = 0$ and $τ_p$ with height $H_1$) is a sinc function,
where $\text{sinc}(x) = \frac{\sin(x)}{x}$. The width of the central peak of the sinc function is $2/τ_p$. The
shorter the duration of the hard pulse, the greater the range of frequencies excited.

Applying a gradient magnetic field causes the spins to spread out in frequency
space. A “soft” pulse excites just a slice of these spins, corresponding to a specific
location. By changing the slice of excited spins, a complete three-dimensional image of
two-dimensional images can be obtained. The method of obtaining slice selection is
apparent from the discussion of hard pulses. Excite the spins with a sinc pulse of
Figure 1.3. Using truncated sinc pulses results in rounding of the rectangular slices in frequency space. The relationship between the widths of the sinc pulse in time domain to the width in frequency space is evident.
relatively long duration, a few milliseconds, with an applied gradient during the pulse. If the time from the peak to the first zero of the sinc pulse is \( \tau_s \), then a region of \( \frac{4}{\tau_s} \) is excited in frequency space. This relationship is shown in Figure 1.3. In MRI it is impossible to excite the spins with an infinite durational pulse required for a complete sinc function. Usually the sinc pulse is terminated after an even number of zeroes, either four or six are commonly used. This inexact sinc pulse results in rounding of the rectangular excitation in frequency space but the effects do not significantly affect the selection of the desired spins. Methods are available to remove this affect.

The simplest method to excite a slice is to center the desired region at zero frequency relative to the oscillator. Then a sinc pulse can be applied which will excite the spins of width \( \frac{4}{\tau_s} \), centered at zero. Alternately, the sinc function can be convoluted with an oscillating cosine function that then will excite the spins centered at the oscillating frequency of the cosine function. To make the slice more selective two methods are available. The first lengthens the sinc pulse duration, and the second increases the gradient strength and further spread the NMR signal.

The sinc pulse has an unwanted effect on the NMR signal. Assuming the signal starts at the center of the sinc pulse, the signal accumulates a phase of \( e^{\frac{-\pi \mu_s}{2}} \). The accumulated phase attenuates the amplitude of the NMR signal and must be removed to maximize the signal. The first method to remove the extra phase involves reversing the field gradient after the sinc pulse for a time \( \tau_p/2 \). The accumulated phase after the gradient reversal is then zero. Alternatively, a hard 180° pulse can be applied.
Figure 1.4. An extra phase is added during slice selection that attenuates the NMR signal. To eliminate the extra phase two methods are available. The first method in part a, uses a gradient of the opposite magnitude to cancel the accumulated phase for half the time of the excitation pulse. In part b, the extra phase is removed with a π pulse and application of the same gradient of half the time.

immediately after the sinc pulse. This changes the accumulated phase during the sinc pulse to $e^{-j\frac{2\pi}{\tau}}$ as the effect of a hard 180° pulse changes $\overrightarrow{k} \rightarrow -\overrightarrow{k}$. The gradient is again applied of the same sign as applied during the sinc pulse for a time $\tau/2$ so that the total phase is zero. These two methods for zeroing the accumulated phase of the sinc pulse are
illustrated in Figure 1.4. Since the NMR spins are not a linear system, the refocusing gradients should be 52% of the time the gradient was applied during the selective excitation and of the opposite magnitude. Slice selection has one major drawback as compared to full three-dimensional imaging, the signal amplitude continues to decay by T₂ effects during the selective excitation, and this magnetization decay is irrecoverable. In Figures 1.5 and 1.6 are two of the major types of pulse sequences for imaging and their methods of transiting k-space. Both of these methods require slice selection to image in three-dimensions.

1.3.2. PROJECTION RECONSTRUCTION

Figure 1.5 shows a polar raster of k-space and is commonly referred to a Projection Reconstruction (PR). The 2D plots in k-space can be repeated to obtain a complete three-dimensional picture of the object by varying the slice selection in the z direction and repeating the 2D acquisition. In PR, one direction of the gradient is used for slice selection, while the remaining two gradients are turned on at the same time with different magnitudes. The read gradients, defined as being on during signal acquisition, transverse k-space in the radial direction as the sum of the squares of the two gradients.
Figure 1.5. The pulse sequence shown is the method for Projection Reconstruction. This method uses two read gradients and no phase encoding to acquire the image. This method has advantages and disadvantages to Fourier Imaging that are explained in the text. Projection Reconstruction (PR) acquires $k$-space in polar coordinates.
Selective Excitation

rf

- Z Slice Gradient
- Y Phase Gradient
- X Read Gradient

Acquire

Figure 1.6. Fourier imaging using the direct Fourier transform to reconstruct the image making this method the easiest to implement. Cartesian coordinates are used in acquiring k-space making direct Fourier transform possible.
By successively changing the relative magnitudes of the two read gradients, a polar raster in k-space is acquired. \( |G| = (G_r^2 + G_z^2)^{1/2} \) and \( \tan \phi = G_r/G_z \).

Upon completion, there are two methods to attain an image from the acquired data. The first method uses direct interpolation to convert the polar raster into a Cartesian raster and then a direct Fourier transform can be applied. However, this method leads to significant artifacts in the image. The second method is the projection reconstruction where the image is interpolated in real space where simple nearest-neighbor shift leads to fewer imaging artifacts. The real space image is obtained from a Fourier transform

\[
\rho(r) = \int S(k, \phi) e^{-2\pi i \cdot r \cdot k} dk
\]  

(1.13)

where \( S(k, \phi) \) is the signal acquired during the experiment.

For each position of the profile, \( \rho(r) \), is calculated, then for the Cartesian coordinate, \( r = (x \cos \phi + y \sin \phi) \), the nearest value of \( \rho(r) \) is assigned. The PR method is a back-projection and interpolation process encoding the image in a purely by frequency variations. One primary advantage of this method of image reconstruction is the real time display of the acquired images.
1.3.3. FOURIER IMAGING

Figure 1.6 is the Fourier Imaging method (FI), this method relies solely on the Cartesian coordinate system. One gradient is used for the slice selection, the second gradient, called the phase gradient, applies an accumulated phase modulation to the signal. The third gradient is on during signal acquisition and is referred to as the read gradient. The phase gradient is repeatedly changed, and the signal is acquired during each increment of the phase gradient with the read gradient on during acquisition. The FI image reconstruction uses both phase and frequency modulations to acquire the image. Fourier transforming processes the image.

\[ p(x, y) = \int \int S(k_x, k_y) e^{2\pi i (k_x x + k_y y)} dk_x dk_y \]  

The method has advantages and disadvantages. The reconstruction of the image is easily applied by direct Fourier transform of the acquired time domain signals. The disadvantage is the necessary delay in starting the acquisition, resulting in loss of signal amplitude due to \( T_2 \) effects.

Noise is added during reconstruction of the MRI image. The different rasters in Figures 1.5 and 1.6 determine noise differently. The polar raster has an increased signal to noise, explained in Section 1.5.1, over the Cartesian raster by approximately 10%. Weighting of the noise is different because of the size difference of the “boxes” in the two rasters. The Cartesian boxes are all identical, while the polar boxes vary with
distance from the origin. The smaller boxes in the Polar rasters near the origin cause the increase in SNR as the noise at the edge of the raster where the image has zero intensity is weighed less in reconstruction of the image. Of course, both PR and FI can be expanded to full three-dimensional acquisitions with the benefit of increased signal since there will be no decay due to T₂ during slice selection. Also, it is possible to attain 2D images with two phase gradients in Fourier Imaging with some additional signal processing.

1.3.4. ACQUIRING SIGNALS WITH ECHOES

The above pulse sequences only acquire the FID of the signal, and consequently the Fourier transform of the signal is handicapped. Digitizing back-to-back FID's or spin echoes can double the Fourier transform spectrum amplitude. Besides the advantage in the signal to noise in the frequency domain, acquiring echoes removes the dispersion curve (commonly called the imaginary) portion of the Fourier transformed signal. A symmetric time domain signal yields a real Fourier transform and has the added benefit of acquiring information from all four quadrants in k-space. The FID method only acquires information in the first and fourth quadrant of k-space.

Another advantage of using echoes to acquire images in k-space is that the gradients have a necessary finite rise and fall times and switching transients. The gradient takes a finite time to reach the steady linear gradient strength and by acquiring
echoes no significant signal is acquired at the beginning or end of the gradient pulses. When using echoes, the signal is small at the start and finish of the gradient pulses as the echo forms at the middle of the gradient pulse. This removes an additional source of artifacts and imaging errors. Echoes also have the benefit of insuring only the selected spins from the soft pulse or slice selection refocuses. Spins from outside the selective pulse do not refocus properly, and contribute no signal when echoes are used.

The dispersion curve in the frequency domain can have artifact affects on the acquired image. The elimination of the dispersion curve removes the phasing of the spectrum to calculate the real spin density. Figure 1.7 demonstrates a pulse sequence using echoes. As the spin density is ideally a real function, acquiring spin echoes results in a real symmetric signal.

For example, in two-dimensional MRI Fourier Imaging, the FID signal is, in the most basic form.

\[
S(t_1, t_2) = ke^{i\lambda_1} e^{\frac{i\omega_1}{\tau_1}} e^{\frac{i\omega_2}{\tau_2}}. \tag{1.15}
\]

where \( k \) is a constant depending on spin density. Fourier transforming Equation 1.15 with respect to \( t_1 \) and \( t_2 \).

\[
F(\omega) = \begin{pmatrix}
  i \\
  \frac{i}{(\omega - \Omega_1) + \frac{i}{T_1}} \\
  \frac{i}{(\omega - \Omega_2) + \frac{i}{T_2}}
\end{pmatrix}
\tag{1.16}
\]

which can of course be decomposed into a real absorption curve and imaginary dispersion curve for each individual transform. The problem with acquiring just the FID of the.
Figure 1.7. Acquiring the MRI image with spin echoes has advantages over two quadrant imaging, the
method used when acquiring FID's. The echoes remove gradient transients and remove imaginary
cross terms from the image. The figure demonstrates echoes in the read dimension only, but echoes
are possible in the indirect dimension as well. Full echo imaging can also increase the signal to noise
per scan.
signal is now apparent: the real Fourier transform has components that are actually imaginary in origin. These imaginary components are not indicative of the image.

However, the Fourier transform of an echo is real and symmetric. Now integrating Equation 1.15 from negative infinity to infinity for a spin echo versus zero to infinity for a FID

\[
F(\omega) = \left( \frac{2}{T_1} \right)^2 \left( \frac{2}{T_2} \right)^2 \frac{1}{(\omega - \Omega_1)^2 + \left( \frac{1}{T_1} \right)^2} \left( \frac{1}{(\omega - \Omega_2)^2 + \left( \frac{1}{T_2} \right)^2} \right) \quad (1.17)
\]

that is completely real with no cross terms leading to additional real components. Spin echoes yield a doubling of the signal to noise, allow full four quadrant k-space imaging, ignore rise and fall time effects of the gradients, and remove the problems of imaginary terms multiplying together to produce unwanted real terms. For these reasons, spin echoes are the preferred method of imaging. Of course, it is also possible to use the phase gradients to obtain echoes in the indirect dimension as well.

1.3.5. SIGNAL CONTRAST

There are two main contrasting methods available in MRI, T₁ and T₂. The longitudinal and traverse relaxation times provide natural contrasting mediums in
imaging. Since different portions of the cell have different relaxation times, a pulse sequence can be constructed to highlight the differences in relaxation times between the regions in the sample to be imaged. The magnetization in the transverse direction is

\[ M = M_0 \left( 1 - 2\exp\left( -\frac{T_R - T_E}{2T_1} \right) \right) + \exp\left( -\frac{T_R}{T_1} \right) \exp\left( -\frac{T_E}{T_2} \right) \]  

(1.18)

for a spin echo pulse sequence where \( T_E \) is the time of the echo and \( T_R \) is the repetition time for each pulse sequence. For a gradient echo, the magnetization is

\[ M = M_0 \left( 1 - \exp\left( -\frac{T_R}{T_1} \right) \right) \exp\left( -\frac{T_E}{T_2} \right) \]  

(1.19)

where a 90°-degree pulse forms a gradient echo, after a negative gradient is applied for a time \( \tau \) and then a positive gradient is applied for a time \( 2\tau \). This pulse sequence produces an echo at time \( 2\tau \) as the accumulated phase returns to zero. see Section 5.3.3.

Inspection of Equation 1.18 and 1.19 demonstrates by varying \( T_R \), the signal is weighted by the longitude relaxation time, \( T_1 \). However by varying \( T_E \) while holding \( T_R \) constant but greater than \( T_1 \), a map of \( T_2 \) values can be shown in the image.
1.4. CELLULAR COMPOSITION AND RESOLUTION REQUIREMENTS

Most MRI images are made from $^1$H atoms due to atom’s abundance in biological organisms, most notably water. Combining the abundance of hydrogen with the relatively large gyromagnetic ratio, $\gamma = 42.5$ MHz per Tesla, makes $^1$H MRI the best choice for microscopic imaging.

A typical animal cell ranges in size from 10 to 30 μm and plant cells from 10 to 100 μm. Cells are composed of many different structures. Figures 1.8 and 1.9 show the differences between plant and animal cell structures while Table 1.1 lists the relative dimensions of the structures. To achieve resolution inside a biological cell, Table 1.1 demonstrates resolution should be approximately one cubic micron. One-micron resolution will allow numerous voxels, a 3D pixel, across the cell’s dimensions. The size of the cell is $(m \Delta r)^3$ where $m$ is the number of points to be imaged in one dimension and $\Delta r$ is the voxel resolution.

The chemical composition of a plant or animal cell assists $^1$H MRI imaging. Ninety-nine percent of the weight of a cell is composed of Carbon, Hydrogen, Nitrogen, Oxygen, Phosphorous, and Sulfur all of which can be observed in at least one isotope with NMR. Table 1.2 lists the chemical composition of a typical cell. The high concentration of cellular water, 70% by weight, and thus hydrogen atoms, increases the number of atoms in each voxel and the signal per voxel.
Figure 1.8. A typical plant cell showing interior structure and relative dimensions.

<table>
<thead>
<tr>
<th>Component</th>
<th>Average Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Cell Size</td>
<td>1 to 100 ( \mu m )</td>
</tr>
<tr>
<td>Nucleus</td>
<td>3 to 10 ( \mu m )</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>1 to 10 ( \mu m )</td>
</tr>
<tr>
<td>Chloroplasts</td>
<td>2 ( \mu m ) by 5 ( \mu m )</td>
</tr>
<tr>
<td>Lysosomes</td>
<td>0.2 to 0.5 ( \mu m )</td>
</tr>
<tr>
<td>Peroxisomes</td>
<td>0.2 to 0.5 ( \mu m )</td>
</tr>
<tr>
<td>Cell Walls in Plants</td>
<td>0.1 to 10 ( \mu m )</td>
</tr>
<tr>
<td>Golgi Apparatus</td>
<td>&gt;50 nm</td>
</tr>
<tr>
<td>Ribosomes</td>
<td>30 nm</td>
</tr>
<tr>
<td>Cellular Membrane</td>
<td>4 to 5 nm thick</td>
</tr>
<tr>
<td>Nuclear Pores</td>
<td>1 nm</td>
</tr>
</tbody>
</table>

Table 1.1. Cellular Structure and average dimensions for components in a typical cell\(^2\).
Figure 1.9. A typical animal cell showing the interior structure and relative dimensions.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Percent of Weight</th>
<th>Number of Types of Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>70</td>
<td>1</td>
</tr>
<tr>
<td>Inorganic Ion</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Sugars</td>
<td>3</td>
<td>200</td>
</tr>
<tr>
<td>Amino Acids</td>
<td>0.4</td>
<td>100</td>
</tr>
<tr>
<td>Nucleotides</td>
<td>0.4</td>
<td>200</td>
</tr>
<tr>
<td>Lipids</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>Other Small Molecules</td>
<td>0.2</td>
<td>~200</td>
</tr>
<tr>
<td>Macromolecules</td>
<td>22</td>
<td>~5000</td>
</tr>
</tbody>
</table>

Table 1.2. Water is the most common molecule inside a cell composing approximately 70% of the weight in a typical cell. As a majority of the remaining molecules also contain Hydrogen, it makes Hydrogen the preferred atom to use in MRI images.  

27
Resolution in hospital MRI machines is on the order of one cubic millimeter. Current resolution limits are approaching the required one cubic micron limit with MRI images of large cells with low resolution already done\(^2^3\). Roffmann\(^2^4\) reports resolutions of 9.4x9.4x150 \(\mu\text{m}\). Schoeniger\(^2^5\) has achieved 20x20x100 \(\mu\text{m}\). Cho\(^2^6\) 15x15x100 \(\mu\text{m}\), and Bowtell\(^7^7\) has reported 4.5x4.5x100 \(\mu\text{m}\) and Morris\(^2^8\) (6\(\mu\text{m}\))^3. The preceding images were all done on plant cells except for Morris. Plant cells, especially plant stems, vary slowly in one dimension allowing for thick image slices due to uniformity of the plant, unlike animal cells. Current MRI microscopy requires large regions of the cell to be averaged for each voxel. True micro-cellular imaging has not yet been achieved in MRI.

1.5. RESOLUTION LIMITATIONS

Attaining the desired one-micron resolution in all three spatial dimensions requires advancement in imaging hardware as well as data processing. The obstacles are the signal to noise ratio (SNR) for each voxel, \(T_2\) effects, diffusion effects, gradient requirements, and magnetic susceptibility artifacts.
1.5.1. SIGNAL TO NOISE RATIO

The signal in NMR and MRI is generated by the spins relaxing in the magnetic field and producing an emf in the detection coil. In our experiments a solenoid coil is used for both excitation and detection of the nuclei. To calculate the emf induced in the coil, the first step is to find the flux $\Phi$, through the solenoid produced as the spins relax to equilibrium.

$$\Phi = \sum_{n,m} \int \tilde{B} \cdot d\tilde{s} = \sum_{n,m} \int \tilde{\nabla} \times \tilde{A} \cdot d\tilde{s} \quad (1.20)$$

where the integral is over the sample volume. Using Stoke's theorem and the expression for the magnetic potential $A$, Equation 1.20 can be rewritten as

$$\Phi = \sum_{n,m} m \frac{\mu_n}{4\pi} \int \frac{\tilde{r} \times d\tilde{l}}{|r|} \quad (1.21)$$

Inspection of Equation 1.21 reveals that the flux is equal to the magnetic field per unit current times the magnetic moment. $\Phi = m \frac{B}{i}$ of the coil, independent of the spins. This is the principle of reciprocity: the flux produced by a spin is equivalent to the field per unit current produced by the coil at the location of that one spin.

The emf in the solenoid is the time derivative of the flux and can be shown to be

$$\text{emf} = \omega_0 m \frac{\mu_0 L}{\sqrt{V_{coil}}} \quad (1.22)$$

where the substitution $\frac{B}{i} = \frac{\mu_0 L}{\sqrt{V_{coil}}}$ has been used; $L$ is the inductance of the solenoid.
The first obstacle in obtaining micro-cellular images is the signal to noise ratio (SNR). The signal from the $^1$H atoms must be greater than the background noise. The signal, the numerator in Equation 1.23, is the emf produced by the magnetic moments of the nuclei. The noise term is the denominator of the second part of Equation 1.23 and will be discussed below. Several authors\textsuperscript{20-22} derive expressions for SNR.

$$SNR = K\eta M_i \sqrt{\frac{\mu_n Q\omega_n V_c}{4kT_{re}} B} = \frac{KV_{sample} \omega_n M_i (\frac{H_1}{i})}{\sqrt{4kT_{re} BR}} .$$ \hspace{1cm} \text{ (1.23)}$$

where $\eta$ is the sample-filling factor equal to $V_{sample}/V_c$ and $V_c$ is the coil volume and $K$ is a scaling factor. $M_i$ is the magnetization of the spins, $B$ is the bandwidth of the filter, $k$ is Boltzman's constant, and $T_{re}$ is the effective coil temperature. $Q = \omega L/R = \Delta \omega / \omega$ is the quality factor of the rf coil where $L$ is the inductance, $R$ the resistance of the coil, and $\Delta \omega$ is the frequency spread at resonance. The nuclear magnetic moment, $m$, is given by the Curie law:\textsuperscript{24}$

$$m = \eta M_i V_c = \frac{N\gamma^2 h^2 l (I + 1) H_n}{3kT_{sample}} .$$ \hspace{1cm} \text{ (1.24)}$$

where $N$ in the number of spins and $I$ is the spin of the nucleus, $\frac{1}{2}$ for Hydrogen.

The current per unit field, $H_1$, is largest for a solenoid coil and more efficient than saddle-shaped\textsuperscript{25}, birdcage\textsuperscript{24} or planar\textsuperscript{25} rf pickup coils. The solenoid field per unit current\textsuperscript{26} is

$$\frac{H_1}{i} = \frac{\mu_0 n}{d_{coil}} \sqrt{1 + \left(\frac{h}{d_{coil}}\right)^2} = \frac{\mu_0 L}{\sqrt{V_c}} .$$ \hspace{1cm} \text{ (1.25)}$$
where \( n \) is the number of turns, \( d_{\text{coil}} \) the coil diameter, and \( h \) is the height of the coil. The field per unit current represents the coupling between the spins and the rf pickup coil. The SNR can now be expressed as

\[
SNR = \frac{1}{4kT_{\text{amp}}} \frac{K \omega_0^2 N \gamma h^2}{\sqrt{4kT_{\text{eff}}}} \left( \frac{\mu_0 \mu}{d_{\text{coil}} \sqrt{1 + \left( \frac{h}{d_{\text{coil}}} \right)^2}} \right).
\]  

(1.26)

The dependence of the SNR to \( \gamma \). \( \omega_0 = \gamma H_0 \), makes \(^1\)H imaging, with hydrogen's large \( \gamma \) and cellular concentrations, the nuclei of choice in MRI.

The SNR formula conveyed in Equations 1.23 and 1.26 can be difficult to use. Shifting one parameter often has effects on other variables, so quadrupling the coil volume while keeping the filling factor constant may not double the SNR. The variables \( Q \) and \( K \) might also vary. The SNR is only valid for order of magnitude estimations. Table 1.3 demonstrates how the variables are coupled

Table 1.3 demonstrates how the variables are coupled

reducing the voxel size from one cubic millimeter resolution in hospital MRI machines to the required micro-cellular resolution will result in a signal loss of \( 10^9 \). The volume to be imaged has been reduced by a factor of one billion.

The optimal SNR, assuming the number of \(^1\)H atoms in one cubic micron is \( 7 \times 10^{10} \), a 50 \( \mu \)m coil, temperature of 300 K, an amplifier noise temperature of 70 K and a static field of 9 T is

\[
SNR = \frac{\pi}{8} \frac{\omega_0^2 T_{\text{amp}} N \gamma h^2}{kT_{\text{amp}}} \sqrt{\frac{\mu_0}{kT_{\text{eff}} V_c}} = 2.5
\]  

(1.27)
Table 1.3. The variables in the signal to noise ratio are inter-related and cannot be changed arbitrarily without effecting other variables. The relative coupling between variables is shown in the table.

With a SNR of 2.5, the spins from one cubic voxel can be observed, ideally. Equation 1.27 required making the resistance an adjustable parameter to increase the Q of the coil and that is not experimentally practical.

1.5.2. RECOVERING SIGNAL TO NOISE

To overcome the immense loss of signal, several things can be done. The first method to recover SNR is reducing the coil diameter from a meter in hospital MRI to 50 microns to yield an increase in SNR. A $10^4$ increase in SNR can be achieved with rf coil diameters of reduced sizes. This reduction comes from the inverse relationship of the
coil diameter to the SNR. Rogers offers a new method for producing microcoils that may eventually reduce the size of the coils further using micro-contact printing.

Increasing the static magnetic field results in additional accretion of SNR. The dependence of the SNR on the field squared gives rise to an addition factor of $10^3$, by raising the field from a few tenths of a Tesla to ten Tesla. The remaining factor of $10^2$ lost in the SNR will have to be recovered by a reduction in the noise, signal averaging and pulse sequence improvements.

1.5.3. SOURCES OF NOISE

There are many sources of noise in an rf circuit. Johnson or thermal noise, amplifier noise, and sample noise are all potential noise sources. Johnson noise is caused by random motions of electrons in the circuit resulting in a noise voltage of

$$V_{jn} = \sqrt{4kTRB}.$$  \hspace{1cm} (1.28)

The amplifier noise is calculated by replacing the temperature term in the Johnson noise with an effective temperature, a combination of the circuit temperature and the noise temperature of the amplifier. Good power amplifiers have noise temperatures around 70 K, less than room temperature of 300 K, and may be cooled to further reduce the noise temperature of the amplifier.
1.5.3.1. CAPACITIVE SAMPLE NOISE

The sample in the rf coil creates an effective resistance in the circuit from capacitive and inductive effects. The capacitive losses arise from the resistance in the rf coil creating potential differences across the sample. The capacitance is greatest between the individual wires in the coil but the stray electric fields produce an effective capacitance across the sample. The dielectric power loss due to the sample is proportional to the self-capacitance of the coil. Comparing the power stored in the self-capacitance of the coil with the power stored in the tuning capacitor, see Section 3.3.2, in the resonance circuit, results in the proportion.

\[
\frac{P_{\text{self}}}{P_{\text{tuning}}} \propto \frac{C_{\text{self}}}{C_{\text{tuning}}}. \quad (1.29)
\]

The self-capacitance scales with the diameter of the coil and is approximately 2 pF per centimeter of the diameter. The tuning capacitor is typically a few tens of picofarads and consequently for a 50-micron diameter coil, the stored power in the self-capacitance is negligible. The negligible power loss can be equated to an effective resistance through \( P = I^2 R_{\text{cap}} \). The noise due to the capacitive resistance, \( R_{\text{cap}} \), will be unimportant compared to the Johnson noise of the rf coil.
1.5.3.2. INDUCTIVE SAMPLE NOISE

The inductive resistance\(^\text{11,26,48}\) is determined by the sample’s dimensions. The effective resistance due to inductive effects and can be calculated by equating the power loss in the sample with \(P = I^2 R_{\text{ind}}\). The effective resistance due to the \(H_1\) field on a cylindrical sample of radius \(b\) and length \(l\) is

\[
R_{\text{ind}} = \frac{\pi \omega \mu \mu_0 n l b^4 l \sigma}{16 d_{\text{coil}}^2 \left(1 + \left(\frac{h}{d_{\text{coil}}}\right)^2\right)}.
\]  

The conductivity of biological cells\(^\text{11}\) is about 1.2 (ohm-meters)\(^{-1}\). The inductive resistance varies with the fifth power of its dimensions making the inductive resistance negligible. In traditional MRI machines the sample noise overwhelms the thermal noise since the dimensions of the sample are larger. In MRI microscopy, the Johnson noise will dominate. The rf coil’s resistance of a few hundred milliohms overwhelms the effective resistance due to capacitive and inductive effects.
1.5.3.3. AC RESISTANCE AND JOHNSON NOISE

The noise due to the resistance in the rf coil is dependent on DC and AC effects and must be minimized to increase the SNR. The DC resistance is the resistivity of the wire times the ratio of the length of the wire to the cross sectional area. AC effects increase the resistance of the coil due to the skin depth effect and the proximity effect. The skin depth is

$$\delta = \sqrt{\frac{2}{\mu \omega \sigma}},$$  \hspace{1cm} (1.31)

where $\mu$ is the permeability, $\omega$ is the frequency and $\sigma$ is the conductivity. The skin depth effect reduces the effective cross sectional area since the rf field can not penetrate the entire wire, effectively restricting the current to flow in a cylindrical shell of width $\delta$. The proximity effect is an additional factor of interference between the AC field and individual wires. The induced eddy currents flow in wedges of the cross section of the wire, out on the edge and in at the center to oppose the changes in the AC field. Eddy currents are closed loops and do not change the total current, but restrict the effective area, see Figure 1.10.
Figure 1.10. The fields from neighboring wires in a solenoid coil creates eddy currents that further restrict the effective cross sectional area of the wire beyond the skin depth effect. The fields cause eddy currents to flow in at the center of the wire and out along the outer edge of the wire restricting the effective area.

The total effective resistance can be modeled\textsuperscript{13,54-55} by

\[ R_{\text{r}} = R_d \left[ F + \eta \sigma G \left( \frac{d}{s} \right)^2 \right]. \]  

(1.32)

where \( F \) is the skin depth effect, \( \eta \sigma \) is a geometrical factor dependent on coil dimensions. \( G \) is the proximity effect, \( d \) is the wire diameter and \( s \) is the spacing between wire centers.

Figure 1.11 show how \( F \) and \( G \) scale with wire diameter and the frequency of the rf pulse.
presented as a look-up table in Butterworth's papers. The $u_{\text{eff}}$ is also presented as a table. Butterworth does an excellent job in explaining proximity effects and calculating effective AC resistance of single layer and multilayer rf coils.

Wire spacing and coil dimensions effect the SNR through variations in the resistance of the coil. The optimal wire spacing is predicted to have a broad maximum at $d/s = 0.6$. The SNR attains maximum value when the wire diameter is approximately the skin depth of the copper wire, $\delta = 3.3 \mu m$ at 400 MHz. Unfortunately the minimum wire
diameter available commercially is 5 \mu m in diameter and minimum working diameters is approximately 10 \mu m. Figure 1.12 shows the calculated AC resistance for five turns copper coils with different wire diameters.

Multilayer coils increase the turns per length and consequently the field per current term in the SNR. The tradeoff arises in the proximity effects increasing the total resistance of the coil. If smaller wire diameters or other processes become available, the SNR can be increased. The criterion is that the number of layers times the diameter of the wire must be less than the skin depth. The increase in the inductance from multilayer coils will also aid with tuning the resonance circuit and increase the effective filling factor of the coil.

1.5.3.4. LIMITING CASES FOR SIGNAL TO NOISE

The resistance of the coil modeled in Equation 1.32 has limiting values for small and large values of \( z = d/\delta \) for a solenoid coil with spacing between wire centers at two diameters. In the skin depth regime of \( z \) greater than eight the

\[
\text{SNR} \propto \frac{\omega_0^{1/4}}{d_{\text{coil}}}.
\]  

(1.33)

For small values of \( z \), less than two, the current distribution becomes uniform and the
Figure 1.12. The calculated AC resistance is shown above for various wire diameters for a five turn coil, keeping the spacing between adjacent turns equal to two wire diameters. For small wires, the resistance increases dramatically as the skin depth is approached.

The limiting equations illustrate methods to improve the SNR but most microcoils do not fall within either range of $z$ for validity. The smallest wire commercially available is at the upper limit of the small $z$ regime. The optimal coil will be as small as possible wrapped with the smallest wire available and optimal wire spacing with the fewest numbers of turns to cover the sample. Any decrease in the resistance must be balanced to ensure the inductance is large enough to allow tuning of the resonance circuit.

\[ SNR \approx \frac{\omega_0^2}{\sqrt{nd_{cm}}} \]  

(1.34)
Engineering an rf coil with a 50 μm diameter can be accomplished by using a micropipette as a coil form, see Chapter 3. The pipette will aid in positioning the cell in the coil through capillary action.

1.5.3.5. OTHER METHODS FOR INCREASING SIGNAL TO NOISE

Reducing circuit resistance and the eliminating stray inductance from wires leads to the use of circuit boards. By using circuit boards, the lengths are minimized between capacitors and the inductors in the resonance circuit and will improve the SNR. The signal to noise ratio can be further improved by experimental repetition, over the one scan SNR, by adding the experiments together. The overall signal to noise grows as the square root of the number of repetitions.

The use of superconducting rf coils would decrease the temperature value in the Johnson noise and increase the SNR. However, the large static magnetic fields involved in NMR and the necessity of keeping the biological sample above the freezing point makes this method unworkable. McFarland has another solution to reducing the Johnson noise. The circuit and the sample are kept at two different temperatures. Improving the SNR can be achieved by reducing the temperature of the circuit while keeping the cell above freezing. Nitrogen gas is passed at 300 K between the rf coil and the sample, keeping the sample above freezing. A SNR improvement of 10 has been observed with
this method, but engineering difficulties remain. The nitrogen gas has a tendency to vibrate the sample and reduce resolution.

1.5.4. T₂ LIMITED RESOLUTION

Each voxel in the image has a frequency width, corresponding to the change of the Larmor frequency across the voxel. The Full Width at Half Max (FWHM) of the signal must not be larger than the frequency width of the voxel or the Rayleigh criteria must not be violated to resolve individual voxels. The frequency bandwidth over a voxel is

\[ \Delta f_{\text{voxel}} = \gamma G \Delta r \]  

(1.35)

and varies with the strength of the gradient. The bandwidth of the signal is dependent on the transverse relaxation time.

\[ BW = \frac{1}{\pi T_{\text{eff}}} \]  

(1.36)

Combining Equations 1.35 and 1.36 results in a condition for frequency encoding.

\[ \Delta r = \frac{2}{\gamma GT_{\text{eff}}} \]  

(1.37)

\( T_{\text{eff}} \) is the effective T₂ time with the gradients applied, typically much smaller than T₂. As Equation 1.37 is derived based on frequency encoding, it is only applicable to the read-out direction of Fourier Imaging or Projection Reconstruction.
\[ T_2 \text{ is approximately } \approx 100 \text{ ms for intracellular water giving a linewidth of } 3 \text{ Hz. If} \]

\[ T_2 \text{ is too short the signal FWHM is wider than the frequency bandwidth of the voxel. As} \]

\[ T_2 \text{ increases, the peaks narrow and resolution increases as the peaks in Fourier space} \]

\[ \text{become smaller than the voxel size. The real limiting factor is the magnitude of } T_2 \text{ that is} \]

\[ \text{inherent on the sample and cannot be changed: this is called } T_2 \text{ limited resolution. } T_2 \]

\[ \text{limited resolution also limits the duration of the MRI experiment: all phase encoding and} \]

\[ \text{slice selection must be completed before the NMR signal has decayed to zero. Using the} \]

\[ \text{optimal value of the bandwidth makes the SNR, see Equation 1.27, proportional to } T_2. \]

\[ \text{The additional factor of } \sqrt{T^2} \text{ comes from the quality factor in the coil. Longer } T_2 \text{ times} \]

\[ \text{increase the SNR, but unfortunately } T_2 \text{ is not an adjustable parameter.} \]

1.5.5. BANDWIDTH LIMITED RESOLUTION

FI MRI imaging requires phase encoding to distinguish two different regions in space. The phase difference between two different regions in the sample must be distinguishable. The phase difference between two voxels in the image with the gradient applied during \( T_{\text{acq}} \) is.

\[ \Delta \phi = \gamma G\Delta r T_{\text{acq}} \quad \text{(1.38)} \]
where $\gamma$ is now in radians per second. The minimum detectable phase varies with reconstruction algorithms\textsuperscript{19,20,25} and for Fourier transform reconstruction the minimum detectable phase is $\pi$. The bandwidth-limited resolution is

$$\Delta r = \frac{\pi}{\gamma G T_{\text{acq}}}.$$  \hspace{1cm} (1.39)

where the acquisition time is the time during which the gradients are applied. Equation 1.39 brings the first requirement on the strength of the gradient and more specifically on the product of $GT_{\text{acq}}$.

1.5.6. DIFFUSION LIMITED RESOLUTION

In all liquids, molecules move randomly through the sample volume. Random wandering of the atoms during signal acquisition can decrease the resolution of the image. As the atoms diffuse through the sample, the magnetic field variations due to the applied gradients cause precession at differing frequencies. Signal broadening is the result of differing precession rates. The effect can be seen through a simple derivation by Carr and Purcell\textsuperscript{15}. Consider a 1D (z axis) model of diffusion. If the nucleus undergoes one random jump of length $\zeta$ in a time $\tau$, the field the nucleus would see after $j$ jumps is

$$H_z(j\tau) = H_z(0) + G\zeta \sum_{i=1}^{j} a_i.$$ \hspace{1cm} (1.40)
where $a_i$ is a random variable equal to $\pm 1$. The accumulated phase difference after a time $t = N\tau$ compared to a stationary nucleus is

$$
\phi_n = \phi - \phi_0 = \gamma \tau \sum \left[ H_z(j\tau) - H_z(0) \right].
$$

(1.41)

This sum can be simplified.

$$
\phi_n = G\zeta\gamma \sum \sum a_i = G\zeta\gamma \sum (N+1-j)a_i = G\zeta\gamma \sum ja_i.
$$

(1.42)

where the last simplification was made because $a_i$ is a random variable. The rms deviation of the accumulated phase is found by summing the series.

$$
\langle \phi_n^2 \rangle = \frac{G^2\zeta^2\gamma^2\tau^2N(N+1)(2N+1)}{6}.
$$

(1.43)

In the large $N$ limit and using the Einstein-Smoluchowski relation for the bulk diffusion constant, $D = \zeta^2/2\tau$, rms phase becomes

$$
\langle \phi_n^2 \rangle = \frac{2G^2\zeta^2Dt}{3}.
$$

(1.44)

where the time in Equation 1.44 is only during signal acquisition and not the total time.

Typical values of the bulk diffusion constant for free water are approximately $2.2\times10^{-5}$ cm$^2$/s. The diffusion for intracellular water may be about 70% of free bulk diffusion constant.
1.5.6.1. BROADENING DUE TO DIFFUSION

The accumulated phase difference serves to broaden and attenuate the signal. The ensemble-averaged phase modulation is \( \exp(i\phi) = \exp\left(\frac{\langle \phi^2 \rangle}{2}\right) \) resulting in an attenuation of the signal by diffusion of

\[
M = M_0 \exp\left[ -\frac{G^2 \gamma^2 DT}{3} \right].
\] (1.45)

The attenuation causes broadening of the signal by

\[
\Delta f_D = 0.6 \left( \frac{\gamma G^2 D}{3} \right)^{1/4}.
\] (1.46)

where \( \Delta f_D \) is the FWHM of the signal. This linewidth is inherent independently of the acquisition time. The broadening due to diffusion limits the resolution of individual voxels,

\[
\Delta r_D = 0.6 \left( \frac{D}{3\gamma G} \right)^{1/4}.
\] (1.47)

Increasing the strength of the gradient improves the resolution limit imposed by diffusion effects.

The diffusion resolution can be calculated by another method. The bandwidth through one voxel must be larger than rms movement during the signal acquisition, providing a limit on voxel width of
Equations 1.47 and 1.48 are equivalent to within a factor of two. Comparing Equations 1.39 and 1.48 results in a conflict on the dependence of acquisition time. To satisfy both equations simultaneously, \( T_{\text{aq}} \) will have to be small and the gradient large. A relationship between \( T_{\text{aq}} \) and the strength of the gradient can be derived.

\[
\Delta r_p = \sqrt{\frac{2DT_{\text{aq}}}{3}}.
\]  \hspace{1cm} (1.48)

The diffusive resolution limit requires an acquisition time of one millisecond and the required gradient to attain one-micron resolution is 11.6 T/m. Diffusion effects can also be used to increase image contrast across an impermeable boundary\(^{24,25}\). The different diffusion rates enhance the boundary and can be used as a contrasting mechanism.

1.5.6.2. REDUCING DIFFUSION EFFECTS WITH SPIN ECHOES

During a spin echo pulse sequence, see Figure 1.2, two times of length \( \tau \) occur. The attenuation of the signal after a spin echo due to \( T_2 \) and diffusion effects is

\[
M(2\tau) = M_0 \exp \left[ -\frac{2\gamma^2 G^2 \tau^3}{3} \right] \exp \left[ -\frac{2\tau}{T_2} \right] = M_0 \alpha. \hspace{1cm} (1.50)
\]
after combining the attenuation through each time interval $\tau$. $M_0$ is the equilibrium magnetization and $\alpha$ the attenuation factor. The magnetization at $t = 2\tau$ is identical to the magnetization at $t = 0$, expect the signal has been attenuated by a factor $\alpha$.

The Carr-Purcell' echo sequence adds additional $\pi$ pulses every odd multiple of $\tau$ to the end of the spin echo sequence, see Figure 1.13. Using this sequence to only acquire the center of the echo makes this sequence even more advantageous. This echo sequence diminishes the effect of diffusion on the attenuation of the signal. The attenuation of the magnetization after $n$ echoes is then $\alpha^n$, and magnetization is given by

$$M(2n\tau) = M_0 \exp \left[ -\frac{\gamma^2 G^2 D (n2\tau) \tau^2}{3} \right] \exp \left[ -\frac{n2\tau}{T_2^*} \right] \exp \left[ -\frac{n2\tau}{T_1} \right]$$

(1.51)

at the center of the echo. The echo center in each echo of the Carr-Purcell pulse sequence is attenuated by $e^{-\frac{n2\tau}{T_2^*}}$ instead of $e^{-\frac{2n\tau}{T_2}}$. With $T_{2^*} < T_2$, the Carr-Purcell sequence removes the field inhomogeneities as well as the diffusive attenuation.

It is important to note the diffusive phase loss takes place independently in each time interval $\tau$ and the loss is proportional to $(2n\tau)\tau^2$. This allows for $\tau$ to be arbitrary small, minimizing the diffusive attenuation, while keeping the product of $2n\tau$ constant. The Carr-Purcell sequence can reduce the diffusive effect to an arbitrarily small size while keeping $T_2$ effects constant. Varying $\tau$ and keeping $2n\tau$ constant allows many echoes to be acquired with the same total elapsed time. The sequence with more echoes in the same time will have a smaller diffusion effect. The Carr-Purcell echo train allows
Figure 1.13. The pulse sequence for a Carr-Purcell-Meiboom-Gill pulse sequence is shown. A π/2 pulse along the x-axis is applied first and the remaining π pulses are applied 90° out of phase, as compared to the π/2 pulse, along the y-axis. The CPMG pulse sequence removes accumulated errors due to imperfect π pulse unlike the Carr-Purcell pulse sequence. The echoes form at odd multiples of τ, in-between each pair of π pulses.

Signal acquisition for longer times since the attenuation due to field inhomogeneities and the diffusion is reduced. Imperfections in the T_{20} and T_{180} times accumulate in a basic Carr-Purcell sequence added further attenuation in the echo train. In the Carr-Purcell-Meiboom-Gill sequence, the accumulated imperfections can be shown to be zero by keeping the phase of the π/2 pulse orthogonal to the phase of the π pulse.

1.5.7. MAGNETIC SUSCEPTIBILITY LIMITED RESOLUTION

Since the cell is an inhomogeneous sample, different regions in the cell have distinct magnetic susceptibilities due to varying concentrations of organic and inorganic molecules. Table 1.4 lists the molar magnetic susceptibility of some cellular molecules.
These susceptibility differences create their own field gradients. Adding the susceptibility effect, the frequency in the rotating frame becomes.

\[ \omega(\vec{r}) = \gamma \vec{G} \cdot \vec{r} + \gamma \Delta H_{0,\text{avg}}(\vec{r}) \quad (1.52) \]

where \( \Delta H_{0,\text{avg}} \) is the difference in the field from the average field caused by the susceptibility differences. Equation 1.52 is naive. The boundary effects are not included, but the equation will suffice in describing the image distortion.

The acquired signal in k-space and its Fourier transform are

\[ S(\vec{k}) = \iint \rho(\vec{r}) \exp[i \gamma \Delta H_{0,\text{avg}}(\vec{r}) \vec{r}] \exp[i 2\pi \vec{k} \cdot \vec{r}] d\vec{r} \quad (1.53) \]

\[ \rho(\vec{r}) = \iiint S(\vec{k}) \exp[-i 2\pi \vec{k} \cdot \vec{r}] d\vec{k} \quad (1.54) \]

It is now obvious that the spin density no longer accurately reflects the true image, see Equation 1.14. Susceptibility artifacts result from the method of image reconstruction, where either Polar or Cartesian raster is employed. In both cases, the variance in the magnetic field is on the order of \( \Delta H_{0,\text{avg}}/G \). In geranium cells, spherical air cavities of 10 \( \mu \text{m} \) in diameter have susceptibility gradients of 20 T/m. The susceptibility gradients are much larger than the applied imaging gradients of 10 T/m.

In model systems the susceptibility effect is readily apparent. In Figure 1.14 spheres and cylinders of different susceptibility than the medium in which they are embedded are subject to normal and transverse magnetic fields. The variations in the field cause susceptibility effects that are easily observed. Reduction of the susceptibility effects could be accomplished by making the inhomogeneities in the field small compared to the size of the magnetic gradient.
Figure 1.14. Magnetic susceptibility differences, $\chi_1$ and $\chi_2$, can create imaging artifacts if not properly removed. The figure demonstrates the effects of having two different susceptibility materials in a magnetic field and the different effects caused by the orientation of the magnetic field.

Susceptibility effects not only introduce image artifacts but can also attenuate the signal. The variance in the local field produces gradients and diffusion effects. The attenuation of susceptibility induced diffusion after a spin echo is

$$M = M_0 \exp \left[ -\frac{2\gamma^2 \Delta H \tau \tau_d}{3} \right].$$

(1.55)

where $\tau_c$ is a correlation time measuring how long the molecules take to diffuse across the distortion. $\tau_c$ is analogous to the diffusion coefficient. Since susceptibility effects produce large time independent gradients, the accumulated phase difference can be
refocused with a spin echo pulse sequence. A spin will accumulate an extra phase of $e^{\theta}$ due to the susceptibility after the $\pi/2$ pulse in a time $\tau$. The $\pi$ pulse changes the accumulated phase to $e^{-\theta}$ and during the next interval $\tau$ and the accumulated phase returns to zero at the center of the echo.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Formula</th>
<th>Type</th>
<th>Molar Susceptibility (x10^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(cgs units)</td>
</tr>
<tr>
<td>Water</td>
<td>H_2O</td>
<td></td>
<td>-13.6</td>
</tr>
<tr>
<td>Glucose</td>
<td>C_6H_{12}O_6</td>
<td>Sugar</td>
<td>-103</td>
</tr>
<tr>
<td>Glycerol</td>
<td>C_3H_5O_2</td>
<td>Sugar</td>
<td>-57.06</td>
</tr>
<tr>
<td>Fructose</td>
<td>C_6H_{12}O_6</td>
<td>Sugar</td>
<td>-99.2</td>
</tr>
<tr>
<td>Ribose</td>
<td>C_5H_{10}O_5</td>
<td>Sugar</td>
<td>-84.6</td>
</tr>
<tr>
<td>Sucrose</td>
<td>C_{12}H_{22}O_{11}</td>
<td>Sugar</td>
<td>-189.1</td>
</tr>
<tr>
<td>Palmitic Acid</td>
<td>C_{16}H_{32}O_2</td>
<td>Fat</td>
<td>-198</td>
</tr>
<tr>
<td>Stearic Acid</td>
<td>C_{18}H_{36}O_2</td>
<td>Fat</td>
<td>-220</td>
</tr>
<tr>
<td>Oleic Acid</td>
<td>C_{18}H_{32}O_2</td>
<td>Fat</td>
<td>-208</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>C_{27}H_{48}O</td>
<td>Lipid</td>
<td>-284.2</td>
</tr>
<tr>
<td>Amine</td>
<td>C_{4}H_{11}N</td>
<td>Protein</td>
<td>-56.8</td>
</tr>
</tbody>
</table>

Table 1.4. The magnetic susceptibility varies dramatically for different compounds potentially found inside cellular organisms. Magnetic susceptibility inhomogeneities can lead to unwanted field gradients and distortion of MRI images.

Susceptibility differences can come from other places beside inhomogeneities in the cell. Susceptibility mismatches in the medium that stabilizes the cell in the rf coil can also have drastic effects on the linearity of the gradient. Susceptibility matching liquids can reduce these field distortions. The susceptibility mismatch between the wire in the rf coil and the sample can also limit resolution and has led to development of zero susceptibility wires. A zero susceptibility wire can be manufactured by coating the paramagnetic Copper with a diamagnetic element. Similar to diffusion enhanced
resolution at barriers. susceptibility effects can also be used to contrast regions\textsuperscript{51,70-72} of different susceptibilities.

The CPMG pulse sequence effectively removes many of the undesirable effects in microimaging. The diffusive attenuation is reduced. By acquiring only the center of the echoes in the echo train, the attenuation due to field inhomogeneities is eliminated and the echoes refocus any susceptibility by broadening. The CPMG can also be used to increase the signal to noise ratio, see Section 4.5.2.1. For these reasons, the CPMG pulse sequence will be used throughout the imaging experiments presented here to attain the highest possible resolution.

1.6. GRADIENT COILS

Large gradients are required to increase the bandwidth of individual voxels, and remove diffusive and susceptibility effects. Micron resolution of a biological cell rests on the ability to engineer gradient coils that produce gradients of around 10 T/m. are linear over the sample region and can be switched in very short times. A large gradient implies a large efficiency, defined to be gradient strength per ampere of current. A review of gradient coil construction can be found in Turner\textsuperscript{73} and Carlson\textsuperscript{74}. Usually highly linear gradient coils are constructed using a distributed current method. This method\textsuperscript{53-76} entails solving a boundary value problem subject to limitations on inductance and linearity and
transferring the solution to discrete wires. Since the gradient strength is proportional to the inverse of the square of the size of the coil\cite{10}, these methods are too complicated at the necessarily small diameters and can be avoided.

Short switching times require small inductance in the gradient coil or the use of screening methods. Eddy currents arise in rapidly changing fields through mutual inductance. Minimizing mutual inductance effects reduces the switching time and ensures field linearity by reducing eddy current induced magnetic fields. Shielding can be accomplished by two methods, passive and active. Passive screening\cite{11} involves placing the gradient coil in a metallic cylinder with the thickness much greater than the skin depth. Active screening\cite{12} involve constructing a coil within a coil. These coils, commonly called a quadrupole coil\cite{13}, consist of an inner coil to produce the gradient and an outer coil to reduce the magnetic field outside the coil to zero. The small size of the gradient coils in microimaging will reduce the requirement for active gradient shielding.

1.7. EXPERIMENTAL SETUP AND OVERVIEW

Figure 1.15 pictures the experimental setup used in the imaging experiments and a schematic of the experiment is shown in Figure 1.16. The data was acquired with an Apollo Tecmag capable of acquiring discrete complex points and rf and gradient
modulation. The TTL controls of the Tecmag are used to control the gradients and the PIN diode duplexer. The duplexer is needed to switch between the transmit and receive portions of the experiment as we are using the same rf microcoil for both aspects. The PIN diode duplexer is explained in Chapter 2. The microcoils, discussed in Chapter 3, and the gradient coils, Chapter 4, are inside the shield can of the probe, also discussed in Chapter 3. The gradient power supply is explained in Chapter 6 along with a discussion on performance and switching times. Chapter 7 contains images obtained with this experimental apparatus. Finally, Chapter 8 is a discussion of future directions and experiments and other methods for improving the resolution in MRI and related topics.
Figure 1.16. A block diagram of the experimental apparatus used for MRI.
1.8. LIST OF REFERENCES


2.1. INTRODUCTION

The microcoil regime requires substantial changes to the conventional NMR apparatus. In this chapter, the focus is on the special requirements of the "duplexer" electronics\textsuperscript{14}. The duplexer plays two roles. In the "transmit" mode it routes the NMR transmitter or excitation pulses to the probe tank circuit and microcoil, while isolating these same pulses from the receiver preamplifier. In the subsequent "receive" mode, the duplexer routes the small NMR signal to the preamplifier. During receive mode, the duplexer must also effectively isolate the transmitter amplifier output from the probe and preamplifier, blanking any noise from the transmitter. In this experiment we have
assumed that the same rf coil is being used in both the transmit (excitation) and receive (detection) modes.

2.2. NMR PASSIVE DUPLEXER

A conventional NMR duplexer is a passive device, shown in Figure 2.1a. It employs crossed diode pairs, labeled numbers 1 and 2 in the figure, in order to switch automatically between transmit and receive modes. During the transmit mode, the high power transmitter amplifier is on, putting out rf with amplitude of 10's to 100's of volts. Thus both diode pairs, numbers 1 and 2, are effectively zero impedance, as normal silicon diodes have turn on thresholds of approximately 0.7 volts. rf power then flows freely from the transmitter to the probe. The power, however, is blocked from the receiver preamplifier because that pathway appears as \( \lambda/4 \) to (effective) ground. During the receive mode the NMR signal does not bias the diodes, typically the NMR signal voltage is much smaller than the turn on threshold for the diode, and thus both diode pairs become high impedance. The NMR signal from the probe then has only one pathway available, from the probe to the preamplifier. Furthermore, the diodes isolate any noise from the transmitter during signal acquisition from the probe and preamplifier.
Figure 2.1. Block diagram of the conventional passive (A) and active (B) duplexer. Also given in (B) is a table with the state of each switch (S₁, S₂, etc.) in the transmit and receive modes of the duplexer.
The passive device duplexer described above and shown in Figure 2.1a performs quite well in conventional NMR experiments. In our work on 383 MHz proton NMR using microcoils, however, the passive system is highly inadequate for two main reasons. First, because the microcoils have such small volumes, the required transmitter power is quite low. While conventional experiments employ power levels of 10-1000 W, such high levels would be unnecessary and even destructive if used in a microcoil NMR system. For microcoils, typical power levels are ~0.03 W, giving 90° pulse times of 5μs. These power levels correspond to voltages of approximately 1 V, voltages of the same order of magnitude as the diode characteristic voltages of ~0.7 V. Therefore, during the transmit mode the diodes are not driven unambiguously to their “on” state, and thus the passive duplexer circuit does not operate as envisioned.

A second major problem with the passive circuit is that the typical diodes employed have capacitance of a few pF, which cannot be neglected at frequencies as high as 383 MHz. For example, a stray capacitance of 2 pF for a single diode gives impedance \((\omega C)^{-1}\) of only 100 Ω for a crossed pair at 383 MHz, while the desired impedance is significantly greater than 50 Ω, which is the characteristic impedance of NMR electronics.
2.3. PIN DIODE BASED DUPLEXER

In the following sections the complete design and construction techniques for an actively controlled PIN diode-based duplexer that satisfies all requirements is presented. During the transmit mode, the insertion loss of power going from the transmitter to the probe is measured as only 0.8 dB, while the isolation between the transmitter amplifier and the preamplifier is greater than 32 dB, as compared with only a few dB for the conventional circuit at this frequency. During the receive mode, the isolation between transmitter and preamplifier is greater than 68 dB (−10 dB in the conventional circuit), while the insertion loss between the probe and the preamplifier is only 0.6 dB (−2 dB in the conventional circuit). The system can easily be switched between transmit and receive modes in a time <1μs using transistor-transistor-logic (TTL) pulses.

2.3.1 OVERVIEW OF ELECTRONICS

The duplexer circuit block diagram is given in Figure 3.1b. Shown in the figure are four switches, S1, S2, S3, and S4, and the state of each switch for both transmit and receive modes. There are only two differences between the block diagrams of the active and passive circuits. First, in the active circuit, actively controlled switches replace
Second, the extra switches $S_2$ and $S_3$ and two $\lambda/4$ cables are added for improved isolation of the transmitter amplifier from the receiver preamplifier during receive mode and to provide a high impedance path for the signal back to the transmitter. The most important difference, of course, is the replacement of passive diodes with PIN diode-based active switches.

A PIN diode is a silicon semiconductor consisting of a normal PN diode with a layer of intrinsic silicon in-between the P and N layers of the diode. This intrinsic layer has a finite thickness and area and when biasing current is passed through the diode, holes and electrons are injected into the intrinsic region of the PIN diode. The carrier lifetime, $\tau$, of the holes and electrons in the intrinsic region determines the low frequency cutoff limit of the diode, $f_c = (2\pi\tau)^{-1}$.

At rf frequencies below the cutoff frequency, a PIN diode acts as a normal PN diode and as rf is passed through the diode, significant distortion occurs. Near the cutoff frequency, the rf suffers some distortion as the PIN diode acts a linear resistor with a small nonlinear component. The PIN diode is essentially a pure linear resistor at frequencies above ten times cutoff frequency. A DC biasing current can control the resistance value, it is this characteristic that makes the PIN diode ideal at rf frequencies.

With a positive forward bias DC current, the diode is "on" and acts as a very small resistor, and thus can pass rf with minimal attenuation. This linearity of the PIN diode allows the low power rf pulses required for micro-coil NMR to pass without distortion or significant attenuation. Conversely, with negative voltage across the diode, the diode is "off", and the diode attains high input impedance. Furthermore, in the off
state the PIN diode has a very small capacitance, typically a few hundred femtofarads. The small capacitance occurs because of the intrinsic region, which results in a large effective spacing between the charge regions of the diode, relative to the PN diode with no intrinsic region. The smaller stray capacitance of the PIN diode is due to the decrease in parallel plate capacitance, allowing it to be used as a rf switch up to frequencies much higher than 100 MHz, the typical limit for conventional diodes.

Figure 2.2a and 2.2b show the PIN diode series switch circuits used for S₁, and for S₂, S₃, and S₄ respectively. In switch S₁, two PIN diodes are placed back to back in the RF path. To open the switch, a bias current of 35 mA is applied at the position indicated. Conversely, to close the switch, a reverse bias voltage of -24 V is applied. The LC tank circuits shown in Figure 2.2a are tuned to the rf frequency, 383 MHz, and at resonance serve as rf chokes, preventing the rf from being shorted out to ground or to the DC bias pulse generator. The operation of S₂, S₃, and S₄ is quite similar to that of S₁.

The two importance characteristics in choosing a PIN diode are the series resistance, Rₛ, and the junction capacitance, Cᵢ. The ideal insertion loss for an “on” PIN diode switch in series, as in Figure 3.2a and isolation for an “off” switch are given as:

\[
\text{Insertion (dB)} = 20 \log \left( 1 + \frac{R}{2Z₀} \right) \tag{2.1}
\]

and

\[
\text{Isolation (dB)} = 10 \log \left( 1 + \left[ \frac{\omega Cᵢ}{2Z₀} \right]² \right) \tag{2.2}
\]

where Z₀ is the characteristic impedance of the NMR spectrometer, usually 50 Ω.
A Hewlett-Packard HSMP-3890 PIN diode was chosen to best attain low \( R_s \) and \( C_j \). The typical series resistance for a fully “on” diode is 2.5 \( \Omega \), and the junction capacitance for the “off” diode is 200 \( \text{fF} \). The carrier lifetime of this PIN diode is 200 ns, providing a cutoff frequency of 800 kHz, well below the operating frequency of 383 MHz. Using biasing currents of 35mA for the “on” state voltage -24V for the “off,” we measured, for the circuit of Figure 3.2a, an insertion loss of 0.5 dB for the “on” state and isolation of 36 dB for the “off,” both in reasonable agreement with the specified \( R_s \) and \( C_j \) values.

The four switches are combined to form the NMR duplexer, the full circuit diagram is given in Figure 2.3. The TTL controlled biasing circuit is given in Figure 2.4. When the pulse is being transmitted, PIN’s 1, 2, and 5 are biased with positive current while PIN’s 3 and 4 are biased with negative voltage. PIN’s 1 and 2 form an rf switch, see Figure 2.3, but now coupled with PIN number 3 provide higher isolation between the transmitter and receiver during signal acquisition. The rf pulse is transmitted through the series switch and propagates to the T-junction between the probe and the preamplifiers. Since PIN number 5 is on, there is a \( \lambda/4 \) path to ground and consequently high impedance. This allows for the pulse to travel to the probe with minimal losses while protecting the pre-amplifiers from high power rf pulses.
A. Switch S1

![Circuit diagram for switch S1]

B. Switches S2, S3, S4

![Circuit diagram for switches S2, S3, S4]

Figure 2.2. Circuit diagram for the switches (A) S1 and (B) S2, S3, and S4, as they appear in Figure 2.1B.
During signal acquisition PIN's 3 and 4 are biased positively while the remainder are biased negatively. The signal returning from the probe encounters two $\lambda/4$ to ground in series, obstructing the path returning to the transmitter because of the high impedance. However, the path to the preamplifiers has relatively low impedance and the signal propagates to the preamplifiers with minimal losses. This configuration has an added advantage that the signal does not pass through any blocking capacitors in the path to the preamplifiers, reducing some attenuation of the signal. The transmitter and the receiver are isolated during signal acquisition by the back-to-back PIN diodes in series. Additionally, PIN number 3 is shunted to ground and remaining rf from the transmitter passing through the switch is grounded. The $\lambda/4$ to ground by PIN number 4 provides high impedance for any remaining rf from the transmitter, reducing CW rf interference with both the probe and preamplifiers during signal acquisition.

2.3.2. CONSTRUCTION METHODS AND REQUIREMENTS

At the operating frequency of 383 MHz the rf wavelength is quite short (2.5 ft in free space), and thus careful attention must be paid to lead lengths. We found that a tidy and carefully planned construction was essential\textsuperscript{13,14}. The entire circuitry of Figures 2.3 and 2.4 is mounted on double-sided one-ounce 0.014 inch thick copper-clad PC board with G-10 substrate of thickness 1/16 inch. Rf microstrips were formed by lines on the
top of the board and a ground plane that covers the entire bottom side of the board. All
biasing circuit components are surface mounted to reduce the holes into the substrate of
the circuit board. Impedance matching the NMR duplexer to the remaining NMR system
requires that the duplexer have 50 Ω impedance. see Section 2.3. By varying the width of
the microstrip, the impedance of the transmission line can be calculated.

Board layout in rf electronics is critical. ground planes on the topside of the board
are in all cases separated from rf traces by at least three trace widths to insure constant
impedance. The ground plane area on the top of the board is connected to the bottom
side by wrapping copper tape around the edges and soldering it to the board along the
board’s outer perimeter. rf traces should be straight to reduce additional losses in the
duplexer. When biasing circuitry must cross any rf traces, the paths should be
perpendicular to reduce coupling and losses in the system.

Additionally, stray inductance in the board layout, often a few nanohenries
drastically reduces the effectiveness of λ/4 transmission lines. Location of all rf grounds
on the topside of the circuit board should be located close to the edge of the circuit board.
This will reduce the rf path to ground as the rf is not properly grounded until it
encounters the ground shield on the backside of the circuit board; copper tape around the
edges of the circuit board helps to minimize this length. Even with these additional
precautions, several nanohenries of stray inductance remain. In section 2.4 the effects of
and solutions to stray inductance are explained. The layout of the duplexer, complete
with the biasing circuitry, is shown in Figure 2.5.
Figure 2.3. Full circuit diagram of the duplexer, but omitting the biasing circuitry, which is given in Figure 2.4.
Figure 2.4. Diagram of the biasing circuit, which takes an input TTL and outputs -24 Volts (on TTL 0) or +35mA (on TTL 1) for “bias 1” (as labeled in the circuit in Figure 2.3) and the opposite for “bias 2”.
Figure 2.5. Picture of the board layout, with scale. Board includes the duplexer circuitry of Figure 2.3 and biasing circuitry of Figure 2.4. The remaining area of the PC board is reserved for other aspects of the experiment, including rf preamplifiers.
2.4. 50 Ω TRANSMISSION LINES

The rf traces of the circuit board must also be designed to have the characteristic impedance of 50 Ω. For a microstrip trace of width w. and copper thickness t. on a substrate of height h. the characteristic impedance has been given by Rosenstark15:

\[
Z_0 = \frac{120\pi}{\sqrt{\varepsilon_{re}}} \left[ \frac{w}{h} + 1.393 + 0.667 \ln \left( \frac{w}{h} + 1.444 \right) \right]^{\frac{1}{2}} . \tag{2.3}
\]

where \(\varepsilon_{re}\) is the effective dielectric constant and \(w_e\) is the effective width of the traces, both given below. Equation 3 is valid only if \(w/h > (2\pi)^{-1}\), generally met for common PC boards. The effective width to height ratio appearing above is altered slightly from the direct \(w/h\) ratio because the electromagnetic energy is partially stored in the dielectric and partially in the vacuum space above. The expression is given in terms of the thickness \(t\) of the Cu strip16:

\[
\frac{w_e}{h} = \frac{w}{h} + \frac{1.25}{\pi} \frac{t}{h} \left[ 1 + \ln \left( \frac{2h}{t} \right) \right] . \tag{2.4}
\]

For the same reason, the effective dielectric constant appearing in Equation 2.3 is not directly equal to the dielectric constant of the substrate. The effective dielectric constant is given by

\[
\varepsilon_{re} = \frac{\varepsilon_e + 1}{2} + \frac{\varepsilon_e - 1}{4.6} \frac{t}{h} - \varepsilon_e - 1 \frac{t}{h} \frac{1}{4.6} \frac{w}{h} \sqrt{2 + 10 \frac{h}{w}} . \tag{2.5}
\]
where $\varepsilon_r$ is the dielectric constant of the substrate material. This effective dielectric constant must be used in calculating the $\lambda/4$ length.

$$\frac{\lambda}{4} = \frac{c}{4f\sqrt{\varepsilon_r}} \quad (2.6)$$

where $c$ is the speed of light and $f$ is the frequency of the duplexer. 383 MHz.

In full consideration of these constraints, the geometry of our PC board, and the G-10 dielectric constant $\varepsilon_r = 4.35$ (at 500 MHz), Equation 2.6 calls for trace widths of 2.97 mm, and we used this throughout the rf portion of the design. Figure 2.6 demonstrates the dependence of the trace width to the impedance of the microstrip transmission lines. Narrower trace widths with consequently higher impedance were used for the DC biasing circuitry to further reduce rf losses in the duplexer.

2.5 $\lambda/4$ TRANSMISSION LINES AND STRAY INDUCTANCE

Despite best efforts to construct ideal $\lambda/4$ microstrips, inevitably adjustments are needed. In estimating the needed $\lambda/4$ length we approximate the diodes at the ends of the cables as lumped elements. Inevitably though because of finite lead lengths and stray inductances it is necessary to make adjustments.

The load at the end of a transmission line can greatly affect the overall impedance characteristics of that transmission line. Consider a transmission line of length $l$, with
Figure 2.6. The dependence of the trace width in forming microstrip transmission lines. For the board configuration given in the text, a trace width of 2.97 mm gives the impedance of 50 Ω.

complex load impedance, $Z_0$ shorted to ground. The total complex impedance network can be transformed into.

$$Z_{\text{transformed}} = Z_0 \frac{Z + iZ_0 \tan(kl)}{Z_0 + iZ_0 \tan(kl)}$$  \hspace{1cm} (2.7)

where $Z_0$ is the characteristic impedance of the transmission line, usually 50 Ω, and $k = 2\pi/\lambda$. 

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Stray inductance in the circuit causes a load impedance of \( Z_i = i\omega L \). A stray inductance of a few nanohenries leads to a dramatic effect on the input impedance of the transmission line. The stray inductance is usually unavoidably located in leads to the diodes and the distance to the ground plane on the backside of the circuit board. Figure 2.7 demonstrates the consequences of a few nanohenries of stray inductance on the transformed impedance of the network, where the magnitude of the network impedance is plotted versus changes in wavelength of the transmission line from \( \lambda/4 \). The stray inductance has no effect on the shape of the impedance curve: the inductance creates an effective length change in the \( \lambda/4 \) line. This effective length change causes the attenuation of a \( \lambda/4 \) line to decrease, as small length changes in the transmission line have drastic effect on the magnitude of the impedance.

To correct for the stray inductance, adjustable capacitors, with range 6.5-25 pF, are included in the circuit as shown in Figure 2.3. Johanson brand surface mount 2320 series capacitors were used for this purpose. The success of this approach suggests that the capacitors effectively tune out stray inductance in the shunt to ground, making a series LC short for the rf. The capacitors also have the added benefit of isolating the DC bias currents/voltages from the rf electronics.
Figure 2.7. The impedance of a \( \lambda/4 \) to ground through a stray inductor can radically change the effective impedance. The magnitude of the complex impedance is plotted against the change in the effective length from \( \lambda/4 \) of the transmission line. To read the effective impedance of the transmission line, the value is read at the zero point for the particular curve, thus a \( \lambda/4 \) line to ground with three nanohenries of stray inductance is only \(-2000\) ohms instead of infinite with no stray inductance.
2.6. TUNING THE RF DUPLEXER

Placing a continuous wave rf source in the 'rf in' port of Figure 3 and measuring the rf at both the probe and preamplifier's port easily accomplish tuning the variable capacitors. The capacitor for PIN number five is tuned until the rf seen by the preamplifier port has been minimized. Switching the rf source to the probe position and monitoring the remaining ports, the remaining capacitors can be tuned. To tune capacitor from PIN number three we first remove the capacitor from PIN number four and tune for maximum signal due to a \( \lambda/2 \) at the rf in port. Next, we replace the variable capacitor from PIN number four and tune for a minimum at the 'RF in' port.

2.7 NMR DUPLEXER EXPERIMENTAL RESULTS

During the pulse phase of a NMR experiment, the duplexer exhibits isolation of 32 dB between the pulse to the probe and the pulse to the preamplifiers. The tuned \( \lambda/4 \) length cause sufficient isolation of the high power pulse and protects the preamplifiers from damage. In transmit mode the pulse was measured to have an insertion loss of 0.8 dB at the probe port, but this is not a crucial loss since in practice one can simply increase the transmitter power. In receive mode the RF signal from the probe has a measured
insertion loss of 0.6 dB from the probe to the pre-amplifier port, while isolation of the transmitter from the preamplifiers is greater than 68 dB during signal acquisition. This high degree of isolation is quite helpful if the transmitter amplifier does not have adequate blanking.

Switching the duplexer between transmit and received modes is limited in speed only by the finite carrier lifetime of the PIN diodes, approximately one microsecond. This is more than adequate for typical NMR experiments involving either liquid or solid samples.
2.8. LIST OF REFERENCES


3.1. INTRODUCTION

The most formidable obstacle to high resolution MRI is sensitivity\(^{14}\). As the image resolution increases, the "pixel" size, and the volume of sample corresponding to each pixel ("voxel"), decreases. With fewer nuclear spins in each voxel, the signal to noise ratio is degraded. The problem of sensitivity, however, can be addressed for small samples by using microcoils\(^{17}\). Observing a fixed sample volume, a cell of a few hundred cubic microns, with a large receiver coil of one centimeter in diameter results in a filling factor of \(10^{-9}\) and a consequently similar reduction in the signal to noise ratio. However, using a coil of the approximate cellular dimension results in an advantageous
filling factor approaching one, and an increase in the signal to noise by a factor of one billion.

In this chapter, the coil form, namely the pipette, the winding of the coil and the MRI probe used in the experimental work will be described. In addition, the problems of choosing the correct capacitor, tuning the LC tank circuit, and the effects of the microcoils having a small quality factor, Q, will be addressed.

3.2. PIPETTE SAMPLE TUBE

In order to hold a liquid NMR sample of dimensions ~100 μm or less, it is necessary to develop a sample container that has the following desirable features: it must have appropriately small dimensions so that a large "filling factor" can be achieved, and it must be easily manipulated. To meet these requirements we have settled on glass quartz capillary tubes (Sutter Instrument Company) of O.D. 1 mm and I.D. 0.70 mm. and length 100 mm. These capillary tubes are then "pulled," using a Sutter Instruments P-2000 "Micropipette Puller." The "pulling" operation clasps the tube at each end, and then heats the tube to temperatures sufficient to melt the quartz midway along its length using a carbon dioxide laser. The CO₂ laser provides several advantages over traditional heated filaments: laser heat is clean, leaves no metallic residue, and can be turned off instantly. The mid-region of the tube heats, stretches, and tapers to smaller diameters. The laser intensity or heat duration of the laser pulse, the area of the pipette heated and
the tension applied to the capillary tube are all controlled by the user and can be adjusted
to form the desired length and angle of the taper.

The instrument then breaks the tube into two pieces by pulling in such a way to
maintain straightness. The final product is two "micropipettes", one of which is
illustrated schematically in Figure 3.1. Diameters much less than 20 μm can be pulled
with this machine, but fragility limits the minimum diameters to ~20 μm for our
applications. The unpulled region of the glass micropipette tube allows for easy use and
mounting as the thickness makes this region rigid. The tensile strength of quartz glass is
superior to other glasses and allows for larger working ratios of the inner diameter to the
outer diameter of the micropipette.

Quartz sample tubes are superior to other glasses and coil forms as quartz absorbs
less electromagnetic radiation due to a low dielectric constant. Superior coils forms have
the following characteristics: low magnetic susceptibility, high quality factor Q, and
devoid of NMR signals of interest. The loss tangent tabulates dielectric losses.

\[
\tan \delta = \frac{\pi}{Q} .
\] (3.1)

or the loss factor.

\[
\text{Loss Factor} = \varepsilon_d \tan \delta .
\] (3.2)

where \(\varepsilon_d\) is the dielectric constant of the material. The low dielectric losses for quartz
make it the best coil form available, see Table 3.1, where loss tangents for several
materials are tabulated. Quartz is a crystal of silicon and oxygen containing no hydrogen
and unlike silicon aluminum or borate glasses where impurities exist in the glass: quartz
Figure 3.1. A quartz capillary tube is pulled to smaller diameters with a P2000 micropipette puller. The tapered region provides the location to wide the coil of various outer diameters and serves as the coil form. The unpulled portion of the pipette is rigid, allowing for easy manipulation and mounting.

<table>
<thead>
<tr>
<th>Material</th>
<th>Dielectric Loss at 500 MHz</th>
<th>Dielectric Loss at 1 MHz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartz</td>
<td>0.03</td>
<td>0.0002</td>
</tr>
<tr>
<td>Macor</td>
<td>-0.1</td>
<td></td>
</tr>
<tr>
<td>Soda Lime Glass</td>
<td></td>
<td>0.061</td>
</tr>
<tr>
<td>Borosilicate</td>
<td></td>
<td>0.005</td>
</tr>
<tr>
<td>Pyrex-7740</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrex-7070</td>
<td>-0.2</td>
<td></td>
</tr>
<tr>
<td>Silicone</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Teflon</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Si3N4</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>-5</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.1. The dielectric loss tangent and magnetic susceptibility for several potential coil forms are shown\(^\text{12}\). The dielectric loss tangent is measured at 400 K and 500 MHz. The dielectric properties at 1 MHz are from Corning. The dielectric property of water is provided as a comparison to the remaining materials.

is comparably pure\(^\text{12}\). Impurities in the dielectric affect the NMR signal as they can allow additional adverse relaxation mechanisms.
3.3. rf MICROCOIL CIRCUITRY

The winding of the microcoils requires the use of a microscope and extreme patience to insure careful wire spacing and low stray effects. In the following section, the winding process of the microcoil and the placement of the coil on a PC board are explained. Throughout the experiment, the PC board layout has changed and evolved and several versions are shown with the benefits and shortcomings of each explained. In the experiments presented, the microcoil is used for both the receiver and the transmitter in the NMR tank circuit.

3.3.1. WINDING THE COIL

Although it is possible to wind coils by hand, we have since improved upon that process through construction of a winding apparatus, shown in Figure 3.2. Because the coil diameter needed is too small to be seen by the unaided eye, all winding is accomplished under a microscope. One end of the wire, usually copper, with a diameter of 10 or 20 μm (Goodfellows or California Fine Wire), is glued (Norland Optical, #68) onto the large diameter end of the micropipette. The glue cures with UV light allowing
for placement and adjustments of wires before the glue cures, leaving approximately a foot of wire glued to the micropipette.

Next, the wire is ‘threaded’ into a small hole, ~200 μm, on the end of the "winding attachment", as shown in Figure 3.2, and the large diameter end of the
micropipette is tightened in the drill chuck. The remaining wire is hung off the end of the winding attachment and a fishing weight is clamped on the wire to provide tension, perpendicular to the axis of the micropipette. The wire is then loosely wrapped, until the approximate diameter of the coil to be constructed is encountered in the gradual taper of the micropipette.

As the user turns a crank handle, the linear carriage holding the winding attachment travels on a ¼"-20 threaded rod, moving the carriage one inch in twenty turns of the crank handle. Simultaneously the drill chuck rotates with the crank handle at a gear ratio determined by the user, who can choose among a discrete set of values. A small gear is located on the drive shaft with the drill chuck, and larger (more teeth) gear is located on the ¼"-20 threaded rod. A drive belt connects these two gears, see Figure 3.2. Theoretically, in order to maximize signal to noise it can be shown\(^{13,15}\) that the spacing between the centers of two adjacent turns of wire should be equal to two wire diameters. As an example, for 20 µm diameter wire, one needs 1 single revolution of the drill chuck for 40 µm of linear motion of the carriage. This can be accomplished with a gear ratio of 30 (30 turns of the drill chuck for every one turn of the crank handle. Table 3.2 lists the possible combinations of small and large gears and the resulting wire spacing for the coil winder machine.

Now, with the winding between each turn set, the desired number of turns, keeping the wire spacing constant, is wound and glued to the micropipette with the optical glue. Choosing the appropriate glue is important as choosing a coil form with low tangent losses. However, glues have other properties that must be considered: the
ease of use and cure temperatures. Table 3.3 lists several glues suitable to adhere the coil windings to the quartz pipette.

Norland Optical 123K epoxy was chosen to attach the coil to the pipette. This epoxy was chosen for it’s relatively low dielectric properties but especially for the room temperature cure time of a few minutes with UV light. The remaining glues, though some of lower loss tangents, are all two-part mixtures requiring heated cure temperature significantly above room temperature of up to several hours. Subsequent experiment measurements demonstrate the losses in the coil system are dominated by wire losses of the microcoil and not by dielectric losses of the glue characterized by Q measurements, section 3.7, that were independent of the adhesive used for similarly designed coils. The

<table>
<thead>
<tr>
<th>Large gear (# of grooves)</th>
<th>10 groove Small Gear</th>
<th>12 groove Small Gear</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>102 μm</td>
<td>121.9 μm</td>
</tr>
<tr>
<td>36</td>
<td>71 μm</td>
<td>84.7 μm</td>
</tr>
<tr>
<td>44</td>
<td>58 μm</td>
<td>69.3 μm</td>
</tr>
<tr>
<td>48</td>
<td>53 μm</td>
<td>63.5 μm</td>
</tr>
<tr>
<td>60</td>
<td>42 μm</td>
<td>50.8 μm</td>
</tr>
<tr>
<td>72</td>
<td>35 μm</td>
<td>42 μm</td>
</tr>
<tr>
<td>80</td>
<td>32 μm</td>
<td>38 μm</td>
</tr>
<tr>
<td>90</td>
<td>28 μm</td>
<td>34 μm</td>
</tr>
<tr>
<td>100</td>
<td>21 μm</td>
<td>25 μm</td>
</tr>
<tr>
<td>120</td>
<td>21 μm</td>
<td>25 μm</td>
</tr>
<tr>
<td>130</td>
<td>19.5 μm</td>
<td>23 μm</td>
</tr>
</tbody>
</table>

Table 3.2. Wire spacing a function of gear choice for the winding machine. After deciding on the desired wire spacing, this table allows the user to choose the appropriate gears to attain the desired wire spacing. For example, for 20 μm wire and spacing between centers of adjacent turns equal to 40 μm, a 60 groove large gear and a 10 groove small gear should be chosen.
Table 3.3. The dielectric properties of selected epoxies. The dielectric properties of the glue determine the losses the glue contributes due to the absorption of electric field energy. The loss tangent was measured at the indicated frequency and generally increases for higher frequencies. However, the ease of use of the glue must be taken into consideration. All data presented was obtained from the manufacturers.

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Description</th>
<th>Loss Tangent</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emerson + Gumming</td>
<td>Stycast 1269</td>
<td>0.005</td>
<td>1 MHz</td>
</tr>
<tr>
<td>Epoxy Etc.</td>
<td>20-3060</td>
<td>0.01</td>
<td>100 Hz</td>
</tr>
<tr>
<td>Epo-Tek</td>
<td>301-2</td>
<td>0.038</td>
<td>1 MHz</td>
</tr>
<tr>
<td></td>
<td>353ND</td>
<td>0.038</td>
<td>1 MHz</td>
</tr>
<tr>
<td></td>
<td>H54</td>
<td>0.001</td>
<td>1 MHz</td>
</tr>
<tr>
<td></td>
<td>H61</td>
<td>0.016</td>
<td>10 kHz</td>
</tr>
<tr>
<td></td>
<td>H67MP</td>
<td>0.02</td>
<td>10 kHz</td>
</tr>
<tr>
<td></td>
<td>377</td>
<td>0.01</td>
<td>10 kHz</td>
</tr>
<tr>
<td>Norland Optical</td>
<td>123K</td>
<td>0.0315</td>
<td>1 MHz</td>
</tr>
<tr>
<td>Master Bond</td>
<td>EP30</td>
<td>0.044</td>
<td>100 MHz</td>
</tr>
<tr>
<td></td>
<td>EP21LV</td>
<td>0.024</td>
<td>300 MHz</td>
</tr>
</tbody>
</table>

1. 2 Part Epoxy, Heat Cure
2. 1 Part Epoxy, UV Cure

weight is then removed from one lead and the wire on the top of the micropipette is cut and unwound. This lead was glued to the unpulled region of the pipette. The process results in a microcoil adhered to the micropipette with two wire leads. Figure 3.3 shows several coils wound on a micropipette. Figure 3.4 shows a coil with the glue applied.
Figure 3.3. Several coils are shown that were constructed with the aid of the winding machine. The first lead to the coil is hanging below each coil. The remaining lead still needs to be unwound on the right side of the coil to form the second lead. The first coil is five turns constructed with 20 \( \mu \text{m} \) diameter copper wire. The coil is \(-100 \mu \text{m}\) in length and \(-75 \mu \text{m}\) in diameter. The second coil is made from 18 \( \mu \text{m} \) copper wire. In this coil, the wire is insulated allowing the turns to be touching their neighbors. The coil is \(-75 \mu \text{m}\) in diameter and \(-50 \mu \text{m}\) in length. The third coil is made from 20 \( \mu \text{m} \) diameter copper wire and is five turns. The length of the coil \(-100 \mu \text{m}\) and the diameter is \(-50 \mu \text{m}\). The fourth coil is constructed from 10 \( \mu \text{m} \) copper wire and is five turns. The length of the coil is \(-45 \mu \text{m}\) and the diameter is \(-25 \mu \text{m}\). This last coil is one of the smallest constructed for the NMR/MRI experiments.
3.3.2. NMR RESONANCE CIRCUITS

The usual NMR resonant tank circuit consists of an inductor (the receiver and transmitter coil) in parallel with the “tuning” capacitor. This LC parallel circuit is then placed in series with a “matching” capacitor. This set-up enables one to couple energy into and out of the circuit, matching the circuit impedance to the 50 Ω coaxial cables that are typically used. Figure 3.5 presents the two NMR resonance circuits used in this experiment.

In Figure 3.5a, resonance occurs when the tuning capacitor in combination with the parallel inductor resonant at the desired frequency.

\[
\omega_0 = \frac{1}{\sqrt{L(C_m + C_f)}} \tag{3.3}
\]
A.

To 50 W
Transmission Line

**L**

C\text{m}

C\text{t}

Ground

B.

To 50 W
Transmission Line

R

L

C\text{t}

Ground

Figure 3.5. The first resonance circuit in part A is the normal matching and tuning circuit used in NMR. However, due to the small inductances and larger resistances in microcoils, the matching capacitor is not always necessary and the circuit in part B can be used.

where \( C_\text{m} \) is the matching capacitance and \( C_\text{t} \) is the tuning capacitance. The resonance condition occurs when the imaginary impedance is forced to zero. The matching capacitor can then be used to match the impedance of the transmission line, usually 50 \( \Omega \), with the impedance of the NMR tank circuit. An inductor and capacitor in series creates an rf short, while a inductor in parallel with a capacitor creates an infinite rf impedance. The combination of parallel and series capacitance allows for a middle value to be chosen, 50 \( \Omega \). The matching capacitor can be calculated by forcing the real part of the impedance to be \( Z_0 = 50 \Omega \) and is given by (in the large Q limit).
In microcoil NMR, the matching capacitor is not always required. The absence of the matching capacitor allows for a simplified design and minimum lead lengths to and from the microcoil. For this modified set-up one can show that the impedance at the resonance frequency \( \omega = (LC_0)^{1/2} \) is given by \( Z = Q^2 R \), where \( R \) is the coil resistance and \( Q \) is the quality factor of the coil, defined by \( Q = \frac{\omega L}{R} \). The inductance of the coil is \( L \), see section 3.6.3. Fortuitously, this impedance is of order 50 \( \Omega \) for the typical values of \( Q \) and \( R \), eliminating the need for a matching capacitor. See section 3.6.2 for further discussion of tuning NMR circuits, section 3.7 for \( Q \) measurements and section 3.6.4 for consequences of imperfect matching conditions.

\[
C_m = \frac{1}{QZ_0\omega_0^2L}.
\]  

(3.4)

3.3.3. RF CIRCUIT BOARDS

Several versions of the rf board have been constructed in the evolution of the experimental apparatus. The three versions that are discussed in this section are shown in Figure 3.6. The rf board is a 1/32" thick double-sided copper laminated FR-4 PC board in each version. The board contains the NMR microcoil and tuning and matching (if required) capacitors. Versions two and three have significantly better rf characteristics with less stray inductances, and the third version is currently in use in the experiment. However, version one is explained briefly as some data included in Chapter 5 is taken
Figure 3.6. There were three main versions of the rf board used in the experiments. The board in part A is the oldest version and relied on variable capacitors to tune and match the rf microcoil. This version had more stray inductance than versions B and C. In the last two versions, the variable capacitors have been replaced with fixed capacitors and the ground shield on top of the board has been removed. The only difference between B and C is that the SMA connector has been moved closer to the microcoil limiting the lead length.

with this version of rf board. The center pin of the SMA connector in all three versions is connected to a 50 Ω microstrip transmission line\textsuperscript{20}, see section 2.4, of width \textendash 1.4 mm. In version one, the SMA connector connects the top and bottom ground planes of the rf
board. Circuit lengths to and from the coil are minimized as much as possible considering the constraints on the system, allowing access for both the rf rigid coaxial cable connected to the SMA on the rf board and two tuning rods for the variable capacitors. The first version of the rf board uses variable capacitors, Johanson 2320 series, to tune and match the microcoil to the 50 Ω transmission line.

In order to place the micropipette as close as possible to the relevant connection on the board, a slot of width 1.5 mm (somewhat larger than the 1 mm diameter of the micro-pipette) is cut into the board, and the micropipette is inserted into this slot. To accommodate the tapering of the micropipette, the width of the slot is also tapered, in a way such that the coil is placed quite close to the board edge. In this way we can eliminate close to 1 mm of lead length. The slot in the first version is cut through the rf board and one lead from the coil is soldered to trace on top of the rf board and remaining lead is soldered to the ground plane on the reverse side.

In subsequent versions, the slot is cut only 0.5 mm deep into the rf board. This "tapered slot width" scheme is illustrated in Figure 3.7. The depth of the slot matches the radius of the un-stretched segment of the micropipette, as shown in Figure 3.1. Therefore, the center of the micropipette now lies in the top plane of the board in versions two and three. Figure 3.8 shows an microcoil and the taper slot width on an rf board. In the new design both leads going to the coil start in the top plane of the board, while in version one, one lead was soldered on the top plane and the was soldered on the bottom ground plane. With both leads of the microcoil in the top plane of the rf board, the overall sum of the leads to and from the coil is shorter by roughly the thickness of the
Figure 3.7. Tapering the slot holding the micropipette allows the shortest possible lead length to the microcoil. Minimizing lead length is important to reduce the effects of stray inductance.

board. Shorter leads on the microcoil improve the rf characteristics of the resonance circuit. see section 4.6.3.

Finally, in the current design we have eliminated the ground plane that was previously on the top of the PC board. In the new design there is a ground plane only on the bottom of the board. A copper plug is used as a feed-through from the top of the board to the ground plane on the bottom. Also in the latest generations of the rf board the variable capacitors have been removed and only a single fixed capacitor (size 0402 or 0505) is soldered less than 1 mm from the coil, in order to maximally localize the rf tank
Figure 3.8. The tapered slot on an rf board is shown. The microcoil, too small to be seen, is tuned with a 0402 capacitor. The 50 W stripline connects the microcoil to the SMA connector.

The very short distance between the capacitor and coil has reduced the stray inductance in our circuit from -10 nH to -1.4 nH from the second and third version as compared to the first version. The third version of the rf board is largely identical to the second version, but the 50 Ω microstrip has been shortened to reduce the attenuation of the NMR signal to the SMA connector. A discussion of stray inductance issues is given in section 4.6.3.
3.3.4. COIL POSITIONING WITH RESPECT TO GRADIENT SYSTEM

The rf coil and NMR sample must be positioned carefully with respect to the gradients, in order that the sample will experience a highly uniform gradient. In particular, estimates of section 5.2 suggest that the sample should be placed within ~200 μm of the center of the gradient coils. In this discussion below, we assume that the rf coil has been wound and glued onto the micropipette. Figure 3.9 shows a representative view of the gradient system, completely discussed in Chapter 5. However, the gradient system has a geometric center where the change to the static magnetic field, B₀, is zero and the coil must be placed within that region. A discussion of the placement of the microcoil on the rf board is included here for completeness of preparation of the rf board.

One can think of this positioning in two stages. First, one must designate and mark a position with respect to the rf board that is to be the center of the gradient coils. The center of the gradient field in the vertical dimension is the top plane of the rf board. Horizontally, we it will be located 50 μm away from the edge of the slot, as pictured in Figure 3.7, so that a micropipette of outer diameter 100 μm will be centered correctly when it lies flush to the edge of the board. To specify the remaining horizontal coordinate, a hash mark is machined into the board perpendicular to the direction of the slot. This hash mark is controlled by a computer controlled milling machine in conjugation with the geometry of the gradient system. The three mounting holes on the rf board are used as references positions when machining the rf boards to the gradient system, as both the gradients and rf board are mounted on the same component.
Figure 3.9. Three different orientations of the gradient boards are shown above. The gradient boards are shown for completeness in discussing the microcoil placement and are discussed in Chapter 5.

With the desired coil and sample location, with respect to the rf board, now identified and marked, we proceed with a method to affix the coil and sample to this location. At this stage the winding of the coil on the micropipette, described above, is complete, and the micropipette remains in the drill chuck of Figure 3.2. The rf board is attached to the "positioning attachment", and this positioning attachment now replaces the "winding attachment" of Figure 3.2. In this configuration the plane of the rf board is automatically oriented parallel to the axis of the micropipette. The "positioning attachment" can be used to manipulate the x, y, and z coordinates of the rf board in a controlled manner. With this configuration, and with the aid of a magnifying glass, the coil can be moved to the desired location. The unpulled region of the micropipette is then glued to the board with Norland Optical #68, a UV curing glue. The wire leads are
soldered to the top of the board using low-temperature indium solder to ensure that the delicate copper wire leads are not melted or cut. High temperatures cause serious damage to the delicate copper wires.

The assembled gradients and rf circuitry is shown in Figure 3.10. The axis of the micropipette tube containing the coil is mounted parallel to the y axis with respect to the gradients. Both the gradients and the rf board are mounted on acrylic piers of appropriate heights to ensure the rf coil is in the exact vertical center of the gradient coils in the vertical dimension. Though accurate machining and planning the rf board is placed in the geometrical center of the gradients.

3.4. NMR/MRI PROBE

Figure 3.11 shows the two probes used in the experimental process. The first probe is used in conjunction with the first two version of the rf board, and the second probe is used only with the remaining third version of the rf board. The first probe has tuning rods to control the variable capacitors of the first version of the rf board. The construction of the probes is identical except for material and location of the rigid coax. The rigid coax has been moved closer to the center of the probe to accommodate the third version of the rf board.

The probes is inserted into an Oxford Instruments superconducting 9 Tesla, vertical, room temperature bore magnet, with bore diameter 101 mm. Large diameter
Figure 3.10. The rf board slides in-between the two planes of the gradient board. Both the gradient board and rf board are mounted on the same acrylic mounting piece. The mounting piece is machined to ensure that the rf board is vertically centered in-between the gradient planes.

cables. Belden 9114, are used to connect the three independent gradient amplifiers to each of the three (floating) gradient coils. Additionally, this type cable (Belden 9114) transmits rf pulses to the microcoil tank circuit, and NMR signals from the tank circuit to the preamplifiers. A home-built duplexer, see Chapter 2, was specially designed to route, in the appropriate directions, the very low power rf pulses used in microcoil NMR and the NMR signal. This cable serves the dual purpose of having a low resistance per unit length and a low attenuation of the rf pulse and NMR signal at 400 MHz. The low resistance\(^{21}\) in both the center (3.9 m\(\Omega/m\)) and the outer conductors (3.6 m\(\Omega/m\)) allow for
The probe in part A was used for both versions 1 and 2 of the rf board. The probe in part B is used only with version 3 of the rf board. The microcoil and gradients are contained in the shield can.

A maximum gradient current to be transferred to the probe for a given power supply voltage. Additionally, the coaxial nature of the cable renders magnetic force interaction with the static magnetic field in the superconducting magnet negligible. The low attenuation (0.11 dB/m) permits the maximum NMR signal amplitude to reach the preamplifiers.

The gradient coils, Figure 3.9, and rf receiver coil tank circuit, Figure 3.5 are both located inside the "shield can" labeled in Figure 3.11. Rigid coaxial, 0.141 in. (Precision Tube) diameter is connected to the rf circuitry and is chosen for the low attenuation at
383 MHz. RG-58A (Belden) cable is connected from the top of the probe to each of the three SMA's of the gradient coils. On top of the probe, the RG-58A cable is terminated in a N-type connector for easy connection to the Belden 9114 cable discussed earlier. The assembly is then mounted to the probe and fixed into position in the magnet through the remaining four holes shown in Figure 3.10.

The construction material used in the probes also varies between the two versions. The shield can serves the purpose of isolating the high frequency components of the gradient pulses from the static magnetic field of the superconducting magnet. The ratio of the skin depth of the lowest frequency component of the gradient pulse to the thickness of the can should be much less than one. This condition is met for frequencies greater than 1 kHz (pulses less than 1 ms in duration) since the thickness of the can is approximately one centimeter. The first probe consists of brass body and copper shield can, while the second probe is all aluminum. Aluminum was chosen for the shield can over the copper for the lighter weight, even though some shielding loss does occur due to the decrease in conductivity. Also, copper alloys can contain significant iron impurities but the aluminum alloy 6061 is not ferromagnetic. Ferromagnetic components inside the static magnetic field wildly distort the homogeneity of the magnetic field and should be avoided. For a complete discussion of magnetism and probe construction see papers by Doty.1121.
3.5. RF CONNECTORS

The rf connectors used inside the shield must be non-magnetic. This requirement is extremely difficult to satisfy. Standard practice in manufacturing the coax connectors is to coat the body of the connector, either stainless steel or brass, with a nickel underplate. The nickel underplate is used to allow the easy deposition of a gold layer on the outer surface of the connector. However, both nickel and stainless steel are magnetic and can have homogeneity consequences in the static magnetic field. These types of connectors should be avoided if possible.

Non-magnetic connectors are available but extremely difficult to locate. It is possible to custom manufacture connectors with a copper underplating over a brass body with the standard outer plating of gold. Additionally, a material called white bronze is available from AMP, another non-magnetic alloy. The white bronze, however, does not allow for a gold outer plating to be applied.

For the PC mount SMA connectors used for both the connections to the rf board and gradients a custom manufactured part was ordered from Johnson Components. Part number 142-0701-202 is Brass/Cu/Au and number 142-0701-204 is Copper/Silver. The first part was used in this experiment. To connect the rigid coax to the rf board part number 142-0694-002 was used and is also Brass/Cu/Au connector. The flexible coax connector from the probe to the gradient boards was ordered from AMP, part number 1082032.
3.6. TUNNING AND CAPACITORS

For this section, the discussion of tuning will not deal with version one of the rf board and variable capacitors specified, however, the discussion is applicable. In versions two and three of the rf board, tuning was accomplished with chip capacitors. The chip capacitors were either 0402 (0.040 in. by 0.020 in.) or 0505 (0.055 in. by 0.055 in.). Tuning was assessed by sending rf to the probe through a directional coupler and monitoring the reflected amplitude. For each coil successive values of capacitance were tried until a value was found that achieved tuning at the fixed frequency of 383 MHz. The solenoid approximation for the inductance of the microcoil.

\[ L = \mu_0 n^2 V_{\text{coil}} \]  

where \( n \) is the turns per unit length of the coil. Typical values of inductance for the microcoil are 0.5-3 nH requiring capacitance between 20 and 110 pF to tune at the resonance frequency of 383 MHz. To ensure the smallest possible leads lengths between the microcoil and the capacitor, the capacitor was soldered approximately 1 mm from the microcoil, see Figure 3.12. The small lead lengths have adverse effects on the stray inductance in the circuit, see section 4.6.3.

The circuit in Figure 3.5b is the resonance circuit used for “small” microcoils. these coils are typically less than 100 \( \mu \)m diameter with fewer than seven turns. The “small” microcoils do not generally require a matching capacitor. “Larger” microcoil can require the addition of a matching capacitor in the circuit. see Figure 3.5a. In this case.
the 50 Ω microstrip transmission line on the rf board is cut and an additional chip capacitor is soldered across as the matching capacitor. The matching capacitor should be placed close to the LC tank circuit.

3.6.1. VARIABLE CAPACITORS

For the first version of the rf board variable capacitors were used from Johanson Manufacturing, series 2320. Typically, a 10 pF variable tuning capacitor was used while matching value was approximately 15 pF. However, Q values for these capacitors are typically ~50 or less indicating a significant quantity of inductance in the capacitor as the
capacitor approaches self-resonance. Any amount stray inductance becomes comparable to the inductance of the microcoil as even a few nanohenries can dominate the total inductance in the circuit. Figure 3.13 shows the Q plot for 2320 series of capacitors provided by Johanson Manufacturing. The stray inductance in these capacitors proved detrimental to the signal to noise ratio and consequently the next version of the rf board uses chip capacitors. The variable capacitors reduced the signal to noise ratio by an approximate factor of 100 compared with similar studies done with chip capacitors in Chapter 4 as the stray inductance decreased from ~10 nH to ~1.4 nH.

3.6.2. CHIP CAPACITORS

Chip capacitors solve the problems of stray inductance. A typical 0402 capacitor has ~250 pH of inductance\(^\text{1}\) comparable to the microcoil, but no longer dominant. However, chip capacitors have additional problems similar to the problems of rf connectors. The solder pads on the edge of the capacitor typically have a layer of nickel in-between the silver connection to the electrodes and the solder platting on the outside. This layer of nickel does cause significant homogeneity problems in the static magnetic field due especially to the closeness of the capacitor to the microcoil creating a effective \(T_2^*\) of less than 200 \(\mu\)s from ~10 ms in our magnet.

To measure the magnetic susceptibility of the chip capacitors several different capacitors from different manufactures where placed in a superconducting SQUID. The
Figure 3.13. The Q values (vertical scale) are shown as a function of frequency (horizontal scale) for different 2320 series capacitors. The two versions used in version 1 were the 2320-4 and 2320-5 both of which have poor Q's at 400 MHz that indicates significant stray inductance in the capacitor. This information was provided by the manufacturer.

The capacitors tested are: Panasonic ECJ 0402 capacitors with nickel inner plating, Metuchen 0402 capacitor with Palladium/Silver termination, and American Technical Ceramics Series 100 A Non-Magnetic 0505 capacitors. The results are shown in Table 3.4. The 0402 capacitors are paramagnetic while the 0505 ATC 100 A capacitors are diamagnetic. Even the removal of the nickel inner plating in the Pd/Ag 0402 chip does not remove the magnetism from the capacitor.

The ATC 100 A chip capacitors provide the best choice for insuring magnetic field homogeneity. The volume magnetic susceptibility of both copper and FR-4 are both
<table>
<thead>
<tr>
<th>Capacitor</th>
<th>Magnetic Susceptibility</th>
<th>Normalized Magnetic Susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>0402 Panasonic ECJ Series</td>
<td>2.34E-3</td>
<td>1</td>
</tr>
<tr>
<td>Metuchen 0404 Pd/Ag</td>
<td>5.94E-4</td>
<td>0.253</td>
</tr>
<tr>
<td>ATC 100 A non-magnetic</td>
<td>-1.45E-3</td>
<td>-0.0575</td>
</tr>
</tbody>
</table>

Table 3.4: The magnetic susceptibility data is presented for several different chip capacitors. The normalized magnetic susceptibility assumes the 0402 Panasonic ECJ has a value of one. The remaining capacitors are normalized to that value and adjusted for the volume difference in the ATC 100 A series. The ATC 100 A non-magnetic capacitors are diamagnetic instead of paramagnetic like the 0402 chip capacitors.

diamagnetic and are similar to the magnetic susceptibility of the 0505 chip capacitor. Since the capacitor, copper and the FR-4 have similar magnetic properties, the magnetic field gradients formed due to the magnetic susceptibility differences are smaller and the $T_1$ returns to approximately 10 ms as compared to using the 0402 chip capacitors. The 0505 capacitors have flaws as well: there increase size required the planes of the gradient boards to be further apart, increasing the current demand to attain large gradients. Additionally, the 0505 capacitors have approximately 350 pH of stray inductance, slightly more than the smaller packaged 0402 capacitors. Figures 3.14a provides Q dependence on frequency for the 0505 ATC capacitors and Figure 3.14b shows the self-resonance frequency for the capacitor as an indication of stray inductance in the capacitor. The effect on the linewidth by the 0402 capacitor and the 0505 capacitor by local magnetic field homogeneity is shown in Figure 3.15. The same coil was tuned with different types of capacitors resulting in a large difference in the linewidth of the Fourier transformed spectrum. Some data presented in Chapter 4 use the 0402 chip capacitors, but the capacitor of choice is the ATC 100 A 0505 (non-magnetic) capacitors for the superior homogeneity effects. Figure 3.16 shows a rf coil tuned with a 0505 capacitor.
Figure 3.14. The ATC Series 100A capacitor have superior magnetic properties. In part A, the Q dependence as a function of capacitance is shown and the self-resonance frequency is shown in part B. This information was provided by the manufacturer.
Figure 3.15. The field inhomogeneity caused by the 0402 capacitor is demonstrated by the increased linewidth of the spectrum. The 0505 capacitor causes a more homogeneous magnetic field. The same microcoil and rf board were used and tuned with each capacitor.
3.6.3. TUNING THE RESONANCE CIRCUIT

The impedance of the circuit in Figure 3.5b can be calculated from the impedance of individual components. For capacitors the impedance is \( X_C = -i\omega C \) and for inductors the impedance is \( X_L = i\omega L \). The resonance condition for the tuning circuit, as defined by forcing the impedance to be real can be shown to be.

\[
1 - \omega^2 CL = \frac{r^2 C}{L} = 0. \tag{3.6}
\]

Figure 3.16. A microcoil is tuned with a 51 pF 0505 ATC Series 100 A capacitor.
The resonance of the circuit then occurs at

$$\omega = \left( \frac{LC}{\sqrt{1 + \frac{1}{Q^2}}} \right)^{1/2}$$

which reduces to the normal resonance condition for large $Q$ values.

At resonance, the impedance can be shown to be

$$Z = \frac{r^2 + \omega^2 L^2}{r}.$$  \hspace{1cm} (3.8)

and this reduces to $Z = Q^2 r$ in the limit of large $Q$. This resonance circuit does use the matching capacitor to impedance match the LC tank circuit to the 50 $\Omega$ transmission line possibly causing power reflections of the excitation pulse and the NMR signal.

### 3.6.4. POWER LOSS IN UNMATCHED RESONANCE CIRCUITS

The standing wave ratio (SWR) is a measure of the mismatch between the transmission line and the load at the end of the transmission line$^{25-27}$. The SWR is not the ratio of forward to reflected power but the ratio of the maximum to the minimum value of the voltage. The SWR is given by.

$$SWR = \frac{V_r + V_c}{V_r - V_c}$$

\hspace{1cm} (3.9)
where $V_f$ is the forward voltage and $V_r$ is the reflected voltage. In terms of power by the relation $P = V^2/R$, the

$$SWR = \frac{\sqrt{P_f} + \sqrt{P_r}}{\sqrt{P_f} - \sqrt{P_r}}.$$  \hspace{1cm} (3.10)

where $P_f$ is the forward power and $P_r$ is the reflected power. An additional method of defining impedance mismatch is to define a reflection coefficient, $\rho$ as

$$\rho = \frac{V_r}{V_f} = \frac{SWR - 1}{SWR + 1}. \hspace{1cm} (3.11)$$

Under ideal matching condition, the reflected power is zero, resulting in a SWR of one.

Assume the transmission line terminates in a load of $R_0 + R_s$, where $R_0$ is equal to the characteristic impedance of the transmission line, $R_0 = Z_0$, and $R_s$ is the extra load (either positive or negative). Figure 3.17 depicts the set-up described above. As an example, if the total load is 60 $\Omega$ and $Z_0$ is 50 $\Omega$ then $R_s$ is 10 $\Omega$.

The current in the circuit, assuming $R_s$ is positive, is given by

$$I = \frac{V_f}{Z_0 + R_0 + R_s}. \hspace{1cm} (3.12)$$

where $V_f$ is the forward voltage in the circuit. The reflected voltage is then the voltage drop across the extra load, $R_s$. The reflected voltage $V_r$ is

$$V_r = \frac{V_f R_s}{Z_0 + R_0 + R_s}. \hspace{1cm} (3.13)$$

The maximum and minimum voltages in the circuit are

$$V_{\text{max}} = V_f \left(1 + \frac{R_s}{2R_0 + R_s}\right). \hspace{1cm} (3.14)$$
The standing wave ratio is

\[ SWR = \frac{R_i}{Z_0} \left( \frac{2R_i}{2Z_0 + R_i} \right) = R_i \frac{Z_0}{Z_0} \left( \frac{2Z_0 + R_i}{2R_i} \right) \]

where \( R_i \) has been defined at the actual load of the circuit. If \( R_i \) is negative, \( R_i \) is replaced with \(-R_i\) in Equation 3.12 and then \( SWR = Z_0 / R_i \). The SWR is simply the ratio of the characteristic impedance to the load impedance in the form required to ensure \( SWR \geq 1 \).
The power loss is usually expressed in dB, and is expressed by

\[ dB = 10 \log \frac{P_i - P_r}{P_r} \]  

(3.17)

where dB > 0 represents a power gain and dB < 0 represents a power loss. Using Equation 3.17 and Equation 3.10 yields the power loss as a function of the SWR.

\[ dB = 10 \log \frac{4 \text{SWR}}{(\text{SWR} + 1)^2} \]  

(3.18)

Figure 3.18 shows the relationship between the power loss and the standing wave ratio.

Measuring the forward and reflected voltages in the NMR circuit\(^{11}\) yields a SWR of 1.2 to 2.0. Typical power losses in the NMR circuit with microcoils without a matching capacitor yield power losses between 0.1 dB and 0.5 dB. The effect of poor matching with the transmission line has only a small effect on the power transmission to and from the circuit and the transmission line.

### 3.7. MEASURING THE Q OF A MICROCOIL

Using Figure 3.5b. and the requirement that the voltage drop around the circuit to be zero, results in the equation.

\[ \frac{q}{C} + L \frac{di}{dt} + iR = 0 \]  

(3.19)

where q is the charge on the capacitor and i is the current in the circuit. Differentiating the equation with respect to time gives.
Figure 3.18. The standing wave ratio can be measured to determine the power loss in the unmatched circuit. For SWR's less than 2 (the SWR is plotted on a log scale) the power loss is not significant to warrant the addition of a matching capacitor.

\[
\frac{i}{LC} + \frac{d^2i}{dt^2} + \frac{R}{L} \frac{di}{dt} = 0. \tag{3.20}
\]

Solving this equation,

\[
i(t) = e^{-\omega_0 \sqrt{\frac{1}{4Q^2} - 1}} \left( Ae^{-\omega_0 \sqrt{\frac{1}{4Q^2} - 1}} + Be^{-\omega_0 \sqrt{\frac{1}{4Q^2} - 1}} \right) \tag{3.21}
\]

where A and B are constants determined by initial conditions. It is interesting to note that in the low Q limit, a frequency shift occurs that is not observed in normal NMR limit of
high Q's characterized by the exponentials inside the parenthesis. In the limit of high Q, the exponentials simplify to $e^{\pm i \omega t}$. 

The term in parenthesis in Equation 3.21 is an oscillating function, but the exponential term outside indicates a decaying echo envelope. By measuring the decay of the envelope, it is possible to measure the Q of the coil. A NMR pulse to the coil causes the coil to "ring" after the pulse. The ringing of the NMR pulse decays as the exponential in Equation 3.21. Measuring the $\sqrt{e}$ time of the decay results in a value for Q.

$$Q = \frac{t_r}{\omega_0} = \pi f t_r$$  (3.22)

where the substitution of $\omega_0 = 2\pi f$ has been made. Figure 3.19 shows the ring down of a typical microcoil, a coil of five turns, made from 20 μm copper wire with spacing between adjacent turns equal to twice the wire diameter. The $\sqrt{e}$ time is 8 ns resulting in a Q of 9.6 at 383 MHz. Measured Q's varied from 5 to 20 in our microcoils, with the largest coils possessing the highest values.

Now, with Q measurements and the theoretical resistances from section 1.6.3.3, an approximate value of the impedance of the LC tank circuit can be found from Equation 3.8. The average value of Q for many microcoils is approximately 10, with resistances of ~0.5 Ω, the impedance is very close to 50 Ω for the average microcoil. This provides justification for removing the matching capacitor from the circuit.

Additionally, calculating Q from the relation $Q = \frac{\omega_0 L}{R}$ results in a values comparable to the method described above. Using the inductance given in Equation 3.5

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Figure 3.19. The ringdown of a five turn microcoil constructed with 20 μm diameter copper wire. The length of the microcoil is ~100 μm and the diameter is ~100 μm. The 1/e ring down time was measured to be 8 ns giving a Q of ~10. The rf on the left of the figure is the remains of the rf pulse used to excite the spins in the microcoil.

for a five-turn coil made from copper wire of 20 μm diameter with wire spacing equal to twice the diameter, results in an inductance of 1.4 nH. Using Figure 1.12, a theoretical value of the resistance of 0.6 Ω is found. Adding inductance of the microcoil to the average value of the stray inductance, 1.4 nH, found in all the microcoils, see chapter 4 for an analysis of stray inductance, is the total inductance in the LC circuit. Calculating Q from the total inductance, microcoil plus the strays, and theoretical resistance gives a value of ~11. comparable to the experimental values. The agreement signifies that the losses in the coil are dominated by the resistance in the wire and not any dielectric losses from the quartz coil form or the epoxy bonding the coil to the micropipette.
3.8. LIST OF REFERENCES


Belden Wire and Cable.


4.1. INTRODUCTION

In principle, there are great advantages that can be gained in the signal to noise ratio (SNR) of nuclear magnetic resonance (NMR) experiments on very small samples (having spatial dimensions 100 μm or less) if one employs unusually small receiver coils, "microcoils", that are of similarly small dimensions. The dimensions of microcoils (tens to hundreds of microns) contrast with those of the receiver coils typically used in commercial systems (millimeters to centimeters). In recent years, several investigators have developed and advanced the technology of microreceiver coils and applied it to important problems of NMR spectroscopy and imaging.
Microcoils can be used to work towards the goal of proton ($^1$H) MRI of single biological cells with spatial resolution of 1-2 microns in all three spatial dimensions. (Note that MRI has successfully imaged very large biological cells with size of order 1 mm and with resolution in the 10's of \( \mu m \). The goal is to image cells of more typical size \( \sim 10-100 \ \mu m \), with 1-2 \( \mu m \) resolution.) The most formidable obstacle to high resolution MRI is the need for adequate signal to noise ratio (SNR). As the attempted image resolution increases, the number of nuclear spins within each voxel decreases, along with the SNR. Microcoils are essential to overcome this loss and to achieve maximum resolution. Microcoil NMR however is not straightforward. The microcoil is ideally thought of as a discrete element of a resonant tank circuit, tuned with capacitors that are adjusted to match the NMR resonance frequency. Given the tiny dimensions of microcoils, careful design is required in order that the energy stored in the resonant circuit is concentrated in the capacitors and coil, rather than in effective "stray" elements.

Using the design described in Chapter 3 and version 2 of the rf board, eleven coils were built having a range of coil volumes from \( 10^4 \) to \( 10^5 \ \mu m^3 \) (diameters from \( \sim 1 \ \text{mm} \) to as small as \( 20 \ \mu m \)). The signal to noise for each coil was then experimentally measured. For the smallest coils the signal to noise obtained appears adequate for proton magnetic resonance imaging microscopy with resolution of \( (1 \mu m)^3 \). Specifically, the SNR is approximately one for a single scan applied to a volume of approximately \( 2 \mu m \times 2 \mu m \times 2 \mu m \) \( (\sim 10 \ \text{femtoliters}) \) of water, or \( \sim 7 \times 10^{11} \) proton spins \( (\sim 1 \ \text{picomole}) \). This SNR was obtained using coils of dimensions less than 100 \( \mu m \) with an applied field of 9 Tesla (resonance frequency 383 MHz), and applying the Carr-Purcell-
Meiboom-Gill (CPMG) sequence\textsuperscript{14,15} to obtain an effective decay time $T_2$ of $\sim 1$ s. This minimum number of detectable spins is an order of magnitude smaller than the reported values obtained using the new technology of magnetic resonance force microscopy.

$\sim 10^1$ protons at room temperature\textsuperscript{16}.

Along with the results from the SNR microcoil measurements, the dependence of SNR on several important parameters is also included. These parameters are the wire spacing between adjacent turns, the resistance of the coil wire, and the length of the coil's "leads" to the microcoil. The effects of varying the preceding attributes and the corresponding effect on the measured signal to noise ratio are presented.

\subsection*{4.2. THEORITICAL CONSIDERATIONS}

The NMR signal is an electromotive force (emf) induced in the receiver coil by precessing nuclear magnetic moments. The signal competes with background Johnson noise due to resistance losses in the receiver coil\textsuperscript{2,4,17-21}. see Chapter 1. The time domain SNR for a solenoid coil with the sample fully enclosed is given by:\textsuperscript{23}

$$\frac{Signal}{Noise} = \frac{\omega_0^2 N \gamma h^2}{4 k_B T_{\text{sample}}} \sqrt{\frac{\mu_0 Q}{4 k_B T_{\text{en}} B V_{\text{coil}}}}.$$  \hspace{1cm} (4.1)

$V_{\text{coil}}$ is the coil volume, $N$ is the total number of spins, and $Q$ is the quality factor of the resonant circuit. $\omega_0$ is the NMR resonance frequency, $k_B$ is Boltzman's constant, and $B$ is
the receiver bandwidth. $T_{\text{sample}}$ is the temperature of the sample, and $T_{\text{eff}}$ is a temperature characterizing the noise of the system. Typically, with losses occurring predominantly in the rf receiver coil, one can take $T_{\text{eff}}$ equal to $T_{\text{nd}} + T_{\text{amp}}$, where $T_{\text{amp}}$ is the noise temperature of the preamplifier, typically ~80-100 K for low noise commercial preamplifiers. (Note that a "noise figure" of 1dB corresponds to an amplifier noise temperature of ~75K.) SNR in Equation 4.1 is defined as the time domain signal amplitude of the NMR free induction decay at its peak, divided by the root mean square Johnson noise. Equation 4.1 also gives SNR in Fourier domain, defined as the peak height in Fourier domain divided by root mean square noise, if one substitutes a frequency of order of the signal linewidth $\sim T_{\text{eff}}^{-1}$ (see discussion in section 4.5.4) for the bandwidth B.

Of course, multiple factors in Equation 4.1 influence SNR. However, constraining our attention to the goal of observing live biological cells having a fixed size and composition, and performing the experiment at room temperature, then the options are more limited. One tries to maximize Q, but in practice Q is limited by the resistivity of the metal used in the rf coil at room temperature, see Section 3.7. One can attempt to cool the coil while maintaining the sample at room temperature, but inevitably this also requires a larger volume coil$^{18}$. There is only one variable that may be varied over a large range for this situation, and that is the volume of the coil, $V_{\text{vol}}$, which is constrained only in that it must be large enough to contain the sample. For biological cells of dimension 10-100 $\mu$m one may reduce $V_{\text{vol}}$ drastically from its typical values (dimensions of mm's
to cm's) and still contain the sample. This is the microcoil NMR approach that which we pursue here.

While it appears simple to decrease the volume of the coil and thereby obtain SNR benefits as predicted in Equation 4.1, in practice great care must be taken. The assumption of Equation 4.1 is that all energy stored in the rf resonant circuit resides in the capacitors and the rf coil, with no "stray" energy and loss residing in, for example, the leads from the capacitors to the coil. As one moves to smaller coils, one expects that the leads and other "stray" effects may become more important. Thus, a tidy, compact design and construction will be crucial.

4.3. PREPARING A SERIES OF MICROCOILS

In order to test the dependence of the signal to noise on coil volume predicted by Equation 4.1, copper coils were wound with diameters ranging from 1 mm to 20 μm. The coils were wound with spacing between centers of adjacent wires equal to twice the diameter of the wire. The coil wire diameters used were either 10 or 20 μm copper wire (Goodfellows or California Fine Wire), except for the coils having the two largest coil volumes, where wire diameters of 60 μm were used. The number of turns in the coil varied from 2 to 10. The coils were soldered and placed on the rf board, version 2, and tuned to the resonance frequency of 383 MHz with Panasonic ECJ 0402 chip capacitors.
Tuning was assessed by sending rf to the probe through a directional coupler and monitoring the reflected amplitude. For each coil successive values of capacitance were tried until a value was found that achieved tuning at the fixed frequency of 383 MHz. See Chapter 3 for a complete discussion of tuning and coil winding.

4.4. STRAY INDUCTANCE IN THE MICROCIRCUIT

The stray inductance in the microcoil circuitry limits the effective filling factor of the microcoil, limiting the signal to noise ratio. To measure the stray inductance for each coil constructed, as described above, the capacitance required for tuning at 383 MHz was measured. From that measurement, the effective total inductance of each tank circuit can be inferred, according to the relation \( \omega_0 = \left( L_{\text{total}} C_0 \right)^{-1} \). Then for each inductor an estimate of its inductance, without the stray inductance from the leads, using the infinite solenoid approximation \( L_{\text{enl}} = \mu_0 n^2 V_{\text{enl}} \), where \( n \) is the number of turns per unit length can be determined.

Figure 4.1 shows the measured total inductance \( L_{\text{total}} \) and the inductance \( L_{\text{enl}} \) vs. the capacitance required to tune the microcoil, \( C_0 \). In the case where \( Z_0 = 50 \Omega \neq Q^2 R \), a matching capacitor, a 0402 series ECJ Panasonic, was added in series to the tank circuit, see Figure 3.5a. and \( C_0 = C_{\text{match}} + C_{\text{inl}} \). The matching capacitor was required for only the three largest coil volumes. The difference between the two curves is the stray
Figure 4.1. Capacitance required to tune each coil vs. the calculated inductance of each coil. The theoretical curve is the capacitance expected in the absence of stray inductance, according to the relation $\omega = \left( L_{\text{ind}} C \right)^{-1}$. As the inductance is reduced by going to smaller volume coils, the experimentally measured capacitance approaches an asymptotic value of ~120pF.

To illustrate the difference between the two curves, Figure 4.2 shows the total inductance plotted against the inductance from the microcoil with a non-zero y-intercept corresponding to the average stray inductance. The stray inductance is from lead lengths, capacitors, and rf feedthroughs to the ground plane.
The behavior observed in Figure 4.2 is that $L_{\text{meas}}$, the measured total inductance, varies linearly with the calculated inductance of the coil, $L_{\text{coil}}$, but with a non-zero intercept of 1.4 nH. For the very smallest inductances the tuning capacitance saturates to a value of ~120 pF, again illustrating the effect of a stray inductance of 1.4 nH. Note that $\left[\omega_c L\right]^{-1} = 120 \text{ pF}$ for a frequency of 383 MHz and inductance 1.4 nH.

The clear interpretation of Figure 4.2 and analysis presented above is that the "stray" inductance, presumably from leads, contributes a fixed value of approximately 1.4 nH. This is a rather small value of stray inductance compared to all but the smallest microcoils. For example, a coil of diameter 100 μm and length 100 μm, with 5 turns will have inductance of ~2.5 nH. Thus, the small microcoil circuitry is successful without being dominated by the stray effects.

A reasonable source of the remaining stray inductance is the leads to the coil. The inductance of a straight wire in nH is given by:

$$L_{\text{w}} = 0.2\left[\ln\left(\frac{l}{d}\right) - 0.75\right]$$

where $l$ is the length and $d$ the diameter of the wire, both given in millimeters. The last factor, 0.75, tends towards unity as the frequency increases. Our leads have length of order 1 mm, with 10 μm diameter wire, with an estimated stray inductance of ~0.8 nH. The other significant source of stray inductance is in the capacitors themselves. The Q for the 0402 capacitors is not infinite at resonance frequency and thus has a characteristic inductance of 200 to 300 pH. The remaining stray inductance is in the rf board itself:
Figure 4.2. The measured inductance for 11 coils vs. the calculated inductance for each coil based on its known geometry, but without including inductance contributions from lead lengths. The measured inductance was obtained by measuring the capacitance required to tune each coil to a resonant frequency of 383 MHz. The non-zero intercept of -1.4 nH suggests that the leads, of length of order 1 mm, contribute stray inductance of order 1.4 nH, which agrees with expectations.

Traces on the board and the copper feedthrough to the rf ground plane both of which add additional lead lengths.
4.5. MEASURING THE SIGNAL TO NOISE RATIO AND DEPENDANCE ON COIL VOLUMES

The SNR measurement on the eleven constructed micro-coils and microcircuits is explained in the following sections. In addition to the measurement of the individual coils, important theoretical and experiment considerations are discussed: Carr-Purcell-Meiboom-Gill pulse sequence, theoretical signal to noise calculations and Fourier space bandwidths.

4.5.1. EXPERIMENTAL PROCEDURES

Eleven microcoils, wrapped on micropipettes, and mounted on microcircuits were prepared and tuned as described above. Each micropipette, with microcoil wrapped on it, was dipped in water, so that water completely filled the micropipette. The radius and length of the cylindrical volume of water inside each microcoil was measured using a microscope and the volume of water inside the coil was calculated. On multiple occasions, it was tested and confirmed that no signal was present when the micropipettes were not dipped in water, or when the water had evaporated.

The goal is to find the signal to noise that would be observed in one scan on a sample containing 1 μm$^3$ of water. For all coils, however, a larger volume of water was
present in the micropipette and within the coil, and the measured signal to noise was normalized by dividing by the actual volume of water in the coil, measured in μm$^3$. In all cases, water was also present outside of the solenoid microcoil, because the cylindrical micropipette extended fully through the coil. The estimated signal from the water coming from outside the micropipette is at most a few percent of the total and is considered negligible.

Multiple scans were accumulated and averaged ("signal averaging"). The SNR measured after multiple scans was normalized by dividing by the square root of the number of scans.

4.5.2. CARR-PURCELL-MEIBOOM-GILL (CPMG)

The "Carr-Purcell-Meiboom-Gill (CPMG)" pulse sequence was employed$^{1,26}$ to measure the signal to noise ratio. The CPMG sequence, illustrated in Figure 4.3, consists of a spin echo sequence, see Chapter 1, followed by a train of repeated 180° pulses that repeatedly re-form the echo. The spacing between 180° pulses was typically 1.2 ms, and typically, 4096 echoes were formed and recorded. The pulse durations $T_{as}$ and $T_{180}$ typically of order 5 μs and 10 μs respectively. In all cases the signal sizes were measured as a function of pulse duration to find the correct values of $T_{as}$ and $T_{180}$.
Figure 4.3. The Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence and the procedure used here to assess signal amplitude and SNR. The CPMG sequence: a 90° pulse along the x axis in the rotating frame, followed by a delay \( \tau \), then a train of 180° pulses, separated by 2\( \tau \), along the rotating frame y axis. Echoes form between each pair of 180° pulses.

The CPMG sequence acts to effectively increase the time duration of the NMR signal from a time \( T_2 \), which is limited by magnetic field inhomogeneity in our system, to a much longer time \( T_1 \), which characterizes the irreversible decay of the amplitude of each successive echo. \( T_2 \) is typically one second. The echoes are recorded in the CPMG train for a time duration \( t_{\text{max}} \), which is taken to be equal to or somewhat longer than \( T_2 \). The echoes toward the end of this time interval \( t_{\text{max}} \) are diminished in height relative to those at the beginning. Figure 4.4a shows the echoes of a CPMG pulse sequence. In the figure, there are too many echoes to be individual discerned and only the echo envelope is visible. The CPMG echo train was obtained in 100 scans on a sample of water having volume \( 1.4 \times 10^7 \mu m^3 \), using a coil of 9 turns, length 280 \( \mu m \), diameter 100 \( \mu m \), and wire diameter 20 \( \mu m \). In Figure 4.4b, the first several echoes of the Figure 4.4a are shown.

To compare the effects of the CPMG pulse sequence on the linewidth of the Fourier spectrum, the free induction pulse sequence also must be examined. Consider a
Figure 4.4. In part A, the entire CPMG train of echoes, containing 4096 echoes with a total of 2,097,152 total points, only the envelope of the train can be discerned here. The envelope decays with a time constant, $T_2$, of ~1 s. In part B, the first few digitized CPMG echoes in a train of 4096 total echoes (512 points digitized per echo with dwell time 2 $\mu$s), with $2\pi=1134$ $\mu$s. Total digitization window for each echo is 1024 $\mu$s. Digitizer is not turned on during the 180° pulses and for a short time before and after the "dead time". The rf oscillator frequency is chosen such that the NMR frequency is offset by an amount equal to the reciprocal of the digitization window, $(1/1024)\mu s^{-1} = 976.5$ Hz; thereby the train of echoes is not only an almost periodic function (with period 1024 $\mu$s) but also a function that is nearly continuous from one echo digitization window to the next.
free induction decay experiment consisting of a 90° pulse of width $T_{\text{en}}$ and immediate acquisition of the signal\textsuperscript{13,15,27}. The resulting NMR signal is

$$s(t) = e^{i\Omega t} e^{-t/T_2^*}.$$ \hspace{1cm} (4.3)

where $\Omega$ is the difference in frequency of the NMR resonance frequency and the oscillator. In Equation 4.3, the signal decays with a time of $T_2^*$ due to the inhomogeneities of the magnetic field. Fourier transforming Equation 4.3 gives the usual NMR Lorentzian functions of the absorption (real part) and dispersion curves (imaginary part) of the frequency spectrum:

$$A(\omega) = \frac{1}{T_2^* \left( \frac{1}{T_2^*} + \frac{1}{\Omega} \right)}.$$ \hspace{1cm} (4.4)

and

$$D(\omega) = -\frac{1}{\Omega \left( \frac{1}{T_2^*} + \frac{1}{\Omega} \right)}.$$ \hspace{1cm} (4.5)

The linewidth of the absorption curve of Equation 4.4 is $\Delta \omega = 2/T_2^*$ and can approach ~1 kHz. Since the integral over $\Omega$ remains constant, as the linewidth decreases the height must increase. This decrease in linewidth, by allowing $T_2^* \rightarrow T_2$, increases the signal to noise in frequency space.

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This section shows the mathematical derivation of the Fourier transform of the Carr-Purcell-Meiboom-Gill pulse sequence. Figure 4.5 is a representation of the first few echoes of the CPMG pulse sequence demonstrating the variables used in the derivation. The time $T$ is the time acquired for each echo and $f(t)$ is the signal from each echo, assumed the same for each echo. Only the right half of the first echo is acquired.

The Fourier transform of the echo train is

$$S(\omega) = \left[ \int_0^T f(t) e^{i\omega t} dt + \sum_{n=1}^{\infty} \int_{\frac{nT}{2}}^{\frac{(n+1)T}{2}} f(t) e^{i\omega t} e^{-\frac{i\omega nT}{2}} dt \right] e^{\theta}.$$  \hspace{1cm} (4.6)

where $\theta$ is an arbitrary phase. The integral and can be rewritten as

$$S(\omega) = \left[ \int_0^T f(t) e^{i\omega t} dt + \sum_{n=1}^{\infty} e^{i\omega nT} \int_{\frac{nT}{2}}^{\frac{(n+1)T}{2}} f(t) e^{i\omega t} e^{-\frac{i\omega nT}{2}} dt \right] e^{\theta}.$$ \hspace{1cm} (4.7)

by a change in variables. An assumption has been made in writing Equations 4.6 and 4.7. The signal height of each echo only decays at the 'center' of the interval in Equation 4.7. and the decay of the echo height is not continuous, but discrete. Defining $F(\omega)$ to be the Fourier transform of $f(t)$ and assuming that $f(t)$ is symmetric about zero, Equation 4.7 can be written as a geometric series. The first few terms of the series are:

$$S(\omega) = \left[ F(\omega) \left[ 1 + e^{i\omega T} e^{-\frac{T}{2T}} + e^{2i\omega T} e^{-\frac{2T}{2T}} + \ldots \right] - \frac{F(\omega)}{2} \right] e^{\theta}.$$ \hspace{1cm} (4.8)
Figure 4.5. The variables used in the derivation in section 4.5.2.1. are shown here for clarification. Each echo is digitized for a time $T$ and the echo envelope decays with a time constant $T_2$.

The geometric series $a + ar + ar^2 + \ldots$ can be summed to $\frac{a}{1-r}$ for $r$ less than one, as is the case in Equation 4.8. The series sums to

$$S(\omega) = \left[ F(\omega) \left[ \frac{1}{1 - e^{-\frac{\tau}{T_2} \omega}} \right] - \frac{1}{2} \right] e^{\theta}.$$  

(4.9)
and by phasing the spectrum with \( \theta = \frac{\pi}{2} \). Equation 4.9 becomes only real as the imaginary part of \( S(\omega) \) is zero. The real part of \( S(\omega) \) is:

\[
S(\omega) = F(\omega) e^{\frac{r}{T}} \frac{\cos(\omega T)}{\cos(\omega T)}
\]  \hspace{1cm} (4.10)

The extremes of Equation 4.10 occur when \( \sin(\omega T) = 0 \). By inspection of Figure 4.6, which is a plot of Equation 4.10, the maximums occur at \( \omega T = 2n\pi \) or

\[
f = \frac{n}{T}
\]  \hspace{1cm} (4.11)

where the relationship between \( f \) and \( \omega \); \( \omega = 2\pi f \) has been used. The maximums occur at integer value of the inverse of the acquisition time for each echo. The peaks of Equation 4.10 occur for every value of \( n \) to make Equation 4.11 valid inside the lineshape of \( F(\omega) \), which provides an envelope function for the CPMG peaks. Figure 4.7 demonstrates the consequences of Equations 4.10 and 4.11 in the Fourier transform of a CPMG pulse sequence.

The height of each CPMG peak can be calculated by substituting Equation 4.11 into Equation 4.10 and solving for \( S(\omega) \). The height of the Fourier transformed peaks are

\[
\text{Height of CPMG Peaks} = F(2n\pi) e^{\frac{r}{T}} \frac{1}{1 - e^{\frac{-r}{T}}}
\]  \hspace{1cm} (4.12)

and in the limit of \( T_2 \to \infty \), the peaks also approaches infinity, similar to the delta function formed by Fourier transforming a infinite sine or cosine wave. However, in the
Figure 4.6. Equation 4.10 has maximums for $\omega T = 2n\pi$ which is evident for the plot of the equation in the figure above.

usual limit of CPMG pulse sequences, $T > T_s$, where several hundred or thousand echoes are acquired during each acquisition cycle. Equation 4.12 simplifies to $T_s/2T$.

From Equation 4.12, the Half-Height-Full-Width (HHFW) of the peaks can be calculated. The HHFW measures the linewidth of the peak as the full width of the lineshape at half the total height of the line. The HHFW of the CPMG peaks is

$$HHFW = \frac{2}{T} \cos^{-1} \left( \frac{1}{2 - e^{-\frac{\tau}{T}}} \right).$$

(4.13)
Again, assuming $T > T_2$, Equation 4.13 reduces to

$$HHFW = \frac{2}{T_2}.$$  \hspace{1cm} (4.14)

where the linewidth of the CPMG peaks is "Lorentzian". The comparison of Equation 4.14 and the result form Equation 4.4 finds the linewidths of the overall NMR line $F(\omega)$ to be similar in form to the linewidth of each CPMG peak. With $T_1' < T_2$, the CPMG
pulse sequence serves to narrow the resonance linewidth, increasing the height of the peak and the signal to noise ratio. The CPMG also serves to “shrink” the effects of the magnetic field inhomogeneities and susceptibilities into just one frequency.

4.5.2.2. MAXIMIZING THE SIGNAL TO NOISE IN A CPMG ECHO TRAIN

The Fourier domain signal of the CPMG echo train is similar to which is obtained in “magic angle spinning (MAS)”\textsuperscript{17} with “sideband” peaks. These “sideband” peaks are separated by a frequency equal to the reciprocal of the time between echo formations (whereas in MAS the peaks are separated by the rotor frequency). To maximize the signal to noise of the CPMG pulse sequence, the sequence should be initiated to give only one CPMG peak. This is accomplished by centering the Fourier transform of a single echo off resonance by $1/T$, where $T$ is the time acquired for each echo in the CPMG echo train. Only one CPMG peak is guaranteed if the acquisition time is short compared to the inhomogeneous decay time $T_2^*$. Analogously, if the linewidth of a single echo is less than $1/T$, then only one major peak will appear, having a linewidth characterized by $2/T_2$. The acquisition time, $T$, should be chosen to satisfy both of the above conditions for the CPMG sequence to yield the maximum possible signal to noise. Figure 4.8 shows
Figure 4.8. The Fourier transform of the CPMG train in Figure 4.4. Since the rf oscillator frequency is chosen such that the NMR frequency is offset by an amount equal to the reciprocal of the digitization window, \((1/1024)\mu s^{-1} = 976.5 Hz\), thereby the train of echoes is not only an almost periodic function (with period 1024 \(\mu s\)) but also a function that is nearly continuous from one echo digitization window to the next. A single major peak is at 976.5 Hz with linewidth of (full width at half maximum) 0.4 Hz.

The resulting lineshape of Fourier transforming the CPMG echoes in Figure 4.4a, the HHFW of the line is approximately 0.4 Hz, whereas the HHFW of the FID \(-1000\) Hz.
4.5.3. MAXIMIZING THE SIGNAL TO NOISE RATIO

Limiting the total time of signal acquisition in the time domain can maximize the signal to noise in the frequency domain. In the time domain, the signal decays, assuming the oscillator is on resonance, by

\[ s(t) = s_0 e^{-t/T_z}, \quad (4.15) \]

where \( s_0 \) is a constant and \( T_z \) is the characteristic transverse magnetization time in the presence of field inhomogeneities. In frequency space, the signal to noise ratio is

\[ \frac{S}{N} = \sqrt{n t_{\text{max}}} \frac{1}{\rho_n}. \quad (4.16) \]

where \( n \) is the number of scans, \( t_{\text{max}} \) is the total time data was acquired in the time domain, \( \bar{s} \) is the time-averaged signal in the time domain and \( \rho_n \) is the square root of the frequency independent power spectral density, to be defined in the following section. \( \bar{s} \) is defined to be

\[ \bar{s} = \frac{1}{t_{\text{max}}} \int_0^{t_{\text{max}}} s_0 e^{-t/T_z} dt. \quad (4.17) \]

Evaluating the integral in Equation 4.17 and substituting into Equation 4.16 yields the signal to noise ratio in frequency space.

\[ \frac{S}{N} = s_0 \frac{T_z}{\sqrt{t_{\text{max}} \rho_n}} \left[ 1 - e^{-t_{\text{max}}/T_z} \right]. \quad (4.18) \]
assuming $t_{\text{max}} > T_z$. Graphing Equation 4.18. in terms of $T = t_{\text{max}} / T_z$, the remaining factors being constant, displays a maximum value at $T = 1.25T_z$, see Figure 4.9. To attain the highest signal to noise in frequency space, the CPMG echo train should be truncated at the time $T_{\text{final}} = nT = 1.25T_z$, where $n$ is the number of acquired echoes. By acquiring longer than the cutoff time, the relative signal to noise in each echo decreases to the amount that it no longer significantly contributes to the signal but only to the noise.

![Graph](image-url)

**Figure 4.9.** The signal to noise versus $t/T_z$. A maximum is reached at $t=1.25T_z$. 

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4.5.4. BANDWIDTH IN FOURIER SPACE

To adequately calculate a theoretical signal to noise ratio in frequency space, an analogous bandwidth to time domain value must be derived. In time domain, the bandwidth is twice the cutoff frequency of the filters used to record the NMR signal, but is not as straightforward to calculate in the frequency domain.

The expression for the frequency independent power spectral density is, see Equation 4.16, is

\[ \rho_s = \sqrt{4k_B T_{\text{sys}} R} \quad \text{(4.19)} \]

where \( k_B \) is Boltzman's constant, \( T_{\text{sys}} \) is the temperature of the coil and system and \( R \) is the characteristic impedance of the coil, usually 50 \( \Omega \). Substituting Equation 4.19 into Equation 4.18 and comparing the result with the Johnson noise:

\[ \text{Johnson Noise} = \sqrt{4k_B T_{\text{sys}} R B} \quad \text{(4.20)} \]

where \( B \) is the bandwidth, yields the frequency space effective bandwidth.

\[ B_{\text{eff}} = \frac{t_{\text{max}}}{T_2} \quad \text{(4.21)} \]

The remaining terms in Equation 4.18 are from the signal.

Equation 4.9 is only valid in the condition that \( t_{\text{max}} \geq T_2 \). For \( t_{\text{max}} \) equal to \( T_2 \) (corresponding to recording each successively diminishing echo until an echo amplitude 1/e times that of the first echo is reached) the effective bandwidth is \( B_{\text{eff}} = T_2^{-1} \). The bandwidth expression in Equation 4.9 was used to calculate the theoretical signal to noise
ratio to compare to the experimental value to theoretical model. Equation 4.1, in testing
the microcoils. The value used in the effective Fourier bandwidth was 1 Hz. If one did
not apply CPMG, but instead only recorded the first echo, then the effective bandwidth to
appear in the SNR expression in Equation 4.1 would be \( B_{\text{eff}} = \left( T_2 \right)^{-1} \), or \(-1 \text{ kHz}\). The
CPMG then provides an enhancement factor in SNR of order \( \sqrt{1000} \approx 30 \).

4.5.5. SIGNAL TO NOISE RESULTS

Signal to Noise was measured using the CPMG pulse sequence for the eleven
coops, spanning four orders of magnitude in volume. The results are shown in Figure 4.10
where the SNR are normalized to 1 scan on 1 \( \mu \text{m}^3 \) of water and is plotted as SNR vs.
\( 1/\sqrt{V_{\text{coil}}} \). This type of plot is chosen because the expression for the SNR (Equation 4.1)
predicts a linear dependence on \( 1/\sqrt{V_{\text{coil}}} \) if other important factors including Q are held
constant. A best-fit line of the form \( \text{SNR} \approx 1/\sqrt{V_{\text{coil}}} \) is shown as well. Remarkably, and
perhaps surprisingly, the data follow this functional form rather well.

Along with this experimental data, for each coil, the theoretically predicted SNR
from Equation 4.1 is also shown, using the experimentally measured Q values and a
bandwidth of 1 Hz. see Section 4.5.4. Measured Q's varied from 5 to 30, with the largest
Figure 4.10. Filled circles: Signal to noise for one scan on one $1 \mu m^3$ of water, as measured for 11 coils with volumes ranging from $10^4 \mu m^3$ (1 mm diameter) to $10^2 \mu m^3$ (20 $\mu m$ diameter), vs. $\sqrt{V_{coil}}$. Closed squares: Theoretically predicted SNR for each coil, using Equation 4.1 and the measured Q values for each coil. Solid line shows the form $S/N \propto \sqrt{V_{coil}}$ that would be expected if each coil had the same Q. For the smallest coils, a SNR of 0.1 is obtained, indicating a SNR of 1 for a single scan on only 10$\mu m^3$ of water.

coops possessing the highest values. There are no adjustable parameters in this theoretical prediction. The very high agreement between experiment and theory is surprising because for the very smallest coils the inductances are $\sim 0.3 \text{ nH}$, less than the measured stray inductance of 1.4 $\text{nH}$.
The SNR vs. $\sqrt{V_{coil}}$ plot demonstrates several important items. First, by going from millimeter diameter coils down to 10's of μm diameter coils one gains a factor of about 100 in SNR. Second, the SNR that can be obtained for the smallest coils is unprecedentedly high. A SNR of ~0.1 is obtained for one scan applied on a 1 μm³ sample of water. Equivalently, the SNR is approximately one for a single scan applied to a volume of approximately $2μm \times 2μm \times 1μm$ (~10 femtoliters) of water, or approximately $7 \times 10^{11}$ proton spins (~1 picomole). This minimum number of detectable spins is an order of magnitude smaller than the best reported value obtained using the new technology of magnetic resonance force microscopy, $10^{15}$ spins. Of course one can signal average, repeating the experiment in a time comparable to the water $T_1$ of a few seconds, to observe still smaller volumes; signal to noise increases as the square root of the number of experiments.

The SNR is also much greater than that reported in previous work of Peck et al. on microcoil NMR. They find for their smallest coils of diameter ~50 μm a SNR of ~$10^{-4}$ for a single scan of 1 μm³ of water, while our measurement indicate ~$10^{-1}$. Certainly the main factor contributing the higher SNR reported here is the application of the CPMG sequence, which enhances the SNR by ~30. A second factor explaining the SNR values being higher than those reported by Peck et al. is that the experiment is at a higher magnetic field of 9 Tesla, while they used a 4.7 Tesla field. This probably accounts for a factor of 2-4. Finally, our tank circuit is more compact, with less stray inductance. Peck et al. and Olson et al. insert a 50 Ω transmission line of length several centimeters.
between the capacitors and the micro-coil, while the capacitors are placed quite close, within distance ~1 mm.

4.6. DEPENDENCE OF SNR ON OTHER FACTORS

Having established the great benefits in signal to noise provided by microcoils in detecting small sample volumes, some additional experimental tests are presented. The parameters investigated are the resistivity of the wire, the wire spacing between turns and the length of the leads to the microcoil.

4.6.1. COIL RESISTIVITY

The resistivity and wire spacing are both expected to affect SNR. as given in Equation 4.1 through their effects on \( Q = \omega L / R \), with \( SNR \propto \sqrt{Q} \). Naively, one might expect that the coil resistance, \( R \), would remain proportional to the resistivity, \( \rho \), through the relation \( R = \rho l / A \), with \( l \) the total length and \( A \) the cross sectional area of the coil wire. At rf frequencies, however this expectation is not met. The resistance of the coil at rf frequencies, \( R_{AC} \), is greater than its DC value, \( R_{DC} \), due to two effects. A brief review
of the two effects are presented here for completeness, see Section 1.6.3.3 for additional information.

### 4.6.1.1. THEORETICAL RESISTANCE OF MICROCOILS

First, the skin depth\(^{15}\) of wire decreases the cross sectional area of the wire and increases the resistance of the coil. The skin depth effect limits the current to flow in a cylindrical shell of width \(\delta\). The skin depth of a wire is

\[
\delta = \sqrt{\frac{2}{\mu \omega \sigma}}
\]

where \(\mu\) is the permeability and \(\sigma\) is the conductivity of the wire. \(\delta=3.3 \mu m\) for copper at 383 MHz. The added AC resistance caused by the skin depth is modeled by\(^{15,14}\).

\[ R_{\text{skin}} = R_{p} F \]

where

\[
F = \left(\frac{d}{2\delta}\right) \frac{J_0 \left(\frac{d}{\delta}\right)}{J_1 \left(\frac{d}{\delta}\right)}
\]

and \(d\) is the wire diameter, and \(J_0\) and \(J_1\) are zero and first order Bessel functions respectively.

Second, the close proximity of wires in adjacent turns of the coil induces eddy currents in neighboring wires that also effectively increase the total resistance of the coil.
through the “proximity effect”, see section 1.6.3.3. Eddy currents are closed loops and do not change the total current in the wire, but restrict the effective cross sectional area of the wire. The net current flow is moved further to the surface of the wire.

A theoretical model for AC resistance in rf coils is shown by Butterworth\(^{14}\), he gives the AC resistance of a ‘few’ turn single layer coil as

\[ R_{AC} = R_{DC} \left[ F + \mu_{tr} G \left( \frac{d}{s} \right)^2 \right] \]  

(4.24)

where \( G \) is the proximity factor, presented as lookup table in Butterworth’s papers as a function of \( z = d/\delta \). The spacing between adjacent wire centers is given by \( s \) and for the coils presented in this paper, \( d/s \) is usually 0.5. \( \mu_{tr} \) is a geometrical factor dependent on coil dimensions and number of turns, also given by Butterworth. For 5.5 turns of 25 \( \mu m \) diameter wire wrapped on a 100 \( \mu m \) diameter coil, the \( \mu_{tr} \) is approximately seven.

AC resistance is shown as a function of the resistivity in Figure 4.11 based on Equation 4.5 for above dimensions, demonstrating the two regimes in resistance. The first regime is when \( d/\delta (\rho<1) \) is small and the current flows uniformly through the cross sectional area of the wire and the resistance is linear in \( \rho \). The second, is when \( d/\delta (\rho>500) \) is large causing the current to be restricted to the surface of the wire and the resistance is proportional to \( \sqrt{\rho} \). At 383 MHz the ratio of \( d/\delta \) for any metal is not in either region but lies in-between the two extremes and consequently the dependence of the SNR on \( \rho \) lines in-between these limits for microcoils at 383 MHz.
Figure 4.11. The two regimes for AC coil resistance. For high resistivities, the resistance is proportional to the resistivity. At low resistivity, the resistance is proportional to the square root of the resistivity. The AC resistance is calculated for a five-turn microcoil and 20 μm diameter wire with spacing between turns set at twice the wire diameter.

4.6.1.2. EXPERIMENTAL RESISTANCE MEASUREMENTS

To test the impact of wire resistivity upon SNR, a set of six 5.5 turn coils were wound with 25 μm diameter wire using various metal with resistivities ranging from 1.7 to 50 Ω-cm. The coils wound were made from copper (ρ=1.69 Ω-cm), gold (ρ=2.2 Ω-cm), brass (ρ=6.4 Ω-cm), platinum (ρ=10.6 Ω-cm), platinum/iridium alloy (ρ=20 Ω-cm).
and Manganin ($\rho=50$ $\Omega$-cm). The coils were then tuned with a parallel capacitor in a LC circuit to the resonant frequency of 383 MHz. Despite the very similar geometry of the six coils, it was observed that systematically lower capacitance values were required to tune the coils having high resistivity, see Figure 4.12. This finding is not surprising: while the resonant frequency in the limit of high $Q$ is given by $\omega_0 = \sqrt{LC_0 / \omega}$, where $C_0$ is the capacitance required to tune a “high” $Q$ coil. The more general expression is

$$\omega_0 = \sqrt{\frac{1}{LC(1+\frac{1}{Q^2})}}.$$  \hspace{1cm} (4.25)

where the required capacitance is related to $C_0$ by

$$C_{\text{min}, Q} = \frac{C_0}{1 + \frac{1}{Q^2}}.$$  \hspace{1cm} (4.26)

The signal to noise for each coil was measured using a free induction decay sequence on samples of water inside each micropipette. Corrections were applied to the SNR for small variations in water volume within the various coils.

Figure 4.13 shows the measured SNR vs. microcoil resistivity. Also shown (solid curve) is the theoretically expected dependence of SNR on resistivity based on Equations 4.1 and 4.5 and the dependences of $F$ and $G$ on $z$ as given by Butterworth. $u_{\text{eff}}$ is taken as 7, appropriate (again following Butterworth) for the geometry of this coil. The observed experimental dependence of SNR on $\rho$ is much stronger than predicted: the dashed line shown is a power law fit of the experimental data of the form $S/N \propto \rho^{-0.75}$, while the Butterworth theory predicts an exponent varying between -0.25 to $-0.4$ over this resistivity range.
Figure 4.12. The tuning capacitance changes from the expected high Q limit and the value of the tuning capacitance is smaller for high resistivity metals. \( C \) is the capacitance that is expected in the high Q limit and \( C' \) is the value that tuned the microcoil to resonate at 383 MHz. The difference between these two values is plotted on the vertical scale. The high Q limit is valid for metals such as copper and gold, but for other metals the low Q's reduce the value of the tuning capacitor.

4.6.2. SIGNAL TO NOISE DEPENDANCE ON WIRE SPACING

Next, the dependence of SNR on the wire spacing between adjacent turns is tested. The parameters in Equation 4.5 used are \( F=3.39; \ u_{\text{eff}}=7; \ G=0.932 \), values appropriate for the coil geometry used here as prescribed by Butterworth. Among NMR practitioners it is widely believed, based on the Butterworth theory, that the solenoid geometry yielding optimum SNR performance has turn spacing \( s \) between the centers of
Figure 4.13. Signal to noise vs. micro-coil resistivity is shown for a constant coil geometry. SNR is normalized to one for a Cu coil and measured for several micro-coils made of various metals, as labeled. Each has 100 μm outer diameter, 360 μm length, and 5.5 turns of wire. Wire diameter in each case is 25 μm. Also shown (solid curve) is the theoretically expected dependence of SNR on resistivity based on Equation 4.1 and 4.24, the Butterworth expression for the AC resistance of a coil.

The parameters in Equation 4.24 are F=2.39; μ,=7; G=0.932, appropriate for the coil geometry used here as prescribed by Butterworth. The observed experimental dependence of SNR on ρ is much stronger than predicted. The dashed line is a power law fit of the experimental data of the form $S/N \propto \rho^{-0.755}$.

Adjacent wires equal approximately 2d, where d is the wire diameter. This experiment tested the conception for the microcoil circuit and found that the greatest SNR is obtained for wire spacing d/s approaching one, the limit in which the wires are touching. The signal to noise was once again tested using a Free Induction Decay experiment.
To test the dependence of SNR on wire spacing, a series of Copper coils using 20 μm diameter wire were prepared. Each coil has length 280 μm, outer diameter 100 μm; but each coil has a different number of total turns. Figure 4.14 shows, for each of these coils, the experimentally measured signal to noise ratio vs. microcoil d/s. Also shown is the theoretically predicted dependence of SNR on d/s, as obtained from Equation 4.1 and 4.5. The theoretical prediction shows a broad maximum for d/s~0.6, which corresponds to a wire spacing (center to center) of ~1.7 wire diameters. Experimentally though, a closer wire spacing approaching one provides a greater SNR. The experimental result gives a SNR some ~60% greater for d/s approaching one than for d/s=0.6. This unexpected result might reflect effects of “stray” elements in the circuit; however, the inductances of the coils made here are rather larger than the estimated stray of 1.4 nH. For example, the coil here with d/s~0.6 has an estimated inductance of ~2.5 nH.

4.6.3. SIGNAL TO NOISE EFFECTS ON LEAD LENGTHS

Inevitably as one uses microcoils of smaller dimensions, the issue of lead length arises. For example, for a coil of 5.5 turns and outer diameter of 100 μm, the total length of wire within the coil itself is 1.73 mm. Intuitively one expects that it will be desirable to keep lead lengths small compared to this length of wire within the coil.
Figure 4.14. Closed circles: Experimentally measured signal to noise ratio vs. micro-coil d/s, where d is the wire diameter and s the spacing between the centers of adjacent micro-coil turns. Data are normalized to one for the micro-coil having the greatest SNR. Each micro-coil has length 280 µm, outer diameter 100 µm, and is made with Cu wire of 20 µm diameter. It is found experimentally in this case that SNR is maximized for d/s approaching one, the limit in which adjacent wires would be touching each other. Solid curve: Theoretically predicted dependence of SNR on d/s, as obtained from Equations 4.1 and 4.24 as in Figure 10. The theoretical prediction shows a broad maximum for d/s=0.6, which corresponds to a wire spacing (center to center) of ~2 wire diameters, a recipe familiar to NMR practitioners. Experimentally though we find here that a closer wire spacing provides greater SNR. Note that the inductance estimated for the coil having d/s=0.6 and these coil dimensions is 2.5 nH, larger than but comparable to the estimated stray inductance of ~1.4 nH.

In order to test this expectation, a Copper coil was prepared with dimensions of 25 µm diameter and wire turn spacing d/s=0.5. One lead on the coil was intentionally left unnecessarily long and the leads were attached to the microcoil to the circuit board and tuned it by iteratively trying different capacitance values. Figure 4.15 shows the coil with
Figure 4.15. This is the coil used to test the effect of stray inductance. Notice one lead is extremely long approximately 1.2 cm in this photo. The lead was cut and resoldered several times to measure the signal to noise as a function of lead length.

an extremely long lead and Figure 4.16 shows the capacitance required to tune the microcircuit at each lead length. The lead length information is plotted as a ratio of the lead length to the total length of the wire in the microcoil, leads plus the coil. It can be shown that the decreases in capacitance is due to the added inductance in lead length and not a significant change in Q using Equation 4.2. Butterworth's theory and the relation $Q = \omega L/R$. Q remains approximately constant over the range of increasing stray inductance.
Figure 4.16. For longer leads and an increase in stray inductance, the tuning capacitance decreased as expected.

First, the signal to noise is measured for the first length and then the lead is clipped shorter and the signal to noise measurement is repeated. Continually repeating with successively shorter leads, until a minimum lead length of 600 μm is reached, the total for both leads that can be adequately manipulated and soldered. The results of SNR vs. ratio of lead length to the total length of the microcoil are given in Figure 4.17. As expected the SNR diminishes greatly as the lead length becomes long compared to the length of the wire in the coil. SNR using lead lengths of ~1 cm are more than 90% smaller than the SNR for the short leads of 600 μm. The signal to noise data has not been compared to a theoretical model.
Figure 4.17. Measured SNR vs. lead length, for a Cu micro-coil of outer diameter 100 μm, with 5.5 turns of 25 μm diameter wire (coil wire length 1730 μm). To make the measurement the coil was first prepared with leads much longer than necessary. SNR was first measured; then the leads were repeatedly clipped and re-soldered, and SNR measured. SNR for the shortest leads is normalized to one.
4.7. LIST OF REFERENCES


CHAPTER 5

TRI-AXIAL MAGNETIC FIELD GRADIENTS

5.1. INTRODUCTION

As discussed below, for ultra-high resolution imaging, gradient magnitudes of greater than 10 Tesla/m are necessary along all three axes x, y, and z (for gradients $G_x = dB_x / dx$, $G_y = dB_y / dy$, and $G_z = dB_z / dz$). The field gradient is, of course, a tensor. However, for high field imaging only the z component of the applied gradient field, where z is the direction of the applied static field $B_z$, has any substantial effect on the NMR frequency. Therefore, following custom, 6 of the 9 tensor components are ignored and the gradient is written as a vector. In this chapter, the development of the necessary gradient system and its use with the microcoil NMR apparatus is discussed and explained. The developed gradient system obtains gradient fields higher than the
required 10 T/m, typically 20 T/m or greater, over a cubic volume of (600 \mu m)^3. In addition to the fundamental obstacle of signal to noise ratio, discussed in Chapter 4, however, there is also a technical challenge, the need for rapidly switchable magnetic field gradients with strength sufficient to overcome the problem of diffusion of the spins during signal acquisition. The gradient power supply will be discussed in detail in Chapter 6, including the switching characteristics of the gradients.

In all MRI schemes, the spatial origin of signal intensity is determined by measurement of signal frequency or phase accumulation. The precession frequency of a nucleus is directly proportional to the local value of the applied magnetic field. Then, with a known spatially varying applied field, the frequency specifies a location. For a strong, uniform magnetic field $B_0$ applied parallel to the $z$ axis, and a gradient $G = \left[ (\partial B_z / \partial x) \hat{i} + (\partial B_y / \partial y) \hat{j} + (\partial B_z / \partial z) \hat{k} \right]$, the NMR resonance frequency of a spin located at position $\vec{r}$ is given by:

$$\omega(\vec{r}) = \gamma \left( B_0 + \vec{G} \cdot \vec{r} \right)$$

(5.1)

where $\gamma$ is the nuclear gyromagnetic ratio ($2\pi \times 42.5759 \text{MHz/T}$ for protons).

In order to measure a spin’s location with precision $\Delta x$, one must necessarily measure the frequency with a corresponding precision $\Delta \omega$. By the uncertainty principle, that measurement requires a minimum acquisition time duration, $\Delta T_{acq} \geq 1/\Delta \omega$. For high resolution, then, one requires that the product of the gradient strength and the time of measurement, $\Delta T_{acq}$, be large. In particular, the resolution $\Delta x$ is given by $^{24}$:

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\[
\Delta x \geq \frac{\pi}{\gamma G_t T_{\text{aq}}} \tag{5.2}
\]

If, however, the spins diffuse spatially during the acquisition, then of course the frequency and spatial resolution may be further limited, according to:

\[
\Delta x \geq \sqrt{\frac{2DT_{\text{aq}}}{3}} \tag{5.3}
\]

where \(D\) is the diffusion constant, approximately \(2.2 \times 10^{-5} \text{ cm}^2/\text{s}\) for water. The diffusion for intracellular water may be about \(70\%\) of the bulk diffusion constant.

It is clear from Equations 5.2 and 5.3 that in order to achieve high resolution it is necessary to acquire quickly, with a short \(T_{\text{aq}}\), to minimize the impact of diffusion in Equation 5.3, while maintaining a large product \(\gamma G T_{\text{aq}}\), so that the frequency can be adequately resolved, as in Equation 5.2. The only way to achieve both goals is to use strong gradients. A spatial resolution of \(\sim 1 \mu m\) is the desired goal in order that meaningfully image biological cells of typical dimension \(\sim 10-100 \mu m\) can be obtained. Combining Equations 5.2 and 5.3 results in a relationship between the desired one-dimensional resolution, \(\Delta r\), and the required gradient strength:

\[
G = \frac{2\pi D_{\text{cellular}}}{3\gamma (\Delta r)^3}. \tag{5.4}
\]

A plot of Equation 5.4 is given in Figure 5.1, demonstrating the high gradient fields required. The problem of diffusion, Equation 5.3 then requires \(T_{\text{aq}} \leq 1 \text{ms}\), using the bulk
Figure 5.1. Higher resolutions in MRI require ever increasing gradient strengths. For resolutions less than 2 μm in one dimension, the required gradient strengths increase rapidly.

The gradient magnitudes required (order ~10 T/m or 1000 G/cm) are quite large as compared with typical values employed in clinical imaging (~1-10 G/cm) and in current MRI microscopy work (typically a few hundred G/cm). On the other hand, much larger gradients as 250,000 T/m are obtained, for example, over very small volume regions near the ferromagnetic tips of the newly developed NMR force microscopes. The unique features of the gradients reported here are that (1) they are triaxial (i.e., $\partial B_x/\partial x$, $\partial B_y/\partial y$, and $\partial B_z/\partial z$), (2) they are adequately uniform over (600 μm)³ as compared with
the sample dimensions of ~100 μm, and (3) they are rapidly switchable from positive to negative with variable magnitude, so that the rich array of MRI pulse/gradient sequences that have been developed may be used. Chapter 6, the discussion of the gradient power supply will thoroughly discuss the switching characteristics and magnitude adjustments of the gradient pulses.

5.2. GRADIENT SYSTEMS

Two different gradient systems were developed depending on the type of capacitor used to tune the microcoil. The differences are required due to the thickness of the capacitors themselves that must fit in-between the gradient boards. The 0402 capacitors have a thickness of 0.020", while the 0505 capacitors require a clearance of 0.057". See Chapter 3 for a complete discussion on capacitors. The clearance restrictions require two different dimensions for the gradient coils geometry that is explained below. However, since the gradient field is proportional to the inverse of the separation of the gradient coils, the 0402 gradients will produce stronger gradients than the 0505 gradients for a given current through the gradient coils.

The 0402 gradient boards of Figure 5.2 can produce gradients, applied to a water sample in the micropipette described in Chapter 3. $G_x = \frac{\partial B_z}{\partial x}$, $G_y = \frac{\partial B_z}{\partial y}$, and $G_z = \frac{\partial B_z}{\partial z}$, where $z$ is the direction of the static field $B_0$. The vertical gradient $G_z$ is
Figure 5.2. Top, bottom, and side views of the gradient coil platform are shown. SMA connectors give a size scale. The gradient coils are made with 30-gauge wire-wrap wire and lie in the planes of two pc boards, which are displaced vertically by 1.5 mm. The counter-wound circular coils on the top and bottom planes of the gradient board produce the vertical gradient $G_z$. Rectangular gradient coils, labeled in the figure, produce the horizontal gradients $G_x$ and $G_y$. The rf tank circuitry board is inserted between the two gradient boards, in the gap that is visible in the "side" view in the figure.

achieved using two counter-wound circular loops, connected in series, with one on each of the two gradient boards. The horizontal gradients $G_x$ and $G_y$ are each achieved using "rectangular" configurations, to be described more fully below. Again (for both $G_x$ and $G_y$) there are coil patterns on each of the two boards, connected in series.
5.2.1. ANTI-HELMHOLTZ COILS FOR G₂

The anti-Helmholtz coil is composed of two loops of wire; in one loop, the current proceeds clockwise and in the other loop counterclockwise. Figure 5.3 shows the current pattern for the anti-Helmholtz coil. The magnetic field along the axis, z direction, for this configuration can be written as:

\[ B_z = \frac{1}{2} \frac{\mu_0 I r_z^2}{\left( r_z^2 + \left( \frac{1}{2} d + z \right)^2 \right)^{3/2}} - \frac{1}{2} \frac{\mu_0 I r_z^2}{\left( r_z^2 + \left( \frac{1}{2} d - z \right)^2 \right)^{3/2}}. \]  

(5.5)

The optimal configuration is found by expanding the derivative of Equation 5.5 with respect to z in a series expansion in terms of z.

\[ \frac{dB_z}{dz} = -48 \frac{\mu_0 I r_z d}{(4r_z^2 + d^2)^{3/2}} - 1920 \frac{\mu_0 I r_z d (d^2 - 3r_z^2)}{(4r_z^2 + d^2)^{3/2}} z^2 \]

\[ -26880 \frac{\mu_0 I r_z d \left( 10r_z^4 - 10r_z^2d^2 + d^4 \right)}{(4r_z^2 + d^2)^{3/2}} z^4 + O(z^6), \]  

(5.6)

where the first term is the desired constant gradient along the axis of the coils. The second term in the first non-linear term in the expansion and for optimal configuration of the anti-Helmholtz coil with only one set of loops this term is forced to be zero. The solution presents a restriction on the ratio of the diameter to the radius of the loops, namely

\[ d = \sqrt{3} r. \]  

(5.7)
The magnetic field along the axis for the optimal configuration for a single pair of loops is shown in Figure 5.4.

The magnetic field as a function of the position \((r,z)\) for one loop of wire \(i\) is

\[
B(r, z) = \frac{\mu_0 I}{2\pi} \frac{1}{\sqrt{(r_c + r)^2 + z^2}} \left[ K(u) + \frac{r_c^2 - r^2 - z^2}{(r_c - r)^2 + z^2} E(u) \right].
\]  

(5.8)

where \(K(u)\) and \(E(u)\) are elliptical integrals of the first and second kinds respectively and \(r_c\) is the loop radius. The argument in the elliptical integrals, \(u\), is

\[
u = \sqrt{\frac{4r_r}{(r_c + r)^2 + z^2}}.
\]  

(5.9)
Figure 5.4. The magnetic field gradient of an optimally configured anti-Helmholtz coil. The gradient field is highly linear at the center of the two loops of wire.

The magnetic field gradient defined over the entire volume of the two loops comprising the anti-Helmholtz pair is

\[ \frac{dB_z}{dz} = \frac{dB_z}{dz} \left( r, z - \sqrt{3}r \right) - \frac{dB_z}{dz} (r, z) \]

and is shown in Figure 5.5 in units of \( \mu_0 I \) and for a loop radius of one unit. The magnetic field at the geometric center of the two loops is

\[ G_z = \frac{48\sqrt{7} \mu_0 I}{343 r^2} \]  \hspace{1cm} (5.10)

where the constant has an approximate value of 0.64. The gradient is adequately linear, within 5%, in a sphere of radius 0.6\( r_0 \).
5.2.2. RECTANGULAR COILS FOR $G_x$ AND $G_y$

The gradient profiles of the long, rectangular coils $G_x$ and $G_y$, which are visible in Figure 5.2, are not easily visualized. In order to understand these coil configurations consider the schematic of Figure 5.6. In Figure 5.6, the rectangles are approximated as having infinite length to width ratio, and illustrate the current flow through a plane perpendicular to the long direction of the rectangle (taken for the moment to be x). The
Figure 5.6. The rectangular gradient coils producing $G_x$ and $G_y$ can be understood in terms of eight long, parallel wires, oriented and carrying current as shown. The current direction is indicated for each wire, either into or out of the page. The $z$ component of the magnetic field is shown as a function of position as the field changes along the $y$-direction providing the desired gradient. Of course, this configuration can be rotated 90° to achieve a $z$ component magnet field gradient along the $x$-direction.

The magnetic field near the center of the configuration is pictured. One can see from this illustration that the $z$ component of magnetic field will vary linearly as one moves along the $y$ direction, and hence we have the desired gradient $G_z = \partial B_z / \partial y$.

The variables used to optimize the gradients are shown in Figure 5.7 as well as the current direction in each wire to ensure a symmetric field. The ratios $w/z_n$ and $y/z_n$ can be optimized to achieve the strongest linear gradient possible. The eight wires used to configure the gradient coil and assumed to have the magnetic field of

$$B(y,z) = \frac{1}{2} \frac{\mu_0 I (y - y_0)}{\pi \left( (y - y_0)^2 + (z - z_0)^2 \right)}$$

(5.11)

where the wires are considered infinite compared to the sample dimensions in the $x$ direction. The wire is at position $(y_0, z_0)$. The total magnetic field then is the sum of Equation 5.11 for all eight wires taking care to sum properly over the direction of current in the wire.
Figure 5.7. The current configuration used to produce \( G \). This configuration has two parallel planes, located a distance \( 2z_0 \) apart, each containing two loops of current. The loops in one plane lie directly above (below) the loops in the other plane; however, in the figure we have displaced the planes so that both can be visible. Parameters \( w \), \( y_c \), and \( 2z_0 \) are defined in the figure, and values are given in Table 5.1.

\[
B_{\text{pair}}(y, z) = \sum_{\text{pairs of planes}} \frac{\pm}{\text{current direction}} \frac{\mu_0 I^* (y - y_0)}{2\pi \left( (y - y_0)^2 + (z - z_0)^2 \right)} 
\]

(5.12)

where the sum is over the pairs of coordinates \( \left( y + \frac{w}{2}, \pm z_0 \right) \), \( \left( y - \frac{w}{2}, \pm z_0 \right) \), \( \left( -y + \frac{w}{2}, \pm z_0 \right) \), and \( \left( -y - \frac{w}{2}, \pm z_0 \right) \). For this symmetric relation, the field at \((y, z) = (0, 0)\) is zero for all ratios of \( w/z_0 \) and \( y_c/z_0 \) that is the prime consideration to form a linear gradient. It can be shown that the in the expansion

\[
B_j(y, z) = B_j(0, 0) + y \frac{dB_j(0, 0)}{dy} + y^2 \frac{d^2B_j(0, 0)}{dy^2} + y^3 \frac{d^3B_j(0, 0)}{dy^3} + \cdots
\]

the zeroeth and second order terms in \( y \) are zero. The first order term is the desired linear gradient.
leaving the highest non-zero term the third derivative of the magnetic field. This term can be made zero by manipulating the ratios $w/z_0$ and $y/z_0$.

There are four numerical solutions to the forcing the third derivative to zero; only two of the solutions are geometrically different. The solutions are shown in Figure 5.8. The two solutions are evaluated for the maximum gradient strength in Figure 5.9. Using Figure 5.9, the maximum magnitude for the linear gradient $\frac{dB}{dy}$ occurs for the ratio

$$\frac{w}{z_0} = 1.55 \quad (5.13)$$

corresponding to

$$\frac{y}{z_0} = 1.19 \quad (5.14)$$

in Figure 5.8. Together these two ratios provide the optimal configuration to get the strongest linear gradient from these eight wires. Using the optimum ratios, the field gradient is shown in Figure 5.10. This derivation holds for the assumption of infinite wires in the $y$ direction and to evaluation of $G_i$ instead of $G$. The gradient strength at the center of the wire configuration is

$$G_i = G_y = 0.46 \frac{\mu_0 I}{z_0} \quad (5.15)$$

where the optimal ratios have been used to evaluate the magnitude. The gradient is sufficiently linear within a cube of side length $0.4z_0$. 

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Figure 5.8. There are two solutions to force the third order term in the expansion of the rectangular gradients to zero, indicated by the two curves. For each value of \( y/z_0 \), there is at least corresponding value of \( w/z_0 \) that causes the third order term to be zero.

Figure 5.9. Using the solutions found in Figure 5.8, the field gradient was calculated for each configuration of the \( y_0 \) and \( w \). Examination of the graph above shows that there is an optimal configuration for the gradients to give the strongest linear field.
Figure 5.10. The field plot is shown for the optimally configured rectangular gradient system. The gradient is sufficiently linear with a cube of 600 μm on a side.

5.2.3. THE COMPLETE GRADIENT SYSTEM

An Autocad™ rendition for the construction of the gradient boards is shown in Figures 5.11 and 5.12. Figure 5.11 is the gradient system compatible with the 0402 capacitors while Figure 5.12 is the system used with the 0505 capacitors. In both cases, a
Figure 5.11. A rendition of the 0402 gradients systems is shown. The separation between the planes in the gradients only fits the 0402 capacitors. Since the gradient separation is smaller than the 0505 gradients, these gradients can obtain stronger gradients.

0.070-inch sheet of G-10 is machined with a 0.025-inch end mill to follow the winding pattern of the gradient coils. The spacing between the two planes is fixed with a spacer to ensure proper z, for the G. The G gradient was wound in a groove 0.060 inch deep by 0.025 inch wide on both planes of the gradient system. Next, the G gradient was wound in a groove 0.040 inch deep by 0.025 inch wide, with different proportions of the rectangles corresponding to the increased separation between the planes of the gradient coils. Last, the G gradient was wound in a groove 0.020 inch deep by 0.025 inch wide.
Figure 5.12. The 0505 gradients are very similar in design to the 0402 gradients. The only differences are slightly different geometries of the gradient coils due to the larger separation of the planes necessary to fit the 0505 capacitors.

with radii to match the planar separation. Table 5.1 provides the dimensions for each of the gradient systems.

<table>
<thead>
<tr>
<th></th>
<th>0402 Gradients</th>
<th>0505 Gradients</th>
</tr>
</thead>
<tbody>
<tr>
<td>$G_x$</td>
<td>$z_n$</td>
<td>1.51</td>
</tr>
<tr>
<td></td>
<td>$w$</td>
<td>2.33</td>
</tr>
<tr>
<td></td>
<td>$y$</td>
<td>1.79</td>
</tr>
<tr>
<td>$G_y$</td>
<td>$z_n$</td>
<td>2.02</td>
</tr>
<tr>
<td></td>
<td>$w$</td>
<td>3.12</td>
</tr>
<tr>
<td></td>
<td>$y$</td>
<td>2.40</td>
</tr>
<tr>
<td>$G_z$</td>
<td>$z_n$</td>
<td>2.52</td>
</tr>
<tr>
<td></td>
<td>$r$</td>
<td>2.91</td>
</tr>
</tbody>
</table>

Table 5.1. The dimensions in millimeters for the different gradient systems are shown above. The 0402 gradient system and the 0505 system are shown. Since the initial spacing between the planes is smaller to accommodate the smaller 0402 capacitors, the overall dimensions of the 0402 gradients are also smaller.
In the grooves that are milled for the gradient coils the gradient wiring is inserted. Either 30 gauge wire-wrap wire or 24 gauge varnish insulated copper wire is used, each with an outer diameter of 0.020 inch. One end of the wire in each coil is soldered to the center pin of a SMA PC-mount vertical connector on the lower plane of the gradient system. The wire is then positioned in the groove and wound on both planes and secured with Loctite 420 (superglue) and the remaining end is soldered to the body of the same (floating) SMA connector on the top plane. The wire for the deepest coil, in our case \( G \), is laid first, followed by \( G_s \) and \( G_t \). The SMA connectors are also fixed to the gradient boards with superglue. This method allows the current to be carried in a coaxial cable, with current flowing down the center conductor and up the outer conductor minimizing any magnetic force interaction with the static magnetic field in NMR. The required currents to achieve 12 T/m in each gradient are in Table 5.2.

<table>
<thead>
<tr>
<th></th>
<th>0402 Gradients</th>
<th>0505 Gradients</th>
</tr>
</thead>
<tbody>
<tr>
<td>( G )</td>
<td>47.1 Amps</td>
<td>75 Amps</td>
</tr>
<tr>
<td>( G_s )</td>
<td>84.1 Amps</td>
<td>120 Amps</td>
</tr>
<tr>
<td>( G_t )</td>
<td>126 Amps</td>
<td>169 Amps</td>
</tr>
</tbody>
</table>

Table 5.2. The current required for the high gradient field is shown in the table above for each gradient system. The price of increased field homogeneity with the 0505 capacitors is the increase in current required to achieve 12 T/m gradient strength.

A discussion on maximum currents and duty cycle of the gradients as well as the gradient power supply is in Chapter 6. The \( G \), and \( G_s \) gradients wrapped with 30 gauge wire typically have resistances ~ 0.140 ohms. If they were wrapped with 24 gauge wire,
the resistances were lower, approximately 0.030 ohms. The G, gradients with 30 gauge wire have resistances of approximately 0.040 ohms but if they were wrapped with 24 gauge wire, the resistance was only ~0.005 ohms. The gradients reported here have inductance estimated to be of order ~0.1 μH.

As evident in the table, the price of field homogeneity with the 0505 capacitors requires higher currents to achieve the same gradient magnitudes. A 0505 gradient system wound with 24-gauge wire is shown in Figure 5.13. the SMA connectors used to attach the gradient to the probe are non-magnetic. see section 3.5. An assembled view complete with the gradients, rf board, and the pillar system used to position the boards in the vertical dimension complete with a side view profile of the system is shown in Figure 5.14. Chapter 3 contains information of winding the coils and positioning the coil with respect to the gradient system.

5.3. GRADIENT TESTING

Two samples were constructed to test the gradient strengths for the three gradient coils G₁, G₂, and G₃: first, a (non-ideal) cylinder of water, and second, a (non-ideal) cylinder of water containing a 20 μm Polystyrene Divinylbenzene bead (Duke Scientific Corporation). In both cases the axes of the cylinder is parallel to the y-axis of the
Figure 5.13. The 0505 gradient system shown here is wrapped with 24 gauge wire. All three gradient coils are wrapped on the two planes of the gradient system. The picture at the left is the top view, the center picture is the bottom plane and then the right picture is a side profile showing the gap between the two planes to allow the capacitor to fit.

gradient system. The 0402 gradient system with version one of the rf board was used in testing the gradient strengths.

5.3.1. (NON-IDEAL) CYLINDER OF WATER

Using the gradients on a finite sample of know geometry allows for the measurement of the gradient strength as the gradients map the spin density. In this example, a 'non-ideal' (to be explained below) cylinder is used. A gradient applied perpendicular to the axis will yield an elliptical lineshape, while a gradient parallel to the
Figure 5.14. An assembled view of the 0505 gradients complete with an inserted rf board is shown in the top portion of the figure. The piers of the acrylic mounting piece are designed to ensure that the rf board is vertically centered in the gradient field. A side profile showing the position of the gradients relative to the rf board is shown as a side profile in the bottom portion of the figure. In order to keep the spacing between the planes as small as possible while at the same time allowing the 0505 capacitor to fit in-between, a 0.005" groove was cut out of the inside surface of the bottom gradient board.

axis yields a rectangular lineshape. The first sample is a (non-ideal) cylinder of water of length 160 μm and diameter 50 μm. This sample, shown schematically in Figure 5.15, is.
Figure 5.15. Schematic of the (non-ideal) cylindrical sample of water, which is used to obtain the lineshapes of Figures 5.17, 5.18, and 5.19 is shown above. This sample is located inside the micropipette. The water is confined on one end by a 50 μm diameter fiber optic glass rod, and on the other end by glue. These interfaces are not perfectly flat. Additionally, the diameter tapers by ~10% over the length of the sample.

ideally, a finite length cylinder of water. However, as discussed below, the shape of the water sample is only approximately cylindrical.

The sample was prepared by dipping narrow end of the micropipette in water, causing the water to enter because of surface tension effects. Sealing the narrow end of the pipette with glue forms the first wall of the cylinder of water. This confines the water on one side. Then, a 50 μm fiber optic glass rod is inserted into the opposite (wide) end of the micropipette, until it cannot be inserted any further. The reason that the rod cannot be inserted further is that its O.D. (outer diameter), 50 μm, is at that point equal to the tapering I.D. of the micropipette. As the glass rod is inserted it displaces water, and when it is fully inserted a cylinder of water results which is 50 μm in diameter. and, in this
particular case. 160 μm in length. The large diameter end of the micropipette in then sealed with glue to prevent evaporation of the water.

The resulting idealized cylinder of water is however non-ideal for three reasons. First, the interfaces between the water and the glue and between the water and the fiber optic glass rod may not be perfectly flat planes. Second, there is a taper in the I.D. of the tube. Third, the water outside the cylinder can still be observed with the NMR. As the coil axis is parallel to the y-axis of the gradients, these effects are more noticeable when applying the G\text{y} gradient. From optical microscope observations, we estimate this taper as approximately 10% over the 160 μm length. The micro-pipette is then wrapped in a coil of five turns of 20 μm copper wire, wire spacing equal to twice the diameter of the wire, and attached to an rf circuit board, as described in Chapter 3.

To test gradient strengths we apply a spin-echo pulse sequence on the sample with a single gradient (x, y, or z direction exclusively, where y is the axis of the cylinder and z the axis of the static field B_0) pulsed on throughout the experiment. The spin-echo sequence is shown schematically in Figure 5.16. The spin-echo sequence consists of a 90° pulse, followed by a wait time τ, then an 180° pulse, another wait time τ, and then acquisition of the second half of spin echo. Figure 5.17a shows the acquired second half of the spin echo obtained while passing 85 Amps through the G\text{y} gradient coil. 240 scans are averaged. Figure 5.17b shows the Fourier transform “lineshape” of the time domain signal of Figure 5.17a. Below we discuss the interpretation of this lineshape, and other lineshapes in which various currents were applied to the G\text{x}, G\text{y}, and G\text{z} gradient coils.
Figure 5.16. Schematic diagram of the spin-echo sequence used to test the gradient coils $G_x$, $G_y$, and $G_z$. The sequence consists of a 90° pulse, followed by a wait time $\tau$, then a 180° pulse, another wait time $\tau$, and then acquisition of the second half of spin echo. The gradient is applied throughout the sequence.

Figure 5.18 shows the observed lineshapes obtained with currents of 14, 85 and 159 Amps applied through the $G_x$ gradient coil. The lineshape expected in this measurement, on a cylinder of water, with a gradient applied perpendicular to the cylinder y axis (either along x or z), is effectively a plot of $P(x)$ vs. $x$, where $P(x)dx$ is defined as the amount of water having $x$ coordinate between $x$ and $x+dx$. For a cylinder, such a plot would have the shape of a semi-circle (if vertical axis scaling is chosen to make it so). The semi-circular shapes of Figure 5.18 confirm this expectation. The total linewidth of the lineshapes in Figure 5.16 is simply related to the respective gradient strengths and the diameter $D$ of the water cylinder, according to:

$$\text{Linewidth} = \gamma GD$$  \hspace{1cm} (5.16)

For the 85 Amp curve of Figure 5.18 the measured total linewidth is $45.000\pm1000$ Hz and using Equation 5.16 gradient of $G_x = 21\pm1$ T/m is calculated. This compares with
Figure 5.17. In part A, the acquired second half of the spin echo was obtained while passing 85 Amps through the \( G_z \) gradient coil and 240 scans are averaged. In part B is the Fourier transform "lineshape" of the time domain signal of in part A.

the theoretical estimate based on Equation 5.15 of \( G_z = 21 \) T/m for such a current, and for the \( G_z \) gradient parameters \( z_z = 1.51 \) mm. Clearly, the gradient is acting as expected, and providing a gradient of adequate strength for our needs.
In Figure 5.19, the $G_y$ curve for 99 Amps, the measured total linewidth is $20,000 \pm 1000$ Hz, resulting in a gradient of $G_y = 9.4 \pm 0.5$ T/m. This compares with the theoretical estimate based on Equation 5.10. of $G_y = 9.4$ T/m for such a current, and for the $G_y$ gradient parameters of $r = 2.91$ mm. The coil constructed to supply the $G_y$ gradient demonstrates that adequate gradients strengths can be achieved of the expected values.

Application of gradients along the $x$ and $z$ directions of our cylinder (with cylindrical axis $y$) yield similar, semi-circular lineshapes as expected. For gradients applied along the cylindrical axis $y$, however, one expects a very different lineshape. The function $P(y)$ is expected to have rectangular shape. Results for gradients along $y$, $G_y$, direction is shown for the cylindrical sample in Figure 5.20. The expected rectangular
Figure 5.19. Lineshape of the cylinder is shown when applying the $G_y$ gradient, perpendicular to the axis of the cylinder. The lineshape is approximately the expected semi-circular shape.

Figure 5.20. Lineshape of the cylinder is shown when applying the $G_x$ gradient, parallel to the axis of the cylinder. For an ideal cylinder, we expect a rectangular lineshape. The measured lineshape deviates from rectangular. This deviation most likely results from tapering of the cylinder radius and from observation of water outside the cylindrical region.
lineshape is not observed. We suspect that this might result from the non-ideality of the cylindrical sample shape, including the tapering of the cylinder radius along the cylinder axis, the lack of a perfect cut-off at the ends of the cylinders, and observation of water that extends beyond these cut-offs.

5.3.2. (NON-IDEAL) CYLINDER OF WATER WITH 20 \( \mu \text{m} \) POLYSTYRENE BEAD INSERTED.

Because the non-ideality of the cylindrical water sample resulted in the unexpected lineshape of Figure 5.20, a second sample was prepared to characterize the \( G_z \) gradients. The sample was constructed with water diameter 50 \( \mu \text{m} \) (as in the first sample), with length 60 \( \mu \text{m} \), but with a polystyrene bead of 20 \( \mu \text{m} \) in diameter (Duke Scientific) contained in the cylinder of water. The solid polystyrene bead is not expected to contribute to the proton's NMR signal, as the solid hydrogen atom's \( T_1 \) is much shorter than that of a liquid. This sample was wrapped in a five-turn coil with 20 \( \mu \text{m} \) copper wire and attached to a rf circuit board.

The resulting lineshape for this sample, for a current of 141 Amps through the \( G_z \) coil, is shown in Figure 5.21. The lineshape shows the expected dip in signal amplitude that results from the water displaced by the bead. The linewidth of the dip, 18.000\( \pm \)1000
A 20 um polystyrene bead was placed in a similar cylinder of water to demonstrate the strength of the $G_s$ gradients. Since, the NMR signal is from the hydrogen in the surrounding water, the polystyrene bead creates a dip that can be used to measure the gradient $G_s$.

Hz. yields the gradient magnitude $G_s$ according to the Equation 5.16. The inferred gradient of $21\pm2$ T/m compares well with that expected from theory of $20$ T/m, according to Equation 5.15. $z_s = 2.02$ mm and a bead diameter of 20 $\mu$m.

5.3.3. GRADIENT ECHO PULSE SEQUENCE

As a test of the linearity of the gradients constructed for the microcoil system, a gradient echo pulse sequence was used. The pulse sequence used for the gradient echo is
in Figure 5.22. The spins are excited first by a 90° pulse, and then a positive magnitude gradient is applied in the G₁ gradient (0402 version) with a current of 16.5 amps or 4.2 T/m. As evidenced in the signal in the time domain, see Figure 5.23, the signal accumulates a phase of $e^{2\pi G_1 t}$ during the first gradient pulse. As the spins dephase, the signal amplitude decreases during the one-millisecond pulse to zero magnitude in a millisecond.

At the one-millisecond mark, the gradient is reversed and a negative magnitude gradient is applied during the following two milliseconds. During the negative gradient pulse the spin accumulate the phase $e^{-2\pi G_1 t}$, and when the accumulated phase returns to zero, the signal amplitude grows to a maximum, the echo effect, and continues to dephase again due to the continuing negative gradient pulse. The change in magnitude after the FID to the center of the gradient echo is due to the normal exponential decay due to $T₁^*$. The gradient echo only occurs if the gradients are linear and constant in magnitude but in opposite directions.
Figure 5.22. The pulse sequence used to acquire a gradient echo is shown. The gradient is first turned on positive for a time $\tau$ and then switched negative. An echo forms when the signal removes the phase accumulated when the gradient was positive. The gradient echo sequence can be used to demonstrate the linearity of the magnetic field gradients. $\tau$ is one millisecond.

Figure 5.23. The NMR signal resulting from the pulse sequence in Figure 5.22 is shown. The FID is acquired immediately after the $\pi/2$ pulse and the gradient echo returns at $2\tau$ as expected demonstrating the linearity of the field gradients. Only the signal envelope is visible on this scale.
5.4. LIST OF REFERENCES


CHAPTER 6

GRADIENT POWER SUPPLY

6.1. INTRODUCTION

A custom designed gradient power amplifier was constructed to drive the current in the gradient coils that are discussed in Chapter 5. The requirements for the gradient power supply in MRI are: fast switching, reversible currents (positive and negative gradients) of adjustable duration, an amplitude of greater than 100 amps, and a workable duty cycle. Short pulses, on order of a millisecond, balances the current requirement of hundreds of amperes into a workable duty cycle and represents the major challenge in designing the required power supply. The power supply designed for this experiment is constructed around Power MOSFETS.
6.2. POWER MOSFETS

A Metal-Oxide-Semiconductor-Field-Effect-Transistor or MOSFET\(^+\) is cross-sectioned in Figure 6.1. Enhancement mode MOSFETS are non-conductive until a gate-source, \(V_{GS}\), is applied. This type of MOSFET\(^+\) has no channel to conduct the electrons until the gate voltage is applied. A positive gate-source voltage creates a conducting channel by pulling carriers from the n-channel material and holes from the p-channel material into the upper layers of the substrate under the gate. As the gate-source voltage is increased, the layer of conduction under the gate increases causing the "on" resistance of the MOSFET to decrease. A typical MOSFET may have thousands of sources conducting in parallel.

The dependence of the "on" resistance or \(R_{ds}\) (drain-source resistance) on the gate-source voltage allows the MOSFET to be used in two major different manners. By applying a large gate-source voltage, approximately 15 Volts, the MOSFET has switching characteristics. In this configuration the MOSFET is "on": the full "on" resistances is a few milliohms with full gate-source voltage. Second, the MOSFET can be used as a variable resistor by varying the gate-source voltage on the MOSFET. The second methods allows for a method to set the current in the circuit with a voltage control. The gate-source voltage and the resulting current is shown for the two MOSFETS used in the power supply in Figure 6.2. The IRF1010E is used as a switch in
Figure 6.1. A cross section of a typical MOSFET is presented. It is important to note that there are potentially thousands of channels in a typical MOSFET. An application of positive gate-source voltage creates a conducting channel between the source and the drain.

The circuit, explained fully below, and the IXFK110N07 is used as the current setting MOSFET. As the gate-source voltage increases the IXFK110N07 permits increasing levels of current to flow making it the perfect variable resistor unlike the IRF1010E that has negative slope at high gate-source voltages, above 10 volts, not shown in Figure 6.2.

The internal construction of a MOSFET also leads to a parasitic diode anti-parallel to the MOSFET transistor. The diode is a normal PN diode between the N-type drain layer and P-type body of the MOSFET. To stop the parasitic diode from becoming biased, manufacturers place an external diode in parallel with the parasitic diode.
Figure 6.2. The gate-source voltage affects the current that can pass between the source and the drain. In part A, the IRF1010E gate-source voltage curve is shown while the IXFK110N07 gate-source curve is shown in part B. These graphs are from the manufacturers of each MOSFET.
MOSFETS were chosen for their very fast switching times. The switching time is only limited by the time it takes the carriers to flow from the source to the drain, theoretically a few hundred picoseconds. Since the MOSFET is not effected by carrier lifetimes, there is no turn-off delay. As soon as the voltage decreases, the MOSFET begins to turn off. These characteristics make the MOSFET an ideal component to build a gradient power supply around. A gradient pulse in MRI ideally has zero rise and fall times with a minimum of voltage switching transients.

6.2.1. PARASTIC CAPACITANCES

Power MOSFETS have severe parasitic capacitances, as shown in Figure 6.3, and must be controlled properly to attain fast switching characteristics. The gate-source capacitance is the largest of the parasitic capacitances and limits the turn-on and turn-off times of the MOSFETS drastically. This capacitance is typically ~3000 pF for the IRF1010E and ~10,000 pF for the IXFK110N07. The gate-source, $C_{gs}$, gate-drain, $C_{gd}$, and source-drain, $C_{sd}$, are typically specified as the input capacitance: $C_{iss}$, output capacitance, $C_{oss}$, and reverse transfer capacitance, $C_{rss}$. The $C_{iss}$, $C_{oss}$, and $C_{rss}$ are the values typically listed in the manufactures data sheets. The following relations relate these capacitances:

\[ C_{iss} = C_{gs} + C_{gd} \]  

(6.1)
The parasitic capacitances in a MOSFET are severe and must be properly dealt with to attain fast switching times. The three parasitic capacitances typically associated with MOSFET are shown. The extra PN diode to counteract the parasitic diode is also shown.

\[ C_{gs} = C_{gd} \]  \hspace{1cm} (6.2)

and

\[ C_{gs} = C_{ds} + C_{gd} \]  \hspace{1cm} (6.3)

The capacitances are shown for both MOSFETS in Figure 6.4.

The \( C_{gs} \) capacitance is a result of the overlap between the source and channel regions by the gate. The gate-source capacitance is independent of the applied voltages. The gate-drain capacitance comes from two parts. First, the depletion region immediately under the gate adds capacitance. Second, the capacitance develops in the overlapping regions between the silicon and the gate. The \( C_{gd} \) is nonlinear with respect to the voltage. The \( C_{ds} \) capacitance is associated with the diode between the drain and the source and varies inversely with the square root of the drain-source voltage.
Figure 6.4. The parasitic capacitances are shown in part A for the IRF1010E MOSFET as function of the drain-source voltage. The IXFK110N07's parasitic capacitances are shown in part B. These graphs are taken from the manufacturers.
6.2.2. MILLER EFFECT

The input capacitance, Equation 6.1, is not the total capacitance seen by the input voltage into the MOSFET. The Miller Effect\textsuperscript{14} affects all field effect transistors (FET) and depends on the voltage gain of the particular FET. The voltage gain is defined as the ratio of $V_{gs}/V_{gs'}$. This voltage gain leads to an increase in the input capacitance.

$$C_{in} \approx C_{gs} + \left(1 + \frac{|V_{gs}|}{V_{gs'}}\right) C_{gd}, \quad (6.4)$$

and for large voltage gains in the MOSFET, the input capacitance is significantly larger than the gate-source parasitic capacitance. The $C_{gd}$ is a nonlinear function of the voltage and provides the feedback between the input and output of the MOSFET. The gate-drain capacitance is also called the Miller capacitance as it can cause the total capacitance to be larger than the sum of the static capacitances\textsuperscript{10}.

Since the exact total input parasitic capacitance is not known, the data sheets typically provide a total gate charge\textsuperscript{4}, $Q_g$. In addition, the gate-source charge, $Q_{gs}$, and the $Q_{gd}$, or the Miller charge is also provided. The total gate charge for the IRF1010E is approximately ~120 nC, and the IXFK110N07 has a total gate charge of ~500 nC. The total gate charge is relatively insensitive to voltages and applied currents and provides an easy way to determine the necessary power to drive the MOSFET. Multiplying the total gate charge by the desired switching frequency results in the current required to switch
the MOSFET. A gate-charge waveform is shown in Figure 6.5 demonstrating the effects of all three types of gate charges and the effects on the gate-source voltage.

6.3. GATE DRIVING CIRCUIT

After determining the desired switching speed of the MOSFET, a driving circuit to supply the current must be designed. A single operational amplifier (op-amp) generally provides only a current of few tens of milliamps, below the requirements for fast MOSFET switching. While a MOSFET is a voltage-controlled device, a high current drive is required, beyond a single op-amp circuit, to adequately charge and discharge the capacitances of the MOSFET. For this purpose a push-pull transistor pair, or complimentary emitter-follower, was used in conjunction with an op-amp driving circuit, see Figure 6.6.

When the MOSFET is turned on, a pulse is emitted from the op-amp to the gates of both transistors. The top or push transistor (NPN) is turned on draining charge from the storage capacitor and power supply and transferring the charge to the effective capacitor in the MOSFET through the resistor, $R_{on}$. As the charge is transferred, the gate-source voltage increases and turns the MOSFET on. During the pulse, the current ceases to flow as the capacitor is charged, keeping the gate-source voltage constant. At turn off, the pull transistor (PNP) turns on as the NPN transistor turns off. The PNP transistor pulls the
Figure 6.5. The parasitic capacitances do not allow for proper design of the driving circuits. The Miller effect causes the stray capacitance to increase and makes the gate charge the more important parameter for designing the drive circuit. The voltage on the gate goes through three different phases. First, the gate-source charge is charged and then the Miller charge must be charged. During the time it takes the Miller charge to charge, the gate voltage does not rise. Finally, after the Miller charge is charged, the gate-source voltage continues to rise.

charge out of the MOSFET through both resistors, $R_{on}$ and $R_{off}$, as the diode is forward biased, turning the MOSFET off as the gate-source voltage returns to zero.

The resistors, $R_{on}$ and $R_{off}$ in Figure 6.6 can be used to control the turn on and turn off times. As the resistance values increase, the current delivered to the MOSFET decreases changing the turn on and turn off times. The resistances cannot be arbitrary reduced to zero, as $dv/dt$ restrictions in the gate-source voltage rates in the MOSFETS induce failure. The diode used must be a fast response diode, usually type 1N4148, and allows the turn off effective resistance to be lower than the turn on resistance.
Figure 6.6. The drive circuit used in the gradient power supply is shown above. A push-pull transistor pair, that is chosen depending on the standoff voltage required, powers each MOSFET. The 100 μF capacitor allows peak currents of several amperes to be used. The two resistors in parallel with the diode can be used to manipulate the turn on and turn off times of the MOSFET.

The gate driver circuitry must be low impedance to insure maximum current transfer to the MOSFET. For this reason $R_{on}$ was chosen to be 10 Ω and $R_{off}$ was 22 Ω, giving an effective $R_{off}$ resistance of approximately 7 Ω, without violating any MOSFET restrictions. The transistors must have high current capability and high current gain. As the current is only flowing during turn on and turn off times, power dissipation factor of the transistors is not important. Zetex transistors, see Table 6.1 for specifications, where chosen depending on the required voltage standoff that is discussed in section 6.5. All the mentioned transistors are capable of handing at least 4 amps of pulsed current.
<table>
<thead>
<tr>
<th>Transistor</th>
<th>Type</th>
<th>Collector-Emitter Voltage (V)</th>
<th>Peak Pulse Current (A)</th>
<th>Continuous Current (A)</th>
<th>Turn On Time (ns)</th>
<th>Turn Off Time (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZTX 1053A</td>
<td>NPN</td>
<td>75</td>
<td>10</td>
<td>3</td>
<td>90</td>
<td>750</td>
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<tr>
<td>ZTX 1051A</td>
<td>NPN</td>
<td>40</td>
<td>10</td>
<td>4</td>
<td>100</td>
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<td>PNP</td>
<td>-70</td>
<td>-4</td>
<td>-2</td>
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<td>750</td>
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<tr>
<td>ZTX 1151A</td>
<td>PNP</td>
<td>-40</td>
<td>-5</td>
<td>-3</td>
<td>140</td>
<td>300</td>
</tr>
</tbody>
</table>

Table 6.1. Characteristics of the transistors used in the gradient power supply are presented. The “top” MOSFET in the H-bridge has a larger standoff voltage than the lower MOSFETS. The “top” MOSFET are the 75-volt transistors, while the remaining MOSFETS use 40-volt transistors. All transistors used have high peak current and fast turn on times, the most important specifications in choosing the proper transistor for the current driver for a MOSFET.

6.4. MOSFET THERMAL CHARACTERISTICS

Manufactures generally specify a maximum DC current the MOSFET can accommodate without failure. However, the maximum current specified is for a single pulse of approximately one millisecond even though DC current is claimed. The maximum MOSFET current also decreases as a function of the case temperature. For these reasons, adequate care must be taken in discerning the MOSFET characteristics from manufactures data sheets.
Figure 6.7 shows the effects of increasing case temperature on the two MOSFETS used in the gradient power amplifier. The IXFK110N07 has flat temperature dependence up to one hundred degrees Celsius, making this MOSFET ideal for acting as a variable resistor. To keep all the MOSFETs at constant temperature, adequate air-cooling and the use of heat sinks on all of the MOSFETs is required. However, the most important method for controlling case temperatures is low duty cycle, defined as the ratio of time on to time off of the MOSFET. In this application, duty cycles were kept at approximately 0.1%, or one millisecond pulses every second.

In addition to failure caused by the changes brought on by high case temperature to the conduction properties of the MOSFETs it is possible to destroy the silicon junctions inside. The safe operating range of the IRF1010E is shown in Figure 6.8. Even when adequately heat sunk, air-cooled, and with low duty cycles, long pulses can still destroy the MOSFET. Thermal destruction of the MOSFET is still possible by demanding the MOSFET to dissipate too much power even when using adequate cooling and low duty cycles. Demanding pulses significantly greater than one millisecond at the high current desired for large gradient fields has dire effects on the IRF1010E MOSFETS as shown in Figure 6.8. The IXFK110N07 is significantly more thermally rugged to long current pulses than the IRF1010E from experimental tests and working experience. The durability of the current setting MOSFET is necessary as this MOSFET will be conducting longer than the switching MOSFETS as discussed in the section below.
Figure 6.7. The case temperature of the power MOSFET affects the current that is allowed to flow between the drain and the source. The case temperature effects on the current for the IRF1010E is shown in part A, while the IXFK110N07 case temperature effects are shown in part B. The IXFK110N07 is used as the current setting MOSFET because the current is independent of the case temperature, making it an ideal MOSFET for a voltage controlled resistor. These graphs are provided by the manufacturers.
Figure 6.8. Not only is the case temperature important in designing with power MOSFETS, but the internal characteristics must be investigated. Long pulses, greater than 1 ms, at high currents can greatly damage the IRF1010E as shown in the graph above. Care must be taken to not damage the individual silicon junctions from overheating before the heat can be transferred to the case. The graph is provided by the manufacturer.

6.5. H BRIDGE CONFIGURATION

The gradient coils are placed electrically in the center of a MOSFET “H” bridge composed of IRF1010E MOSFETs. These transistors are operated as switches and are driven by individual op-amps circuits. In series with the bridge is an IXFK110N07 that sets the desired current through the coil by adjusting the gate-source voltage through a
user adjustable 10 turn potentiometer. The “H” bridge configuration provides the current switching, producing either positive or negative currents depending on the desired direction. A TTL pulse from the Apollo Tecmag is used to control the positive current with another TTL pulse to control the negative current. In this way, since the same current setting components are used for both current directions in the magnetic field coils, identical pulse shapes are obtained for both positive and negative directions. In addition to setting the gate-source voltage, the current setting MOSFET is also pulsed “on” with a user controlled gate-source voltage when either TTL signal is used through a summing op-amp. Pulsing all three MOSFET, two from the “H” bridge and the current setting MOSFET, drastically reduce switching transients and improves the settling time of the current to a constant level. At first glance, it might appear that the current setting MOSFET does not need to be pulsed along with the H-bridge MOSFETS. However, not pulsing this MOSFET induces large over-shoots in current during turn on and turn off, more severe at turn on. These switching problems last over 50 μs and can reach 300% of the desired current. The switching transients encountered by not pulsing are not narrow current spikes; the area under the “glitch” becomes a significant percentage of the area in the desired pulse shape. In Figure 6.9 a schematic of the basic circuit is shown.
Figure 6.9. The basic H-bridge circuit is shown. Channel A and B provide the method to attain positive and negative gradients. The gradient coil "floats" in the center of the H-bridge.

6.6. GRADIENT POWER SUPPLY

The complete circuit diagram is shown in Figure 6.10. To achieve the necessary gradients, currents of \(-150\) Amps are required. The maximum output voltages of the current sources that we have constructed are 36 Volts. Three identical circuits are used.
Figure 6.10. The circuit diagram for the gradient power supply is shown (2 pages).
for \( G_x \), \( G_y \), and \( G_z \) coils. A TTL logic sequencer sets direction, either positive or negative, and duration of the current pulse. TTL signals are received by an input transistor and terminated with 50 \( \Omega \), fixing any input signal to the driver at approximately 5 volts. An internal op-amp is used to convert the "saturated" logic pulses into the appropriate operating point for the bridge MOSFETs.

MOSFETs gate-source voltages of approximately 10 Volts are required to attain minimum on resistance in the MOSFET. For this reason an additional power supply, +30 V is needed in top MOSFET in the "H" bridge. This MOSFET is switched from ground to +30 V when "on" keeping the gate voltage above the maximum source voltage of approximately 20 volts. The 36 volts of the external power supply, across the capacitors, is referenced to -16 volts of the internal power supply. This leaves +20 volts on the high side of the H-bridge. The bottom MOSFET in the bridge is floated on the current path to insure a gate source voltage in excess of 10 volts. All five MOSFETS are driven from driver circuits as discussed in section 6.3.

The current setting MOSFET is referenced to -16 Volts in the current path and a voltage shifting op-amp is used to shift the TTL pulse to -16 Volts in the "off" state of the gradient power supply. The desired current is set by a 10-turn potentiometer setting the gate-source voltage of the MOSFET. An adjustable 1 k\( \Omega \) resistor is in series with one TTL receiver before the summing op-amp to account for unbalanced op-amp gain and resistor values.

An external capacitor bank, of 120-4,700 \( \mu \)F. 50 V capacitors provide stored current for the gradient pulse. However, the ripple current of the capacitors limits the
current available to the gradient coil and required the capacitor bank to be connected in parallel to increase the total available current. In addition to the capacitors in the bank, 16-4,700 μF capacitors are on the gradient board to limit any voltage drops before the current is pulsed. An independent external power supply, 36 V, in the high current path provides the power to charge the capacitor bank and is connected to the internal power supply (dual 16 V) at the low voltage side at the bottom of the current path. It is important for the external capacitors to function that care is taken to keep the “internal” resistance of the bank small.

The pulse is monitored by an op-amp configured as a differential amp across gradient coil. Using the gradient coil as a shunt resistor removes an additional resistor in the current path increases the maximum current. An addition of a known sense resistance in series with the gradient coil was originally used to measure the current. Resistors between 10 and 100 mΩ where tested and all drastically effected the switching behavior of the gradient power supply. The best waveforms (squarest) were acquired by removing the sense resistor and measuring the voltage difference across the gradient coil. The output of the differential amplifier is available to be displayed by an oscilloscope.

The gradient coils have resistance of ~30 mΩ, and in connection with coaxial cable carrying the current, the total resistance is ~180 mΩ. The ~180 mΩ resistance of the gradient coil system and the 36 V of the external power supply are the only limiting factors prohibiting even higher current values in excess of the 200 Amps currently available. A picture of the gradient power supply is shown in Figure 6.11.
Figure 6.11. One gradient power supply circuit is shown. The adjustable knob controls the amplitude of the gradient and the TTL lines control the direction, positive or negative.

The current is pulsed inside the 9 Tesla magnetic field in Figure 6.12, for three different values of current, corresponding to three different voltages across the gradient coil for 500 μs. In this case, the Gx gradient was pulsed, but similar waveforms occur for all three gradients. It is important to realize that the measured voltage in Figure 6.12 has been reduced by a factor of ten due to the voltage divider in the differential current measuring amplifier. As the transients increase at lower currents, rise time can be sacrificed to decrease the effect by increasing $R_{on}$ in the driver circuits of the MOSFET drivers. With the high gradient currents required, the gradients are usually run between the middle and highest curve in Figure 6.12, eliminated the need for changing $R_{on}$.
The resistance for the \( G_y \) coil, including all cables, is \( \sim 115 \, \text{m}\Omega \). This corresponds to a current of 188 amps in the highest curve, 73 amps in the middle curve, and 34 amps in the lowest curve in Figure 6.12. In the magnetic field, current spikes last for less than \( -5 \, \mu\text{s} \) with a total of \( -10 \, \mu\text{s} \) settling time to the desired current. At higher currents, the voltages spikes at turn "on" actually decrease but turn "off" spikes increase. There is also a turn on delay of \( -10 \, \mu\text{s} \) but this is compensated by a turn off delay \( \sim 10 \, \mu\text{s} \) keeping the total pulse duration at the desired length. The rise time for the highest current is shown in
Figure 6.13, the rise time is 5 µs, while the rise time for the middle pulse is 3.5 µs. The spike for low current makes measuring the rise times difficult in this case.

Applying a "correction pulse" can compensate the switching transients. A pulse of the same direction (positive or negative) is applied for approximately 50 µs on the opposite side of the 180° pulses in a spin echo. This method cancels out the accumulated phase from the switching transients in the image. The "correction pulse" method is illustrated in Figure 6.14. To complete discussion of the gradient amplifier, Figure 6.15 demonstrates the behavior of the gradient power supply when switching from positive to negative currents. The switch occurs in ~10 µs. The gradients were set to switch from positive to negative with no time in-between, consequently the second pulse has been shortened in duration from the desired 500 µs. Usually, operational limitations require ~25 µs of "dead" time in-between switching the gradient from positive to negative to remove artificially shorting the second pulse.

Currents of hundred of amperes can be pulsed through the gradient coils, for times not greatly exceeding one millisecond, powered by the gradient power supply. The gradient power supply, built around MOSFETS, provides a method to rapidly switch the current direction, adjust the magnitude of the current, and duration of the pulse. The configuration of the MOSFETS enable the high gradient fields to be obtained that are necessary in MRI microimaging.
Figure 6.13. Rise time for the highest current pulse in Figure 6.12 is ~5 μs. Rise times decrease for lower currents until spikes make measurements difficult.

Figure 6.14. The "spikes" at the beginning and the end of the gradient pulse can be corrected by the application of an additional pulse after the 180° pulse. The correction pulse is effectively subtracted from the phase encoding period, removing the "spikes". Typically, the correction pulse is ~ 50 μs.
Figure 6.15. The gradients were switching with no time between the pulses. Each direction was turned on for 500 µs. The inset figure shows the switching time of ~10 µs. Usually, to avoid artificially shorting the second pulse, a "dead" time ~25 µs is placed between pulses when the direction of the current is switched.
6.7. LIST OF REFERENCES

CHAPTER 7

MRI IMAGING AND MICROIMAGES

7.1. INTRODUCTION

To properly phase encode with an echo train requires a phase cycling method to remove unwanted imaging artifacts. The phase cycling technique is called the PHAPS sequence and is explained in section 7.2.6. Besides, signal to noise limitations, the maximum k-space vector is the other leading factor in resolution and the effects of limiting the magnitude of the vector to finite values is explored in section 7.2. In the remaining sections, MRI microimages are shown and explained. Current resolutions are approximately 100 \( \mu m^3 \) and are better than a factor of 2 than previous images. However,
the resolutions reported here is not the overall limit of the experimental apparatus, just
the latest current resolution limit.

7.2. PHASE ENCODING WITH ECHO TRAINS

Acquiring an image with phase encoding and echo trains causes imaging artifacts unless special pulse sequences are used. A normal Carr-Purcell' (CP) or Carr-Purcell-
Meiboom-Gill' (CPMG) pulse sequence induces a mirror or virtual image at the negative
frequency of the desired real image; the imaged is mirrored across zero frequency. The
mirror effect can place the virtual image directly on top of the real image, creating a
disastrous imaging artifact. In addition to the mirror image, zero-frequency artifacts are
also created. To remove the artifacts, a Phase-Encoding-Phase-Alternating\textsuperscript{3,6} (PHAPS)
pulse sequence is used. The general pulse sequence used to encode the MRI images is
shown in Figure 7.1, minus the phase cycling information. To create images, one
dimension is selectively excited with a soft $\pi/2$ pulse, while the remaining two
dimensions are phase encoded; the gradient is turned on for varying times to transverse $k$-
space in each dimension. The time after the selective excitation and the first $\pi$ pulse is
held constant to remove any unintentional $T_2$ contrast. As the gradients "on" time
increases, an extra time delay becomes shorter, keeping the total time between the $\pi/2$
and $\pi$ pulse constant. After the phase encoding, the echoes are repeatedly formed with an
Figure 7.1. The imaging pulse sequence used for all the images presented in this chapter is shown above. The phase encoding times extend from -640 μs to +660 μs and was acquired as echoes in both indirect dimensions. The echo train continues for hundreds of echoes. Negative times for the gradients were obtained by having the phase encoding on the opposite side of the first \( \pi \) pulse, not shown, instead of opposite magnitude gradient.

The echo train. The PHAPS phase cycling sequence is necessary to remove the imaging artifacts and is explained in the following section.
7.2.1. ROTATION MATRICES

To help facilitate the explanation of the imaging artifacts when echoes trains are used: it is necessary to introduce the following rotation matrices. For rotations, $\theta$, about the x-axis, a vector is multiplied by the following matrix.

\[
\begin{bmatrix}
1 & 0 & 0 \\
0 & \cos \theta & \sin \theta \\
0 & -\sin \theta & \cos \theta
\end{bmatrix}.
\]  
(7.1)

Rotations about the y-axis and z-axis are

\[
\begin{bmatrix}
\cos \theta & 0 & \sin \theta \\
0 & 1 & 0 \\
-\sin \theta & 0 & \cos \theta
\end{bmatrix}
\]  
(7.2)

and

\[
\begin{bmatrix}
\cos \theta & -\sin \theta & 0 \\
\sin \theta & \cos \theta & 0 \\
0 & 0 & 1
\end{bmatrix}
\]  
(7.3)

respectively. These rotation matrices can be used to simulate the effects of rf pulses. Both $\pi/2$ and $\pi$ pulses have imperfections that can be modeled with the above rotation matrices. rf pulses in NMR and MRI are never perfect and the errors can lead to unwanted effects$^3$. Rotations about the z-axis correctly model the extra phase added by application of the gradients pulses when phase encoding. To explain the imaging artifacts, it is sufficient to limit to the on-resonance condition with infinitely short rf pulses$^3$ and an initial magnetization of one. Elimination of the both the zero frequency
artifact and mirror imaging requires the careful combination of the Carr-Purcell and Carr-Purcell-Meiboom-Gill echo trains.

7.2.2. CARR-PURCELL (CP) ECHO

The Carr-Purcell echo train is $90^\circ, -180^\circ, -180^\circ, -\ldots$ pulse sequence. The phase of the initial $\pi/2$ pulse is the same phase as the following $\pi$ pulses. To account for the imperfections in the pulses, a pulse of angle $\phi$ is applied, ideally a $\pi/2$ pulse, to the equilibrium magnetization assumed to be along the $z$-axis. The imperfect rf pulses are the results of $H_1$ field inhomogeniety, rf penetration depth and imperfect measurement. The rotation results in the vector

$$\vec{M} = \begin{bmatrix} 0 \\ \sin \phi \\ \cos \phi \end{bmatrix}. \quad (7.4)$$

Next, the phase encoding gradient rotates the magnetization about the $z$-axis with an angle $\psi$, resulting in the resulting vector.

$$\vec{M} = \begin{bmatrix} -\sin \psi \sin \phi \\ \cos \psi \sin \phi \\ \cos \phi \end{bmatrix}. \quad (7.5)$$

After the first $\pi$ pulse, assumed to be an angle $\theta$ to account for imperfections in the rf pulse, the magnetization is
The resulting magnetization vector is vastly different from the result with perfect applied rf pulses.

\[
\mathbf{M} = \begin{bmatrix} -\sin \psi \sin \phi \\ \cos \theta \cos \psi \sin \phi + \sin \theta \cos \phi \\ -\sin \theta \cos \psi \sin \phi + \cos \theta \cos \phi \end{bmatrix}
\]  \hspace{1cm} (7.6)

It is evident from Equation 7.6 that the imperfection in the \( \pi/2 \), pulse leads to additional terms in the \( y \) and \( z \) components of the magnetization after the first \( \pi \), pulse and these terms do not depend on the phase encoding gradient and \( \psi \). As these components are independent of the \( \psi \), they appear as a DC offset in the phase encoding direction and an artifact will arise in the Fourier transform at \( \omega \) equal to zero. This is the explanation for the zero frequency artifacts.

To cancel the zero frequency artifacts, the pulse sequence \( 90^\circ, -180^\circ, -180^\circ, ... \) is added to the sequence \( 90^\circ, -180^\circ, -180^\circ, ... \). The \( 90^\circ, -180^\circ, -180^\circ, ... \) sequence gives a magnetization vector for the first echo of

\[
\mathbf{M} = \begin{bmatrix} -\sin \psi \\ -\cos \psi \\ 0 \end{bmatrix}
\]  \hspace{1cm} (7.7)

\[
\mathbf{M} = \begin{bmatrix} -\sin \psi \sin \phi \\ \cos \theta \cos \psi \sin \phi + \sin \theta \cos \phi \\ \sin \theta \cos \psi \sin \phi + \cos \theta \cos \phi \end{bmatrix}
\]  \hspace{1cm} (7.8)

and when added to Equation 7.6, the DC offset in the phase encoded direction is canceled resulting in the magnetization vector.
\[ \vec{M} = \begin{bmatrix} -2\sin\psi\sin\phi \\ 2\cos\theta\cos\psi\sin\phi \\ 2\cos\theta\cos\phi \end{bmatrix}. \quad (7.9) \]

By adding the two CP sequences, the DC artifact is removed from the image.

### 7.2.3. CARR-PURCELL-MEIBOOM-GILL (CPMG) ECHO

The CPMG pulse sequence similarly produces zero frequency artifacts in imaging. The CPMG pulse sequence of \(90^\circ, -180^\circ, -180^\circ, -\ldots\) results in a magnetization vector

\[ \vec{M} = \begin{bmatrix} -\cos\theta\sin\psi\sin\phi + \sin\theta\cos\phi \\ \cos\psi\sin\phi \\ \sin\theta\sin\psi\sin\phi + \cos\theta\cos\phi \end{bmatrix}. \quad (7.10) \]

after the first \(\pi\) pulse. The DC offset is again evident in Equation 7.10 in the \(x\) and \(z\) component like the CP sequence and Equation 7.6.

Applying a similar correction to the CPMG pulse sequence of \(90^\circ, -180^\circ, -180^\circ, -\ldots\) produces the magnetization vector of

\[ \vec{M} = \begin{bmatrix} -\cos\theta\sin\psi\sin\phi - \sin\theta\cos\phi \\ \cos\psi\sin\phi \\ -\sin\theta\sin\psi\sin\phi + \cos\theta\cos\phi \end{bmatrix}. \quad (7.11) \]
Adding Equations 7.10 and 7.11 eliminates the zero frequency artifact and results in the magnetization of

\[
\mathbf{\tilde{M}} = \begin{bmatrix}
-2 \cos \theta \sin \psi \sin \phi \\
2 \cos \psi \sin \phi \\
2 \cos \theta \cos \phi 
\end{bmatrix}.
\] (7.12)

similar to the method in the Carr-Purcell echo.

7.2.4. THE CARR-PURCELL-MEIBOOM-GILL (CPMG) ECHO TRAIN

Following section 7.2.3, adding the 90° -180° -180° -... sequence to the 90° -180° -180° -... sequence removes the zero frequency artifacts. The magnetizations of the first four CPMG echoes are:

\[
\mathbf{\tilde{M}}_{\text{CPMG Echo 1}} = \begin{bmatrix}
-2 \cos \theta \sin \psi \sin \phi \\
2 \cos \psi \sin \phi \\
2 \cos \theta \cos \phi 
\end{bmatrix}.
\] (7.13)

\[
\mathbf{\tilde{M}}_{\text{CPMG Echo 2}} = \begin{bmatrix}
-4 \cos^2 \theta \sin \psi \sin \phi + 2 \sin \psi \sin \phi \\
2 \cos \psi \sin \phi \\
-2 \cos \phi + 4 \cos^2 \theta \cos \phi 
\end{bmatrix}.
\] (7.14)

\[
\mathbf{\tilde{M}}_{\text{CPMG Echo 3}} = \begin{bmatrix}
-8 \cos^3 \theta \sin \psi \sin \phi + 6 \cos \theta \sin \psi \sin \phi \\
2 \cos \psi \sin \phi \\
-6 \cos \theta \cos \phi + 8 \cos^3 \theta \cos \phi 
\end{bmatrix}.
\] (7.15)
and

\[
\begin{bmatrix}
-16\cos^4 \theta \sin \psi \sin \phi + 16\cos^2 \theta \sin \psi \sin \phi - 2\sin \psi \sin \phi \\
2\cos \psi \sin \phi \\
-16\cos^2 \theta \cos \phi + 16\cos^4 \theta \cos \phi + 2\cos \phi
\end{bmatrix}. 
\] (7.16)

The first echo in the CPMG echo train is correct, there is no mirror image and the resulting MRI image is real. However, the second echo is mirrored with no real images. This process alternates, odd echoes are real and the even echoes are mirrors. By assuming perfect \(\pi/2\) and \(\pi\) pulses in Equations 7.13-7.16, the mirror effect becomes evident. Defining \(\vec{M}_{\text{CPMG, Echo 1}}\) as \([-\sin \psi \; \cos \psi \; 0]\), the first four echoes reduce to

\[
\vec{M}_{\text{CPMG, Echo 1}} = \begin{bmatrix} 1 \\ 1 \\ 0 \end{bmatrix}. 
\] (7.17)

\[
\vec{M}_{\text{CPMG, Echo 2}} = \begin{bmatrix} -1 \\ 1 \\ 0 \end{bmatrix}. 
\] (7.18)

\[
\vec{M}_{\text{CPMG, Echo 3}} = \begin{bmatrix} 1 \\ 1 \\ 0 \end{bmatrix}. 
\] (7.19)

and

\[
\vec{M}_{\text{CPMG, Echo 4}} = \begin{bmatrix} -1 \\ 1 \\ 0 \end{bmatrix}. 
\] (7.20)

with this pattern repeating for even and odd echoes throughout the echo train. The magnetization along the \(x\) direction continually changes sign.
The effects of the magnetization changing sign are easily understood by assuming the x magnetization is real and the y magnetization is the imaginary component of the NMR signal. When the real component of the magnetization "leads" the imaginary component, the frequency of the Fourier transform is positive relative to the oscillator. However, when the imaginary component "leads" the real component, the frequency of the Fourier transform is negative. The changing of the "lead" component in the magnetization is exactly what is happening to the magnetization during each CPMG echo. The cosine "leads" a sine wave, while a negative sine wave "leads" a cosine wave as demonstrated in Equations 7.17 to 7.20. The image is mirrored across the frequency of the rf oscillator: if the real image occurs at positive frequencies then mirror image occurs at negative frequencies.

7.2.5. CARR-PURCELL (CP) ECHO TRAIN

Following the discussion in the previous section, the first four echoes of the Carr-Purcell echo train are found by adding $90, -180, -180, -...$ and $90, -180, -180, -...$:

$$
\tilde{M}_{\text{CP,Echo}} = \begin{bmatrix}
-2\sin \psi \sin \phi \\
2\cos \theta \cos \psi \sin \phi \\
2\cos \theta \cos \phi
\end{bmatrix}.
$$  \hspace{1cm} (7.21)

239
\[
\begin{bmatrix}
-2\sin\psi \sin \phi \\
2\cos^2 \theta \cos \psi \sin \phi - 2\cos \psi \sin \phi \\
-2\cos \phi + 4\cos^2 \theta \cos \phi 
\end{bmatrix}, \quad (7.22)
\]

and

\[
\begin{bmatrix}
-2\sin\psi \sin \phi \\
8\cos^2 \theta \cos \psi \sin \phi - 6\cos \theta \cos \psi \sin \phi \\
-6\cos \theta \cos \phi + 8\cos^3 \theta \cos \phi 
\end{bmatrix}, \quad (7.23)
\]

\[
\begin{bmatrix}
-2\sin\psi \sin \phi \\
16\cos^2 \theta \cos \psi \sin \phi - 16\cos^2 \theta \cos \psi \sin \phi + 2\cos \psi \sin \phi \\
-16\cos^2 \theta \cos \phi + 16\cos^4 \theta \cos \phi + 2\cos \phi 
\end{bmatrix}. \quad (7.24)
\]

Inspection of Equations 7.21-7.24 reveals the mirror imaging effect similar to the CPMG echo train. In the CP echo train, the y-magnetization is alternating sign. It is important to note that the even echoes in the CP echo train have an opposite sign of the even echoes. Without the phase encoding rotation, the odd echoes are positive and the even echoes are negative, see Figure 7.2. Neither the CPMG nor the CP echo train removes the mirror effect, but a combination of the two sequences can remove both the zero frequency artifact and the mirror images.

It is well known in NMR and MRI that in the Carr-Purcell echo train, error accumulates in the \( \pi \) pulses and effectively causes the echo train to decay in a time significantly shorter than \( T_2 \). The accumulated error is \( n\delta \theta \) in the \( n \)th echo with an error of \( \delta \theta \) in each \( \pi \) pulse in the echo train. To eliminate the accumulated error Meiboom and Gill modified the pulse sequence so the phase of \( \pi \) pulse is different than the phase of the \( \pi/2 \) pulse by 90°. The modification removes the accumulated error for the even echoes.
and returns the decay of the echo envelope to $e^{-\gamma \delta}$. Consequently, a method of removing the mirror image needs to be developed without increasing the decay of the echo envelope or affecting the signal to noise ratio.
7.2.6. PHASE-ALTERNATING-PHASE-SHIFT (PHAPS) ECHO TRAIN

The magnetization components after an imperfect 180° pulse can be split into two parts. Computation of the magnitude of the transverse components of the first echo in Equation 7.13 and 7.21 result in the magnitude being split into two parts. The first part has magnitude  \( \frac{1}{2} \sin \phi (1 + \cos \theta) \) at an angle of \( -\psi \) with respect to the axis of rotation and the second part has a magnitude of \( \frac{1}{2} \sin \phi (1 - \cos \theta) \) at an angle of \( \psi \) with respect to the axis of rotation. The second part of the magnetization represents the magnetization being unchanged by the application of the 180° pulse and consequently a real image. Adding the CP echoes to the CPMG echoes will result in a purely mirrored image while subtracting the echoes results in only a real image. The first four real echoes, formed by subtracting the CP and CPMG echoes are:

\[
\vec{M}_{\text{Real Echo 1}} = \begin{bmatrix}
2 \sin \psi \sin \phi (\cos \theta - 1) \\
2 \cos \psi \sin \phi (\cos \theta - 1) \\
0
\end{bmatrix}.
\]

\[\text{(7.25)}\]

\[
\vec{M}_{\text{Real Echo 2}} = \begin{bmatrix}
4 \sin \psi \sin \phi (\cos^2 \theta - 1) \\
4 \cos \psi \sin \phi (\cos^2 \theta - 1) \\
0
\end{bmatrix}.
\]

\[\text{(7.26)}\]
Real Echo:
\[
\begin{bmatrix}
2\sin\psi \sin \phi (4 \cos^2 \theta - 3 \cos \theta - 1) \\
2\cos\psi \sin \phi (4 \cos^2 \theta - 3 \cos \theta - 1) \\
0
\end{bmatrix}
\]  \hspace{1cm} (7.27)

and
\[
\begin{bmatrix}
16 \cos^2 \theta \sin \psi \sin \phi (\cos^2 \theta - 1) \\
16 \cos^2 \theta \cos \psi \sin \phi (\cos^2 \theta - 1) \\
0
\end{bmatrix}
\]  \hspace{1cm} (7.28)

By adding the echoes of the CP and CPMG echo train, mirror images are formed:
\[
\begin{bmatrix}
-2 \sin \psi \sin \phi (\cos \theta + 1) \\
-2 \cos \psi \sin \phi (\cos \theta + 1) \\
4 \cos \psi \cos \phi
\end{bmatrix}
\]  \hspace{1cm} (7.29)

\[
\begin{bmatrix}
-4 \cos^2 \theta \sin \psi \sin \phi \\
4 \cos^2 \theta \cos \psi \sin \phi \\
4 \cos \phi (2 \cos^2 \theta - 1)
\end{bmatrix}
\]  \hspace{1cm} (7.30)

\[
\begin{bmatrix}
-2 \sin \psi \sin \phi (4 \cos^2 \theta - 3 \cos \theta + 1) \\
-2 \cos \psi \sin \phi (4 \cos^2 \theta - 3 \cos \theta + 1) \\
4 \cos \psi \cos \phi (4 \cos^2 \theta - 3)
\end{bmatrix}
\]  \hspace{1cm} (7.31)

and
\[
\begin{bmatrix}
-4 \sin \psi \sin \phi (4 \cos^4 \theta - 4 \cos^2 \theta + 1) \\
4 \cos \psi \sin \phi (4 \cos^4 \theta - 4 \cos^2 \theta + 1) \\
4 \cos \phi (8 \cos^4 \theta - 8 \cos^2 \theta + 1)
\end{bmatrix}
\]  \hspace{1cm} (7.32)

It is interesting to note that the real images all have zero z magnetization.
The above calculations have all been preformed with assumptions: the spins are on resonance, rf pulses have no pulse duration and the spin system contains only one spin. A more complete mathematical representation with a distribution of spins results in differences compared with the single spin case. However, the single spin case does demonstrate the two imaging artifacts and their removal through phase cycling of the echo trains. In a distribution of spins the first echo is always a real image; the mirror image does not appear until the second echo. With distributed spins, the real and mirror images have different magnitudes in the second echo, with the dominant image being mirrored, magnitude $2\sin^2\frac{\theta}{2}$ with only a small real component magnitude, $\sin^2\theta$. This behavior is not clearly shown when only one spin is considered.

When only the CPMG pulse sequence is used, the first echo contains a real image and the second echo contains only a mirror image. For echoes later in the sequence, the mirror and real images mix, with even echoes having a larger mirror component and odd echoes a larger real component. After many echoes in the train, the magnitudes of the real and mirror images become equal. The PHAPS phase cycling method removes the mirror images and zero frequency artifacts from the MRI image without changing the signal to noise ratio of the real image and preserving the signal averaging benefits from the four scans required in the PHAPS sequence.
7.2.7. PHAPS PHASE CYCLING

Using phase cycling to remove the zero frequency artifacts and the mirror images has been demonstrated in the preceding section. To further remove unwanted effects from imperfect $\pi$ pulses, the $\pi/2$ pulse is rotated 180° in phase and the experiment is repeated with the experiments either being added to remove zero frequency artifacts or subtracted to remove the mirror images. The phase shift of the $\pi/2$ pulse is necessary to remove Free Induction Decays that arise from the imperfect $\pi$ pulses in the echo train. Table 7.1 lists the proper phase cycling to remove the imaging artifacts.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Phase of $\pi/2$ pulse</th>
<th>Phase of all $\pi$ pulse</th>
<th>Acquisition Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>x</td>
<td>x</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>x</td>
<td>y</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>x</td>
<td>-x</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>x</td>
<td>-y</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-x</td>
<td>x</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>-x</td>
<td>y</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>-x</td>
<td>-x</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>-x</td>
<td>-y</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 7.1. The phase cycling to properly remove all imaging artifacts when phase encoding with an echo train.

The first few echoes from a PHAPS sequence are shown in Figure 7.3. The even echoes have smaller magnitude than the odd echoes until later in the echo train. As the real and mirror images mix, the difference between even and odd echo heights disappears later in
Figure 7.3. The first 10 echoes, indicated by the numbers on top of the figure, are shown for the PHAPS sequence. Notice the even echoes initially have a small amplitude but grow in later echoes. A small second echo indicates the mirror image has been effectively removed from the imaging sequence. Only one “period” of the echo is acquired per echo and then, 15 μs later, another π pulse is applied and another echo acquired. This process is repeated hundreds of times to acquire the entire echo train.

The resulting phase encoded images with the PHAPS sequence results in only a real image being produced.
The Fourier transform of a function is \( F(\omega) = \int_{-\infty}^{\infty} f(t)e^{-i\omega t} dt \). When the limits of integration change due to requirements of a finite data acquisition, the effects on the Fourier transform can induce severe rounding of the edges in the image as well as Gibbs artifacts. It is impossible to acquire data for an infinite amount of time due to storage limitations and most importantly the desire to acquire the image in a reasonable amount of time. To accomplish the image acquisition, the data is truncated after a "reasonable" amount of time to minimize the rounding effects.

In Figure 7.4a is a sinc function shown as a function of time. When Fourier transformed, with limits of integration of minus infinity-to-infinity, results in a perfect square function as seen in Figure 7.4b. However, when the limits of integration are changed and the "Fourier" transform becomes \( F(\omega) = \int_{-\tau}^{\tau} f(t)e^{-i\omega t} dt \), the consequences are seen in Figure 7.5. In this figure, eight different limits of integration have been performed and the effects on the "square" function are shown. The limits of integration are 1.3.5.10, 20, 50, and 100, and 500 radians. Notice the rounding at the edge of the square pulse and the Gibbs ringing at the edges of the function. Similarly, the rounding of the image occurs during selective excitation when a truncated sinc pulse is used.

To calculate the effective limit of integration in the Fourier transform from the MRI imaging sequence, the linewidth of the sample (in each dimension), in Hz at the applied
Figure 7.4. In part A, a sine pulse is shown. Using a complete Fourier transform, limits of integration from negative infinity to positive infinity results in a perfect square pulse as shown in part B.

Gradient strength, and the longest imaging time in k-space for each dimension are needed.

The effective limit of integration is

\[
\frac{HHFW}{2} \cdot 2\pi \cdot \text{Longest Time} = \text{Limit of Integration} \quad (7.33)
\]
Figure 7.5. The effect of truncated limits of integration on a sinc pulse. The limits of integrations are, in order, 1, 3, 5, 10, 20, 50, and 100, and 500 radians. Notice the artificial rounding that occurs on the edges when the limits of integration are truncated as well as the Gibbs ringing on the edges.

where the time in Equation 7.33 is the longest time the gradients are on during phase encoding. Once the effective limit of integration is determined, comparison with Figure 249
7.5 demonstrates the artificial rounding of the image. The rounding due to the truncation of the Fourier transform limits resolution in the image and must be considered when calculating the resolution of the MRI images. Usually, limits of 20 or greater radians are acceptable for producing sharp edges in MRI. Either increasing the gradient strength or increasing the applied gradient “on” time, \( k = \frac{1}{2\pi} \gamma G t_{\text{on}} \), can accomplish increasing the effective limit of integration. In the imaging method, Figure 7.1. \( t_{\text{on}} \) is the largest time where the gradient is “on” during the phase encoding. The maximum k-space value limits the resolution in all MRI images and must be balanced with the necessity of limiting the image acquisition time.

### 7.4. MRI MICROIMAGING PHANTOMS

To acquire an image, a PHAPS echo train was acquired, 2-dimensional, for each point in k-space (typically a 64 by 64 array) and signal averaged for 16 scans per point unless otherwise mentioned. The data acquisition for each echo was 32 points with a 16 \( \mu \text{s} \) dwell time for each of the 128 echoes per echo train. The image takes ~20 hours, and is only limited by the duty cycle allowed by the gradient power supply. The delay between acquisitions cannot be less than one second to avoid damage to the MOSFETS.
Each echo train is then Fourier transformed after leftshifting the echo train to the center of the first echo. The FT of each echo train results in a single frequency spectrum and the data point corresponding to that frequency, with the phase encoding information, is then extracted and written to a new file. The new file is two-dimensional, with 64 points in 64 files 1D records, one point per each value of k-space. This new file then undergoes a 2D Fourier transform to present the image. See Appendix A for scripts to automate processing with the Apollo Tecmag.

Constructing MRI microimaging phantoms is extremely difficult. The problem with imaging very small objects is constructing the phantoms in the first place. The objects in the length scale required, typically less than 50 microns, limits the potential imaging phantoms to spheres and rods.

7.4.1. PYREX RODS IN A CYLINDER OF WATER

One problem with imaging the hydrogen in free water is the very long $T_1$ and $T_2$ times. Typically, $T_1$ is approximately ~2 seconds and $T_2$ is ~1 second. The long $T_1$ times restricts the repetition rate of the successive experiments. To decrease the effective $T_1$ time, a 0.01 Molar solution of Cu(NO₃)₂•2.5H₂O solution was added to the water producing $T_1$ times less than 200 ms and $T_2$ times less than 20 ms.
For the first microsample, a coil was wrapped with six turns with 20 μm copper wire, with an outer diameter of 100 μm and an inner diameter of 77 μm. All measurements were obtained optically with a microscope. Water was then placed in the micropipette through capillary action. A 25 μm diameter Pyrex rod was then inserted into the micropipette, parallel to the axis of the microcoil, displacing some water inside the microcoil. Imaging the water inside the microcoil, in slices perpendicular to the axis, should yield a circle of water 77 μm in diameter with a 25 μm diameter “hole” in the image.

Figure 7.6 was acquired with an imaging scheme similar to Figure 7.1, but with no selective pulse, a “hard” 90° pulse was used instead. The $G_z$ gradient was 4.5 T/m and the $G_z$ gradient was 3.5 T/m, with the axis parallel to the x direction. The resulting image is 2-dimensional, with the depth of imaging approximately 200 μm, the length of the microcoil. The image was scaled to show the expected circular shape of the outer diameter. The maximum time the gradients were on was 660 μs for each gradient with 20 μs steps from -640 μs to 660 μs in a 64 by 64 array.

In Figure 7.7, the full imaging scheme was used as shown in Figure 7.1 with a selective pulse excitation of approximately half of the spins, ~100 μm deep. The transverse gradients strengths are 6 T/m for $G_y$ and 8 T/m for $G_z$. The sample used was different than the sample in Figure 7.6, accounting for the position change of the Pyrex rod inside the microcoil. The image was scaled to show the expected circular shape of the outer diameter. The maximum time the gradients were on was 660 μs for each gradient with 20 μs steps from -640 μs to 660 μs in a 64 by 64 array.
The first image of a Pyrex rod inside a cylinder of water is shown at the top of the figure. The rod is not perfectly round due to the fact that the rod is not completely parallel to the axis of the cylinder. See the text for imaging parameters. The image dimensions are shown in the schematic below the image.

In both the preceding images, the rod does not appear as a perfect circle because the rod was not perfectly parallel to the axis of the coil. This imperfection caused the
Figure 7.7. A second Pyrex rod sample was constructed and imaged by the parameters in the text. This sample is once again oval due to the fact that the rod was not completely parallel to the axis of the coil. Unlike Figure 7.6, this image was created with a selective pulse of 100 μm in depth. Image dimensions are shown on the bottom schematic.

"hole" to become oval and blurred. Additionally, the Pyrex rods caused significant relaxation of $T_1$ and $T_2$ that degraded the image. Pyrex rods can contain magnetic...
impurities in the glass, significantly shorting $T_1$ and $T_2$, and increasing the difficulty in imaging due to $T_2$ effects. Originally, Pyrex micropipettes were used a coil form but were discarded in favor of quartz micropipettes due to the adverse effects on $T_1$ and $T_2$.

7.4.2. QUARTZ RODS IN A CYLINDER OF WATER

The next sample was constructed by using the pipette puller to pull short quartz (~1 mm diameter) rods to smaller diameters of less than 50 μm. A 1 mm diameter quartz rod was pulled in a manner similar to the method used to construct the micropipettes. The quartz rod was then inserted into the small end of the micropipette and both ends were sealed with optical glue to prevent the water with Cu(NO$_3$)$_2$·2H$_2$O added, from evaporating. The quartz rods are not perfectly uniform but their properties are superior to the Pyrex rods in the previous samples. Additional effects reducing $T_1$ and $T_2$ are not present when using quartz rods.

A ~35 μm diameter quartz rod was inserted into a 5 turn coil constructed with 20 μm diameter copper wire. The coil had a length of 250 μm, an outer diameter of 90 μm and an inner diameter of 75 μm. The rod is not fully inserted into the coil, leaving regions in the coil with no rod and regions in the coil with the rod. Figure 7.8 shows a representation of the constructed sample.
Figure 7.8. This a rendition of the sample used for the next three images. In the first image, Figure 7.9, a slice 100 µm in depth centered on the left side of the sample encompassing both a the rod and the region without the rod. Figure 7.12 is a slice of just the rod and Figure 7.13 is a slice centered in the region without the rod.

The first image taken with this sample, Figure 7.9, was a wide slice of ~100 µm in length including regions were the rod is present and not present. The gradient strengths for both $G_x$ and $G_z$ were 6 T/m and the longest duration of the gradients was 660 µs. The resulting image clearly shows the inner diameter wall of the micropipette and an lower averaged region at the location of the rod. This region has lower intensity since the rod displaces the water, but not zero intensity since the rod is not fully inserted into the coil. A horizontal slice profile of the sample is shown in Figure 7.10 in the region were there is no rod showing sharp edges and a flat profile inside the coil. A vertical slice is shown in Figure 7.11 showing the two plateaus in the sample corresponding to areas with and without the rod. Both of these plateaus have a relatively flat profile.
Figure 7.9. The quartz rod is not completely inserted into the coil and the excited slice includes regions with and without the rod. The resulting image shows the two regions, with the darker region where the rod is located.
Figure 7.10. A horizontal projection of the image in Figure 7.9 is shown corresponding to the white horizontal line in the figure. Notice the steep edges corresponding to the edge of the cylinder and the flat profile throughout the sample.
Figure 7.11. The vertical profile of the image in Figure 7.9 shows two plateaus corresponding to regions with the rod (lower plateau) and without the rod (higher plateau). The lower plateau is not zero since part of the excited slice includes regions without the rod.
To demonstrate the selectivity of the slices in the image, a 25 \( \mu \text{m} \) wide slice was taken of the sample in a region were the rod is located, see Figure 7.12. The image clearly shows the “hole” at the edge where the rod is located. In Figure 7.13, the slice, 25 \( \mu \text{m} \) wide, was centered on a region without the rod and the resulting image clearly shows a circle indicating the water inside the inner diameter of the micropipette. Both 25 \( \mu \text{m} \) wide slices was taken with \( G_y \) and \( G_z \) still at 6 T/m and longest duration for the gradients was 660 \( \mu \text{s} \).

7.4.3. POLYSTYRENE BEADS

Many beads, mean diameter of 20 \( \mu \text{m} \), were placed inside a micropipette and the small end of the micropipette was sealed with optical glue. A glass rod was inserted in the unpulled side of the micropipette and packed the beads in the pipette. The resulting sample was completely filled with beads, each bead touching at least two other beads, see Figure 7.14. An image was taken, Figure 7.15, with \( G_y \) and \( G_z \) at 7 T/m and 7.3 T/m respectively and the longest pulse length of the gradients of 660 \( \mu \text{s} \). The image is difficult to predict since an optical view perpendicular to the axis of the coil is impossible to attain. The image used slice selection with a slice width of 25 \( \mu \text{m} \) and clearly shows
Figure 7.12. A slice of 25 microns in depth was excited in the region only including the rod. The rod forms a void in the image. Some zero frequency artifacts are present in the image. An axial profile of the sample is shown below. The lower plateau corresponds to the position of the rod and the high region on the left to the location where the rod is no longer inserted into the coil.
Figure 7.13. A 25 micron slice was excited in a region without the rod resulting in the circular image of the water density. The one-dimensional axial profile is shown below indicating the slice selection (through in the profile) corresponding to the water profile. Comparison with Figure 7.12 demonstrates that the high point of the profile was excited corresponding to the region without the rod.
Figure 7.14. Many polystyrene beads of diameters 15-30 microns were placed inside a quartz micropipette with a rod inserted (at left) to compact the beads and prevent any motion. The coil location is indicated and a 5 turn coil, 100 μm in length and 70 μm in diameter was constructed and Figures 7.15-7.17 were acquired with this sample.

The next two images, Figures 7.16 and 7.17 are MRI microimages of the sample. However, this time the slice widths are only 14.8 μm wide. For both images, the G_y gradients were 5.5 T/m and the G_z gradient was 9.1 T/m. The G_x gradient was 17 T/m for slice selection. The slice selection in Figure 7.17 is displaced 7 μm from the image in
Figure 7.15. A 25 micron slice of the sample in Figure 7.14 is shown. Several beads are visible in the image.

Figure 7.17, so some of the same beads are visible in both images. The beads in these images are clearly visible. The images are oval due to the unbalanced gradients. A horizontal projection is shown in Figure 7.17 and a vertical projection in Figure 7.18 of the image in Figure 7.16. These two images demonstrate the resolution in each dimension, which is discussed in the following section.
Figure 7.16. A 15 micron slice was excited in the sample of Figure 7.14. At least three beads are visible in this image.
Figure 7.17. A 15 micron slice was excited in the sample of Figure 7.14. However, this slice was shifted 7 microns from the position of the image in Figure 7.16. The two "large" (maybe more) beads are visible in both images with the bead on the left in the first image but no longer in this image.
Figure 7.18. A horizontal profile of the image in Figure 7.17 is shown above through the "big" hole in the upper half of the image. The edges of the image can be used as a guide for determining resolution, as they ideally should have infinite slope. The actual slope can be used to estimate the spatial resolution in the y-direction of the image. The signal to noise ratio for this image is approximately seven.
Figure 7.19. A vertical slice through the image in Figure 7.17 is shown. The edges of the sample can be used to estimate the resolution of the image, see text. The signal to noise for the image is approximately seven.
7.5. MRI RESOLUTION

Resolution in MRI is difficult to quantify. Usually in MRI resolution is quoted to be far superior that actually visible as most experimentalists use the voxel dimensions as the resolution. While voxel dimensions calculated from the gradient strengths in all three directions provide a lower limit on resolution, they do not qualify for the resolution limitation. The voxel size is usually smaller than the resolution in the image. The resolution is limited by largest k-space vector and signal to noise in addition to the applied gradient strengths that determine voxel resolution.

To estimate the resolution, the slope on the falling edges of the sample profiles, see Figures 7.18 and 7.19, where used. Ideally, the slope should be a vertical line with infinite slope but due to SNR limitations and finite k-space vectors, rounding does occur. From the rounding on the edges of the sample a rough estimate of resolution can be obtained for the image in Figure 7.17. Estimated resolution for the image is $14.8 \mu m \times 2 \mu m \times 3.5 \mu m$ or $\sim 100 \mu m^3$. The hundred cubic micron resolution is currently the best obtained, but is definitely not the lower resolution limit for the system. However, the resolution reported here is among the best ever reported for Magnetic Resonance Microscopy.
There are several factors that are currently limiting the resolution in the system and both arise from hardware limitations of the Apollo Tecmag and computers in general. The file size of each image taken is 128 MB, already large, but to completely image in 3-dimensions, the file size would have to be 150 GB. Clearly, this is not feasible. To limit the file size, after 128 echoes no further data is recorded. However, there is still significant signal present after 128 echoes; the file size limitation prevents the complete acquisition of the echo train. A sample acquisition of a PHAPS echo train is shown in Figure 7.20.

In addition to the file size problem, the digital receiver in the Tecmag acquires three zeroes at the end of each acquisition of an echo. The zeroes effectively convolute a repeated square wave on top of the NMR signal, degrading the SNR of the FT of the PHAPS echo train. The zeroes also add to the data file size as this information is still saved with the complete file. In our current pulse sequence, not only are the last three points zero, but also the first point in each echo is also bad due to digitizer recovery limitations. Four out of the 32 points acquired for each echo effectively add systematic noise to the image. Figure 7.21 demonstrates the zeroes in the acquisition of a PHAPS sequence. The forced acquisition of the zeroes by the Apollo Tecmag forces over-sampling as compared to the Nyquist criteria and also increases the file size.
Figure 7.20. A single PHAPS echo train is shown above. Notice that the signal is not zero at the end of the 128 echoes displayed. The data file size limitation leads to a decrease in the maximum possible signal to noise ratio for the image.

Digitizer Zeroes the Data

Figure 7.21. A few echoes of the PHAPS echo train in Figure 7.20 are shown. The arrows indicate the location of the "zeroes" acquired by the Tecmag. The zeroes increase the file size and limit the signal to noise as now the signal is convoluted with a repeating square pulse. The echo train ideally should be a continuous damped sine wave.

Decreasing the number of acquired points per echo to the Nyquist criteria would result in a serious convolution with the repeating square wave and reduce signal to noise.

Figures 7.17 to 7.19. show that the acquired image is always placed in only one quadrant of k-space. The reason for this restriction is evident from the addition and subtraction of string of π pulses. Ideally, the resulting signal from adding and subtracting 271
the pulse train of \( \pi \) pulses should only be noise. However, it seems that the Tecmag has small variations, less than 1\%, in rf power output effecting the flip angle of each pulse. The sequence of addition and subtraction of the pulse train results in imperfect cancellation of the NMR signal and adds "signal" to regions where there should be no "signal". This effect causes additional noise in the system as a DC baseline and the zero frequency spikes after Fourier transform usually appear in both dimensions of the image. Removal of this Tecmag problem will allow for the image to be centered in k-space, limiting the number of points in k-space to successfully acquire the image. The reduction of the number of data points could lead to a 32 x 32 data matrix and significantly smaller file size and a corresponding decrease in the minimum image acquisition time.

True 3-dimensional imaging will increase the signal to noise as every scan contributes to the signal. To accomplish 3D imaging, a "script" in Visual BASIC was written, but not completely implemented, to correct for the first two hardware limitations. The script is in Appendix A. The script effectively acquires each PHAPS echo train, removes the zeroes from the file and after Fourier transforming the file, saves only the one required data point corresponding to the value at the desired frequency. This method allows the file size limitation to be circumvented as the PHAPS echo trains do not have to be individually saved. Since file size is not a problem with the script, it allows for complete acquisition of the entire echo train increasing the potential SNR. Full 3D imaging will acquire additional work and programming to be fully implemented.
7.7. DISCUSSION ON IMAGING

While the 100 μm³ resolution presented here is an improvement over the current resolution in MRI microscopy, it does not represent the lower limit for the MRI system. The signal to noise ratio is approximately seven for the image in Figure 7.19, indicating signal to noise can be still be sacrificed to increase resolution through the use of larger gradients or a more selective soft pulse. The resolution of a 100 μm³ is also extremely conservative as compared to previous authors, see Chapter 1, as they often use voxel dimension as their resolution. Several "technical" glitches centered on the Apollo Tecmag need to be solved to further increase the resolution in the MRI microscopy experiments. Work will continue to probe the limits of resolution with MRI imaging phantoms (rods and beads) until a limit of resolution based solely on SNR is reached. Concurrently, the imaging of biological cells will occur.
7.8. LIST OF REFERENCES


8.1. INTRODUCTION

While Magnetic Resonance Imaging has disadvantages as compared to optical microscopy, mainly imaging acquisition time and resolution, it does have some important advantages as well. MRI offers different mechanisms to further enhance the image by increasing the contrast between two different regions of the image. Perhaps the most important potential for this increase in resolution presented is the possibility for localized chemical information through magnetic resonance spectroscopy (MRS) or the imaging of motion inside the cell. Diffusion weighted imaging perhaps can provide insight into the transport behavior inside a cell. Beyond the resolution enhancement presented, there is
the potential for further increase in resolution by 2 - 3 orders of magnitude through a sequence known as DESIRE. Diffusion Enhanced Signal Intensity and Resolution permitting nanometer resolution inside biological cells.

8.2. IMAGING CONTRAST MECHANISMS

Traditional MRI imaging is more difficult to implement than optical microscopy for imaging cells but does offer some superior attributes. First, the cell does not have to be died or sliced and killed to be imaged as in optical microscopy. MRI allows for the imaging of living cells in a full three-dimensional picture, whereas optical microscopy offers only two-dimensional views. Several contrasting mechanisms are available in MRI: spin density, $T_1$ and $T_2$ differences between tissues, magnetic susceptibility differences, magnetization transfer, diffusion and other injected contrast agents.

The most common contrast mechanisms in MRI are $T_1$ and $T_2$ and are previously discussed in section 1.3.5. $T_1$ differences between tissues offer a method to determine regions of tissue boundary inside the sample. Tissues with longer $T_1$ times will have a lower intensity in the MRI image as compared to tissues with shorter $T_1$ times. $T_1$ and $T_2$ weighted images are commonly used to discern white matter, gray matter and cerebrospinal fluid in MRI images of the brain in hospital MRI images. $T_1$ contrast can also be used to enhance detail of vascular flow.
Many diseased regions of a sample have higher $T_2$ values than surrounding healthy tissue, offering a method of localization of the disease. Since $T_2$ is usually significantly shorter than $T_1$, a small change in $T_2$ can have a large percent change and result in a more significant contrast than changes in $T_1$. MRI imaging offers great clinical advantageous over optical microscopy in terms of determining the location of diseased tissues.

Magnetic susceptibility differences inside the sample can also be used as a contrasting mechanism. Susceptibility differences across a boundary create magnetic field gradients and can be exploited to enhance the boundaries between regions in the sample. The susceptibility effects are due to phase differences accumulated during by moving across boundaries with localized magnetic field differences causing phase differences across the boundary. Together, these contrasting mechanisms provide information that is not available in optical microscopy. The ability of imaging living organisms without alteration can make MRI imaging superior to optical imaging.

8.3. DYNAMIC MRI MICROSCOPY

The first use of dynamic imaging occurred in 1964 by Stejkal and Tanner. Together, these two people were the first to use NMR to measure diffusion constants. Using an effective phase encoding gradient, $g$, with zero time integral accumulates a
phase proportional to the velocity in the signal. The effective phase encoding gradient, g, is not to be confused with the spatially encoding gradients, G.

Analogous to k-space, motion is measured in q-space, where q is the Fourier conjugate variable to the motion. Using dynamic MRI microscopy\cite{1,3} it is possible to measure velocity, acceleration and other higher order derivatives of the motion using MRI dynamic imaging. To measure velocity, the topic of discussion in this section, the first moment of g must be non-zero, and p is defined to be the first moment of g.

\[ \bar{p} = \gamma \int_0^r \int g(t) dt. \quad (8.1) \]

The phase shift generated with a zero time-integral effective gradient field is proportional to the moment times the velocity.

Applying both the spatially encoding gradient and velocity encoding gradient results in the modulated signal.

\[ S(k,q) = \int \rho(r) e^{i2\pi k} \int P(r,r',\Delta) e^{i2\pi q(r' - r)} drdr'. \quad (8.2) \]

where P is the probability a spin a point r moves to r' in a time \( \Delta \). Using the Fourier transform on Equation 8.2, yields the velocity motion as well as the random diffusion information. In Figure 8.1 is the pulse sequence to measure velocity information and in Figure 8.2 is a pulse sequence that removes the velocity information yielding only the diffusion motion. These pulse sequences are called pulsed-gradient-spin-echoes (PGSE).

Measuring diffusion constants and flow at the intracellular level has the potential to add a new tool to biology. Transport behavior inside the cell can possibly be visualized and quantified like never before. MRI dynamic imaging with the improved
Figure 8.1. This PGSE pulse sequence incorporates both k-space and q-space information allowing the visualization of the velocity flow throughout a region of spatial interest. This pulse sequence uses the projection reconstruction technique (PR) to encode the spatial information. The pulsed gradients, $g$, are varied in duration and/or magnitude to sample the complete q-space.

resolution presented can allow for intracellular diffusion studies, and local velocity and acceleration measurements inside living cells.
Figure 8.2. This PGSE sequence removes the velocity information with a double echo technique leaving only the self-diffusion information. Phase shifts due to coherent motion are refocused with the double echo.

8.5. MRI CHEMICAL SPECTROCOPY

To accomplish MRI microscopy, several modifications to the existing hardware will have to be made beyond what is presented in the previous seven chapters. Currently, linewidths of the hydrogen in water are typically ~100 Hz or about 0.5 ppm in the 9 Tesla magnetic field used in these experiments. To further reduce linewidths and increasing the spectral resolution, several modifications can be made. Significant line broadening is the result of the capacitor's location close to the rf coil.
To remove the effect of the capacitor, the capacitor can be placed at the "magic angle" with respect to the rf coil and the static magnetic field. Assuming the capacitor is a dipole, this configuration would reduce the field distortions at the coil location. However, this method will probably result in longer lead lengths to the coils and possible decrease the signal to noise. Another modification, is to remove the physical capacitor entirely from the circuit and use striplines to make capacitors directly on the rf board. This method has the added advantage of possible removing some additional stray inductance, as about 1/3 of the stray inductance in the rf board is present in the capacitor. Stripline techniques will possibly increase the difficulty in tuning the rf coils but will be rewarded with lower stray inductances and lower field inhomogeneities. Since, the striplines will be constructed out of the copper all ready on the circuit board, no further materials of differing magnetic susceptibilities will be introduced.

In addition to removing the capacitors, susceptibility matching fluids can be added in the "can" of the probe. The magnetic field is uniform inside a cylinder of the uniform magnetic susceptibility. Since the probe is made of differing materials, all of which are diamagnetic, adding a liquid in the "can" that is also diamagnetic will create more uniform magnetic fields. Usually, FC-43 is added as the magnetic susceptibility matching liquid. However, this liquid is expensive and evaporates rather quickly.

Fromblin, or fluorinated pump oils, offers a better alternative to FC-43. The higher molecular weights of the fluorinated pump oils decrease the volatility of the liquid and make them significantly cheaper. Y04 grade Fromblin (Ausimont) has a molecular
weight of 1500 amu and a density of 1.87 g/cm³. Fromblin has the following chemical structure.

\[
CF_i \left[ O - CF - CF_2 \right] \left[ \begin{array}{c} \vdots \rule{0.8cm}{0.15em} \rule{0.8cm}{0.15em} \\ CF_i \end{array} \right]_{m} - [O - CF_2]_n - O - CF_i
\]

where \( m \) and \( n \) are numbers that indicate the number of times the chain is repeated. Higher molecular weight of the Fromblin corresponds to higher values of \( m \) and \( n \). For the Y04 grade Fromblin, \( m \sim 7.7 \) and \( n \sim 1.1 \), on average. The diamagnetic volume magnetic susceptibility can be found using Pascal’s method and is approximately \(-0.667 \times 10^{-6}\) very similar to Copper’s \(-0.768 \times 10^{-6}\) (cgs units). The use of susceptibility matching liquids and the removal of the capacitors has the potential to dramatically narrow the linewidths as compared to the current hardware configuration.

After improving the spectral resolution in the probe, chemical spectroscopy is possible. MRI spectroscopy can provide localized spectral information allowing the possibility of intracellular chemistry to be explored. It is this possibility that offers the greatest insight into the cellular biology and will provide information that is not available in any other methods. Localized chemical information from inside the cell is the best method to attain new information about cells and is a goal for subsequent research.

Several different pulse sequences can be used to attain localized chemical information. ISIS (Image Selective in-vivo Spectroscopy) was specifically designed for Phosphorus NMR. It uses an 8 experiments phase cycle to select the desired voxel of interest relying on an addition-subtraction principle. This method is more effective on larger sample volumes due to the addition and subtraction errors. DIGGER (Discrete
Isolation from Gradient-Governed Elimination of Resonances is a modification to the ISIS sequence and uses a sin-sinc pulse to excite the slices resulting in lower errors on small volumes. 

The STEAM sequence (Stimulated Echo Acquisition Mode) relies on a stimulated echo formed by three orthogonal selective (sinc pulses) 90° pulses. Three 90° pulses form a stimulated echo. Only the voxel "seeing" all three selective pulses forms an echo and the spectral information is acquired in absence of the gradients. This method produces localized chemical information. Other pulse sequences can be added to suppress the water signal resulting in only the spectrum from non-water molecules. An improvement on the STEAM sequence is the PRESS sequence (Point Resolved Spectroscopy): the pulse sequence is shown in Figure 8.3. Both STEAM and PRESS have advantages and disadvantages as compared to the each other. PRESS is less susceptible to motion and diffusion artifacts and gives a signal to noise advantage of twice that over the STEAM sequence. The STEAM sequence works better with short T2 than the PRESS sequence due to the shorter required times between rf pulses in a stimulated echo. Both of these sequences provide spectral information in a single scan. The PRESS and STEAM sequences provide the opportunities to acquire chemical information inside cells and with the improved resolution presented in the previous chapters.
Figure 8.3. The PRESS technique acquires chemical information in a single voxel in only one scan through the use of three selective pulses. Only the voxel that "sees" all three selective pulses will produce a signal during acquisition. Additional despoiling gradient pulses may be required to destroy unwanted magnetization.

8.6. DESIRE SEQUENCE

If a sample is placed in an inhomogeneous magnetic field with linear gradients in all three spatial directions, rf pulse in presence of the gradient can saturate the resonance in a certain region of the sample. This technique is known as "hole-burning". The spins inside the saturated region will then begin to diffuse in throughout the sample. The diffusion rates are limited by the properties of the sample, diffusion constants and barriers, and relaxation rates of both the transverse and longitudinal magnetization. Molecules from outside the saturated regions can diffuse into the region and become
saturated with the addition of another rf pulse. If this process is repeated many times, an amplification of the excited region is possible as the number of saturated spins increase. This sequence is known as the DESIRE sequence, or Diffusion Enhanced Signal Intensity and Resolution, which exploits diffusion to magnify the effective signal from a given voxel by as many as 2-3 orders of magnitude\(^1\)\(^-\)\(^2\).

In order to explain mechanism of this method, first pre-suppose that, using a sophisticated sequence of time dependent gradients and time dependent rf phase and amplitude, it is possible to apply saturating pulse (one which destroys the z-component of magnetization) to only a small volume region within a biological cell. For example, let us suppose that we can destroy the magnetization everywhere within a sphere of diameter 1 \(\mu\)m. (The volume of this sphere \(V_{\text{uum}}\), which is given by \(0.52 \mu m^3\).) Schematically, this is depicted as a black circle in Figure 8.4, labeled “1” in the figure.

After 1 ms, essentially all of the spins initially inside the sphere will have diffused to the outside, being replaced by “fresh” spins which have not yet experienced the pulse. This new situation is represented by the situation labeled “2” in the Figure 8.4. Next, we apply another pulse to the 1 \(\mu\)m diameter sphere, flipping now the new spins which have entered (situation “3” in the Figure 8.4). Waiting again for a time of 1 ms, the sphere refreshes with (mostly) new, unflipped spins. This sequence can be cycled for an arbitrary number of times. Ultimately there is limited effectiveness in taking the total time duration beyond the proton \(T_1\)-Is, so we expect on the order of 1000 cycles.

For \(N\) cycles, the remarkable thing is that the total number of flipped spins will correspond to a volume of almost \(N\) times the volume \(V_{\text{uum}}\). The number is only “almost”
Figure 8.4. Schematic diagram of the concept of "Pure Desire" sequence, as described in the text. It can be used to magnify the signal from a voxel. Only two pulses are illustrated, but in practice, hundreds of pulses could be applied, magnifying the effect by 2-3 orders of magnitude.

...because spins present in the sphere are not exclusively "fresh," unflipped spins.

Mathematical simulation of the DESIRE sequence starts with the diffusion equation.

\[
\frac{\partial \vec{M}}{\partial t} = D \nabla^2 \vec{M}
\]  

(8.3)

where \(D\) is the diffusion constant for intercellular water and \(\nabla\) is the Laplacian operator.

The diffusion equation can be added to the Block Equations. Equations 1.5-1.7, to form the Block-Torrey Equations. Assuming the sample of interest is an infinite sphere, and the saturation occurs in a sphere of diameter 1 micron (radius \(\text{a}^{'}\)) at the center. Equation 8.3 can be solved to
find the magnetization in the sphere at later times. The solution to Equation 8.3 for a spherical volume is:

\[ M(r,t) = \frac{1}{2r(\sqrt{\pi Dt})} \int_{0}^{\infty} f(r') r' \left( e^{-\frac{(r-r')^2}{4Dt}} - e^{-\frac{(r+r')^2}{4Dt}} \right) dr' \] (8.4)

where \( f(r') \) is the initial conditions of the sample. For the saturated spins, \( f(r) = 0 \) for \( r < a \) and \( 1 \) for \( r > a \). The numerical factor is Equation 8.4 normalizes the volume so the magnetization in the whole sphere is one.

A time of 1 ms is allowed to evolve and then Equation 8.4 is integrated again, changing the initial conditions to the magnetization at 1 ms. This process is repeated for \( N \) times, each time using the “old” magnetization as the “new” initial conditions after forcing the magnetization for \( r < a \) to zero. To determine the magnetization effects on the entire sample after \( N \) iterations, a computer code in FORTRAN was written and is in Appendix B. The results are given and described in Figure 8.5. If we then apply a 90-degree pulse and observe an NMR signal from the entire sample, it will be reduced by an amount corresponding to this volume reduction of almost \( NV_{\text{num}} \). Thus, it is possible to enhance signal to noise ratio by 2-3 additional orders of magnitude beyond what has already been attained using microcoils.

Now, clearly, some protons within a biological cell do not diffuse. Then of course their signals would not be amplified by the DESIRE sequence. An image built from DESIRE would then be based on some kind of product of proton density and diffusivity. The diffusivity then serves not only as an enhancer of signal to noise ratio but also as a powerful effective contrast agent. The DESIRE sequence demonstrates the
Figure 8.5. Theoretical prediction of S/N enhancement from the DESIRE sequence applied to a 1 μm diameter sphere of water (volume $V_{1\mu m}$), within a much larger water sample. The prediction is obtained by numerically solving the diffusion equation with appropriate initial and boundary conditions. Spins within $V_{1\mu m}$ are saturated once every millisecond. Plotted is reduction of the NMR signal from the entire sample, in units of $V_{1\mu m}$, after N pulses have been applied, vs. the “Pulse Number” n. The destruction of signal from the entire sample is measured in units of $V_{1\mu m}$. For the parameters used, which are appropriate for water and for the volume resolution that we seek, the plot predicts signal amplification by at least two orders of magnitude.

feasibility of significantly increases the spatial resolution in Magnetic Resonance Imaging to the nanometer level.
8.7. GRADIENT POWER SUPPLY MODIFICATIONS

Modifying the gradient power supply to accept control from the Tecmag will allow amplitude modulation of the gradients. The Apollo Tecmag supplies a differential +/-10 Volt output for control of external gradients. The differential output can be used to replace the voltage setting potentiometer of the current gradient power supply and is necessary to use the DESIRE sequence. In addition to the DESIRE sequence, using the Tecmag to control the magnitude of the gradients will allow for shorter phase encoding times since the time the gradients would be on is now constant. This method can save time in phase encoding the image and can shorten the refocusing pulse duration of the selective pulse by increasing the amplitude of the refocusing gradient. By shortening the total time of the pulse sequence, \( T_2 \) effects limiting the signal to noise ratio can be even further limited.

8.8. CONCLUSIONS

The hardware deigned for MRI microscopy, rf circuitry and microcoils, gradients and the gradient power supplies and the rf switching circuits all function together and demonstrate the potential for even further increase in MRI resolution. While current
resolution limit is approximately 100 μm³. This is not the ultimate limit of the hardware constructed, but a limit of the Apollo Tecmag. These limitations, discussed in Chapter 7, have the potential to be removed with further work and software modifications. Further resolution enhancement is possible without any additional modification to the MRI hardware constructed, but the hardware modification suggestion in Chapter 7 can lead to applications into cellular biology and chemistry. The potential for intracellular chemistry should be the next future focus for the experimental apparatus constructed for MRI microscopy.
8.9. LIST OF REFERENCES


A.1. INTRODUCTION

The Apollo Tecmag can be automated through Visual BASIC computer programs. These programs simplify data processing and can be used to automate the entire data acquisition process. Several programs written to process the data and a preliminary program for data acquisition for complete 3-Dimensional imaging is presented in the following sections. The associated "forms" for the Visual BASIC programs are not shown.
A.2. LEFTSHIFT PROGRAM

The leftshift processing function built into the Apollo Tecmag only works for files up to two-dimensions. To leftshift a three-dimensional file, like the files used to acquire the MRI images, the following program must be used.

```vba
Dim Data As NTNMR.Document
Dim App As NTNMR.Application
Dim Points As Long
Dim Nx As Long, Ny As Long, Nz As Long
Dim N2D As Long

Private Sub Command1_Click()
    Dim objectpath As String
    objectpath = App.GetActiveDocPath
    Set Data = App.GetObject(objectpath)

    Nx = Data.GetNMRParameter("Points 1D")
    Ny = Data.GetNMRParameter("Points 2D")
    Nz = Data.GetNMRParameter("Points 3D")

    N2D = Ny * Nz
    Points = Text1.Text
    Form1.Caption = "Dimensioning..."
    Data.ResetDimensionInfo Nx, N2D, 1, 1
    Data.LeftShift Points, True
    Data.ResetDimensionInfo Nx, Ny, Nz, 1
    MsgBox("Finished LeftShift")
End Sub
```
A.2. EXTRACT PROGRAM

The data processing requires an "extraction" of a single data point from each Fourier transformed PHAPS echo train. The extracted data point is then written to a new file called "Extract.tnt". This file is then redimensioned as a 2-dimensional file, thus reducing the raw 3D file to a 2D file containing only the phase encoded information, for 2D Fourier transformation for display of the image.

Dim Data As NTNMR.Document
Dim App As NTNMR.Application
Dim DataPoint As Variant
Dim i As Long, k As Long, Extract As Long
Dim Nx As Long, Ny As Long, Nz As Long
Dim Ntotal As Long, Num As Long

Private Sub Command1_Click()
  CreateObject("NTNMR.Application")
  Set App = GetObject("NTNMR.Application")
  objectpath = App.GetActiveDocPath
  Set Data = GetObject(objectpath)

  Nx = Data.GetNMRParameter("Points 1D")
  Ny = Data.GetNMRParameter("Points 2D")
  Nz = Data.GetNMRParameter("Points 3D")

  Dim DataFile() As Double
  Dim PointData() As Variant
  Num = Ny * Nz * 2
  ReDim DataFile(Num) As Double

  Ntotal = Nx * Ny * Nz
  Data.ResetDimensionInfo Ntotal, 1, 1, 1

  Extract = Text1.Text
  i = Extract
  k = 0
If Extract > Nx Then
    MsgBox ("Extract data point must be less than Nx")
End
End If

Form1.Caption = "Entering Loop"
Screen.MousePointer = vbHourglass

Do While (i < Ntotal)
    PointData = Data.GetDatapoint(i)
    DataFile(k) = PointData(0)
    DataFile(k - 1) = PointData(1)
    k = k - 2
    i = i + Nx
Loop
Form1.Caption = "Writing New Data File"
Screen.MousePointer = vbDefault
MsgBox ("Finished Extracting Data, Writing New File")

Data.OpenFile "D:\NTNMR\Data\DerekNTNMR\Extract.tnt"
Data.SetData Ny " N/ * N/ * 2. DataFile
Data.ReSetDimensionInfo Ny, Nz, 1, 1

Form1.Caption = "Done"
End
End Sub

A.3. ZERO PROGRAM

Since the Apollo Tecmag acquires zeroes at the end of each data acquisition of a single echo in the PHAPS echo train, this program was written to shift the zeroes to the end of each 1D file.
Dim Acq As Long
Dim Num As Long, Nz As Long

Private Sub Command1_Click()

CreateObject("NTNMR.Application")
Set App = GetObject(), "NTNMR.Application")
objectpath = App.GetActiveDocPath
Set Data = GetObject(objectpath)

Dim ZeroPoints() As Double
Dim PointData() As Double

Nx = Data.GetNMRParameter("Points ID")
Acq = Data.GetNMRParameter("Acq. Points")
Num = L * Nx

For Dim ZeroPoints(Num) As Double
For Dim PointData(Num) As Double

i = 1
k = 1
l = 1

PointData = Data.GetData
Do While i <= Num
   k = k + 1
   l = l + 1
   ZeroPoints(k) = PointData(i)
   ZeroPoints(k - 1) = PointData(l - 1)
   k = k - 1
   l = l - 1
   Loop
i = i + 1 'Skip 2*4 Points in array (zeros)
l = 1
Loop

Data.SetData L * Nx, ZeroPoints

End
End Sub
The following script was written to include the three previous scripts. In addition, this script circumvents the data file size limitation by processing each PHAPS echo train before acquiring the next data file. This method removes the necessity of saving every single echo train, greatly limiting the file size and will allow for complete acquisition of each echo train independent of file size limitations. The gradient durations and delay tables are automatically calculated and passed to NTNMR for each acquisition. The data file, containing the extracted data points, is saved as “Out.tnt”. This script can be expanded to allow full 3D imaging in the future.

```
Dim Data As NTNMR.Document
Dim App As NTNMR.Application
Dim Shift As Long
Dim Phase As Long
Dim Nx As Long, Ny As Long, Nz As Long
Dim Acq As Long
Dim I As Long, k As Long, t As Long
Dim s As Long, t As Long
Dim Gyincrcmcnt As Long, Gzincrcmcnt As Long
Dim NumPoints As Long, PointExtract As Long
Dim ShiftZero As Long

Private Sub Command1_Click()
    CreateObject("NTNMR.Application")
    Set App = GetObject("NTNMR.Application")
    objectpath = App.getActiveDocPath
    Set Data = GetObject(objectpath)
    Gyincrcmcnt = Gyiner.Text
    Gzincrcmcnt = Gziner.Text
    Nx = GzPoints.Text
    Nz = GzPoints.Text
```
Shift = ShiftPoints.Text
Phase = PhaseShift.Text
PointExtract = PointExt.Text
ShiftZero = Zero.Text

Dim Extract! i As Double
Dim ZeroPoints! i As Double
Dim Delay1(I) As Long
Dim Delay2(I) As Long
Dim Delay3(I) As Long
Dim Delay4(I) As Long
Dim Delay5(I) As Long
Dim Delay6(I) As Long
Dim Delay7(I) As Long
Dim Delay8(I) As Long

Nx = Data.GetNMRRParameter("Points 1D")
Acq = Data.GetNMRRParameter("Acq. Points")
Num = 2 * Nx
NumPoints = Ny * Nz * 2

ReDim Extract! NumPoints As Double
ReDim ZeroPoints(Num) As Double
ReDim Delay1(Ny) As Long
ReDim Delay2(Ny) As Long
ReDim Delay3(Ny) As Long
ReDim Delay4(Ny) As Long
ReDim Delay5(Nz) As Long
ReDim Delay6(Nz) As Long
ReDim Delay7(Nz) As Long
ReDim Delay8(Nz) As Long

Initialize ZeroPoint Array

j = 1
Do While j <= Num
  ZeroPoint(j) = 0
  j = j + 1
Loop

Initial Gradient increments

k = 1
Do While k <= Nz
  If k = 1 Then
    Delay3(k) = (Ny / 2) * Gyincrement + 50
  Else
    Delay3(k) = (Ny / 2) * Gyincrement + 50
  End If
  k = k + 1
End Do

Form1.Caption = "Initializing Gradient Arrays..."
Delay 4(t) = 50

Else
   Delay 3(t) = Delay 3(t - 1) - Gyincrement
   Delay 4(t) = Delay 4(t - 1) + Gyincrement
End If

Else
   Delay 1(t) = Delay 1(t - 1) + Gyincrement
   Delay 2(t) = Delay 2(t - 1) - Gyincrement
   Delay 3(t) = 50
   Delay 4(t) = (Ny / 2 - 1) * Gyincrement + 50
End If

End If

t = t - 1

Do While (I <= N / 2)
   If I <= Ny / 2
      Delay 5(i) = 50
      Delay 6(i) = (Ny / 2) * Gyincrement + 50
   Else
      Delay 7(i) = Delay 3(s - 1) - Gyincrement
      Delay 8(i) = Delay 4(s - 1) + Gyincrement
      End If
   End If

End If

s = s * I

Do While (s <= Ny)
   If s <= (Ny / 2)
      Delay 5(s) = 50
      Delay 6(s) = (Ny / 2) * Gyincrement + 50
   Else
      Delay 7(s) = Delay 3(s - 1) - Gyincrement
      Delay 8(s) = Delay 4(s - 1) + Gyincrement
      End If
   End If

s = s * I

Do While (t <= N)
   Data.SetTable "Gz1", Delay5(s)
   Data.SetTable "Gz2", Delay6(s)
   Data.SetTable "Gz3", Delay7(s)
   Data.SetTable "Gz4", Delay8(s)
   Form2.Text2.Text = s
   s = s + 1
End Do While (t <= Ny)
Data.SetTable "Gy1", Delay1(t)
Data.SetTable "Gy2", Delay2(t)
Data.SetTable "Gy3", Delay3(t)
Data.SetTable "Gy4", Delay4(t)
Form2.Text1.Text = t
   t = t + 1

' Begin Acquisition

Data.ZG
Data.CheckAcquisition
Do While Not Data.CheckAcquisition
   Loop
   t = 1
   k = 1
   l = 1

'Shift out Zero Points

PointData = Data.GetData
Do While t <= Num
   Do While (l <= (Acq - ShiftZero) And i <= Num - 2)
      ZeroPoints(k) = PointData(i)
      ZeroPoints(k + 1) = PointData(i + 1)
      k = k + 2
      i = i + 2
      l = l + 1
      Loop
      i = i + 2 * ShiftZero
      t = t + 1
      Loop

Data.SetData "Nx, ZeroPoints"
Data.LeftShift Shift, True
Data.BaselineCorrection
Data.Fourier Transform
Data.PhaseCorrection Phase, 0

Data.Save
DataPoint = Data.GetData(PointExtract)
Extract(p) = DataPoint(p)
Extract(p + 1) = DataPoint(1)
p = p + 2

Loop
   t = 1
   Loop

Form2.Hide

Form1.Caption = "Writing New Data File..."
Daia.OpcnFüo ’ D :\N T N M R D ataVD crokN TN M R \O ut.tnl"
Data.SoiData NumPoints, Extract
Data.Save
Data.ReSetDim ensionlnfo Ny. N z. 1. 1
Data.Save
End
End Suh
[’ m ate Suh Com m and2_Click( )
End
End Suh

A.5. M IC R O C O IL PROGRAM

To aid in the tuning the microcoils, the following program was written which
calculates the value of the tuning capacitor based on stray inductance, coil deminsions.
and the number o f turns o f the microcoil. This program does not interact directly with
NTNMR.

Private Suh cm dC apCalc_Clickl i
Dim reFreq .-\s Double, relnduct .As Double
Const PI = 3.14159265
reFreq = 383.43 " 1 0 ^ 6
I f txtlnductance = "" Then G o T o W rong Values
relnduct = CD hl(txiInductance.Text)
' Capacitance in picofarads
txtCapReq.Text = C D b l(((2 * rcFreq * P I) ' 2 * relnduct * 10 '' f-9 ))

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(-1 ) * 10 '' ( 12))


Module

WrongValues:
    MsgBox "Please enter a valid number." & vbExclamation
End Sub

Private Sub cmdInductance_Click()
    Dim reTurns As Double, reLength As Double, reDiam As Double
    Dim reLeads As Double, reWDiam As Double
    Dim chkFin As Boolean
    Dim reCoil As Double, reSLeads As Double

    chkFin = chkFinite.Value

    ' Convert Numbers to mks units
    If txtTurns.Text = "" Or txtLength.Text = "" Or txtDiameter.Text = "" Then GoTo WrongValues
    reTurns = CDbl(txtTurns.Text)
    reLength = CDbl(txtLength.Text) * 10 ^ (-6)
    reDiam = CDbl(txtDiameter.Text) * 10 ^ (-6)

    If txtLeads.Text = "" Then
        reLeads = 0
    Else
        reLeads = CDbl(txtLeads.Text) * 10 ^ (-6)
    End If

    If txtWireDiam.Text = "" Then
        reWDiam = 0
    Else
        reWDiam = CDbl(txtWireDiam.Text) * 10 ^ (-6)
    End If

    Const PI = 3.14159265
    Const mu = 4 * PI * (10 ^ (-7))

    ' reCoil is the inductance of the coil in Henries
    If chkFin Then
        reCoil = CDbl(mu * reTurns ^ 2 * PI * (reDiam / 2) ^ 2 * reLength / (reDiam ^ 2 + reLength ^ 2))
    Else
        reCoil = CDbl(mu * reTurns ^ 2 * PI * (reDiam / 2) ^ 2 * reLength / (reLength ^ 2))
    End If

    ' reSLeads is the inductance of the leads in microhenries
    ' If statement allows for calculation of only coil inductance in left blank
    ' reLeads is changed into mm for formula

    If (reWDiam = 0 Or reLeads = 0) Then
reSLeads = 0
Else
    reSLeads = CDbl(0.0002 * (reLeads * 10 ^ 3) * (Log(4 * reLeads / reWDiam) - 0.85))
End If
Total inductance is returned in nanohenries

txtInductance.Text = CDbl(reCoil * 10 ^ (9) + reSLeads * 10 ^ (3))
Exit Sub
WrongValues:
    MsgBox "Please enter a valid number.", vbExclamation
End Sub
Private Sub cmdNotes_Click()
    frmNotes.Show
End Sub
Private Sub cmdStray_Click()
    Dim reFreq As Double, reIndue As Double, reCap As Double
    Const PI = 3.14159265
    reFreq = 383.53 * 10 ^ 6
    If txtInductance.Text = "" Then GoTo WrongValues
    reIndue = CDbl(txtInductance.Text) * 10 ^ (-9)
    reCap = CDbl(txtCap.Text) * 10 ^ (-12)
    txtStray.Text = CDbl((2 * reFreq * PI ^ 2 * reCap) ^ (-1) - reIndue) * 10 ^ 9
    Exit Sub
WrongValues:
    MsgBox "Please enter a valid number.", vbExclamation
End Sub
APPENDIX B

DESIRE COMPUTER SIMULATION CODE

B.1. INTRODUCTION

The computer code to calculate Figure 8.5 was written as two FORTRAN codes. The program uses the Equation 8.4 to calculate the magnetization at every given point in a sphere, using the magnetization at time of 1 ms as the initial condition for the subsequent pulse, see section 8.6. The magnetization as a function of position, the output for the first program, is then integrated to determine the total magnetization enhancement effect due to the DESIRE sequence in the second FORTRAN program.
B.2. DESIRE MAGNETIZATION PROGRAM

The following program was written to calculate the magnetization as explained in section 8.6. The code is in FORTRAN.

```
program will take a set of initial conditions corresponding to a pulse destroying the magnetization for r>a. The magnetization is then allowed to diffuse throughout a sphere of radius R for a time T. Then the magnetization is again destroyed for r>a. This process is repeated N times. The result is a function of magnetization as function of r at time T.

Derek Seeber
August 9, 1999
Diffusion Program v1.1

double precision inc, Posit, Mag, a, R
double precision S, T, X
double precision int, Posit1, Mag1

integer l, p, m, Points
integer N, j, q, w, k

parameter(Points=32000)

dimension Posit(Points), Mag(Points), Posit1(Points)
dimension Mag1(Points)

a=0.5d0 'Radius in which magnetization is destroyed!
R=250 'Outer Radius limit, only values to R/2 are correct!
T=1.0d-3 'Evolution Time after pulse!
inc=R/Points

N=100 'Number of times magnetization destroyed!
x=1.0d-10 'Non zero startin point, avoid divide by zero error!

'Initialize Position Array and initial magnetization
'Only need to pass points that are r>=a

write *, * 'Diffusion Program'

j=0
int=x

do 10 l=1,Points
```
i = init
if (Posit1 >= 1) then
  Posit1 = init
  Mag1 = 1.0d0
else
  j = j + 1
  Mag1 = 0.0d0
endif
mu = init + in
10 Continue

open(unit=89, file='pulse1.dat', status='unknown')
open(unit=90, file='pulse2.dat', status='unknown')
open(unit=91, file='pulse3.dat', status='unknown')
open(unit=92, file='pulse4.dat', status='unknown')
open(unit=93, file='pulse5.dat', status='unknown')
open(unit=94, file='pulse6.dat', status='unknown')
open(unit=95, file='pulse7.dat', status='unknown')
open(unit=96, file='pulse8.dat', status='unknown')
open(unit=97, file='pulse9.dat', status='unknown')
open(unit=98, file='pulse10.dat', status='unknown')
open(unit=99, file='pulse11.dat', status='unknown')
open(unit=100, file='pulse12.dat', status='unknown')

do 10 p = 1, N
  write (*, '*') ' Pulse #', p
  do 20 m = 1, Points
    call QTRAP(Posit1, Mag1, Points-m, Posit(m), T,a,R,S)
    Mag(m) = S
 20 Continue

' Pulse: destroy magnetization!
' Transfer magnetization to Mag1 array for evolution during next
' pulse. Keeping magnetization 1 for large r, the if statements
' keep the magnetization monotonically increasing. Since the function
' is not integrated to infinity, the function drops off for values
' approximately R/2. When the function starts to decrease, the
' Magnetization is reverted to equilibrium value of 1

' Find where magnetization is equal to 1.0!
k = 0
100 Continue

do 70 w = 1, Points
  if (1.0d0 - Mag(w) > 1.0d-10) then
    goto 71
  else
    k = k + 1
  endif
70 Continue
if(k.eq.Points) write(*,*),'Error, I never reached'

71 Continue

'Magnetization is then 1.0 until R'
do 72 q=k+1.Points
   Mag(q)=1.0d0
72 Continue

do 33 m=1.Points
   if (p.EQ.1) write(89,*) Positt(m), Mag(m)
   if (p.EQ.5) write(88,*) Positt(m), Mag(m)
   if (p.EQ.2) write(90,*) Positt(m), Mag(m)
   if (p.EQ.4) write(91,*) Positt(m), Mag(m)
   if (p.EQ.6) write(92,*) Positt(m), Mag(m)
   if (p.EQ.8) write(93,*) Positt(m), Mag(m)
   if (p.EQ.10) write(94,*) Positt(m), Mag(m)
   if (p.EQ.20) write(95,*) Positt(m), Mag(m)
   if (p.EQ.30) write(96,*) Positt(m), Mag(m)
   if (p.EQ.40) write(97,*) Positt(m), Mag(m)
   if (p.EQ.50) write(98,*) Positt(m), Mag(m)
   if (p.EQ.60) write(99,*) Positt(m), Mag(m)
   if (p.EQ.70) write(100,*) Positt(m), Mag(m)
   if (p.EQ.80) write(101,*) Positt(m), Mag(m)
   if (p.EQ.90) write(102,*) Positt(m), Mag(m)
   if (p.EQ.100) write(103,*) Positt(m), Mag(m)
33 Continue

'Make magnetization zero for rea'
do 30 q=1.Points
   if(Positt(q).ge.10) then
      Mag(q)=Mag(q)
   endif
30 Continue

40 Continue

end

SUBROUTINE QTRAP(P,M,XX,T,A,B,S)

'This subroutine uses the trapezoid rule to integrate the diffusion
effect in the sphere

double precision XX,T,A,B,S
double precision EPS, OLDS
double precision P, M

integer JMAX, J, No

dimension P(No), M(No)

'EPS is the allowable error value!'
PARAMETER EPS=1.0D-7, JMAX=200

OLDS=-1.0D0
DO 11 J=1,JMAX
   CALL TRAPZD(P,M,NO,XX,T,A,B,S,J)
   IF(ABS(S-OLDS),LT,EPS*ABS(OLDS)) RETURN
   OLDS=S
11   CONTINUE
   WRITE(*,'(A)') 'Too many step is QTrap'
END

SUBROUTINE TRAPZD(P,M,NO,XX,T,A,B,S,N)
   'Evaluates the function and computes the area under the curve by the
   trapezoidal rule. returns to QTRAP to see if error condition is met

   DOUBLE PRECISION XX,T,A,B,S
   DOUBLE PRECISION INC, DEL, SUM
   DOUBLE PRECISION M, P, First, Last
   DOUBLE PRECISION Ans, Y

   INTEGER N, IT, TMN, No
   DIMENSION P(No1, M(No1)

   IF(N.EQ.1) THEN
      CALL FUNC(P,M,NO,XX,T,A,First)
      CALL FUNC(P,M,NO,XX,T,B,Last)
      S=0.5D0*(B-A)*(First+Last)
      IT=1
   ELSE
      TMN=IT
      DEL=(B-A)/TMN
      INC=A+0.5D0*DEL
      SUM=0.0D0
      DO 12 J=1,IT
         CALL FUNC(P,M,NO,XX,T,INC,Ans)
         SUM=SUM+Ans
         INC=INC+DEL
12     CONTINUE
      S=0.5D0*S+(B-A)*SUM/TMN
      IT=2*IT
   ENDIF
   RETURN
END
SUBROUTINE INTERP(P,M,N,X,Y)

' Given an array P of length N, and given a value X, returns a value
' Y, where Y is a linear approximation to the value at X.

double precision P, M, X, Y

integer N, J, JL, JU, JM

dimension P(N), M(N)

JL=0
JU=N-1

IF(X.GT.P(N)) then
  Y=1.0d0
  Return
end if

IF(JU-JL.GT.1) then
  JM=(JU-JL)/2
  IF(P(N).GT.P(JM)).EQV.(X.GT.P(JM)) then
    JL=JM
  else
    JU=JM
  endif
  goto 10
end if

10 J=JL

'Linear approximation to Y at X!'
Y=(M(J)+1-M(J))/((P(J+1)-P(J))*(X-P(J))+M(J))

RETURN

end

SUBROUTINE FUNC(P,M,No.XX.T.X,Ans)

'This is the function being integrated over!

double precision XX, T, X
double precision Y, D, Pi, CONST
double precision P, M, Ans
double precision EXP1, EXP2

integer No, JJ

dimension P(No), M(No)

call INTERP(P,M,No.X,Y)
D=22.00d0  !Diffusion in microns^2/s!
Pi=3.1415926d0  

CONST=0.5d0*(1.0d0/XX)*(1.0d0/(sqrt(Pi*D*T)))  
EXP1=exp(-((XX-X)**2)/(4.0d0*D*T)))  
EXP2=exp(-((XX+X)**2)/(4.0d0*D*T)))  
A n s = X*Y*Const*(EXP1-EXP2)  

Return  
End  

B.3. INTEGRATION PROGRAM

This program takes the output from the program in section B.3 after the desired number of saturation pulses, and integration the magnetization to find the enhancement effect of the DESIRE sequence. This program is also written in FORTRAN.

' This program will read in a data file produced by diffusion1.f. It contains the magnetization as a function of position. This program will then integrate that function over the volume of a sphere and give an answer to the amount of magnetization destroyed.

double precision S, Posit, Mag  
integer j, Points  
parameter Points=1585  
dimension Posit(Points), Mag(Points)  
open(unit=50, file= 'pulse1.dat', status= 'unknown')

do 12 j=1, Points  
   read(50, *) Posit(j), Mag(j)  
12 Continue  

Call QTRAP(Posit, Mag, Points, 0.0d0, 0.0d0, S)

write(*,*) 'Answer'. S  
311
SUBROUTINE QTRAP(P,M,No,A,B,S)

'This subroutine uses the trapezoid rule to integrate the magnetization decrease throughout the sphere

double precision A,B,S
double precision EPS, OLDS
double precision P, M

integer J, JMax, No
dimension P(No), M(No)

'EPS is the allowed error value'
parameter EPS=1.0d-6, JMax=20)

OLD=-1.0d30

DO 11 J=1,JMAX
   CALL TRAPZD(P,M,No,A,B,S,J)
   if (ABS(S-OLDS)*LT EPS*ABS(OLDS)) RETURN
   OLDS=S
11 Continue

WRITE (*,*) 'Not enough steps in Qtrap'

end

SUBROUTINE TRAPZD(P,M,No,A,B,S,N)

'Evaluates the function and computes the area under the curve until error condition is met

double precision A,B,S
double precision INC,DEL,SUM
double precision M,P,First,Last
double precision Ans

integer N, IT, TMN, No
dimension P(No), M(No)

IF(N.EQ.1) then
   call FUNC(P,M,No,A,First)
   call FUNC(P,M,No,B,Last)
   S=0.5d0*(B-A)*(First+Last)
   IT=1
else
TNM=IT
DEL=(B-A)/TNM
INC=A+0.50d0*DEL
SUM=0.0d0
DO 12 J=1,IT
   CALL FUNC(P,M,N,INC,Ans)
   SUM=SUM+Ans
   INC=INC-DELT
12   CONTINUE
S=0.50d0*(S+(B-A)*SUM/TNM)
IT=2*IT
END

SUBROUTINE INTERP(P,M,N,X,Y)
!Given an array of length N and given a value X, returns a value
!Y, where Y is a linear approximation to X

DOUBLE PRECISION P, M, X, Y
INTEGER N, J, JL, JU, JM
DIMENSION P(N), M(N)

JL=0
JU=N+1
IF(X.GT.P(N)) THEN
   Y=1.0d0
   RETURN
END IF

IF(JU-JL.GT.1) THEN
   JM=(JU+JL)/2
   IF((P(N).GT.P(JM)).EQV.(X.GT.P(JM))) THEN
      JL=JM
   ELSE
      JU=JM
   END IF
   GO TO 10
END IF

J=JL

!Linear approximation!
Y=((M(J+1)-M(J))/(P(J+1)-P(J)))*(X-P(J))+M(J)
IF(Y.GT.1.0d0) Y=1.0d0
END
SUBROUTINE FUNC(P,M,No,X,Ans)

double precision X, Y, Pi
double precision P, M, Ans

integer No

dimension Pi(No), Mt(No)

call INTERP(P,M,No,X,Y)

Pi=3.1415926
Ans=1.0d0-Y*4.0d0*Pi*X**2)

Return
end
LIST OF REFERENCES


Kepro Circuit Systems Inc.


Belden Wire and Cable.


