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Remotely Detected Nuclear Magnetic Resonance of Microscopic Flow for Chemical and Biological Analysis

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Remotely Detected Nuclear Magnetic Resonance of Microscopic Flow for Chemical and Biological Analysis

by

Thomas Zdzislaw Teisseyre

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Bioengineering

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
Dla mojej matki
I write these acknowledgements on the morning of February 28, 2012, my mother’s birthday. Today she turns 57. This work is dedicated entirely to her. She has been my guide from the time when she would drive me around in our little blue Fiat during Sopot’s stormy winters, to our dramatic move to the States, and finally to my inevitable move across the country to California. She gave up her ownership of a burgeoning electronics company to take on the unenviable task of maintaining a hair salon in Florida and switching roles to what started at minimum wage. From that time, she’s worked tirelessly to get me to where I am now and sacrificed much to generate the output of this work. I’ve had it easy because of these sacrifices and am eternally grateful.

I’d like to thank my love, Meredith. She has been the support I’ve needed to spend all our weekends on writing these pages. She is endlessly dedicated to my work, success, and happiness. There is no one who more selflessly on a daily basis has contributed to making this happen. Meredith, you can have any of this you want.

Every member of my close family lives in the same town in Poland. The distance has made keeping in touch throughout my education quite difficult. They do make it much easier, however, with the constant cheering. If there’s ever any concern that I’ve strayed too far from close family, it’s obliterated when I think of the veritable parade that comes to greet me at the airport when I touch down
to visit. The ringing support from, particularly my grandmother Krystyna, but also my cousins Mariusz, Ania, Krystian, Kryszystof and Alicja, my uncle Krszystof, my aunt Irena, and of course our newest addition Mateusz can be heard here from 6,000 miles away.

Quite often I will look up and cock my head to the side and wonder how it is that my two closest friends live, not only on the west coast, but within several miles of my San Francisco apartment. Jason is always the most interested in the progress of my research. If my dissertation was little league and he was my dad, he’d be at every game. Mitchell brings his tireless confidence to each conversation. In questions about life and love and cinema, there’s not a more authoritative source. Regardless of how busy or distracted we get, they are family, and I’m thankful to have them close.

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I’ve given up on an argument. He’s been involved in each of these. And of course, there’s the ever present and seemingly omnipotent Charlie. A constant guide, I’m quite convinced that I wouldn’t have been able to finish college without his reinforcing attitude.

Of course none of this would have been possible without the Pinenuts. Having been an engineer before coming to Berkeley, I never thought I’d get along with chemists. In fact, all through college, I can’t say I was particularly interested in chemistry. Ironically, I found myself in a lab within the Berkeley Chemistry Department doing my dissertation work and being fascinated by the concepts. They took me in with open arms. I can’t imagine picking a better or more akin group of people to have worked with. Essentially all the ideas in this dissertation have been vetted by my closest colleague Nick Halpern-Manners. We’ve shared research and publication success, frustration, and long hours at the CMRR in the most frigid of condition (for me at least). Our lead throughout all this was Vik Bajaj, whose natural leadership drove the content of this dissertation. I’d also particularly like to thank Nick Graziani for many discussions about the science and design. His affinity for and strong grasp of the most complex subjects is remarkable. I would not have been able to arrive at the final subject of this dissertation without my initial foray into xenon biosensors. Team biosensor, and particularly Todd Stevens, Monica Smith, and Matt Ramirez helped to shape my final research direction. I would also like to acknowledge Dan Kennedy for his substantial contribution to this work. The
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I conclude these acknowledgments with the paterfamilias, Alex Pines. I had heard of Alex when I was at Yale. A close colleague of mine was raving about the lab. I could immediately tell that there must be something special going on in Berkeley. After researching the publications and concluding that Alex’s work was, in fact, nuts, I decided that this is where I should go. These rumors were confirmed when I first met Alex in his old office in Hildebrand Hall. He’s the only person I know whose intellect does not temper, even in the least bit, his spirit and enthusiasm. He is a force of nature, and I am humbled to have been under his tutelage for these 5 years.
Remotely Detected Nuclear Magnetic Resonance of Microscopic Flow for Chemical and Biological Analysis

by

Thomas Zdzislaw Teisseyre

The topic of this research is nuclear magnetic resonance (NMR) and magnetic resonance imaging (MRI) with remote detection for microfluidics. While NMR has long been capable of providing rich spectral information for an array of application, its primary limitation is sensitivity. To combat this in samples with poor filling factor, remote detection NMR was developed in the Pines’ Lab. This work builds on this platform to achieve a substantial signal enhancement over conventional techniques. These developments focus on data acquisition schemes and novel applications, sub-Nyquist data sampling, and hardware design and fabrication. The application areas include the study of flow in microfluidic chips, chromatographic separations, and live animal measurements. The results of these developments and applications include high resolution images of flow, multidimensional velocity measurements, as well as spectral analysis.
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CHAPTER 1: INTRODUCTION

1.1 MOTIVATION

Effective analytical techniques for lab-on-a-chip devices are central in determining device design and to the development of parallel and robust assays. Traditionally, this includes miniaturized analogs of large scale laboratory techniques based on mass spectrometry or optical spectroscopy. Common analytical schemes include velocimetry and spectroscopic assessments of fluid composition. For the former, fast optical techniques such as fluorescence particle tracking or particle image velocimetry (PIV) have been demonstrated in microscale forms. While offering high spatial resolution and accurate depictions of mixing properties, these techniques have limited spectroscopic integration and require exogenous chromophores. Such restrictions limit utility in chemical analysis and biologically relevant systems, respectively. Concurrently, label-free techniques have been developed for chemical sensing in microfluidic environments. These analyses are sometimes application specific, such as attenuated total reflection-Fourier transform infra-red (ATR-FTIR) spectroscopy or the more typical surface enhanced Raman spectroscopy (SERS). While highly sensitive, these methods are incapable of three-dimensional velocimetry, have limited imaging flexibility, and are difficult to apply to complex analytes such as biological macromolecules, emulsions, and whole blood samples.

We have therefore sought a generic tool that is capable of probing both chemistry and fluid dynamics without perturbing the analyte or dynamics of interest. In macroscopic
applications, nuclear magnetic resonance (NMR) and magnetic resonance imaging (MRI) can image and quantify the constituents of a complex sample non-invasively with virtually unlimited depth of penetration. Simultaneously, magnetic resonance techniques can provide fully integrated three-dimensional velocimetric information at microscale resolution. NMR velocimetry has been used to study complex fluid mechanics in porous media, experimental biofluids, and mixing in microfluidic channels. Additionally, significant progress has been made toward incorporating the rich spectral information provided by NMR onto microfluidic platforms. These capabilities are equally applicable to opaque samples and fabrication strategies.

NMR does, however, suffer from low sensitivity, relying most commonly on the low thermal polarization of water protons, which is about 1:10^5. This limitation is compounded in the microfluidic realm where only a fraction of the imaging volume is of interest and magnetic field inhomogeneities create spectral broadening. To elaborate, the sensitivity of an inductive detector is proportional to the magnetic flux it encloses, making the traditional NMR experiment insensitive to microfluidic or microporous structures. In these scenarios, the fluid volume occupies only a small fraction of the entire detector volume. To overcome these obstacles, we have proposed remotely detected NMR (RD-NMR). Here, spatial and chemical information within the microfluidic device is encoded in the magnetic degrees of freedom. The flow then carries the information to a single optimized point detector, such as a microcoil, creating sensitivity improvements of 2-3 orders of magnitude. This general strategy can be easily applied to any microfluidic geometry. The improvement in temporal and spatial
resolution makes microfluidic NMR practical and allows complete integration of spectroscopy, imaging, and velocimetry on one platform. The tradeoff for this wealth of information is added dimensionality, which requires extended acquisitions. However, hardware, acquisition, and processing modifications can greatly optimize the technique. These can be based on both common MRI methods as well as those unique to the remote detection modality. Furthermore, applying RD-NMR to answer biological and chemical questions further characterizes the modality and highlights potential areas of improvement.

1.2 AIMS

The purpose of this work is to investigate the use of RD-NMR as an integrated analytical technique capable of both quantifying velocities in microscale flows and spectroscopically assessing their chemical composition in applications ranging from chromatography to vascular measurements in live animals. This is accomplished with the development of fast acquisition schemes, application specific probe designs, and generalizable encoding strategies.

The specific contributions of this work include:

1.) Improved RD-NMR acquisition times utilizing compressed sensing with prior information about the chip geometry in both the reconstruction and sampling schedule.
2.) Application of RD-NMR to imaging chromatographic columns and detecting fast separations of small molecules.

3.) Integrated design of a microfluidic chip with a remote stripline detector for improved detection volume flow control.

4.) A novel implantable coil design and fabrication strategy for vascular measurements in live animals.
CHAPTER 2: NMR AND MRI

2.1. NUCLEAR MAGNETIC RESONANCE

To date, NMR is the most powerful, informative, and malleable of all forms of spectroscopy. Its phenomenal application potential, non-invasive nature and unlimited penetration depth can be attributed to the low electromagnetic energy involved. These advantages have spawned a separate discipline, MRI, where new discoveries are pushing the forefront of medical research. This includes high resolution imaging techniques that provide microstructural anatomical details as well as spectroscopic insight into intricate physiological pathways. Simultaneously, fundamental developments in multidimensional NMR spectroscopy yield unprecedented information about molecular structure and function at the small molecule, meso, and macroscopic scales. Many of these results are made possible with the use of NMR’s toolbox of pulse sequences, which adds additional flexibility. Finally, engineering developments, both in industry and academia have resulted in magnets with ultrahigh magnetic fields and application-optimized radiofrequency arrays. The most notable aspect of all these developments is their occurring more than 65 years after the NMRs discovery. More so than most mature branches of science, NMR research is thriving and further developing with each new application.

Many excellent texts provide an all-encompassing and detailed description of NMR and MRI theory [1-8]. An introduction into the most pertinent topics and techniques used in this work will be discussed here.
2.1.1. **Classical Description**

A thorough quantum mechanical understanding of the underlying physics of the experiment allows one to investigate the details of NMR phenomenon. However, for much of this work, the classical analogue provides an equally valuable framework by which to explain experimental results. Included here is a simple introduction based on first principles.

To begin our description of angular momentum, we will consider a very straightforward case of a charge moving in a circle as illustrated in Figure 2.1.1. This spinning charge example will allow us to develop an understanding of angular momentum and magnetic moments. Furthermore, it can be used to derive the simple Larmor relationship which defines the most fundamental principle in spin precession underlying NMR.

![Figure 2.1.1: Point charge q moving with a velocity v around a loop of radius r.](image)

A charge $q$, rotating about a loop of radius $r$ with a velocity $v$ creates a current, or charge per unit time (Coulombs/second=Amperes). Note that the time for the particle to travel around the loop $t=2\pi r/v$. In equation form:
The magnetic moment created by this current loop is the electric current over the area of the loop. Combining this with the classical equation for angular momentum, \( L \), gives the relationship for the magnetic dipole moment, \( \mu \):

\[
\mu = \left( \frac{q}{2m} \right) L = \gamma L \quad (2.2)
\]

Here the magnetic moment, \( \mu \), is related to the angular momentum, \( L \), by the gyromagnetic ratio, \( \gamma \). While this is the classical gyromagnetic ratio, it also holds true for the quantum mechanical case of orbital angular momentum.

Finally, placing the rotating charge in a magnetic field will result in a torque \( T \). The torque on a magnetic dipole moment is

\[
T = \mu \times B_0 = \gamma L \times B_0 \quad (2.3)
\]

And it causes a precession about the magnetic field, which is described by

\[
\frac{d\mu}{dt} = \mu \times \gamma B_0 \quad (2.4)
\]

Where the precession frequency is

\[
f = \frac{\gamma}{2\pi} B_0 \quad (2.5)
\]

In NMR, this equation can be used to determine the Larmor frequency of precession of a nucleus in a magnetic field. The gyromagnetic ratio is a constant and is unique to each type of atom. For \(^1\text{H}, \gamma/2\pi \approx 42.58 \text{ MHz/Tesla} \) and \(^{13}\text{C}, \gamma/2\pi \approx 10.71 \text{ MHz/Tesla} \)
Lastly, a concept that will return in the quantum mechanical description is the potential energy of the magnetic moment in a magnetic field.

\[ E = -\mu \cdot B \]  \hspace{1cm} (2.6)

Therefore, for a magnetic field along the longitudinal axis, \( z \), the combination of Equation 2.2 and Equation 2.6 results in an equation that relates angular momentum and potential energy:

\[ E = -\gamma L_z B_0 \]  \hspace{1cm} (2.7)

2.1.2. **Nuclear Spin**

The concept of nuclear spin is abstract, but typically conveyed with the spinning top analogy. If we consider a spinning nucleus to be analogous to a spinning top, then nuclear spin can seem like a very real thing. One can envision this scenario to picture precession and angular momentum. Initially, the top is completely aligned vertically and possesses enough energy to spin constantly without any motion. Once there is a slight dissipation of energy, or something tips the top away from its vertical axis, the top exhibits a secondary frequency superposed on its primary frequency. This is precession. The top seems to defy the gravitational force by virtue of its angular momentum or spinning energy and does not fall. However, the truth of the matter is that spin is less tangible. It is in fact an inherent property of each nucleus. Along with mass, charge, and magnetism, nuclear spin is actually a physical quantity of the atom and quite unlike an event, like the spinning of a top.
2.1.3. **Quantized Angular Momentum and the Zeeman Effect**

![Energy vs Magnetic Field Graph](image)

*Figure 2.1.2: Zeeman splitting in the presence of an increasing magnetic field.*

With this basic mental image of spinning tops and at least an intuitive sense of what is real and what is not, we can move into a more formal description of spin characteristics.

Unlike the continuous classical analogue, the angular momentum of a nuclear spin is quantized. This means that it comes in discrete packets, or levels, and is unlike anything in the perceptible world. Quantum angular momentum takes the following form:

\[
L = \left( \frac{\hbar}{2\pi} \right) \sqrt{I(I + 1)}
\]  
\[
(2.8)
\]

Where Planck’s constant, \( \hbar = h/2\pi = 1.054 \times 10^{-34} \), and \( I \) is the nuclear spin quantum number and takes on only integer values. Particles with spin can take on \( 2I+1 \) sublevels. Thus a particle with \( I = 1/2 \), such as \( ^1\text{H} \) and \( ^{13}\text{C} \) can take on 2 energy levels. These levels are degenerate, meaning that they have the same energy, unless placed in a magnetic field. Furthermore, they are represented by a second quantum number \( m \) and the spin angular momentum can be written as

\[
L_z = \hbar m
\]  
\[
(2.9)
\]
When placed in a magnetic field, the states are split by the Zeeman effect illustrated in Figure 2.1.2. For spin \( \frac{1}{2} \) nuclei \( m = \pm \frac{1}{2} \), and the energy difference between the two states can be determined by combining Equation 2.7 and 2.9 to yield

\[
\Delta E = \frac{1}{2} \gamma \hbar B_0 + \frac{1}{2} \gamma \hbar B_0 = \gamma \hbar B_0 = hf \quad (2.10)
\]

This relationship is central NMR and defines a sensitivity constraint of the experiment.

The energy difference between the spins is directly proportional to the spin frequency, which according to the Larmor relationship in Equation 2.3, is dependent on magnetic field and the gyromagnetic ratio. These parameters can be modified in the experiment with the use of higher magnetic fields and different nuclei, respectively. In the next section, we explain how this basic spin, splitting, and energy difference directly impacts the signal that is seen in the NMR receiver.

2.1.4. **Bulk Magnetization**

Here we discuss how the effect of the magnetic field on each of the individual spins affects the bulk magnetization of the sample. This is a bulk effect on net magnetization moment, \( M \), and it is completely dependent upon the energy difference explained in Equation 2.10.
A static magnetic field has an effect on both the nuclear and bulk magnetization of the sample. The individual spins have a tendency to precess about the static field. This effect was outlined above. The individual spin states can be described as either parallel, spin-up, or anti-parallel, spin down, with the static field in $\alpha$ or $\beta$ states respectively, as illustrated in Figure 2.1.3. In this case, $\alpha$ is the lower energy configuration and $\beta$ is the higher energy configuration. Spins preferentially orient in the lower energy configuration, and their distribution is given by the Boltzmann relationship:

$$\frac{n_\alpha}{n_\beta} = e^{-\Delta E/kT} \quad \text{(2.11)}$$

Meanwhile, as a result of this small preference for the parallel state, the bulk magnetization, $M$, tends to align with this magnetic field creating a net magnetization moment which can be detected by the NMR receiver.

And the bulk magnetization $M_0$, in one of its simplest forms is

$$M_0 = \frac{nB_0\gamma^2\hbar^2}{4kT} \quad \text{(2.12)}$$
Where $k$ is Boltzmann’s constant, $T$ is temperature, and $n$ is the number of nuclear spins per unit volume. This magnetization is directly related to the signal in the NMR receiver. To summarize this Equation the magnetization scales directly with the number of spins in the sample, the magnetic field, the gyromagnetic ratio and inversely with temperature. In sensitivity limited, detector optimized applications MRI and NMR developments focus on enhancements defined by Equations 2.11 and 2.12. While reducing the sample temperature is impractical, it is possible to increase magnetic fields, utilize different nuclei, or change the population distribution with hyperpolarization techniques.

Furthermore, the net magnetization vector is the sum of all magnetic dipole moments. Its precession can be characterized by Equation 2.4 and modified to include this fact:

$$\frac{dM}{dt} = M \times \gamma B_0 \quad (2.13)$$

This fundamental precessional relationship forms the basis of the Bloch Equation which will be discussed in later sections. Equation 2.13 only illustrates the precessional behavior that results from the static magnetic field. However, this interaction with the static magnetic field doesn’t generate an electromotive force in the conventional inductive receiver. In order to gain information about the sample, one must manipulate these magnetic dipole moments to generate transverse magnetization. This is accomplished with a secondary field, $B_1$, generated by a radiofrequency transceiver coil.
2.1.5. **Radiofrequency Excitation**

![Diagram of radiofrequency excitation](image)

*Figure 2.1.4: Radiofrequency excitation. At equilibrium in a magnetic field, the magnetization vector of a sample (red) is initially aligned along longitudinal (z) axis. In order for this magnetization to induce a current in an inductor, it must produce a changing magnetic field in the inductor, shown as 4 loops along the transverse plane (x-y).*

Initially, the bulk magnetization vector created by the static magnetic field is oriented along the z axis. In order to induce a current, i.e. receive a signal, in an inductive detector with a magnetic field axis oriented in the transverse plane, the magnetization vector must be rotated, or excited, into the transverse plane. This situation, before magnetic field rotation is depicted in Figure 2.1.4. Here, the longitudinal magnetization, \( M_0 \), is incapable of inducing a current in the coil. This is a basic requirement in all conventional inductively detected NMR and MRI. The nuclear spins are both manipulated and detected, most commonly with the same radiofrequency coil transceiver (transmit and receive), in the transverse plane. This can be accomplished by applying a radiofrequency pulse to the sample that is resonant with the Larmor
frequency of the nuclei. In a simple radiofrequency coil, excitation is accomplished with a linearly polarized transverse field. Circularly polarized excitation is possible with a quadrature coil, but will not be considered in this description.

A linearly polarized field along the x, or i, axis oscillating at the frequency $\omega$ is

$$B_1(t) = 2B_1 \cos(\omega t) i$$  \hspace{1cm} (2.14)

Where $\omega$ is typically the Larmor frequency, or $\omega_0 = \gamma B_0$. This expression is the mathematical equivalent of two circularly rotating magnetic fields, and therefore can be decomposed into

$$B_1(t) = B_1(t)(\cos(\omega t) i - \sin(\omega t) j) + B_1(t)(\cos(\omega t) i + \sin(\omega t) j)$$  \hspace{1cm} (2.15)

With the term on the left representing the left handed, or clockwise, field and the term on the right representing the right handed, or counterclockwise, field. Only the field rotating in the same direction as the nuclei of interest will affect the spins significantly. Nuclei with a positive gyromagnetic ratio, which results in a negative Larmor frequency, precess clockwise. Clockwise precession is most common and only nuclei with positive gyromagnetic ratio are used in this work. The counterclockwise field, indicated by the right handed term, only adds a small effect called the Bloch-Siegert shift, which usually can be ignored.

2.1.6. RELAXATION

Once excited into the transverse plane, the natural response of the magnetization is to decay and return to the longitudinal state. This phenomenon is relaxation and is
characterized by two time constants. The first, called the spin-lattice, or longitudinal relaxation is given the abbreviation $T_1$. The second, called the spin-spin, or transverse relaxation is given the abbreviation $T_2$. While these parameters establish fundamental limits on the linewidth and detection time of some NMR and MRI experiments, they also provide a great deal of information about the sample and are measured in many applications ranging from \textit{in vitro} biomolecular assays to \textit{in vivo} diagnostic imaging. Importantly, the causes and mechanisms for relaxation are multifactorial and vary between samples. This is the topic of detailed statistical mechanics discussions. Here, we consider only a classical description of the bulk effect which is sufficient to form a clear understanding of the experimental results.

The term spin-lattice relaxation to describe $T_1$ is more appropriate to solids, but is nevertheless used for all samples. This form of relaxation occurs as the sample magnetization returns to its equilibrium orientation along the static magnetic field or $z$ direction. This is energetically the magnetic equilibrium. The application of a radiofrequency pulse that reorients the magnetization into the transverse plane disturbs this equilibrium.

$T_1$ is the longer of the two relaxation time constants. It changes with magnetic field, nucleus, temperature, biological tissue, solvent, among other experimental parameters. The bulk sample effect can be described by the following exponential function

$$M_z = M_0 (1 - e^{-t/T_1}) \quad (2.16)$$

Where $M_0$ is the equilibrium magnetization to which $M_z$ is returning after being zeroed.
Spin-spin relaxation, or $T_2$, is the decay of the transverse magnetization after excitation by a radiofrequency pulse. Typically, and particularly for the applications seen in this work, the $T_2$ is on the order of tens of milliseconds. The primary mechanism is the slight variation in precession frequency among individual spins that results from the magnetic fields felt by surrounding spins, thus termed spin-spin relaxation. $T_2$ is described by an exponential decay:

$$M_{xy} = M_0 (e^{-t/T_2}) \quad (2.17)$$

Where the initial magnetization along the transverse dimension is $M_0$. It’s important to note that other factors contribute to the $T_2$ effectively seen in the experiment. This parameter is abbreviated $T_2^*$ and is the experimental reality of $T_2$. Most commonly $T_2^*$ is affected by dephasing caused by magnetic field inhomogeneities. One central concept is that the linewidth of peaks in an NMR spectrum is dictated by the length of $T_2^*$. For highly inhomogeneous samples with a short $T_2^*$, line broadening causes reduced spectral resolution and potential overlap.

2.1.7. BLOCH EQUATION

The guiding equation that characterizes the behavior of net magnetization in a magnetic field is the Bloch equation. The most general form is

$$\frac{d\mathbf{M}}{dt} = \mathbf{M} \times \gamma \mathbf{B} - \frac{M_x \mathbf{i} + M_y \mathbf{j}}{T_2} - \frac{(M_z - M_0) \mathbf{k}}{T_1} \quad (2.18)$$

This formula combines the precessional effect, transverse, and longitudinal effects described above. Here, $\gamma$ is the gyromagnetic ratio, $M$ is the bulk magnetization oriented
along the dimension in the subscript, \( B \) is the external magnetic field, \( T_2 \) is the spin-spin relaxation time constant, and \( T_1 \) is the spin lattice relaxation time constant. The external magnetic field \( B \) is composed of the static field \( B_0 \), the radiofrequency field \( B_1 \), and a gradient field for imaging. In the NMR spectroscopy experiment it is appropriate to omit the latter. The gradient fields will be included in later sections on imaging.

The static magnetic field \( B_0 \) is oriented along the \( z \) dimension. Modifying the first term in the Bloch equation and then representing in matrix form results in the following:

\[
\frac{d\mathbf{M}}{dt} = \mathbf{M} \times \gamma B_0 \mathbf{k} = \begin{bmatrix} i & j & k \\ M_x & M_y & M_z \\ 0 & 0 & \gamma B_0 \end{bmatrix} = M_y \gamma B_0 i - M_x \gamma B_0 j
\]

\[
= \begin{bmatrix} 0 & \gamma B_0 & 0 \\ -\gamma B_0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix} \begin{bmatrix} M_x \\ M_y \\ M_z \end{bmatrix} \tag{2.19}
\]

Expanding this equation to include the left-hand term of the \( B_1 \) field in Equation 2.15, we arrive at the following relationship:

\[
\frac{d\mathbf{M}}{dt} = \mathbf{M} \times \gamma (B_1(t) \cos(\omega t) \mathbf{i} - B_1(t) \sin(\omega t) \mathbf{j} + B_0 \mathbf{k}) \tag{2.20}
\]

Which, in matrix form is represented as

\[
\frac{d\mathbf{M}}{dt} = \begin{bmatrix} 0 & \gamma B_0 & \gamma B_1(t) \sin(\omega t) \\ -\gamma B_0 & 0 & \gamma B_1(t) \cos(\omega t) \\ -\gamma B_1(t) \sin(\omega t) & -\gamma B_1(t) \cos(\omega t) & 0 \end{bmatrix} \begin{bmatrix} M_x \\ M_y \\ M_z \end{bmatrix} \tag{2.21}
\]

The static magnetic field causes precession at \( \omega_0 = -\gamma B_0 \) and the radiofrequency \( B_1 \) field is applied at a frequency \( \omega \).
2.1.8. THE ROTATING FRAME

At this point, the Bloch equations become cumbersome if we don’t consider an alternative frame of reference around which to form an understanding of the experiment. If, instead of observing the spins from a stationary frame, we move to a frame of reference that is rotating at a frequency $\omega$, then the mathematics is simplified. In this rotating frame, the effective field can be represented by the following:

$$\mathbf{B}_e = B_1(t)\mathbf{i} + \left(B_0 - \frac{\omega}{\gamma}\right)\mathbf{k} \quad (2.22)$$

Where the magnitude of the effective field is

$$B_e = \sqrt{B_1^2 + \left(B_0 - \frac{\omega}{\gamma}\right)^2} \quad (2.23)$$

Importantly, the effect of this simplification is to remove the precessional effect of the static magnetic field and allow for a radiofrequency excitation that is no longer rotating about the longitudinal axis. Furthermore, the effective field magnitude when the this new frame of reference rotates at the Larmor frequency, $\omega = \omega_0$, is $B_e = B_1$. In this simplified representation, the magnetization is no longer visualized as a constantly precessing vector. Now, in the presence of a static field, the magnetization is a vector pointing along the longitudinal axis. With the application of a radiofrequency field, the magnetization rotates down to the transverse plane without any superimposed precession frequency.
2.2. **MAGNETIC RESONANCE IMAGING**

In his historic paper in Nature in 1973, *Image formation by Induced Local Interactions: Examples Employing Nuclear Magnetic Resonance*, Paul Lauterbur introduced the world to a new way of looking at NMR and imaging altogether[9]. He begins by stating that “image formation usually requires that the object interact with a matter or radiation field characterized by a wavelength comparable to or smaller than the smallest features to be distinguished”. As an alternative, he suggests that the resolution can become independent of wavelength by taking advantage of induced local interactions. He goes on to show the first, and technically speaking, the last, “zeugmatogram” of a phantom containing two tubes filled with water spatially separated in the magnet. While Zeugmatography didn’t catch on, Magnetic Resonance Imaging did. As explained in the introduction to this work, MRI has since grown into a complex and multidisciplinary field. The basics of image acquisition will be described here.

2.2.1. **GRADIENTS**

The magnetic field gradient is the third applied field and is central to MRI, or the creation of images from the rich magnetic dipole information described above. Simply put, the magnetic field gradient creates frequency variations throughout the sample that can be correlated to position. In the case of conventional NMR on a straight tube of water, spatial differences in the sample are typically uninteresting to image. However, for samples where the spatial distributions of nuclear spins is of interest, such as whole body human imaging, multiplexed spatial encoding schemes, porous media, and
microfluidic chips, the nuclear spin locations provide a wealth of information. Furthermore, in cases of flowing systems, the gradients can create phase contrast between components of different velocities. This is essential for studies that compose this work. Finally, in applications where significant motion causes image blurring, such as cardiac MRI, the gradient pulses can be designed in such a way as to accelerate the image acquisition.

First, we will consider the simplest case of using a gradient field for spatial encoding. The gradient affects the Larmor frequency of a sample in a static field $B_0$ such that it varies linearly across the sample. For a one dimensional, time invariant gradient, $G$, along the x dimension, $G_x$, the Larmor frequency is

$$\omega(x) = \frac{\gamma}{2\pi}(B_0 + G_x x) \quad (2.24)$$

Here, the frequency is influenced by the summed static field and the gradient field, which varies with dimension x. The static field is in units of Tesla or Gauss, while the gradient field is typically in units of T/cm or G/cm.

There are generally two basic types of gradient encoding: frequency and phase, corresponding to the x and the y dimension, respectively. While the terms used to describe these gradients is somewhat of a formality, the distinction is significant and will be described in later sections.
2.2.2. K-space

![Diagram of K-space and two-dimensional Fourier transform](image)

**Figure 2.2.1: K-space and the two-dimensional Fourier transform. K-space on left transformed through 2D Fourier transform into image space.**

Central to an understanding of the use of gradients in MRI, and the frequency-space relationship, is the k-space formalism. K-space is the conjugate, or frequency domain and is related to real space through the Fourier transform. Examples of these two domains for an image are illustrated in Figure 2.2.1. With this simplification in mind, the MRI experiment can be viewed as simply acquiring points, or scanning, k-space. The image is then computed by calculating the Fourier transform. The term image-space will be used to describe the real-world spatial domain and the term k-space describes the conjugate, or frequency domain.

In a subsequent section, a mathematical structure based on the 2D Fourier transform will be used to set the stage for the basic concepts. For more detailed background, the reader is referred to several excellent texts that provide an introduction to the 2D Fourier transform and a general background of signal processing [10-12]. The most relevant description of these concepts, particularly the signal equation, is included in Dwight
Nishimura’s text, and will be described briefly here with slight modifications. Further details are also included from Steve Conolly’s MR Systems course at Berkeley.

2.2.3. SIGNAL EQUATION

As described above, the MRI experiment correlates spatial frequencies that are encoded using a time varying gradient field into positions. The actual signal received in the radiofrequency coil from the sample is therefore an array of frequencies that are related to space through the Fourier transform. More specifically, the signal received is the 2D Fourier transform of the magnetization at some frequency in space. For the 2D case, the signal equation is written as follows

\[ s(t) = \frac{d}{dt} \iint M(x, y) e^{-i\phi(t, x, y)} dx \, dy \quad (2.25) \]

Where the phase \( \phi(t) \) is

\[ \phi(t, x, y) = \int_{0}^{t} 2\pi f(x, y, \sigma) d\sigma = \left[ \gamma B_{0} t + \gamma x \int_{0}^{t} G_{x}(\sigma) d\sigma + \gamma y \int_{0}^{t} G_{y}(\sigma) d\sigma \right] (2.26) \]

This equation states that the phase accrued over space and time is directly related to the integral of frequency, which in turn is made up of factors contributing from the static field \( B_{0} \), as well as the integral of each of the gradients applied over time \( G_{x} \) and \( G_{y} \).

Equation 2.26 demands the following simplification:

\[ k_{x}(t) = \frac{\gamma}{2\pi} \int_{0}^{t} G_{x}(\sigma) d\sigma \quad (2.27a) \]

\[ k_{y}(t) = \frac{\gamma}{2\pi} \int_{0}^{t} G_{y}(\sigma) d\sigma \quad (2.27b) \]
Where \( k \) is the location in k-space in each the x and y dimension. This means that the point in conjugate domain, or k-space, is determined by the integral of the time over which the gradient is applied. This can be interpreted as the application of gradients allowing for the coverage or interrogation of k-space in precise and controlled manners.

With these simplifications, the final signal equation is written as follows:

\[
s(t) = \iint M(x, y)e^{-i\gamma B_0 t}e^{-i2\pi[k_x(t)x+k_y(t)y]} \, dx \, dy = e^{-i\gamma B_0 t}F_{2D}\{M_0(x, y)\} \tag{2.28}
\]

Where \( e^{-i\gamma B_0 t} \) is the carrier frequency determined by the static field \( B_0 \) and \( F_{2D} \) is the two dimensional Fourier transform of, in this case, magnetization. In the rotating frame, the signal is effectively demodulated.

2.2.4. Phase Encoding, Frequency Encoding, and Readout

![Diagram of encoding schemes in MRI]

Figure 2.2.2: Encoding schemes in MRI. Phase encoding, where an individual point by point matrix of k-space points is acquired (a). Frequency encoding, where a gradient is turned on during the receiver acquisition (b).
Variations in k-space coverage through the use of different gradient schemes provides an important tool in the design of MRI pulse sequences. Some basic examples of these variations are illustrated in Figure 2.2.2. In Figure 2.2.2a, the gradient is applied for a fixed amount of time at a fixed amplitude, turned off, and the resulting signal at that point detected. This process is then repeated at different x and y gradient amplitudes in order to acquire a complete sampling of k-space through a process called phase encoding. Once completely sampled, the entire field is Fourier transformed and results in an image. However, and certainly for conventional MRI, this is impractical. It’s possible to read an entire line of k-space, without having to independently sample each point. This is illustrated in Figure 2.2.2b and is called frequency encoding. The x and y gradients are applied simultaneously to reach a certain line of k-space and then the x-gradient is applied while the signal is being detected during readout. Certainly, these two concepts have been generalized in pulse sequences which offer different features or interrogate different samples. However, for the purposes of remotely detected MRI, where the encoded magnetization is stored and then displaced, readout is not a possibility. Therefore our discussion is restricted to phase encoding.

2.2.5. Resolution and Field of View

With a framework for understanding k-space and the role of gradients in MRI, the reader is well-equipped to do a basic calculation before commencing any imaging experiment. Determining the size of the sample to be imaged as well as the level of detail within the image are interrelated properties. In order to gain image-space
resolution, sampling field of view is increased in k-space. In order to gain image-space field of view, the increment between k-space samples is reduced. These concepts are explained in the following equation, which apply to each imaging dimension for a phase encoded gradient scheme:

\[
\delta = \frac{1}{\frac{\gamma}{2\pi} G_{\text{max}} t} \quad (2.29a)
\]

\[
FOV = \frac{1}{\frac{\gamma}{2\pi} G_{\text{incr}} t} \quad (2.29b)
\]

Where \( \delta \) is the resolution, \( FOV \) is the field of view, \( G_{\text{max}} \) is the maximum gradient amplitude, \( G_{\text{incr}} \) is the gradient increment, and \( t \) is the duration of gradient application.

2.2.6. Velocity Encoding

![Diagram of bipolar velocity encoding gradient](image)

Figure 2.2.3: Bipolar velocity encoding gradient. Amplitude is indicated by \( G_x \) with gradient durations \( \Delta t \) spaced by time \( t_s \).
The velocity encoding gradient accomplishes the task of discriminating between nuclear spins that are stationary and those that have been displaced during the application of the gradient. This is accomplished with a bipolar gradient with zero total area, as illustrated in Figure 2.2.3. Upon application of this gradient, spins that have moved accrue a phase while spins that are stationary have not. In order to understand this in more detail, we turn to a description of the phase accrued due to a spatially varying gradient field. Generalizing from Equation 2.26, the phase accrued along a single dimension $x$ is given by

$$
\phi(t, x) = \gamma \int_0^t x(\sigma) G_x(\sigma) \, d\sigma \quad (2.30)
$$

Which can be expanded in a Taylor series

$$
\phi(t, x) = \gamma \sum_{n=0}^{\infty} \frac{1}{n!} \left( \frac{d^n x}{dt^n} \right) \int_0^t G_x(t) \, n \, dt = \gamma x m_0 + \gamma v m_1 + \cdots \quad (2.31)
$$

Where $m_0$ corresponds to the zeroth gradient moment and $m_1$ corresponds to the first gradient moment. Importantly, as described above, $m_0 = 0$ because the phase accrued in the first gradient lobe is refocused by the second, negative lobe, for spins that are stationary. The phase accrued only from the velocity term is

$$
\phi(t, x) = \gamma v \left[ - \int_0^{\Delta t} G_x \, t \, dt + \int_{t_s}^{2\Delta t + t_s} G_x \, t \, dt \right] = \gamma v G_x \Delta t (\Delta t + t_s) \quad (2.32)
$$

This equation establishes a relationship between the phase and the velocity of the spins. In practical terms, the gradient parameters are known and the phase can be determined experimentally. With proper calibration, the velocity can then be calculated for a given
experiment. Furthermore, velocity encoding gradients can be applied simultaneously in all dimensions with corresponding spatial encoding gradients yielding three-dimensional velocimetric maps of flowing systems. Lastly, this general approach of using gradient moment nulling can be used to design gradients to cancel higher order terms, such as acceleration.

2.2.7. DATA SAMPLING

Typically, the topic of sampling comes first in any discussion about data acquisition. Here, it is described last so as to provide a brief introductory teaser for Chapter 5 about compressive data acquisition in remote detection. The reader is referred to several texts for a more in depth introduction [10-12].

The discussion regarding k-space, FOV, and resolution as it pertains to MRI in section 2.2.5 is incomplete without a basis on which to choose a sampling schedule. There are several basic questions involving the minimum number of samples necessary and the spacing between the samples to determine FOV and resolution that are answered with well-defined lower bounds.
We begin with the simplest and most intuitive example of sampling, which is a periodic function (signal) of a certain frequency, $f$, as illustrated on the top of Figure 2.2.4a. The sampling schedule is of utmost importance considering that this analog continuous signal must be digitized for all modern electronics and computation. Sampling schedule in this case means the set of evenly spaced points chosen along the horizontal axis which interrogate the function along the vertical axis. In Figure 2.2.4a, the function is sampled at a high frequency by the points shown in red. When these sampled points are then interpolated with a continuous function, below, the original signal in black is reproduced completely. In many applications, such as those that are data or acquisition time limited such as MRI, it may be desirable to acquire as few points as possible. However, if too few points are used, i.e. a sparser sampling schedule, as shown in Figure 2.2.4b, then the original function, and particularly the frequency, is misrepresented.

In order for the original signal in Figure 2.2.4 to be represented accurately, the sampling schedule must satisfy the Nyquist criterion, which states that the function must be

---

Figure 2.2.4: Digital sampling of a periodic function. Sampling of a periodic function is illustrated above (a) and below (b) the Nyquist criterion.
sampled at least twice per period, or, more succinctly the sampling frequency $f_s$ should be:

$$f_s \geq 2f_{\text{max}} \quad (2.33)$$

Where $f_{\text{max}}$ is the maximum frequency present in the received signal. Since Figure 2.2.4 only contains a signal composed of a single frequency $f_{\text{max}}=f$. In this case, the sampling criterion is clearly visible, and falls somewhere between the sampling schedules illustrated in Figure 2.2.4a and 2.2.4b.

While the Nyquist criterion certainly applies to MRI, the signal is acquired in k-space and then transformed into image space. This requires some adjustment to the interpretation. Certainly much has been written about the underlying sampling theory. However, in order to understand sampling in k-space, one must understand that multiplication in the frequency domain (k-space) translates to convolution in the image domain (image space). Furthermore, the Fourier transform of a comb, or a sampling function, in the frequency domain is a comb in the image domain. Thereby a sampled function in k-space, $f_s(k)$ may be represented as:

$$f_s(k) = f(k) \left[ \frac{1}{\Delta k} \text{comb}\left(\frac{k}{\Delta k}\right) \right] \quad (2.34)$$

Where $\Delta k$ is the sampling interval and $f(k)$ is the unsampled function. The Fourier transform of this is

$$f_s(x) = f(x) * \text{comb}(x\Delta k) \quad (2.35)$$
Where the \( f_s(x) \) is the sampled function in image space and \(*\) represents the convolution of the two functions. Equations 2.34 and 2.35 state that the sampling of a function in k-space corresponds to a replication of the function in real space. The sampled function \( f_s(x) \) will be accurately represented if the replicates do not overlap, or alias. Aliasing may be avoided if the sampling interval in k-space follows the Nyquist criterion, which now may be written as

\[
\Delta k \geq \frac{1}{F_{OV}} \quad (2.36)
\]

The fewer sampled points there are, the more the images will overlap in image space. One such example is illustrated below in Figure 2.2.5. Figure 2.2.5a is sampled at the appropriate k-space interval, and therefore satisfies the Nyquist criterion. Figure 2.2.5b, however, is sampled below the Nyquist criterion, which shows clear overlap of images.
Figure 2.2.5: MRI image sampling in k-space above (a) and below (b) the Nyquist criterion. When the conjugate k-space domain sampling satisfies the Nyquist criterion, the image is reproduced accurately with no aliasing or overlap (a). When there are insufficient samples, aliasing is observed, particularly repetition of the large nose on the left, and posterior aspect of the skull on the bottom right (a).

With this understanding of Nyquist sampling and how it applies to MRI, the final point of note is one of practicality. With the advent of sophisticated spectroscopic imaging sequences and new applications of clinical MRI for various patient groups and functions, image acquisition time becomes a limiting factor. This is particularly true in situations where other faster modalities provide comparable information. Furthermore, for lab-on-a-chip applications, where optical imaging provides nearly instantaneous analysis, MRI certainly suffers from long acquisition times by comparison. Therefore, to improve practicality, research into more sophisticated sampling schemes that maintain image quality will be part of all MRI research. These methods, dubbed compressed sampling, are discussed in Chapter 5.
CHAPTER 3: OVERVIEW OF MICROFLUIDICS

3.1. INTRODUCTION

Micro/nanofluidics has emerged over the past several decades as a result of an interest in expanding the breadth of applications allowed by fabrication techniques established in the semiconductor industry. In this field, the manipulation of small fluid volumes in biomolecular, chemical, or medical processes and measurements improves cross-platform integration and accessibility while decreasing cost and increasing analytical speed. Some examples among the many advantages of miniaturized assays is that they are portable, have multiplexing capabilities, low reagent volumes and finely tunable, customizable physical properties. Many successful implementations of large scale laboratory processes have been demonstrated at the micro and nanoscale. These methods, dubbed Micro Total Analysis Systems (μTAS) or Lab-on-a-Chip (LOC) are made possible by the unique physics that arises when fluidics enters the micro-regime. The pertinent topics within microfluidic physics, microfabrication techniques, and current applications will be discussed here. The reader is also referred to general texts that provide a comprehensive overview of the entire field [13-18].

3.2. BASIC PHYSICS OF MICROSCOPIC FLOWS

3.2.1. NAVIER STOKES

The governing equations for fluid mechanics are based on Newton’s second law of motion. The Navier Stokes relationships are highly general, and a detailed discussion
and derivation is included in many great texts [18, 19]. A general, vector form of these equations for incompressible fluids is written here:

\[
\rho \left( \frac{dv}{dt} + v_x \frac{dv}{dx} + v_y \frac{dv}{dy} + v_z \frac{dv}{dz} \right) = \rho g - \nabla P + \eta \nabla^2 v \quad (3.1)
\]

Here, \( \rho \) is density, \( v \) is velocity corresponding to the subscripted dimension, \( g \) is the gravitational force vector, \( P \) is pressure, and \( \eta \) is viscosity. The left of the equation represents the acceleration, or inertial forces. For simplification, the nonlinear inertial terms disappear in the case of microfluidic flows. The right represents a summation of gravity, pressure, and viscous forces respectively. The viscous force dominates in microfluidic flow regimes and will be described in more detail. Furthermore, dividing the inertial force by the terms on the right side of the equation results in dimensionless numbers, which define flow characteristics.

### 3.2.2. Shear Stress, Viscosity, and Newtonian Fluids

![Figure 3.2.1: Shear stress on a two-dimensional fluid element. A force at the top of the fluid, \( \tau \), creates a velocity distribution, \( v \), along the element and deforms the fluid a distance \( \Delta x \). Here, the fluid element is shown at two times, \( t=0 \), and \( t=T \), before and after application of the shearing force.](image)

Figure 3.2.1: Shear stress on a two-dimensional fluid element. A force at the top of the fluid, \( \tau \), creates a velocity distribution, \( v \), along the element and deforms the fluid a distance \( \Delta x \). Here, the fluid element is shown at two times, \( t=0 \), and \( t=T \), before and after application of the shearing force.
The two-dimensional deforming fluid volume illustration in Figure 3.2.1 explains the necessary relationships between shear stress, strain, viscosity and thereby the fundamental definition of a Newtonian fluid. This scenario is illustrated for the two-dimensional simplified case but is generalizable to more realistic scenarios. The fluid element in Figure 3.2.1 is shown in blue on the left at $t=0$. A shear force along the top edge of the fluid element creates velocity increasing from 0 at the bottom to $v$ at the top and shears the fluid element as shown on the right. Some relevant parameters that characterize flow in this scenario include shear stress, shear strain, shear rate, and viscosity. Shear stress, $\tau$, is the force on the liquid per unit cross sectional area ($F/A$). Shear strain, $\varepsilon$, is the displacement of the fluid along the wall under the force relative to width of the channel ($\Delta x/y$) the derivative of which is the rate of shear strain, or shear rate ($dv/dy$). Lastly, viscosity, $\eta$, is a measure of the fluids resistance to deformation rate, relating shear stress to shear rate according to Equation 3.2, or Newton’s law of viscosity:

$$\eta = \frac{\text{shear stress}}{\text{shear rate}} = \frac{\tau}{dv/dy} \quad (3.2)$$

Different fluids are classified according to this basic relationship. In the case where the relationship between shear stress and shear rate is linear, the fluid is considered Newtonian. Here, the viscosity does not change with varying shear stress. This is the case for water, where viscosity is always constant and independent of applied shear stress. If however, the shear stress depends on the shear rate, the fluid is non-Newtonian. The viscosity changes with applied force, as it does in quicksand or blood.
The mechanics of non-Newtonian fluids are typically more complex. In this work, while certain NMR parameters are studied in the blood of live animals, the fluid mechanical studies are restricted to Newtonian fluids.

3.2.3. The Reynold’s Number

The Reynold’s number is the dimensionless metric by which the predictability of a given flow is determined. It defines the characteristics of flow based on simple quantifiable parameters. Specifically, flows at high Reynolds number are turbulent while those at low Reynolds number are laminar. As discussed above, in microfluidic systems we expect flow characteristics that are laminar precisely because of this relationship:

\[
Re = \frac{\rho dv}{\eta}
\]  

Where, \( \rho \) is density, \( d \) is a characteristic length, \( v \) is flow velocity, and \( \eta \) is viscosity. As shown, the Reynold’s number is the ratio between the inertial forces, \( \rho dv \), in the Navier Stokes’ Equation and the viscous forces. For small diameters and low velocity, as is present in microfluidic devices, the viscous forces dominate and the flow pattern is predictable, or laminar.

As an example, for water in a 150 \( \mu \)m circular channel flowing at 20 cm/s, \( Re \approx 30 \). While highly dependent on flow geometry and conditions, a general approximation is that the turbulent-laminar transition occurs at \( Re \approx 2300 \). As the Reynolds number increases above this value and flow becomes turbulent secondary flow elements such as eddy currents begin to appear. This complexity is avoided at small dimensions (small \( d \)) and
low flow rates (small v) and allows for precisely controllable flow characteristics. While this simplified flow is beneficial for some applications, it causes difficulties in others that require fast mixing.

3.2.4. Poiseulle Flow

In all cases of fully developed, pressure driven, Newtonian fluid flow, as those described within this work, the laminar flow profile takes on a characteristic parabolic shape. This can be readily derived from Equation 3.2 and by considering the scenario in Figure 3.2.1 with some modifications. Consider the same fluid element flowing between two parallel plates. The shear force is now no longer an unidentified force on the top of the fluid but caused instead by the plate on both the top and bottom surfaces of the fluid element. The shear stress profile along the cross section of a liquid flowing between two parallel plates is at a maximum closest to channel walls and drops to 0 in the middle of the channel. Specifically, the integral of this shear stress profile, which yields velocity, is 0 at the walls of the channel and a maximum in the middle. For cylindrical channels, the profile is described by:

\[
v_x(r) = 2V \left(1 - \left(\frac{r}{R}\right)^2\right) \quad (3.4)\]

Where \(v_x(r)\) is the velocity along the flow direction \(x\), relative to the distance away from the channel wall, \(r\), \(V\) is the mean velocity, and \(R\) is the channel radius.
3.2.5. Dispersion

![Figure 3.2.2: Taylor dispersion. In this two-dimensional depiction, a rectangular slice of fluid is first deformed into a parabola and subsequently blurred into a plug over time.](image)

It’s important to note that the parabolic flow profile develops over time. Therefore a strip of fluid that spans the cross section of the channel, very much like an encoded slice of an NMR experiment that is transverse to the channel, will become stretched with fluid at the center moving ahead of that at the walls. This scenario is depicted in Figure 3.2.2. This convective spreading combines with diffusion across the channel that blurs the parabolic profile, causing a Gaussian like plug to form along the flow direction. This superposition effect is called Taylor Dispersion and dominates over diffusion alone in most flowing microfluidic applications.

3.3. Detection Methods

The majority of detection and analytical methods for microfluidics are largely based on optics. Comprehensive reviews of the optical literature as it pertains to microfluidics are available [14, 20, 21]. Certainly, the conventional optical techniques such as direct visible light imaging, absorption and fluorescence detection make up the majority of methods widely adopted in lab-on-a-chip. While highly beneficial and necessary, the limited
information provided by the standard methods along with the reliance on labeling strategies make them unlike the NMR methods developed herein and will therefore not discussed further. Here, we consider a pair of pertinent examples, namely Raman spectroscopy and particle image velocimetry (PIV) to illustrate the differences from NMR and difficulties faced in microfluidic analytics to date. The complexity of integrating a comprehensive chemical assessment along with a technique for measuring velocity on the microscale limits most research to choosing one over the other. Optimizing the scheme to gain optical access and matching the refractive properties of the microfluidics chip adds a subsequent layer of design that must be considered.

As a comparison, recent developments with NMR utilizing optimized microcoils both separate from and integrated onto the microfluidic chip will be briefly outlined below. To date, these optimized strategies have not yet been integrated with remote detection NMR techniques at high field. Certainly the combination of optimized highly sensitive detectors and signal amplification provided by remote detection will serve to enhance both developments.

3.3.1. RAMAN SPECTROSCOPY

Raman scattering is an inelastic interaction that occurs when photons collide with a molecule and lose energy resulting in a characteristic Stokes’ shift. The scattering effect combined with appropriate detection strategies results in a spectrum that serves to characterize the molecule of interest. While these Raman spectra can be rich with information, the limited number of photons that exhibit this effect results in a weak
signal. Subsequent research has revealed, however, that certain surfaces are known to amplify the effect, allowing for a more commonly used technique in microfluidics, dubbed surface enhanced Raman spectroscopy (SERS). These surfaces feature metallic nanostructures with sharp edges or gaps with enhanced local electromagnetic fields that serve to amplify the Raman signal. This signal enhancement afforded by the substantial research in engineered substrates makes SERS one of the leading label-free analytical techniques being developed in biology, chemistry and microfluidics.

A thorough review of the SERS literature, background, and most recent developments is available [22]. Some examples of the technique development can be found in the Luke Lee lab at Berkeley, which has explored thermal effects on the SERS signal [23] as well as developed a highly uniform self-organized nanopore array[24]. Much of the broader research can be categorized into two primary subspecialties: understanding the mechanistic nature of the SERS effects and developing novel nanoassemblies for various applications. In the microfluidic realm, SERS assemblies have been integrated directly onto the chip. Custom designed chips with functionality that matches the analysis are utilized to detect an array of bioanalytes. The limitation here is that the substrates potentially limit the microfabrication flexibility and require specific chip designs. The design constraints for RD-NMR are quite different. While the technique is general, and can be applied to any fabricated microfluidic design with all available materials, the specific flow properties often need to be modified to optimize the RD-NMR acquisition. The details of this restriction will be discussed in later sections. SERS does however have a lower limit of detection. In some fundamental studies
comparing different substrates in the mid 90’s, the SERS technique showed sensitivities of single to hundreds of femtomoles [25]. The RD-NMR microcoil used in the experiments described here is only capable of tens of millimolar detection, at volumes which equate to nearly $1 \times 10^6$ femtomoles. While this can certainly be improved dramatically with optimization and highly sensitive magnetometer detectors, it does speak to the strength of the SERS technique. The combination of SERS and microfluidics holds great potential for future development with both new substrates, microfluidic geometries, and the miniaturization of all large scale laboratory analytics.

3.3.2. **PARTICLE IMAGE VELOCIMETRY**

Particle image velocimetry is capable of assessing and quantifying fluid flow in a broad range of length scales [26]. Most commonly, this technique characterizes macroscopic scale turbulent flow features. Micro PIV and particle tracking velocimetry (PTV) utilize microscale particles in the flow to measure local and macroscopic scale fluid velocities. These techniques are capable of quantifying fluid patterns and characterizing microfluidic channel design. Much of the work surrounding micron scale PIV systems have been completed at a nearby junior college [27-29]. Here the study design typically features an epifluorescent microscope, CCD imaging and cross-correlation algorithms to track particle motion between stroboscopically acquired images. The primary advantage of PIV over NMR or MRI based velocimetric techniques is fast acquisition times. Particle tracking in microscale flows does suffer from limited generalizability. It is, to-date, a niche field with limited development in biological and chemical lab-on-a-chip assays.
As a comparison, NMR is inherently sensitive to the displacement of nuclear spins and is capable of providing high resolution, quantitative and detailed velocity information [30-36]. Additional benefits include the capability of conducting these measurements label-free and in materials that do not require optical access. The potentially long acquisition times and equipment demand reduces NMR practicality, making it a highly uncommon tool in microfluidics labs. In this work, we develop techniques that are aimed at expanding the scope of NMR in microfluidic velocimetry.

3.3.3. NMR IN MICROFLUIDICS

NMR offers a high degree of modularity compared to most detection schemes. The radiofrequency coil can be either at the outlet or directly on the chip. This choice is dictated by the specific application. The former method, where optimized microcoils with low limits of detection (high sensitivity) have been used to detect the output of chromatographic micro separations have largely been developed by Webb et al [37-40]. Here, high resolution spectra are used to distinguish chemical species as they come off the chromatographic column. The complex mixture characterization can be done in two orthogonal dimensions: the chemical shift NMR spectral dimension and the chromatographic elution dimension. The details of chromatographic separations and NMR detection both at the micro and macro scales will be discussed in Chapter 7. Additional novel coil designs with on-chip fabrication strategies that extend beyond chromatography have also been proposed in flowing and stationary samples [41-43]. While the microsolenoid coil and on-chip fabricated stripline used in these studies
incorporated several design principles to optimize limit of detection, this was not a focus. The results, in all cases are acquired at high concentrations. Coil optimization and fabrication strategies for improving sensitivity in microfluidic and lab-on-a-chip platforms beyond that inherently allowed by RD-NMR will dramatically expand the potential scope of this work.
CHAPTER 4: REMOTE DETECTION

4.1. INTRODUCTION

Remotely Detected NMR (RD-NMR) and MRI (RD-MRI) were introduced in order to improve the SNR of imaging or spectroscopy of samples with a small detectable volume[44]. Some of the earliest conceptions of these types of systems were porous media, microfluidic chips, and the human brain. In conventional experiments, a coil encloses the entire sample, making the relative contribution of the region of interest (ROI) small. In an ideal case, one would wrap a volume matched microcoil around each ROI and acquire the necessary information. Certainly the invasiveness of this approach along with the lack of generality makes it impractical. RD-NMR is a general hardware solution to this problem for a flowing or displaceable system. It is possible to improve the SNR by both placing the entire sample in a large coil and including an additional remote volume matched coil at the flow outlet. With these two coils, RD-NMR effectively separates the encoding and detection steps allowing for the optimization of both. In conventional high field applications where sensitivity is limited, the experiment benefits from the macroscopic sample encoding as well as from the microscopic SNR optimized detection.

After its introduction in the Pines’ lab, the technique saw rapid development [45-47], quantification [48], application [33, 49-53], and optimization [34]. Most notably, both microfluidic [34, 47, 50, 51] and porous media [33, 49] experiments demonstrated
substantial improvements in sensitivity by 10\(^6\), spatial resolution of 15\(\mu\text{m}\) [47] and temporal resolution <1ms [54]. Further developments with low field NMR and sensitive magnetometers illustrate the potential portable application of RD-NMR [55-58]. These low field approaches will be particularly practical in developing microfluidic low-cost analytical schemes. The techniques developed in this work can be integrated with these low field hardware advancements to yield an integrated solution.

4.2. THE BASICS

4.2.1. FUNDAMENTAL PULSE SEQUENCE

![Diagram of remote detection pulse sequence](image)

Figure 4.2.1: Basic remote detection pulse sequence. The sequence (below) is shown with a schematic of the experimental setup of a microfluidic chip that is spatially arranged according to the location of each of the corresponding pulses. Encoding occurs on the chip and stroboscopic detection occurs in the remote coil.

Figure 4.2.1 illustrates the first and simplest remote detection pulse sequence. The pulse sequence is placed alongside a schematic of the experimental setup that shows the microfluidic chip, inlets, outlet, and remote coil. The pulse sequence and experimental
setup are meant to correspond spatially and temporally, i.e. encoding occurs on the chip first and detection occurs in the remote coil second. The microfluidic chip is placed in a large volume radiofrequency coil for encoding inside the high field magnet (not shown). The sample, in most cases water, is driven into the microfluidic chip with pressure or a syringe pump and flows continuously throughout the experiment. As the sample flows through the chip, information is encoded with the volume coil, stored, and then transported to the remote coil for detection. The following sections will explain each of these stages in detail.

4.2.2. Encoding and Storage

Here, encoding is the process by which spatial, chemical, or velocity information is stored into the magnetic degrees of freedom of the nuclear spins. While typically the entire remote detection probe, including the microfluidic chip and remote microcoil is placed into the volumetric encoding region, the only relevant information is on the microfluidic chip itself. As illustrated in Figure 4.2.1, encoding occurs with two 90 degree pulses. The first pulse tips the magnetization into the transverse plane. The spins can then evolve over a period $t_{\text{enc}}$, and then are tipped by the second pulse along the longitudinal axis for transport. This second, storage pulse, is essential to the remote detection method. By tipping the magnetization back along the longitudinal axis, it can then be transported to the remote microcoil and is only susceptible to long lived $T_1$ relaxation. Without the storage pulse, the encoded information would decay with $T_2$, which is significantly shorter, particularly in these inhomogeneous systems with
magnetic susceptibility variations across the chip as well as those resulting from flow. With the storage pulse, the encoded information has 3-5 seconds to arrive at the detection, which is sufficient for all the applications discussed in this work.

4.2.3. Travel

After encoding, the continuously flowing system transports the information to the remote microcoil. Since the remote microcoil is spatially displaced from the encoding region, there is a travel time associated with transport. The magnetic properties of the fluid after storage were discussed above: the stored magnetization decays with T1. During this time, the most relevant physical property to be considered are governed by the physics of microscopic flows. The dominant factor is Taylor dispersion, or the blurring of the encoded region as a result of shear forces and diffusion, as discussed in Section 3.2.5. This effect is illustrated in some of the data included in this work. Specifically, Taylor dispersion contributes to the overlap of images acquired along the travel dimension. Without this blurring effect, one would expect each RD-MRI acquisition to result in perfectly separated plugs of fluid separated by time as the encoded region flows through the remote microcoil. Before we continue with a discussion of imaging in a remote detection setting, we must consider the variation in FIDs that result from flow and encoding that form the basis of the RD-NMR and RD-MRI acquisition.
4.2.4. STROBOSCOPIC DETECTION AND THE TRAVEL CURVE

![Image of travel curve]

Figure 4.2.2: Travel curve. Remote detection FIDs detected after encoding and storage. The sequence of FIDs makes up the time of flight dimension and characterizes flow through the microfluidic chip.

After leaving the microfluidic chip and traveling to the remote volume matched microcoil, the signal is detected. As discussed above, the storage pulse returns the encoded magnetization into the longitudinal axis. In order to detect in the transverse plane, a 90 degree pulse must be applied in the remote microcoil and then the receiver turned on to record the FID. It’s important to note that the volume of the remote microcoil for most experiments conducted in this work is approximately 100 nL. The total chip volume can be more than an order of magnitude greater. This means that the pulse acquire sequence in the remote microcoil must be looped, in a stroboscopic fashion, n-times, in order to detect the entire encoded signal from the chip. While this adds a dimension to the experiment, dubbed the time-of-flight dimension, it also provides valuable information about the flow characteristics in the microfluidic chip.
The combined sequence of FIDs for a single encoding step on the chip is called the travel curve, and is illustrated in Figure 4.2. This example, composed of 8 FIDs, contains spins that were encoded by the encoding coil, mostly so around the 5th FID, and those that passed through the chip unencoded, i.e. the first and the last. The duration of each FID, as well as the total duration of the entire travel curve, is dictated by the flow rate and calibrated before the travel time acquisition. The goal in calibrating the RD-NMR travel curve is to increment the separation of stroboscopic pulses in the remote microcoil such that they are applied with the passage of each coil volume. The duration of the travel curve should be sufficient to allow all of the encoded information to pass through the remote microcoil.

4.3. A PULSE SEQUENCE FOR IMAGING AND VELOCIMETRY

Most recently [47], a more sophisticated remote detection pulse sequence was developed that incorporates an array of improvements which broaden the potential applicability of this technique. The most recent version of the sequence, which is used in this work, will be discussed in the following sections. All discussions about the current pulse sequence pertain to Figure 4.3.1.
Figure 4.3.1: Remote detection imaging pulse sequence with position, velocity encoding and phase cycling. The lines of the pulse sequence are detection (remote microcoil), encoding (volume coil), and gradients (G) in each dimension. Specific gradients are labeled along the horizontal axis where $G_{ss}$ is slice selection, $G_{pe}$ is phase encoding, $G_{ve}$ is velocity encoding, and $G_{shim}$ is gradient shimming during detection.

4.3.1. SLICE SELECTION

As discussed previously, the first 90 degree pulse tips the magnetization to the transverse plane for further encoding. This excitation can be region specific with the use of a shaped pulse in the presence of a slice selection gradient. In this implementation, slice selectivity is introduced along the longitudinal, or z dimension. Practically, in microfluidic applications, the slice selective gradient serves the purpose of only exciting signal on the microfluidic chip. All chips have inlet and outlet capillaries which can overlap the FOV and cause aliased signals. Also, the volume of fluid in the inlet and outlet capillaries is substantially higher than on the chip and tends to dominate the image signal received. The longitudinal slice selection pulse can be designed such that it
excludes signal from these capillaries. Furthermore, region selectivity allows specific areas of the chip to be excited and is essential for any multiplexing applications.

4.3.2. **Phase Encoding**

Phase encoding is implemented with biphasic gradient pulses, simultaneously applied in some combination of the three imaging dimensions $x$, $y$, and $z$. Each k-space point that is encoded as a result of its possessing travel time dimensionality and thereby flow information, must be separately encoded in the remote detection scheme. Additionally, frequency encoding would require the magnetization to be oriented along the transverse between encoding and detection, as in a conventional MRI experiment. This, in itself, would enable quadrature detection of a hypercomplex signal, but is not possible in the current experimental setup. The substantial travel time requires storage to maintain encoded information between the chip and detection regions. As such, the time between each phase encode is limited by the total duration of the encoded signal to pass through the detection coil, i.e. the total length of the travel curve. The biphasic nature of the phase encoding gradients creates velocity compensation by nulling this gradient moment, as described above.

Due to the high dimensionality of a remote experiment, reduction in the number of k-space points increases the speed of the acquisition significantly. For some applications, high resolution images may not be required, or conversely, the macro scale chip outline may be known. Using this information to optimize the number and distribution of k-space points, and thereby phase encodes, will be discussed in Chapter 5.
Lastly, Figure 4.3.2 illustrates gradients oriented along 3 orthogonal axes: x, y, and z. This axis system is aligned along the gradient directions and does not compensate for the alignment of the sample. Consider a sample, represented by the blue arrow in Figure 4.3.2, rotated from the gradient axis by some angle \( \theta \) and \( \phi \). In some experiments, it may be necessary to align the image space with the orientation of the sample.

An image space which is aligned to the sample can be achieved by oblique gradients created by using a linear combination of the primary 3 axis gradients determined by a standard three dimensional rotation matrix:

\[
\begin{bmatrix}
G_x' \\
G_y' \\
G_z'
\end{bmatrix} =
\begin{bmatrix}
\cos \phi + \cos^2 \theta (1 - \cos \phi) & -\cos \theta \sin \theta (1 - \cos \phi) & -\sin \theta \sin \phi \\
-\cos \theta \sin \theta (1 - \cos \phi) & \cos \phi + \sin^2 \theta (1 - \cos \phi) & -\cos \theta \sin \phi \\
\sin \theta \sin \phi & \cos \theta \sin \phi & \cos \phi
\end{bmatrix}
\begin{bmatrix}
G_x \\
G_y \\
G_z
\end{bmatrix} \tag{4.1}
\]

Here, \( \theta \) corresponds to the angle along the x-y plane and \( \phi \) corresponds to tilt away from the z-axis, as illustrated in Figure 4.3.2. In order to align image space with the
sample in three dimensions, it’s most practical to first determine θ by acquiring two
dimensional x-y images and rotating only θ in order to align the sample with one axis,
either x or y. Depending on the sample, it may be possible to acquire only one
dimensional images and accomplish this task. With this value of θ, the next angle φ is
then determined by acquiring z-x’ or z-y’ images and adjusting φ.

4.3.3. VELOCITY ENCODING WITH THE REMOTE DETECTION PULSE SEQUENCE

It’s possible to acquire detailed velocity maps that locally quantify the flow
characteristics of a microfluidic chip. As illustrated in Figure 4.3.1, this is accomplished
in the remote detection pulse sequence with a triphasic velocity encoding gradient.
Section 2.2.6 details the mechanism by which a biphasic gradient cancels position. The
triphasic gradient used in the remote detection sequence cancels both acceleration and
position moments. Just as in the case of a biphasic gradient, the total area under the
velocity encoding gradient is zero (+G: -G in the biphasic case and +G : -1.5G : +0.5G in
the triphasic case). The gradient lobes are separated by a delay in order to account for
gradient rise time.

In a typical remote detection velocimetry experiment, the velocity is determined by the
difference in phase from a positive and negative application of the velocity encoding
gradient. This is done for each phase encode, effectively doubling the total experimental
time and adding dimensionality. If the total gradient duration of all three lobes is τ and
the delay between each lobe is α, the phase difference is Δφ, the gradient amplitude is G,
and the gyromagnetic ratio is γ, the velocity v is
This equation relates velocity with the calculated phase difference between the two acquisitions. The velocity can be calculated for each pixel and voxel of an imaging acquisition with the phase encoding gradients yielding quantitative velocity images. Results from experiments incorporating velocity encoding in remote detection have shown previously [33, 34, 47] and will be discussed in detail below.

4.3.4. GRADIENT SHIMMING

An added pulse sequence feature illustrated in Figure 4.3.1 is the low amplitude gradients applied during the detection phase of the experiment. During setup, the sample is aligned in a way that the sweet spot of the magnet, or most homogeneous $B_0$ magnetic field region, is between the microfluidic chip and the remote detection coil. Water filled capillaries are inserted to provide signal for shimming next to the microfluidic chip and the shims are set to maximize the homogeneity around the chip region. If at this point a spectrum is acquired in the remote detection microcoil, the linewidths will be quite broad due to the un-optimized shimming and relatively small sweet spot of the magnets used. To account for this effect, it is possible to move the homogenous region during fluid travel from the encoding to detection region by applying low amplitude linear gradients for the entire duration of the stroboscopic detection. These are illustrated as $G_{\text{SHIM}}$ in Figure 4.3.1. In order to determine these gradient values, one must array each gradient, $x$, $y$, and $z$ individually and acquire spectra for each point in the array. Typically, due to the long duration of application,
gradient values under 1.5 G/cm are used. Importantly, gradient shims were not used in the animal experiments at the University of Minnesota. Horizontal animal magnets typically have sufficiently large homogenous regions to bypass the need for additional gradient shimming.

4.3.5. PHASE CYCLE

The phase of a radiofrequency pulse, in the rotating frame, is the orientation of the pulse’s magnetic field vector in the transverse, or x-y plane. Here we will use a standard convention of phases: 0, π/2, π, 3π/2. In sequences involving more than one radiofrequency pulse, it is possible to cycle the phases of the pulses and receiver, and upon addition of the signal from each step, keep desirable signal and remove unwanted signal. In the remote detection pulse sequence, the phase cycle is used to enable quadrature detection and to only receive information from signals that were encoded. By applying a 4-step phase cycle, the travel curve is made up of positive encoded information on the chip, unlike that illustrated in Figure 4.3.1 from the basic remote detection pulse sequence.
The phase cycle employed is recreated here for reference:

<table>
<thead>
<tr>
<th>Excitation</th>
<th>Refocusing</th>
<th>Storage</th>
<th>Remote Exc.</th>
<th>Receiver</th>
</tr>
</thead>
<tbody>
<tr>
<td>$3\pi/2$</td>
<td>0</td>
<td>$3\pi/2$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$\pi/2$</td>
<td>$\pi/2$</td>
<td>0</td>
<td>$\pi$</td>
<td>$\pi/2$</td>
</tr>
<tr>
<td>$3\pi/2$</td>
<td>$\pi$</td>
<td>$\pi/2$</td>
<td>0</td>
<td>$\pi$</td>
</tr>
<tr>
<td>$\pi/2$</td>
<td>$3\pi/2$</td>
<td>$\pi$</td>
<td>$\pi$</td>
<td>$3\pi/2$</td>
</tr>
</tbody>
</table>

Table 4.3.1: Four step phase cycle used in the imaging and velocimetry remote detection pulse sequence. The columns correspond to excitation, refocusing, and storage in the encoding radiofrequency coil and remote excitation in the remote microcoil. The receiver, which acquires signal in the remote microcoil, is also phase cycled.

Studying this table, it becomes obvious why the signal from unencoded spins cancels to zero. The remote excitation pulse cycles from 0 to $\pi$ phase. Therefore, without excitation, refocusing, or storage, the stroboscopic detection simply cancels out all spins that were not encoded on the microfluidic chip. This could correspond to spins that were not in the encoding region, or even stationary spins or artifacts in the remote microcoil.
CHAPTER 5: COMPRESSION SAMPLING WITH PRIOR INFORMATION

5.1. INTRODUCTION

There are still considerable opportunities to accelerate the acquisition of remotely detected microfluidic MRI. Previously, it’s been noted that microscale systems in which remote detection is most likely to be useful are sparse because the interesting features occupy only a small fraction of the image field of view. This is a disadvantage from the filling factor perspective, but can be beneficial when dealing with data sampling schemes. We thus employed compressed sensing, previously introduced in clinical MRI, to acquire and reconstruct images from a fraction of the data ordinarily recorded in an exhaustively sampled experiment.

5.1.1. COMPRESSED SENSING OVERVIEW

The concept of reconstructing data from insufficient information, or below the Nyquist sampling criteria detailed in section 2.2.7, was introduced by Candes and Donoho, separately, around 2005 [59, 60]. Both groups developed intricate theory to quantitatively prove the underlying assumptions of compressed sensing. These proofs were published and discussed thoroughly. Some of the initial applications of compressed sensing to MRI acquisition were attempted by Lustig et al. [61, 62]. MRI is a natural space for reduced data acquisition. The technique often suffers from long acquisition times when compared to other imaging modalities and the data is acquired
in a conjugate space amenable to a sparsifying transform. For these reasons, the description herein will be restricted exclusively to MRI acquisition and reconstruction.

The illustration in Figure 2.2.4 shows that when there are insufficient samples of a sinusoidal function, the function cannot be represented accurately. In this simplified example, the sinusoid is sampled at regular intervals; the period between samples is constant. The frequency of the undersampled sinusoid is misrepresented. This can be described as an artifact of the sampling scheme. However, if we consider the case where the sampling schedule does not have a defined period, and the signal is sampled at irregular intervals, it’s possible to conceive of a solution where the sinusoid is reproduced exactly, even with insufficient sampling. In this case, the artifacts are independent of the underlying periodicity of the sampling scheme. For more complex signals, undersampled data and reconstruction require a representation of the signal that is sparse and the sampling occurs in a conjugate domain such as k-space. This is compressed sensing, described simply a nonlinear reconstruction technique combined with a random sampling scheme. This will be described in the following sections. In spite of this, the basic concept remains: irregular random undersampling causes incoherent artifacts and an accurate reconstruction of the underlying signal.

5.1.2. THE SPARSE REPRESENTATION AND THE WAVELET TRANSFORM

One of the primary requirements of compressed sensing is that the images have a sparse representation. The different types of sparsifying transforms, including discrete cosine, wavelet, and finite differences have been described previously in the context of
compressed sensing [61, 63]. While transforms such as finite differences are well suited for inherently sparse images, such as angiograms, that only require edge detection, the interesting details of microfluidic devices lie within the bounds of the channel. As such, it has been shown previously that the finite differences does not perform as well in sparsifying images as the wavelet transform for these applications [64]. In this work, the sparsifying transform is the discrete wavelet transform (DWT), and will be focused on exclusively.

The wavelet transform arose from a need to develop a technique that incorporates time dependence in spectral information. Whereas the Fourier transform fully characterizes all of the frequency components of a given signal, it doesn’t give information about when these frequencies appear. That is to say, given time dependent periodic signal with 3 frequency components from time $t=0$ to $t=T$, the Fourier transform will fully characterize the signal from 0 to T, giving all three frequency components in the output spectrum. If however, 2 additional frequencies appear from $t=T$ to $t=2T$, the Fourier transform from $t=0$ to $t=2T$ will contain all 5 of the frequency components. It does not include information about the signal changed that occurred at $t=T$.

The wavelet transform, on the other hand, characterizes the distribution of frequencies temporally, and is described by the following equation:

$$W_f(\tau, m) = \frac{1}{\sqrt{m}} \int_{-\infty}^{\infty} f(t) \varphi \left( \frac{t - \tau}{m} \right) dt \quad (5.1)$$
The idea behind the wavelet transform is to reproduce the signal using a single mother wavelet, $\varphi$. In equation 5.1, $m$ is the scale, and $\tau$ is the shifting increment. This mother wavelet is scaled and shifted to represent the signal for varying scales. The low, or fine, scales correspond to high frequency components. The high, or coarse, scale corresponds to low frequency components. In a signal that is time dependent, the analysis essentially consists of translating the wavelet for each scale through the signal in time, multiplying by the signal, and then integrating. The wavelet transform is then just a representation of the signal for each scale, with accurate reproduction of the signal components temporally. It is now obvious why the one dimensional wavelet transform represents the temporal distributions of frequencies and the Fourier transform does not.

As with the Fourier transform, this basic time dependent continuous example can be extrapolated to multidimensional images quite readily. In this example, the wavelet transform is no longer a plot of frequency vs. time, but instead contains both spatial and frequency information. The wavelet transform of an image is a representation of the horizontal, vertical, and all image components distributed spatially in the transform for each wavelet scale. In this work, this is the sparse representation of image information.

5.1.3. THE POINT SPREAD FUNCTION

To fully understand the effect of subsampling an image, we must assess the input output relationship as it relates to our subsampling function. A linear system can be fully characterized by its response to an impulse, called the impulse response or Green’s
function and in imaging, the point spread function (PSF). While the output of a complex system based on a complex input may be difficult to predict, it’s simple knowing the impulse response; the output of a linear system (such as those considered in this work) is just the convolution of the impulse response with the input. Therefore our system can be described empirically based on simple input-output relationships.

The point spread function is crucial in determining a random sampling scheme. Specifically, it can be used to measure the interference on one pixel by its neighboring pixel caused by undersampling. Relating this to the above description, the PSF of a given subsampling scheme can be used to measure the incoherence of the scheme and calculate the output based on a given input signal. For MRI applications, the transform point spread function fully characterizes the effect of subsampling in k-space on the sparse representation, or wavelet domain. As described in [61], the TPSF calculates the effect on a point at position j in the wavelet domain from a point at position i and is described by

$$TPSF(i; j) = e_j^* φ F_u^* F_u φ^* e_i$$ \hspace{1cm} (5.2)

More specifically, a point in the wavelet domain at i is transformed by the wavelet transform $φ$, and then into k-space by the Fourier transform, $F_u$ where it is subsampled in k-space. This is an accurate representation because the data is actually collected in k-space and subsampling in this domain occurs with the acquisition of fewer data points than required by the Nyquist criterion. Transforming back into wavelet space, the interference on point j can be calculated. The goal of selecting an effective subsampling
scheme is to minimize the TPSF such that the noise is incoherent and therefore there is limited interference between pixels.

5.1.4. DATA RECONSTRUCTION WITH THE L1-NORM

Once a random subsampling scheme in k-space is selected and the data is acquired, compressed sensing requires a nonlinear reconstruction of the image. The l1-norm reconstruction in equation form is written, as follows:

\[
\begin{align*}
\min & \|\psi m\|_1 \\
\text{s.t.} & \|Fm - k\|_2 < \varepsilon
\end{align*}
\]  \hspace{1cm} (5.3a)

Here, \( m \) is the reconstructed image, \( \psi \) is the wavelet transform, \( F \) is the Fourier transform, \( k \) is the acquired data in k-space, and \( \varepsilon \) is some fraction of the noise level. Essentially, the sparse representation of the image (wavelet transform of the image domain), is minimized according to the l1-norm (Equation 5.3a). This minimization of the l1-norm enforces the sparsest representation of the reconstruction. The secondary constraint (Equation 5.3b) enforces consistency with the acquired data controlled by a value \( \varepsilon \) which is set such that the reconstruction converges. For small values of \( \varepsilon \), the reconstruction may take significantly long for a minimal improvement in reconstruction quality. As such, the value may be optimized for a given reconstruction, application, or noise level.
5.1.5. **DATA RECONSTRUCTION WITH ITERATIVE SOFT THRESHOLDING**

![Diagram](image)

**Figure 5.1.1:** Nonlinear reconstruction using iterative soft thresholding in the wavelet domain. The acquired data is transformed into the wavelet domain, thresholded, and then the acquire points which were subject to the threshold operation are replaced in the original data set.

Iterative soft thresholding has been shown to be equivalent to l1-norm reconstruction [65-67], but yields a faster convergence. Here we develop a technique that shows robust reconstruction of synthetic subsampled data. The process flow for this method is illustrated in Figure 5.1.1. Here, the acquired data is transformed into the wavelet domain where it is soft thresholded. The soft threshold is expressed by

\[
(\psi m)_\tau = \begin{cases} 
  \psi_m - \tau |\psi_m|, & |\psi_m| > \tau \\
  0, & |\psi_m| < \tau 
\end{cases} \quad (5.4)
\]

Where data that are below the threshold are set to zero and data above the threshold have the threshold subtracted. The data is then transformed back to k-space. For data consistency, acquired points that were thresholded are replaced in k-space and the process is repeated.

5.1.6. **COMPRESSED SENSING IN RD-MRI AND MICROFLUIDICS**

Previously, we have employed a compressed sensing reconstruction for remotely detected microfluidics with velocity encoding [64]. In those experiments, we achieved
subsampling factors of 8-16 with phase-sensitive velocimetric data, and up to 32 with absolute-valued intensity data. The sampling optimization based on the TPSF and reconstruction methods were developed.

5.1.7. COMPRESSED SENSING WITH PRIOR INFORMATION

In this work, we introduce an improvement to this method which uses our a priori knowledge of the flow geometry to improve the robustness of the reconstruction process with even fewer data. Similar to the approach in [35], our reconstruction minimizes the l1-norm of the compressed representation of the reconstruction to optimize for sparsity while both maintaining consistency with data and enforcing the a priori constraint on the image. We expand this technique and apply it in remotely detected MRI of microfluidic devices, achieving subsampling levels of 128x. We incorporate prior knowledge in the optimization (a) through a soft outline mask outside of which any image intensity is penalized in the constraint function (b) effectively enforcing an irregular field of view:

\[
\begin{align*}
\min & \| \psi m \|_1 \\
\text{s.t.} & \| F m - k \|_2 + s \| M_r m \|_2 < \epsilon
\end{align*}
\]

Here, \( \epsilon \) is set to a fraction of the noise level to force agreement with the data, \( M_r \) is the image space a priori mask, \( s \) is the image-mask compliance multiplier. This image-mask compliance multiplier, \( s \), is a weighting factor that biases the reconstruction towards the real space a priori mask. The compressive transformation is the wavelet transform, \( \psi \), and the data is manipulated in Fourier space, \( F \), minimizing the reconstructed data, \( m \),
while ensuring agreement with the acquired data, k. The relative weighting of the two constraints in (b) is adjustable through an image-mask compliance factor, s, which is empirically optimized to minimize the error. Furthermore, reducing ε improves agreement with the data while increasing reconstruction time. We also show that prior information about the microfluidic chip geometry alters the optimal k-space sampling scheme, resulting in one that is specific to the features of the image. Specifically, the distribution of sampled points in k-space can be modified to minimize reconstruction errors and tailor the acquisition based on prior information. While our mask contains no details of the internal structure of the fluid channels, we show in simulations and experiments that its inclusion in the objective function and in the k-space sampling optimization improves the fidelity with which these internal details are reconstructed.

Using this method, we are able to accurately reconstruct six dimensional MRI images (three spatial and three vector velocity dimensions) of a serpentine microfluidic mixer from an MRI data set that is undersampled by a factor of 128. Since knowledge of flow pathways is a generic feature of all fabricated microfluidic devices, we anticipate that this method will be useful in any MRI experiment in all lab-on-a-chip devices.

5.2. EXPERIMENTAL METHODS

5.2.1. REMOTE DETECTION DATA ACQUISITION

NMR experiments were performed on a 7 T Oxford Instruments superconducting magnet mated to a Varian console probe and gradient coils. Imaging experiments were conducted using magnetic field gradients with nominal maximum values of 100
Gauss/cm along all axes, also produced by Varian, and encoding was performed using a 40 mm Varian volume imaging probe. A complete description of the remote detection experiment and hardware appear elsewhere [27]. Briefly, water flows from a regulated pressure-driven flow apparatus to the chip, which is enclosed by a volume imaging coil through which RF pulses are applied to accomplish spatial and velocity encoding. The encoded spin information is then stored as long-lived longitudinal magnetization, and it travels to a microsolenoid NMR probe for detection. The microfluidic serpentine mixing chip is etched in glass and consists of three channels that terminate in two inputs and one output. The glass microfluidic chip was fabricated by etching a rectangular channel, 150 ± 10 μm in width and 90–120 μm in depth, limited by manufacturing tolerances. While scanning confocal microscopy would have been capable of resolving the precise shape of this channel, no such experiment was attempted here, and hence, information about the cross sectional, or through-plane, dimension was not included in the mask which constrains the image to the known geometry of the chip. The chip outlet was connected to a microcapillary (150 μm) through which its contents reached the microcoil detector, a 12-turn microsolenoid wound around a tube that encloses the capillary. Pressure-driven flow of water, doped with 2% isopropanol to prevent bubble formation, was maintained with a driving pressure of 10 psi for the duration of these experiments.
The NMR pulse sequence for the remote detection experiment is illustrated in Figure 5.2.1. Following slice-selective excitation of only the region of interest, phase encoding of spatial and velocity information is performed in the context of a spin echo, as detailed in [27]. We employ gradient moment nulling to cancel the phase accrued due to the motion of spins during their spatial encoding. The information is then stored as long lived longitudinal magnetization by a terminal $\pi/2$ pulse and detected stroboscopically after flow to the detector. During transport to the detector, a gradient pulse is applied to dephase any residual transverse magnetization that persists due to imperfections in the storage pulse, and the pulses are phase cycled to eliminate the contribution of any unencoded signal. The primary difference from the pulse sequence described in section 4.3 is the absence of gradient shimming. This component was not incorporated when
these experiments were acquired. This same rationale applies to oblique gradient adjustments, however because the experimental results in this section are three dimensional, they do not require precise alignment of the sample with the gradient axis.

In the following experiment, three-dimensional velocity encoded image was acquired with 128x128 points in the plane of the chip and 16 points perpendicular to the plane of the chip. However, because the image acquisition was undersampled by a factor of 128 (vide infra), we recorded only 4096 points instead of the 524,288 points that would be required for each exhaustively sampled hypercomplex image. These images were encoded with velocity information in a separate dimension in which the velocity encoding gradient was switched between positive and negative phase to generate a phase contrast proportional to the velocity. For each point, the total time for the encoded volume to flow through the detection region was 2 s, with each of the 40 stroboscopically acquired FIDs each lasting 50 ms. A separate set of 3D images were acquired for each of the three velocity components: x, y, and z.

5.2.2. IMAGE RECONSTRUCTION

Images were reconstructed using software implemented in MATLAB and the l1-minimization was implemented with an iterative optimizer[68], called from within MATLAB. Following their apodization with a decaying exponential, we transformed and integrated the primary data around the position of the water resonance to yield 40 FIDs for each k space point, constituting a time of flight curve weighted by the k-space interferogram. The data were then arranged into a multidimensional matrix of
128x128x16 k-space points for each of two velocity-encoded dimensions. The points in
the conjugate space that were not sampled were initially replaced with zeros.

Details of our reconstruction scheme for sparse data in the absence of prior
information constraints have been described elsewhere [64] and will only be treated
briefly here. First, sampling tables were determined by choosing an optimal distribution
from among many randomly generated k-space distributions, each weighted towards
the center of k-space. The reconstruction uses an iterative algorithm that constrains the
difference between the image and the measured data and maximizes its sparsity in the
transform (wavelet) domain by minimizing the l1 norm as discussed earlier. It was
implemented in Matlab using the Wavelab v.8.02 [69] package and spgl1 v.1.7 [68] for l1-
norm optimization. Geometric data about the flow geometry were used as added
constraints to this minimization, expressed weakly (with a variable weight, s) in the
objective function by an outline mask outside which image pixel intensities are
constrained to lie below a threshold value. By “outline mask,” we mean that the mask is
a geometrically weak constraint, in that it is completely homogeneous with respect to
the internal structure of the fluid channels and only serves to constrain the edge
topology of those channels within the field of view.

5.2.3. SYNTHETIC DATA RECONSTRUCTION

Reconstruction fidelity is dictated by the strength of this image-mask compliance
multiplier. We therefore explore the possible regimes of scaling in silico by constructing a
model image that is internally structured on several length scales: a 64x64 image of the
University of California, Berkeley, logo, divided into an intersecting line grid (Figure 5.3.1a), and subsampled in the Fourier domain by a factor of two. We simulated experimental noise in the test image by adding normally distributed noise set to 1% of the maximum image intensity. Next, the mask corresponding to this was a similar logo that was homogeneous in its internal structure. During the reconstruction, the image-mask compliance multiplier \( s \) was applied as noted in the above equation and varied for 8 different values ranging from 0-32.

5.2.4. EXPERIMENTAL DATA RECONSTRUCTION

Experimental data in the microfluidic mixing chip were reconstructed separately for each time of flight point. Boundary constraints that incorporate a priori information about the chip geometry were imposed in the form of a image space mask generated from a processed photograph of the chip (Figure 5.3.2a) and aligned to a low resolution scout image. Contrast between the channel and the glass in the photograph was created by injecting dye into the channel. The chip geometry boundaries were determined from the photograph in MATLAB. The channel was identified by thresholded pixels above the average glass pixel value, and assigning these a value of 1. Values below the threshold were assigned to zero. Lastly, the image was Gaussian filtered in k-space to accommodate for registration imperfections. Artifacts were manually removed from the mask photograph and the 2D section was replicated across all 16 slices of the acquired image dimension. The reconstruction was carried out at moderate image-mask compliance value, \( s=2 \), to control relative weighting of compliance to the a priori mask.
and data. The inequality constraint for the l_2-norm constraint was determined by taking a fraction of the average noise value from an early TOF point at which no encoded signal had yet reached the detector.

We next extracted vector velocity information from the reconstructed data. For each component of the velocity, the phase difference between positive and negative gradient acquisitions was determined for each voxel. The data were corrected by subtracting a linear phase in the Z dimension that persists even in a static phantom. Finally, the phase data were converted to velocity units and plotted on a 3D surface as velocity vectors (Figure 5.3.4).

This same experimental data were used for comparison with equivalent reconstructions conducted without the benefit of flow information. Two dimensional projections from the three (spatial) dimensional data were acquired by summing the intensity information across the through plane dimension. These 2D projections images were displayed for an image-mask compliance reconstruction of s=0 (no mask) and s=2 (Figure 5.3.3).

5.2.5. RECONSTRUCTION AND K-SPACE SAMPLING OPTIMIZATION

Lastly, we produced an optimized sampling schedule by varying both the random Gaussian sampling distribution and image-mask compliance (s) variable. The Gaussian sampling distribution, used to determine the k-space gradient values in an experimental subsampling acquisition, was varied by its relative weighting toward the center of k-space. Ideally, k-space sampling captures both high and low frequency components.
However, with significantly fewer sampled points the relative distribution of the points should be optimized to yield minimal reconstruction error and try to match the statistics of the data. Additionally, *a priori* information about the reconstructed geometry changes the optimal sampling scheme by providing additional information in the reconstruction. Second, the image-mask compliance variable (s), which dictates the relative adherence of the data to the *a priori* mask, was also optimized to generate minimal reconstruction error. Both variables were tested in a sample reconstruction of a synthetic dataset that resembles the experimental microfluidic chip. First, a synthetic three dimensional data set was generated from the experimental data as described above for generating the mask. Voxels inside the channel were given a value of 1 and voxels outside the channel were given a value of zero. The resulting synthetic data resulted in a three dimensional image that was, like the experimental data 128x16x128 pixels. Unlike the *a priori* mask, the synthetic sample data only occupied 4 slices of the 16 slice three dimensional dataset. Normally distributed noise was then added to the data set as described previously. The data was Fourier transformed and subsampled with 5 different 128x subsampled randomly generated k-space distributions with various weightings toward the center of k-space. The errors from the best and worst reconstructions were analysed in detail. The two corresponding sampling schemes, which result in the lowest and highest reconstruction errors, are illustrated in the left column of Figure 5.3.5 as two dimensional slices of a three dimensional k-space sampling scheme. The k-space sampling scheme at the upper panel of this figure, which corresponds to the reconstructions in Figure 5.3.5a and Figure 5.3.5b, was more weighted toward the center
of k-space. The scheme illustrated in the bottom panel is a more diffuse distribution with more points weighted to sample higher resolution points in k-space. Each sampling table was restricted to fully sample the centermost points of k-space. The reconstructions were calculated with the same *a priori* mask which outlines the channel geometry as in the experimental reconstruction. An intermediate value of image-mask compliance (s) was used. Each of the reconstructed datasets were quantitatively analysed to determine the error resulting from 128x subsampling and reconstruction. Reconstruction errors were determined by comparing the reconstruction to noiseless input synthetic data. Because our reconstruction algorithm scales the data due to a Fourier transform function convention, the reconstructed image was first normalized, rendering it on same scale as the input data. The errors were calculated by subtracting the normalized reconstructed image from the noiseless fully sampled input synthetic data. For percentage calculations this error was normalized to the noiseless fully sampled input synthetic data. The sampling scheme which yielded the smallest average reconstruction error in the channel was determined to be the optimal sampling schedule. We then investigated the errors resulting from varying the image-mask compliance variable(s) for a dataset subsampled with this optimized sampling scheme. As with the Cal logo, reconstructions were calculated for 8 different image-mask compliance multipliers (s), ranging from 0-32. Reconstruction errors were determined as described above, and the optimal image-mask compliance value was selected to be the reconstruction with least average error within the channel.
The same in silico model was used to assess the effect of increasing the noise level on the reconstruction. The goal was to determine whether the reconstruction was denoising or biasing the data with a random Gaussian noise distribution. The results are shown in Figure 5.3.6.

5.2.6. **Iterative Thresholding**

Because this kind of reconstruction is computationally intensive, we also implemented an iterative soft thresholding scheme with a priori information. This scheme has been introduced previously and shown to be equivalent to l1-norm minimization, but is much faster to compute [65-67].

Using this technique, the data is directly multiplied by the image space a priori mask to enforce the prior knowledge constraint in image space. In the sparse, wavelet domain, the data is then soft thresholded by a value $\tau$, here empirically determined, as defined by the following function:

$$
(\psi m)_\tau = \begin{cases} 
\psi m - \frac{\tau(\psi m)}{|\psi m|}, & |\psi m| > \tau \\
0, & |\psi m| < \tau
\end{cases} \quad (5.6)
$$

Lastly, in k-space, the acquired data that is known, i.e. acquired during the experiment, is replaced if it was removed by the thresholding operation. This sequence is then iterated until reconstruction quality is optimized. For reconstruction of the University of California logo, we used 50 iterations to complete the reconstruction. These results are shown in Figure 5.3.7.
5.3. RESULTS AND DISCUSSION

5.3.1. SAMPLE DATA RECONSTRUCTION

Figure 5.3.1: Illustrative example of sample data reconstruction scheme. A 64x64 Cal logo containing a grid and noise (1%, see text) (a) was Fourier transformed and 2x subsampled (b). The subsampled data are then reconstructed using a soft mask (upper left of Fourier transformed image) to incorporate a priori constraints on the geometry. The mask is applied with different weights during the reconstruction. The illustration shows the effect of increased mask scaling starting with no prior knowledge (c), constrained with s=2 (d), and overconstrained with s=32 (e).

To investigate the effects of varying the image-mask compliance constraint on the fidelity of reconstruction, we used a synthetic image consisting of an internally structured feature placed upon a blank image field, as described in Figure 5.3.1. Direct Fourier transformation of the subsampled k-space representation of this image (Figure 5.3.1a) resulted in unacceptable artifacts (Figure 5.3.1b). This linear transformation was not expected to be an acceptable solution of the inherently ill-posed inverse problem,
and indeed the resulting artifacts distort the internal structure at all spatial wavelengths and similarly obscure the background.

Next, utilizing a compressed sensing reconstruction without *a priori* information (Figure 5.3.1c) improves the reconstruction quality significantly. Both the vertical and horizontal high density grid lines become more apparent, but the upper left grid is unclear. The image also exhibits characteristic wavelet artifacts and significant noise in the image background.

The imposition of geometrical constrains in the reconstruction dramatically improves the result, at least within an appropriate range of weighting parameters (Figure 5.3.1b). Near the optimal image-mask compliance scaling, Figure 5.3.1c, middle, the entire grid is accurately reconstructed at all wavelengths, and the background is also correctly rendered. For high mask-image compliance scaling values, however (Figure 5.3.1c bottom), we note a degradation in reconstruction quality. In the case, the reconstruction overemphasizes agreement with the mask relative to the acquired data. In particular, incoherent artifacts (noise) are folded into the region of interest and distort its internal structure.
5.3.2. SERPENTINE MIXING CHIP

Figure 5.3.2: Comparative figure illustrating benefits of a priori masking. Image of the microfluidic serpentine mixer (a), image resulting from direct Fourier transformation of subsampled data (b), applied mask generated from serpentine chip photograph (c), and reconstructed surface of the chip geometry in which the mask was used during the reconstruction (d). All surfaces were cropped to include only the first 90 points along the longitudinal direction to avoid image wrapping from the outlet capillary.

To probe the limits of our technique, we applied it to multidimensional velocimetric imaging of a serpentine microfluidic mixer (Figure 5.3.2a). We have acquired velocity-encoded images and, from them, derived intensity images by summing the absolute values of these velocity-encoded data. Figure 5.3.2 illustrates the directly Fourier transformed three dimensional image (b), corresponding three dimensional mask (c), the image reconstructed with our algorithm, including the imposition of a known geometrical constraint (d). The reconstructed three dimensional image, illustrated as a surface layer, is an accurate qualitative depiction of the overall chip geometry, including
the proportions of the rectangular channels. The image has relatively few artifacts considering the degree of subsampling involved.

Figure 5.3.3: Images of the serpentine mixing chip 128x subsampled and reconstructed without (a) and with prior information (b). Priori information was a mask during the reconstruction with an optimized scaling parameter. The three dimensional image is displayed here as a projection along the orthogonal dimension, not illustrated.

This reconstruction is also illustrated as a two-dimensional projection along the orthogonal direction (Figure 5.3.3). The two dimensional image is shown for both the reconstruction performed without any mask biasing the results towards a known geometry (Figure 5.3.3a) and with the optimal mask and mask scaling value (Figure 5.3.3b). The image reconstructed with the mask clearly shows reduced artifacts and an improved representation of the chip geometry.
Figure 5.3.4: Subsampled three dimensional, vector velocity field superimposed on an image of the chip surface. All planes of the velocity field are displayed for this perspective of the 3D volume. The three dimensional surface model, reconstructed from intensity data, is show in red. Arrows correspond to velocity vectors and are show in blue. The spacing of the velocity vectors is dictated by a grid corresponding to the acquired voxels. There is asymmetric flow in each inlet due to differing lengths of capillary tubing that supply the chip from a source at common pressure (a). The velocity direction changes at each turn in the chip (b). The average linear velocity for the straight segments of flow in the microfluidic chip was 19.04 cm/s with a standard deviation of 1.82 cm/s.

Figure 5.3.4 shows the three dimensional surface with a three dimensional velocity vector field superimposed. The input volumetric flow rate is biased more heavily to the lower branch (Fig 5.3.4a), a consequence of an asymmetry in the flow pathway leading to the microfluidic chip. In this image, the overall direction of flow is consistent with our expectations, including at turns and channel boundaries (Figure 5.3.4b). The mean linear velocity within the straight region of the channel is 19.04 cm/s with a standard deviation
of 1.82 cm/s. This closely matches previously published results [64], as well as the expected linear velocity for a rectangular channel 125 μm x 150 μm with a volumetric flow rate of 250 μL/min. This is the expected volumetric flow rate at 10 psi for this system.

5.3.4. RECONSTRUCTION ERROR AND SAMPLING MASK OPTIMIZATION

Figure 5.3.5: Subsampling mask optimization. Optimization and error calculations by reconstruction of synthetic three dimensional 128x subsampled data are shown. Reconstructions were done without (a) and with (b) prior information for an optimized sampling schedule. Sampling optimizations were done by varying the weighting toward the center of k-space (c). Errors are illustrated in one slice of real space images (middle column) for the entire region. A quantitative error assessment of the interior of the channel is shown in histograms (left column). Percentage error is binned and shown on
The vertical axis of each histogram. The percentage of pixels within each error bin is shown on the left vertical axis and the cumulative percentage is shown with a red line referenced to the right vertical axis. Each histogram also contains a reference line showing the cumulative percentage of pixels that fall within 20% error.

The manner in which a fixed number of data points are distributed in the conjugate space significantly impacts the reconstruction quality. We therefore optimized the sampling distribution with a three-dimensional synthetic data set resembling the microfluidic chip. Reconstruction fidelity was calculated quantitatively by comparing the reconstruction with the synthetic input data. In Figure 5.3.5, we show the results of image reconstruction in a three dimensional synthetic data set subsampled by a factor of 128 in the Fourier domain, illustrating only the center slice (128x128) of the full data set (128x16x128). Synthetic data were created using five different 128-fold k-space subsampling schemes, two of which are shown in the left column. We display both differences in intensity as a function of reconstruction parameters (with and without noise) and quantify these errors by means of histograms, illustrating that reconstructions without (Figure 5.3.5a) and with prior information (Figure 5.3.5b) are dramatically different. Reconstruction at this high subsampling ratio yields images with significant artifacts outside the channel and large errors within the channel, unless prior information constraints are used (Figure 5.3.5b). In this case, the majority of pixels are included within 10% error and over 90% of pixels fall within 20% error. This is in contrast to Figure 5.3.5a, where less than 60% of pixels are within 20% error.

The sampling table optimization is also illustrated in Figure 5.3.5b and Figure 5.3.5c, which differ qualitatively in the degree to which the sampling is biased towards the
center of k-space. Biasing the sampling away from the center of k-space increases reconstruction error. In this case, while the mask provides low resolution information, the microfluidic chip is lacking internal structure, as is the case in velocity encoding images. Therefore, the most accurate reconstruction representation is still represented by maintaining sufficient low resolution components. While our optimization here is empirical, our results illustrate the potential of achieving higher levels of subsampling by rigorous optimization of the sampling scheme to take advantage of the prior information about microfluidic geometry.

\textbf{Figure 5.3.6:} Synthetic reconstruction of with varying noise levels. The figure illustrates a case where reducing signal to noise ratio affects reconstruction quality.
Lastly, we assess the effect of noise level on reconstruction accuracy with the same *in silico* model to ensure that the reconstruction method is not inaccurately biasing the output image. This is illustrated in Figure 5.3.6. As expected, introducing high noise into the reconstruction image is reflected in the error. This demonstrates that the method is not prone to denoising or biasing the data if the noise is random.

5.3.5. **Iterative Thresholding**

![Figure 5.3.7: Iterative reconstruction with prior information. Reconstruction of a 64x64 2x subsampled University of California, Berkeley logo. Each of the 3 images illustrate iterations in the thresholding and masking algorithm which eventually converges to a final solution.](image)

Lastly, we demonstrate in Figure 5.3.7 the results of the iterative thresholding reconstruction scheme with *a priori* masking, which was used to improve reconstruction speed. Here, after 50 iterations, we show significant equivalence to l1-norm reconstruction.

5.4. **Conclusions**

We have demonstrated a method to acquire and reconstruct MRI images of microfluidic devices for which there is some prior knowledge of the flow geometry. Logical extensions of this technique include the encoding of chemical information in
microfluidic chemical assays. In those cases, prior knowledge about the NMR spectrum may be similarly integrated into the reconstruction to allow for a higher degree of subsampling. In all cases, we have not yet exploited this prior information in the optimal design of sampling schedules or the devices themselves, and this may result in further savings in acquisition time.
CHAPTER 6: MICROFABRICATED STRIPLINE DETECTOR

6.1. MOTIVATION

While the signal sensitivity enhancement in RD-NMR has been quantified [48] and substantial proof of concept executed, further improvements and optimization to the technique hardware have yet to be discussed. With the current experimental hardware design, which will be discussed below, the signal enhancement offered by the improvement in filling factor is counteracted by the necessary travel time from encoding region to detection. This limits the utility of RD-NMR in applications that require slow flows and short T1 species, both of which are common in lab-on-a-chip devices. Furthermore, the large distance from the encoding region to the detection region may limit the accuracy of flow assessment through RD-NMR because of Taylor dispersion discussed in Section 3.2. Fortunately, these apparent limitations may readily be overcome with an optimized experimental setup made possible with the use of lithographic methods common in the fabrication of microfluidic devices.

This section briefly introduces first experiences with a planar microfabricated stripline for detection in place of the solenoid wrapped around the capillary outlet. We propose and implement a design that both reduces the travel time from encoding to detection and also thereby minimizes dispersive effects in RD-NMR experiments. This preliminary work forms the basis of future work to optimize the RD-NMR method and
enhance applicability to a wide array of real-world lab-on-a-chip devices. The method also promotes integration into existing lab-on-a-chip infrastructure by enabling modularity and optimized hardware fabrication.

6.2. **CONVENTIONAL REMOTE DETECTION EXPERIMENTAL SETUP**

![Diagram of conventional remote detection probe](image)

*Figure 6.2.1: Conventional remote detection probe. On the left, the microfluidic chip is contained in a delrin holder and the flow is provided by a PEEK capillary. The output flow exits the chip via the same capillary and travels into a brass shield which contains the resonant circuit. A schematic of the interior of the copper hat is shown at the right. The flow enters the detection microcoil which has the PEEK capillary threaded through the interior. The coil itself is immersed in a liquid (FC-43 Fluorinert), for susceptibility matching. The microcoil is connected to the resonant circuit with capacitors and a circuit board mounted on a delrin support.*

The current experimental hardware design has been described in detail previously [47] and will only be discussed here briefly. The chip is encased in a delrin holder which is then placed in a 40 mm commercial RF probe inserted into the bottom of the magnet for encoding. The delrin holder is attached to a home-built probe (Fig. 6.2.1) that contains the resonant circuit and is inserted through the top of the magnet. There are several
microfluidic chips used with varying flow patterns, all which contain one outlet that is attached to a microcapillary that is threaded into the homebuilt probe that uses a 16 turn microsolenoid for detection. The distance from the outlet of the microfluidic chip to the microsolenoid is approximately 5 cm. This distance is restricted by the probe dimensions and accessibility to the resonant circuit. This design was ideal for fast flow applications characteristic of the initial proof of concept studies. Here the relaxation from travel time resulted in only minimal signal loss. In real world scenarios, such as the microchromatographic columns discussed in Chapter 7, this probe design proved to be suboptimal and posed limitations to the generalization of the technique.

6.3. STRIPLINE PROBE DESIGN OVERVIEW

Figure 6.3.1: Exploded view of modified probe design of RD-NMR hardware with stripline and circuit. The probe consists a stripline circuit (shown on the right), which is enclosed in the chip holder and covered by the chip faceplate. The resonant circuit containing tunable capacitors (not shown) is placed inside the brass shield and attached to a delrin support which attaches to the remainder of the probe.
In contrast to the previously established setup, we have designed and fabricated an integrated stripline detector directly bonded to the microfluidic channel. The stripline replaces the 16 turn solenoid previously discussed. This design dramatically improves proximity of the encoding and detection regions. Furthermore, the stripline properties can be custom tailored to the specific application based on flow rate, channel dimensions and inductance/capacitance properties. Because of the ease of fabrication, the detection region (consisting of a stripline detector as well as channel interface) can be generalized into a plug-and-play module that is interchangeable between different microfluidic chips.

An exploded view of the general probe setup is illustrated in Figure 6.3.1. Here, the resonant circuit is similar to that in the conventional NMR probe. The circuit board contains variable capacitors for tuning and matching (Newark, Palatine, IL, 1-30pF, not shown) and protrudes through the brass shield connecting via solder directly to the stripline circuit. The opposing end of the resonant circuit serves as a mount for the SMA connector which then connects to the coaxial cable leading out of the magnet and to the console. Both parallel and series gaps on the resonant circuit design allow for the placement of chip capacitors for fine tuning and matching. The brass shield covers the circuit and connects the delrin support with the chip holder. A faceplate screws onto the chip holder and supports the connectors that interface with the microfluidic chip.
Figure 6.3.2: Detailed view of stripline and microfluidic channel contained in chip holder. The microfluidic channel outlet is directly bonded to the stripline for detection. The stripline consists of a saddle like design with connector pads to interface with the resonant circuit (Fig 6.3.1). Inlet and outlet ports correspond to screw holes on the faceplate (Fig 6.3.1) which allow for interface with input and output peak capillaries and connectors.

A more detailed top view of the microfluidic chip and stripline setup inside the delrin holder is illustrated in Figure 6.3.2. The microfluidic channel etched in PDMS is directly bonded onto a stripline circuit. The stripline circuit and chip are both placed in the chip holder and microfluidic connectors are supported by the delrin faceplate (Figure 6.3.1). The outlet of the microfluidic chip overlaps the stripline immediately after the encoding region, removing the need for a connecting PEEK capillary between encoding and detection. The flow moves the encoded information directly to the segment of the chip with detection channel adhered above the stripline detector. In this part of the chip, it may be possible to include a modular component, where the stripline and adhered channel can be connected to any number of chips or flow geometries. The connector
pads on the stripline are directly soldered to the resonant circuit board protrusion which extends through the brass shield via solder.

6.4. MICROFLUIDIC CHIP AND STRIPLINE FABRICATION

The stripline probe and microfluidic chip microfabrication procedure was developed by Daniel Kennedy in the Pines Lab. All fabrication was conducted in the BNC in Stanley Hall at UC Berkeley. The probe was made of resonant circuitry and a stripline on the same printed circuit board (PCB). The microfluidic chip was made of PDMS and bound onto the PCB to interface with the stripline directly.

PCBs were prepared according to the following protocol. The PCB was cleaned using acetone rinse, followed by IPA rinse, followed by water rinse, followed by N\textsubscript{2} dry.

Shipley S1818 photoresist (Dow Chemical Corporation, Midland, MI) was spun for 10s at 500rpm (100rpm/s ramp), followed by 30s at 2500rpm (300rpm/s ramp). This gave the photoresist a thickness of \sim 2 microns. The PCB was then baked at 120° C for 2.5 min to polymerize S1818 and then exposed under a 365nm UV source. Clear areas on photomask allow UV to hit S1818, depolymerizing it. Average exposure energy was \sim 80\text{mJ/cm}^2 (~16s at 5mW/cm\textsuperscript{2} UV intensity). The PCB was then washed with 1:1 CD30 developer:water solution to remove depolymerized S1818. Wash time was \sim 1.5min.

The PCB was immersed with photoresist in 100% ferric chloride solution and etched until all copper was removed from areas not covered by photoresist. Etch time was approximately 25 minutes without heating. The PCB was rinsed with water and the
photoresist was removed with acetone and washed with IPA and water. The PCB was then dried with N₂.

The PCB was then coated with a thin layer of PDMS for improved binding to the microfluidic chip. First, PDMS monomer and PDMS curing agent were mixed 10:1 (volume:volume) and stirred for ~3min until milky white color. The mixture was placed in a vacuum dessicator, the vacuum was pulled, and allow to degas 1-2 hours. The PDMS was spin coated on photoresist for ~10s at 500rpm (100rpm/s ramp), followed by 1min at 6000rpm (300rpm/s ramp). This gave the PDMS a thickness of ~35 microns. The PCB was then heated to 60°C for 4 hours until hardened.

Microfluidic chips were made in PDMS on a silicon wafer mold. The silicon wafer was cleaned using acetone rinse, followed by IPA rinse, followed by water rinse, followed by N₂ dry.

SU8 2100 was spun for 10s at 500rpm (100 rpm/s ramp), followed by 30s at 3000rpm (300 rpm/s ramp). This produced a 100μm thick coating. The PDMS was baked for 5min at 65°C, 20min at 95°C. It was then exposed to 240mJ/cm² UV energy (48s exposure at 5mW/cm² UV intensity). It was then baked for 5min at 65°C, 10min at 95°C and developed in SU8 developer for 10min. It was then annealed at 150°C for 1 hour.

PDMS monomer (in liquid form) with PDMS curing agent were mixed 10:1 (volume:volume). A 4” diameter wafer and 10g of PDMS monomer resulted in a 1mm thick chip. The mixture was stirred ~3min until a milky white color and placed in a vacuum dessicator, and allowed to degas for ~1-2 hours. About 30min before degassing
was done, the SU8 mold was placed in a different dessicator with a small amount of trichloro(1,1,2,2-perfluorocytl)silane under vacuum. The degassed PDMS was poured onto the silanized wafer and heated to 60°C for 4 hours until hardened.

The PDMS was pulled off the SU8 mold and the resulting chips were cut to size (using a razor) inlet and outlet holes were punched. The channel side of the PDMS was cleaned with methanol and water, then dried with N2. The same was done to the PDMS side of the PCB, then dried with N2. The two pieces to be bonded (PDMS-coated PCB and PDMS) were placed into a reactive ion etching (RIE) plasma system. Vacuum was turned on and the surfaces were exposed to O2. The RF system was turned on, creating an oxygen plasma and exposing both pieces to oxygen plasma. The system was opened to atmosphere. The stripline and channels were aligned and bonded under slight pressure. The assembly was allowed to sit at ~60°C for several hours to ensure a good bond.

6.5. STRIPLINE EFFICIENCY AND SENSITIVITY COMPARED TO CONVENTIONAL SOLENOID

Here, we investigate the sensitivity and coil efficiency of the current stripline setup as compared to the conventional solenoid wrapped around a PEEK capillary. These results should suggest areas of potential improvement in both stripline design, as well as optimization of geometrical channel-stripline matching.
Figure 6.5.1: Relative sensitivity of solenoid and stripline setup after application of a 90° pulse. Both solenoid (left) and stripline (right) spectra are shown for the same scaling level. Because of a broad PDMS background signal in the stripline spectrum (~1.5 kHz), a short 3ms delay was introduced between pulse and acquisition. For accurate comparison, the same delay was introduced in the solenoid experiment.

In order to test coil sensitivity and efficiency, a simple pulse-acquire experiment following a 90° pulse calibration was conducted. As illustrated in Figure 6.5.1, the stripline signal results in a significant broad peak upfield (~1.5kHz) of the primary signal (0 kHz). This signal can be attributed to the PDMS, and consequently has a relatively short transverse relaxation time. In order to reduce the broad signal, we introduce a brief delay of 3ms between pulsing and acquisition in this experiment. For accurate comparison, the delay was also included to acquire the solenoid spectrum in Figure 6.1.4.

As a relative assessment of the sensitivity of the two coils we compare 90 degree pulse calibrations. At the same power, the solenoid had a 90 degree pulse width of 0.8 μs, whereas the stripline had a 90 degree pulse width of 3 μs. This difference is to be expected, as the stripline is a planar structure on one side of the channel. Each of the
spectra in Figure 6.5.1 demonstrates the signal after application of the respective 90 degree pulses. Furthermore, computing the relative SNR of the two scenarios results in a SNR difference of ~7.5x, with the solenoid having significantly superior signal.

This reduced sensitivity can be attributed to both a significant difference in filling factor, Q factor, as well as a small difference in volume. For the latter case, the stripline probe consists of a rectangular microfluidic channel that is 100 μm on each side. The solenoid contains a PEEK capillary with an internal diameter of 150μm, making the volume of water contained in the solenoid ~1.8x larger. Furthermore, computational simulations of both scenarios suggest a reduced filling factor in the stripline case using the current geometry. Both of these stripline limitations are to be expected from the first generation design. Potential improvements are highlighted later in this chapter.

6.6. RD-MRI WITH STRIPLINE DETECTOR

![Time of flight RD-MRI images of PDMS microfluidic chip with stripline detector. Each time of flight image is labeled as time after encoding. 8 time of flight points are shown from the total of 30 points in the travel curve. The images are 71x71 covering a FOV of approximately 11 mm in the vertical direction and 22 mm in the horizontal (along the length of the chip), corresponding to a spatial resolution of ~143μm x 286 μm.](image)

Figure 6.6.1: Time of flight RD-MRI images of PDMS microfluidic chip with stripline detector. Each time of flight image is labeled as time after encoding. 8 time of flight points are shown from the total of 30 points in the travel curve. The images are 71x71 covering a FOV of approximately 11 mm in the vertical direction and 22 mm in the horizontal (along the length of the chip), corresponding to a spatial resolution of ~143μm x 286 μm.
Imaging results using the stripline and PDMS microfluidic chip described above are shown in Figure 6.6.1. The most recent pulse sequence described in section 4.3 was used for these experiments. Each time of flight point is labeled as the time after encoding. The 71x71 images resulted in a pixel resolution of 143μmx286μm. There were 30 TOF points acquired in the travel curve, but only 8 representative points separated by 60ms are shown here. The total acquisition time for a single travel curve was 1.8 s. The encoded information arrives at the detection stripline quickly, appearing immediately in the 2\textsuperscript{nd} TOF point after encoding, corresponding to only a 70ms delay between encoding and detection. Importantly, the background signal from PDMS observed in the spectrum of the stripline coil is completely removed using the remote detection phase cycle previously implemented.

6.7. CONCLUSIONS

These preliminary results outline the design considerations and limitations to be pursued in the second generation stripline probe. While significantly lower than a conventional hand wound solenoid, we show sufficiently high SNR to demonstrate RD-MRI with the current setup.

Optimization of the stripline probe should first focus on improving the overall SNR and achieving levels as close as possible to those with the solenoid setup. A dramatic improvement is likely possible by increasing the filling factor of the stripline probe. This can easily be accomplished by reducing the separation of the wires that make up the stripline. Furthermore, matching the volume of the channel to the stripline geometry
will achieve sensitivities similar to those seen in the solenoid setup. Enhancements in channel design, as well as more complicated double sided geometries will result in even further improvements. Recent work with microfabricated RF probes describe the possibility of dramatically improved sensitivity for small volume samples [41-43]. These specific designs can be readily implemented in a remote detection setup with the current probe design. Importantly, these modifications and optimizations are significantly more difficult in the solenoid setup without major adjustments to the hardware and specialized flow modifications.

This design approach dramatically improves detector and chip modularity. The detection region can now be easily fabricated and optimized for each specific application and microfluidic chip. Furthermore, the stripline and channel dimensions can be more closely matched to yield improved SNR and optimized for any number of parameters, i.e. high temporal resolution, optimized flow rate and detection volume.
CHAPTER 7: APPLICATIONS TO FAST CHROMATOGRAPHIC SEPARATIONS

7.1. INTRODUCTION

This work is a first proof of concept to illustrate the utility of RD-NMR in analytical chemistry to monitor separations and assess the design of chromatographic columns. Developments in both direct and remote detection NMR for the characterization of polymer monoliths in capillary columns are presented. First, RD-MRI is used for imaging and velocimetry of the mobile phase. This illustrates both the advantages of remote detection experiments on monolithic columns as well as the extreme conditions under which microimaging can be performed. Direct in-line monitoring of small molecule separations is then carried out under comparable conditions to demonstrate the unique power of magnetic resonance and the advantages of a simultaneous dual coil platform in studying microscale chromatographic processes.

This work suggests the potential of developing a multidimensional technique of completely orthogonal and thereby complimentary analyses which identify the substituents of a complex mixture based on non-redundant physical properties. As a result, the potential for a combined methodology, where NMR is used as a detection scheme to monitor a separation as it occurs on the column, and perhaps to encode the velocities of separated molecules, is a logical progression of this work.
7.1.1. **Chromatography Basics**

![Schematic of Chromatography](image)

**Figure 7.1.1: Schematic outlining the components of a chromatographic separation.** A mobile phase solvent flows through a stationary bed of particles or solid porous material. Molecules are separated based on their interactions with the stationary phase flow off the column in distinct bands of chemical species in the eluate.

Chromatography is an indispensable tool used to both elucidate the constituents of a complex mixture as well as to remove unwanted impurities in samples. The list of potential fields and applications is limitless, including analytical chemistry, biochemistry, chemical synthesis, drug discovery, drug manufacturing, and industrial chemical manufacturing\[70-73\]. In this work, our experiments are specific to high pressure liquid chromatography (HPLC) for the separation of small molecules. Purifications, separations, and analyses are done by taking advantage of the association of the molecules with some substrate or phase. Two phases are employed: mobile and stationary. The mobile phase is a liquid which is pumped, at high pressures, through the stationary phase, which is most commonly a bed of particles or solid porous material. If this stationary phase happens to be hydrophobic with polar mobile phase, then the chromatography is considered reverse-phase (RP-HPLC). This is by far the most common form of chromatography and the discussion here is focused exclusively on RP-HPLC.
Consider a non-polar molecule, such as an aromatic hydrocarbon, dissolved in a hydrophilic mobile phase, such as a mixture of organic solvent with water, flowing through a stationary porous bed, as illustrated in Figure 7.1.1. In RP-HPLC, the hydrocarbon has some affinity for the stationary phase and will be adsorbed and thereby impeded by its interactions. These interactions are highly specific to the molecule and their composite effect, as the mobile phase makes its way through the stationary phase, is unique. Consequently, each of the components of a mixture of several different molecules, with varying polarity will exhibit unique bulk properties. As each of the molecules of a certain species is delayed by the mobile phase by different amounts of time, the composite effect is a separation of the sample into bands of chemical species. In equation form, the overall retention factor, \( k \), of a given molecule, \( m \), is given by

\[
k_m = \frac{n_{\text{stationary}}}{n_{\text{mobile}}}
\]  

(7.1)

Where \( n_{\text{stationary}} \) is the number of moles of \( m \) in the stationary phase and \( n_{\text{mobile}} \) is the number of moles in the mobile phase. This equation states that as the equilibrium concentration of a molecule in the stationary phase increases, the retention factor increases. Molecules of higher retention factor are expected to elute off the column over longer periods of time. Therefore, the experiment is simple, and injecting molecules of different polarities will result in their separation into distinct bands at the outlet of a chromatographic column. This scenario is illustrated below in Figure 7.1.2a. In this diagram, the spatial dimension is represented on the horizontal axis and the concentration is on the vertical
axis. However, this simple explanation is contrary to experimental reality, and just as dispersion causes a blurring of bands in a laminar flow, discussed in section 3.2.5, a similar mechanism of band broadening occurs in flow through porous media.

7.1.2. Band Broadening

![Diagram of band broadening](image)

**Figure 7.1.2**: Band broadening after a chromatographic separation and after injection. An injected plug and separation is represented as Gaussian functions with the spatial dimension represented on the horizontal axis and concentration along the vertical. In an ideal example, an injected plug is separated into its substituents with no broadening (a). Experimentally, the separated species are broadened on the column (b) and the injected plug is broadened prior to entering the column (c).

Before delving into the events that occur on a chromatographic column to a band of analyte, it’s important to consider what happens ahead of the column. An ideal injection of a complex mixture would come in the form of a narrow plug with uniform concentration that is somehow optimized in volume to the capacity of chromatographic
stationary phase. In this scenario, the mixture would arrive at the column as uniformly as possible, interact, and only be susceptible to the broadening on the column itself, as illustrated in Figure 7.1.2b. This ideal scenario optimizes the separation between species and maximizes the concentration of a given species within a plug. However, this is rarely the case. In microchromatographic applications, such as those discussed below, a plug of a complex mixture injected into the mobile phase is susceptible to Taylor dispersion, as discussed in section 3.2.5 prior to arriving at the column. As the distance traveled between the injection site and column increases, the dispersion increases. The injected uniform plug then arrives at the column as a blurred band with a Gaussian concentration distribution, as illustrated in Figure 7.1.2c. This reduces the quality of the separation and maximum concentration of each species by effectively spreading each of the separation bands at the outlet. In high field NMR-chromatography applications such as those discussed in this work, dispersion before the column is virtually unavoidable. The automated injector must be placed a safe distance from the high field magnet, thereby increasing the travel time. This effect can, however, be minimized with the use of a small interior diameter capillaries or by reducing the travel time from injection to the column. The latter can be accomplished with portable permanent magnet arrays or shielded high field magnets. It may also be possible to do a manual injection with a non-magnetic injector directly into the column in the high field magnet. Automating this process is only limited by commercially available components.

Band broadening within the column (Fig. 7.1.2b and Fig. 7.1.2c) was described originally by Van Deemter et al. [70]. The overall effect can be attributed to several underlying
mechanisms: multiple path flow, longitudinal diffusion, and resistance to mass transport in the stationary and mobile phase. The first effect involves the motion of molecules of a given species through the column. Any two molecules can take different paths through the column, and therefore some molecules will elute later than the same type of molecule which takes a different path and undergoes fewer interactions. A separation band is made up of molecules that have taken both all paths, and is broadened by the process. The second effect, longitudinal diffusion, is the natural diffusion process of a sample placed in the column under a stopped flow condition. Here, the sample will diffuse in all three dimensions. This effect is reduced with flow and scales inversely with velocity as it is related to the time in the column. Finally, the last cause of band broadening is based on the transport of the molecules from the mobile phase to the stationary phase. It’s important to note that the pores of the stationary phase particles are filled with stationary solvent. Molecules can either enter these pores and then diffuse back out into the mobile phase, or interact with the stationary phase. In both processes, the length of diffusion into the pores or into the stationary phase will vary and of course cause a variance in the amount of time that any given molecule will stay in the column. The effect on the bulk sample is band broadening caused by some combination of all the effects described above.

7.1.3. Detection Methods

UV visible spectroscopy is the most common method of monitoring chromatographic separations in real-time. A detector is placed at the outlet of the chromatographic
column to assess the composition of the eluate in order to distinguish compounds as they come off the column after separation. With this form of detection, the absorption of light is measured and a correlation can be made with the specific analyte. Furthermore, knowing the path-length through the detector, the concentration can be calculated based on absorbance. While this technique is sensitive and provides a high temporal resolution, it requires well-resolved analyte peaks and the use of chemical standards that correlate species as they elute from the column. UV visible spectroscopy is generally nonspecific, providing limited information about differences in species. Lastly, it’s limited to optically transparent medium and impractical for interrogating separation or flow within the chromatographic column.

Mass spectrometry can also be used for real-time detection, providing an additional method to confirm peak assignments. The chemical information provided through mass spectrometry is limited to the mass-to-charge ratio of the individual compounds, unless secondary processes such as fragmentation are utilized. While these secondary processes can yield great insight, they do not provide chemical details of the intact molecules in anon-charged state. In many applications, the output of the chromatographic column necessitates a non-invasive interrogation, limiting the utility of mass spectrometry. Furthermore, just like UV visible spectroscopy, mass spectrometry is incapable of any multidimensional imaging and limited to eluate detection.
7.1.4. NMR in Chromatography

One powerful alternative to these detection methods is NMR, which has been used for imaging [74-76] and spectroscopy of chromatographic columns under both stopped [77, 78] and continuous flow in-line detection modes [39, 79]. In a typical NMR-chromatography experiment, the NMR radio frequency detection coil is positioned at the outlet of a chromatographic column, allowing for continuous monitoring of the effluent. Any columns near the magnetic field must be free of magnetic components, making fused silica capillaries the preferred format. Previous implementations of this concept have been successfully demonstrated for in-line monitoring of microscale separations using particle packed columns [37, 38, 80]. Concurrently, magnetic resonance imaging (MRI) has also been used to image the interior of particle-based chromatographic columns in order to identify flow inefficiencies, to suggest improvements in column design, and to visualize separations as they occur on the columns [40, 74-76]. Although insightful, these studies remain limited due to the low sensitivity and poor temporal resolution of MRI. These restrictions preclude its application to the analysis of microscale flow, such as that exhibited in capillary liquid chromatography [81] or on-chip chromatography [82]. In order to analyze separations and flow properties within microscale chromatographic devices, changes need to be made to the detection scheme.

In previous MRI studies of chromatographic columns, the entire column is placed in a single, large radio frequency coil [74-76, 83, 84]. In this geometry, the fraction of the
detector coil volume that is filled by liquid molecules which give rise to the detectable NMR signal is exceedingly small. As the sensitivity of detection is directly proportional to the filling factor of the coil (the fraction of the coil volume occupied by the sample), a microsolenoid with diameter comparable to the inaccessible microporous features of the column will afford the highest sensitivity [39, 49]. In other words, one way to improve sensitivity would be to wind a microcoil around a specific region of interest inside of the column. While conceptually sound, this is clearly infeasible. As an alternative, remotely detected MRI could prove to be beneficial for chromatographic separations by providing simultaneous imaging with an improved filling factor and spectroscopic detection of the eluate.

Figure 7.1.3: Illustration of the remote detection experiment, as applied to a chromatographic column. The encoding coil, indicated on both sides of the column, encloses the entire volume of the monolith. The microsolenoid detector is placed at the outlet of the column, and provides a sensitivity increase due to the greatly enhanced filling factor for a given voxel (shown in yellow).

A schematic of the remote detection concept as it pertains to a chromatographic column is illustrated in Figure 7.1.3. Since the volume of the remote detection microcoil is
matched to the volume of the chromatographic features of interest, a sensitivity enhancement of several orders of magnitude may be achieved [32, 54]. Fourier transformation of these data will yield any encoded spectroscopic, image or velocity data, as well as a correlated time-of-flight parameter that reflects transport of fluid from the encoding region to the detector. At these scales, remote detection achieves an improvement in acquisition speed of up to 6 orders of magnitude over traditional MRI [32, 48]. These types of enhancements are beneficial for chromatographic applications that require detailed high resolution information about the column interior and flow features as well as small molecule separation detection with high sensitivity. Specifically, due to the low concentrations and poor filling factor involved, RD-NMR can dramatically improve the practicality of NMR for chromatography.

As a limitation, the longitudinal relaxation time (T1) of the encoded spins provides one of the fundamental restrictions to the remote detection experiment. For short T1 nuclei, such as those used in this study, significant signal loss is observed approximately 5 s after encoding. In order to prevent decay of the stored magnetization, relatively fast flow velocity must be used in order to quickly move the fluid from the encoding region to the detector. This specific limitation, while a factor in all RD-NMR experiments, is compounded in a chromatographic setting. Here, the relatively low flow rates, compared to previous studies using RD-NMR [33, 34, 47], as well as the low injection volumes result in generally limited signal from the analyte. In spite of this limitation, the previously described gains in spatial and temporal resolution make this technique very promising for the analysis of capillary chromatographic separations, provided that the
required flow rates can be achieved. Additionally, the remote detection setup is inherently equipped with two separate coils, making it an ideal platform for integrated imaging and in-line spectroscopy in continuously flowing systems. The coupling of remote detection techniques with new developments in fast microseparations, such as porous polymer monoliths, provides a powerful analytical method that can give further insight into both separation dynamics and characterization.

7.1.5. **Monolithic Chromatographic Columns**

![Figure 7.1.4: Cartoon illustrating the difference between a monolithic (top) and particle packed (bottom) chromatographic column. The monolith is made of a single piece of continuous polymer. It is synthesized in situ. The particle packed column is made up of individual spherical particles.](image)

Stationary phases in chromatographic columns are most commonly packed spherical particles that are either porous, superficially porous, or non-porous (Figure 7.1.4b). In these columns, the macroscopic space between particles provide a path for convective flow while microscopic pores within particles enable molecule scale interactions [85]. While each design has it’s unique advantages, particle packed columns generally contain voids which make the columns susceptible to mobile phase stagnation and mass transfer obstacles described above [85, 86]. They also require end frits to hold the particles in place, pose some difficulties with preparation, and have high back pressure. A potential
solution to these concerns would include a uniform stationary phase made up of one piece of porous material. Toward this end, monolithic chromatographic columns were developed in the early 1990s [87-89]. These types of columns are made up of continuous silica or organic polymers and are prepared in situ, making them ideal for microscale capillary applications with low volumes (Figure 7.1.4a). Due to their unique pore structure, traditional polymer monoliths display higher permeability than their particle-based counterparts, while still providing similar separation efficiencies for large molecules[90-92]. These characteristics can allow for faster flow rates and, thus, shorter analysis times than with particle columns. Due to their relative ease of preparation, cost effectiveness, and highly connected porous network that allows convective mass transport to dominate, use of polymer monoliths has grown steadily[93-95]. Despite these advantages, and the availability of polymer monoliths for nearly 20 years, a detailed study of the internal flow dynamics of polymer monoliths has not been reported and the columns have seen limited commercial adoption. One potential reason for this could be that, while polymer monoliths are excellent for the separations of large molecules, the rapid and efficient separations of small molecules have traditionally been a challenge.
Figure 7.1.5: Preparation of a hypercrosslinked polymer monolith. The preparation consists of a radical polymerization followed by hypercrosslinking. In the upper right, images of the internal microstructure of an organic polymer monolith.

Recently, \textit{in situ} hypercrosslinking has been incorporated in chromatographic monoliths by Urban \textit{et al.} resulting in an increase in surface area and more efficient chromatographic separations of small molecules in capillary columns\cite{95, 96}. The preparation consists of two steps. The first step is a radical polymerization using a vinylbenzene derivative such as styrene (Figure 7.1.5). This steps results in a generic monolith polymer for separating large molecules, described above. The second step, developed more recently for chromatographic monoliths\cite{96}, consists of a Friedel-Crafts alkylation which creates a hypercrosslinked polymer with reduced pore sizes and increased surface area that is optimized for small molecule separations.
Due to their ability to quickly and efficiently separate small analytes with low back pressure, hypercrosslinked poly(styrene-co-vinylbenzyl chloride-co-divinylbenzene) monolithic columns are suitable for hyphenated experiments such as combined chromatography RD-NMR. The reduced pressure accommodates faster flow rates on existing equipment, essential for reducing travel time from the encoding on the column to detection in the remote microcoil. Due to their high surface area, the monoliths are still capable of efficient separations at these flow rates. Lastly, easy in situ preparation makes it possible to adjust columns to specific applications and experimental setups by optimizing capillary diameter and column loading capacity.

7.2. EXPERIMENTAL METHODS

7.2.1. CHEMICALS AND MATERIALS

Styrene (99%), vinylbenzyl chloride (mixture of 3- and 4-isomers, 97%), divinylbenzene (80%, technical grade), 2,2-azobisisobutyronitrile (98%), acetonitrile (HPLC grade), water (HPLC grade), 1,2-dichloroethane, benzylalcohol, benzene, and butylbenzene were all obtained from Sigma-Aldrich (St. Louis, MO). The monomers (styrene, vinylbenzylchloride, and divinylbenzene) were purified by passage through a bed of basic alumina to remove the inhibitors. Ferric chloride was purchased from Fisher (New Jersey, NJ). Polyimide-coated 530 μm i.d. fused silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ). The commercial particle packed capillaries (Acclaim phenyl-1, 50 mm x 250 μm i.d., particles 3 μm, average pore size 120 Å) used for ruggedness studies were obtained from Dionex (Sunnyvale, CA).
7.2.2. **Preparation of Monolithic Columns**

The detailed procedure for the generic monolith preparation has been reported previously[95]. In brief, generic monoliths were prepared in capillaries using *in situ* polymerization of mixtures of 21% styrene, 7% vinylbenzyl chloride, and 12% divinylbenzene dissolved in binary porogen solvent containing 19% toluene and 41% 1-dodecanol. Azobisisobutyronitrile (1%, w/w, with respect to monomers) was used as the initiator. The polymerization mixtures were purged with nitrogen for 10 min and then filled into the vinylized capillaries. Both ends of the capillary were sealed with rubber stoppers and the capillary was placed in a water bath. Polymerization was carried out at 70 C for 20 h. Both ends of the capillary were then cut to adjust its length, and the monolithic column was washed with acetonitrile.

7.2.3. **Hypercrosslinking**

The monolithic columns were flushed with 1,2-dichloroethane at a flow rate of 0.25 μL/min for 2 h. A filtered solution of 1 g of FeCl₃ in 20 mL of 1,2-dichloroethane was pumped through the columns at a flow rate of 0.25 μL/min for 2 h. The hypercrosslinking reaction was then allowed to proceed at 90 C for 2 h. The modified columns were washed with water overnight and tested.

7.2.4. **Liquid Chromatography**

LC experiments were performed using an Agilent 1100 system (Agilent, Palo Alto, CA), equipped with a pump, autosampler, and injector. The monolithic capillary column was connected to the injector via an empty 250 cm x 50 μm i.d. connection capillary. The
monolith was attached in series with the NMR system described below. Typically, separations were performed in the isocratic reversed-phase mode, using a mixture of 90% acetonitrile (ACN) and 10% water as the mobile phase.

7.2.5. Remote Detection NMR Spectroscopy and MRI in Chromatographic Monoliths

NMR experiments were performed on a 7 T Oxford Instruments (Oxford Instruments, Oxfordshire, UK) superconducting magnet mated to a Varian console (Varian, Palo Alto, CA). The system is equipped with an SGRAD 88/55/HD/S combined shelf-shielded gradient for microimaging and 18 channel room temperature shim set. For RD-MRI experiments, the monolithic column was centered in a 40 mm Varian volume imaging probe, which served as the encoding coil. The outlet of the column was then connected to the remote microcoil detector by a 5 cm x 150 μm i.d. PEEK capillary (Idex Health & Science, Oak Harbor, WA). The microcoil detector was a 360 μmi.d. 12-turn copper solenoid encased in a cell filled with FC-43 Fluorinert (3M, Maplewood, MN) for susceptibility matching. The column and detection coil were positioned such that both were within the homogeneous region of the magnet. Shimming coils were employed to maximize the homogeneity in the encoding coil. During the microcoil acquisition, the encoding coil-optimized shim values were augmented with precalibrated linear gradients in order to optimize the sweet spot for the detection region. The same microsolenoid was used for both encoding and detection during the small molecule separation experiments.
7.2.6. Remote Detection MRI of Flow Through a Monolith

The mobile phase for these experiments consisted of 100% ACN pumped at a flow rate of 50 μL/min. Initial excitation consisted of a 5 kHz slice selective pulse, typically a sinc waveform, applied in the presence of a slice selection gradient chosen for region-selective encoding of the column. Following excitation into the transverse plane, phase encoding gradients were applied. The gradient amplitudes were taken as linear combinations of the laboratory frame gradients, such that the imaging plane was orthogonal to the direction of flow. The phase-encoding gradients were compensated for spin motion up to first order (velocity)[97]. The experiment incorporated a spin echo to remove any evolution due to static field inhomogeneities. A final π/2 pulse was applied to store the encoded information along the longitudinal axis, where phase evolution ceases and the effects of relaxation are greatly diminished. Since the mobile phase was continuously flowing, the encoded information was transported to the volume matched microsolenoid for detection before decay of the stored magnetization. Because the volume of the microsolenoid is smaller than that of the total encoded volume of the column, it can take up to 5 s for encoded fluid to flow through the detection coil. The time-of-flight dimension reflects the flow time between encoding and detection steps in a remotely detected experiment. It is important to note that this differs from the transit (or retention) time described in the directly detected experiments, which references the time between the initial injection and subsequent direct detection in the microsolenoid. The duration of each acquired free induction decay (FID) was correlated to the coil
residence time, which was \( \sim 80 \) ms. A total of 50 time-of-flight acquisitions were collected during the course of remote acquisition.

The entire encoding and detection sequence was repeated for each conjugate-space point of the image. In this case, we acquired 19 x 19 transverse images with a field of view of \( \sim 760 \) \( \mu \)m and a resolution of \( \sim 40 \) \( \mu \)m. A position- and acceleration-compensated velocity-encoding gradient was added after the refocusing pulse in order to perform high-resolution velocimetry experiments. Two experiments were performed for each phase encoding point, alternating between positive and negative velocity-encoding gradients to encode displacement in the phase of the spins. The complex phases for the velocity encodes were then subtracted to yield an absolute phase difference image, which was later converted to units of velocity. The strengths of these gradients were chosen to avoid phase wrapping.

For a given volumetric flow rate, the reduced void space in the monolith will lead to higher linear velocities than in the open capillary. Velocity calibrations were performed to match the linear velocities of the monolith and open capillary used for the imaging studies. First, the average linear velocity along the direction of flow was encoded. The encoded volume comprised fluid throughout the entire column, with a flow rate of 50 \( \mu \)L/min. This was done by taking an array of velocity-encoding gradient values and calculating the phase difference between the complementary positive and negative lobes (vide infra). For each gradient value, 50 time-of-flight FIDs were acquired and then inverted to give a spectrum. The phase of the corresponding spectrum resulted in plots
of the relative phase at each time-of-flight point. The slope of the linear region of the phase plot is indicative of the flow rate in the column. These plots were averaged across the linear region and over all of the time-of-flight points to give a characteristic phase at the chosen flow rate. Flow rate was varied through the capillary in order to match the average linear velocity with the value calculated for 50 μL/min flow through the column. A match in linear velocities was found between the 50 μL/min volumetric flow rate in the monolith and a 135 μL/min flow rate in the capillary.

7.2.7. REMOTE DETECTION IMAGE PROCESSING

RD-MRI acquisitions consisted of 19 x 19 phase-encoded points, each containing 50 stroboscopically acquired FIDs (80 ms each). These data were analyzed in Matlab (Mathworks; Natick, MA). The individual FIDs were first Fourier transformed, giving a frequency spectrum with peaks for water and acetonitrile. Complex summation was carried out across the ACN peak to yield a single k-space value for each phase encode. Two-dimensional Fourier transformation was then performed across the array of k-space values, resulting in 50 19 x 19 images, one for each time-of-flight point. Velocities were determined by taking the phase for each k-space point and subtracting the positive and negative velocity lobe acquisition. To convert from the measured phase difference into the corresponding velocity, one first expands the formula for accrued phase in a series of moments, which correspond to contributions from position, velocity, acceleration. This was discussed previously in Section 2.2.6
For a velocity-encoding gradient that is compensated for position and acceleration, the only relevant term is the second integral, and we can carry out the appropriate integration across our gradient waveform (which has three lobes: +G, 3/2G, and +1/2G) to arrive at the formula, which converts between phase difference and velocity. In the case of a three-lobe position- and acceleration compensated gradient with lobe amplitudes as described above, a total duration for all three lobes of $\tau$, and a brief delay between each lobe of R (not counted toward $\tau$), the formula is described above.

7.2.8. **NMR Spectroscopy of Small Molecule Separations**

![Diagram of remote detection LC-NMR setup](image)

**Figure 7.2.1:** Schematic of the remote detection LC-NMR setup. The pump and injector are connected to the column, inside the 7 T magnet, via a long capillary. Fluid flows through the column, which is enclosed in the encoding volume coil (not shown), and into the remote microsolenoid detector.

The separation experiments integrated the built-in HPLC injector into the remote detection flow setup, as shown in Figure 7.2.1. The separations were carried out at a flow rate of 35 μL/min. The 0.5 μL sample consisted of benzyl alcohol, benzene, and butylbenzene at a ratio of 1:1:7 (1.1:1.3:5.0 M). Upon injection, the spectrometer was triggered to acquire spectra every 250 ms after a preliminary 35 s delay to account for
travel through the 250 cm capillary from the injector to the column. Using the autosampler, this sequence was repeated 256 times to increase the signal-to-noise ratio, although an unambiguous view of the separation can be achieved in a single scan, as shown in Figure 7.2.1. An extended sequence of 60 injections was also conducted to examine the injection reproducibility.

7.3. RESULTS AND DISCUSSION

![Figure 7.3.1: Axial images illustrating intensity and velocity for acetonitrile flowing through the open capillary (top) and monolith (bottom). Five characteristic time-of-flight points were selected and the corresponding images are ordered by their travel time. Conditions: columns 90 mm x 530 µm I.D.; mobile phase 100% ACN, capillary flow rate 135 µL/min, monolith flow rate 50 µL/min.](image-url)
Although NMR studies of mobile phase flow dynamics in particle-based columns have been demonstrated [74, 75, 83] there are no comparable studies of fluid flow within monolithic columns. While both of these column formats enable chromatographic separations, they possess fundamentally different pore structures and may not produce the same flow profile.

A series of two-dimensional remotely detected images in an empty 530 μm i.d. capillary and an organic polymer monolith is shown in Figure 7.3.1. These images, which are axial with respect to the direction of flow, show both intensity and velocimetry data. Five of the 50 acquired time-of-flight points are shown, labeled by the time from storage pulse to arrival in the microsolenoid (increasing from left to right). Fluid that is encoded closest to the outlet of the column arrives at the microsolenoid detector first and is therefore detected in earlier times of flight. The progression of images gives a view of the flow profile and dispersion within each system and therefore insight into the overall flow behavior. The hypercrosslinked monoliths have a reduced interstitial volume compared to the open capillary. As a result, the linear flow velocity within the monolithic column is higher than for the empty capillary at the same volumetric flow rate. To provide an equivalent comparison, the volumetric flow rate of the open capillary was increased in order to match the linear velocity to the flow inside of the monolith, as described in the Experimental Section. As would be expected, the first encoded packet of fluid takes longer to arrive in the monolith experiment (1.30 s for the open capillary and 1.90 s for the monolith) since the flow is slower in the capillary connecting the column to the detection region. The time for the entire encoded region to
travel through the microsolenoid detector is $\sim 2.25$ s in both cases. Because the flow of the mobile phase is non-turbulent, the early time-of-flight points can be attributed to the liquid, which was closest to the detector at the time of encoding and which therefore arrived at the detector first. This is quantified in the velocity images, which show that fluid in earlier time-of-flight points has higher linear velocities in both the monolith and capillary. The average linear velocities decrease along the time-of-flight dimension from $\sim 4$ cm/s in early time-of-flight points down to $\sim 2$ cm/s for later points.

The most significant differences between the two experiments are seen in the flow profile. As expected, a parabolic flow profile is observed in the open capillary, with fluid in the center of the capillary appearing earlier and with higher velocity than fluid near the boundaries. This behavior is characteristic of laminar flow and a no-slip boundary condition. The observed profile is substantially different in the monolith, which has a nearly plug-like profile across the entire radial dimension of the column. These differences illustrate that the unique monolithic pore structure serves to disrupt flow and evenly distribute the mobile phase across the column. It has been demonstrated that the tortuous morphology in monolithic columns can negatively impact the radial distribution of mobile phase across the column[98]. However, it is clearly shown that the monolith instead acts as a continuous natural frit, distributing the mobile phase evenly.
Figure 7.3.2: Histograms of the velocity data for both capillary and organic polymer monolith. Summed and individual time-of-flight histograms are shown, corresponding to the same data and conditions found in Figure 7.3.1.

Histograms of the velocity along the direction of flow (Figure 7.3.2) can provide quantitative insight into the flow behavior. The histograms are shown for the same time-of-flight points as in Figure 7.3.1, as well as for the sum of all time-of-flight points. The individual time-of-flight histograms are scaled to their respective summed histogram, which has its largest bin set to a value of 1. The summed histogram for the empty capillary data shows a clear peak and a significant number of pixels with velocities far from the mean. The additional shoulder seen in this distribution is representative of the large quantity of rapidly moving fluid at the center of the capillary, which retains a noticeably higher velocity than the fluid at the edges (Figure 7.3.1).

Significantly, the range of velocities observed for the empty capillary is much wider than with the monolithic column, which exhibits a relatively flat distribution of velocities that rapidly drops off on both sides of the histogram. This is consistent with uniform flow across the monolithic column and is unlike the flow within an open capillary, where
wall interactions lead to significant drag and a wider velocity distribution. Interestingly, the open capillary shows a maximum velocity that is approximately twice as large as the average, a feature that is characteristic of laminar flow. The individual time-of-flight points show far narrower distributions (and, thus, more uniform velocities) in the monolith, further reinforcing these conclusions. These results are consistent with a relatively uniform flow distribution within the monolith, promoted by homogeneous interactions with the high surface area of the nanoporous material. The enhanced surface area and connectivity within hypercrosslinked organic monoliths are the primary characteristics that lead to the plug-like flow. These same features accommodate fast, efficient separations of small molecules at relatively high concentrations.

Having successfully demonstrated the application of RD-MRI to monolithic columns, investigations into achieving meaningful separations under these unusual flow conditions were performed. As mentioned previously, a rapid flow rate is required to transport the material from the encoding region to the detection coil before the encoded information is lost to relaxation. These conditions run contrary to what is required to produce efficient chromatographic separations, as dictated by the van Deemter curve. Thus, the separation of small molecules using a polymer monolith at high flow rates is a significant accomplishment.

Part of the challenge in monitoring a chromatographic separation with NMR comes from the experimental setup. Most notably, magnetic components of the HPLC must be
placed at a distance from the superconducting magnet, as they can cause inhomogeneities in the magnetic field and lead to potential physical hazards. As a result, an extended length of empty capillary is required between the HPLC hardware and column, which adds undesirable dispersion to the injection. Additionally, our home built microsolenoid detector is not fully optimized for sensitivity and requires signal averaging and relatively large injections to achieve adequate signal-to-noise ratio. However, injecting a large volume or a concentrated sample may lead to overloading of the stationary phase.

Figure 7.3.3: Two-dimensional plot illustrating the separation of benzyl alcohol, benzene, and butylbenzene using a hypercrosslinked monolithic chromatography column. The horizontal axis corresponds to the NMR chemical shift, while the vertical axis represents the transit time of compounds undergoing chromatographic separation determined by the time until detection in the microsolenoid. The data are shown using 1, 16, and 256 signal averages. Conditions: column, 90 mm x 530 μm i.d.; mobile phase, 80:20% ACN and water;
flow rate, 40 μL/min; back pressure, 245 bar; sample, benzyl alcohol, benzene, and butylbenzene (1:1:7, 1.1:1.3:5.0 M); injection volume, 0.5 μL.

In spite of these challenges, a fully resolved separation of benzyl alcohol, benzene, and butylbenzene is achieved using a 0.5 μL injection at a high flow rate. A two-dimensional plot showing the aromatic region of the detected NMR spectrum versus the time until detection (indicative of the elution time) is shown in Figure 7.3.3. The chemical shift is shown along the horizontal axis and transit time (retention time) along the vertical axis. While signal is observable from these compounds after a single acquisition, the data shown here are averaged over 16 repeated experiments. By using NMR detection, exact assignment of the eluting compounds can be made based on the chemical shifts of the protons. This is particularly advantageous in situations where multiple components fully or partially coelute, causing overlap in the chromatographic dimension that may still retain distinguishable peaks in the spectral axis. Combining NMR detection with chromatography allows users to observe the identity and quantity of each component as it elutes and is a powerful tool when optimizing conditions for fast, efficient separations. A clear separation is achieved using the model mixture discussed here, where the doublet with the largest upfield shift is characteristic of the butylbenzene protons and the two earlier peaks belong to the benzene and benzyl alcohol protons. Further characterization of the mixture is provided along the transit time dimension, which complements the chemical shift information and allows for optimal identification of compounds.
Figure 7.3.4: Two dimensional plot illustrating reproducibility of separations on monolithic columns with 60 repeated injections of a benzyl alcohol and benzene mixture.

In addition to their separation capabilities under far from optimal flow conditions, the monolithic columns were found to be very rugged. A series of 60 sequential sample injections (∼300 column volumes) showed less than 2.1% RSD (relative standard deviation) in the travel time from injector to detection coil (Figure 7.3.4). This is particularly notable in that the monoliths were subjected to flow rates of greater than 20 µL/min and pressures in excess of 240 bar for several weeks of continuous use. For comparison, two 50 mm x 250 µm i.d. commercial columns packed with 3 µm silica C18 particles were subjected to the same flow and pressure conditions applied to monoliths
of comparable dimensions. After only 3 days of use, the particle packed columns showed visible voids at the top, while the monoliths showed no physical signs of degradation. The flexibility and low cost of preparing monoliths in nonmagnetic capillary formats far exceed that of comparable particle-based systems, making them very advantageous for hyphenated NMR techniques.

7.4. CONCLUSIONS

Using LC-NMR, a wealth of information can be attained for the characterization of both separations and flow. The additional advantages of remote detection allow information to be encoded within fluids while they are still on the column and provide a tremendous sensitivity enhancement when detecting small volumes of flowing liquid. The requirements of the remote detection are well-suited to the study of hypercrosslinked organic polymer monoliths, which allow for fast flow rates and efficient separations of small molecules. These monoliths are shown to be robust media for the rapid separation of small molecules. Further, in separating the polarization, encoding, and detection steps of an NMR experiment, remote detection enables truly portable LC-NMR instrumentation. Our ongoing work, focusing on the use of portable NMR spectrometers with tailored permanent magnet arrays [99, 100] will obviate the principal restrictions of size and expense.
CHAPTER 8: IMPLANTABLE COILS FOR IN VIVO VASCULAR SPECTROSCOPY AND IMAGING

8.1. INTRODUCTION

8.1.1. EXPERIMENTAL MOTIVATION

One in vivo manifestation of a sample with poor filling factor is the brain. The MRI RF coil is designed to contain the entire brain volume while the region of interest may be confined to a single voxel within this volume. The SNR limitation created by this geometrical restriction results in an inability to directly image or characterize the neuronal firings that cause global and regional brain functions. Specifically, it may require the synchronized firing of large number of neurons to be able to detect any induced current in an MRI experiment. Teasing apart this microscale activity, as well as the metabolic function, and flow information based on the bulk signal received in the RF coil is indeed a difficult task. These events are further confounded by the cerebral vasculature, which is dense and highly branched.

The cerebrovasculature is a complex network of vessels that perfuse the tissue and satisfy the high metabolic requirements of functioning neurons. Much remains unknown about this underlying intricate physiology. Analytical schemes for measuring perfusion, flow, connectivity, and function have rendered significant gaps in our ability to directly correlate experimental results and physiological reality. A clear example of this is the
blood oxygen dependent (BOLD) signal, where the interconnected hemodynamic and neural response is still poorly understood after much study [101-104].

Two mechanisms by which to isolate variables and characterize parameters in brain physiology experiments is to regionally define the area of interest with hardware and to create metabolic specific contrast with the use of tracers. Of particular relevance is the contrast mechanism enabled by hyperpolarized $^{13}$C compounds. This MRI method has drawn much interest recently due to the high achievable signal, long T1 relaxation times under physiological conditions, and adaptability to a wide array of compounds and metabolites. Furthermore, injecting any tracer, including a $^{13}$C labeled compound increases the signal to noise ratio and provides improved contrast from the region or analyte of interest.

Building upon prior remote detection and cerebral hyperpolarized 13C studies lays the groundwork for future RD-MRI in live animals with the development of implantable radiofrequency coils. These coils are used to collect measure signals in rat vasculature at the jugular vein and common carotid artery. Furthermore, the coils are used for phantom RD-MRI experiments using hyperpolarized 13C. The primary contributions of this work include a first attempt at 13CRD-MRI, the development of a robust, highly reproducible fabrication and design of an implantable RF coil, and a platform for measurement of MRI parameters in blood vessels (such as T1 and metabolite flux).
8.1.2. **Brief Overview of Brain Vasculature**

The rat is a natural experimental model for studying microscopic flow in live animals with remote detection. The brain perfusion parameters, fast travel times, well established surgical and hyperpolarization protocols match nicely to the remote detection techniques described above. Specifically, the relevant blood vessel diameters for placement of a remote detection coil are amenable to surgical implantation with existing protocols. The small travel distance from encoding in the brain to detection in the remote coil, as well as the established injection and distribution of hyperpolarized tracers with long relaxation times overcomes the T1 restriction inherent in the remote detection technique. Several excellent references contain details about the rat [105] and human [106, 107] nervous system physiology and anatomy. This section is only a brief overview of the vasculature, necessary for the experiments described below.

The brain’s arterial supply is provided by the common carotid and vertebral arteries. The common carotid arteries originate at the arch of the aorta (left) and the brachiocephalic artery (right). They then bifurcate into the internal and external carotid arteries. The vertebral arteries originate from the subclavian arteries which also originate at the aortic arch.
The experiments described in this chapter are largely motivated by previous work in the Pines’ lab with oscillating electrical currents [108]. That work sought to develop a technique that could be used to image brain activation directly. This was accomplished in vitro by detecting oscillating electrical currents similar to those seen in brain activations using NMR methods based on a resonant mechanism with applied RF pulses. The remote detection experiment illustrated in Figure 8.1.1 was an experimental phantom which crudely mimics the in vivo condition in the brain. Here, a microcapillary
phantom with flow through an applied electrical field (Figure 8.1.1a) resulted in distinct contrast (Figure 8.1.1c and Figure 8.1.1d) in the region of the applied field. The conventional remote detection pulse sequence was used with an added resonant spin lock pulse before the phase encoding gradients. When the strength of the spin lock pulse matched the frequency of the oscillating electrical currents, the spin magnetization had a detectable difference in phase from the non-resonant condition. Remote detection was used to illustrate the utility of using flow to achieve a significant SNR improvement over a bulk measurement in a volume coil. A natural extension of these results is an assessment of the in vivo applicability of remote detection. Then potentially to build on this in order to zoom into specific functional areas with the improved sensitivity offered by remote detection for imaging induced magnetic fields from oscillating currents in neurons.

8.1.4. IMPLANTABLE RF COILS

Here, we briefly discuss work at the University of Minnesota that formed the foundation for the design of the implantable coils described herein [109]. In this study by Zhang et al., changes in $^{17}$O labeled water signal was used to determine the arterial input function using region-defined (REDE) implanted vascular RF coils. These coils were of comparable size to the coils used in this study, at ~1mm in diameter and 11mm in length. The coils were placed around the rat carotid artery and only detected signals on the interior of the artery.
These previous studies outlined the design objectives of the coils used here. The primary physiological obstacle to overcome was to develop a coil that reduced the likelihood of venous collapse. This is possible by fabricating coils of a constant, reproducible diameter. Printed pre-fabricated coils allow for design customization specific to the surgery at hand. Furthermore, minor variations in coil geometry or design features do not require major alteration of the coil fabrication protocol or surgical procedure.

8.1.5. Hyperpolarized C-13 Tracers As Metabolic Tracers

We utilize hyperpolarized C-13 as a tracer to create contrast and amplify signal from injections in live animals. The techniques and theory of hyperpolarization have been discussed in detail previously [110-121]. In these studies, the hyperpolarization of various nuclei, i.e. 3He, 13C, 129Xe, has improved the sensitivity of in vitro molecular imaging, and in vivo animal and human studies showing high resolution anatomical details, cancer metabolism, and cerebral perfusion among other applications.

The most common substrate used in recent years has been [1-13C]pyruvate, where researchers have monitored its conversion to [1-13C]lactate, [1-13C]alanine, and [1-13C]bicarbonate in vivo. One reason for this wide adoption in cancer is the ubiquitous Warburg Effect, which states that the dominant energy producing pathway in cancer is glycolysis. Cancer cells both uptake higher amounts of glucose and produce excessive lactate irrespective of tissue oxygenation. Therefore, the local tumor environment has a higher concentration of lactate and can be localized with appropriate tracers, such as [1-13C]pyruvate. More accurately, the lactate to pyruvate ratio has been instructive in
locating regions of altered metabolism and subsequently tumor identification [114, 122, 123].

A second and particularly thorough approach involves the monitoring of lactate and pyruvate levels as inputs to kinetic models [117, 124]. These previous attempts focus on modeling [1-13C]pyruvate dynamics in cell culture bioreactors that mimic physiological function in the hopes of elucidating in vivo mechanisms. To date, little work has investigated in vivo metabolic kinetics because of the complexity of the parameters involved, including concentration and polarization levels.

One particular parameter that is relevant to both remote detection and all in vivo metabolism studies with [1-13C]pyruvate is the longitudinal relaxation time, T1. In a hyperpolarized environment, the T1 characterizes the signal decay, or return to equilibrium magnetization from the hyperpolarized state. Accurate in vivo measurements of hyperpolarized [1-13C]pyruvate, beyond simple studies in blood samples, are essential for understanding the underlying substrate kinetics and informing kinetic models.
8.2. **Experimental Methods**

8.2.1. **Overview of Experimental Setup**

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*Figure 8.2.1: Implantable coil experimental setup overview. The hyperpolarized sample was injected into a syringe pump that pumped into an injector, that was placed near the animal inside the magnet. The implanted coil is shown on the common carotid artery. The rat was placed on an animal holder, placed on a ventilator, and blood gases were monitored.*

The implantable radiofrequency coils were designed and assembled in the Pines Lab at Berkeley. Preliminary tests were conducted to assess experimental feasibility on water and $^{13}$C enriched samples. All remote $^{13}$C phantom and animal experiments were conducted at the University of Minnesota, Twin Cities in the Center for Magnetic Resonance Research under the guidance of Professor Malgorzata Marjanska. *In vitro*
blood T1 experiments were conducted by Dr. Marjanska and are included here for comparison.

For *in vivo* animal experiments, the experimental setup consisted primarily of 4 categories of instrumentation: hyperpolarization, injection, physiology, and detection. These components are illustrated in Figure 8.2.1. The radiofrequency coil was implanted and the rat was placed on a home-built holder. The hyperpolarized substrate was transferred via a syringe pump located close to the hyperpolarizer into an injector, located on the animal holder close to the rat in the magnet. The injector then administered the hyperpolarized substrate and was detected using the implanted radiofrequency coil. The rat was placed on a ventilator and physiology was monitored throughout the experiments.
8.2.2. Probe Design and Fabrication

Figure 8.2.2: Implantable RF coils. A schematic of a single coil is shown (a). Here, the top layer is outlined in black and the bottom layer is outlined in yellow. Thru-holes are shown in red. An image of the actual printed sheet of copper containing many RF coils of varying dimensions designed in a saddle configuration is also shown (b). The coils were printed on two sides with inductor on one side and thru-holes leading to coil leads on the back side. Also included on the back side was a passive copper shield. The coils feature large through holes around the periphery for sutures. Also shown is a fully assembled coil with twisted pair leads, epoxy for support, (c). The twisted pair was bent to fit around a blood vessel and then extend out of the tissue and connect to the resonant circuit (not shown).

Implantable coils were fabricated on a flexible printed circuit board (PCB) using a proprietary low loss tangent substrate (CuFlon from Polyflon Co, Norwalk, CT). A CuFlon sheet is made up of a double sided layer of plated copper separated by a thin
layer of polytetrafluoroethylene (PTFE) (Figure 8.2.2b). 159 coils of varying dimensions were printed on a 9 in. x 9 in. single sheet of CuFlon. Each individual coil spanned the front and back layers of the sheet. Layers were electrically connected by plated thru-holes. Fabrication began with a ½ oz. (.7 mils) sheet of CuFlon onto which the patterns were printed and thru-holes were drilled. The final sheet consisted of 1.5 oz. copper (2.1 mils) with 1 oz. in the thru-holes. All copper traces were finished with an immersion tin to help in soldering and to reduce board oxidation.

The coils were designed in AUTOCAD (Autodesk, Inc., San Rafael, CA). Figure 8.2.2 illustrates a schematic of an individual coil (a), a sheet of printed coils (b) and a fully assembled coil (c) with leads that connect to a resonant circuit with capacitors (not shown). The coil design that best matched the rat anatomy and proved to be optimized for the surgical procedure had overall dimensions of 0.4 in. in length and ranged in width from 0.45 in. to 0.58 in. This includes the entire body of the coil including shielding. The actual geometry of the printed coil trace on the top layer was 0.23 in. in length and 0.17 in. in width. Looking more closely at Figure 8.2.2a, the layers are color-coded as follows: the front layer in black, the back layer in yellow, and thru-holes in red. The front layer featured a printed saddle coil with a supporting horizontal strap across the leads. Unplated thru-holes around the periphery of each individual coil (8.2.2a and 8.2.2b) were used to thread sutures during implantation to hold the coil in place on the blood vessels and maintain a cylindrical configuration during experiments. Plated thru-holes shown on the printed coil traces (8.2.2a), allowed for electrical contact between the layers, and led to the back side of the coil that contained the printed leads with contact
pads. The fabricated coil can be easily bent due to the flexibility of the material. After bending the coil around a cylindrical support, the printed contact pads on the back layer were then soldered to a twisted pair of braided lead wire (8.2.2c). The twisted pair connected the coil to a resonant circuit. In addition to the leads and connector pads, the back layer also consisted of a passive shield of copper that covered the entire back side of the coil outside of the printed leads. The outline of the back layer between the copper shield and PTFE is shown as a rectangular shape in yellow in Figure 8.2.2a. The back of the leads and connector pads on the back layer are also visible in the picture of the printed sheet (8.4b) because the thin layer of PTFE is transparent. The leads on this back layer were supported by both the thin layer of PTFE, as well as the horizontal strap on the top layer. For added support, the soldered interface between the printed connector pads and twisted pair was coated with epoxy. The epoxy covered the back layer of exposed PTFE and was supported by the shield (also on the back layer).

8.2.3. 13C TRACER HYPERPOLARIZATION

For remote detection phantom experiments, aliquots (~150 μL) of the mixture of 283.9 mg of urea (Isotec, Miamisburg, OH) dissolved in 60% glycerol (0.4626 g): 40% H2O (0.2353 g) mixture with 15 mMTris[8-carboxyl-2,2,6,6-tetra(1-hydroxyethyl)]-benzo[1,2-d:4,5-d0]bis(dithiole-2-yl)methyl] sodium salt (OX63 trityl radical) were placed into liquid helium and hyperpolarized by dynamic nuclear polarization (DNP) (HyperSense, Oxford Instruments, UK) in a field strength of 3.35 T at approximately 1.4 K for 4 h (time constant ~4300 s). 13C-urea samples were dissolved with D2O.
Separately, for in vivo T1 experiments, aliquots (~10 μL) of pure [1-13C]pyruvic acid (Isotec, Miamisburg, OH) and 15 mMTris[8-carboxyl-2,2,6,6-tetra(2-(1-hydroxyethyl))-benzo[1,2-d:4,5-d0]bis(dithiole-2-yl)methyl] sodium salt (OX63 trityl radical) were placed into liquid helium and hyperpolarized by DNP in a field strength of 3.35 T at approximately 1.4K for 90 min (τ~700 s).[1-13C]pyruvate samples were then dissolved in 0.32 mM Na2EDTA solution, 40 mM TRIS buffer, 40 mMNaOH and 0.32 mMNa2EDTA solution (buffered solution) to produce 4 mL of hyperpolarized solutions at a concentration of ~35 mM and a pH of 7.

8.2.4. HYPERPOLARIZED13C PHANTOM EXPERIMENTS

Phantom experiments using hyperpolarized 13C and the implantable coils were conducted in order to assess injection timing, rate, coil sensitivity, and flow stability during injection. [1-13C] urea was hyperpolarized according to the protocol described above. 116.7 mg of urea were injection into the flow phantom that consisted of a looped region of 1/16” capillary placed in the field of a homebuilt surface coil designed for brain imaging in rats. The encoding region consisted of approximately 25 cm of tubing. The distance from the end of the injection region to the detection region was approximately 8 cm. For detection, the implantable coils described above were sutured around the 1/16” tubing.

The pulse sequence consisted of a single adiabatic inversion encoding pulse (invpat.25) with a pulse width of 2 ms and a power of 45dB. Immediately after encoding, continuous detection pulses were applied for 4 seconds. During the same injection, a
control experiment was conducted with identical detection pulses but with the encoding pulse turned off.

8.2.5. **Animal Preparation and Coil Implantation**

![Diagram of Animal Holder](image)

**Figure 8.2.3: Animal holder for in vivo MRI experiments.** The animal is placed pronate on the curved surface. The holder is illustrated in a three-dimensional view (a) and a front view (b). A tube with flowing warm water was positioned on top of the animal and supported by the heating tube holder holes for maintaining temperature. The leads for the temperature probe were threaded through the temperature probe holder. The leads leading from the jugular coil were placed through the indicated hole and soldered to the resonant circuit board below the holder.

Animal experiments were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Minnesota. Male Sprague-Dawley rats with a weight range of 260-320g (Charles River Laboratories, Inc.) were placed in an induction chamber ventilated with 5% isoflurane. Once unconscious, rats were quickly removed and xylocaine was applied in the throat using a cotton swab, and a 14-gauge catheter was carefully inserted between the vocal cords. The catheter was then connected to the
ventilator providing a 70%:30% N₂O₂ mixture and 1.8% isoflurane. The rat was placed supine and the common carotid artery (CCA) was exposed with a midline neck incision. After the CCA was dissected free from surrounding nerves and fascia, the radiofrequency coil was placed around CCA and secured by suture to a natural position. Femoral vein and both arteries were cannulated for infusion of hyperpolarized substances, blood pressure monitoring, and blood sampling, respectively. Previous studies at the University of Minnesota have shown that the femoral vein cannulation resulted in reduced line obstruction over caudal vein cannulation. The femoral vein also allowed rapid injection, improving the signal observed from the hyperpolarized substance. Once the surgery was complete, the incisions were closed and the animals were placed in a home-built animal holder (8.2.3), and the head position was fixed using ear rods and a bite-bar. The implanted neck coil was then connected to the resonant circuit mounted on the underside of the animal holder. Body temperature was maintained at 37 °C using a heating pad with warm water circulation. Blood gases were measured every 20 min to ensure stable physiological conditions. Cardiac pulsation and blood pressure were monitored constantly throughout the experiment.

The hyperpolarized solution, i.e.[1-13C] pyruvate (2.3 mL, 34.5 mM) were injected intravenously using the separator/infusion pump into the anesthetized rat. Injection started~11 s after dissolution and lasted for 6 s.
8.2.6. T\textsubscript{1} MEASUREMENT IN \textit{IN VITRO} BLOOD SAMPLES

The data used to obtain T\textsubscript{1} values for [1-\textsuperscript{13}C] pyruvate in blood samples at 9.4 T were acquired using an 18mm outer diameter spherical glass bulb (Wilmad-Labglass, Buena, NJ) into which 1.5 mL of blood mixed with 0.3 mL of hyperpolarized solution was injected. Data were acquired using the radiofrequency coil assembly consisting of an inner \textsuperscript{13}C linearly polarized surface coil (12 mm diameter) and a \textsuperscript{1}H quadrature surface coil (two loops of 14mm diameter) built according to previously described design\textsuperscript{[125]} and a small flip angle pulse-acquire (4.5° at the coil center, T\textsubscript{R}=1.5 s, 160 scans) with \textsuperscript{1}H WALTZ-16 decoupling.

8.2.7. HYPERPOLARIZED \textsuperscript{13}C ANIMAL EXPERIMENTS

Animals were prepared according to the protocol described above. For these studies we investigated the T\textsubscript{1} of [1-\textsuperscript{13}C] pyruvate in arterial blood. All \textsuperscript{13}C data were acquired on a 9.4T, 31 cm horizontal bore magnet (Magnex Scientific, Oxford, United Kingdom) interfaced with a Varian Digital Drive console (Varian, Palo Alto, CA, USA). The magnet was equipped with a gradient insert capable of reaching 450 mT/m in 200 μs (Resonance Research, Inc., Billerica, MA). All spectra were acquired with 32,000 complex points and a spectral width of 50 kHz. Animal positioning was checked with the \textsuperscript{1}H quadrature surface coil placed on the head and gradient echo images (T\textsubscript{R}=60ms; T\textsubscript{E}=3.9ms; matrix=256x128; slice thickness=2 mm). Spectra were acquired using the implantable radiofrequency coil and a pulse-acquire sequence (T\textsubscript{R}=600ms, 160 scans).
8.2.8. **Data Analysis**

Remote detection phantom experiments were processed with the technique described above, yielding a single travel curve. For *in vivo* T1 relaxation analysis, data were processed in MATLAB using the curve fitting tool. Data were first apodized with a in the time domain with an exponential and then each spectrum was Fourier Transformed. The real spectrum peak was integrated and the points before maximum signal were removed. The resulting points were fit to a mono-exponential decay function. *In vitro* data were processed in Origin 8.6 (Origin Lab Corporation, Northampton, MA).

8.3. **Results**

8.3.1. **Remote Detection Phantom Study: Hyperpolarized $^{13}$C Urea Travel Curve with Automated Injection**

![Image of travel curves](image)

*Figure 8.3.1: Hyperpolarized $^{13}$C urea travel curve in phantom. For the same injection, a travel curve with (a) and without (b) an encoding pulse. The same implantable coil designed for live animals was used for detection and a homemade rat surface coil was used for encoding.*
Hyperpolarized $^{13}$C urea was used to obtain a travel curve in a 1/16” capillary phantom. These experiments verified injection, timing, and coil sensitivity as a pre-requisite to the in vivo experiments. Since the pulse sequence lacked a storage pulse and phase cycle as in previously studies, the single inversion storage pulse simply caused a decay of the signal. Figure 8.3.1 illustrates the results with (a) and without (b) an inversion pulse. The remote coil, which was the same design as used for the in vivo experiments, was continuously pulsing. A clearly detected travel curve is visible in Figure 8.3.1a. The time on the horizontal axis is the time after encoding. Signal begins to decay approximately 0.5 s after encoding. The travel curve persists for approximately 3 seconds total. The signal decay in Figure 8.3.1b is indicative of signal loss. This spectrum was acquired during the same injection as Figure 8.3.1a but was the second of the series.

8.3.2. Hyperpolarized [1-$^{13}$C]Pyruvate: Arterial T1 Measurements

Hyperpolarized [1-$^{13}$C] pyruvate experiments were conducted to assess metabolite dynamics in vivo and characterize the constraints of conducting remote detection experiments.
Spectra obtained in vivo with the implantable coil placed around the CCA. The top spectrum is the signal obtained 22.8 s after the injection began. The bottom spectrum shows the signal after 7.8 s. [1-13C] pyruvate and [1-13C] pyruvate hydrate is visible earlier in the acquisition. [1-13C] lactate appears at the later time point. Repetition time (TR)=0.6 s, pulse width (pw)=120 μs, line-broadening = 10 Hz.

Spectra characteristic of the signal seen in the implantable coil are shown in Figure 8.3.2. The spectra obtained showed substantially high SNR to detect signal from [1-13C]pyruvate (172 ppm) and [1-13C]pyruvate hydrate (181 ppm) early in the acquisition. These signals are clearly visible approximately 7.8 seconds after the acquisition began. [1-13C]lactate (185 ppm) begins to appear later in the acquisition (22.8 s) after the animal metabolized the injection. The [1-13C]pyruvate signal amplitude is significantly reduced at 22.8 s.
Figure 8.3.3: T\textsubscript{1} measurements with implantable coil placed on common carotid artery over a 55 second time course. 3 data sets from the same animal are shown after 3 separate injections of 13 mg of hyperpolarized [1-\textsuperscript{13}C] pyruvate (pyr). The [1-\textsuperscript{13}C]pyruvate data are shown as green diamonds and fit with a blue line representative of a monoexponential. [1-\textsuperscript{13}C] pyruvate hydrate (pyrhyd) data are shown as purple diamonds and fit with a red line representative of a monoexponential. The specific T\textsubscript{1} values calculated for each compound per injection are shown overlaid on the graph. The standard deviation for each T\textsubscript{1} is indicative of the fit 95% confidence interval.1\textsuperscript{st} injection: R\textsuperscript{2}(pyr)=0.986, R\textsuperscript{2}(pyrhyd)=0.963, 2\textsuperscript{nd} injection: R\textsuperscript{2}(pyr)=0.957, R\textsuperscript{2}(pyrhyd)=0.713, 3\textsuperscript{rd} injection: R\textsuperscript{2}(pyr)=0.962, R\textsuperscript{2}(pyrhyd)=0.728.

In order to assess metabolite dynamics and fully characterize the in vivo T\textsubscript{1} of hyperpolarized [1-\textsuperscript{13}C]pyruvate, spectra were obtained and the integrated signal for each spectrum was plotted over a time course of 55 seconds. The results of this analysis are shown in Figure 8.3.3 for three separate injections into the same animal with the implantable coil wrapped around the CCA. The T\textsubscript{1} ranges from approximately 6.3-8.9
seconds with 95% confidence for [1-13C]pyruvate. For [1-13C]pyruvate hydrate, the range of potential T1 values 3.5-8 seconds with 95% confidence. The larger data variance for [1-13C]pyruvate hydrate is because of the lower SNR and therefore reduced goodness of fit. With slightly different fits using the same data in Origin 8.6, the average T1 value was determined to be 7.5 ± 1.3 seconds for [1-13C]pyruvate and 5.1 ± 1.1 for pyruvate hydrate. Here, the standard deviation corresponds to data uncertainty between injections.

8.3.3. HYPERPOLARIZED [1-13C] PYRUVATE: IN VITRO T1 MEASUREMENTS

In vitro T1 experiments were conducted by Dr. Malgorzata Marjanska at the University of Minnesota Center for Magnetic Resonance Research in order to assess differences from the in vivo scenario and draw parallels to previously published studies. In this set of experiments, the T1 for [1-13C]pyruvate and [1-13C]pyruvate hydrate in whole blood was 31±1 seconds and 23±1 seconds, respectively. These times are markedly longer than the in vivo experiments shown in section 8.3.2.

8.4. DISCUSSION

Remote detection phantom experiments with hyperpolarized 13C urea proceeded as expected with the anticipated results. Certainly, the travel curve observed in Figure 8.3.1a has been demonstrated in multiple publications and with many different nuclei, including hyperpolarized samples. These experiments are included here to emphasize the importance of proper sample and instrument calibration. Here, we verified detection coil sensitivity, injection settings, spectrometer settings for multiple coils, and pulse parameters. While these experiments have not yet demonstrated conclusive results in
live animals, there is substantial evidence that they are, in fact possible. Preliminary data (not shown) demonstrate potential travel curves with injections of $^{13}$C urea, encoding with a volumetric head coil, and detection with the implantable coils at the jugular veins.

The hyperpolarized [1-$^{13}$C]pyruvate experiments revealed interesting results regarding the underlying dynamics of hyperpolarized substrates in the blood. The apparent T$_1$ of hyperpolarized [1-$^{13}$C]pyruvate is significantly shorter when detected with an implantable RF coil wrapped around the CCA than during in vitro experiments in whole blood. This could have a substantial impact on modeling the conversion of pyruvate to other metabolites. As seen in many imaging studies, the apparent T$_1$ in the brain, liver, and other organs, is more similar than it is in vitro.

Importantly, by closely assessing the spectrum received in the implantable coil, we notice a conversion of [1-$^{13}$C]pyruvate to [1-$^{13}$C]lactate. The [1-$^{13}$C]lactate signal is lower in the whole blood experiment. This may be attributed to differing lactate dehydrogenase (LDH) activity and a higher metabolic output from living organ systems with higher energy demands.

As noted above, the apparent T$_1$ of hyperpolarized [1-$^{13}$C]pyruvate was much shorter in live animals than in whole blood samples. The mechanism of relaxation in a living animal as compared to a whole blood sample certainly requires more study. The varying rates of relaxation in different compartments, as well the diffusion and interaction of pyruvate in these environments could contribute to reduce observable T$_1$. Additionally,
oxygenation levels and tissue uptake could certainly account for the faster rate of signal decrease. After correcting for these expected concentrations effects, the apparent $T_1$ is still shorter \textit{in vivo} than \textit{in vitro}, suggesting alternative mechanisms and requiring further future study.

8.5. \textbf{CONCLUSIONS}

The experiments discussed herein outline the potential utility of implantable RF coils for studying metabolic flux in live animals. Furthermore, we outline the general experimental setup and potential $T_1$ constraints when extending these studies to remote detection mode. The nature of the study will dictate, the nucleus used. For tracer based remote detection studies, $^{13}$C urea serves as an ideal compound to generate contrast. It is not metabolized and exhibits a longer $T_1$ and overall higher SNR in live animals when detected with an implantable coil around an artery or vein (data not shown). For metabolic modeling, $[1-^{13}]$pyruvate is an interesting compound to study input and output function for metabolic modeling at the artery or vein, respectively. In all cases, implantable coils provide an effective means by which to regionally define an area of interest and zoom in on a smaller subset of the multitude of physiological parameters that define large scale metabolic activity.


APPENDIX I: STRIPLINE REMOTE DETECTION PROBE

A1.1: Stripline remote detection probe microfluidic chip holder faceplate.
A1.2: Stripline remote detection probe microfluidic chip holder base.
A1.3: Stripline remote detection probe brass shield/chip support.
A1.4: Stripline remote detection probe circuit support.
A1.5: Stripline remote detection probe circuit board.
APPENDIX II: IMPLANTABLE VASCULAR COIL

A2.1: Two variations of flexible PCB implantable vascular coil.
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