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Progress in Microfluidic Nuclear Magnetic Resonance

by

Daniel Joseph Kennedy

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Biophysics

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA, BERKELEY

Committee in charge:

Professor Alexander Pines, Co-Chair
Professor David E. Wemmer, Co-Chair
Professor Lydia Sohn
Professor Jeffrey A. Reimer

Fall 2014

Progress in Microfluidic Nuclear Magnetic Resonance

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by

Daniel Joseph Kennedy

Abstract

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University of California, Berkeley

Professor Alexander Pines, Co-Chair
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Nuclear magnetic resonance (NMR) has matured into an extremely powerful technique, with applications ranging from chemical analysis to molecular biology to medical diagnostic imaging. Unfortunately, the hardware associated with this technique has traditionally been large, immobile, expensive, and required frequent specialized maintenance. Microfluidic technology has allowed for a great reduction in complexity and expense for most common analytical techniques; however, little work has been done to leverage the advantages of microfluidics and nuclear magnetic resonance in a single device. In this thesis, I describe advances toward the goal of creating small, portable, inexpensive, easy-to-use NMR microfluidic instrumentation. First, I describe projects relating to the use of ^{129}Xe chemical sensors on microfluidic devices. I demonstrate a silicon and glass microdevice which allows the production and optical detection of hyperpolarized ^{129}Xe at low magnetic fields. The device enhances the NMR sensitivity of ^{129}Xe experiments by a factor 10^4 on hardware that is a factor of 10^6 smaller in size and power requirements than current polarizers. I describe techniques for patterning arrays of ^{129}Xe chemical sensors on microfluidic devices and using them to detect chemicals at picomolar sensitivities. These technologies may be integrated to produce highly integrated portable devices for detecting arbitrary chemicals in complex fluids. Due to the use of NMR as the detection modality, they may be used on dirty samples which cannot be interrogated through the use of optical spectroscopy or mass spectrometry, such as biofuels or whole human blood. The second part of this thesis deals with a method of encoding imaging information using arrays of thin magnetic films instead of magnetic field gradients. This technique obviates the need for large instrumentation in imaging experiments and may allow for greater portability, further increasing the practicality of microfluidic NMR experiments. These advances represent a significant step toward microscale NMR technology.

To whom it may concern.

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In the brief span of four months that marked the time between the end of my undergraduate studies and the beginning of my graduate ones, my research interests changed drastically. When I first applied to Berkeley, I had intended to work in the field of structural biology and protein folding. As the time preceding my westward journey narrowed, I found myself drawn more and more to a new interest in the development of hardware and methodology. Upon arriving at the campus, I felt a twinge of panic as I realized that my old list of potential thesis advisors was completely irrelevant to my newly chosen field. I am first and foremost grateful to Susan Marqusee, who, in addition to being a phenomenal first-year graduate adviser, first introduced me to the work of Alex Pines.

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Symbols

Variables

a_0	z component of initial acceleration
$\mathbf{B}$	magnetic field vector
B_0	static azimuthal magnetic field
$\mathbf{B}_1$	RF pulse field vector
B_1	RF pulse field strength
$\hat{\mathbf{B}}_1$	RF pulse field vector in the rotating frame
B_{eff}	effective magnetic field due to chemical shift
B_{Rb}	magnetic field detected by Rb
B_{Stark}	fictional field due to AC Stark effect
B_{Xe}	magnetic field detected by Xe
d_{coil}	inductive coil diameter
dI/dt	rate of photon absorption by alkali vapor
E	potential energy
f	alkali oscillator strength
F	total angular momentum
F_{amp}	pre-amplifier noise figure
FOV_x	field-of-view in x dimension
FOV_y	field-of-view in y dimension
g_N	nuclear g-factor
g_S	Lande g-factor
$\mathbf{G}$	gradient vector
G_x	x gradient strength
G_y	y gradient strength
$G_{y,max}$	maximum gradient in the phase-encoded dimension
$G_{y,min}$	minimum gradient in the phase-encoded dimension
G_z	z gradient strength
h_{coil}	height of inductive coil
$\mathbf{I}$	nuclear spin operator
I_z	azimuthal nuclear spin operator
I_-	nuclear spin lowering operator
I_+	nuclear spin raising operator
k_x	x component of k-space
k_y	y component of k-space
K	equilibrium dissociation constant
K_{coil}	inductive coil geometric factor
l	length of beam path
l_{MFP}	mean free path
L_{coil}	coil inductance
m	mass

$m(x, y)$	nuclear spin magnetization distribution
$\mathbf{M}$	nuclear spin magnetization vector
M	nuclear spin magnetization
M_0	equilibrium magnetization
M_{Rb}	^{87}Rb magnetization
M_x	x component of magnetization
M_{Xe}	^{129}Xe magnetization
M_y	y component of magnetization
M_z	z component of magnetization
n	atomic density
n_{alkali}	alkali atomic density
n_{Rb}	Rb atomic density
n_{Xe}	^{129}Xe atomic density
N	number of nuclear spins
$N_{-1/2}$	number of nuclear spins in the -1/2 spin state
$N_{+1/2}$	number of nuclear spins in the +1/2 spin state
N_x	number of imaging points in the frequency-encoded dimension
N_y	number of imaging points in the phase-encoded dimension
O_{CryA}	cryptophane-A occupancy
OD_0	on-resonance optical depth
p	gas pressure
p_{N_2}	nitrogen partial pressure
p_{Xe}	xenon partial pressure
P	nuclear spin polarization
P_0	equilibrium polarization
P_{alkali}	alkali longitudinal electron spin polarization
P_{OP}	optical pumping power
P_{Rb}	Rb longitudinal electron spin polarization
P_x	transverse alkali polarization along optical pumping axis
P_{Xe}	^{129}Xe polarization
P_y	transverse alkali polarization perpendicular to optical pumping axis
P_z	longitudinal alkali polarization
q	electric charge
q_N	nuclear slowing-down factor
Q	quality factor
Q_{gas}	gas leak rate
$\mathbf{r}$	position vector
r	distance
R_{coil}	coil resistance
R_{flow}	loss of polarization due to gas flow
R_{OP}	rate of optical pumping
R_{SD}	loss of polarization due to spin destruction collisions
R_{SE}	loss of polarization due to spin exchange collisions
R_{wall}	loss of polarization due to wall collisions
R_γ	rate of decay after optical pumping

$s(t)$	NMR signal
$\mathbf{S}$	electronic spin vector
S_z	azimuthal electronic spin operator
S_{noise}	magnetometer system noise
S_-	electronic spin lowering operator
S_+	electronic spin raising operator
SNR_{rms}	root-mean square signal-to-noise ratio
t	time
T	temperature
T_1	longitudinal or spin-lattice relaxation time
T_2	transverse or spin-spin relaxation time
T_2^*	inhomogeneous spin-spin relaxation time
T_2^{inhom}	inhomogeneous contribution to the spin-spin relaxation time
T_{coil}	inductive coil temperature
TE	spin echo time
v_0	z component of initial velocity
v_{RMS}	root-mean square velocity
V	volume
V_{cell}	alkali cell volume
V_{coil}	volume of inductive coil
V_{sample}	sample volume
W_k	k-space bandwidth
x	horizontal location
y	vertical location
z_0	z component of initial position
$[CryA]$	concentration of CryA in water
$[H_2O]$	concentration of H_2O
$[Xe@CryA]$	concentration of Xe in CryA
$[Xe@H_2O]$	concentration of Xe in water
α	probability of electron-nuclear overlap
γ	gyromagnetic ratio (angular frequency units)
γ_I	electronic gyromagnetic ratio
γ_S	nuclear gyromagnetic ratio
γ	gyromagnetic ratio (frequency units)
δ	imaging resolution
$\delta(\mathbf{r})$	Dirac delta function
δ_{bound}	^{129}Xe chemical shift of chemically-bound sensor
δ_{ppm}	chemical shift in parts-per-million
δ_{skin}	skin depth
$\delta_{unbound}$	^{129}Xe chemical shift of unbound sensor
δ_x	imaging resolution in the frequency-encoded dimension
δ_y	imaging resolution in the phase-encoded dimension
δB_0	magnetic field variation
δS	magnetometer signal variation

Δz	thickness of excited slice
$\Delta\nu_{coil}$	bandwidth of receiver
$\Delta\nu_{Rb}$	FWHM of Rb optical line
$\Delta\omega_0$	Larmor frequency variation
η	filling factor
$\eta_{transfer}$	spin transfer efficiency
θ	azimuthal rotation angle
κ_{SE}	spin exchange coefficient
κ_0	alkali-noble gas Fermi-contact interaction enhancement
μ_r	relative permeability
μ_S	spin magnetic moment
μ_{Xe}	^{129}Xe magnetic moment
ν_0	center frequency of alkali D1 transition
ν_{light}	detuned laser frequency
ν_{Xe}	^{129}Xe NNMR frequency
ϕ	phase
$\phi(x, y)$	phase distribution
ρ	electrical resistivity
σ	chemical shift shielding factor
σ_0	on-resonance optical cross-section
τ_{90}	90° pulse time
τ_{IR}	inversion recovery delay time
τ_{meas}	measurement time
τ_{rise}	electronic rise time
τ_{RF}	RF pulse duration
τ_{ve}	velocity encoding time
ω	angular frequency
ω_0	Larmor frequency
ω_{eff}	effective Larmor frequency due to chemical shift

Physical Constants

c	speed of light = $2.998 \times 10^8 \text{ m s}^{-1}$
d_{Xe}	diameter of a xenon atom = $4.32 \times 10^{-10} \text{ m}$
e	elementary electric charge = $1.609 \times 10^{-19} \text{ C}$
h	Planck's constant = $6.626 \times 10^{-34} \text{ J s}$
$\hbar$	reduced Planck's constant $\hbar = h/2\pi = 1.05 \times 10^{-34} \text{ J s}$
k_B	Boltzmann constant, $1.381 \times 10^{-23} \text{ J K}^{-1}$
m_p	proton mass = $6.669 \times 10^{-27} \text{ kg}$
r_e	classical electron radius = $2.818 \times 10^{-15} \text{ m}$
$\hat{x}$	unit vector in the x direction (lab frame)
$\hat{x}'$	unit vector in the x direction (rotating frame)

$\hat{y}$	unit vector in the y direction (lab frame)
$\hat{y}'$	unit vector in the y direction (rotating frame)
$\hat{z}$	unit vector in the z direction (lab frame)
$\hat{z}'$	unit vector in the z direction (rotating frame)
γ_e	electron gyromagnetic ratio = 1.761×10^{11} rad s ⁻¹ T ⁻¹
μ_0	vacuum permeability = $4\pi \times 10^{-7}$ T m A ⁻¹
μ_B	Bohr magneton = 9.274×10^{-24} J T ⁻¹

Abbreviations

AC	alternating current
AMR	anisotropic magnetoresistance
CE	capillary electrophoresis
CMR	colossal magnetoresistance
CPMG	Carr-Purcell-Meiboom-Gill experiment
CryA	cryptophane-A
CVD	chemical vapor deposition
DFB	distributed feedback laser
DNA	deoxyribonucleic acid
DNP	dynamic nuclear polarization
DOTA	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid
EPI	echo-planar imaging
FID	free-induction decay
FOV	field-of-view
FT	Fourier transform
FWHM	full width at half maximum
GMR	giant magnetoresistance
HF	hydrofluoric acid
HWHM	half width at half maximum
HWP	half-wave plate
IR	inversion recovery
LOD	limit of detection
LPCVD	low-pressure chemical vapor deposition
MEOP	metastability exchange optical pumping
MRI	magnetic resonance imaging
NIR	near infrared
NIST	National Institute of Standards and Technology
NMR	nuclear magnetic resonance
PBS	polarizing beam splitter
PBSa	phosphate buffered saline
PCB	printed circuit board
PCR	polymerase chain reaction

PEEK	polyether ether ketone
PHIP	parahydrogen induced polarization
PVD	physical vapor deposition
ppb	parts-per-billion
ppm	parts-per-million
QWP	quarter-wave plate
RD	remote detection
RF	radiofrequency
RGA	residual gas analyzer
RWA	rotating wave approximation
SABRE	signal amplification by reversible exchange
SDS	sodium dodecyl sulfate
SEOP	spin-exchange optical pumping
SNR	signal-to-noise ratio
SQUID	superconducting quantum interference device
ssDNA	single-stranded deoxyribonucleic acid
STP	standard temperature and pressure
TE	spin echo time
TMP	turbomolecular pump
TMR	tunnel magnetoresistance
TOF	time-of-flight
UHMW-PE	ultrahigh molecular weight polyethylene
UHV	ultra-high vacuum
UV	ultraviolet
VCSEL	vertical-cavity surface-emitting laser
XBMS	xenon-based molecular sensor
2DFT	two-dimensional Fourier transform imaging

Chapter 1

Introduction

Since its discovery nearly 70 years ago by Bloch, Hansen, and Packard [16], and Purcell, Torrey, and Pound [129], nuclear magnetic resonance (NMR) spectroscopy has been developed into a technique of fundamental importance in the physical and biological sciences. It has been utilized in an enormous number of applications ranging from the confirmation of small molecule syntheses [176] to the determination of protein [179, 180] and nucleic acid structures [51] at atomic-level resolutions to the analysis of semiconductors [183] and other solid-state materials. The NMR phenomenon has also led to breakthroughs in imaging since magnetic resonance imaging (MRI) was invented in the early 1970s by Lauterbur [94] and Mansfield [103, 54]. As the magnetic resonance technique which is best known to the public, MRI has developed into a massive field in its own right, allowing diagnostic medical imaging [58] which is non-invasive and does not present a health hazard due to the use of ionizing radiation, unlike other techniques such as X-ray computed tomography (CT) [22]. The magnetic resonance techniques provide a means of analysis which yields tremendous information about a sample's physiochemical environment in a non-invasive manner which does not perturb the sample. They have become indispensable to modern scientific research, resulting in a total of four Nobel Prizes over the years.

Despite their usefulness in a huge number of fields, NMR and MRI suffer from a number of weaknesses which limit their applicability to numerous other applications in which they might otherwise be extremely useful. Compared to other analytical techniques, such as optical spectroscopy and mass spectrometry, NMR and MRI produce signals which are very weak. This is due to the very low energy of the nuclear spin transitions on which NMR and MRI rely, as well as the low efficiency of inductive detection which is typically used to detect the magnetic resonance signal. Because of these limitations, advances in NMR and MRI have historically trended toward the development of spectrometers utilizing progressively higher magnetic fields in order to increase both the magnetic resonance signal and the detection efficiency. Generation of these large magnetic fields, which now exceed field strengths of 20 T, or 400,000 times the strength of the Earth's magnetic field, is accomplished using superconducting magnets which are very large, immobile, and expensive. Reliance on such hardware has precluded the use of NMR and MRI for applications which require small,

portable, and inexpensive detection platforms, such as any applications which might require real-time analysis in the field.

This drawback of NMR and MRI technology is particularly problematic in light of recent developments in the miniaturization of numerous other analytical techniques. In particular, the development of microfluidics technology [175] has revolutionized chemical and biochemical analysis and is now routinely utilized in fields ranging from chemical biology [173] to chemical synthesis [46] to petrochemistry [18]. In the biomedical industry alone, more than 200 companies throughout the world develop microfluidic point-of-care diagnostics and research tools. Applications of these technologies are commonplace in both academia and industry; they include blood analysis systems, cell-based assays, immunoassays, the polymerase chain reaction (PCR), and drug discovery, among many others [182]. In all of these cases, microfluidics has offered a means of greatly reducing the size, complexity, and cost of the instrumentation used in such fields. Key to this trend has been leveraging the production techniques perfected over the course of six decades by the semiconductor industry. The microfluidic revolution has greatly increased the applicability of these techniques by making devices portable, allowing tools to be brought to samples of interest rather than the other way around, and by making them inexpensive, allowing their use in areas and populations which would normally be economically prohibitive. Microfluidics also allows the use of very small amounts of reagent volumes (typically less than 1 μL) due to the very small dimensions of microfluidic devices. This has the added benefit of generating little chemical waste, which has become particularly important in an era which emphasizes the development of “green” techniques.

Although microfluidics has had an enormous impact on many fields in biochemical and chemical analysis, its application to NMR and MRI has been very limited. Until the turn of the millennium, applications combining microfluidics and NMR were essentially non-existent [70]. The first microfluidic NMR experiment attempted to monitor chemical electrophoresis (CE) on a microfluidic chip using a planar radiofrequency (RF) microcoil [167]. Poor coil sensitivity made it impossible to detect the CE analytes, but allowed detection of water and ethanol due to the high concentrations of hydrogen atoms in these molecules. Subsequent improvements in planar coil design allowed for the NMR detection of chemical reactions on a microfluidic device [105, 174]. Development of the stripline detector [8] solved issues related to inhomogeneous broadening which arose with the initial microcoil designs. Parallel development of remote detection techniques [112] utilizing external solenoid detection coils culminated in the application of NMR methods to the imaging of microfluidic channels [106] and the analysis of microfluidic flow [76]. Since these developments, remote detection NMR has found numerous uses in the analysis of microfluidic devices [6].

Despite the use of NMR and MRI in the analysis of microfluidic devices, the application of these techniques to microfluidics has mostly been an afterthought. Few devices have been designed to intentionally utilize NMR and MRI detection principles. The vast majority of those that have been designed with such schemes in mind have, for the most part, required the use of very high magnetic fields and the attendant large, immobile, and expensive superconducting magnets. A notable exception here is the development of a small-scale NMR

instrument which utilizes a small magnet in place of a large superconducting one [161].

This thesis is concerned with the development of small, inexpensive, and portable microfluidic devices designed with NMR and MRI detection principles in mind; in particular, we examine a number of microfluidic instruments and methodology which might allow the use of xenon-based molecular sensors (XBMS) for the detection of arbitrary chemicals in complex fluids. It is organized as follows. In Chapter 2, we develop a basic description of NMR and MRI. In Chapter 3, we examine the development of a microfabricated source of hyperpolarized ^{129}Xe which operates at very low magnetic fields. In Chapter 4, we consider experiments related to the patterning and detection of XBMS on microfluidic devices. In Chapter 5, we consider a method of encoding MRI information without the need for electronically pulsed magnetic field gradients and their attendant large and immobile instrumentation. Finally, in Chapter 6, we provide an analysis of the future prospects for the instruments and methods described in the previous chapters.

Chapter 2

Principles of NMR and MRI

A complete treatment of NMR spectroscopy requires the powerful machinery of quantum mechanics. Though sublime and worthwhile, this description of NMR is both unnecessary for the purposes of this thesis and has been reviewed in detail by numerous other authors. The majority of this thesis can be understood using only a classical description of NMR, which will be presented in this chapter. A few quantum mechanical results will be introduced as needed throughout the thesis. Basic results from quantum mechanics can be found in texts by Cohen-Tannoudji [32], Sakurai [137], and Shankar [151]. A full treatment of the quantum theory of angular momentum can be found in the text by Rose [132]. Readers who are interested in the full quantum mechanical description of NMR should refer to introductory texts by Keeler [86] and Levitt [96]. Advanced treatments may be found in texts by Abragam [1] and Ernst [47]. Readers interested in the principles of MRI should consult the introductory text by Nishimura [119] or the advanced text by Bernstein [12].

This chapter is organized as follows. In Section 2.1, we consider nuclear spin, the intrinsic angular momentum associated with certain atomic nuclei. In Section 2.2, we discuss the Zeeman interaction which gives rise to energy-level transitions between different nuclear spin states. In Section 2.3, we consider how the Zeeman interaction gives rise to a bulk nuclear magnetization which can be detected by magnetic field sensors; in Section 2.3.1, we consider advanced methods by which this magnetization can be significantly enhanced through so-called “hyperpolarization” techniques. In Section 2.4, we study the interaction between the nuclear magnetization and an externally applied magnetic field, which gives rise to Larmor precession of the magnetization about the direction of the magnetic field. In Section 2.5, we develop the Bloch equations, a set of coupled differential equations which describe the classical evolution of the bulk nuclear spin magnetization in a magnetic field. In Section 2.6, we consider the mechanisms which give rise to decay of the nuclear spin magnetization; T_1 relaxation is considered in Section 2.6.1, while T_2 relaxation is considered in Section 2.6.2. In Section 2.7, we consider the spin echo pulse phenomenon which allows for the reversal of the significant decay in magnetization which arises due to spatial inhomogeneities in the magnetic field. In Section 2.8, we put these basic elements together to provide a description of the NMR experiment. We consider some basic NMR pulse sequences, including the

measurement of T_1 and T_2 (Section 2.8.1); we also consider alternative means of detecting the NMR signal (Section 2.8.2). In Section 2.9, we turn our attention to the development of MRI techniques; we consider the “k-space” perspective and how it allows encoding of information in the phase of the nuclear magnetization (Section 2.9.1), the attainable imaging resolution and field-of-view (Section 2.9.2), and the use of magnetic field gradients to select an NMR signal only from particular locations in space (Section 2.9.3). Finally, in Section 2.10, we consider the remote detection technique which is indispensable to the experiments described in this thesis; we describe the acquisition of travel curves which allow addition of a time dimension to the MRI experiment (Section 2.10.1), phase encoding and velocity encoding in the remote detection experiment (Section 2.10.2), and the method by which the remote detection experiment is detected (Section 2.10.3).

2.1 Nuclear Spin

The NMR signal is based entirely on the interactions of nuclear magnetic moments (often referred to in the NMR parlance as nuclear spins) with their environments. As an entirely quantum mechanical phenomenon, spin has no equivalent in classical mechanics. It was first experimentally discovered in 1922. While testing the Born-Sommerfeld hypothesis that the direction of angular momentum is restricted to lie only along certain directions, Stern and Gerlach demonstrated that the unpaired valence electron of silver atoms could only take on discrete values of angular momentum [56]. Physicists attempted to ascribe this previously unknown form of angular momentum to self-rotation of electrons orbiting the atomic nucleus; however, these calculations revealed that the particle would have to rotate faster than the speed of light in order to agree with experimental results. In non-relativistic quantum mechanics, spin is taken as the intrinsic angular momentum associated with a subatomic particle and is well-described by the spinor formalism introduced by Pauli [124]. In 1928, the introduction of the relativistic Dirac equation allowed for the first prediction of the electron spin [41]. Quantum electrodynamics introduced a small correction to Dirac’s calculation to account for the interaction of the electron magnetic moment with vacuum fluctuations and brought about an unprecedented level of accuracy in the calculation of this value [23].

In contrast with the electronic case, nuclear spin magnetic moments are notoriously difficult to calculate. For this reason, we will follow the usual treatment in non-relativistic quantum mechanics, which takes the spin to be an intrinsic and experimentally determined angular momentum associated with a subatomic particle. Nuclear spins follow the same basic behavior as electron spins. They can take on half-integer and integer values, ranging from 0 (no spin angular momentum, e.g. ^4He) to $1/2$ (the simplest case from the NMR perspective, e.g. ^1H , ^{13}C , ^{15}N , ^{31}P , and ^{129}Xe) to 7 (^{176}Lu). In general, the spin magnetic moment μ_S of a subatomic particle is proportional to the electric charge q , the mass m , and

the spin vector $\mathbf{S}$:

$$\boldsymbol{\mu}_S = g \frac{q}{2m} \hbar \mathbf{S} \quad (2.1)$$

Here, g is the Lande g-factor of the subatomic particle and $\hbar$ is the reduced Planck constant. In NMR, the magnetic moment is instead written in terms of the gyromagnetic ratio γ :

$$\boldsymbol{\mu}_S = \gamma \hbar \mathbf{I} \quad (2.2)$$

Here, $\mathbf{I}$ is the nuclear spin. The gyromagnetic ratio (given in units of rad s T^{-1}) is proportional to the g-factor g_N of the nucleus, the elementary electric charge e , and the mass m_p of a proton:

$$\gamma = g_N \frac{e}{2m_p} \quad (2.3)$$

For convenience in calculations, the gyromagnetic ratio is often given in units of Hz T^{-1} and denoted as γ :

$$\gamma = \gamma/2\pi \quad (2.4)$$

Table 2.1 provides a list of the spins and gyromagnetic ratios of nuclei which are commonly used in NMR.

Any nucleus with a non-zero spin will give rise to a magnetic moment. The interactions between this magnetic moment and nearby magnetic fields give rise to the NMR signal. These fields may be generated by other nuclei or by an external source, making NMR a gauge of both internal and external interactions.

2.2 The Zeeman Interaction

The dominant interaction in NMR is typically that between a nuclear magnetic moment and a static external magnetic field $\mathbf{B}$. Classically, the potential energy E of a magnetic moment in a magnetic field is:

$$E = -\boldsymbol{\mu}_S \cdot \mathbf{B} \quad (2.5)$$

Table 2.1: NMR Nuclei and Their Gyromagnetic Ratios

Nucleus	I	γ (10^6 rad s $^{-1}$ T $^{-1}$)	γ (MHz T $^{-1}$)
^1H	1/2	267.513	42.576
^2H	1	41.065	6.536
^3He	1/2	203.789	32.434
^{13}C	1/2	67.262	10.705
^{14}N	1	19.331	3.077
^{15}N	1/2	-27.116	-4.316
^{17}O	5/2	36.264	5.772
^{19}F	1/2	251.662	40.052
^{23}Na	3/2	70.761	11.262
^{27}Al	1/2	69.763	11.103
^{29}Si	1/2	-53.188	8.465
^{31}P	1/2	108.291	17.235
^{129}Xe	1/2	73.997	11.777
^{131}Xe	3/2	22.089	3.516

If we assume that the external magnetic field is a constant field directed along the z axis ($\mathbf{B} = B_0 \hat{\mathbf{z}}$), as is typically the case in NMR and MRI, and refer back to Equation 2.2, we get:

$$E = -\gamma \hbar B_0 I_z \quad (2.6)$$

Here, the azimuthal nuclear spin operator I_z is defined by its action on a nuclear spin state; when the operator is applied to a spin state $|I m_I\rangle$, it will return the state $m_I |I m_I\rangle$. Thus, the potential energy of the interaction between the nuclear magnetic moment and the magnetic field depends on the nuclear spin. Since the work in this thesis is performed entirely on spin-1/2 systems, we will consider only this case. For a spin-1/2 nucleus, $m_I = \pm 1/2$; assuming a positive gyromagnetic ratio (as is typically the case; for the opposite case, we can simply change the sign), the energy difference ΔE between the -1/2 higher energy state and the +1/2 lower energy state is:

$$\Delta E = \gamma \hbar B_0 \quad (2.7)$$

The energy difference between the -1/2 state aligned against the magnetic field and the +1/2 state aligned with the magnetic field is known as the Zeeman effect; it is the dominant interaction at the magnetic field strengths generally encountered in NMR. The Zeeman effect is illustrated in Figure 2.1. Recalling Planck's equation $\Delta E = \hbar \omega$, we arrive at another result:

$$\omega_0 = -\gamma B_0 \quad (2.8)$$

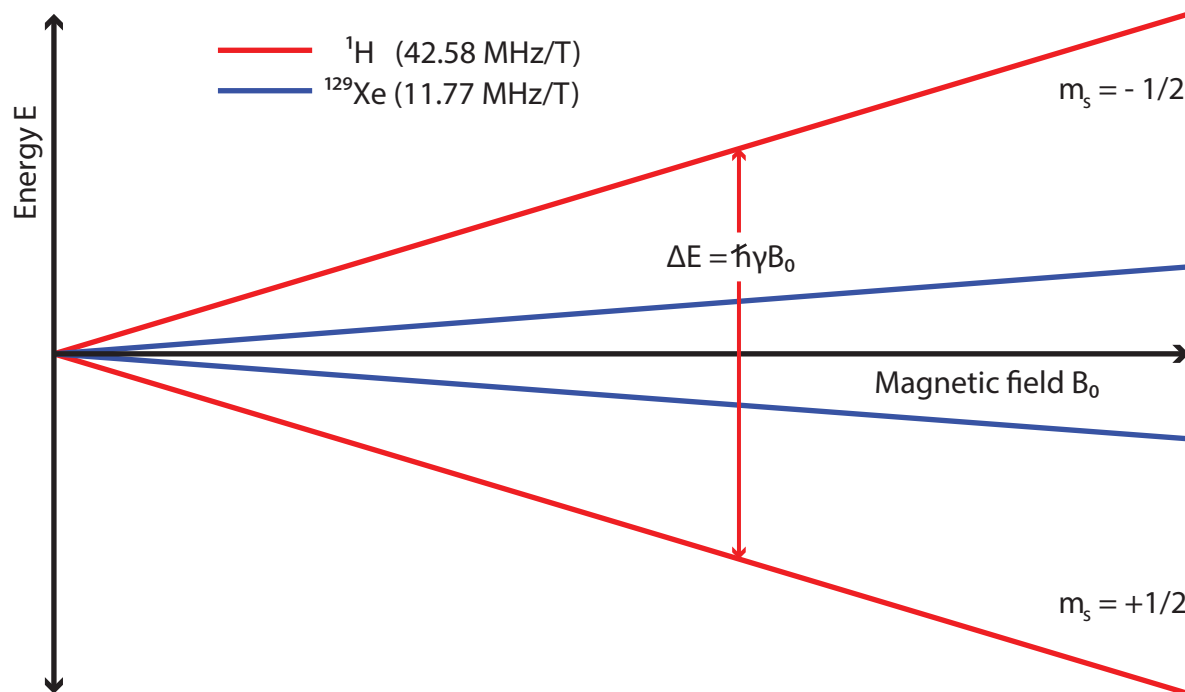


Figure 2.1: The Zeeman Effect. The energy level splitting between $-1/2$ and $+1/2$ spin states increases linearly with the magnetic field.

Here, ω_0 , the Larmor frequency, is the angular frequency of radiation required to excite transitions from the lower Zeeman energy level to the higher level.

2.3 Magnetization and Nuclear Spin Polarization

The NMR signal does not result from the nuclear magnetic moment generated by a single nuclear spin, which is very small. Rather, it is produced by a large ensemble of nuclear spins acting to produce a macroscopic magnetic moment which can be manipulated with RF pulses and detected with a magnetic field sensor. The equilibrium magnetization M_0 due to N nuclear spins in a magnetic field is:

$$M_0 = \frac{N\gamma\hbar}{2k_B T} P_0 \quad (2.9)$$

Here, k_B is the Boltzmann constant and T is the temperature. The equilibrium nuclear spin polarization P_0 is the difference in populations between the $-1/2$ energy state and the

+1/2 energy state; it is easily calculated using the Boltzmann distribution:

$$\begin{aligned}
 P_0 &= \frac{N_{-1/2} - N_{+1/2}}{N_{-1/2} + N_{+1/2}} \\
 &= \frac{N \exp\left(\frac{-E_{-1/2}}{k_B T}\right) - N \exp\left(\frac{-E_{+1/2}}{k_B T}\right)}{N \exp\left(\frac{-E_{-1/2}}{k_B T}\right) + N \exp\left(\frac{-E_{+1/2}}{k_B T}\right)} \\
 &= \frac{\exp\left(\frac{\gamma \hbar}{2k_B T}\right) - \exp\left(\frac{-\gamma \hbar}{2k_B T}\right)}{\exp\left(\frac{\gamma \hbar}{2k_B T}\right) + \exp\left(\frac{-\gamma \hbar}{2k_B T}\right)}
 \end{aligned} \tag{2.10}$$

Here, $N_{-1/2}$ and $N_{+1/2}$ are the number of nuclear spins in the -1/2 and +1/2 spin states, respectively; $E_{-1/2}$ and $E_{+1/2}$ are the energy of the -1/2 and +1/2 spin states, respectively. At room temperature, even the largest technically achievable magnetic fields produce an energy level difference which is only a very small fraction of the thermal energy. Therefore, we can expand the exponentials in a Taylor series and truncate at first order to obtain a simplified expression for the equilibrium polarization:

$$P_0 = \frac{\gamma \hbar B_0}{2k_B T} \tag{2.11}$$

From this, we can make several important observations. Firstly, an increase in the static magnetic field leads to an increase in the equilibrium polarization and the basic NMR signal. This is one reason for which the trend has been toward the use of superconducting magnets producing ever larger magnetic fields. Secondly, a decrease in temperature yields higher nuclear spin polarization as there is less thermal energy available to induce transitions between the +1/2 and -1/2 spin states. This effect is exploited in certain cryogenic experiments, but cannot be leveraged for many chemical and biological samples which must remain near room temperature in order to maintain the interesting properties which motivate experimentation in the first place. Finally, the equilibrium polarization at room temperature is very small. Even utilizing the highest commercially available magnetic fields of 23.5 T, the equilibrium polarization is only $\approx 8 \times 10^{-5}$. This is a very small value and is one of the reasons for the general lack of sensitivity in NMR. At lower magnetic fields, the polarization is even lower; for instance, at the Earth's magnetic field of 50 μ T, the equilibrium polarization is only $\approx 2 \times 10^{-10}$. This low polarization generally precludes the observation of an NMR signal at low magnetic fields, at least as long as the system remains at the thermal equilibrium value at the Earth's magnetic field.

2.3.1 Non-Equilibrium Hyperpolarization

A significant portion of NMR’s sensitivity limitation can be overcome by preparing non-equilibrium spin state distributions which persist longer than the duration of the NMR experiment. These methods generally produce a substantially greater population of nuclei in one spin state than the other and enhance the polarization (and thus the NMR signal) by two or more orders of magnitude. Numerous methods exist to produce these distributions for different nuclei.

Dynamic nuclear polarization (DNP) exploits the much larger gyromagnetic ratio of unpaired electrons in free radicals as compared to either ^1H nuclei (often referred to as “protons” in the NMR parlance) or ^{13}C nuclei, the nuclei for which this technique is typically employed. The electron’s much larger gyromagnetic ratio means that it is much more highly polarized at thermal equilibrium. If the electron couples with nearby nuclei, electron polarization can be transferred to these nuclei by applying microwave pulses at the electron Larmor frequency [122]. In this case, the upper limit to the signal enhancement is the ratio of the electron gyromagnetic ratio to that of the nucleus of interest; this is a signal enhancement of approximately 660 for protons and approximately 2600 for ^{13}C . Additional enhancements can be obtained by cooling a solution containing free radicals and the substance of interest to further increase the electron spin polarization. In this case, the nuclear spin polarization can approach a value of 1, which represents a more than four order of magnitude increase in signal over the polarizations obtained at high magnetic fields [34]. Although this method requires cryogenic cooling, it does not require the extremely low temperatures which would be required to obtain a very large nuclear spin polarization directly. To directly obtain a proton polarization of near unity, it is necessary to cool the sample to around 1 mK; due to the much higher electron gyromagnetic ratio, the same proton polarization can be obtained by cooling the sample to around 1 K and exploiting the DNP effect. This obviates the need for sophisticated cryogenic systems capable of reaching millikelvin temperatures. Readers interested in the principles and applications of DNP should consult the extensive review from the Griffin research group [102].

The parahydrogen-induced polarization (PHIP) technique is a method of producing hyperpolarized spin distributions by exploiting the symmetry properties of molecular hydrogen (H_2). Hydrogen exists in two isomers, orthohydrogen and parahydrogen. The former is a triplet system composed of the states in which both hydrogen atoms are in the $-1/2$ spin state, in which both hydrogen atoms are in the $+1/2$ spin state, and the symmetric combination of states in which one hydrogen atom is in the $-1/2$ spin state and the other is in the $+1/2$ spin state. With three energy sublevels, orthohydrogen acts like a spin-1 system. Parahydrogen is a singlet system consisting of the anti-symmetric combination of states in which one hydrogen atom is in the $-1/2$ spin state and the other is in the $+1/2$ spin state. With only one energy sublevel, parahydrogen acts like a spin-0 system, and is thus NMR inactive. A sample of nearly 100% parahydrogen can be created by cooling hydrogen gas to 20 K in the presence of an activated carbon or iron oxide catalyst. The parahydrogen can then be warmed to room temperature, where it will remain in the parahydrogen state for up to a day. The warm, long-lived parahydrogen can then be introduced into another

molecule through a hydrogenation chemical reaction. If the two hydrogen atoms bind to magnetically inequivalent sites on the target molecule, a particular spin state will be favored at each location. In this manner, parahydrogen can be used to place hyperpolarized protons at particular locations on a molecule. The hydrogenation reaction can take place at either high magnetic field [20] or low magnetic field [128]. Work using catalysts containing transition metal centers, known as SABRE (signal amplification by reversible exchange) reagents, has allowed for the generation of hyperpolarized protons without requiring a net chemical reaction [3]. Using these techniques, the polarization of protons in various chemical species can be increased by nearly four orders of magnitude over the polarizations attained at high magnetic fields.

Finally, hyperpolarization has been achieved by transferring angular momentum from photons, which can be prepared into nearly pure spin states, to nuclei. In particular, extensive work has been performed to impart hyperpolarization to noble gas nuclei, particular ^{129}Xe and ^3He . Hyperpolarization is imparted in a two-step process known as spin-exchange optical pumping (SEOP) [171]. In the first step, optical pumping, circularly polarized photons from a laser are cast onto a vapor of alkali metal atoms, usually rubidium (Rb) or cesium (Cs). The laser light is tuned to an optical transition of the valence electron of the alkali atom and induces an electronic transition. Because the circularly polarized light is composed of photons in a single spin state (say, $+1$), absorption of a photon also implies a change in spin state from $-1/2$ to $+1/2$ for alkali electrons which start in the $-1/2$ state; electrons initially in the $+1/2$ state are unaffected. Decay back to the ground electronic state leaves an excess of alkali valence electrons in the $+1/2$ state, as compared to the equilibrium spin state distribution. During spin exchange, collisions between polarized alkali atoms and unpolarized noble gas atoms lead to a transfer of polarization to the nuclei of noble gas atoms. In this manner, noble gas polarizations of approximately 0.9 have been obtained under special conditions [118]. This thesis deals extensively with applications involving hyperpolarized ^{129}Xe . Further discussion of spin-exchange optical pumping and hyperpolarized ^{129}Xe is therefore deferred to Chapter 3.

For completeness, there exists a second method for producing hyperpolarized ^3He . In metastability exchange optical pumping (MEOP), the unique energy level structure of ^3He is exploited to allow direct optical pumping of the ^3He nucleus [33]. The 2^3S excited electronic state, a metastable state, is populated by collisions with electrons produced by a plasma discharge. In the metastable state, hyperfine coupling between the ^3He nucleus and the electrons gives rise to very strong entanglement between the ^3He nuclear and electron spin states. Thus, angular momentum orientation in the electrons gives rise to simultaneous angular momentum orientation in the nucleus. Gas-phase collisions give rise to angular momentum exchange between optically pumped ^3He and ^3He which remained in the electronic ground state. This method is capable of achieving polarizations of nearly 1 with very modest laser power requirements. The interested reader should consult the review by Batz [11].

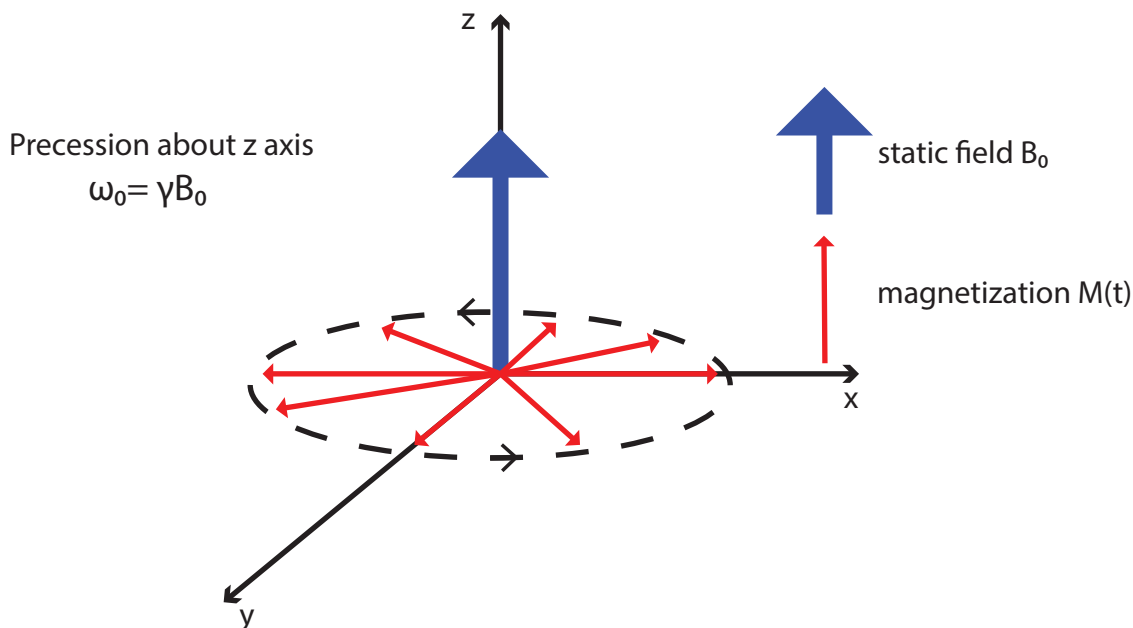


Figure 2.2: Larmor Precession. A nuclear magnetic moment with a component in the xy plane rotates about a magnetic field directed along $\hat{z}$ at the Larmor frequency ω_0 .

2.4 Larmor Precession

Whether utilizing a thermally polarized spin distribution or a hyperpolarized one, the spin dynamics of the NMR experiment remain the same. Here, we investigate how nuclear spin magnetization evolves in the presence of a magnetic field. We employ a classical vector description of the magnetization, rather than examining the evolution of the density matrix, as is done in a quantum mechanical analysis of the system. The classical description is adequate for the NMR experiments described in this thesis.

The nuclear spin magnetization can be mathematically expressed as a vector which is oriented along the direction of the magnetic field (taken to be in the $\hat{z}$ direction by convention) at thermal equilibrium. The magnetization vector can be manipulated by applying RF pulses on resonance with the Larmor frequency of the nuclei; these pulses have the effect of rotating the magnetization vector. Upon applying an RF pulse which rotates the magnetization vector into the plane transverse to the magnetic field, the magnetization vector will begin to precess about the magnetic field. This phenomenon is known as Larmor precession; the frequency of rotation is equal to the Larmor frequency (the product of the gyromagnetic ratio and the magnetic field, defined in Equation 2.8). Figure 2.2 illustrates Larmor precession about a magnetic field directed along $\hat{z}$. This rotating magnetization can be detected with an inductive pickup coil (or alternate means of detection), giving rise to the NMR signal. Detection methods are discussed in Section 2.8.2 after development of the spin dynamics.

2.5 The Bloch Equations

The equations of motion for the evolution of spin magnetization were introduced by Felix Bloch in 1946 and bear his name [16]. They describe the evolution of magnetization $\mathbf{M}$ in a magnetic field $\mathbf{B}$:

$$\frac{d\mathbf{M}}{dt} = \gamma \mathbf{M} \times \mathbf{B} \quad (2.12)$$

These differential equations are easily solved in a number of cases. We first examine the solution to magnetization evolving in a plane perpendicular to a static magnetic field - Larmor precession. Here, we let $\mathbf{B} = B_0 \hat{\mathbf{z}}$. We can rewrite the Bloch equations:

$$\frac{dM_x}{dt} = \gamma M_y B_0 \quad (2.13)$$

$$\frac{dM_y}{dt} = -\gamma M_x B_0 \quad (2.14)$$

$$\frac{dM_z}{dt} = 0 \quad (2.15)$$

This set of coupled differential equations can be solved using standard techniques. If we take the initial magnetization to be along $\hat{\mathbf{y}}$ ($M_x(0) = 0$, $M_y(0) = M_0$, $M_z(0) = 0$), the Bloch equations are solved by:

$$M_x = M_0 \sin(\gamma B_0 t) \quad (2.16)$$

$$M_y = M_0 \cos(\gamma B_0 t) \quad (2.17)$$

$$M_z = 0 \quad (2.18)$$

This is the Larmor precession described in the preceding section. The magnetization precesses around $\hat{\mathbf{z}}$, the direction of the applied magnetic field, at the Larmor frequency. Our second important example concerns the spin dynamics in response to an RF pulse $\mathbf{B}_1$ on resonance with the Larmor frequency oscillating along the x-axis:

$$\mathbf{B}_1 = B_1 \cos(\gamma B_0 t + \phi) \hat{\mathbf{x}} \quad (2.19)$$

The RF pulse field is typically rewritten in terms of counter-rotating components in order to simplify the process of solving the systems dynamics:

$$\mathbf{B}_1 = \frac{1}{2}(B_1 \cos(\gamma B_0 t + \phi) \hat{\mathbf{x}} + \sin(\gamma B_0 t + \phi) \hat{\mathbf{y}}): \text{ resonant} \\ \frac{1}{2}(B_1 \cos(\gamma B_0 t + \phi) \hat{\mathbf{x}} - \sin(\gamma B_0 t + \phi) \hat{\mathbf{y}}): \text{ non-resonant} \quad (2.20)$$

The two counter-rotating components describe rotating vectors in the transverse plane, rotating in opposite directions. The resonant component matches the direction of the Larmor precession, while the non-resonant component does not. Thus, the resonant component has a much greater effect on the nuclear magnetization. In the vast majority of circumstances (including all of the situations considered in this thesis), the non-resonant component can be ignored. This is known as the rotating wave approximation (RWA). Utilizing the RWA, we can write the Bloch equations for an RF pulse:

$$\frac{dM_x}{dt} = \gamma(M_y B_0 + \frac{1}{2}M_z B_1 \sin(\gamma B_0 t + \phi)) \quad (2.21)$$

$$\frac{dM_y}{dt} = -\gamma(M_x B_0 - \frac{1}{2}M_z B_1 \cos(\gamma B_0 t + \phi)) \quad (2.22)$$

$$\frac{dM_z}{dt} = \gamma(\frac{1}{2}M_x B_1 \sin(\gamma B_0 t) - \frac{1}{2}M_y B_1 \cos(\gamma B_0 t)) \quad (2.23)$$

2.5.1 The Rotating Reference Frame

The Bloch equations for an RF pulse can be drastically simplified if a change of reference frame is made. We make a change to a reference frame rotating at the Larmor frequency by taking linear combinations of the axes in the laboratory frame which oscillate at the Larmor frequency. In the rotating reference frame, an RF pulse oscillating at the Larmor frequency appears stationary in time. Using primed variables to refer to quantities measured in the rotating reference frame, we can rewrite the pulse as:

$$\mathbf{B}'_1 = \frac{1}{2}B_1 \hat{\mathbf{x}}' \quad (2.24)$$

Substituting this into the general Bloch equation, we get the Bloch equations in the rotating frame for a RF pulse:

$$\frac{dM_x}{dt} = 0 \quad (2.25)$$

$$\frac{dM_y}{dt} = \frac{1}{2}\gamma B_1 M_z \quad (2.26)$$

$$\frac{dM_z}{dt} = -\frac{1}{2}\gamma B_1 M_x \quad (2.27)$$

Taking the initial magnetization to be along $\hat{\mathbf{z}}$ ($M_x(0) = 0$, $M_y(0) = 0$, $M_z(0) = M_0$), the Bloch equations are solved by:

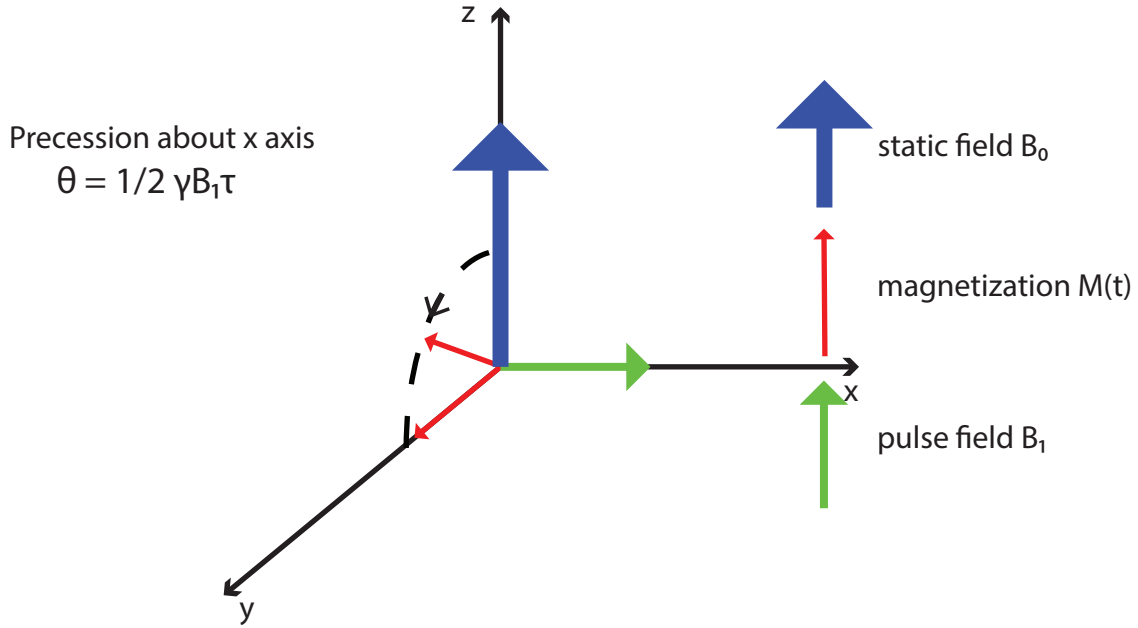


Figure 2.3: A 90° Pulse. The resonant component of the RF pulse tips nuclear spin magnetization from its equilibrium alignment along $\hat{z}$ to the $\hat{x}'$ direction in the rotating frame.

$$M_x = 0 \quad (2.28)$$

$$M_y = M_0 \sin\left(\frac{1}{2}\gamma B_1 t\right) \quad (2.29)$$

$$M_z = M_0 \cos\left(\frac{1}{2}\gamma B_1 t\right) \quad (2.30)$$

The result is similar to that obtained in the case of a static magnetic field. The magnetization precesses around $\hat{x}'$, the direction of the applied magnetic field. By applying an RF field for a duration τ_1 , one may obtain an azimuthal rotation of the magnetization by an angle θ :

$$\theta = \frac{1}{2}\gamma B_1 \tau_1 \quad (2.31)$$

Rotations of 90° and 180° are frequently encountered in NMR and MRI. Specialized pulse sequences make use of different rotation angles. Figure 2.3 shows rotation of magnetization to the xy plane.

2.6 Nuclear Spin Relaxation

To this point, we have taken an idealized view of the dynamics of a system of nuclear spins. We have considered only their interaction with external magnetic fields and have ignored interactions between the spins and their surroundings. These interactions lead to relaxation and decay of the NMR signal, and are generally separated into T_1 relaxation processes and T_2 relaxation processes. We will examine the effects that such processes have on the dynamics of a system of nuclear spins and rewrite the Bloch equations to account for these effects.

2.6.1 T_1 Relaxation

Generation of an NMR signal requires nuclear spin magnetization to be moved from the longitudinal direction (along $\hat{z}$) into the transverse (xy) plane by applying RF pulses. This produces a nuclear spin configuration which is not at thermal equilibrium, as the spins are not aligned in their equilibrium configuration along $\hat{z}$. Magnetization in the xy plane means that the ensemble of nuclear spins exist in a superposition of $-1/2$ and $+1/2$ states, and are therefore higher in energy than they would be at thermal equilibrium. Any mechanisms that act to re-orient the ensemble of nuclear spins will tend to realign the magnetization, with a bias toward alignment of the magnetization along its equilibrium direction.

This bias toward the thermal equilibrium spin configuration is often referred to as longitudinal relaxation, spin-lattice relaxation, or T_1 relaxation. The phrase “longitudinal relaxation” comes from the fact that this processes tends to move nuclear magnetization into the longitudinal $\hat{z}$ direction, aligned with the magnetic field. The phrase “spin-lattice relaxation” comes from the mechanism by which this process takes place. The system of nuclear spins is not isolated in a static environment. Rather, it experiences magnetic fluctuations caused by the motions of other nuclei in a surrounding “lattice”. These surrounding nuclei are constantly changing position due to vibrations and rotations of the molecules of which they are a part. These constant fluctuations may produce transient magnetic fields with components which lie in either $\hat{x}'$ or $\hat{y}'$. These will induce transitions with a bias to return the magnetization towards its thermal equilibrium direction. The phrase “ T_1 relaxation” reflects the fact that the recovery of longitudinal magnetization M_z often follows an exponential trend with time constant T_1 . Assuming that all nuclear spin magnetization is initially in the transverse plane ($M_z(0) = 0$):

$$M_z = M_0(1 - \exp(-t/T_1)) \quad (2.32)$$

T_1 relaxation is dependent on the magnetic field. Shorter T_1 time constants are encountered when the period of rotation about $\hat{z}$ ($\approx 1/\omega_0$) is roughly equal to the correlation time for the processes causing relaxation. This decrease in T_1 results from the ability to more easily induce energy fluctuations under these circumstances. For protons in water at high

field strengths of a few tesla, T_1 is usually 3-5 seconds. For hyperpolarized ^{129}Xe , T_1 may range from a few seconds for ^{129}Xe dissolved in blood [15] to a few hours in the solid phase [55].

Long T_1 relaxation times may have deleterious effects on an NMR experiment. Due to the weakness of the NMR signal, many applications require that an experiment be repeated hundreds or thousands of times in order to increase the signal-to-noise ratio (SNR). In order to ensure that each of these experiments is equivalent to all of the others, it is necessary to allow the magnetization to return to its thermal equilibrium value after each experiment; this is usually accomplished by waiting a period of approximately $5 \times T_1$, when more than 99% of the magnetization has realigned along $\hat{z}$. In other applications, it is necessary to perform a number of different experiments in which some parameter is changed between each experiment; these applications also require waiting a period of approximately $5 \times T_1$ between successive experiments. If the duration of NMR signal acquisition in each experiment is short compared to the wait time between experiments, most of the instrument time will be spent as dead time waiting for the next experiment to start. This may increase the time needed for an NMR application by orders of magnitude in some cases.

It is possible to alleviate the difficulties posed by long T_1 time constants using a number of techniques. By implementing experiments which utilize smaller RF pulse durations, one can decrease the amount of time required to perform each experiment. For instance, use of a 30° pulse in place of a 90° pulse decreases the wait time between experiments by a factor of 3. It also decreases the SNR of each experiment by a factor of 2, since the magnetization generated in the xy plane during an RF pulse is proportional to the sine of the pulse angle. If each experiment generates a strong NMR signal, it may be worthwhile to take the penalty in SNR while gaining an advantage in signal acquisition time. Another possibility is to alter the T_1 of a sample by introducing an impurity, such as a paramagnetic metal complex like gadolinium (III) chloride, GdCl_3 . Such additives carry large numbers of unpaired electron spins, which create a large magnetic moment and facilitate relaxation. These paramagnetic agents dramatically decrease T_1 , allowing for a substantial decrease in the requisite wait time between successive NMR experiments.

It should be noted that long T_1 times are not always problematic. Any application which requires long-term storage of spin magnetization may benefit from having a long T_1 relaxation time. In particular, long T_1 times are of extreme importance to remote detection (RD) NMR, which is used extensively in the work in this thesis. RD-NMR operates by encoding information in the spin magnetization in one location and transporting that information to another location where it can be detected with far greater sensitivity. This requires that the nuclear spin magnetization stay out of thermal equilibrium until it can be moved to the detector location. As long as the magnetization can be moved to the detector location in a period of time faster than T_1 , the RD-NMR experiment can be carried out. For RD-NMR, it is therefore beneficial to use a system with a long T_1 relaxation time. The principles of RD-NMR are discussed in Section 2.10.

2.6.2 T_2 Relaxation

While the nuclear spin magnetization has a component in the transverse xy plane, the individual spins feel slightly different magnetic fields due to their different physiochemical environments. If these fields have a component along $\hat{z}$, the various spins will precess at slightly different rates. As the spins precess at their various rates, they tend to “spread out” in the transverse plane (mathematically, they develop different phase angles), a process known as decoherence. This has the effect of decreasing the amplitude of the transverse magnetization. This decoherence is often referred to as transverse relaxation, spin-spin relaxation, or T_2 relaxation. The phrase “transverse relaxation” comes from the fact that this process tends to orient nuclear magnetization within the transverse xy plane, perpendicular to the magnetic field. The phrase “spin-spin relaxation” comes from the fact that the relaxation is due to interactions which act on multiple spins. The phrase “ T_2 relaxation” reflects the fact that the decoherence of the transverse magnetization components M_x and M_y often follows an exponential trend with time constant T_2 . Assuming that all nuclear spin magnetization is initially directed along $\hat{x}$, ($M_x(0) = M_0$, $M_y(0) = 0$, $M_z(0) = 0$):

$$M_x = M_0 \sin(\gamma B_0 t) \exp(-t/T_2) \quad (2.33)$$

$$M_y = M_0 \cos(\gamma B_0 t) \exp(-t/T_2) \quad (2.34)$$

It is important to note that there are two different physical processes which lead to decoherence of the transverse nuclear spin magnetization. The first of these is due to magnetic field fluctuations which arise due to the random molecular motions of nearby nuclei, similar to the mechanism which gives rise to T_1 relaxation. This form of relaxation, referred to as “homogeneous relaxation”, cannot be avoided or corrected for. Additional relaxation arises as a result of magnetic field inhomogeneity caused by more ordered sources. Such sources include poor shimming of the NMR magnet (the magnet produces slightly varying fields at different locations in space), the presence of paramagnetic or ferromagnetic impurities, or differences in magnetic susceptibilities between different materials used in the NMR experiment. These effects are collectively referred to as “inhomogeneous relaxation” processes, and can be represented by a time constant T_2^{inhom} . The net transverse relaxation, referred to as T_2^* relaxation, is a combination of the intrinsic T_2 relaxation and the inhomogeneous relaxation:

$$\frac{1}{T_2^*} = \frac{1}{T_2} + \frac{1}{T_2^{inhom}} \quad (2.35)$$

As in the case of T_1 relaxation, T_2 relaxation places limitations on the NMR experiment. Generally, long T_2 relaxation times are desired, as this time constant determines the period of time for which an NMR signal may be sampled. Longer sampling times lead to greater resolution of the various components of the NMR signal. The effects of T_1 and T_2 relaxation on the NMR signal are shown in Figure 2.4.

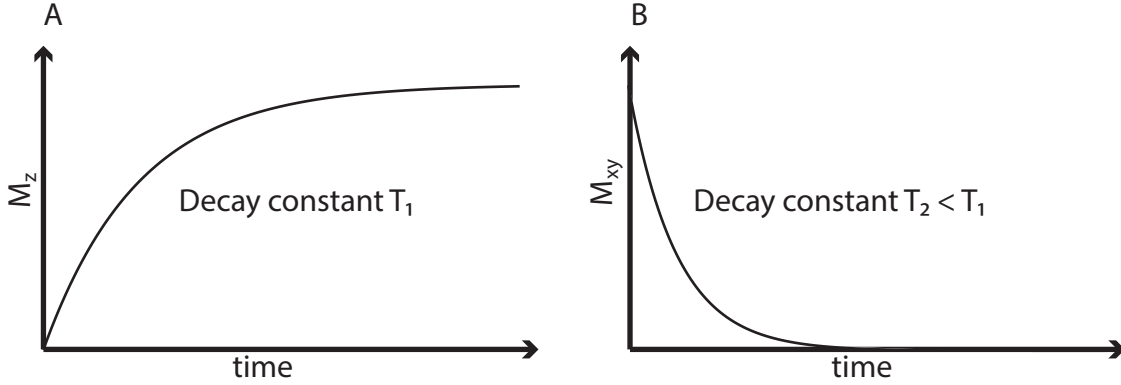


Figure 2.4: T_1 and T_2 Relaxation. A) Non-equilibrium magnetization in the xy plane relaxes to its equilibrium alignment along $\hat{z}$ with time constant T_1 . B) Magnetization in the xy plane decays with time constant T_2 .

Having discussed the relaxation processes, we are now in position to rewrite the Bloch equations to account for these processes:

$$\frac{d\mathbf{M}}{dt} = \mathbf{M} \times \gamma \mathbf{B} - \frac{M_x \hat{x} + M_y \hat{y}}{T_2} - \frac{(M_z - M_0) \hat{z}}{T_1} \quad (2.36)$$

Solution of this equation yields the dynamics discussed in the preceding Sections 2.6.1 and 2.6.2.

2.7 The Spin Echo

There is an inherent difference between homogeneous relaxation processes and inhomogeneous relaxation processes. The former are due to random fluctuations which cannot be anticipated; because of this, they cannot be corrected for. The latter, however, are much more systematic. If the local magnetic field inhomogeneities remain constant or vary in a predictable manner, it is possible to implement techniques to remove the effects of these interactions from the NMR signal.

The NMR pulse sequence capable of removing these effects is the spin echo experiment, invented in 1950 by Hahn [64]. Immediately after applying an RF pulse to generate magnetization in the xy plane, all of the transverse spin magnetization is aligned. If the various nuclear spins feel different azimuthal magnetic fields ranging from $B_0 - \Delta B_0/2$ to $B_0 + \Delta B_0/2$, the spins at low field will precess at a slower rate and accumulate a phase of $\gamma(B_0 - \Delta B_0/2)TE/2$ after a time $TE/2$. The spins at high field will precess at a higher rate and accumulate a phase of $\gamma(B_0 + \Delta B_0/2)TE/2$, resulting in a phase difference of $\gamma\Delta B_0TE/2$ between the nuclear spins at the highest magnetic field and those at the lowest magnetic field. At this point, an RF pulse is applied to generate a rotation of the spins by 180° . This inverts the positions

of the nuclear spins, so that we now have a phase difference of $-\gamma\Delta B_0TE/2$ between the nuclear spins at the highest magnetic field and those at the lowest magnetic field. During another time period $TE/2$, the spins which precess at a higher rate will begin to catch up to those which precess at a lower rate, which they now lag. During this time, the nuclear spins accumulate another phase difference of $\gamma\Delta B_0TE/2$, resulting in a total phase difference of zero. After a total time TE (known as the spin-echo time), the nuclear spin magnetization has recohered, removing the inhomogeneous contribution to the transverse relaxation. This recoherence is known as the spin echo, as it causes a signal which would otherwise fade away due to T_2^* processes to be regenerated after a time TE .

Spin echoes are ubiquitous elements in NMR pulse sequences, as they allow signal losses to be minimized and provide exceptional cancellation of artifacts which arise due to the various magnetic field inhomogeneities described above. They are central to nearly every NMR pulse sequence employed in this thesis, especially in RD-NMR, where they are used to recover the full NMR signal immediately prior to storage of magnetization in preparation for transport to the detector.

2.8 The NMR Experiment

Having spent a good amount of time describing the dynamics of the various processes which occur during an NMR experiment, we are now in position to describe the signal produced during the NMR experiment. The general NMR experiment proceeds as follows. A sample of interest is placed in a static magnetic field and develops a macroscopic magnetization along the direction of the applied magnetic field. RF pulses are applied at the Larmor frequency of the nuclei to move the magnetization vector into the xy plane. The magnetization vector precesses about the longitudinal magnetic field, generating a time-varying magnetic flux. This time-varying magnetic flux induces an electromotive force in an inductive RF pickup coil surrounding the sample. The signal, known as the free-induction decay (FID), is then amplified and digitized for post-processing.

If we applied an RF pulse exactly on resonance with nuclear spins which all experienced the same magnetic field, we would obtain an FID consisting of a sine wave oscillating at the frequency of the RF pulse. The FID would decay in time due to a combination of T_1 and T_2 relaxation processes. Allowing different nuclear spins to experience different magnetic fields (so that, say, one population of spins precesses at the applied RF frequency ω_0 and another precesses at a frequency $\omega_0 + \Delta\omega$), the FID would be a linear combination of sine waves, with one oscillating at a frequency ω_0 and the other oscillating at $\omega_0 + \Delta\omega$. The coefficients in the linear combination would be proportional to the number of nuclear spins experiencing each magnetic field. As we add different interactions, so that numerous populations of spins precess at numerous different magnetic fields, the FID becomes extremely complicated. More information can be obtained by performing a Fourier transform (FT) on the FID in order to

obtain a frequency spectrum $F(\omega)$:

$$F(\omega) = \int_{-\infty}^{\infty} f(t) \exp^{-i\omega t} dt \quad (2.37)$$

The nuclear spins experience numerous different interactions which cause them to precess in the presence of slightly different magnetic fields. This enables experiments which can be used to determine the connectivity of atoms within a molecule, measure bond distances, and so on. Though many interactions are amenable to study by NMR, most of these are not necessary to understand the work presented in this thesis. We shall therefore only consider the chemical shift.

Chemical shift manifests as a slight change in the Larmor frequency of a population of nuclear spins due to interactions with their electrons. The motion of electrons surrounding a nucleus can be viewed as a small current, which creates a small magnetic field. This magnetic field either adds to the magnetic field felt by the nuclear spins (Van Vleck paramagnetism) or subtracts from it (Landau and Langevin diamagnetism) [141]. The extent to which a nucleus is affected by the electronic motion is represented by a shielding factor σ . This shielding factor perturbs the local magnetic field felt by the nuclear spins, so that they feel an effective magnetic field B_{eff} :

$$B_{eff} = B_0(1 - \sigma) \quad (2.38)$$

Given this effective magnetic field, the spins also display an effective Larmor frequency ω_{eff} :

$$\omega_{eff} = \omega_0(1 - \sigma) \quad (2.39)$$

Chemicals shifts are typically on the order of tens of Hz to a few kHz, in large magnetic fields which are on the order of tens to hundreds of MHz. In order to standardize NMR experiments across many different magnetic field strengths, the chemical shift δ_{ppm} is typically given in units of ppm (parts per million):

$$\delta_{ppm} = \frac{\omega_{eff} - \omega_0}{\omega_0} \times 10^6 \quad (2.40)$$

2.8.1 Measurement of T_1 and T_2

T_1 is typically measured using the inversion recovery (IR) experiment. In this experiment, magnetization aligned along the $\hat{z}$ direction is tipped into the $-\hat{z}$ direction by applying a 180°

pulse. During a delay period τ_{IR} , magnetization relaxes back toward thermal equilibrium with time constant T_1 . After this delay, a 90° pulse is applied to obtain the projection of the longitudinal magnetization on the xy plane. The amplitude of the FID is recorded and τ_{IR} is incremented. The resultant curve of FID magnitude versus delay time is exponential with time constant T_1 .

T_2 is typically measured using the Carr-Purcell-Meiboom-Gill (CPMG) experiment [28, 108]. This experiment uses a series of 180° pulses to create numerous spin echoes. The amplitude of each spin echo decays with the transverse relaxation time T_2 . This time constant may be extracted from the resultant curve of spin echo intensity versus time.

2.8.2 Alternative Detection Modalities

NMR experiments conducted in large magnetic fields typically employ inductive RF pickup coils to detect precessing nuclear spin magnetization. The SNR per unit volume V has been derived using the principle of reciprocity [77]. For a common solenoid coil, the result is:

$$\frac{SNR}{V} = \frac{\omega_0^2 n / d_{coil} \sqrt{1 + (h_{coil} / d_{coil})^2}}{\sqrt{n^2 d_{coil} \omega_0^{1/2} / h_{coil}}} \quad (2.41)$$

$$\propto \omega_0^{7/4}$$

Here, d_{coil} is the diameter of the coil and h_{coil} is its height. From this, we note that an increase in the static magnetic field leads to a substantial increase in the SNR of the NMR experiment. A factor of ω_0 is due to the increasing thermal polarization at higher magnetic fields. The other factor of $\omega_0^{3/4}$ is due to the detection coil itself. As an inductive detector, the detection coil becomes more sensitive with increasing magnetic flux. This is another reason for which the trend has been toward the use of superconducting magnets producing ever larger magnetic fields. It also limits the application of inductive pickup coils in low magnetic fields. Fortunately, a number of alternative magnetic field sensors exist which allow sensitive detection of NMR signals at low magnetic fields.

The earliest non-inductive measurements of an NMR signal utilized a superconducting quantum interference device (SQUID) [166, 178]. These devices operate using the Josephson effect, or the quantum mechanical tunneling of electric current in an insulator placed between two superconductors in the absence of an external electromagnetic field. A SQUID uses two Josephson junctions in parallel in a superconducting loop. In the absence of a magnetic field, an applied current splits evenly between the two branches. If a small magnetic field is applied, a current begins to circulate in the loop and establishes a magnetic field which acts to cancel the external flux. The induced current is in the same direction as the applied

current in one Josephson junction and the opposite direction in the other junction. The induced current, and thus the magnetic field, can be measured by taking the difference between the currents in the two Josephson junctions. SQUID devices can be made very small and can measure magnetic fields as small as $3 \text{ fT Hz}^{-1/2}$ [45]. Unfortunately, SQUID sensors typically require liquid helium in order to produce a superconductivity effect. High-temperature SQUID devices utilizing liquid nitrogen have been explored; however, these devices are much less sensitive.

NMR signals can also be detected using magnetoresistive devices [169]. These devices measure a change in the electrical resistance of a thin film material when it is subjected to a magnetic field. Numerous types of these sensors exist, each of which is based on a different mechanism: giant magnetoresistance (GMR), colossal magnetoresistance (CMR), tunnel magnetoresistance (TMR), anisotropic magnetoresistance (AMR). For instance, the AMR effect arises due to changes in the scattering cross-sections of atomic orbitals in response to a magnetic field. These devices can be made extremely small and do not require cryogenic cooling. However, they are orders of magnitude less sensitive than SQUID devices, reaching sensitivities only as low as $100 \text{ pT Hz}^{-1/2}$.

A final class of magnetic field sensor used to detect NMR signals is the alkali vapor atomic magnetometer [181, 95]. These devices operate on the principle of magneto-optical rotation. A circularly polarized laser tuned to an electronic transition of an alkali metal vapor transfers polarization to the valence electron of the alkali metal, creating a large magnetic moment in a phenomenon known as optical pumping. If the alkali atoms experience a magnetic field transverse to the direction of their magnetic moment, the magnetic moment will precess about this field. A linearly polarized laser, composed of a superposition of left and right circularly polarized components, is sent into the alkali vapor transverse to the optical pumping beam. The oppositely polarized components have slight different indices of refraction in the alkali vapor, causing them to travel at slightly different speeds through the alkali vapor; this phenomenon is known as circular birefringence. This has the effect of changing the coefficients of the linear combination and causes a phase to accumulate over time. This manifests as a change in the direction of linear polarization which is proportional to the magnetic field. This rotation of the linear polarization can be measured very sensitively using numerous techniques. Alkali vapor magnetometers have achieved exquisite sensitivities below $1 \text{ fT Hz}^{-1/2}$ [90]. In contrast to SQUID devices, they do not require cryogenics. The main weak point of alkali atomic magnetometers is limitations to their miniaturization: these devices cannot be made smaller than approximately 1 mm^3 without sacrificing sensitivity [62]. Alkali atomic magnetometers are utilized in work presented in this thesis and will be further explored in Chapter 3.

2.9 The MRI Experiment

In contrast to NMR spectroscopy, MRI rarely relies on intramolecular interactions. In its most common implementation in medical imaging, the target nuclear species is usually the

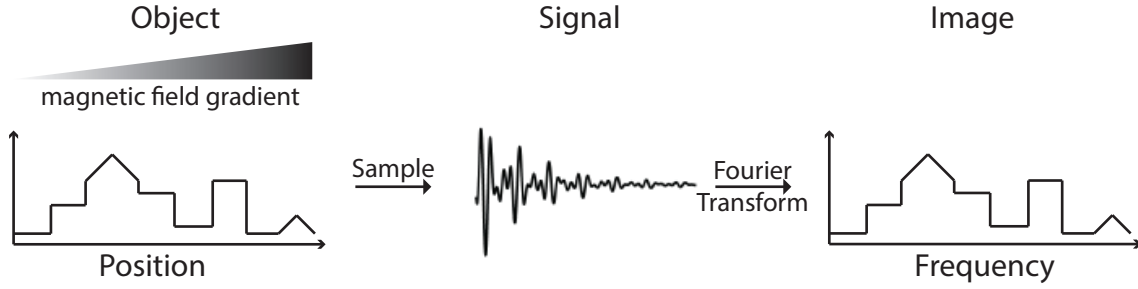


Figure 2.5: Frequency Encoding. A spatially-varying magnetic field $B_z = B_0 + G_x x$ is applied. This has the effect of mapping the Larmor frequency to position x . The NMR signal from each location x is proportional to the number of nuclear spins at that location; the net NMR signal is a linear combination of the NMR signal from the spins located at each position. Fourier transformation yields a cross-section of the nuclear spin distribution.

protons in water. As the protons in water are all chemically equivalent, an NMR spectrum of water yields exactly one peak. For spectroscopic purposes, this is extremely uninteresting. For imaging purposes, however, it can be exploited to serve as a spatial probe. The fundamental insight is to create a linear map between a magnetic resonance parameter, such as the Larmor frequency, and location in space. This can be accomplished by creating spatially-varying magnetic fields of the form:

$$\mathbf{B} = (B_0 + \mathbf{G} \cdot \mathbf{r})\hat{\mathbf{z}} \quad (2.42)$$

This creates a spatially-varying Larmor frequency:

$$\omega = \omega_0 + \gamma \mathbf{G} \cdot \mathbf{r} \quad (2.43)$$

Here, $\mathbf{G} = G_x \hat{\mathbf{x}} + G_y \hat{\mathbf{y}} + G_z \hat{\mathbf{z}}$ is referred to as a magnetic field gradient. The effect is to create a Larmor frequency which is dependent on nuclear spin position. The usefulness of gradient techniques can be most easily understood by imagining a one-dimensional MRI experiment. If we apply a magnetic field gradient in the direction of $\hat{\mathbf{x}}$, the magnetic field becomes $\mathbf{B} = (B_0 + G_x x)\hat{\mathbf{z}}$ and the Larmor frequency becomes $\omega = \omega_0 + \gamma G_x x$. After the nuclear spin magnetization is tipped into the xy plane, the spin populations at the various locations precess at Larmor frequencies tied to their location. The FID is a linear combination of all of the various Larmor frequencies from each location, with the coefficients of each component being the linear density of nuclear spins at each location. Fourier transforming the FID yields a one-dimensional cross-section of the object. An illustration of this scheme, known as frequency encoding, is shown in Figure 2.5.

2.9.1 Phase Encoding and k-space

The frequency encoding technique works well for one-dimensional imaging applications. It can be extended to acquire images in two or three dimensions by rotating the direction of the applied gradient to cover a circle (for two-dimensional imaging) or a sphere (for three-dimensional imaging). This technique, known as projection-reconstruction, has some benefits, such as an intrinsic robustness against imaging artifacts associated with patient respiration [57]; however, it requires a separate experiment to sample each gradient orientation, leading to long image acquisition times. The time required to obtain an image can be significantly reduced by employing phase encoding techniques. We will examine this technique for the two-dimensional case; the generalization to three dimensions is straightforward. Consider a distribution of nuclear spins described by a function $m(x, y)$. Applying a 90° pulse tips the magnetization in the direction of $\hat{\mathbf{x}}'$. In the absence of a magnetic field gradient, the magnetization remains aligned along $\hat{\mathbf{x}}'$, fixed in the rotating frame. Applying a gradient G_x , the Larmor frequencies of various populations of nuclear spins change, so that they acquire a time- and position-dependent phase $\phi(x, t) = \gamma G_x x \tau_x$, where τ_x is the length of time the G_x gradient is applied. The resulting NMR signal is given by $m(x, y) \exp(-i\gamma G_x x \tau_x)$. If we apply simultaneous gradients G_x and G_y , the NMR signal $s(t)$ is given by:

$$s(t) = \int_x \int_y m(x, y) e^{-i\gamma G_x x \tau_x} e^{-i\gamma G_y y \tau_y} dx dy \quad (2.44)$$

This can be simplified by introducing the reciprocal space, commonly referred to in MRI parlance as k-space. The $\hat{\mathbf{x}}$ component k_x is given by:

$$\begin{aligned} k_x &= \frac{\gamma}{2\pi} G_x \tau_x \\ &= \gamma G_x \tau_x \end{aligned} \quad (2.45)$$

The $\hat{\mathbf{y}}$ component k_y is given by:

$$k_y = \gamma G_y \tau_y \quad (2.46)$$

Then, the signal equation becomes:

$$s(t) = \int_x \int_y m(x, y) e^{-i2\pi k_x x} e^{-i2\pi k_y y} dx dy \quad (2.47)$$

This is just the two-dimensional Fourier transform between the frequency-space (the

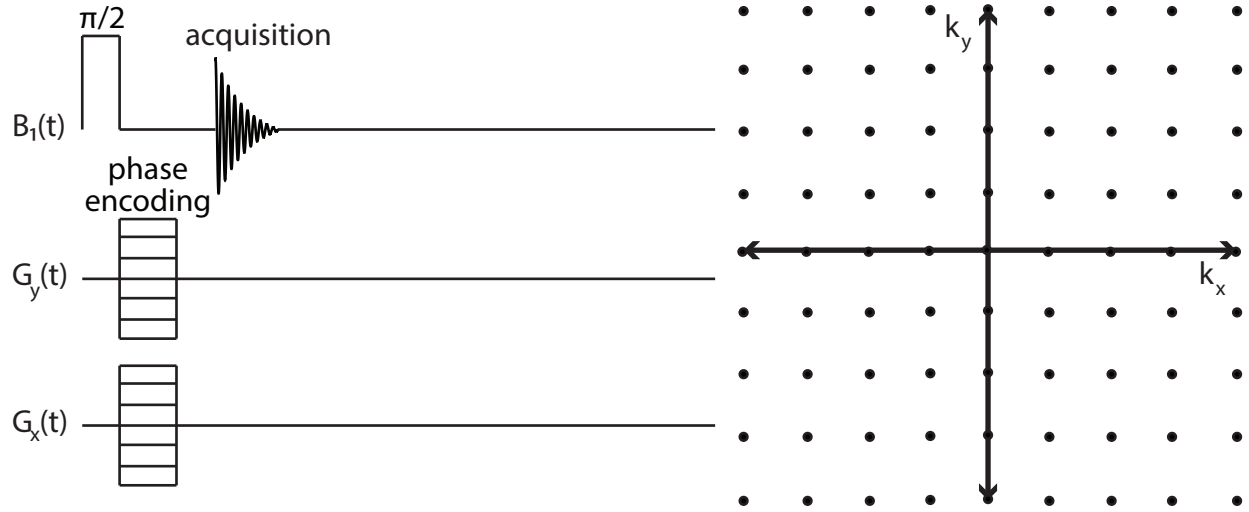


Figure 2.6: Imaging by Pure Phase Encoding. After excitation by an RF pulse, gradient G_x and G_y are turned on for a period of time. The amplitude of the FID at each point in (k_x, k_y) is recorded as each point in k-space is obtained. The resultant k-space trajectory is a series of single points.

time-dependent NMR signal $s(t)$) and k-space (the position-dependent spin encoding spatial frequencies k_x and k_y):

$$s(t) = \int_x \int_y m(x, y) e^{-i2\pi k_x x} e^{-i2\pi k_y y} dx dy \quad (2.48)$$

The spin distribution $m(x, y)$ can be recovered by obtaining NMR signals from each point in k-space and applying a Fourier transform. Since it is impossible to sample every possible point in k-space, the challenge in MRI experiments is to obtain enough data points in k-space to allow accurate reconstruction of the image. MRI pulse sequences employing phase encoding are typically differentiated by the manner in which they sample k-space. The simplest manner of sampling k-space is to use a “pure phase encoding” scheme, whereby a number of experiments are conducted: in each experiment, gradients G_x and G_y are applied for some time τ_x and τ_y , and the NMR signal is acquired. In each successive experiment, G_x and G_y are varied, until every possible point in k-space is sampled. This technique suffers from requiring a long amount of time to acquire the image. However, it is necessary for the remote detection MRI experiment, described in Section 2.10.2. It is also employed in spectroscopic imaging techniques, where an NMR spectrum of the chemical composition is reconstructed at each location in an image [153]. Figure 2.6 shows the pulse sequence and k-space trajectory for the pure phase encoding method.

The acquisition time of an image can be significantly decreased by a combination of phase encoding and frequency encoding in the two-dimensional Fourier transform (2DFT) experiment. For a two-dimensional experiment, a phase encode is performed in one dimension

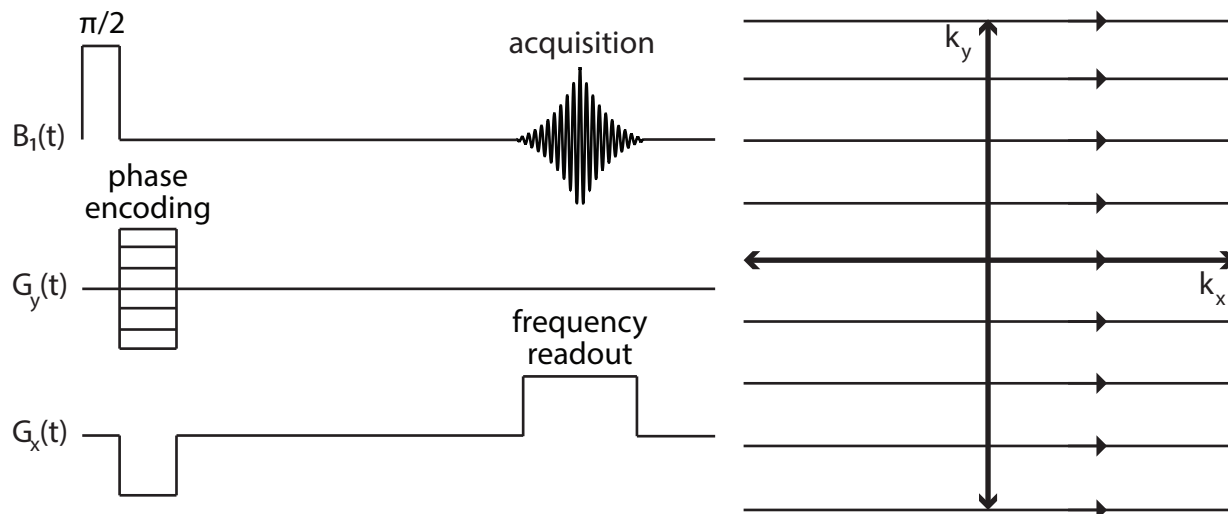


Figure 2.7: Two-Dimensional Fourier Transform (2DFT) Imaging. After excitation by an RF pulse, a gradient G_y is turned on for a period of time, giving a particular value of k_y . A gradient G_x is then turned on during readout of the nuclear magnetization, providing the FIDs at a series of values of k_x . After a “horizontal line” of k-space values has been obtained, the experiment is repeated with a different value of k_y , until a suitable amount of k-space has been filled. The resultant k-space trajectory is a series of horizontal lines.

(usually the y dimension) to reach a particular value of k_y . After the phase encode, a gradient in the x dimension is turned on, allowing sampling of that dimension through the frequency encoding mechanism. This gives NMR signals for a range of k_x values at a fixed value of k_y . The G_y gradient is then incremented, allowing k_x values to be obtained for another value of k_y . The k-space data is acquired by acquiring successive horizontal lines in k-space. Figure 2.7 shows the 2DFT pulse sequence and k-space trajectory.

Additional techniques exist to speed up the MRI experiment by allowing more rapid acquisition of k-space data. For instance, echo-planar imaging (EPI) allows the acquisition of data for all points of k-space in a single experiment [157] utilizing a switching G_x gradient, incrementing G_y gradients, and multiple spin echoes. EPI allows extremely quick acquisitions of k-space data; however, it is extremely susceptible to imaging artifacts. The EPI pulse sequence and k-space trajectory are shown in Figure 2.8.

2.9.2 Resolution and Field-of-View

Unlike other imaging modalities, MRI is generally a near-field technique (some exceptions exist, but they are beyond the scope of this thesis). The wavelength of the RF radiation used to induce spin transitions is on the order of a few meters. For instance, the wavelength in free space at 1.5 T (a commonly used magnetic field strength for clinical applications) is approximately 4.7 m. This is far larger than any sample of clinical interest. Thus, all

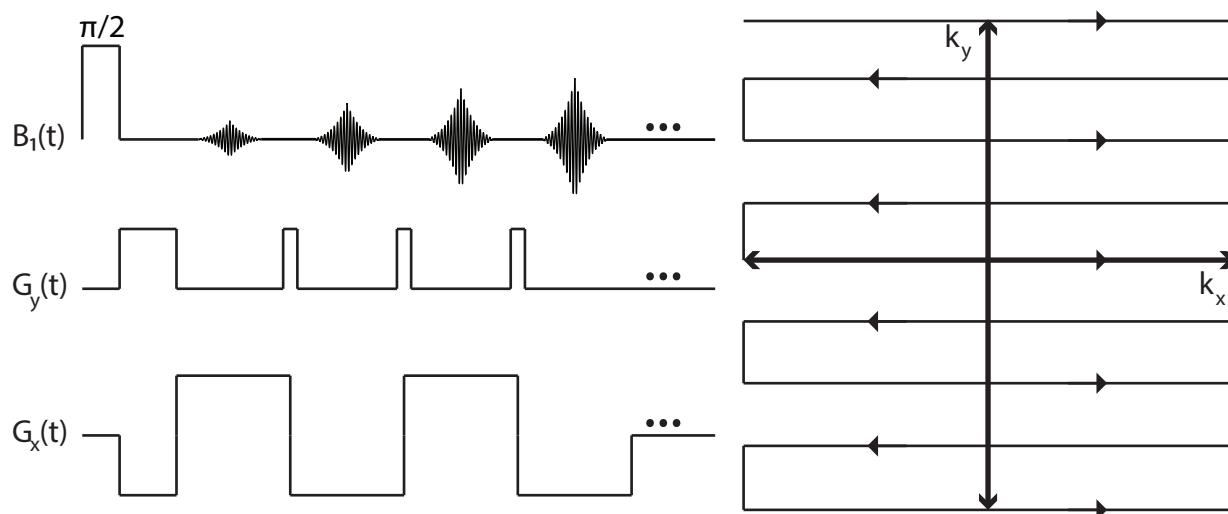


Figure 2.8: Echo Planar Imaging. After excitation by an RF pulse, a gradient G_y is turned on for a period of time, giving a particular value of k_y . A gradient G_x is then turned on during readout of the nuclear magnetization. In contrast to the 2DFT sequence, the readout does not stop at the end of a line in k -space. Instead, G_y is turned on again for a short time, providing a new value of k_y . G_x is given the opposite value, so that readout progresses in the opposite direction of decreasing k_x . Spin echoes are applied each time $k_x = 0$ in order to maintain a strong NMR signal. The resultant k -space trajectory is a series of alternating horizontal lines obtained in one pass.

interactions occur in the near field. Because of this, the usual Fraunhofer diffraction equation which governs optical imaging does not hold and the resolution is not limited by the wavelength. This is a very good thing, given that diffraction limited resolutions of a few meters are not useful for many interesting applications. Instead, the resolution of an MRI experiment is limited by the strength of the magnetic field gradient which can be applied. The Fourier uncertainty principle gives a relationship between the imaging resolution δ and the bandwidth of k-space W_k :

$$\delta W_k = 1 \quad (2.49)$$

For a typical 2DFT experiment featuring phase-encoding in y , the bandwidth will be the difference between the highest and lowest spatial frequencies applied: $W_k = \gamma G_{y,max} \tau_y - \gamma G_{y,min} \tau_y$. If the experiment covers k-space symmetrically, then $G_{y,max}$ and $G_{y,min}$ are equal and opposite and we find that $W_k = 2\gamma G_{y,max} \tau_y$. Thus:

$$\delta_y = \frac{1}{2\gamma G_{y,max} \tau_y}: \text{phase encoding} \quad (2.50)$$

For frequency encoding in x , sampling in k-space is generally achieved by applying the same readout gradient G_x for each phase encoding step in y . Because a single-sided gradient is used during the readout period, the expression for resolution in x is similar to that for resolution in y , but for the absence of a factor of 2:

$$\delta_x = \frac{1}{\gamma G_x \tau_x}: \text{frequency encoding} \quad (2.51)$$

The other relevant parameter in imaging, the field-of-view (FOV) is given by multiplying the spatial resolution by the number of samples taken in k-space, N_x and N_y . For the phase-encoded dimension, we have:

$$FOV_y = \frac{N_y}{2\gamma G_{y,max} \tau_y}: \text{phase encoding} \quad (2.52)$$

For the frequency-encoded dimension, we have:

$$FOV_x = \frac{N_x}{\gamma G_x \tau_x}: \text{frequency encoding} \quad (2.53)$$

Modern MRI equipment allows an experimenter to simply enter desired values for the FOV (typically taken to be slightly bigger than the object being imaged) and number of

data points in each dimension. Gradient and timing values are then calculated to give the desired imaging properties.

2.9.3 Slice Selection

Three-dimensional images may be obtained by a simple extension of the 2DFT scheme introduced in the previous Section 2.9.1. One could simply introduce another phase encoding dimension. More common is to separate the imaging task into slices through the third dimension. This is accomplished by applying a “shaped” 90° pulse, the Fourier transform of which contains the desired frequency profile which is to be tipped into the xy plane. By applying a gradient G_z for a time τ_z in the third dimension, a slice of the sample may be excited for imaging in the xy plane. By varying the frequency content of the pulse, two-dimensional images may be “stacked together” to form a three-dimensional image. The thickness of the excited slice Δz is given by:

$$\Delta z = \frac{1}{\gamma G_z \tau_z} \quad (2.54)$$

2.10 Remote Detection

While standard methods of detecting NMR signals are fantastic for obtaining signals from a standard-sized tube filled with liquid, they are not well-adapted to detecting signals from fluids flowing through microfluidic channels. A microfluidic device is often a rectangular prism a few centimeters long, a few centimeters wide, and approximately 1 mm thick (owing to production by wafer fabrication methods). It is filled with one of more fluidic channels which may vary greatly in length (anywhere from microns to centimeters), but which typically have widths and depths of $1\ \mu\text{m}$ to $100\ \mu\text{m}$. In a standard NMR experiment, a signal RF inductive coil (generally a solenoid) would be placed around the entire microfluidic device in order to both provide RF pulses and to receive the NMR signal. In order to enclose the entire microfluidic device, this receiver coil would need to have a length and diameter of a few centimeters. This leaves us with a large mismatch between the volume of our detection coil and the volume of the fluid being detected. The root-mean square signal-to-noise ratio SNR_{rms} of an NMR experiment is given by [77]:

$$\begin{aligned} SNR_{rms} &= K_{coil} \eta \sqrt{\frac{\mu_0 Q \omega_0 V_{coil}}{4 F k_B T_{coil} \Delta \nu_{coil}}} \\ &\propto \eta \sqrt{V_{coil}} \end{aligned} \quad (2.55)$$

Here, K_{coil} is a numerical factor (≈ 1) which is dependent on the coil geometry, η is

the so-called “filling factor” (the ratio of volume integral of B_1^2 in the sample to the total volume integral of B_1^2 , $\eta = \int_{\text{sample}} B_1^2 dV / \int_{\text{allspace}} B_1^2 dV$; for the special case of a solenoid, $\eta \approx V_{\text{sample}}/2V_{\text{coil}}$), M is the nuclear magnetization, μ_0 is the vacuum permeability, Q is the quality factor of the coil (a measure of the frequency selectiveness of the resonant coil; $Q = \omega_0 L_{\text{coil}}/R_{\text{coil}}$ where L_{coil} is the coil’s inductance and R_{coil} is the coil’s resistance), ω_0 is the Larmor frequency, V_{coil} is the volume of the receiver coil, F is the noise figure of the preamplifier, k_B is Boltzmann’s constant, T_{coil} is the temperature of the coil, and $\Delta\nu_{\text{coil}}$ is the bandwidth of the receiver.

From the perspective of a microfluidic NMR experiment, the receiver coil volume V_{coil} and the filling factor η are the most important factors in the signal-to-noise equation. We might be tempted to think that the SNR increases with an increasing coil volume. However, the SNR is more strongly dependent on the filling factor, which becomes very small in microfluidic experiments. For illustration, consider a 100 μm long microfluidic channel with a circular cross-section and a diameter of 50 μm , which is typical of the microfluidic channels employed in this thesis. Suppose that this channel is part of a microfluidic device which is 20 mm wide and 30 mm long; once again, this is typical of the microfluidic devices employed in this thesis. For such an experiment, we might try to detect the NMR signal using a solenoid with a diameter of 20 mm and a length of 30 mm. In this case, we would get a filling factor $\eta \approx 10^{-7}$.

If, instead, we could place a small solenoid with a diameter a little bigger than 50 μm and a length of 100 μm around the microfluidic channel, we would get a filling factor $\eta \approx 0.5$. Taking into account the loss in SNR due to the smaller coil volume (and accounting for the worse quality factor in a small solenoid), such a coil would increase the SNR by a factor of approximately 2000 as compared to the large volume coil considered in the previous paragraph. Therefore, one manner of increasing the SNR in a microfluidic NMR experiment would be to place a small solenoid at the location of each volume of interest on the microfluidic device. Even if we ignore the extreme difficulties involved in fabricating hundreds or thousands of these solenoids on a microfluidic device, the NMR experiment would suffer from cross-talk between the various receiver channels and the need to replicate the mixers, amplifiers, and other electronic circuitry for each receiver channel.

An elegant solution to this problem is the use of remote detection methods. This technique has been extensively developed in the Pines laboratory for the past decade and applied to numerous systems which utilize fluid flow [112, 106, 76, 60, 67, 61, 71, 72, 73, 6, 66]. The fundamental insight of remote detection is that the traditional NMR experiment can be split into two steps, encoding and detection, which can be separated both in space and in time. The first part of an RD-NMR or RD-MRI experiment, the encoding step, is carried out using a large inductive coil which encloses the entire system under study (e.g. a microfluidic chip). This coil can be used to apply pulses and encode chemical, spatial, velocimetric, and other information in the phase of the nuclear spin magnetization of a flowing fluid. The coil is also used to project the phase information along $\hat{z}$, so that the magnetization decays with the long T_1 relaxation time instead of the much shorter T_2^* relaxation time as it is transported to another location for detection. After the encoding step, the liquid flow to a separate

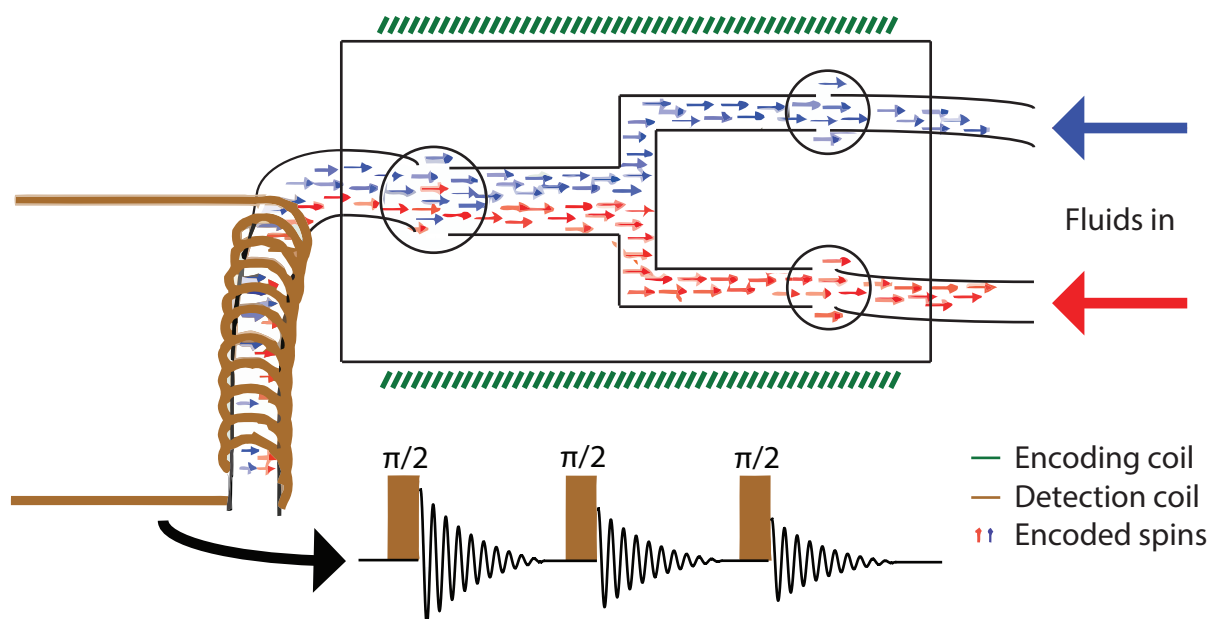


Figure 2.9: The Remote Detection NMR Setup. Fluid flows into a region of interest, where it is subjected to a series of encoding pulses from the encoding coil. Imaging information may be added through the use of magnetic field gradients. The magnetization is stored along $\hat{z}$ and transported by flow to a capillary which is surrounded by a micro-solenoid coil with a large filling factor, where the NMR signal is detected.

detection coil, such as a micro-solenoid wrapped under a capillary tube. Because this coil has a volume similar to that of the fluid being detected, the sensitivity of the experiment is significantly enhanced, usually by 2-3 orders of magnitude. Moreover, the separate encoding and detection coils allow for the individual optimization of each piece of hardware, including the use of the alternative detection modalities discussed in Section 2.8.2. Fourier transformation of the NMR signal will yield the spectroscopic or imaging data which has been encoded, as well as an additional time-of-flight (TOF) parameter. The basic RD-NMR setup is shown in Figure 2.9.

2.10.1 Remote Detection Travel Curves

The simplest RD-NMR experiment utilizes no encoding of spectroscopic or spatial information and consists only of a 90° excitation pulse, optional 180° spin-echo pulse, 90° storage pulse, and a series of 90° detection pulses. When combined with phase cycling that cancels out signal from unencoded nuclear spins, this experiment gives a series of FIDs arrayed along the TOF dimension. Stroboscopic 90° detection pulses are continuously applied. Initially, there is no encoded fluid in the region of the detection coil and no FID is obtained. As encoded fluid begins to arrive at the detection coil, the FIDs grow in amplitude. They then decline in amplitude as the last of the encoded fluid flows out of the detection coil. The travel curve experiment does not yield spectroscopic or imaging information, but does pro-

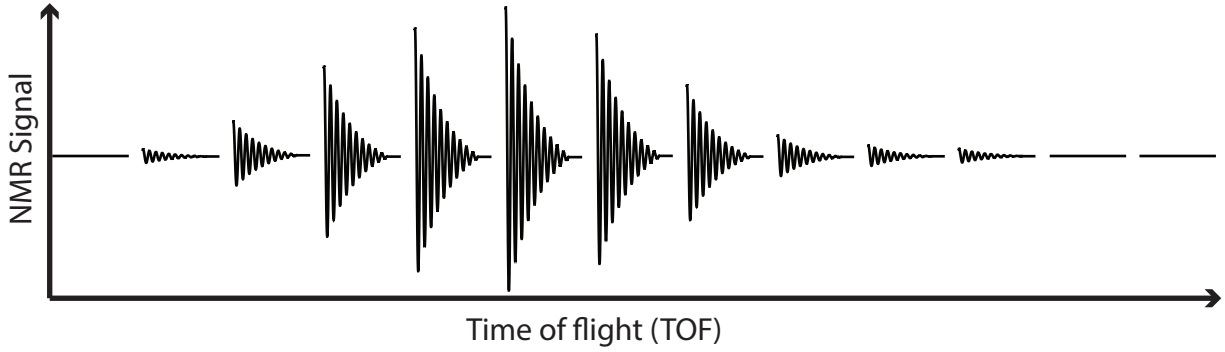


Figure 2.10: Remote Detection Travel Curve. The amplitude of the FIDs remains at zero until the first encoded fluid packets arrive at the detection coil. As encoded fluid arrives at the detection coil, the amplitude of the FID increases and hits a maximum value. It then decreases back to zero as the final encoded fluid packets arrive at the detection coil.

vide invaluable calibration information for further remote detection experiments. The flow rate may be altered to avoid excessive signal losses due to T_1 relaxation as the fluid flows through the microfluidic chip to the detector. The total duration of the signal acquisition can be increased if the travel curve has not reached its baseline level or decreased if too long of a period has passed with no FID. Finally, the time interval between 90° pulses in the stroboscopic acquisition can be set to allow enough time for each fluid packet to flow out of the detection coil prior to beginning the next signal acquisition. The travel curve experiment is shown in Figure 2.10.

2.10.2 Phase Encoding and Velocity Encoding

Following the 90° excitation pulse, phase encoding imaging gradients are applied in a manner very similar to those performed in a typical MRI experiment. However, additional steps must be taken to mitigate imaging artifacts which arise due to phase errors accumulated during flow through a magnetic field gradient. It is also possible to include phase-compensated velocity-encoding gradient pulses in the RD-MRI experiment. The theory of gradient moment nulling is described in reference [127]. We consider the major results here. The time-dependent phase of nuclear spins moving through a gradient field can be written:

$$\phi(t) = \gamma \int_0^t \mathbf{G}(\tau) \cdot \mathbf{r} d\tau \quad (2.56)$$

This phase can be expanded into a sum of moments corresponding to position, velocity, acceleration, etc. by utilizing a Taylor series expansion. We look at the $\hat{z}$ component; the analysis for the components along $\hat{x}$ and $\hat{y}$ are exactly analogous. Given a fluid packet with

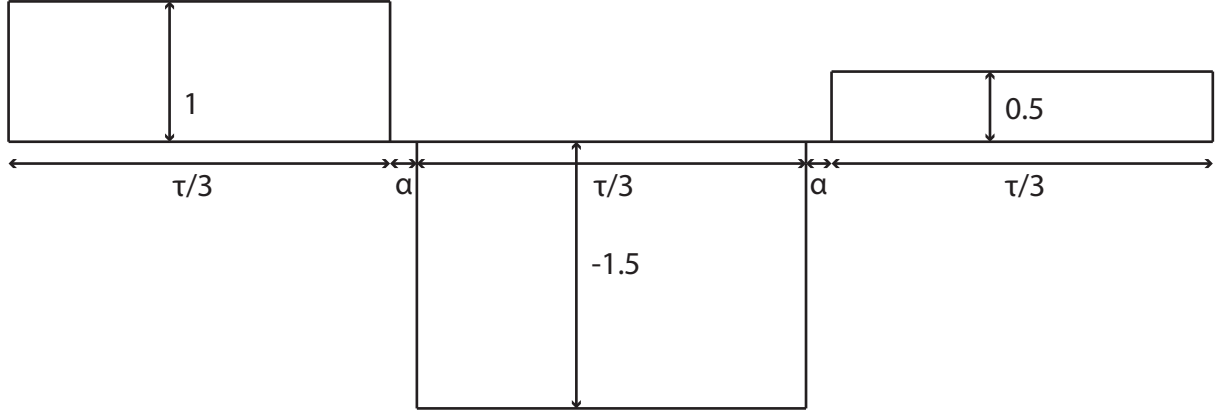


Figure 2.11: Velocity-Encoding Gradient Waveform. The velocity-encoding gradient waveform consists of three lobes of equal duration with relative amplitudes 1 : -1.5 : 0.5.

initial position z_0 , initial velocity v_0 , initial acceleration a_0 , etc., the phase can be written:

$$\phi(t) = \gamma z_0 \int_0^t G_z(\tau) d\tau + \gamma v_0 \int_0^t G_z(\tau) \tau d\tau + \frac{1}{2} \gamma a_0 \int_0^t G_z(\tau) \tau^2 d\tau + \dots \quad (2.57)$$

By choosing the proper gradient waveforms, it is possible to selective null any of these moments. Nulling of n moments requires the use of a gradient waveform with $n + 1$ gradient lobes. Two-lobe gradient waveforms allow nulling of the position in a velocity-encoding experiment, while three-lobe waveforms allow nulling of the position and the acceleration. If we take the phase difference between two experiments which employ gradients of opposite sign, the average velocity in each imaging voxel can be calculated. In the case of a three-lobe velocity-encoding experiment, we employ the gradient waveform shown in Figure 2.11. In this waveform, the relative amplitudes of the gradient lobes are 1 : -1.5 : 0.5. Each of the lobes is turned on for the same period of time $\tau_{ve}/3$. An additional time τ_{rise} (typically about 100 μ s) is included to allow for the gradients to rise to their full values. Integrating the velocity moment over the time $\tau_{ve} + 2\tau_{rise}$, we get the velocity associated with a particular phase:

$$v_0 = \frac{2\pi\phi}{360\gamma G_z} \left(\frac{\tau_{ve}^2}{9} + \frac{\tau_{rise}\tau_{ve}}{3} \right)^{-1} \quad (2.58)$$

By employing a wide range of velocity-encoding gradient values, it is possible to sample the full velocity distribution within each imaging voxel and create a “q-space” inverse-velocity distribution [27]. The velocity field is then recovered by inverse Fourier transformation.

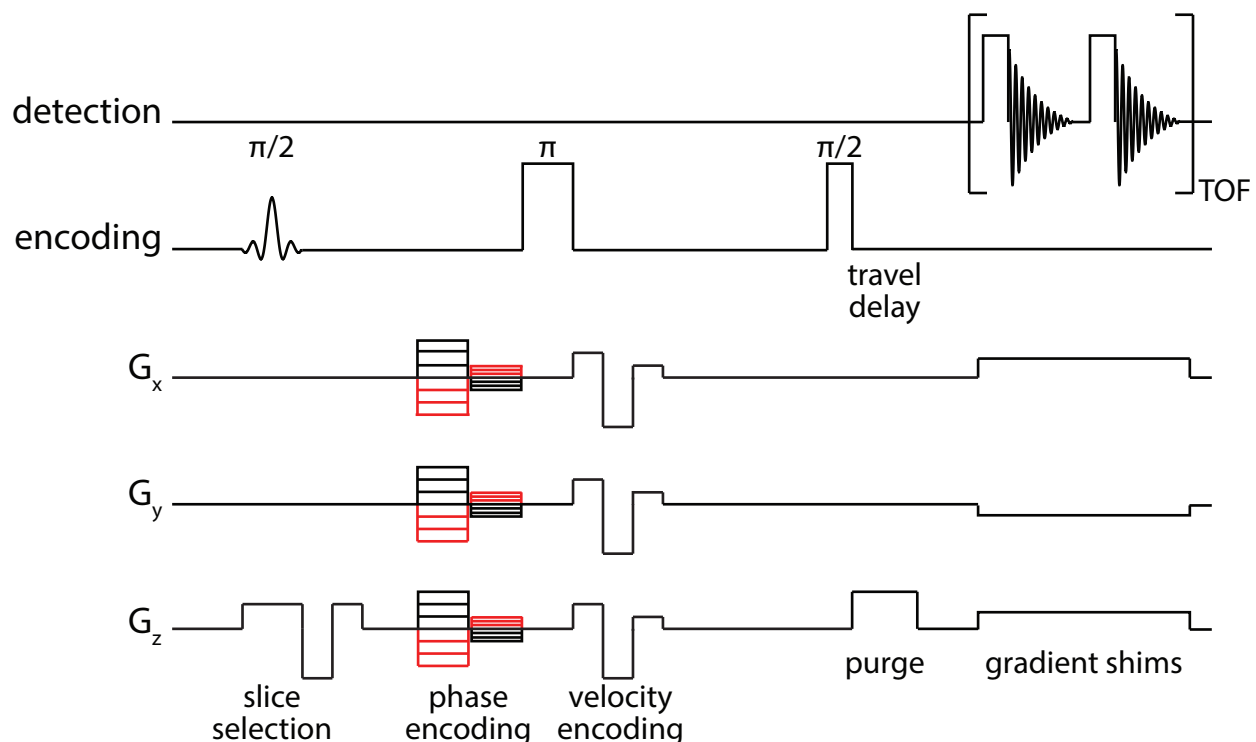


Figure 2.12: Remote Detection MRI Pulse Sequence. In the encoding step, nuclear spins are pushed into the transverse plane with a 90° pulse, which may be a shaped pulse in the presence of a magnetic field gradient to allow slice selection. Following the excitation, spectroscopic or imaging information is encoded in the nuclear spin magnetization in the same manner as a traditional NMR or MRI experiment. The magnetization is projected along $\hat{z}$ by employing a 90° “storage” pulse. The encoded fluid travels to a detection region in the presence of a crush gradient, which destroys any remnant magnetization in the xy plane. The encoded fluid is detected stroboscopically in the detection coil.

2.10.3 Detection

After spectroscopic, spatial, and velocimetric encoding, the nuclear spin magnetization is rotated 90° to lie along $\hat{z}$, which allows the magnetization to decay with the long T_1 time constant as it is transported to the detection region. This storage pulse maps a two-dimensional variable (the $\hat{x}$ and $\hat{y}$ components of the magnetization) onto a one-dimensional variable (magnetization along $\hat{z}$). In order to recover both components, a four-step phase cycle is employed. As the fluid travels to the detector, a crush gradient is applied along $\hat{z}$ to dephase any magnetization which remains in the xy plane. The detection occurs with a stroboscopic series of 90° pulses, followed by reception and amplification of the NMR signal. A representative RD-MRI experiment, employing position encoding, velocity encoding, crush gradients, and stroboscopic detection, is shown in Figure 2.12.

Chapter 3

A Microfabricated ^{129}Xe Polarizer

In Section 2.3.1, we briefly considered the many orders of magnitude enhancement in the NMR signal which is achievable using various hyperpolarization schemes. In this chapter, we focus on the miniaturization of instrumentation for implementing one of these methods, SEOP of ^{129}Xe gas. We examine a novel microfluidic device which has been micromachined from silicon (Si) and Pyrex glass and is capable of both producing hyperpolarized ^{129}Xe gas and detecting the NMR signal using an integrated alkali vapor atomic magnetometer. When integrated with the microfluidic XBMSs discussed in Chapter 4, this device may allow for the highly sensitive detection of arbitrary chemicals in complex mixed fluids. Although the miniature ^{129}Xe polarizer has been developed with application to XBMSs in mind, we forgo a discussion of these sensors until Chapter 4 in an attempt to maintain generality of the work and avoid relegating it to a particular application.

The chapter is organized as follows. In Section 3.1, we motivate this work by examining previous applications of hyperpolarized ^{129}Xe . In Section 3.2, we consider the SEOP method by which hyperpolarized ^{129}Xe is produced; we develop the mathematics of the optical pumping (Section 3.2.1) and spin exchange (Section 3.2.2) processes and examine the design of current large-scale ^{129}Xe polarizers (Section 3.2.3). In Section 3.3, we examine the operation of the alkali vapor atomic magnetometers used in this work. In Section 3.4, we consider the development of a first-generation microfabricated ^{129}Xe polarizer constructed from Si and glass; we describe the design (Section 3.4.1), fabrication (Section 3.4.2), and experimental setup (Section 3.4.3) before turning our attention to results concerning the production and remote detection of hyperpolarized ^{129}Xe (Section 3.4.4), characterization of the hyperpolarized ^{129}Xe (Section 3.4.5), characterization of the device's NMR parameters (Section 3.4.6), and analysis of the role played by diffusion (Section 3.4.7), ending with a comparison of the first-generation device to previous large-scale ^{129}Xe polarizers (Section 3.4.8). In Section 3.5, we consider a second-generation device which has been designed to overcome limitations of the first-generation device; we describe the new device design (Section 3.5.1), improvements in fabrication of the device (Section 3.5.2), improvements in the procedure for filling the device with an alkali metal (Section 3.5.3), improvements in the gas handling system (Section 3.5.4), improvements in the optical system (Chapter 3.5.5), and the implementation of

Table 3.1: NMR Properties of Stable and Long-Lived Isotopes of Xenon

Isotope	Abundance	Stability	I	γ ($10^6 \text{ rad s}^{-1} \text{ T}^{-1}$)
^{124}Xe	0.095%	Predicted $\beta\beta$ Decay [123]	0	N/A
^{126}Xe	0.089%	Predicted $\beta\beta$ Decay [123]	0	N/A
^{128}Xe	1.910%	Stable	0	N/A
^{129}Xe	26.401%	Stable	1/2	73.997
^{130}Xe	4.071%	Stable	0	N/A
^{131}Xe	21.232%	Stable	3/2	22.089
^{132}Xe	26.909%	Stable	0	N/A
^{134}Xe	10.436%	Predicted $\beta\beta$ Decay [123]	0	N/A
^{136}Xe	26.909%	$\beta\beta$ Decay: $2.165 \times 10^{21} \text{ y}$ [2]	0	N/A

imaging capabilities (Chapter 3.5.6).

This chapter contains material previously published in reference [84]. It has been reproduced here with the consent of its coauthors.

3.1 Applications of ^{129}Xe Gas in NMR and MRI

Xenon is the heaviest of the stable noble gases, with an atomic number of 54. Xenon consists of five observationally and theoretically stable isotopes, three observationally stable isotopes predicted to undergo double beta decay, one isotope which has been observed to undergo double beta decay with a half-life of more than 10^{21} years, and more than 30 radioactive isotopes with half-lives ranging from approximately 100 nanoseconds to just over one month. Of the nine isotopes which are stable or persist for a very long period of time, just two have a non-zero nuclear spin and are capable of producing NMR signals. ^{131}Xe is a spin-3/2 nucleus with a very large nuclear quadrupole moment, which gives it a very short longitudinal relaxation time of less than 50 ms when dissolved in most solvents [100]. Although this makes ^{131}Xe difficult to use in NMR experiments, a few studies have been performed using this isotope [160]. However, the spin-1/2 ^{129}Xe is far more commonly used in NMR studies, due to its unique NMR properties. Table 3.1 tabulates the important NMR properties of the nine stable or long-lived isotopes of xenon.

The ^{129}Xe isotope has been employed extensively in NMR and MRI experiments. It has a number of properties which make it an ideal NMR probe. ^{129}Xe is a spin-1/2 nucleus and thus undergoes no quadrupolar or higher order interactions. This leads it to have very long relaxation times in the solid [55], liquid[131], and gaseous[4] phases, and even when dissolved into numerous solvents[121]. Compared to most gases, xenon is very soluble in many different solvents [126], allowing it to be used as a probe in many different chemical environments. As an inert gas, xenon does not react with most molecules (though it does form compounds with chlorine, fluorine, oxygen, and a few heavy metals), making it essentially non-perturbing when introduced into a sample. Since xenon makes up a minuscule part of

the atmosphere (a little less than 9 ppm mole fraction), it is not naturally present in most samples of interest; this means that, in contrast to proton NMR and MRI studies, ^{129}Xe experiments have no appreciable background signal to deal with. It has a relatively high natural isotopic abundance (making up more than a quarter of all xenon in existence) and a relatively high gyromagnetic ratio (about one fourth that of protons). The large electron cloud is highly polarizable and therefore displays a large NMR response to changes in its physiochemical environment, making it a very sensitive reporter of its physical and chemical environment. This large electron cloud also allows for strong hyperfine interactions between ^{129}Xe and optically pumped alkali atoms, allowing for substantial hyperpolarization through SEOP.

These advantages have been exploited in numerous ^{129}Xe NMR studies. The earliest applications focused on imaging of the lung [114], which is very difficult to do with proton MRI due to the low proton density in the lung and short T_2^* times which arise due to magnetic field inhomogeneities caused by the many alveolar-air interfaces [159]. Other studies have utilized hyperpolarized ^{129}Xe imaging in the brain [162]. For a review of these studies, see the recent article by Mugler [113]. These imaging studies mainly took advantage the 4-5 order of magnitude enhancement in the ^{129}Xe NMR signal achievable by hyperpolarization techniques, but did not exploit the numerous other advantages listed in the previous paragraph. Later use of ^{129}Xe for NMR applications included imaging of other biological tissues, probing the structure and dynamics of molecules in solution, surface studies, and transfer of polarization from hyperpolarized ^{129}Xe to other nuclei; for a review of this work, see the article by Goodson [59]. More recent work has focused on combining hyperpolarized ^{129}Xe with XBMSs for the targeted detection of biomolecules [155, 145]. Readers interested in these studies should consult the recent review by Schröder [144]. XBMSs are also discussed in Chapter 4.

Work has been performed to utilize hyperpolarized ^{129}Xe in microfluidic devices [76]. However, the large-scale and non-portable nature of current ^{129}Xe hyperpolarization hardware precludes the direct integration of this technology into microfluidic platforms. Most ^{129}Xe polarizers have been designed to produce litre-sized volumes of polarized gas [44, 136]. These systems, designed for clinical magnetic resonance imaging or the large-scale imaging of materials, are necessarily bulky, expensive, and non-portable. This forces a microfluidic experiment, which is intended to be conducted on small, portable instrumentation, to rely on lab-scale hardware, and removes many of the advantages of turning to microfluidics in the first place. In order to overcome these limitations, we engineered a device capable of producing hyperpolarized ^{129}Xe at volume scales compatible with typical microfluidic applications.

3.2 Production of Hyperpolarized ^{129}Xe

As discussed in Section 2.3.1, SEOP can be used to greatly enhance the ^{129}Xe NMR signal by creating a long-lived non-equilibrium nuclear spin distribution. The SEOP method

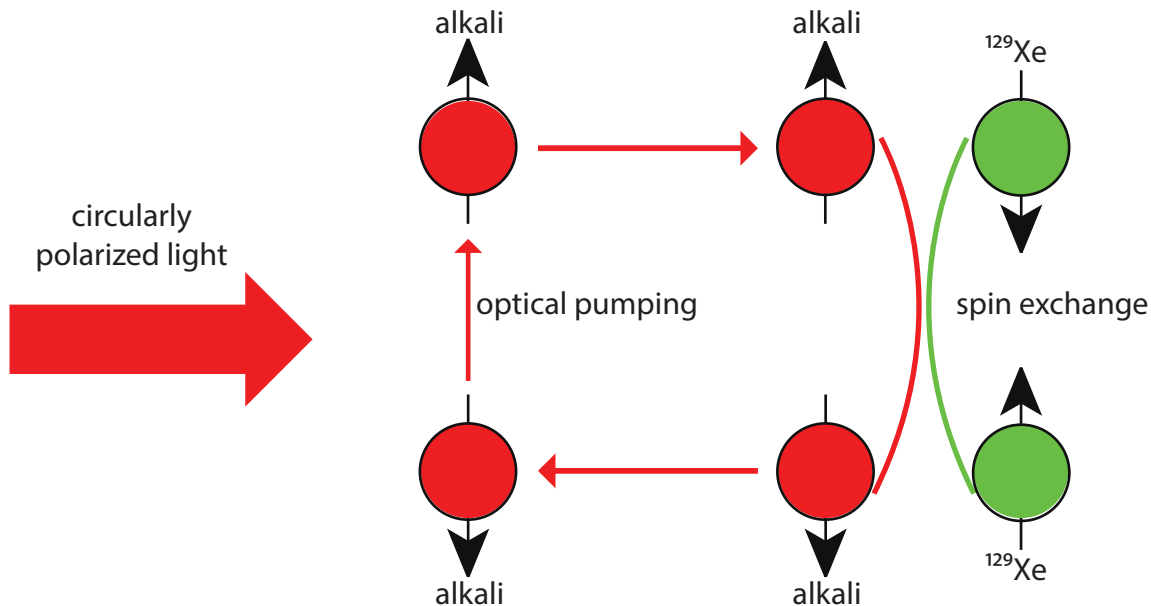


Figure 3.1: Spin-Exchange Optical Pumping (SEOP). Angular momentum is transferred from circularly polarized laser light to the ^{129}Xe nucleus through the optical pumping and spin-exchange processes.

was invented in the 1970s by William Happer [68]. It is a two-step process by which angular momentum is transferred from photons, which can easily be prepared into pure spin states, to nuclear spins. In the first step, optical pumping, angular momentum is transferred to the valence electron of an alkali atom by irradiating an alkali vapor with circularly polarized laser light tuned to an electronic transition of the metal. In the second step, spin exchange, collisions between the spin-polarized valence electrons and noble gas atoms allows for the transfer of polarization to the noble gas nuclei. This scheme is illustrated in Figure 3.1. In the following sections, we develop the mathematics of the SEOP process; in doing so, we use results obtained in the comprehensive treatment by Walker and Happer [171].

3.2.1 Optical Pumping of Alkali Atoms

In the optical pumping step, angular momentum is transferred from a flux of circularly polarized photons to the valence electron of a population of alkali metal atoms. Alkali metals are utilized for a number of reasons. Because these atoms have only a single valence electron, they can be reasonably well-approximated as a one-electron system, greatly simplifying the mathematical description. Their electronic ground state is spherical, further simplifying the mathematics. Moreover, the valence electron is easily polarized, as only transitions from the spherical s ground state are available. These transitions generally lie in the near infrared (NIR) part of the electromagnetic spectrum, for which diode lasers are readily available. Finally, the alkali valence electron can be optically pumped into a non-equilibrium spin state distribution with a relatively long lifetime, allowing later efficient transfer of polarization

Table 3.2: Alkali Electronic Transitions

Alkali Isotope	^{39}K	^{41}K	^{85}Rb	^{87}Rb	^{133}Cs	Reference
Isotopic Abundance (%)	93.3	6.7	72.2	27.8	100	
Nuclear Spin	3/2	3/2	5/2	3/2	7/2	
Nuclear g factor	0.261	0.143	0.539	1.827	0.732	[5]
D1 Vacuum Wavelength (nm)	770.1	770.1	795.0	795.0	894.6	[92]
D2 Vacuum Wavelength (nm)	766.7	766.7	780.2	780.2	852.3	[92]
D1 Oscillator Strength	0.324	0.324	0.332	0.332	0.347	[111]
D2 Oscillator Strength	0.652	0.652	0.668	0.668	0.721	[111]

from the alkali to the noble gas. Recently results have demonstrated alkali polarization lifetimes in excess of 1 minute using specially coated cells [7]. Of the alkali metals, rubidium (Rb) and cesium (Cs) are typically utilized due to their high vapor pressures and large hyperfine couplings with noble gas nuclei. Cs will typically allow the attainment of higher noble gas polarizations due to its high spin-exchange cross-section with noble gases; however, diode lasers producing light at the wavelengths of the electronic transitions of Rb have been historically more available. For that reason, noble gas polarizers have typically used Rb instead of Cs, though this may change as diode lasers are developed at wavelengths conducive to optical pumping of Cs.

Any ^{129}Xe polarizer, large or small, will generally require a few important elements. Rb or Cs must be trapped in a “pumping cell” (typically a few grams in a cylinder or sphere of Pyrex or other borosilicate glass for large-scale polarizers, or a few milligrams in a rectangular prism of Si and Pyrex for our small-scale polarizers). This cell is often coated with silane compounds to reduce relaxation which occurs due to interactions between the alkali atoms and the walls of the pumping cell. The Rb or Cs must then be heated to a temperature above 80 °C to ensure a high enough vapor pressure to allow efficient transfer of polarization to the noble gas. A static magnetic field (typically between 1 mT and 10 mT for large-scale polarizers and between 1 μT and 10 μT for our small-scale polarizer) is required to lift the degeneracy of the magnetic Zeeman sublevels of the alkali metal electronic states and allow optical pumping. A polarizer requires a diode laser tuned to an electronic transition of the alkali metal and various optical components to shape the beam and impart circular polarization. Finally, a mixture of Xe and N₂ (which sometimes includes He as well) gases is provided to the pumping cell; the N₂ gas is included to quench radiative transitions to the ground state, which act to reduce the polarization.

The laser beam may be tuned to excite alkali metal electrons from the $^2\text{S}_{1/2}$ ground state to either the $^2\text{P}_{1/2}$ excited state (D1 transition) or the $^2\text{P}_{3/2}$ excited state (D2 transition). Figure 3.2 shows the alkali electronic energy levels, magnetic sublevels, and the D1 and D2 transitions. Table 3.2 gives the natural isotopic abundances, nuclear spins, nuclear g factors, vacuum D1 and D2 transition wavelengths, and oscillator strengths for the alkali isotopes which are commonly employed in optical pumping.

The polarization optics alter the beam to consist only of photons possessing either -1 (right-handed circular polarization) or +1 (left-handed circular polarization) units of angular

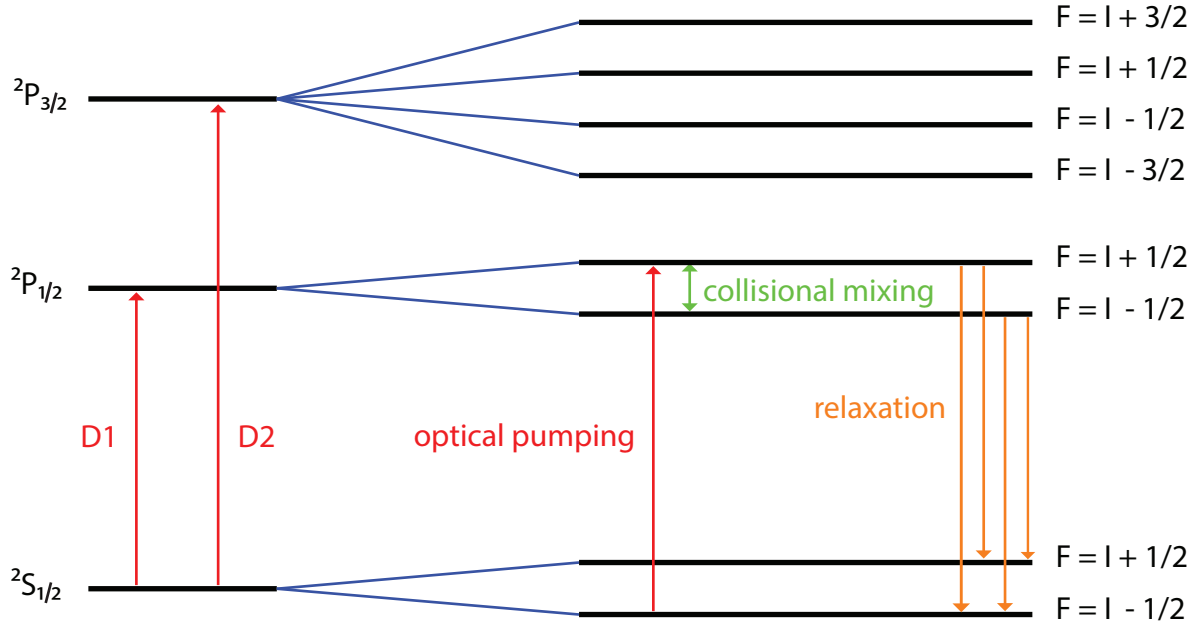


Figure 3.2: Alkali Electronic Energy Levels. Alkali atoms have a $^2S_{1/2}$ ground state and $^2P_{1/2}$ and $^2P_{3/2}$ electronic excited states; these electronic energy states are split into magnetic sublevels by the Zeeman interaction. Transitions into the $^2P_{1/2}$ state are referred to as D1 transitions, while transitions into the $^2P_{3/2}$ state are referred to as D2 transitions.

momentum. For the purposes of ease in developing the mathematics, we assume that the beam is left circularly polarized; the mathematical treatment requires only a sign change for right circular polarization. In this case, alkali atoms are excited from the electronic ground, $-1/2$ spin state to the electronic excited, $+1/2$ spin state; atoms which began in the electronic ground, $+1/2$ spin state are unable to absorb a left circularly polarized photon because such a transition would fail to conserve angular momentum. While in the excited state, collisions between the alkali atom and other gas molecules allow for transitions between the $-1/2$ and $+1/2$ spin states, effectively mixing the two states and equalizing the populations of alkali electrons in the electronic excited, $-1/2$ spin and electronic excited, $+1/2$ spin states. Over time, the excited alkali electrons decay to the ground state (retaining their spin state) with a rate of decay R_γ :

$$R_\gamma = \frac{3}{3 + 7.5p_{N_2}} \quad (3.1)$$

Here, p_{N_2} is the partial pressure of the nitrogen in the cell in units of kilopascals. Over time, the continuous cycle of excitation and decay to the ground state results in a buildup of ground state electrons in the $+1/2$ spin state. This creates a non-equilibrium polarization

of the alkali valence electron spin. The alkali polarization P_{alkali} is given by:

$$P_{alkali} = \frac{R_{OP}}{R_{OP} + R_{SD}} \quad (3.2)$$

Here, R_{OP} is the rate at which the alkali metal vapor becomes polarized via optical pumping and R_{SD} is the rate of relaxation due to spin-destruction collisions, or any collision between alkali metal atoms which leads to a loss of the spin orientation provided by optical pumping. Numerous mechanisms exist which cause spin destruction. For instance, collisions of alkali metal atoms with the glass walls of a pumping cell will lead to a loss of spin order as the alkali electrons are reoriented by the magnetic field fluctuations produced by impurities in the glass [68]. Collisions between alkali metal atoms and N_2 molecules or Xe atoms can lead to a loss of spin order through spin-rotation interactions, in which angular momentum is transferred from the electronic spin to the rotational states of the other body [69]. Collisions between alkali atoms can also give rise to spin-destruction interactions, though the mechanism for this process is not fully understood [171]. These effects are each important in their own right, as they point the way toward engineering methods of decreasing R_{SD} and increasing P_{alkali} . However, from a mathematical standpoint, they are exactly equivalent; for the purpose of simplicity in the dynamics, all of these interactions can be treated with a single variable. By utilizing a high optical pumping rate (high laser power) and minimizing the spin-destruction interactions, it is possible to achieve alkali electron polarizations greater than 90%.

3.2.2 Spin Exchange with ^{129}Xe

Once polarization has been transferred from the circularly polarized laser light to the valence electron of the alkali metal atoms, it can be further transferred to the ^{129}Xe nuclei through repetitive collisions between the polarized alkali metal atoms and the noble gas atoms. There are two mechanisms for this transfer of spin orientation. The transfer can occur either while a polarized alkali atom and a ^{129}Xe atom are bound together through van der Waals forces; this van der Waals molecule is then later broken up by a third body (typically a N_2 molecule). The transfer can also occur through hyperfine interactions resulting during a short-lived collision between a polarized alkali atom and a ^{129}Xe atom. The first mechanism is pressure-dependent and is extremely efficient at low pressures, on the order of a few Torr [171]. At higher pressures, the second mechanism is dominant. All of the devices examined in this thesis operate at high gas pressures; for this reason, we will only consider spin exchange by the second mechanism. Figure 3.3 shows the two mechanisms of spin exchange between an alkali atom and a ^{129}Xe atom.

In binary collisions, the polarization exchange between the alkali electron spin $\mathbf{S}$ and the

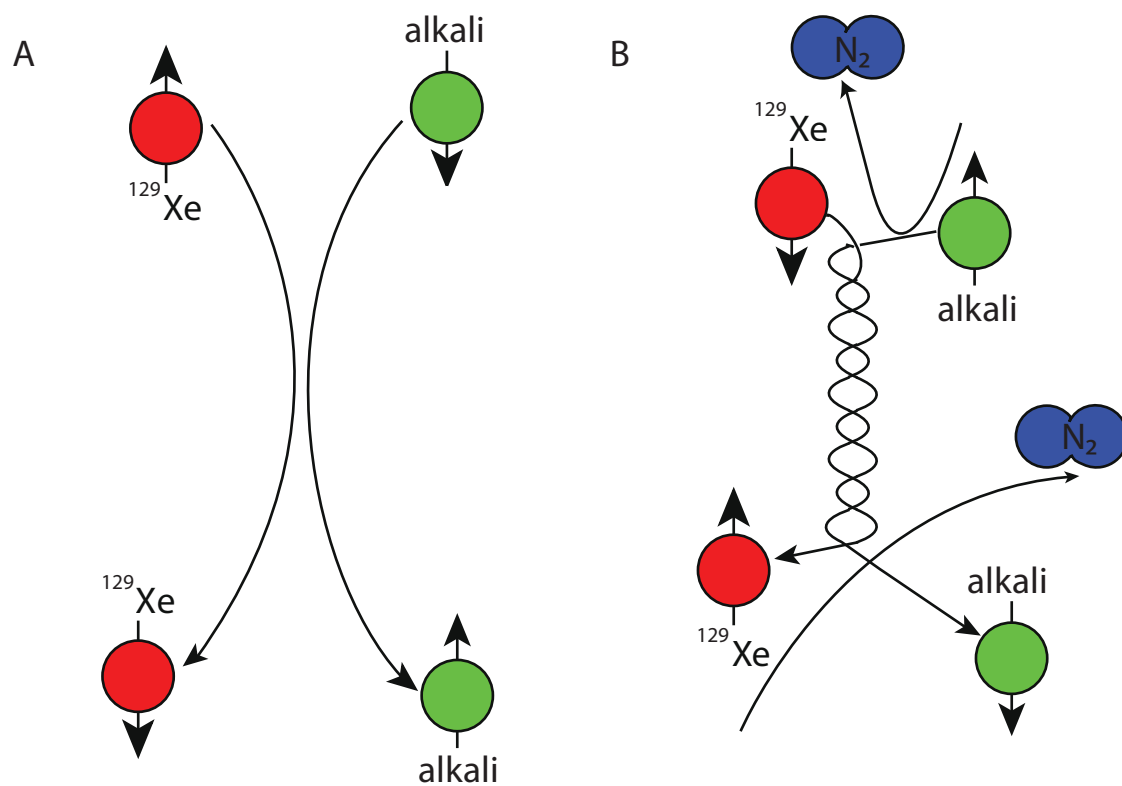


Figure 3.3: Spin Exchange Mechanisms. A) Transfer of polarization from an alkali valence electron to a ^{129}Xe nucleus by formation of a van der Waals molecule and subsequent breakup by a N_2 molecule. B) Transfer of polarization from an alkali valence electron to a ^{129}Xe nucleus by hyperfine coupling during a binary collision.

Table 3.3: Alkali- ^{129}Xe Spin Exchange Coefficients

Alkali Atom	κ_{SE} at 0 T, 100 °C ($\text{cm}^3 \text{ s}^{-1}$)
Rb	2.2×10^{-16} [69]
Cs	4.1×10^{-16} [82]

^{129}Xe nuclear spin $\mathbf{I}$ is described by:

$$\alpha \mathbf{S} \cdot \mathbf{I} = (S_+ I_- + S_- I_+) + S_z I_z \quad (3.3)$$

Here, the quantum mechanical operators $S_{\pm}$ and $I_{\pm}$ are the raising and lowering operators for the electron spin and the nuclear spin, respectively; they act to raise a -1/2 spin state electron or nucleus to the +1/2 spin state, or to lower a +1/2 spin state electron or nucleus to the -1/2 spin state. The operators S_z and I_z give the spin projections along $\hat{\mathbf{z}}$. α is the probability of finding an unpaired alkali electron at the position of the ^{129}Xe nucleus:

$$\alpha = \frac{8\pi}{3} \gamma_S \gamma_I \hbar^2 \delta(r) \quad (3.4)$$

Here, γ_S and γ_I are the gyromagnetic ratios of the alkali electron and the ^{129}Xe nucleus, respectively; $\delta(r)$ is the Dirac delta function; and r is the distance between the ^{129}Xe nucleus and the alkali electron.

From these considerations, we can see the effect of a binary collision. If an electron in the -1/2 spin state encounters a ^{129}Xe nucleus in the +1/2 spin state, the two bodies will swap angular momentum, so that the electron ends up in the +1/2 spin state and the nucleus ends up in the -1/2 spin state. If the initial spin states are reversed, the opposite end state is encountered in each body. If the electron and nucleus begin in the same spin state (either both -1/2 or both +1/2), no interaction occurs. Since we have taken our laser to be left circularly polarized, the net result of optical pumping is to prepare a population of alkali atoms in the +1/2 electronic spin state; the net result of spin exchange is to prepare a population of ^{129}Xe atoms in the -1/2 nuclear spin state. The transfer of spin orientation occurs at a rate R_{SE} :

$$R_{SE} = \kappa_{SE} n_{alkali} \quad (3.5)$$

Here, κ_{SE} is the alkali-noble gas spin exchange coefficient and n_{alkali} is the alkali vapor density. Table 3.3 gives the spin exchange coefficients between the alkali metals which are commonly used for SEOP (Rb and Cs) and ^{129}Xe .

The resultant polarization is determined by a balance of the rate of polarization transfer through spin exchange collisions and the rate of polarization loss to the cell walls R_{wall} . There are other mechanisms which lead to a loss ^{129}Xe polarization, such as collisions between two

^{129}Xe atoms. However, these relaxation rates are generally small in comparison to those imparted by collisions with the cell walls; we therefore ignore them in our treatment. Since the optical pumping process takes place on a timescale ($\lesssim 1$ ms) much shorter than that of spin exchange ($\gtrsim 1$ s), we can assume that the alkali polarization attains its equilibrium value essentially instantaneously. Given an equilibrium alkali polarization P_{alkali} , we obtain the following expression for the time-dependent ^{129}Xe nuclear polarization P_{Xe} following the start of optical pumping:

$$P_{\text{Xe}} = P_{\text{alkali}} \frac{R_{\text{SE}}}{R_{\text{SE}} + R_{\text{wall}}} (1 - \exp(-(R_{\text{SE}} + R_{\text{wall}})t)) \quad (3.6)$$

The long-run equilibrium polarization is given by:

$$P_{\text{Xe}} = P_{\text{alkali}} \frac{R_{\text{SE}}}{R_{\text{SE}} + R_{\text{wall}}} \quad (3.7)$$

3.2.3 Current Large-Scale ^{129}Xe Polarizers

^{129}Xe polarizers can be divided into two general classes: batch mode and continuous flow polarizers. Batch mode polarizers operate by filling a pumping cell with a quantity of ^{129}Xe (which also consists of other isotopes of Xe, N_2 , and sometimes He) and providing continuous optical pumping of the alkali metal vapor. This allows for a buildup of ^{129}Xe magnetization to near the equilibrium value, yielding a very high polarization. The polarized ^{129}Xe gas is then frozen in a cold finger, allowing it to be used at a later time. Frozen hyperpolarized ^{129}Xe has an extraordinarily long lifetime, persisting for approximately 3 hours when stored at 77 K and as long as 8 days when stored at 4 K [55]. This technique is particularly prevalent in medical imaging applications; polarized ^{129}Xe can be produced in a large production facility, frozen in a medically-relevant quantity, and delivered to a patient when needed. For all of the applications described in this thesis, it is necessary to operate the polarizer under continuous gas flow; therefore, we focus on this type of polarizer. A schematic for a typical continuous flow ^{129}Xe polarizer is shown in Figure 3.4.

The first continuous flow ^{129}Xe polarizers was introduced in 1996 [44]. Since then, there has been a great amount of interest in the development of this type of polarizer, resulting in continual design improvement [133, 148, 189, 136, 49, 143, 91]. Initially, ^{129}Xe polarizers utilized total gas pressures on the order of 10 atm. More recent designs have utilized total gas pressures of a few atmospheres or even sub-atmospheric pressures. Of particular interest is the work of Ruset, in which it was found that using a pressure of 0.8 atm and an optical pumping beam directed anti-parallel to the direction of the gas flow yielded ^{129}Xe polarizations of approximately 60%. This increase is achieved by a combination of more efficient optical pumping and an increased spin exchange rate from the contribution of the van der Waals mechanism at low pressures.

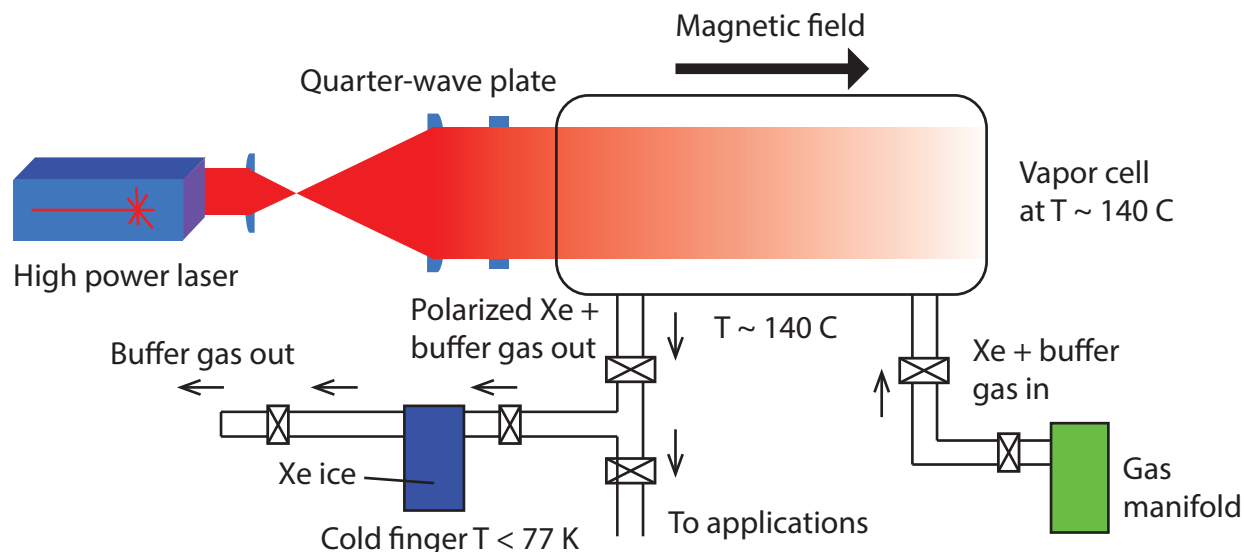


Figure 3.4: Continuous Flow Polarizer. Linearly polarized light from a high power laser is expanded, circularly polarized by a quarter wave plate, and directed to a cell filled with alkali metal. The cell is heated to increase the alkali vapor density. A magnetic field is applied to split the Zeeman sublevels. A gas mixture containing ^{129}Xe is flowed into the cell and undergoes hyperpolarization by SEOP. The gas mixture continuously flows through the polarizer.

Though they are capable of producing high volumes of highly polarized ^{129}Xe , current polarizers are bulky, non-portable, and expensive, and therefore not well suited for use with microfluidic applications. They are designed to produce liter-sized volumes of polarized gas and require large vapor cells and high power lasers utilizing 10 W to 300 W of optical power, while the demands of microfluidics require polarizers producing only microliters of polarized gas while drawing low power (less than 1 W). We now turn our attention to a small-scale polarizer better suited for microfluidics applications.

3.3 Alkali Vapor Atomic Magnetometers

At magnetic fields less than approximately 1 T, it is not feasible to detect the NMR signal using an inductive pickup coil due to the low magnetic field sensitivity, as discussed in Section 2.8.2. AVAMs are an attractive alternative for sensitive detection of the NMR signal at low magnetic fields. They operate by measuring the precession of alkali metal valence electron spin magnetization in the presence of a magnetic field. Sensitivity enhancement is achieved by employing strong optical pumping of the alkali metal valence electron, as described in Section 3.2.1. For a complete treatment of AVAM physics, the interested reader should consult the book by Budker [26] or the excellent thesis by Seltzer [146]. Here, we use only the results pertinent to the magnetometers employed in miniaturized ^{129}Xe polarizers.

The Bloch equation for an ensemble of alkali atoms is often written in terms of a polarization vector $\mathbf{P}_{alkali}$ instead of a magnetization:

$$\frac{d\mathbf{P}_{alkali}}{dt} = \gamma \mathbf{P}_{alkali} \times \mathbf{B} \quad (3.8)$$

Here, the gyromagnetic ratio of the alkali electron spin is given by $\gamma = \gamma_e/q_N$, where q_N is the nuclear slowing-down factor which arises due to coupling of the electron with the alkali nuclear spin. The alkali atoms are subject to relaxation by spin-destruction collisions with buffer gas atoms (typically N_2) and the cell walls and by spin-exchange collisions with other polarized alkali atoms. Take the direction of the optical pumping beam to be along $\hat{x}$. In this case, the transverse and longitudinal relaxation rates are given by:

$$\frac{1}{T_1} = \frac{R_{SD}}{q_N} \quad (3.9)$$

$$\frac{1}{T_2} = \frac{R_{SD} + R_{SE}}{q_N} \quad (3.10)$$

The Bloch equations for an ensemble of polarized alkali atoms can be written as follows:

$$\frac{dP_x}{dt} = \omega_0 P_y - \frac{P_x}{T_2} + R_{OP} \quad (3.11)$$

$$\frac{dP_y}{dt} = -\omega_0 P_x - \frac{P_y}{T_2} \quad (3.12)$$

$$\frac{dP_z}{dt} = -\frac{P_z}{T_1} \quad (3.13)$$

The ensemble of electron spins experiences torques due to the magnetic field and re-orientation by optical pumping, as well as T_1 and T_2 relaxation. At low magnetic fields, optical pumping and alkali spin decoherence occur much more quickly than fluctuations in the magnetic field. Thus, most of the electron spins in the ensemble will fail to complete a single rotation in the transverse plane before they are reoriented by optical pumping or decohere. At any given time, we thus have an alkali electron magnetization vector which has components both in the longitudinal direction and in the transverse plane. This results in a steady state magnetization vector. Our magnetometers utilize the transverse components of the alkali magnetization; the steady state solutions to transverse components of the alkali polarization are given by:

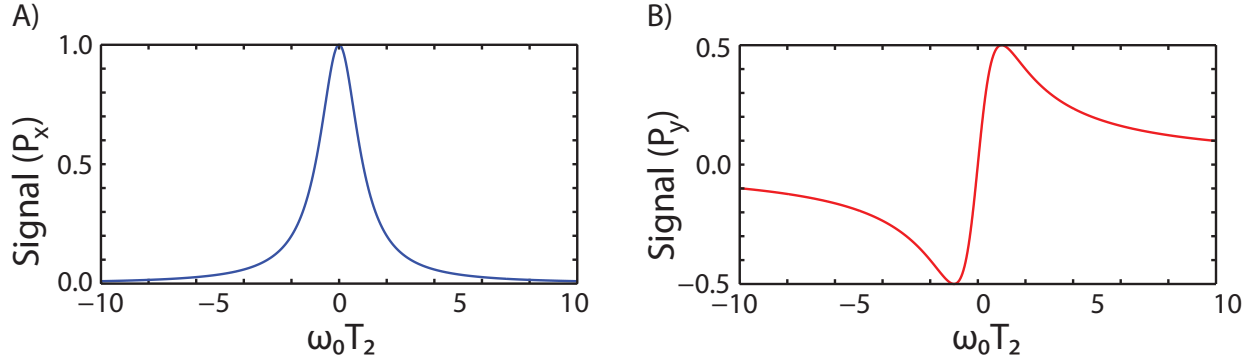


Figure 3.5: AVAM Magnetic Field Response. A) Signal due to component along the optical pumping axis. B) Signal due to component perpendicular to pumping axis.

$$P_x = R_{OP} \frac{\frac{1}{T_2}}{\frac{1}{T_2} + \omega_0^2} \quad (3.14)$$

$$P_y = R_{OP} \frac{\omega_0}{\frac{1}{T_2} + \omega_0^2} \quad (3.15)$$

The magnetometer signals obtained by optically probing the spin states along $\hat{x}$ and $\hat{y}$ are shown in Figure 3.5. The P_x signal yields an absorptive Lorentzian lineshape, while the P_y signal yields the corresponding dispersive Lorentzian lineshape. The dispersive component is particularly useful for measuring magnetic fields close to zero. This is because changes in the magnitude of the magnetic field δB_0 are related to changes in the magnetometer signal amplitude δS by the slope of the magnetic field-magnetometer signal curve:

$$\delta S = \left(\frac{\partial S}{\partial B_0} \right) \delta B_0 \quad (3.16)$$

We measure the magnetic field response using the dispersive component because it has a highly linear regime with a large slope near zero magnetic field, resulting in large magnetometer signals for small variations in the magnetic field. If we take the minimum detectable signal to be the system noise S_{noise} , we find the magnetometer sensitivity to be:

$$\delta B = S_{noise} \left(\frac{\partial S}{\partial B_0} \right)^{-1} \quad (3.17)$$

Thus, the magnetometer sensitivity is experimentally determined by measuring the system noise S_{noise} and the slope of the dispersive component of the magnetometer signal.

Fundamentally, the magnetometer sensitivity is limited by spin projection noise:

$$\delta B = \frac{1}{\gamma_e} \sqrt{\frac{1}{n_{alkali} V_{cell} T_2 \tau_{meas}}} \quad (3.18)$$

Here, γ_e is the electron gyromagnetic ratio, n_{alkali} is the alkali number density, V_{cell} is the cell volume, and τ_{meas} is the measurement time. Thus, for a fixed cell volume and measurement time, the magnetometer sensitivity may be increased by increasing the alkali vapor density, which is a strong function of the cell temperature. The dynamic range of the magnetometer is limited by the width of the dispersive zero-field resonance. Microfabricated AVAMs with cells volumes of a few mm^3 have achieved sensitivities of 5 fT $\text{Hz}^{-1/2}$ with bandwidths of tens of Hz [62].

The zero-field magnetometer described above may be implemented by using two orthogonal laser beams (one for optical pumping and one to probe the optical response). The probe signal may be monitored either by measuring the optical absorption or the optical rotation, though the optical rotation method is employed more often. When using the two-beam setup to measure optical rotation, the probing beam is linearly polarized. A balanced polarimeter consisting of a waveplate, a polarizing beam splitter, and two photodiodes is used to detect the optical rotation. When no magnetic field is present, the waveplate is adjusted to give equal signals in the two photodiodes. When a magnetic field is present, the two circular components of the probing light travel through the alkali vapor at slightly different speeds, due to circular birefringence. This causes the polarization direction to rotate by an angle which is proportional to the magnetic field. When the light hits the beam splitter, it is no longer sent to the two photodiodes with equal intensities; instead, one of the photodiodes sees a slightly greater flux of probing light. By subtracting the photodiode signals, the polarization angle can be measured while eliminating much of the common-mode noise.

In miniaturized and portable magnetometers, it has been more practical to use the same laser beam to carry out both optical pumping and probing, generally employing optical absorption measurements [149, 150, 89, 138, 110]. These measurements apply a modulation to the optical frequency in order to shift the center of the zero-field resonance which arises from a fictional magnetic field B_{Stark} due to the AC Stark shift:

$$B_{Stark} = \frac{I}{h\nu_{light}} \frac{\sigma_0}{\gamma_e} \left(\frac{2(\nu_{light} - \nu_0) \Delta\nu}{\Delta\nu^2 + 4(\nu_{light} - \nu_0)^2} \right) \quad (3.19)$$

Here, σ_0 is the on-resonance optical cross-section of the alkali metal, γ_e is the electron gyromagnetic ratio, ν_0 is the center frequency of the alkali D1 transitions, and ν_{light} is the detuned laser frequency. The magnetometer signal is obtained using a lock-in amplifier locked to the modulation frequency. This allows for a substantial reduction of low-frequency noise in the magnetometer signal.

3.4 First-Generation Miniature ^{129}Xe Polarizer

The first-generation miniature ^{129}Xe polarizer was developed in collaboration between the Pines laboratory and the laboratory of John Kitching at the National Institute of Standards and Technology (NIST).

3.4.1 Microfabricated Polarizer Design

The chip, fabricated from Si and glass, consists of four chambers, each 1 mm thick and several millimeters wide, connected by micro-channels, as shown in Figure 3.6. The two outer chambers act to stabilize the gas flow as it moves from a gas handling manifold operating in the chaotic flow regime to the microfabricated device which operates in the laminar flow regime; the input chamber also serves to hold an additional reservoir of Rb for cleaning of the gas as it enters the device. The two inner chambers work to polarize and optically detect ^{129}Xe gas. As gas flows through the device, a fraction of the ^{129}Xe atoms in the pump chamber (see Figure 3.6) becomes polarized through spin exchange collisions with optically pumped ^{87}Rb atoms at a pumping rate proportional to the ^{87}Rb polarization, P_{Rb} , and the spin-exchange rate, $R_{\text{SE}} = \kappa_{\text{SE}} n_{\text{Rb}}$, where n_{Rb} is the ^{87}Rb atomic number density and κ_{SE} is the ^{87}Rb - ^{129}Xe spin-exchange coefficient [81]. Under gas flow, polarized ^{129}Xe atoms exit the pump chamber and move through the connecting channel into the probe chamber where the NMR signal is measured. The two narrow grooves in the middle of the device provide thermal insulation between the two sides of the device, allowing the pump chamber and probe chamber to be heated to different temperatures. Except where otherwise stated, both larger ^{87}Rb atomic density and stronger laser illumination are utilized in the pump chamber to ensure that the pumping rate of ^{129}Xe in the pump chamber is much larger than that in the probe chamber. In addition to ^{129}Xe and ^{87}Rb , the chip is filled with N_2 gas to serve the dual purpose of quenching radiative relaxation of the optically pumped ^{87}Rb and decreasing the rate of polarization loss due to collisions with the cell walls. We operate the device with 400 Torr of N_2 and 200 Torr of Xe in natural isotopic abundance (26.4% ^{129}Xe).

3.4.2 Device Fabrication

The device consists of a 3 cm x 1 cm x 1 mm Si chip produced from a 100 mm diameter Si wafer; eight devices can be produced on a single wafer. The wafers are cleaned by sequentially sonicating them in baths of acetone, isopropanol, and water for a period of ten minutes in each step. The wafers are further cleaned using the RCA cleaning protocol, which consists of a 10 minute immersion in a 80 °C bath of 5:1:1 water:ammonium hydroxide:hydrogen peroxide, followed by a 15 second immersion in a 2% hydrofluoric acid solution, followed by a 10 minute immersion in a 80 °C bath of 5:1:1 water:hydrochloric acid:hydrogen peroxide. The devices are then rinsed thoroughly with water, dried under N_2 gas, and spin-coated with AZ Chemicals AZ-6600 photoresist with a final spin speed of 1500 rpm for a period

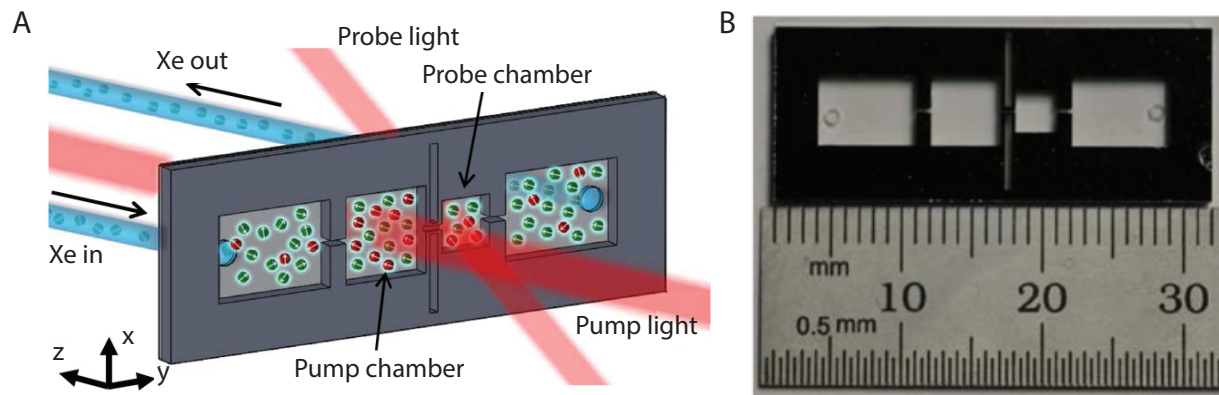


Figure 3.6: Microfluidic ^{129}Xe Polarizer. A) A gas mixture containing ^{129}Xe flows from a gas manifold into the inlet chamber, through the pump and probe chambers, and out of the outlet chamber. The chip is loaded with ^{87}Rb metal. Unpolarized ^{129}Xe atoms entering the pump chamber become polarized through spin exchange with optically pumped ^{87}Rb . The polarized ^{129}Xe then moves downstream, passes through a microchannel into the probe chamber and eventually exits the device through the output chamber. Optical characterization is carried out in the probe chamber. B) The Si chip footprint is 3 cm x 1 cm, with a thickness of 1 mm. The dimensions of the pump and probe chambers are 5 mm x 5 mm x 1 mm and 3 mm x 3 mm x 1 mm, respectively; the channel connecting the pump and probe chambers is 1 mm x 0.3 mm x 0.3 mm. Two tall, narrow grooves are etched from the middle of the chip to provide thermal isolation between the two sides of the device.

of 30 s. They are photolithographically patterned using a 365 nm stepper and developed in AZ Developer solution, which removes unpolymerized photoresist from the areas of Si that are to be etched. Deep reactive-ion etching (DRIE) [104] is used to etch four holes 1 mm deep in each device to form the input, pump, probe, and output chambers, as well as the channels connecting the input chamber to the pump chamber and the probe chamber to the output chamber. Two additional holes are etched through the Si wafer to provide thermal insulation between the two sides of the device and allow the pump chamber and probe chamber to be maintained at different temperatures. The channel connecting the pump and probe chamber is etched in a subsequent DRIE step. The lateral dimensions of the input and output chambers are 8 mm x 5 mm. The input chamber is connected to the pump chamber by a channel that is 1 mm long and 300 μm wide, etched all the way through the Si; a similar channel connects the probe chamber and the output chamber. The lateral dimensions of the pump and probe chambers are 5 mm x 5 mm and 3 mm x 3 mm, respectively; they are connected by a 1 mm long x 300 μm wide 300 μm deep channel. The 300 μm channels are produced in a second round of cleaning, photolithography, and etching. After all holes and channels have been formed, the device is sealed by anodically bonding [35] two slides (each 300 μm thick x 3 cm x 1 cm) of borosilicate glass onto the top and bottom surfaces of the Si chip. The first round of anodic bonding is performed by pressing the Si and glass pieces together and heating to a temperature of 400 $^{\circ}\text{C}$. A potential difference of 1000 V is applied across the pieces. As the anodic bond is formed, the resistivity of the Si-glass interface increases (Si-O₂ forms at the junction) and the electric current across the junction

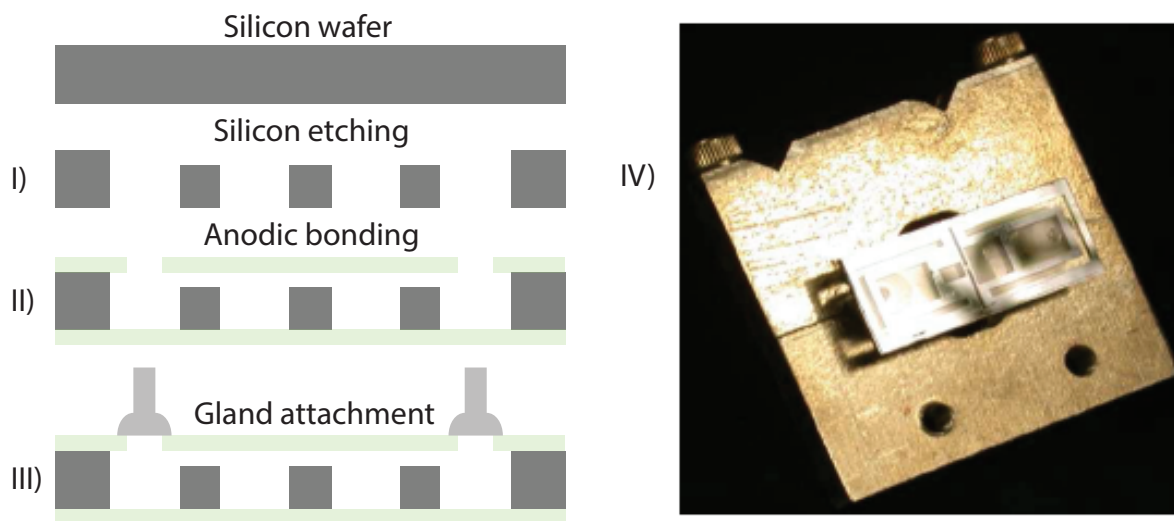


Figure 3.7: Microfluidic ^{129}Xe Polarizer Fabrication. Device fabrication occurs in four steps. I) A Si wafer is etched and diced to form chips with through holes and channels. II) Borosilicate glass windows are anodically bonded to the Si device. III) Stainless steel glands are glued onto the glass windows. IV) The device is clamped in an aluminum holder to prevent torque-induced breakage.

drops. The process is stopped when the current hits 0.01 mA (the lowest reading achievable on the high-voltage power supply), which generally takes about 30 minutes. The pieces are cooled slowly in order to avoid breakage due to the slightly different coefficients of thermal expansion of Si and Pyrex. The bonded wafers are then diced to form chips with one open side. They are further cleaned. Before being bonded to the chip (using the same anodic bonding conditions as during attachment of the first slide), the other glass slide is drilled with holes situated in line with the input and output chambers, through which gas enters and exits the cell. Two non-magnetic stainless steel (grade SS316) 1/8" VCR glands are glued onto the glass slide above the holes using an ultra-high vacuum (UHV) grade epoxy to allow connection of the cell to a gas manifold. This allows connection of the device to the gas handling manifold. Before it is attached to the gas manifold, the device is clamped using a custom-made aluminum holder; without this holder, we found that the polarizer chip broke due to the torques applied at the chip-gas manifold joints. An outline of the device fabrication steps is shown in Figure 3.7; Figure 3.8 provides more detail for the Si etching and anodic bonding steps. All fabrication of the first-generation device was performed at NIST.

Before it is attached to the gas manifold, the chip is filled with isotopically enriched ^{87}Rb vapor, produced by the chemical reaction of barium azide and ^{87}Rb chloride within the input and output chambers, a method developed at NIST [88]. The filling of the device with alkali vapor is as follows: 2 mg of ^{87}Rb chloride are mixed with 10 μL of barium azide dissolved in water. The liquid is then introduced into the input and output chambers of the chip using a microliter pipette and allowed to dry. The cell is attached to the gas manifold system (shown in Figure 3.10 and described in the following Section 3.4.3 and held in place by the aluminum clamp. A turbomolecular pump (TMP) is used to pump the device to a

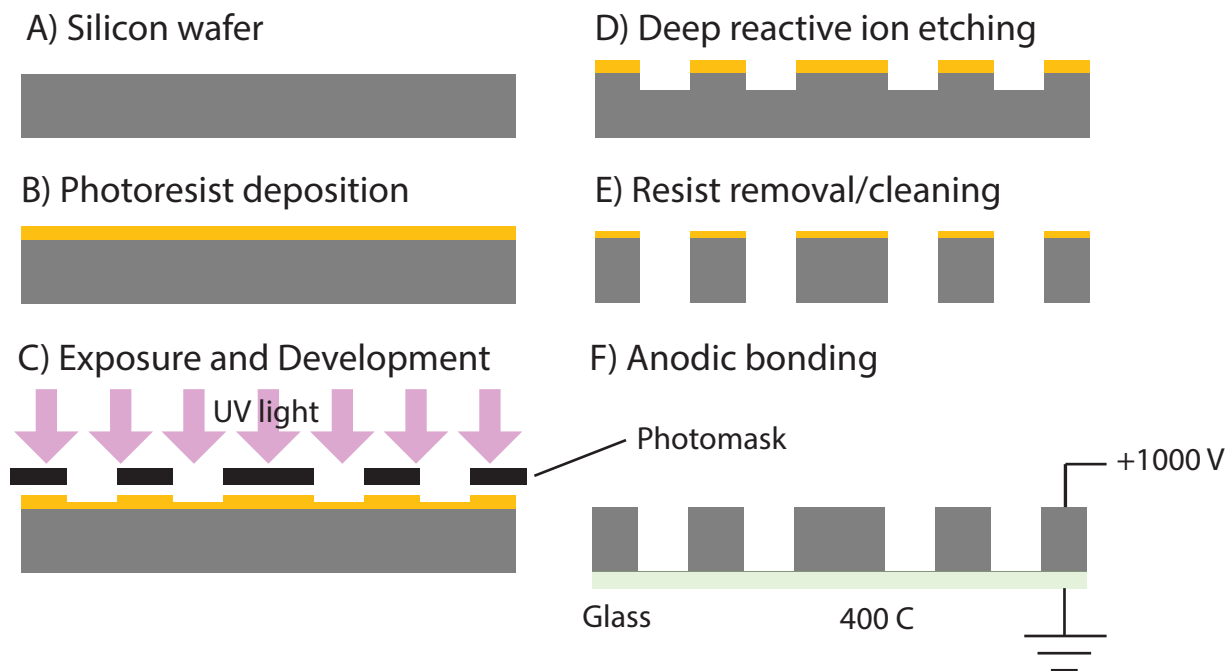


Figure 3.8: Si Etching and Anodic Bonding. A) A round of Si etching and anodic bonding begins with a clean Si wafer. B) The Si wafer is spin coated with a positive photoresist. C) The photoresist is exposed to UV light through a photomask, exposing areas of Si for future etching; depolymerized photoresist is washed away using a chemical developer. D) The exposed areas of Si are etched using the deep reactive ion etching technique. E) The photoresist is removed by dissolution in acetone; the wafer is cleaned using a combination of solvents. F) The Si wafer is bonded to Pyrex by applying anodic bonding at a potential of 1000V and a temperature of 400 °C.

pressure below 10^{-5} Torr, the limit of the vacuum gauge attached to the system. The cell is heated to 90°C using resistive heaters and baked for several hours. After baking, the cell temperature is increased to the activation point of the reaction: $\text{Ba}(\text{N}_3)_2 + 2^{87}\text{RbCl} \rightarrow \text{BaCl}_2 + 3\text{N}_2 + 2^{87}\text{Rb}$. We have observed that the activation temperature occurs around 110°C to 120°C , as measured by thermistors positioned next to the input and output chambers. The activation of ^{87}Rb is confirmed by monitoring the light levels of laser beams transmitted through the pump and probe chambers while scanning the laser wavelengths about the D1 optical transition of ^{87}Rb . Once the presence of ^{87}Rb is established in both chambers, the cell temperature is decreased to 60°C . We then open the valve of the mixing chamber, which has been previously filled with the desired gas mixture, and let this mixture fill the vapor cell. Finally we set the temperature of the cell to the working value. The device operates over a range of temperatures from 110°C to 150°C .

3.4.3 Experimental Setup

Other than the microfluidic device, the experimental setup basically consists of a gas handling manifold, an optical setup, magnetic field coils, heaters, and electronic controllers for the various pieces of equipment. Photographs of the various systems are shown in Figure 3.9.

Prior to activation of the ^{87}Rb metal, the chip is placed inside a two-layer magnetic shield made from mu-metal, an alloy composed of 77% nickel, 16% iron, 5% copper, and 2% chromium or molybdenum which has an extremely high magnetic permeability and acts to screen out ambient magnetic fields. The chip is then attached to the gas manifold shown in Figure 3.10. The gas manifold consists of tanks of ultra-high purity Xe and N_2 gas connected by tubing to getters which remove oxygen and water from the gas to approximately 10 parts-per-billion (ppb) and a mixing chamber. Tubing allows attachment to the inlet and outlet of the microfluidic chip. Fine control of the gas flow rate through the chip is achieved using a leak valve placed immediately downstream from the outlet chamber; gas flow is measured using a gas flow meter. The output gas is collected in a venting chamber. The entire system is connected to a TMP. Various gauges allow measurement of the pressure at various locations throughout the gas manifold. All tubing is 1/4" SS316 tubing provided by Swagelok; all connections are made using 1/4" Swagelok VCR connectors utilizing copper gaskets. When nothing is attached to the gas manifold, it is sealed, pumped, and baked to 150°C to remove atmospheric water vapor from the system and maintain cleanliness. After attachment of the chip, the entire system is pumped to below 10^{-5} Torr. The mixing chamber is then filled to the proper partial pressures of Xe and N_2 . The content of the gas is verified using a residual gas analyzer (RGA).

The optical setup is as follows. Two independently controlled laser beams, tuned to the D1 optical transition of ^{87}Rb at approximately 794.7 nm, irradiate the pump and probe chambers. A distributed feedback laser (DFB) provides the pump laser beam, while light produced by a vertical-cavity surface-emitting laser (VCSEL) is used as the probe laser

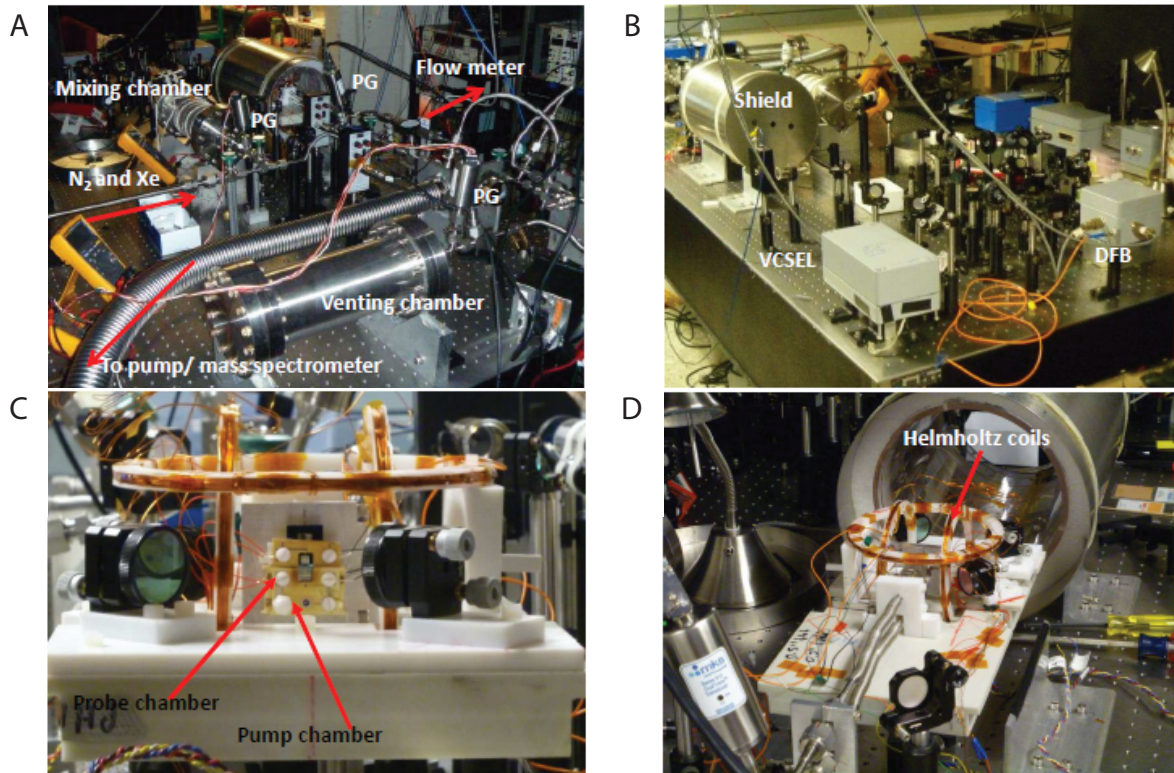


Figure 3.9: Microfluidic ^{129}Xe Polarizer Experimental Setup. The setup consists of A) a gas manifold, B) optics, C) magnetic field controllers, and D) heaters. Electronic controllers for these systems are not shown.

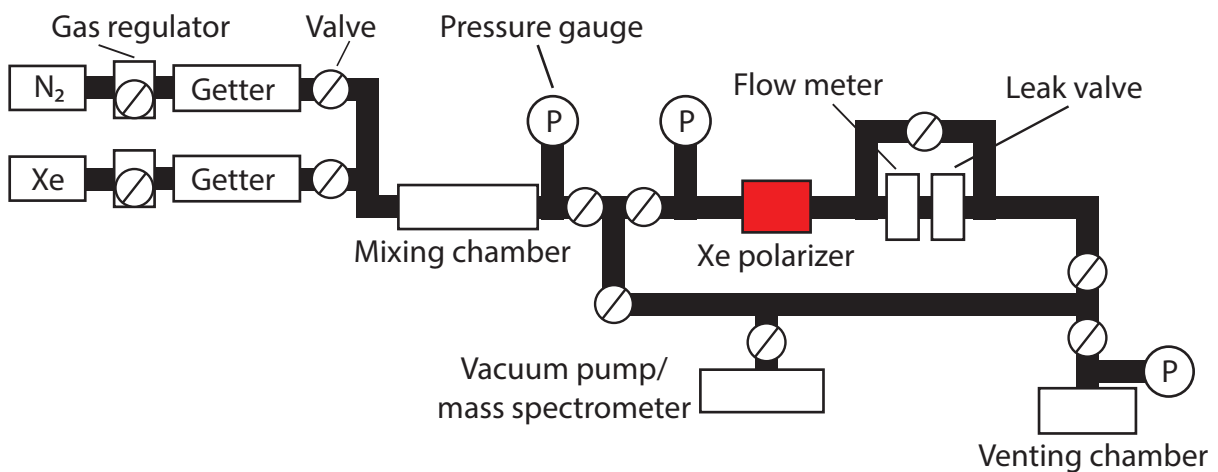


Figure 3.10: Polarizer Gas Manifold. The gas manifold consists of Xe and N_2 gas tanks connected to oxygen and water getters. The gas is mixed in a mixing chamber and flowed into the chip. The flow rate is set using a leak valve and measured using a flow meter. The output gas is collected in a venting chamber. The entire system can be pumped with a TMP.

beam. Each beam is passed through an optical isolator to remove feedback from light reflected off of the later optical components. Each beam is adjusted to the proper power using a combination of a half-wave plate (HWP) and a polarizing beam splitter (PBS). For most of the experiments, the optical power of the pump laser beam was 8 mW, while that in the probe was 100 μW . Each beam is passed through a quarter-wave plate (QWP) to impart circular polarization to the light. The light from each beam is then steered into the magnetic shields using a mirror. The probe beam is directed at a 45° angle through the probe chamber using a pair of mirrors inside of the magnetic shields. An acousto-optical modulator (AOM) is included in the beam path of the pump beam to allow modulation of the pump beam amplitude. After passing through the device, each of the beams is analyzed using a sector photodiode.

The magnetic shields screen out magnetic fields from the earth, building, and laboratory equipment. A set of Helmholtz coils is used to provide transverse magnetic fields in the $\hat{x}$ and $\hat{y}$ directions. A solenoid is used to provide a longitudinal magnetic field in the $\hat{z}$ direction.

The pump and probe chambers are AC heated using two independent sets of surface mount resistors attached to the cell. The temperature at the input and output chamber is measured using two thermistors that are in close contact with the windows of those chambers. The device operates over a range of temperatures from 110 $^\circ\text{C}$ to 150 $^\circ\text{C}$. The ^{87}Rb atomic density in each chamber is characterized by measuring the optical absorption of the laser beams as their wavelengths are scanned across the D1 optical line of ^{87}Rb . From the measured on-resonance optical depth OD_0 , we calculate the atomic density using $OD_0 = \sigma_0 n_{\text{Rb}} l$ with $l = 1$ mm being the length of the beam path inside the pump chamber and $\sigma_0 = 2r_e c f / \Delta\nu$ being the on-resonance optical cross-section where $r_e = 2.818 \times 10^{-13}$ cm is the classical electron radius, c is the speed of light in vacuum, $f \cong 1/3$ is the oscillator strength of the D1 resonance and $\Delta\nu = 8$ GHz is the full-width at half-maximum (FWHM) of the optical line obtained from the fitted optical absorption spectrum.

We use the ensemble of ^{87}Rb atoms in the pump and probe chambers as *in situ* magnetometers. These are zero-field magnetometers sensitive to magnetic fields along $\hat{y}$, as discussed in Section 3.3; they are very similar to those discussed in references [63, 170, 139]. The magnetometers are implemented by introducing a RF field with 7 kHz modulation frequency and 1 μT amplitude along $\hat{y}$. The signals of the magnetometers are extracted by lock-in detection of the transmitted laser intensity at the modulation frequency. The measured noise floors for the pump and probe magnetometers were 5 pT $\text{Hz}^{-1/2}$ and 16 pT $\text{Hz}^{-1/2}$, respectively, with a bandwidth of 40 Hz that is limited by the response of the lock-in amplifiers. This corresponds to a single-shot detection limit of a polarized ensemble of approximately 5×10^{11} ^{129}Xe atoms in each chamber. On resonance, the magnetometers respond linearly to magnetic fields along $\hat{y}$ that are smaller than the half width at half maximum line-width of the magnetometer resonance. For the magnetometers in this work, $\Delta\nu_{\text{HWHM}} = 1$ mT at zero-light levels; it is more than twice as large during optical pumping due to collisions with ^{129}Xe atoms. For each change in device parameters, the linear response of the magnetometers was obtained by extracting the on-resonance slope of the magnetic

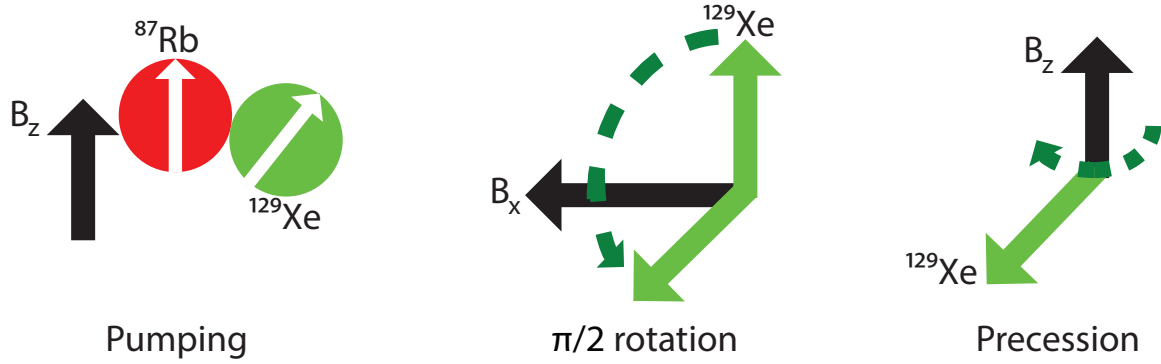


Figure 3.11: Polarizer Pulse Sequence. Optical pumping is carried out in a longitudinal field $B_z = 0.8 \mu\text{T}$. After approximately 10 s of optical pumping, a transverse field of magnitude $5.3 \mu\text{T}$ is turned on for 4 ms to tip the ^{129}Xe magnetization into the $\hat{y}$ direction and initiate precession about $\hat{z}$.

response acquired by scanning the magnetic field along $\hat{y}$. We then used this slope to calibrate the amplitude of the FIDs in magnetic field units. We fit the FID signal in the pump chamber to a single exponentially decaying sinusoid, from which we extracted the amplitude, decay time constant, and Larmor precession frequency of ^{129}Xe .

3.4.4 Production and Detection of Hyperpolarized ^{129}Xe

Using the ensemble of ^{87}Rb atoms in the pump and probe chambers as magnetometers sensitive to magnetic fields along $\hat{y}$ as defined in Figure 3.11, we detected the ^{129}Xe magnetization, and thus its polarization [140], in each chamber as a function of the experimental conditions. The magnitude of the field B_{Rb} sensed by ^{87}Rb due to ^{129}Xe magnetization M_{Xe} is approximately:

$$B_{Rb} = \frac{2}{3} \mu_0 \kappa_0 M_{Xe} \quad (3.20)$$

Here, μ_0 is the vacuum permeability, $M_{Xe} = \mu_{Xe} P_{Xe} n_{Xe}$ with μ_{Xe} , P_{Xe} , and n_{Xe} being the ^{129}Xe nuclear magnetic moment, polarization, and atomic density, respectively. κ_0 represents the enhancement factor due to the hyperfine Fermi-contact interaction between the valence electron of ^{87}Rb and the ^{129}Xe nuclear spin [74], previously measured to be $\kappa_0 \approx 500$ [101]. Measurement of the ^{129}Xe FID in each chamber is made with the magnetometers after rotating the ^{129}Xe longitudinal polarization into the transverse xy plane with a transverse DC magnetic field pulse, as shown in Figure 3.11.

Figure 3.12 demonstrates the efficient transport of polarized ^{129}Xe from the pump chamber to the probe chamber while the gas is flowing. Figure 3.12a shows the measured FID in the pump chamber, obtained by monitoring the transmitted power in the pump beam after the transverse field pulse has tipped the ^{129}Xe magnetization into the $\hat{y}$ direction. Figure

3.12 shows the measured FID in the probe chamber recorded with the pump beam blocked, so that there is no flow of polarized ^{129}Xe into the probe chamber. Under these conditions we observe a weak probe FID with an amplitude of approximately 1 nT, which we attribute to weak SEOP of ^{129}Xe by the polarized ^{87}Rb used as a magnetometer in the probe chamber. In order to mitigate the effect of this weak SEOP on the measured ^{129}Xe signal, we deliberately used oppositely handed photons for optical pumping in the pump and probe chambers, so that the polarized ^{129}Xe produced in the probe chamber was 180° out of phase with that produced in the pump chamber. Figure 3.12c shows that the probe FID changes significantly when the pump beam is unblocked; its amplitude increases and its phase changes by 180° to correspond to the amplitude and phase in the pump chamber observed in Figure 3.12a. This indicates that the detected ^{129}Xe was polarized in the pump chamber and transported to the probe chamber by the flow of gas. A close look at Figures 3.12b and 3.12c reveals that the decay constant of polarized ^{129}Xe in the probe chamber is slightly longer than that in the pump chamber. This feature can be partially explained by noting that freshly polarized ^{129}Xe is continually transferred from the pump chamber to the probe chamber as the probe FID signal is acquired. This leads to a non-exponentially decaying probe FID.

3.4.5 Characterization of ^{129}Xe Polarization

Next, we characterized the ^{129}Xe polarization in the pump and probe chambers as a function of total gas flow rate, alkali atomic density, and pumping power. Figure 3.13 shows the amplitude of the FIDs and the corresponding ^{129}Xe polarization in the pump and probe chambers, which is estimated using Equation 3.20, as a function of total gas flow rate. We observe that the polarization in the pump chamber decreases with flow, while in the probe chamber it builds up, peaks at a flow rate at which the influx of polarized ^{129}Xe is comparable with its spin relaxation rate, and then follows the pump polarization.

The measurements of the ^{129}Xe polarization in the pump chamber, P_{Xe}^{Pu} , and in the probe chamber, P_{Xe}^{Pr} , can be interpreted by slightly modifying Equation 3.7 to account for ^{129}Xe relaxation due to the flow of gas out of each chamber:

$$P_{Xe}^{Pu} = \frac{R_{SE}^{Pu}}{R_{SE}^{Pu} + R_{wall}^{Pu} + R_{flow}^{Pu}} P_{Rb}^{Pu} \quad (3.21)$$

$$P_{Xe}^{Pr} \cong \frac{R_{flow}^{Pr}}{R_{SE}^{Pr} + R_{wall}^{Pr} + R_{flow}^{Pr}} P_{Rb}^{Pr} \quad (3.22)$$

Here, the superscripts Pu and Pr indicate the pump and probe chambers, respectively, and R_{flow} is the inverse of the ^{129}Xe transit time through the chamber.

The solid line in Figure 3.13 corresponds to a fit of the data using Equation 3.21 with R_{SE}^{Pu} as a free parameter and with $P_{Rb}^{Pu} = 0.36$ and $R_{wall}^{Pu} = 0.63 \text{ s}^{-1}$. The determination of

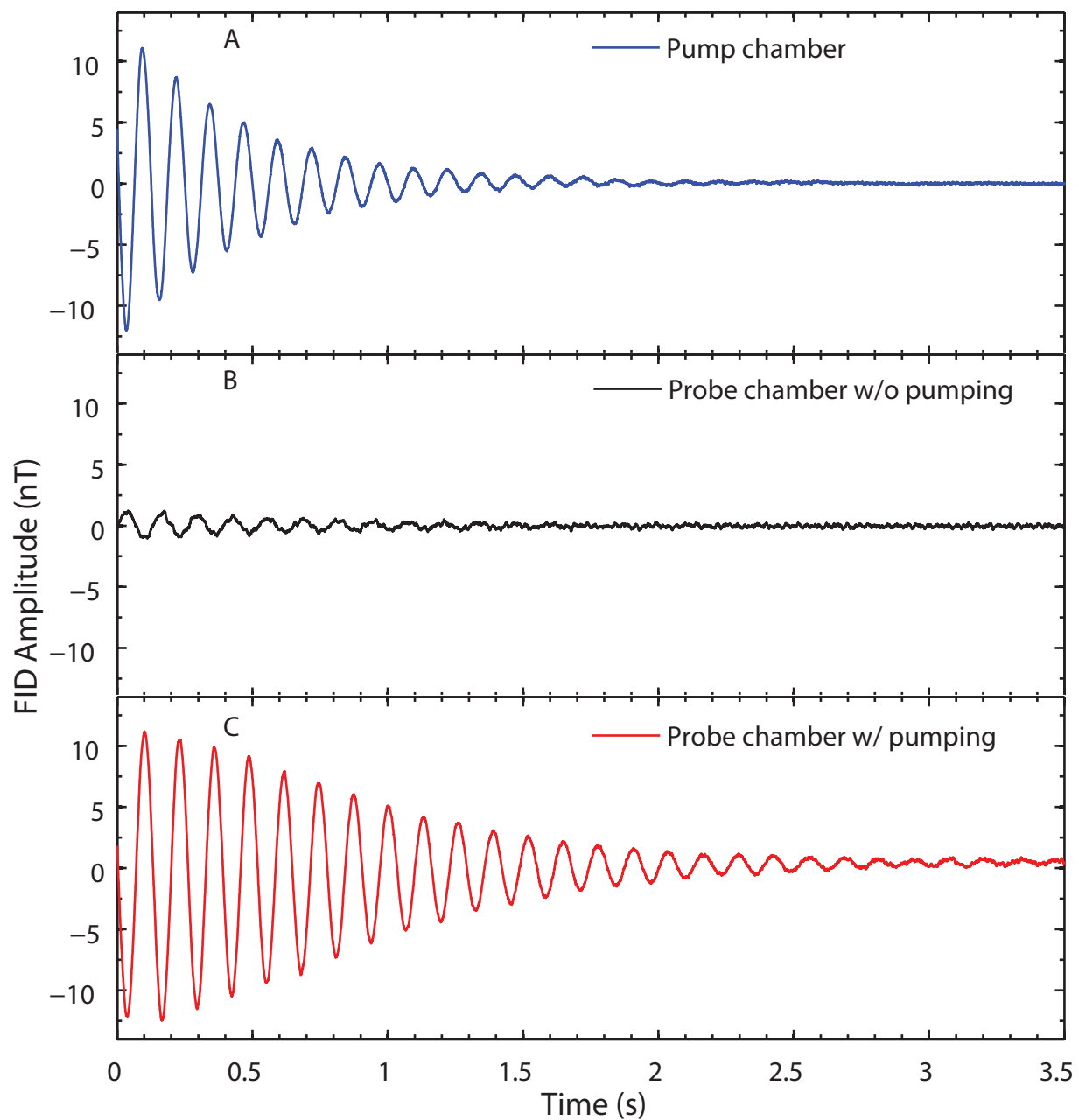


Figure 3.12: Transport of Polarized ^{129}Xe . A) FID of the transverse ^{129}Xe polarization in the pump chamber at a total gas flow rate of $3 \mu\text{L s}^{-1}$. C) FID of polarized ^{129}Xe in the probe chamber with the pump light turned off. D) FID of polarized ^{129}Xe in the probe chamber with the pump light turned on. Note that for this particular experiment the FID obtained in (B) is 180° out of phase with that obtained in the pump chamber (A), due to the use of opposite-handed polarizing photons in the pump and probe chambers.

these numbers is described in Section 3.4.6. From the obtained value for $R_{SE}^{Pu} = 0.012 \text{ s}^{-1}$ and a ^{87}Rb atomic density of $6 \times 10^{13} \text{ cm}^{-3}$ in the pump chamber, which is estimated from the measured on-resonance optical depth, we estimate $\gamma_{SE} = 1.7 \times 10^{-16} \text{ cm}^3 \text{ s}^{-1}$, in agreement with reported values for the spin exchange rate coefficient due to ^{87}Rb - ^{129}Xe binary collisions [81]. The dashed line corresponds to the evaluation of Equation 3.21 using the fitted value for P_{Xe}^{Pu} and $R_{wall}^{Pr} = 0.63 \text{ s}^{-1}$.

Equations 3.21 and 3.22 are useful for understanding some characteristics of our device and guide future designs. We note, though, that they neglect a few effects. Firstly, they assume that the majority of the polarized ^{129}Xe in the probe chamber is there due to the flow of polarized ^{129}Xe from the pump chamber; that is, they assume that the SEOP rate in the probe chamber is small. This is a good approximation for the majority of our experiments; however, it becomes less accurate at low flow rates. More importantly, they neglect diffusion, which is also a good approximation for most of our experiments but again limits the estimates of Equation 3.22 at low flow rates. For example, Figure 3.13 demonstrates that, even when no gas is flowing, the polarization in the probe chamber is larger when the pump laser is on than when it is off. We have performed experiments, which are described in Section 3.4.7, indicating that this is due to the transport of polarized ^{129}Xe from the pump chamber to the probe chamber due to diffusion. Diffusion is expected to be a uniquely important process for polarization transport in microfluidic systems such as those described here, for which the diffusion length is considerably larger than the characteristic size of the chip.

The dependence of the ^{129}Xe polarization in the pump chamber on the alkali atomic density, without gas flow, is shown in Figure 3.14. In our device, relaxation due to ^{129}Xe collisions with the glass walls is much faster than relaxation from spin exchange collisions. Thus, Equation ?? predicts a linear relationship between ^{129}Xe polarization and the ^{87}Rb - ^{129}Xe spin exchange rate, which is proportional to the ^{87}Rb atomic density. This relationship, and a constant ^{87}Rb polarization, explain the linear behavior of the ^{129}Xe polarization for low alkali atomic densities.

At higher alkali densities the ensemble becomes optically thick and, given a finite amount of pumping power, the alkali polarization decreases. As a result the ^{129}Xe polarization no longer increases linearly with alkali density. This limitation can be observed in Figure 3.15, which shows the ^{129}Xe polarization in the pump chamber as a function of pumping power for low and high values of the optical depth OD_0 . At low optical thickness the ^{129}Xe polarization saturates, whereas for the larger optical depth it remains unsaturated at the highest applied optical powers. These results suggest that larger ^{129}Xe polarizations can be achieved by increasing both the ^{87}Rb density and pumping rate.

3.4.6 Characterization of ^{129}Xe NMR Parameters

Figure 3.16 shows the precession frequency of transversely polarized ^{129}Xe in the pump chamber as a function of pumping power, and in the absence of gas flow, for high and low ^{87}Rb on-resonance optical depths. Figure 3.17 shows the relaxation time of transversely

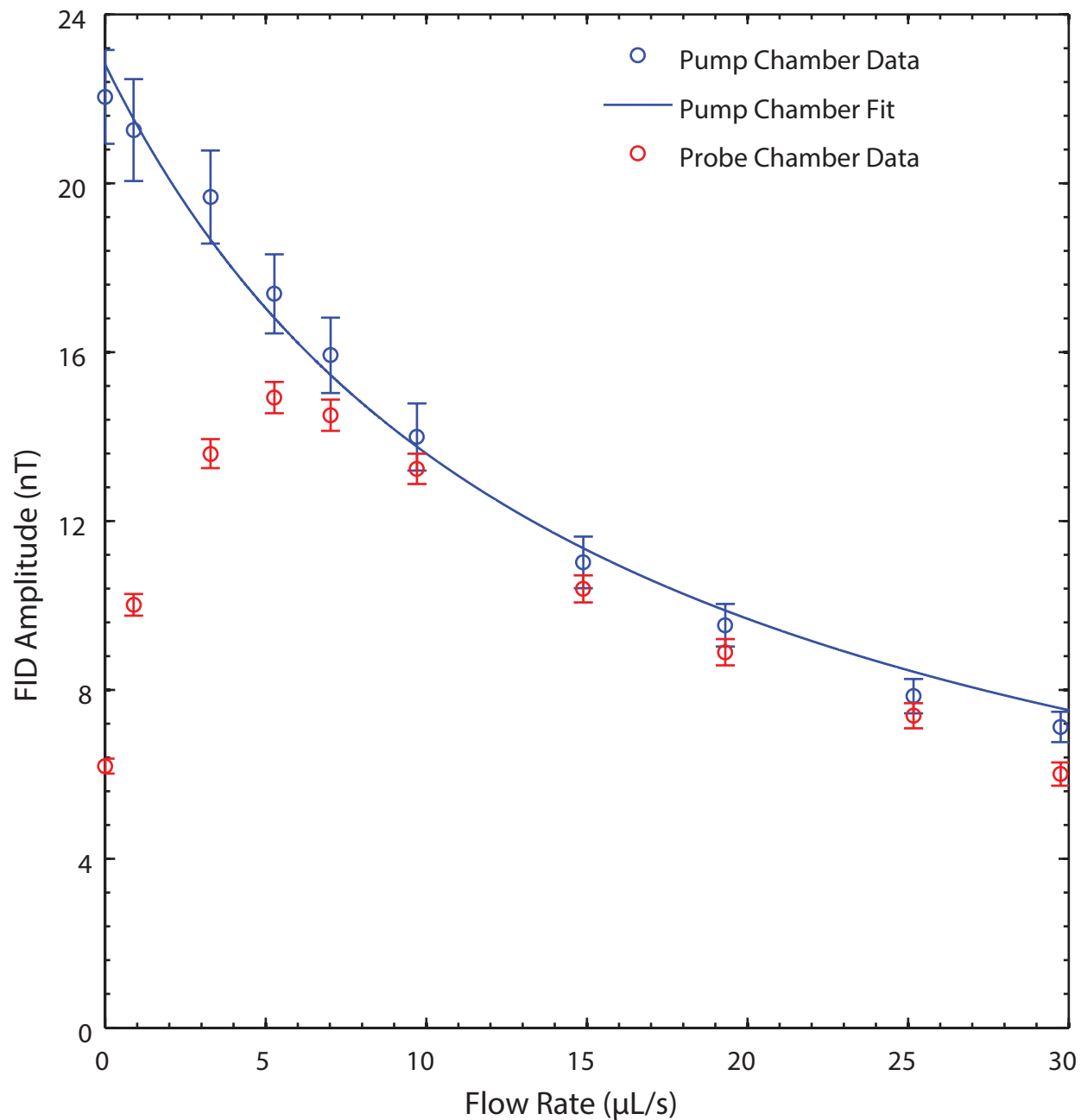


Figure 3.13: Dependence of Polarization Transfer on Gas Flow. ^{129}Xe FID amplitude as a function of total gas flow rate in the pump chamber (blue circles), in the probe chamber with the pump light on (red circles), and with the pump light off (black squares).

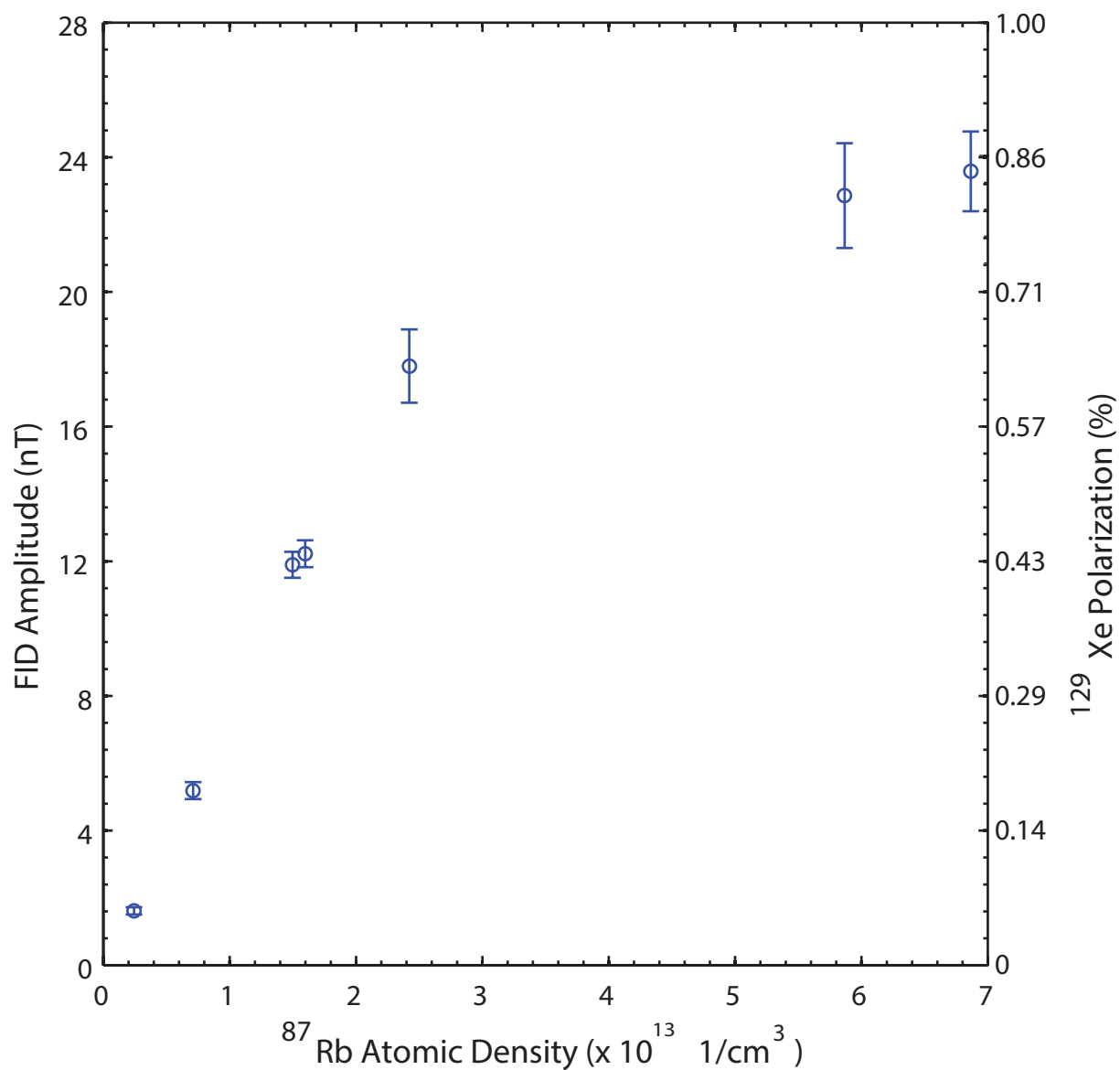


Figure 3.14: Dependence of Polarization on Alkali Density. Pump FID amplitude with no gas flow and 8 mW of pumping power as a function of ^{87}Rb atomic density.

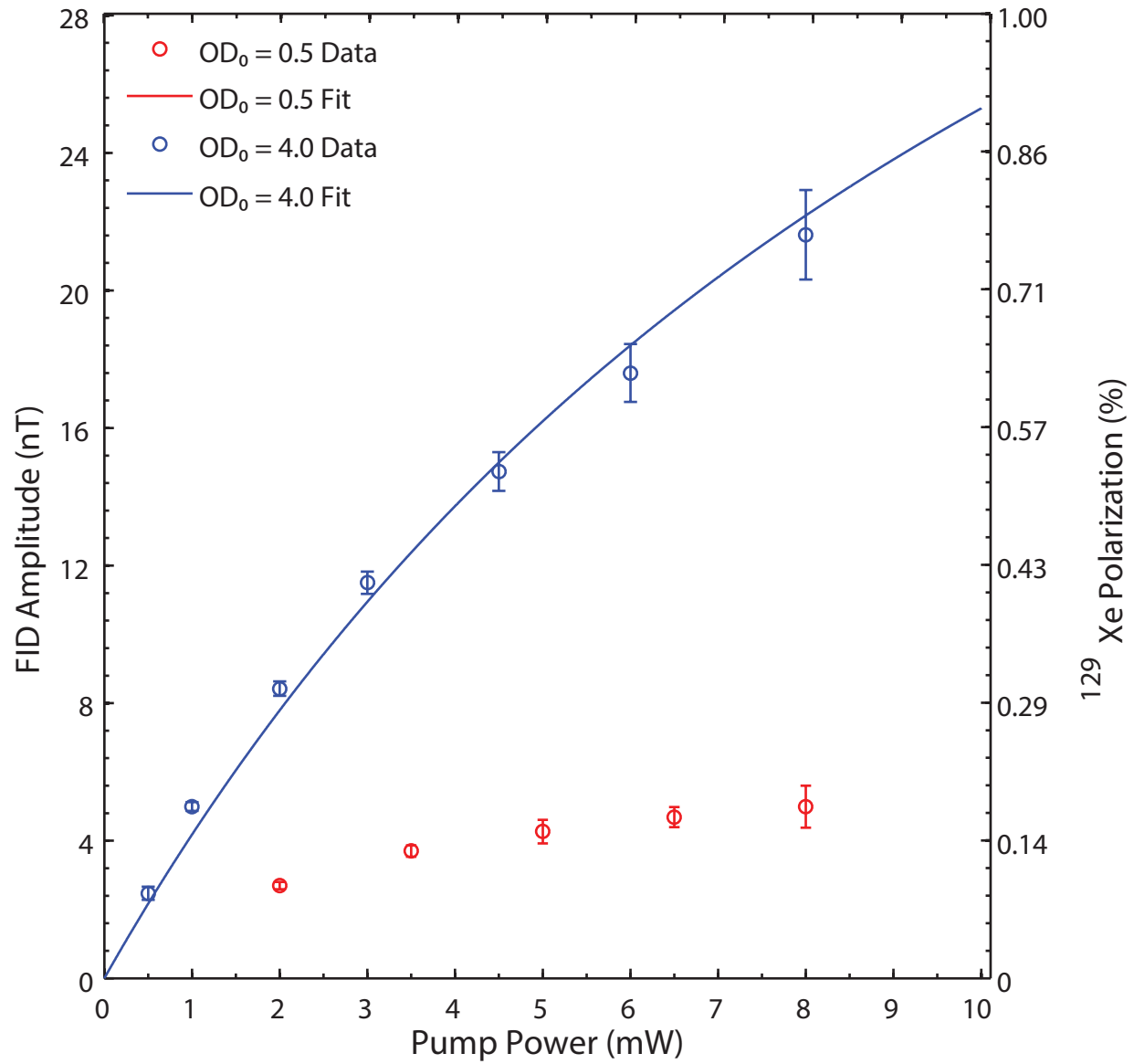


Figure 3.15: Dependence of Polarization on Pumping Power. Pump FID amplitude with no gas flow as a function of optical pumping power for $\text{OD}_0 = 4$ ($n_{Rb} = 6 \times 10^{13} \text{ cm}^{-3}$, shown in blue circles) and $\text{OD}_0 = 0.5$ ($n_{Rb} = 1 \times 10^{13} \text{ cm}^{-3}$, shown in blue circles).

polarized ^{129}Xe in the pump chamber as a function of pumping power, and in the absence of gas flow, for high and low ^{87}Rb on-resonance optical depths. In these figures we can observe additional effects of ^{87}Rb on the ^{129}Xe atoms: namely a frequency shift and faster decay in the ^{129}Xe FID. Just as ^{87}Rb atoms detect the ^{129}Xe magnetization, ^{129}Xe atoms can detect the ^{87}Rb magnetization, which depends on the atomic density of the polarized alkali ensemble. Thus as the ^{87}Rb magnetization increases, the ^{129}Xe precession frequency is shifted. This effect occurs both due to an increase in optical pumping power (which increases the ^{87}Rb polarization) and due to an increase in the ^{87}Rb density (which increases the number of polarized ^{87}Rb atoms able to transfer polarization to ^{129}Xe). The magnitude of the magnetic field B_{Xe} seen by ^{129}Xe due to the ^{87}Rb magnetization M_{Rb} can be approximated by:

$$B_{Xe} = \frac{2}{3}\mu_0\kappa_0 M_{Rb} \quad (3.23)$$

Here, $M_{Rb} = \mu_B n_{Rb} P_{Rb}$, where μ_B is the Bohr magneton. The solid lines in Figure 3.16 correspond to the fit of the data to the model for the ^{129}Xe NMR frequency ν_{Xe} :

$$\nu_{Xe} = \gamma(B_0 + B_{Xe}) \quad (3.24)$$

The dependence of the ^{87}Rb polarization with pumping power P_{OP} is assumed to be of the form $P_{Rb} = aP_{OP}/(1 + aP_{OP})$ [44], where a is some proportionality constant, so that the fitting parameters are a , B_0 , and n_{Rb} . The estimated ^{87}Rb polarization as a function of pumping power is represented by the dashed lines in Figure 3.16. For experimental conditions at high optical depths the ^{87}Rb polarization, and thus magnetization, is not uniform across the cell volume. This non-uniform magnetization ^{87}Rb causes an inhomogeneous magnetic field as sensed by ^{129}Xe , which in turn introduces dephasing and shortens the coherence time T_2^* of the ^{129}Xe ensemble. This behavior is observed in Figure 3.17 which shows the decay time of the ensemble ^{129}Xe FID as a function of pumping power. The solid lines in this figure correspond to a linear fit to the data; the offset for both data fits corresponds to a relaxation rate of 0.63 s^{-1} , from which we infer a ^{129}Xe transverse spin relaxation time of $\approx 1.6 \text{ s}$ in the absence of pumping light. We attribute this spin relaxation to spin destruction collisions of polarized ^{129}Xe atoms with the cell walls.

3.4.7 Diffusion Transport

In this section we show that in the absence of bulk gas flow, polarized ^{129}Xe can be transported by diffusion from the chamber with higher polarization density to the one with lower density. Particularly, we describe experiments demonstrating the diffusion of polarized ^{129}Xe from the probe chamber to the pump chamber, the opposite direction to our usual flow. To achieve this result, the general experimental conditions of our device were as follows: the ^{87}Rb atomic densities in the pump and probe chambers were approximately equal, and the

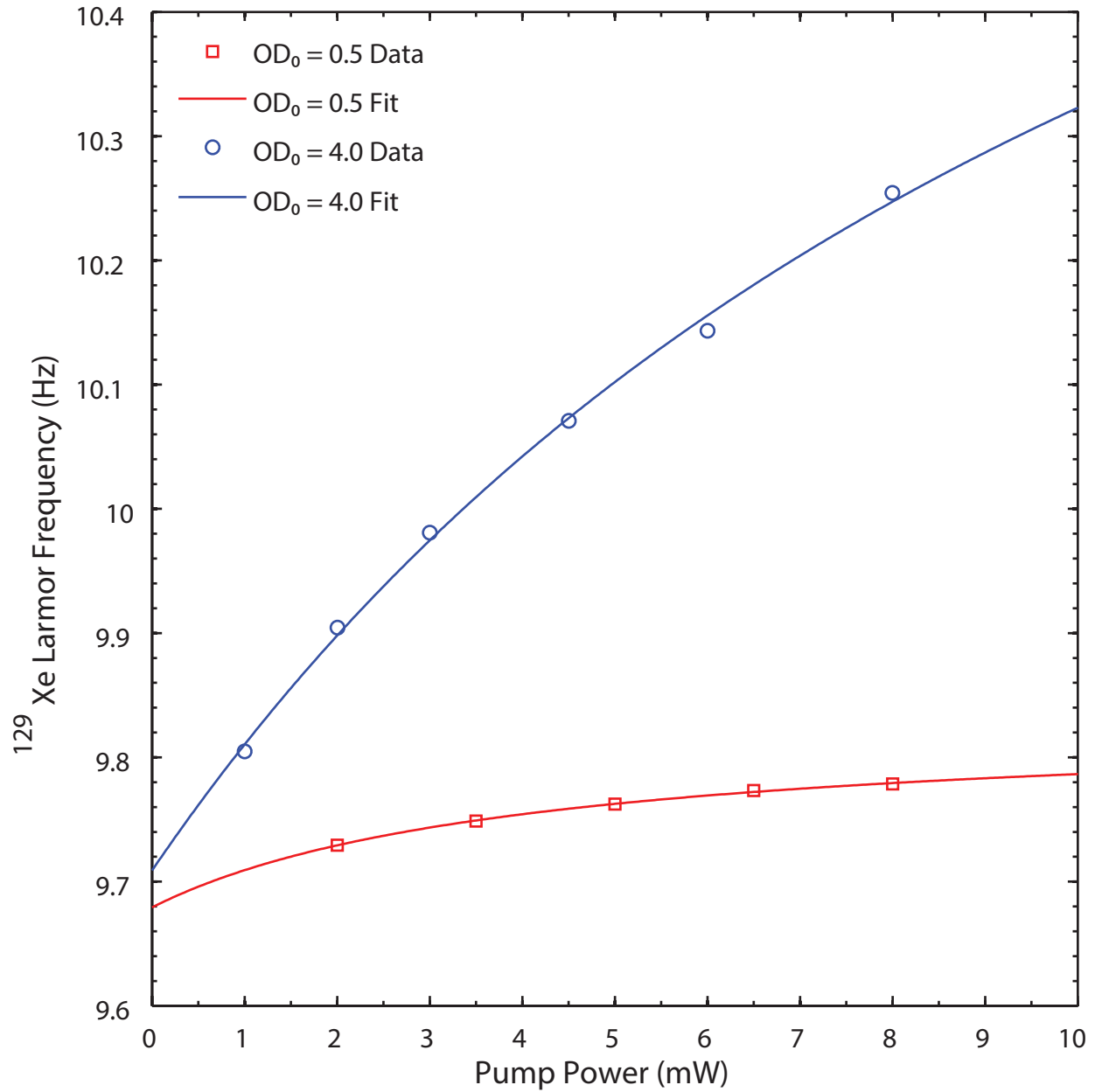


Figure 3.16: ^{129}Xe NMR Frequency. Precession frequency of transversely polarized ^{129}Xe in the pump chamber as a function of pumping power for on-resonance optical depths $OD_0 = 4$ (blue circles) and $OD_0 = 0.5$ (red squares).

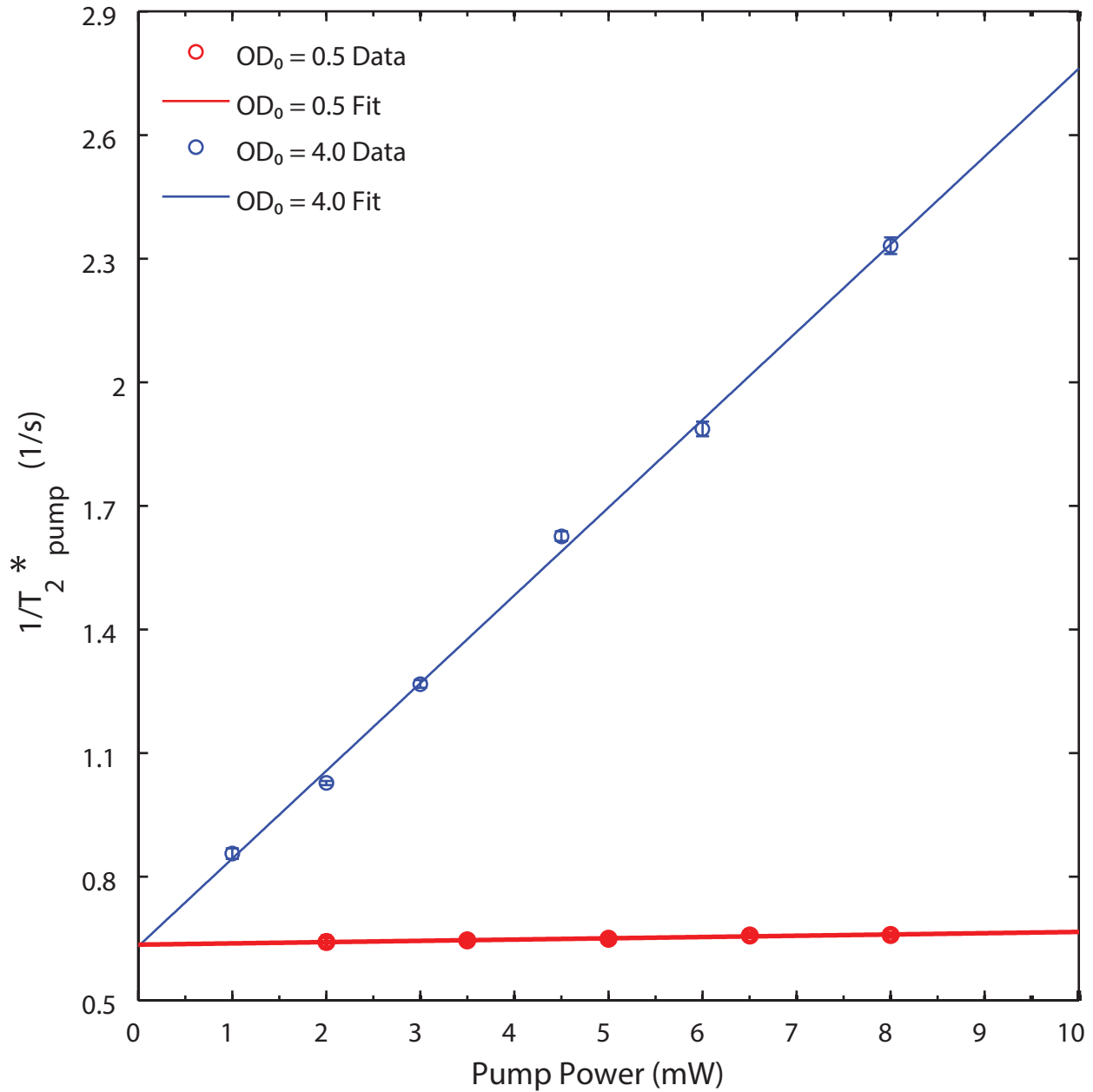


Figure 3.17: ^{129}Xe NMR Relaxation Time. T_2^* of transversely polarized ^{129}Xe in the pump chamber as a function of pumping power for on-resonance optical depths $OD_0 = 4$ (blue circles) and $OD_0 = 0.5$ (red circles).

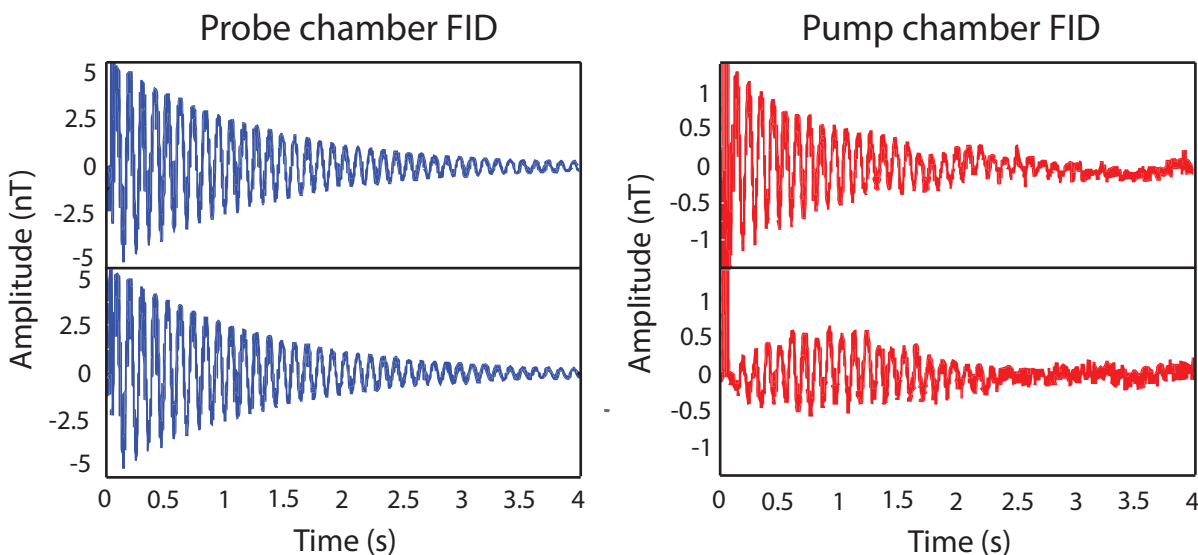


Figure 3.18: FIDs With No Gas Flow, Light On and Off. A) Probe chamber FID with pump light turned off. B) Pump chamber FID with probe light turned off. C) Probe chamber FID with pump light on. D) Pump chamber FID with probe light on.

optical power of the probe beam was much larger than that used for the pump beam. Also, the pump and probe lasers had opposite circular polarizations.

Under these conditions, and for equal polarization destruction rates in each chamber, one would expect the ^{129}Xe polarization in the probe chamber to be much larger than that in the pump chamber, and with opposite direction. This observation is verified in Figures 3.18a and 3.18b, which show the probe FID and pump FID, respectively. The pump FID in Figure 3.18b was taken while the probe light was blocked. In Figure 3.18b we observe that the pump FID is smaller than the probe FID and shifted by 180° . When the probe light is unblocked the pump FID changes significantly, as shown in Figure 3.18d. Unblocking the probe light allows SEOP of ^{129}Xe in the probe chamber, resulting in a diffusive flux of polarized ^{129}Xe into the pump chamber. Due to its opposite spin orientation, the diffused ^{129}Xe interferes destructively with the ^{129}Xe polarization produced in the pump chamber, resulting in a smaller net polarization. This behavior explains the small pump FID amplitude recorded at the beginning of the signal shown in Figure 3.18d. As time progresses, there is a diffusive flux of transversely polarized ^{129}Xe from the probe chamber to the pump chamber resulting in an increase in the pump FID amplitude with time. At later times the diffused transverse polarization decays and the pump FID amplitude decreases. To further verify that this behavior is due to the diffusion of polarized ^{129}Xe from the probe chamber, we performed the following two experiments.

In one experiment we decreased the probe beam optical power and observed the pump FID signal as a function of probe light power. The pump FID signals obtained under these conditions are shown in Figure 3.19. As the probe optical power is decreased, the ^{129}Xe polarization in the probe chamber is reduced. Thus, the flux of diffused polarized ^{129}Xe atoms into the pump chamber decreases as well, resulting in a change in the equilibrium

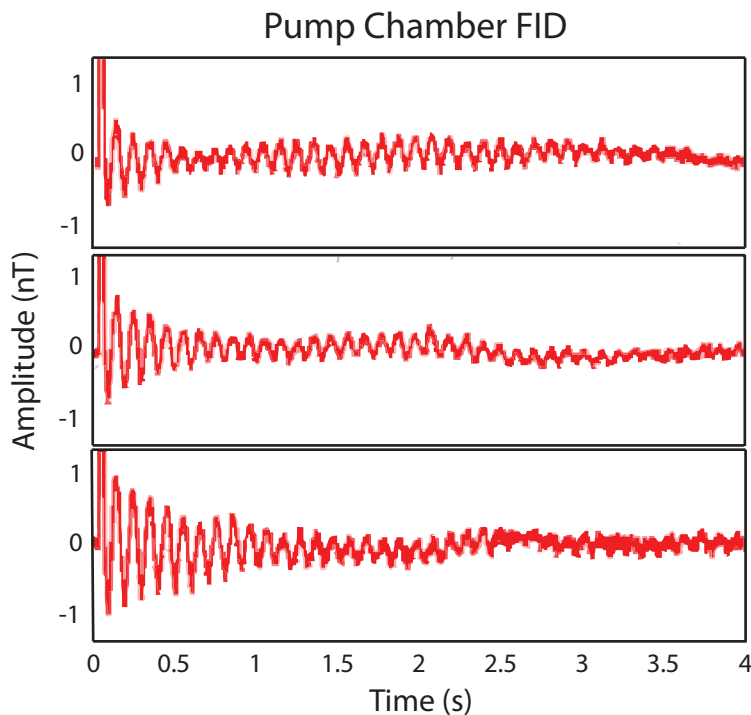


Figure 3.19: Pump FID With No Gas Flow, Varying Pump Power. The optical power of the probe laser beam decreases with (A), (B), and (C). The corresponding optical powers were 3 mW, 2 mW, and 1 mW, respectively.

longitudinal polarization in the pump chamber. After the transverse field is turned on, transversely polarized ^{129}Xe diffuses from the probe chamber to the pump chamber and adds vectorially to the precessing polarized ^{129}Xe already present in the pump chamber. This explains the dynamics observed in Figures 3.19a-c. In Figure 3.19a, the diffusion of transversely polarized ^{129}Xe into the pump chamber results in quick decay of the native pump chamber polarization and establishment of the oppositely phased signal. In Figures 3.19b-c, the smaller polarizations in the probe chamber have a smaller effect on the native pump chamber polarization.

In a second experiment we observed the effects of flowing gas on the pump FID signal. First we operated the device at conditions similar to those under which the FID signals in Figures 3.18c and 3.18d were taken. The probe and pump FID signals obtained under these experimental conditions are shown in Figures 3.20a and 3.20b, respectively. For the pump FID we observe a behavior similar to that displayed in Figure 3.18d. Figures 3.20c and 3.20d show the probe FID and pump FID, respectively, obtained when flowing gas. In our setup the direction of the bulk gas flow is from the pump chamber to the probe chamber. As the gas flow rate is increased, there is a decrease in the amount of ^{129}Xe that diffuses into the pump chamber. For a total gas flow rate of $3\ \mu\text{L s}^{-1}$, we observed that the pump FID signal recovers the structure observed with no gas flow and with the probe beam blocked. This indicates that this flow rate offsets the diffusive flux of polarized ^{129}Xe from the probe chamber to the pump chamber.

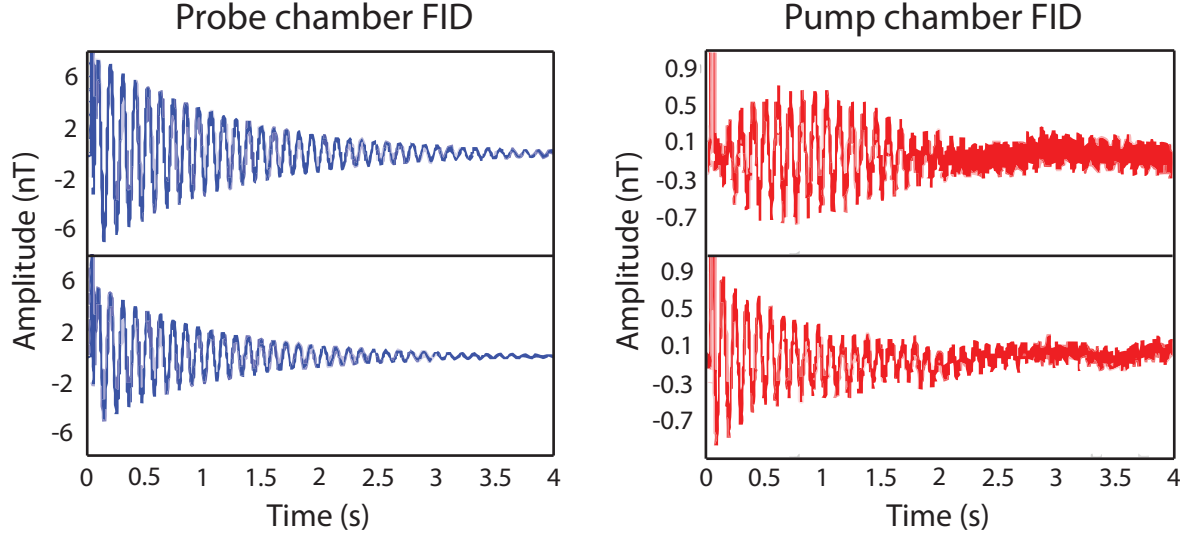


Figure 3.20: FIDs Without and With Gas Flow. FIDs for probe chamber (A) and pump chamber (B) with no gas flow. FIDs for probe chamber (C) and pump chamber (D) with total gas flow rate $3 \mu\text{L s}^{-1}$.

In general, results similar to those described above were observed when we employed strong optical pumping in the pump chamber and weak pumping in the probe chamber with no gas flow. For these conditions, diffusion of polarized atoms from the pump chamber to the probe chamber explains why, with no bulk gas flow, the amplitude of the probe FID displayed in Figure 3.13 is larger when the pump is on than when it is off. These results demonstrate that the transfer of ^{129}Xe polarization occurs due to diffusion even in the absence of bulk gas flow. This type of diffusion transport is used to transfer polarized ^3He between pumping and target chambers in electron scattering experiments [31]. For our device, future experiments will need to be performed to determine whether diffusion has an impact upon the ^{129}Xe polarization ultimately attainable in a microfabricated device and to guide engineering effort related to these effects.

3.4.8 Comparison to Other ^{129}Xe Polarizers

The current polarizations achieved in our device are much lower than the near unity polarizations achieved in some large-scale polarizers [136, 118]. However, larger polarizations are in principle achievable in our device. We note that the ^{129}Xe relaxation of 1.6 s observed in our system is 3 to 10 times faster than previously measured in sealed microfabricated cells with 1 mm^3 volume [43, 42]. In this previous work, ^{129}Xe polarizations on the order of 5% were achieved using similar ^{87}Rb densities and slightly greater light intensities than those used in our device. These observations suggest room for improvement in future designs and motivate further study of the limitations of SEOP in microfabricated confinement volumes. The combination of higher ^{87}Rb density, greater optical pumping power and improved relaxation times may allow for ^{129}Xe polarizations $> 5\%$. The current experiment is designed to

Table 3.4: Comparison Metrics for Various ^{129}Xe Polarizers

	This Work	Ref [118]	Ref [120]
^{129}Xe Polarization	0.45%	30%	8.6%
Total Gas Flow	10 $\mu\text{L s}^{-1}$	N/A	10 mL s^{-1}
^{129}Xe Gas Flow	0.8 $\mu\text{L s}^{-1}$	260 $\mu\text{L s}^{-1}$	260 $\mu\text{L s}^{-1}$
Pumping Chamber Volume	25 μL	0.5 L	0.5 L
Pumping Power	8 mW	170 W	25 W
Absorbed Light	8 mW	90 W	5.9 W
Spin-Transfer Efficiency	0.0038	0.005	0.046
^{129}Xe Polarization Flow Rate / Pumping Power	0.45 $\mu\text{L s}^{-1} \text{ W}^{-1}$	0.45 $\mu\text{L s}^{-1} \text{ W}^{-1}$	0.89 $\mu\text{L s}^{-1} \text{ W}^{-1}$

produce a large magnetization signal at the expense of polarization through the use of a high ^{129}Xe partial pressure; the use of lower partial pressures is expected to trade off magnetization signal for increased polarization, as is the usual case in large-scale polarizers (a notable exception here is the recent development of a large-scale polarizer which achieves very high polarizations even with very high ^{129}Xe partial pressures [118], which seems to have found a “sweet spot” in the polarizer parameters).

An important figure of merit of a polarizer’s performance is its spin-transfer efficiency $\eta_{transfer}$ [14, 171], defined as the ratio between the production rate of polarized ^{129}Xe atoms and the number of photons being absorbed by the alkali ensemble per unit time:

$$\eta_{transfer} = \frac{\frac{d}{dt} P_{Xe} n_{Xe} V_{cell}}{\frac{dI}{dt}} \quad (3.25)$$

Here, V_{cell} is the cell volume and dI/dt is the number of photons absorbed by the alkali ensemble per unit time. Physically, $1/\eta_{transfer}$ represents the number of photons required to polarize the nuclear spin of a ^{129}Xe atom. From the achieved ^{129}Xe polarization of 0.45% at a total gas flow rate of 10 $\mu\text{L s}^{-1}$, and assuming that the 8 mW of incident pumping light is absorbed by the alkali atoms, we estimate that the spin-transfer efficiency of our device is $\eta_{transfer} = 0.0038$. This number is one order of magnitude smaller than the maximum spin-transfer efficiency estimated for the Rb-Xe system due to binary collisions $\eta_{transfer} = 0.047$ [14, 171], but comparable with the spin-transfer efficiency of large-scale polarizers that achieve a polarization on the order of 30% [118]. Table 3.4 compares the estimated spin-transfer efficiency of our device, along with other device parameters, with those of previous polarizers [118, 120]. Another figure of merit shown in Table 3.4 is the ratio of the polarized atom flow rate to the optical pumping power. Based on this metric the production rate per Watt of pumping light for our device is 0.45 $\mu\text{L s}^{-1} \text{ W}^{-1}$. Once again, this is comparable to the production rate in large-scale polarizers [118].

In this work, we employ the ^{87}Rb atoms as *in situ* detectors of the ^{129}Xe magnetization and take advantage of the Fermi-contact interaction to enhance the detection sensitivity to ^{129}Xe by a factor of approximately 500. This approach represents an important feature of

our device that can be used to detect or monitor the ^{129}Xe polarization with high sensitivity at low magnetic fields in cases where the polarized gas can be cleanly extracted from the sample region. If the polarized gas cannot be extracted, as may occur if the gas has been dissolved in a liquid, for example, the device can be easily integrated with microfabricated magnetometers placed outside of the pump and probe chambers. Microfabricated magnetometers have demonstrated sensitivities of $5 \text{ fT Hz}^{-1/2}$ over bandwidths of 100 Hz, more than sensitive enough to detect the magnetic fields due to the currently produced ^{129}Xe polarization, and have been used to detect low-field NMR in microfluidics [95]. This approach may be useful for applications that are not compatible with the presence of alkali atoms in the microfluidic channels.

We note that conventional glassblowing could also be used to make a compact system capable of producing small quantities of hyperpolarized gas. Compared with the microfabricated Si-based system we describe here, we expect such a system to be somewhat larger and to require a higher pumping power, but likely to produce larger quantities of more highly polarized gas, due to the weaker wall relaxation and larger pumping volume. While the microfabricated chip described here is not particularly complicated to fabricate, requiring only simple lithography, etching and bonding, a compact glass-blown system may be simpler to implement in a laboratory setting to carry out scientific experiments on microfluidic systems. However, the microfabricated platform will likely be easier to integrate with other Si-based microfluidic systems due to the common platform material. Microfabrication is likely to be most advantageous in an industrial setting where large numbers of devices might be manufactured. The possibility for large-scale parallel fabrication of devices would likely lead to lower production costs and higher reliability compared with conventional glass-blown systems.

3.5 Second-Generation Miniature ^{129}Xe Polarizer

A second-generation system was designed and built at UC Berkeley in order to improve on design flaws which become evident during testing of the first-generation device, as well as to allow more advanced ^{129}Xe experiments, such as imaging.

3.5.1 Device Design

The second-generation device is again fabricated from Si and glass using a combination of DRIE and anodic bonding. The overall dimensions of the Si pre-form are 4 cm x 2 cm x 1 mm thick, larger than the first-generation device which had overall dimensions of 3 cm x 1 cm x 1 mm. A larger size is used to allow the inclusion of a 1 cm x 1 cm area in which NMR and MRI experiments can be conducted. The device remains composed of 4 chambers. The inlet and outlet chambers have been made much larger (5 mm x 10 mm) to allow for the attachment of glass tubing to one of the glass windows. A 1 mm long x 300 μm wide

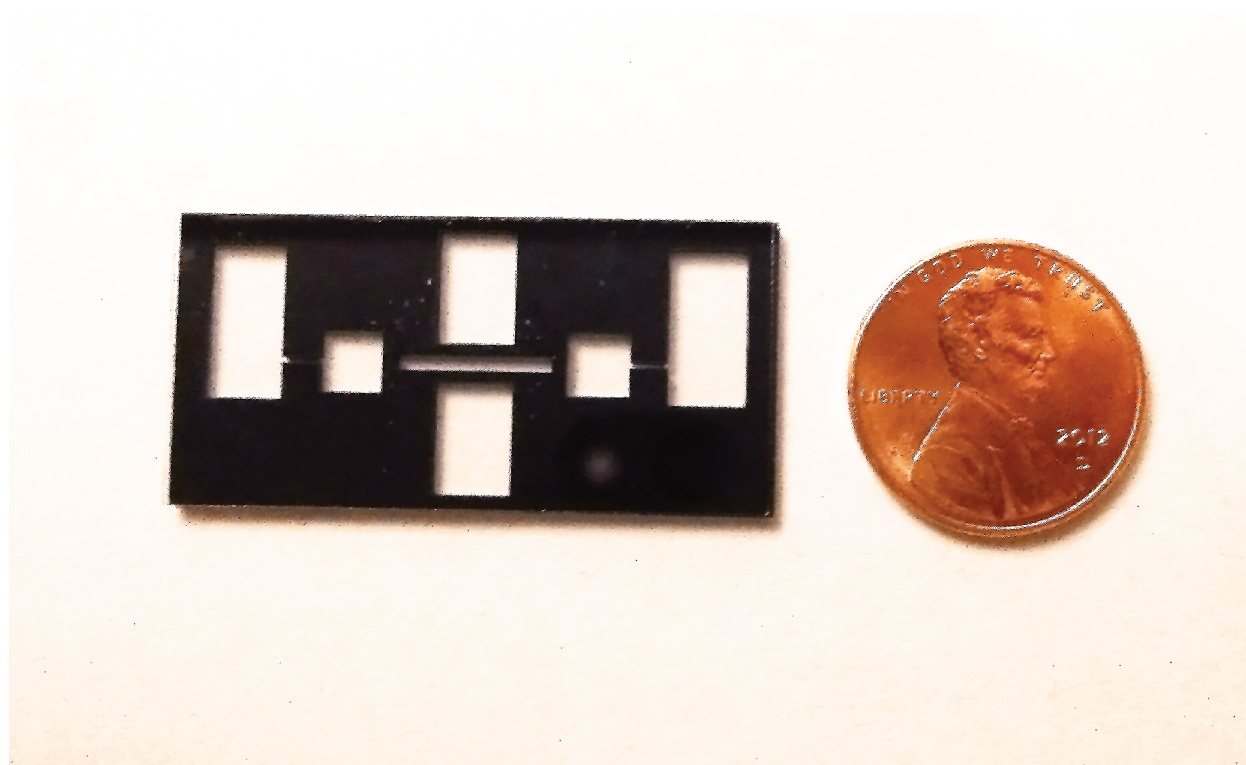


Figure 3.21: Second-Generation Microfluidic ^{129}Xe Polarizer. The Si chip footprint is 4 cm x 2 cm, with a thickness of 1 mm. The dimensions of the pump and probe chambers are 4 mm x 4 mm x 1 mm. Spectroscopy and imaging experiments may be conducted in a 1 cm x 1 cm x 1 mm region between the pump and probe chambers. Two tall, narrow grooves are etched from the middle of the chip to provide thermal isolation between the two sides of the device.

x 300 μm deep channel separates the inlet chamber from the pump chamber and the probe chamber from the outlet chamber. The pump chamber and probe chamber each have lateral dimensions of 4 mm x 4 mm. A 1 cm x 1 cm spectroscopy/imaging region is separated from the pump and probe chambers by 1 mm long x 300 μm wide x 300 μm deep channels. The two sides of the chip are again separated by tall, narrow holes in the Si, thermally isolating the two sides and allowing independent heating of the pump and probe chambers. The second-generation device architecture is shown in Figure 3.21.

3.5.2 Device Fabrication Improvements

In order to decrease gas leaks into the system, the gas flow connections into the inlet and outlet chambers are entirely made of glass. In order to make these connections, we used a homemade jig to align 6 mm outer diameter (OD), thick-walled Pyrex tubing to holes which had been drilled into a 700 μm thick Pyrex plate. The Pyrex plate was heated to 500 $^{\circ}\text{C}$ on a graphite plate to avoid temperature gradients across the glass. A natural gas torch was used to fuse the glass tubing to the glass plate using conventional glassblowing techniques. We

found that glass plates thinner than 700 μm were subject to warping during the glass fusion process, making it impossible to obtain a vacuum-tight seal during the anodic bonding step. The glass tubes were connected using pieces of glass rods as crossbars in order to reduce vibrations that might cause the tubes to break during operation of the device. The tubes were fused (again using glassblowing techniques) onto Pyrex-to-stainless steel connectors fitted with VCR attachments, allowing connection to the vacuum system.

3.5.3 Cell Filling Improvements

As noted in Section 3.4.8, the ^{129}Xe relaxation time of 1.6 s observed in the first generation polarizer was much shorter than the ^{129}Xe relaxation times measured in sealed microfabricated cells with 1 mm^3 volume [43, 42]. One possible reason for this is the rather uncontrolled manner in which ^{87}Rb is added to the first-generation device. Dissolved $^{87}\text{RbCl}$ and Ba_2N_6 salts are pipetted into the pump and probe chambers and react to form ^{87}Rb metal, N_2 gas, and BaCl_2 salt when heated. The pipetting process offers little control over placement of the two salts. We found that a large amount of BaCl_2 salt ended up forming a porous coating of the pump and probe chamber walls; this is in contrast to sealed cells, in which the chemistry may be far more easily controlled via vacuum deposition processes. While we don't expect this salt to be paramagnetic (and thus lead to a direct increase in the ^{129}Xe relaxation rate), we do expect it to adsorb ^{129}Xe and hold polarized ^{129}Xe atoms near the glass walls of each chamber. Since it is believed that the dominant mechanism for ^{129}Xe wall relaxation is due to the presence of paramagnetic impurities in the glass, such absorption may lead to an increased relaxation rate.

In order to avoid the formation of such porous BaCl_2 films in the pump and probe chambers, the second-generation device uses a different method to deposit ^{87}Rb in the chambers. It is based on the photolysis of $^{87}\text{RbN}_3$ to form ^{87}Rb metal and N_2 gas, with no additional products formed [97, 177]. In the second-generation device, we add the $^{87}\text{RbN}_3$ to the input, pump, probe, and output chambers once a single glass window has been anodically bonded to the Si preform. We anodically bond the second glass window after the $^{87}\text{RbN}_3$ has been added, allowing precise placement of the salt in each chamber. We pipet approximately 4 μL of 5.74 M $^{87}\text{RbN}_3$ solution into each chamber while heating the device on a hot plate at 120 $^\circ\text{C}$. This temperature allows the salt to dry without spattering out of each chamber. The volume has been found to be small enough to prevent the solution from being drawn from the chamber by capillary forces. The concentration is chosen to produce 2 mg of ^{87}Rb metal in each chamber. Once the device has been sealed, attached to tubing, and vacuum pumped, the $^{87}\text{RbN}_3$ is converted to ^{87}Rb by illuminating each chamber with a KrF excimer laser emitting 248 nm light. The high-energy light photolyses the $^{87}\text{RbN}_3$. We activate ^{87}Rb metal in the input and output chambers in order to remove any remnant oxygen and water in the device before activating ^{87}Rb in the pump and probe chambers.

Table 3.5: First-Generation ^{129}Xe Polarizations and Relaxation Times

Device	#5	#6	#7	#9	#11
B_{Xe} Day 1	215 pT	18 nT	9 nT	12 nT	19 nT
B_{Xe} Day 2	110 pT	6 nT	725 pT	5 nT	8 nT
B_{Xe} Day 3	N/A	1 nT	N/A	N/A	800 pT
B_{Xe} Day 4	N/A	N/A	N/A	N/A	N/A
Pump T_2 Day 1	215 ms	1.25 s	200 ms	680 ms	1.6 s
Pump T_2 Day 2	110 ms	250 ms	100 ms	210 ms	825 ms
Pump T_2 Day 3	N/A	115 ms	N/A	N/A	250 ms
Pump T_2 Day 4	N/A	N/A	N/A	N/A	N/A
Probe T_2 Day 1	200 ms	720 ms	250 ms	875 ms	1.8 s
Probe T_2 Day 2	95 ms	200 ms	150 ms	625 ms	1.2 s
Probe T_2 Day 3	N/A	95 ms	N/A	N/A	325 ms
Probe T_2 Day 4	N/A	N/A	N/A	N/A	N/A

3.5.4 Gas Manifold Improvements

The first-generation device also suffered from long-term degradation of the hyperpolarized ^{129}Xe NMR signal. For the first few hours following activation of the ^{87}Rb metal, the miniature polarizer produced relatively high ^{129}Xe polarizations and relatively long T_2 relaxation times. Over time, however, the ^{129}Xe polarization and T_2 relaxation times decreased until a ^{129}Xe NMR signal was undetectable after a few days of operation. We have also found that the polarizations and relaxation times varied greatly across devices. In general, a ^{129}Xe signal is not detectable when the T_2 relaxation time in the pump chamber decreases to a value of less than 100 ms; in such cases, relaxation is too fast to allow efficient SEOP to detect a ^{129}Xe NMR signal. In all cases, the devices fail to produce a detectable ^{129}Xe signal after three days of operation. These results are summarized in Table 3.5. Note that numerous devices (e.g. devices #1-4) never produced a detectable ^{129}Xe NMR signal.

We believe that this time-dependent decrease in the ^{129}Xe polarization and relaxation time may be due to oxidation of the ^{87}Rb metal over time, which may occur due to slow leaks of oxygen and water into the polarizer. Since we believe the increased relaxation rates to be due to contact interactions with the cell walls, we begin by calculating how long it takes for a given leak to form a monolayer of $^{87}\text{Rb}_2\text{O}$ on the cell walls. Assuming that the cell is surrounded by normal atmospheric conditions, oxygen will be far more prevalent than water vapor. For this reason, we limit our analysis to the oxidation caused by remnant O_2 gas.

We begin by considering how long a period of time it takes for an O_2 molecule to react with a ^{87}Rb atom for the conditions under which the first-generation device operates. The

mean free path l_{MFP} of an O₂ molecule in xenon gas at pressure p is given by:

$$l_{MFP} = \frac{3k_B T}{\sqrt{2}\pi d_{Xe}^2 p} \quad (3.26)$$

Here, p is the total gas pressure and $d_{Xe} = 4.32 \times 10^{-10}$ m is the diameter of a xenon atom. Given an operating temperature of 150 °C and a total gas pressure of 600 Torr, the mean free path of a ⁸⁷Rb atom is approximately 264 nm. The root-mean square velocity of an O₂ molecule v_{RMS} is given by:

$$v_{RMS} = \sqrt{\frac{3k_B T}{m}} \quad (3.27)$$

Under our operating conditions, this gives a root-mean square velocity of approximately 574 m/s. Thus, an O₂ molecule encounters another body approximately once every 4.6×10^{-10} s. At a ⁸⁷Rb atomic density of 10^{14} cm⁻³, there are approximately 2.5×10^{12} gas-phase ⁸⁷Rb atoms in the first-generation pump chamber. This is in comparison to approximately 1.1×10^{17} Xe atoms and 2.3×10^{17} N₂ molecules. Since Xe atoms and N₂ molecules are about 10^5 times more common than ⁸⁷Rb atoms, a meandering O₂ molecule will collide with a Xe atom or N₂ molecule over 100,000 times more frequently than it will with a ⁸⁷Rb atom. On average, an O₂ molecule will encounter a ⁸⁷Rb atom approximately once every 6.3×10^{-5} s. It will encounter the four ⁸⁷Rb atoms required to form two molecules of ⁸⁷Rb₂O approximately once every 2.6×10^{-4} s.

Following the theory presented in [125], we can calculate the diffusion time of Xe in the first-generation device to be approximately 16 ms for the 1 mm distance between glass walls. We can assume the diffusion time of a ⁸⁷Rb₂O molecule is similar, owing to the similar molecular weights of Xe and ⁸⁷Rb₂O. We therefore conclude that an O₂ molecule destroys four ⁸⁷Rb atoms and is carried to the wall as ⁸⁷Rb₂O a fraction of a second after it enters the cell.

We now turn our attention to how this time scale compares to the entry of O₂ into the cell. Assuming that the unit cell of ⁸⁷Rb₂O has a characteristic length of approximately 6.74×10^{-10} m [188], we find that our glass walls support a total of approximately 1.5×10^{14} ⁸⁷Rb₂O molecules. Since two molecules of ⁸⁷Rb₂O are formed for every molecule of O₂, we only require 7.4×10^{13} molecules of O₂ to enter the system in order to coat both of the glass walls with a monolayer. Approximating all gases in the cell as ideal, we can determine the time required to allow N molecules to enter the system given a gas leak rate Q_{gas} :

$$t = \frac{N k_B T}{Q_{gas}} \quad (3.28)$$

We first consider O₂ leaks from the outside atmosphere into the vacuum system. We

assume that the leak rate when the system is under high vacuum is held to UHV standards (10^{-9} Torr L s^{-1}). Since the leak rate is proportional to the square root of the difference in pressure between the outside world and the inside of our system, we expect a leak rate of approximately 4.5×10^{-10} Torr L s^{-1} under our operating conditions. Under these conditions, formation of a monolayer of $^{87}\text{Rb}_2\text{O}$ takes approximately 10 hours. We also consider O_2 leaks due to the flow of Xe gas through our system. A typical cylinder of ultra-high purity Xe or N_2 contains approximately 1 ppm of gas impurities, including oxygen, water vapor, and hydrocarbons. The getters in the first-generation system are capable of decreasing these impurities to approximately 10 parts-per-billion (ppb). Assuming that 10 ppb of O_2 remains in our gas mixture and a gas flow rate of $5 \mu\text{L s}^{-1}$, this contributes an additional monolayer of $^{87}\text{Rb}_2\text{O}$ every 30 hours.

We are not certain how many layers of $^{87}\text{Rb}_2\text{O}$ are required to degrade the ^{129}Xe polarization and relaxation time. We assume that some three-dimensional structure is required in order to form a porous structure onto which polarized ^{129}Xe atoms may adsorb, but the exact number of layers required remains unknown. Whatever the case may be, the performance of the miniaturized ^{129}Xe polarizer is likely to be improved by substantially increasing the $^{87}\text{Rb}_2\text{O}$ monolayer formation time. In the second-generation system, the gas handling system was improved by using nickel-plated stainless steel UHV gaskets, which are rated to an order of magnitude lower leak rate than the copper UHV gaskets used in the first-generation system. We thoroughly tested each vacuum connection using a helium leak tester. In this manner, the total leak rate of the vacuum system (when pumped to the lowest achievable pressure) was determined to be approximately 5.0×10^{-11} Torr L s^{-1} , an improvement of a factor of 20 over the first-generation system. The second-generation system also uses a more effective getter cartridge to purify the Xe/ N_2 gas mixture to contain less than 1 ppb each of oxygen, water vapor, and hydrocarbons. We expect such improvements to increase the lifetime of the device by more than an order of magnitude.

3.5.5 Optical System Improvements

As discussed in Sections 3.4.5 and 3.4.6, the first-generation system suffered from problems in the design of the optics. Firstly, the available pump laser power prevented the attainment of high ^{87}Rb polarizations at large values of OD_0 . In order to attain higher ^{87}Rb polarizations, the second-generation system includes a DFB pump laser capable of producing 70 mW of power at the pump cell, in comparison to 8 mW for the first-generation system. Secondly, the small size of the first-generation pump laser beam (in comparison to the size of the pumping cell) created a non-uniform distribution of the ^{87}Rb polarization in the pumping cell, leading to faster inhomogeneous relaxation of the polarized ^{129}Xe . To correct this problem, the second-generation system uses an adjustable beam expanding telescope capable of expanding the width of the pump laser beam by a factor of 2-5. During operation of the device, we adjust the beam size until we obtain the best possible ^{129}Xe polarization, which occurs when the laser beam just fills the entire pumping chamber. If the beam is expanded to too great a degree, the pump beam power density decreases, decreasing the

^{87}Rb polarization and thus the ^{129}Xe polarization.

On the detection side, the magnetometer sensitivity of the first-generation device is limited by power fluctuations in the probe laser beam. To increase the magnetometer sensitivity, we implemented a “noise eater” optical feedback circuit, available as a single optical element from Thorlabs. This circuit takes in linearly polarized laser light. The light passes through a liquid crystal retarder and an output polarizer, imparting a slight change to the probe beam polarization. A polarizing beam splitter sends a small and fixed percentage of the beam to a photodiode. The power at this photodiode is continuously monitored and compared to a preset power level. When this value differs from the desired power level, the liquid crystal voltage is adjusted until the optical power reaches the desired level, creating a laser power feedback loop. Using this technique, we achieved a magnetometer sensitivity of $240 \text{ fT Hz}^{-1/2}$ in the probe chamber. This is an improvement of a factor of 30 over the magnetometer sensitivity obtained in the first-generation system and improves the detection sensitivity of the magnetometer to approximately 1.6×10^{10} polarized ^{129}Xe atoms. Experiments to characterize the second-generation polarizer are ongoing as of the writing of this thesis.

3.5.6 Imaging System

The second-generation system was also designed to allow gas-phase MRI experiments on the hyperpolarized ^{129}Xe . We included this capability to allow proof-of-principle experiments demonstrating the utility of the device for integrated production, sophisticated spin manipulation, and detection of hyperpolarized ^{129}Xe gas. The imaging system is based on a cylindrical geometry and is designed to easily slide in and out of the magnetic mu-metal magnetic shields (identical to those used in the first-generation system). The coils are constructed by creating a series of grooves in ultrahigh molecular weight polyethylene (UHMW-PE), which has a high softening point of 130°C and is capable of withstanding the elevated temperatures produced near the miniature polarizer. Copper wire is wound into these grooves to form the pulse and gradient coils. The pulse coils consist of a Helmholtz coil to produce a magnetic field along $\hat{z}$ and a pair of saddle coils to produce magnetic fields along $\hat{x}$ and $\hat{y}$. All of these coils utilize 10 turns of 22 gauge copper wire and are capable of producing magnetic fields of approximately 1 G A^{-1} (1.296 G A^{-1} for B_z , 0.746 G A^{-1} for B_x , and 0.849 G A^{-1} for B_y ; the differences in the B_x and B_y values are due to slight differences in the winding path of these two coils).

The gradient coils consist of a Maxwell coil to produce a gradient $\partial B_z / \partial z$ and a pair of Golay coils to produce gradients $\partial B_z / \partial x$ and $\partial B_z / \partial y$. All of these coils utilize 20 turns of 22 gauge copper wire and produce gradients of approximately $0.5 \text{ G cm}^{-1} \text{ A}^{-1}$ ($0.506 \text{ G cm}^{-1} \text{ A}^{-1}$ for $\partial B_z / \partial z$ and $0.444 \text{ G cm}^{-1} \text{ A}^{-1}$ for $\partial B_z / \partial x$ and $\partial B_z / \partial y$). In order to obtain an imaging resolution of 1 mm , it is necessary to apply gradient pulses in an amount of time which is small compared to the time required for ^{129}Xe to diffuse across an imaging voxel. Given a diffusion time of 16.6 ms for a diffusion length of 1 mm , this means that all gradient pulses should be applied in a period of no more than a few milliseconds. In order to achieve

an imaging resolution of 1 mm with a gradient pulse 1 ms long, it is necessary to apply approximately 10 A of current to the gradient coils. This can be accomplished even with the small wires used in the gradient coils due to the low duty cycle of the gradient coils in an RD-MRI experiment. Each of the coils has a rise time of approximately 10 μs . Imaging experiments are ongoing as of the time of this thesis.

Chapter 4

Microfluidic ^{129}Xe Biosensor Devices

In the previous Chapter 3, we examined the development of miniaturized devices capable of producing and sensitively detecting hyperpolarized ^{129}Xe . Although this device has been designed to be compatible with a broad range of NMR applications, the work was mostly motivated by a desire to produce a microfluidic platform for the high-sensitivity detection of arbitrary components of complex fluid mixtures. In this chapter, we focus on advances in the development of a microfluidic detection platform based on XBMSs.

The chapter is organized as follows. In Section 4.1, we consider the interaction of ^{129}Xe with various molecular cage-like structures to allow the targeted detection of molecules; we examine the cryptophane cages used to host ^{129}Xe atoms (Section 4.1.1), how these cages can be chemically modified to target particular molecules (Section 4.1.2), the use of multiple molecular sensors in a single experiment (Section 4.1.3), and the construction of macromolecular assemblies containing many sensors for substantial enhancement of the SNR (Section 4.1.4). In Section 4.2, we discuss how the sensitivity of a molecular sensing experiment may be enhanced using an indirect detection technique which exploits chemical exchange of ^{129}Xe from inside a sensor molecule, where the concentration is low, to an aqueous environment, where the concentration is much higher. In Section 4.3, we present the development of an apparatus for performing remote detection ^{129}Xe molecular sensing in a large magnetic field; we discuss the design of microfluidic remote detection probes (Section 4.3.1), a double resonance circuit for detection of both protons and ^{129}Xe (Section 4.3.2), a method for dissolving hyperpolarized ^{129}Xe into water (Chapter 4.3.3), system testing experiments (Chapter 4.3.4), and early experiments allowing the detection of hyperpolarized ^{129}Xe on a microfluidic device (Section 4.3.5). Finally, in Section 4.4, we present a microfluidic device which may allow for the simultaneous sensitive detection of an arbitrary number of molecules of interest; we develop the chemistry used to attach the ^{129}Xe molecular sensors to microfluidic devices (Section 4.4.1), provide a method for fabricating the devices (Section 4.4.2), examine the results of early device tests (Section 4.4.3), consider the current limitations to detection sensitivity (Section 4.4.4), and examine future development methods by which the detection sensitivity may be greatly improved (Section 4.4.5).

4.1 ^{129}Xe -Based Molecular Sensors

Bare hyperpolarized ^{129}Xe has found some use in the study of a few biophysical systems. Due to its lipophilicity, ^{129}Xe has been used to investigate the hydrophobic regions of proteins such as myoglobin and hemoglobin [165], lipoxygenase [19], maltose binding protein [135], ribose binding protein [99], and chemotaxis Y protein [98]. However, use of ^{129}Xe NMR for the study of proteins is limited due to the relatively low Xe-protein binding affinities (equilibrium dissociation constant $K < 200 \text{ M}^{-1}$).

In order to overcome the limitations of ^{129}Xe NMR experiments based on the weak natural interaction between Xe and the vast majority of macromolecules, researchers have developed a strategy which involves the use of a Xe host molecule which can be modified to bind to chemical targets with very high binding affinities. Early studies focused on the use of self-assembling dimers [21] or cyclodextrins [10, 154]; however, these hosts suffered from relatively low Xe affinities. Additional work with zeolites [80] and fullerenes [163] yielded much higher affinities, but were limited by the slow rate of Xe exchange into the cavities.

4.1.1 Cryptophane Cages

A host molecule with a suitable Xe binding affinity and fast enough Xe exchange dynamics was found in the class of cryptophane cages. These cages are formed by connecting two cyclotrimeratrylene hemispherical shells which are attached with three alkoxy linkers of varying length to form a nearly spherical shell [52]. They are classified according to the number of methyl groups in each of these linkers; for instance, cryptophane-111 consists of three methoxy linkers, cryptophane-222 consists of three ethoxy linkers, cryptophane-223 consists of two ethoxy linkers and one propoxy linker, etc. Of particular importance in ^{129}Xe NMR has been cryptophane-222, also known as cryptophane-A, and hereinafter referred to as CryA. CryA has an internal volume of $95 \text{ \AA}^3$, compared to the $43 \text{ \AA}^3$ volume of a Xe atom; this is very close to the optimum value of $76 \text{ \AA}^3$ [107] and means that the cage is capable of hosting only a single Xe atom at a time. CryA has relatively fast Xe exchange dynamics, allowing hyperpolarized ^{129}Xe to enter and exit the cage on a useful timescale. It also has a high binding affinity for Xe in both water and various organic solvents [9, 78]. The CryA molecule is shown in Figure 4.1.

The equilibrium dissociation constant K for the Xe-CryA system determines the attainable concentration of Xe in the CryA cage ($[\text{Xe@CryA}]$) given a Xe concentration in water (or other solvent) $[\text{Xe@H}_2\text{O}]$ and the concentration of CryA in water (or other solvent) $[\text{CryA}]$:

$$K = \frac{[\text{Xe@CryA}]}{[\text{Xe@H}_2\text{O}][\text{CryA}]} \quad (4.1)$$

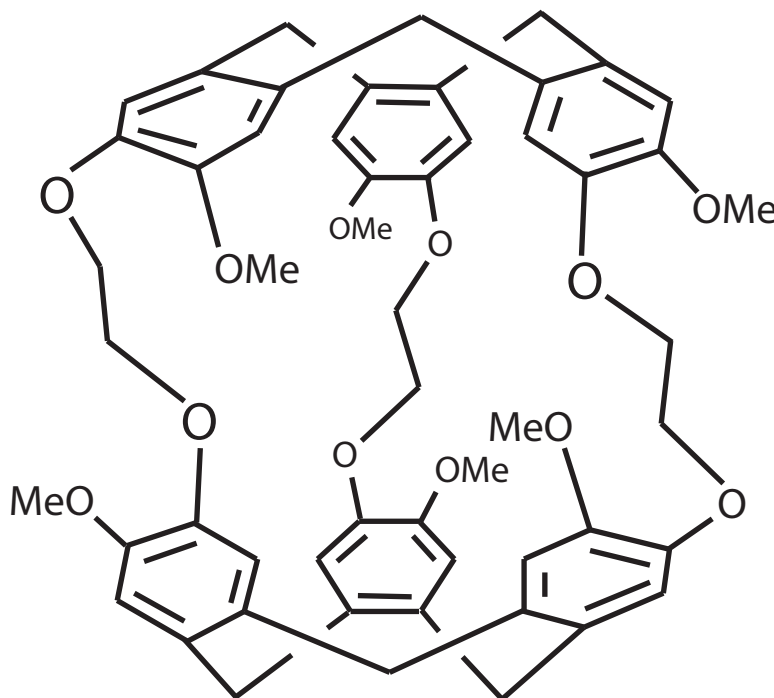


Figure 4.1: Cryptophane-A. The molecule consists of two cyclotrimeratrylene caps connected by three ethoxy linkers.

The Xe-CryA dissociation constant is strongly affected by the size of the cryptophane cage (smaller cryptophanes generally yield higher affinities), the cryptophane surface chemistry, the solvent, and the temperature. While cryptophane-111, the smallest possible cryptophane, has a very high binding affinity, it has very slow Xe exchange dynamics [50]. Bare CryA, which is slightly larger, has a lower binding affinity, but much faster Xe exchange dynamics. It has been modified to contain carboxylic acid groups on the cyclotrimeratrylene caps, greatly increasing the Xe binding affinity. For instance, modification of CryA with three carboxylic acid groups yielded dissociation constants of 17400 M^{-1} in water at 293 K and 30000 M^{-1} in water at 310 K [75]. The addition of three more carboxylic acid groups, for a total of six, greatly increases the water solubility of the cryptophane cage [79]. This increased solubility results in a decreased ^{129}Xe dissociation constant of 6800 M^{-1} in water at 293 K; while this is half as large as the value for the tri-acid CryA, it is still double that of the bare CryA. The chemical exchange time for Xe to migrate in and out of these cages is approximately 70 ms at 293 K and 5 ms at 310 K.

The ^{129}NMR response is greatly different depending on whether a ^{129}NMR atom is in the gas phase, dissolved in a solvent, or encapsulated in a CryA cage. Taking the gas-phase ^{129}Xe to be at a chemical shift of 0 ppm, a hexa-acid CryA cage provides a chemical shift of approximately 65 ppm upfield [79]. Different cryptophane cages give rise to different ^{129}Xe chemical shifts, ranging from 31 ppm for cryptophane-111 [50] to 70 ppm for mono-acid CryA [156]. These results suggest that ^{129}Xe might be sensitive to smaller perturbations in the cryptophane chemical structure. Even deuterium substitutions on the cryptophane cage

can be detected by ^{129}Xe NMR [25]. The largest measured chemical shift in a cryptophane cage of 308 ppm comes from a cryptophane-111 cage complexed with ruthenium (Ru(II)) chelates [48].

4.1.2 Cryptophane Cage Functionalization

While the ^{129}Xe in a cryptophane cage is sensitive to chemical modifications of the cage, it is still extremely limited in the classes of compounds which it can detect. In fact, it is even less sensitive to its environment than bare ^{129}Xe since it may no longer form complexes with large macromolecules while relegated to the inside of a cage. In order to overcome this limitation, researchers in the Pines laboratory modified the CryA cage to contain a chemical targeting moiety which can be easily substituted to detect arbitrary molecules of interest; the resultant molecule is known generally as a ^{129}Xe -based molecular sensor (XBMS). The original XBMS consisted of a mono-acid CryA cage attached to a small peptide (cysteine-arginine-lysine-arginine) which served to increase the solubility of the XBMS in water. This was attached to biotin through a maleimide linker, forming a XBMS with high affinity for the protein avidin [155]. When hosted in a XBMS which is not bound to avidin, the ^{129}Xe chemical shift is approximately 70.2 ppm. When hosted in a XBMS which is bound to avidin, the ^{129}Xe chemical shift moves to approximately 72.6 ppm, representing a shift of 2.4 ppm. The area under each peak is proportional to the population of ^{129}Xe in each environment. Thus, the presence of avidin can be determined by a frequency shift of the ^{129}Xe NMR signal. The relative areas of the bound and unbound peaks allow estimation of the quantity of avidin present. Interestingly, the ^{129}Xe NMR spectra show multiple peaks for both the bound and unbound XBMS; this is due to the different stereoisomers of the XBMS. The ^{129}Xe NMR spectrum for the avidin-biotin XBMS system is reproduced in Figure 4.2 to illustrate the XBMS sensing concept.

Following the development of the first XBMS, additional ^{129}Xe sensors have been developed by other research groups. Targets have included matrix metalloproteinase MMP-7 [172], a 20 base-pair long single-stranded deoxyribonucleic acid (ssDNA) [134], the carbonic anhydrase II enzyme [29], major histocompatibility complex class II protein [142], and rhodamine [53]. Each of these XBMSs achieved a ^{129}Xe chemical shift change of a few ppm. The resultant changes in chemical shift between the chemically-bound sensor (with ^{129}Xe chemical shift δ_{bound}) and the unbound sensor (with ^{129}Xe chemical shift δ_{unbound}) are summarized in Table 4.1. Additionally, XBMSs have been developed to allow detection of integrin transmembrane receptors [147] and cellular transferrin receptors [17] within human cells; to date, NMR studies have not been carried out on these XBMSs.

4.1.3 Multiplexed Molecular Detection

A method for detecting multiple chemical targets using XBMSs based on different cryptophane cages was proposed in the original XBMS paper [155]. The various cryptophane

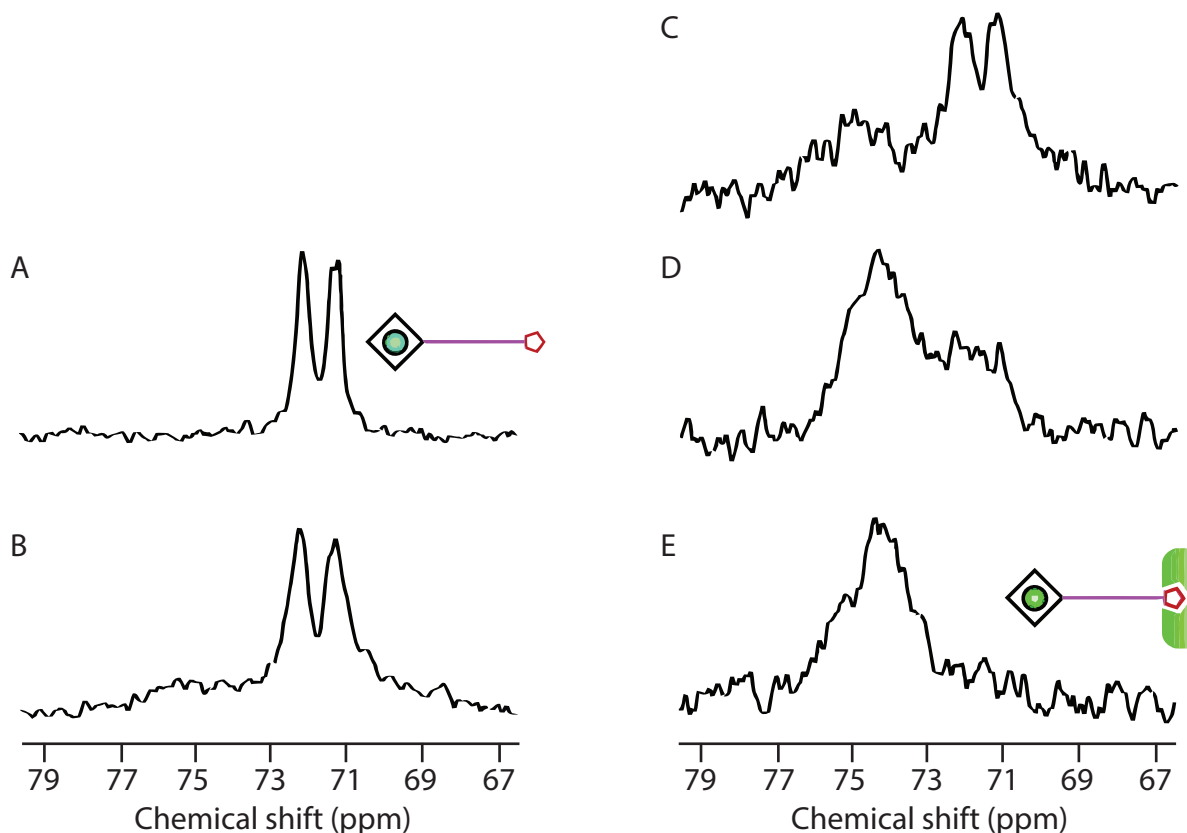


Figure 4.2: Avidin-Biotin XBMS ^{129}Xe NMR Response. The ^{129}Xe chemical shift (given in ppm) shifts from approximately 70 ppm for ^{129}Xe in an unbound XBMS functionalized with biotin (shown as a red polygon) to approximately 72.5 ppm for ^{129}Xe in a XBMS bound to avidin (shown as the green conjugate to the red polygon). The peak at approximately 71 ppm corresponds to ^{129}Xe in an un-functionalized CryA, while the peak at approximately 193 ppm corresponds to ^{129}Xe dissolved in water. A) 0% avidin (300 μM sensor, 0 μM avidin). B) 10% avidin (1800 μM sensor, 170 μM avidin). C) 20% avidin (1800 μM sensor, 350 μM avidin). D) 60% avidin (300 μM sensor, 180 μM avidin). E) 120% avidin (300 μM sensor, 360 μM avidin). Modified from [156].

Table 4.1: XBMS Studies

Target	Sensor	Change in δ
Avidin	Biotin	2.6 [155]
Matrix metalloproteinase MMP-7	MMP-7 substrate	0.5 ppm [172]
ssDNA (20 base pairs)	ssDNA (20 bas pairs)	1.5 ppm [134]
Integrin transmembrane receptor	tetra-arginylglycylaspartic acid	N/A [147]
Carbonic anhydrase II	Triazole-arylsulfonamid	7.5 ppm [29]
Major histocompatibility complex II	Polypeptide (13 residues)	1.0 ppm [142]
Transferrin receptor	Transferrin	N/A [17]
Rhodamine G6	Polypeptide (12 residues)	2.0 ppm [53]

Table 4.2: Cryptophane ^{129}Xe Chemical Shifts

Cryptophane	Solvent	Temperature (K)	^{129}Xe Chemical Shift
Cryptophane-111	$\text{C}_2\text{Cl}_4\text{D}_2$	293	31 ppm [50]
Cryptophane-A	$\text{C}_2\text{Cl}_4\text{D}_2$	293	68 ppm [24]
Cryptophane-223	$\text{C}_2\text{Cl}_4\text{D}_2$	293	60 ppm [24]
Cryptophane-233	$\text{C}_2\text{Cl}_4\text{D}_2$	293	47 ppm [24]
Cryptophane-333	$\text{C}_2\text{Cl}_4\text{D}_2$	293	30 ppm [78]
Mono-acid cryptophane-222	D_2O	293	70 ppm [156]
Hexa-acid cryptophane-A	D_2O	293	64 ppm [79]
Hexa-acid cryptophane-223	D_2O	293	52 ppm [79]
Hexa-acid cryptophane-233	D_2O	293	42 ppm [79]
Hexa-acid cryptophane-333	D_2O	293	35 ppm [79]

cages show very different ^{129}Xe chemical shifts, summarized in Table 4.2. This suggests that one possible manner of multiplexing is to attach different chemical targeting moieties to different cryptophane cages. If the difference in ^{129}Xe chemical shift between each of these cryptophane cages is greater than the chemical shift difference which arises due to chemical binding, each binding event can be easily detected.

Another possibility is to spatially separate multiple XBMSs and employ MRI methods to resolve the various targets. Such a technique was used to simultaneously detect cryptophane-111 and hexa-acid CryA [13]. In this study, the two cryptophanes were spatially separated by dissolving cryptophane-111 in deuterated tetrachloroethane and CryA in water; the immiscibility of these solvents led to spatial separation of the two cryptophanes. They were then imaged using a gradient echo pulse sequence employing frequency-selective RF pulses. A third method of multiplexed detection is presented in the experiments discussed in Section 4.4; this method utilizes patterned arrays of Cry-A XBMSs and frequency-selective RF pulses in the presence of a magnetic field gradient to spatially separate the XBMSs.

4.1.4 Multivalent XBMSs

The detection threshold for a chemical target detected with a XBMS can be drastically enhanced by synthetically increasing the number of cryptophane cages attached to a targeting moiety. This was first accomplished using water-soluble dendrimers which form a complex with CryA through acid-base and hydrophobic interactions [116]. Each complex contained two cryptophane cages, increasing the signal. Additionally, the complex was achiral, eliminating the peak splittings arising due to the cryptophane diastereomers and narrowing the ^{129}Xe NMR peak; these two effects resulted in a total increase in the SNR by a factor of approximately 8.

A more recent innovation has been the use of viral capsids as a means of carrying a large number of cryptophane cages and a single targeting moiety. The viruses are chosen because they contain a large number of repeating protein subunits which are easily chemically modi-

fied to allow covalent attachment of many cryptophane cages. The first use of this technique placed 125 CryA cages on the interior of bacteriophage MS2 [109]. This allowed a significant reduction of the detection threshold for avidin from 10 nM to 700 pM. A further decrease in the detection threshold for avidin was achieved by attaching approximately 1000 CryA cages to the exterior of bacteriophage M13 and utilizing the indirect detection techniques discussed in Section 4.2 [158]. Use of this multivalent sensor allowed for a further decrease in the avidin detection threshold to 230 fM.

4.2 Indirect Detection Through ^{129}Xe HyperCEST

While the XBMSs described in the previous Section 4.1 show great potential for chemical sensing, they suffer from a lack of sensitivity. XBMS studies generally require chemical concentrations of approximately 10 μM for experiments which utilize approximately 10 μL of the sample and are conducted at a field strength of 7.05 T. The exact concentrations required depend on the composition and polarization of the ^{129}Xe gas mixture. Regardless of the experimental conditions, this low sensitivity results from the relatively low population of polarized ^{129}Xe inside the CryA cages at any given time compared to the population of polarized ^{129}Xe dissolved in water. We can determine the relative populations at equilibrium from the disassociation constant:

$$\frac{[\text{Xe@CryA}]}{[\text{Xe@H}_2\text{O}]} = K[\text{CryA}] \quad (4.2)$$

To determine the relative populations, we assume a Xe-CryA dissociation constant of approximately 10000 M^{-1} , similar to those reported in previous CryA studies. In this case, using a CryA concentration of 1 μM , we find that there are 100 times as many Xe atoms dissolved in water as there are inside a CryA cage. If we decrease the CryA concentration to 1 nM, this becomes a factor of 10^5 . Any attempt to use a low concentration of CryA cage leads to a situation in which much more polarized Xe is dissolved in water than occupies the cage.

For completeness, we can determine the extent to which the CryA cages in solution are occupied by a Xe atom. The degree of occupancy of the CryA cage O_{CryA} is defined as the fraction of cage molecules filled by a polarized Xe atom and can be determined using the equilibrium constant K :

$$\begin{aligned} O_{\text{CryA}} &\equiv \frac{[\text{Xe@CryA}]}{[\text{CryA}] + [\text{Xe@CryA}]} \\ &= \frac{K[\text{Xe@H}_2\text{O}]}{1 + K[\text{Xe@H}_2\text{O}]} \end{aligned} \quad (4.3)$$

The concentration of Xe gas in water is given by:

$$[\text{Xe}@H_2O] = p_{\text{Xe}}[H_2O]X_{\text{Xe}} \quad (4.4)$$

Here, p_{Xe} is the Xe partial pressure (in units of atm), $[H_2O]$ is the concentration of water, and X_{Xe} is the mole fraction of Xe dissolved in water. At 25 °C, $[H_2O] \approx 55 \text{ M}$ and $X_{\text{Xe}} = 7.890 \times 10^{-5}$. To determine the occupancy, we again assume a Xe-CryA dissociation constant of approximately 10000 M^{-1} . We further assume that the gas mixture contains 2% Xe gas (with the remaining 98% being either N_2 or a combination of N_2 and He) with a total gas pressure of approximately 75 psi; this is similar to the gas mixture that has been used in XBMS experiments in the Pines laboratory. In this case, we obtain $O_{\text{CryA}} = 81.6\%$. Thus, it is not possible to obtain a great increase in the CryA ^{129}Xe NMR signal by forcing more Xe into the CryA.

In order to obtain lower detection limits, we have a few options. When directly detecting the CryA ^{129}Xe NMR signal, we can increase the SNR by a factor of nearly 4 by using a ^{129}Xe -enriched gas mixture instead of a mixture containing natural isotopic abundance Xe. This method is expensive, as a gas cylinder of 90% enriched ^{129}Xe costs approximately 20 times more than a cylinder containing Xe in natural isotopic abundance (26.4% ^{129}Xe). The CryA ^{129}Xe NMR signal can also be enhanced using the multivalent sensor methods discussed in Section 4.1.4. Finally, the ^{129}Xe polarization can be increased. However, these methods are all fundamentally limited by the relatively low quantity of ^{129}Xe in the CryA cage at any given time.

Another method of improving the detection limit relies on indirect detection based on chemical exchange of ^{129}Xe between the CryA cage, where it exists in low concentration, and water, where it exists in a much higher concentration. This method is known as chemical exchange saturation transfer of hyperpolarized nuclei, or HyperCEST [145]. As discussed in Section 4.1.1, the Xe exchange time into and out of the CryA cage is typically between 10 ms and 100 ms, depending on the temperature. This exchange time is very slow on the timescale of the ^{129}Xe Larmor precession at high magnetic fields. While in the CryA cage, ^{129}Xe atoms have a Larmor frequency which is very different from the Larmor frequency of ^{129}Xe atoms dissolved in water (approximately 193 ppm chemical shift for ^{129}Xe in water versus approximately 65 ppm for ^{129}Xe in CryA). Given this unique resonance frequency and the relatively long time that a ^{129}Xe atom spends in the CryA cage, it is possible to apply frequency-selective RF pulses to target only the ^{129}Xe in CryA, leaving the ^{129}Xe in water unaffected.

In a HyperCEST experiment, an RF pulse tuned to the Larmor frequency of ^{129}Xe in CryA is applied for a period of a few seconds. The power of the pulse is often selected so that an average ^{129}Xe atom experiences a 90° rotation during the time that it spends in the CryA cage. When the ^{129}Xe atom leaves the CryA cage, it is no longer polarized along $\hat{z}$; we refer to this as the ^{129}Xe atom being saturated. Over time, new ^{129}Xe atoms move into the CryA cage and become saturated. They then move into the population of ^{129}Xe

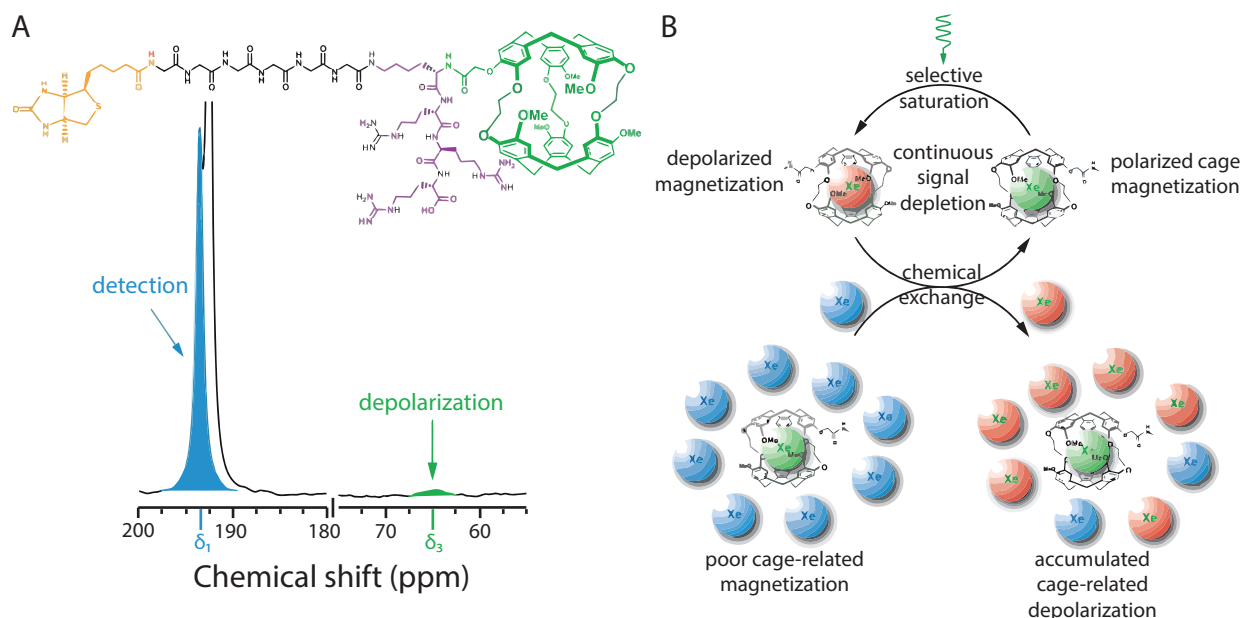


Figure 4.3: The HyperCEST Experiment. A significant improvement of the XBMS detection limit is achieved by selectively saturating ^{129}Xe inside a bound XBMS. As the saturated ^{129}Xe exchanges into water, the water ^{129}Xe NMR signal decreases. The bound XBMS signal is detected as a decrease in the much larger water ^{129}Xe NMR signal. Reproduced from [145].

atoms dissolved in water. As more and more saturated ^{129}Xe atoms move into water, the water ^{129}Xe magnetization is depleted over time. Because the T_1 of ^{129}Xe in water is very long (more than one minute), the saturation pulse can be applied for a long time, allowing for a significant reduction of the water ^{129}Xe NMR signal. At the end of the RF pulse, a 90° pulse is applied, allowing detection of the ^{129}Xe NMR signal. The change in SNR for the water ^{129}Xe NMR signal is far greater than the SNR obtainable during direct detection of the CryA ^{129}Xe NMR signal. This HyperCEST technique allows for detection of a 10 nM concentration of avidin using a XBMS, a significant improvement over the micromolar concentrations required using direction detection methods.

For a given molecular sensing experiment, the exact resonance frequency of the bound ^{129}Xe signal is not known in advance. In order to determine this information, the RF pulse frequency is varied over a wide range which contains both the water ^{129}Xe NMR frequency and the bound XBMS ^{129}Xe NMR frequency. This so-called “z-spectrum” of NMR signal versus RF pulse frequency consists of two regions of signal depletion; one region is centered about the water ^{129}Xe resonance frequency and a much smaller dip is centered about the bound XBMS ^{129}Xe resonance frequency. Once these frequencies have been determined, the HyperCEST sequence consists of two experiments. In one experiment, the RF frequency is set to the resonance frequency of the bound XBMS. In another experiment, the RF frequency is set to an equidistant frequency on the opposite side of the water ^{129}Xe NMR resonance frequency. The water ^{129}Xe signals are then subtracted, yielding the final signal; the second experiment allows for the subtraction of common-mode noise. The HyperCEST experiment is shown in Figure 4.3.

4.3 Remote Detection of Hyperpolarized ^{129}Xe in Water

Before we turned to investigating the use of hyperpolarized ^{129}Xe in microfluidic arrays of XBMSs, we labored to develop a system which would allow the sensitive remote detection of hyperpolarized ^{129}Xe dissolved in a small volume of water. This included the development of an NMR probe capable of detecting the NMR signals from both protons and ^{129}Xe , as well as the development of a method for dissolving ^{129}Xe gas into the water in close proximity to the region in which the NMR signal is encoded. The situation was complicated by the geometry of the Varian Unity Inova 300 superconducting magnet used for these experiments. With shim coils, gradient coils, and an encoding probe in place within the magnet, this system has an inner diameter of 30 mm, which sets the limit on the size of components which may be used for delivering and manipulating the gas prior to integration into a microfluidic device.

4.3.1 Microfluidic Probe Design

The design of the remote detection probe was based on that used in earlier remote detection studies [73]. A solenoid microcoil wound around tubing served as the inductive detector for recording the NMR signal. The coil was housed in a sealed container filled with Fluorinert FC-43, a fluid with a magnetic susceptibility very close to that of the copper from which the detection coil was made. This was included to avoid broadening of the NMR signal due to a copper-air interface. The leads of the coil were soldered to a printed circuit board (PCB) consisting of holes for variable capacitors (1-40 pF) in parallel and in series with the inductive solenoid detector. This formed a tunable resonant LCR circuit which was mounted onto a thick Teflon base to provide rigidity. The active and ground leads of a BNC cable were soldered to copper pads on the PCB to allow the NMR signal to be sent to external electronic amplifiers. The Teflon base was mounted to a brass support with through holes which allowed passage of the BNC cable. The support also contained holes allowing the passage of tuning rods (1/8" diameter G-10 fiberglass rods fitting with ceramic flathead screwdriver tips) to tune the NMR detection electronics. The Teflon base was placed inside of a copper hat which shielded the sensitive pickup circuit from cross-talk arising due to the application of RF pulses at the circuit's resonance frequency. The entire assembly was mounted to a skeleton constructed from aluminum and brass. One end of the skeleton was fitted with an aluminum grounding plate, to which the BNC cable was grounded. The other end of the skeleton was placed in the magnet and covered with an aluminum tube with an outer diameter of 1.25" (28.58 mm) which allowed the remote detection probe to just fit inside the bore of the system. Tubing for the delivery of water and gas were mounted to the outside of this remote detection probe. The entire assembly was placed into the spectrometer from the top of the magnet. The remote detection probe is shown in Figure 4.4.

Initial experiments confirming the remote detection of hyperpolarized ^{129}Xe in CryA in a 1/8" diameter tube were performed by Monica Smith using a resonant circuit tuned to the precession frequency of ^{129}Xe [152]. These experiments utilized mono-acid cryA attached

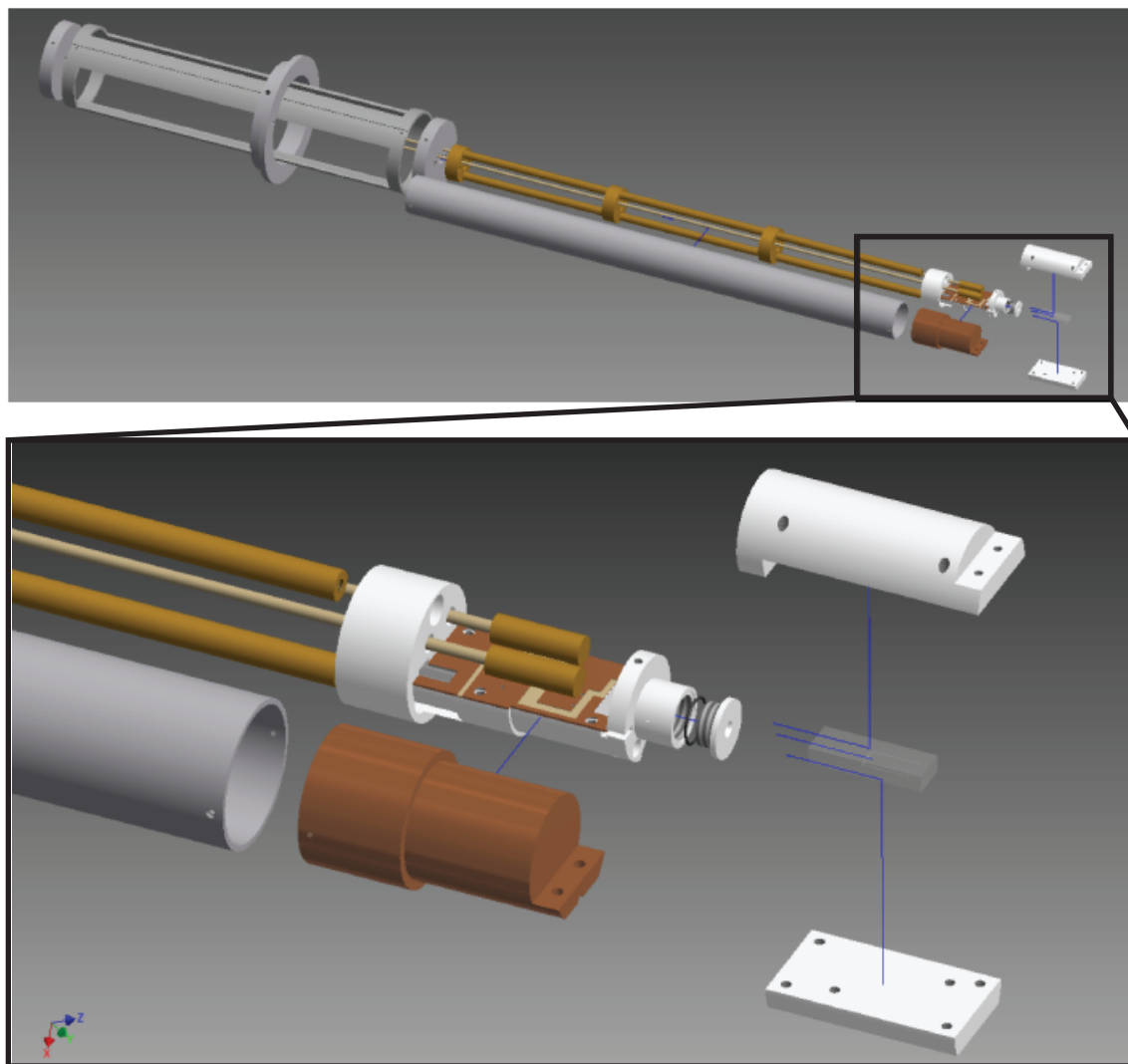


Figure 4.4: Remote Detection Probe. The system is made up of a series of structural supports for positioning the electronics inside the homogeneous magnetic field region of the superconducting magnet. A grounded BNC cable delivers power to the resonant LCR circuit, which is mounted to a Teflon base plate and shielded by a copper hat. The circuit is tuned using fiberglass tuning rods. The remote detection probe is placed face down inside the magnet.

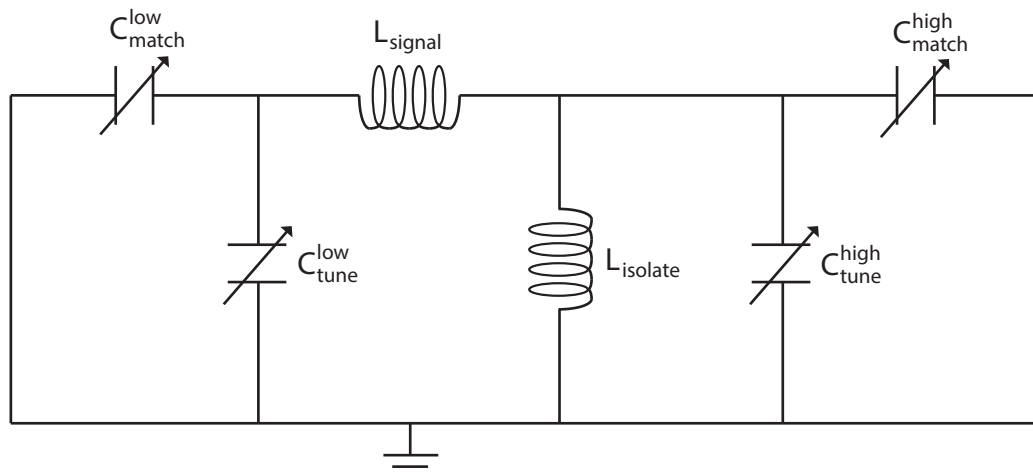


Figure 4.5: Double Resonance NMR Circuit. The circuit consists of two pairs of tuning capacitors placed in parallel with the inductive microcoil and two pairs of matching capacitors placed in series with the inductive microcoil. The two circuits are isolated using a large inductive coil with much lower capacitance than the microcoil used for detection of the NMR signal.

to Advanced Chem Tech TentaGel S-OH beads via a glycine linker; the beads which were trapped by constrictions in the tubing. These experiments were conducted using CryA at an effective concentration of 1.3 mM. The ^{129}Xe polarization obtained using a commercial MITI polarizer was approximately 2%. The gas mixture consisted of 2% natural isotopic abundance Xe, 8% N_2 , and 90% He at a total gas pressure of 60 psi and a gas flow rate of approximate 0.5 SLM.

4.3.2 Double Resonance NMR Probe

From these experiments, it was determined that substantial improvements to the detection sensitivity would need to be made in order to detect the very low numbers of CryA cages which could be placed on a microfluidic device. One such improvement was the implementation of a double resonant circuit capable of detecting both protons and ^{129}Xe . The device was engineered to allow correlation of aqueous Xe time-of-flight information with proton MRI images. Moreover, it allowed for extensive shimming of the strong proton NMR signal from water, increasing the ^{129}Xe detection sensitivity substantially. The microcoil used to detect the proton and ^{129}Xe NMR signals was designed to have a detection volume smaller than that of a typical microfluidic device in order to enable the acquisition of time-of-flight information. Of the many available double resonant microcoil circuit designs, we opted for one which provides efficiency and compatibility with the system’s physical constraints [186]. The circuit design is shown in Figure 4.5.

A single 400 μm inner diameter microcoil made from 18 turns of 32 gauge copper wire was connected to the high frequency (proton) and low frequency (^{129}Xe) channels; the coil

produced an inductance of 195 nH. Each channel was composed of a variable capacitor placed in parallel with the detection coil for tuning and in series for impedance matching. A 1/8" inner diameter isolation inductor made from 4 turns of 16 gauge copper wire was used to isolate the two channels; this coil displayed an inductance of 15 nH. In order to ensure that most of the RF energy is deposited into the sample, the inductance of the isolation coil was much smaller than that of the solenoid used to detect the NMR signal. The variable capacitors had a capacitance range of 1-30 pF and allowed for tuning of the proton channel to the resonance frequency of 299.8 MHz. In order to attain a resonance frequency of 82.9 MHz (^{129}Xe), a 20 pF chip capacitor was placed in parallel with the detection coil in the low-frequency channel. Power was delivered to each circuit via a separate SMA cable attached to the PCB by a soldered edge-mount connector. The quality factor, given by the ratio of the central frequency to the FWHM of the resonance line, was determined to be 1400 for the high-frequency channel and 320 for the low-frequency channel. The homogeneity of the microcoil detector was determined by applying RF pulses of fixed power and variable duration and taking the ratio of the signal intensity following a 810° pulse to that following a 90° pulse; the resultant homogeneity factor of 0.58 is smaller than that of commercial NMR probes, which typically have a homogeneity factor of approximately 0.80.

4.3.3 Gas Dissolution Membranes

In order to detect hyperpolarized ^{129}Xe in water, it is necessary to dissolve the gas into the liquid. Typically, this has been achieved by bubbling the $\text{Xe}/\text{N}_2/\text{He}$ mixture into water using a bubbling apparatus produced by glassblowing. This could not be implemented in our microfluidic system due to the geometry of the system. In order to maintain polarization of the dissolved ^{129}Xe gas, it was necessary to dissolve the gas as close to the microfluidic device as possible. Due to the geometric constraints of the system, a custom dissolution apparatus had to be constructed.

The dissolution apparatus was based upon hydrophobic polypropylene X-50 fibers donated by Membrana, a company which sells a commercial large-scale cartridge system for dissolving various gases into water. These fibers have an inner diameter of 220 μm , an outer diameter of 300 μm , and a porosity of 40%. Water is unable to enter the interior of the fiber due to hydrophobic effects; however, inert gases such as our $\text{Xe}/\text{N}_2/\text{He}$ mixture is able to pass through the fiber and bubble out through the pores. It was found that these membranes are able to reach a Xe concentration in water which is approximately 25% of the value obtained during bubbling. Although this represented a signal decrease by a factor of 4 compared to bubbling, this method was adopted due to its satisfaction of the geometric constraints of the system. The gas exchange system was designed to support a liquid to gas volume ratio of 2:1, similar to that employed in the commercially available cartridge systems.

The homebuilt gas exchange membrane system was built by threading approximately ten X-50 fibers through a 2-3 cm section of 1/8" outer diameter Teflon tubing. The ends of the membranes were then passed through 1/8" brass Swagelok T-style connectors and another



Figure 4.6: Homebuilt Gas Dissolution System. The system consists of bundles of X-50 membrane fibers threaded through 1/8" outer diameter Teflon tubing. The Xe/N₂/He mixture and water flow in opposite directions, allowing the gas mixture to dissolve into the water by means of a concentration gradient.

short piece of 1/8" Teflon tubing. The membranes were fixed to the ends using an epoxy resin in order to form leak-tight seals. After curing, the system was checked by flowing water pressured with 75 psi of N₂ gas. The gas inlet and outlet lines were connected such that the Xe/N₂/He mixture flowed in the opposite direction of the direction of the water flow. This created a concentration gradient which allowed for the dissolution of the gas mixture into water. The homemade gas dissolution system is shown in Figure 4.6.

4.3.4 Detection System Tests

The ^{129}Xe signal in the microcoil was expected to be low, given the low percentage of Xe in the gas mixture, the relatively low polarization, and the relatively low solubility of Xe gas in water. Therefore, the low-frequency channel was tested with a saturated sodium chloride (NaCl) solution containing ^{23}Na ions. ^{23}Na is a spin-3/2 nuclear which is 100% naturally abundant and has a gyromagnetic ratio (11.27 MHz T⁻¹) very close to that of ^{129}Xe (11.77 MHz T⁻¹). At 7 T, its resonance frequency is 79.4 MHz, a frequency to which the low-frequency channel of the double resonance probe is easily tuned. We injected salt water into a 1/8" outer diameter Teflon tube filling the detection region and observed a ^{23}Na NMR signal on the low-frequency channel after signal averaging 16 times.

The experimental parameters determined in the ^{23}Na experiments allowed us to calibrate the NMR experimental parameters for ^{129}Xe studies. The ^{129}Xe signal arising from a 2% Xe, 8% N₂, 90% He mixture at 60 psi, dissolved into water using the homebuilt membrane system, with ^{129}Xe polarized to 2%, was much weaker than that of the ^{23}Na . 1024 signal averages were required to obtain a clear signal with a SNR of 13.4, yielding a single-shot SNR of 0.42.

4.3.5 Detection of Hyperpolarized ^{129}Xe in a Microfluidic Device

To further optimize the system for microfluidic ^{129}Xe experiment, we attempted to detect a ^{129}Xe NMR signal and perform proton-based imaging experiments in a microfluidic device with dimensions similar to those in the biosensor chips discussed in Section 4.4. Two microfluidic chips designed by Elad Harel for previous proton-based remote detection experiments were used in these system tests. The two microfluidic devices are shown in Figure 4.7. The devices were mounted to the remote detection probe using a custom-built chip holder machined from Delrin. Remote detection experiments were conducted by performing the encoding step on ^{129}Xe dissolved in water within the microfluidic device and detecting the magnetization in the microcoil described above. This was accomplished by attaching 360 μm outer diameter tubing to the outlet port using commercially available microfluidic adapters.

The ^{129}Xe NMR signal was acquired by dissolving the $\text{Xe}/\text{N}_2/\text{He}$ mixture at 65 psi into water and flowing through the Y-shaped microfluidic device shown in Figure 4.7a. 512 signal averages produced a SNR of approximately 20, yielding a single-shot SNR of approximately 0.88. Remote detection travel curves were acquired at a liquid flow rate of $9.2\ \mu\text{L s}^{-1}$. This yielded a transit time through the device of approximately 420 ms, as indicated by the ^{129}Xe travel curve. These parameters are useful to bear in mind for future ^{129}Xe XBMS microfluidic studies.

4.4 Microfluidic XBMS Arrays

Given the successful detection of hyperpolarized ^{129}Xe dissolved in water in a microfluidic device, we labored to develop a microfluidic platform for detecting arbitrary chemical analytes using XBMSs. Since the difference in ^{129}Xe chemical shifts between XBMSs bound to targets and those which are unbound is small, it seems unlikely that the chemical shift could be used as a means of determining the binding of multiple different chemical species, due to issues of spectral overlap. Therefore, we decided to design this microfluidic device to utilize both spectroscopy and imaging modalities to detect multiple chemical species. The device uses highly selective spatial patterning of XBMSs in order to deliver XBMSs functionalized for one target to one location and XBMSs functionalized to another target to another location. Because the various XBMSs are located at well-known points in space, spectroscopic imaging techniques can be employed to determine the presence or absence of the target molecule at each known location in space. The basic architecture of a microfluidic device capable of detecting two different chemical analytes is shown in Figure 4.8.

4.4.1 XBMS Attachment Chemistry

The various XBMSs were attached to the microfluidic device using a ssDNA chemistry which has been developed in the laboratory of Professor Matthew Francis at UC Berkeley.

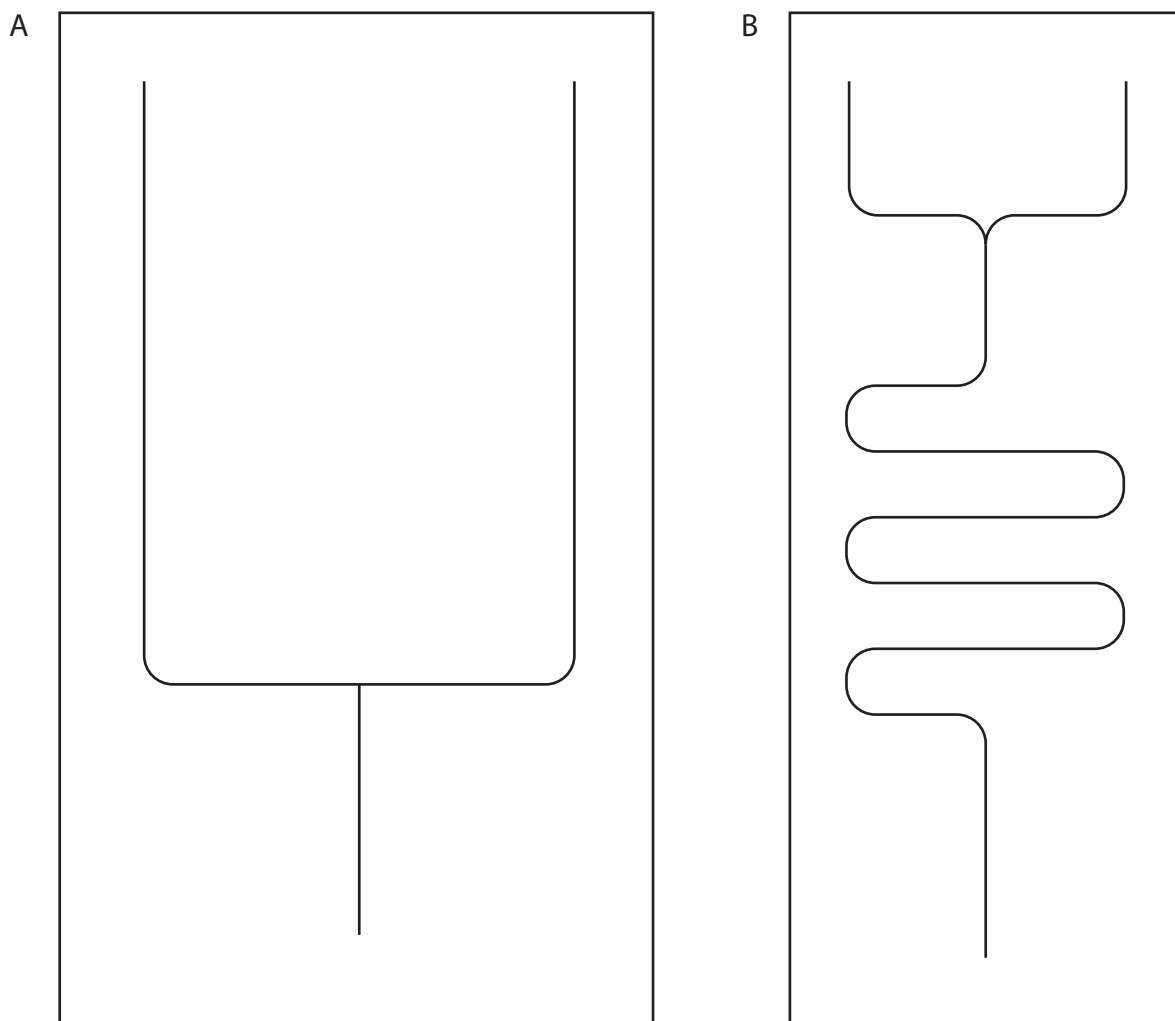


Figure 4.7: ^{129}Xe NMR and Proton-Based Imaging Microfluidics. A) A Y-shaped ship (originally used for proton-based chemical mixing studies) was used in the ^{129}Xe experiments. ^{129}Xe dissolved in water flowed into the device through one inlet port and exited through the outlet port; the second inlet port was blocked with a plug. The device had dimensions of 15 mm wide x 30 mm long x 4 mm thick with microfluidic channels having a semicircular cross-section with a radius of 75 μm . B) A serpentine chip used for proton-based imaging studies. As in (a), one inlet port was blocked with a plug. The device had dimensions of 10 mm wide x 30 mm long x 4 mm thick with microfluidic channels having a semicircular cross-section with a radius of 75 μm .

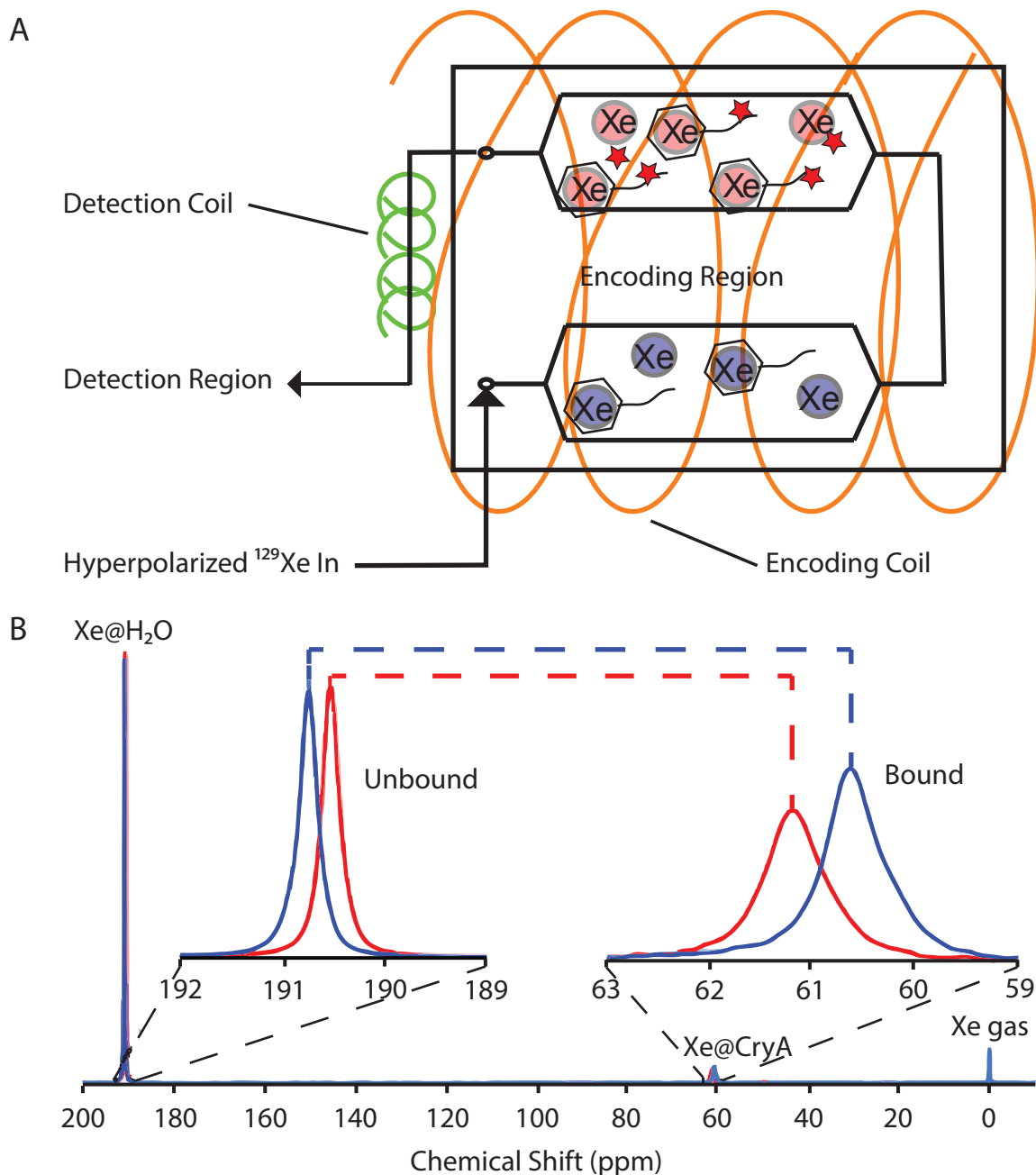


Figure 4.8: Microfluidic XBMS Architecture. Two different XBMSs, designed to bind to two different targets A and B, are attached to well-defined locations in a microfluidic devices. Upon binding of the target molecule A, the XBMS associated with this molecule displays a ^{129}Xe chemical shift. Conducting spectroscopic imaging on the device yields an image in which region A, with the bound XBMS “lights up”, while region B yields no change in signal.

Initially, this method was used to allow the selective patterning of various cells on glass surfaces [30]. In the initial cell experiments, various human cell lines were coaxed to produce surface chemistries which allowed direct attachment of ssDNA. The complementary strand of ssDNA was modified to contain an amine (NH_2) functional group, allowing attachment to glass which had been selectively modified to contain an aldehyde functional group in locations of interest. Upon hybridization of the two strands of ssDNA, the cells remained affixed to the specified locations. Our XBMS microfluidic device was built around a similar chemistry, in which the Pyrex microfluidic devices were modified to contain aldehyde groups. Commercially available NH_2 -ssDNA was then adhered to the device. The complementary strand of ssDNA was attached to an XBMS, which was adhered to the device following DNA hybridization. By utilizing different ssDNA nucleic acid sequences for different XBMSs, localization of each XBMS could be controlled by controlling the placement of each ssDNA sequence.

The microfluidic device was designed to allow the inclusion of an arbitrary number of XBMSs to be included in the imaging region. Due to the need to place specific XBMSs in specific locations, it was determined that the various ssDNA sequences should be added to the device prior to sealing to form microfluidic channels. XBMSs functionalized with the complementary ssDNA could then be flowed into the device after sealing; due to the highly specific nature of DNA hybridization, the XBMSs would be localized in the proper locations (assuming a judicious selection of ssDNA sequences that provides high specificity of binding; commercial tools exist for this purpose). In this manner, the various complementary XBMSs could be attached to the device using an inkjet printer to place the various XBMSs in specific locations; such techniques have been used extensively in other microfluidic devices [40]. This need to pattern the XBMSs prior to bonding of the device places limitations on the microfabrication techniques which may be employed in construction of the microfluidic device. We chose to flow the XBMSs into the device after bonding in order to avoid worries about the low melting point of double stranded DNA, which would have precluded the use of any techniques requiring the use of temperatures above approximately 50°C . Even with this design, we were limited to the use of relatively low temperatures (around 300°C) due to the relatively weak peptide bond formed between the aldehyde-modified glass surface and the NH_2 -ssDNA.

4.4.2 Microfluidic Device Fabrication

The device fabrication procedure proceeded as follows. Pyrex wafers of 100 mm diameter and 1 mm thickness were cleaned by rinsing in acetone, isopropanol, and water. The wafers were then immersed in Piranha solution, a mixture consisting of a volume ratio of 70% sulfuric acid and 30% hydrogen peroxide, for a period of 30 minutes. The wafers were removed from the Piranha solution, rinsed with water, dried under N_2 , and baked at 150°C for a period of 15 minutes to ensure complete dryness. Boxes of these wafers were sent to Valley Design Corporation (Santa Cruz, CA), where the wafers were coated on both sides with a 5 nm thick layer of chromium (to promote adhesion to the glass surface) and a 100

nm thick layer of amorphous polycrystalline silicon. This was accomplished using plasma enhanced chemical vapor deposition (PE-CVD). The Si layer served as a hard protective mask to protect against defects while etching the wafers.

When the wafers returned to Berkeley, they were again cleaned using the method just described. The wafers were then spin coated with Shipley S-1818 positive photoresist using a final spin speed of 1500 rpm and a total spin time of 30 s. This produced a layer of photoresist approximately 2 μm thick. The wafers were baked for 2.5 minutes at 120 $^{\circ}\text{C}$ to polymerize the photoresist. They were then exposed to UV light at 365 nm through a photomask patterned with the locations of the desired microfluidic channels. Exposure of 100 $\mu\text{J cm}^{-2}$ depolymerized the photoresist in the desired areas. A 1.5 minute development in Shipley CD2000 developer cleared the depolymerized photoresist, leaving bare Si in the areas to be etched.

This Si was removed by reactive ion etching (RIE) using sulfur hexafluoride (SF_6). The wafers were loaded into a RIE machine, which was evacuated to a pressure of approximately 5 mTorr. SF_6 was flowed into the chamber under continuous vacuum pumping until a steady pressure of approximately 100 mTorr was obtained. A tuned LCR circuit provided 200 W of RF power to the SF_6 gas, creating a reactive plasma which etched the silicon, leaving bare Pyrex. This was etched to form the microfluidic channels by immersing the wafers in 49% hydrofluoric acid (HF). The HF etch proceeds at a rate of approximately 7.1 $\mu\text{m min}^{-1}$, so that adjustment of the etching time allowed fine control over the depth of the etch (and thus the depth of the microfluidic channels). We attempted to achieve a nominal etch depth of 20 μm , requiring a HF immersion of approximately 2 min, 45 s. The wafers were rinsed thoroughly with water following the HF etch.

Photoresist was then removed from the wafers by immersion in acetone, which dissolved the rest of the photoresist. The wafers were rinsed with acetone, isopropanol, and water and allowed to air dry. Inlet and outlet holes were drilled using a diamond-coated high-speed drill bit. The wafers were then rinsed with acetone, isopropanol, and water, and immersed in Piranha solution for 30 min immediately prior to surface modification and bonding.

During these steps, great care was taken to ensure that the wafers did not become contaminated with dust or other debris. Aldehyde groups were added to the surface of the wafers by exposing them to a vapor of trimethoxysilane aldehyde in a vacuum desiccator for a period of 8 hours. NH_2 -ssDNA (with sequence NH_2 - 5' - TCA TAC GAC TCA CTC TAG GG - 3') was dissolved in sodium dodecyl sulfate (SDS) and phosphate buffered saline (PBSa) and attached to a well in the device using a micropipette. The device was allowed to react for a period of 15 minutes and then carefully dried with a Kim-Wipe. A mixture of sodium borohydride, SDS, and PBSa was added to reduce the amine-aldehyde bond and allowed to react for a period of 15 minutes. The wafer was then washed in SDS and PBSa for a period of 15 minutes. Finally, the wafer was rinsed with acetone, isopropanol, and water, dried under N_2 gas, and baked at 150 $^{\circ}\text{C}$ for 15 minutes to ensure dryness.

The patterned side of the wafer was then placed in contact with a previously cleaned

unpatterned Pyrex wafer and anodically bonded. The layer of Si used as an etch mask served the dual purpose of allowing for a strong anodic bond to be formed between the two Pyrex wafers. Bonding was carried out at a reduced temperature of 275 °C and an increased potential difference of 2500 V. Reduced temperature ensured that the peptide bond was not destroyed, while increased potential difference counteracted lower ion mobility arising due to the reduced temperature. Even with the higher potential difference, the low temperature resulted in very long bonding times of approximately 12 hours. Finally, the bonded wafers were cut into individual devices. A single 100 mm wafer could support six devices with dimensions of 20 mm wide x 30 mm long. The devices were filled with water during storage to prevent degradation of the ssDNA.

4.4.3 Microfluidic Device Testing

The microfabrication procedures described in the previous Section 4.4.2 were tested using optical techniques. These tests were implemented on a device which featured two wells, each 4 mm wide X 19 mm long X 20 μ m deep. The device is shown in Figure 4.9. To test the devices, we left one well unmodified while attaching ssDNA to the other well. In place of bare NH₂-ssDNA, test devices used NH₂-ssDNA modified to contain a fluorophore. This allowed for verification that the XBMS attachment chemistry was able to survive the fabrication conditions. Early testing on three different wafers indicated that the ssDNA was able to survive all of the fabrication steps, as shown by a strong fluorescence signal in the modified well. However, later wafers have failed to show a fluorescence signal. Attempts to determine the cause of this fabrication issue are ongoing at the time of this thesis.

4.4.4 Sensitivity Considerations

Initial calculations suggested that the devices discussed in the previous Section 4.4.2 would have a sufficient XBMS concentration to allow detection of binding of a target chemical species [152]. However, these calculations only considered the concentration of XBMSs in the device; they did not take into account the total number of XBMS molecules, which is the relevant parameter. We therefore conducted a new analysis of the sensitivity of the device. We estimated the XBMS sensitivity of the device by extrapolating results obtained with XBMSs studied in a 5 mm NMR tube which was analyzed using the NMR hardware which would be used for our microfluidic experiments. These studies showed that we were capable of directly detecting (i.e. not using HyperCEST) 50 μ M concentrations of a water-soluble CryA cage dissolved in 450 μ L of water with a SNR of 7.2 in 11 signal averages. This gives a single-shot SNR of approximately 2.2 for a 50 μ M sample. We should thus be able to see a XBMS signal with a SNR of 1 using 450 μ L of a 25 μ M XBMS solution. We took this as the starting point for our calculations.

Using the 25 μ M and 450 μ L numbers, we determined that our experimental setup has a limit of detection (LOD) of approximately 6.8×10^{15} XBMSs. Personal communication

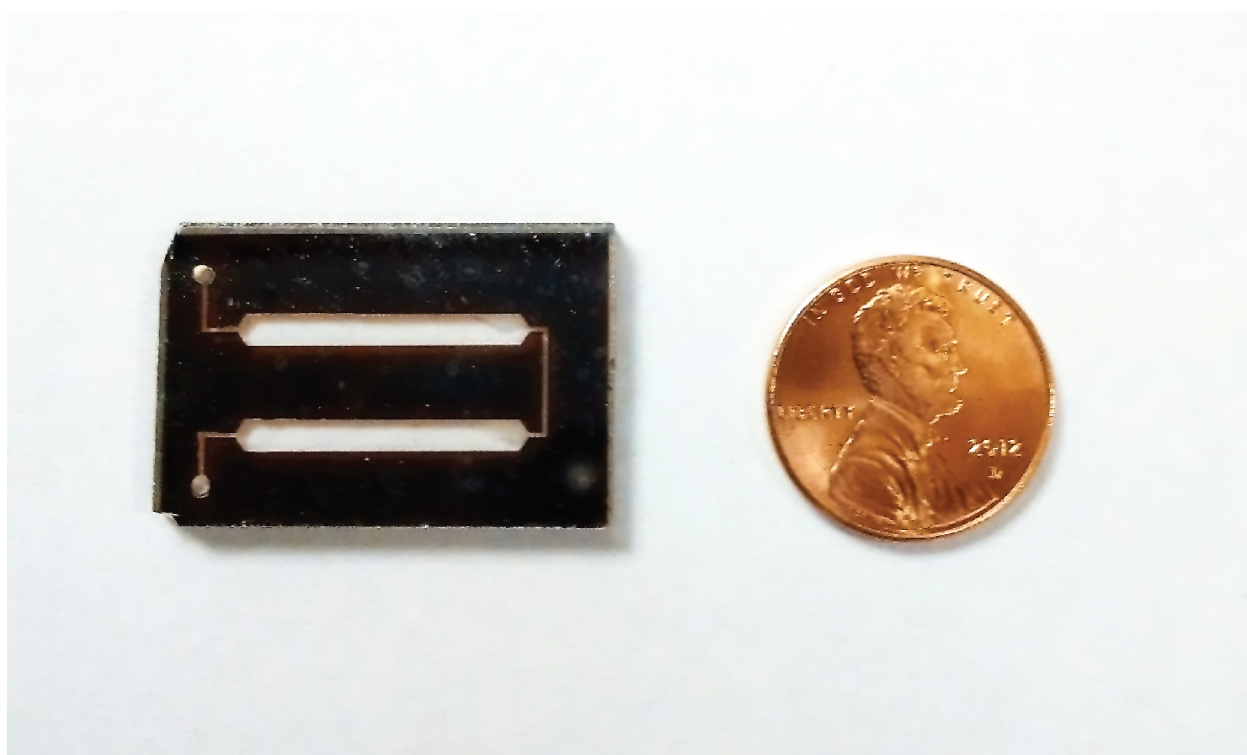


Figure 4.9: Design of Proof-of-Concept Device. The Pyrex device features two wells, each with dimensions of 4 mm wide X 19 mm long X 20 μm deep. One well is left unmodified to serve as a control. The other well is modified to contain ssDNA for binding of a XBMS. Though composed mostly of Pyrex glass, the device appears grey due to a thin film of Si which allows anodic bonding of two Pyrex plates.

with Sonny Hsiao in the laboratory of Matthew Francis has revealed that we are capable of placing approximately 5×10^{11} XBMSs on a surface with an area of 1 cm^2 . Thus, our devices would require a surface area of approximately $2 \times 10^4 \text{ cm}^2$ to accommodate the number of XBMSs required for a molecular sensing experiment on a chip. This is in comparison to the devices actual surface area of 0.76 cm^2 , which is smaller by a factor of more than 4 orders of magnitude. From these calculations, we determined that our initial method of attaching XBMSs directly to the glass surfaces of a microfluidic chip is not a feasible method for conducting microfluidic XBMS experiments.

4.4.5 Sensitivity Improvement

There are a few ways in which the sensitivity of the XBMS microfluidic device can be improved. We can make a minor modification to our fabrication procedure to allow XBMSs to be patterned on both glass surfaces, lowering the LOD by a factor of 2. The ^{129}Xe polarization level of approximately 2% can be increased to at least 30%, which will improve the LOD by a factor of 15. Use of isotopically enriched ^{129}Xe would improve the LOD by a factor of 4. All told, these enhancements would allow a better than two order of magnitude improvement in the device sensitivity. However, this still leaves two orders of magnitude to go. Moreover, improvement of the ^{129}Xe polarization requires substantial modification of the polarizer, while moving to isotopically enriched ^{129}Xe increases the already high gas price by an order of magnitude. These changes should be implemented in the long run; in the short run, we looked to other methods of increasing the device sensitivity.

The low XBMS sensitivity of the device results from the use of a monolayer of XBMSs. We therefore looked to improve the device sensitivity by increasing the number of layers of XBMSs. This can be achieved by attaching large macromolecular structures directly to the well surface (using a slight modification to the chemistry described in Section 4.4.1); these structures can then be functionalized to carry a “payload” consisting of a large number of XBMSs. Previous work has demonstrated the use of a bacteriophage (phage F1) as a molecular scaffold capable of hosting approximately 2500 XBMSs on its surface proteins [158]. Assuming a phage coverage of $5 \times 10^{11} \text{ cm}^{-2}$ and 2500 XBMSs per phage, this improves our LOD by a factor of 2500. By coating both glass surfaces with phage, the LOD is improved by a factor of 5000. Thus, we now require a well with a surface area of 5 cm^2 to achieve a SNR of 1 in a single experiment. This is still nearly an order of magnitude greater than the actual size of our wells. However, signal averaging is now feasible: we estimate a total experiment time of approximately 40 min to achieve a useable SFR of 5 given a transit time of 1 s in the device. Keunhong Jeong in the Pines laboratory was working to synthesize XBMS-laden phage F1 which can be adhered to our glass surfaces at the time of this thesis.

Chapter 5

Spatial Encoding Using Patterned Permanent Magnets

NMR and MRI may be employed as noninvasive measurements yielding detailed information about the chemical and physical parameters that define micro-scale fluid flows. Despite these advantages, magnetic resonance has been difficult to combine with microfluidics, largely due to its low sensitivity when detecting small sample volumes and the difficulty of efficiently addressing individual flow pathways for parallel measurements without utilizing large electric currents (and the attendant large, high-power electronic amplifiers) to create pulsed magnetic field gradients. Here, we consider how RD-MRI employing static magnetic field gradients produced by thin magnetic films can be used to encode flow and overcome some of these limitations. We show how flow path and history can be selected through the use of these thin film labels and through the application of synchronized, frequency-selective pulses. This obviates the need for large electric currents to produce pulsed magnetic field gradients and may allow for further application of NMR and MRI experiments on micro-scale devices.

The chapter is organized as follows. In Section 5.1, we consider techniques for labeling microfluidic flow. In Section 5.2, we describe our novel method for labeling microfluidic flow using RD-MRI and thin films of permanent magnetic materials, which we refer to as the NMR “bar code”, and present our flow phantom for the experiments described in the following two sections. In Section 5.4, we describe experiments allowing distinct regions of an imaging phantom to be individually excited and imaged based on the unique resonance frequencies generated by the permanent magnets. In Section 5.5, we describe a series of experiments allowing flow history to be labeled based on sequential selective excitation of the various regions on the imaging phantom. In Section 5.6, we present computational simulations which demonstrate that this technique may allow spatial resolutions better than a few tens of microns. Finally, in Section 5.7, we consider microfabrication methods which might allow the use of the NMR “bar code” technique on length scales commensurate with those employed in microfluidic experiments.

This chapter contains material which was under peer review for publication at the time of this thesis [65]. It has been reproduced here with the consent of its coauthors.

5.1 Methods for Labeling Microfluidic Flow

Existing methods of producing intrinsically multiplexed microanalytical devices generally involve the assignment of unique, localizable optical signatures to the analytes of interest or to specific regions, flow pathways, or sensor media located on the device. Examples include the development of polystyrene beads implanted with quantum dots to generate unique fluorescence signatures [168] and the striping of optically distinct metals onto microrods [117]. In some cases, these signatures are based on multiple permutable elements that form an address space of many individually recognizable “bar codes” which can be used to distinguish between regions or flow pathways. Such methods rely upon a synthetic, exogenous label element or sensor that forms an adduct with the analyte and are thus difficult to integrate into existing chips and devices. Because the adduct is usually covalent, the label cannot be altered or reset after its initial formation.

A more general solution to the problem of microfluidic labeling should have properties similar to those of an electronic register or memory. Ideally, it should also employ an intrinsic property of the fluid itself as a means of labeling, rather than requiring the use of exogenous molecules. The spin degrees of freedom of the sample fulfill these criteria, as they are addressable and resettable by NMR pulse sequences and do not generally influence the chemistry, transport, or other property relevant to the microfluidic device. Unfortunately, magnetic resonance has been difficult to combine with microfluidics, largely due to its low sensitivity when detecting small sample volumes and the difficulty of efficiently addressing individual flow pathways for parallel measurements. One solution to this problem is to build individual micro-detection and excitation coils for each individual channel used [105]. This is possible for systems consisting of only a few channels which need to be labeled; however, in microfluidic systems which may contain 100 or more channels, the drawback of cross-talk between channels and the complexity of the RF circuitry makes this approach unappealing.

5.2 The NMR “Bar Code” Phantom

As discussed in Section 2.10, remotely detected MRI offers the ability to resolve three-dimensional flow fields in microfluidic devices, models of microreactors, and in nanoporous materials. These measurements overcome many of the sensitivity limitations of microfluidic MRI, but they require pulsed magnetic field gradients that are difficult to make portable, owing to the need for large electric currents which are generated using large electronic amplifiers. An illustration of the need for large electric currents is shown in Figure 5.1, which shows the currents required to obtain various proton imaging resolutions using a small-scale

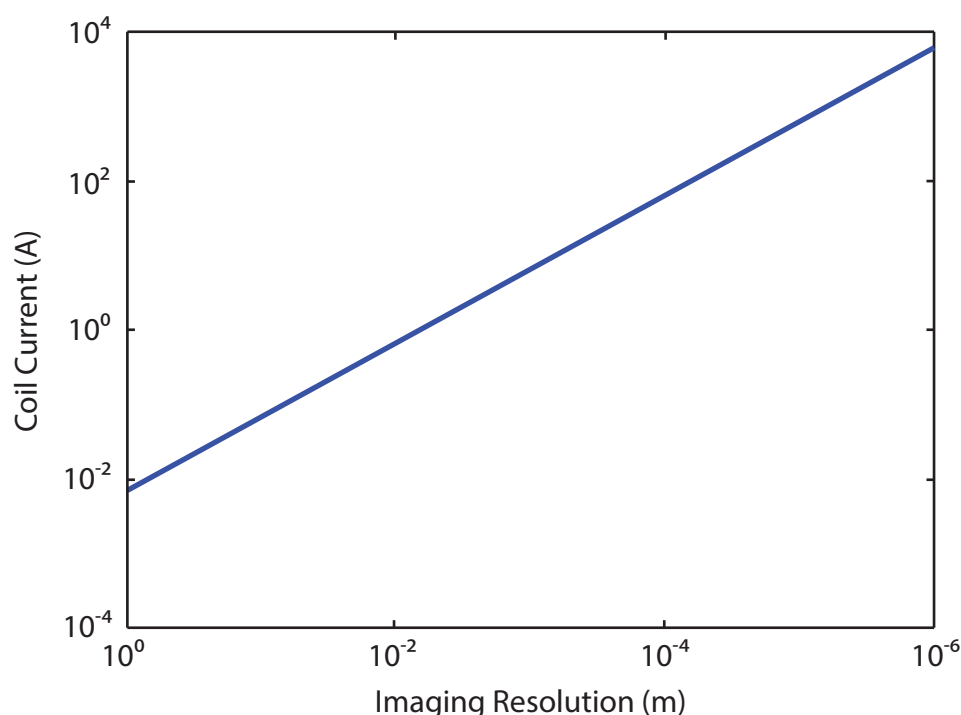


Figure 5.1: Imaging Coil Performance. In order to achieve improvements in the imaging resolution with a given coil, it is necessary to increase the electric current. To achieve imaging resolutions of a few micrometers, it is necessary to use kiloamps of current.

(6 cm diameter) Maxwell coil with 20 turns of wire which produces a magnetic field gradient along $\hat{z}$. In order to achieve imaging resolutions of a few micrometers, the length scale commensurate with microfluidic applications, it is necessary to produce currents of a few kiloamps; such large currents cannot be generated using miniaturized hardware. While it is possible to decrease the requisite current by increasing the number of turns of wire in the coil, this creates additional problems. The inductance L of the coil increases with the square of the number of turns, while the resistance R only increases linearly with the number of turns. Thus, the electronic rise time $\tau_{rise} = L/R$ of the gradient coil increases linearly with the number of turns. This means that any improvement in resolution attained by increasing the number of turns is accompanied by a concomitant worsening of the coil rise time. For the coil considered Figure 5.1, a rise time of approximately 10 μ s is attained with 20 turns of wire. Increasing the number of turns by even a factor of 10 would severely limit the usefulness of the coils in conducting MRI experiments. Therefore, miniaturized magnetic field gradient coils are not suitable for micro-scale NMR instrumentation.

Our alternative method is inspired by the development of molecular imaging reagents incorporating microstructures that alter the local magnetic field, creating frequency-dependent contrast in MRI methods [184]. Here, thin layers of magnetic film are positioned above and below the flow pathway in a region, with a unique number of layers, and hence a unique magnetic field, in each region. In this way, frequency-selective excitation pulses can be applied to individually isolate a desired region and allow localized imaging of selected areas of the flow. This technique also allows a series of frequency-selective pulses in a flowing system

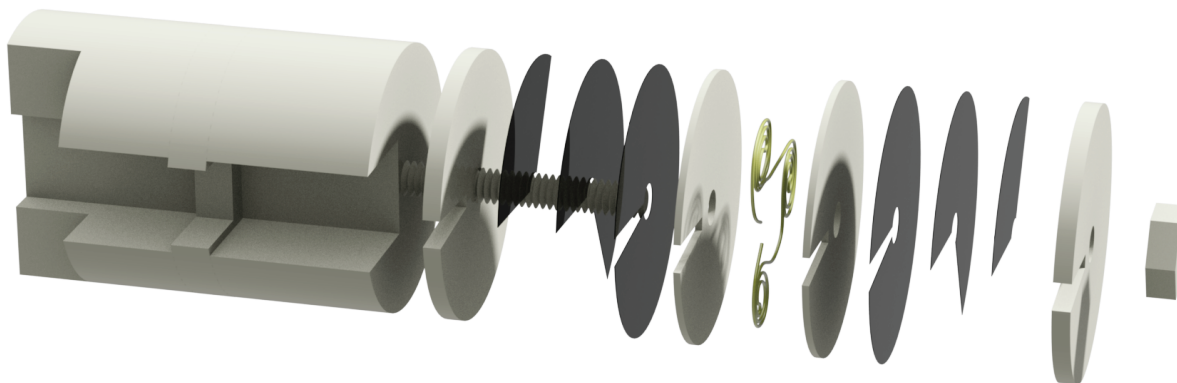


Figure 5.2: The NMR Bar Code Phantom. Water travels through capillary tubing which is wound to form coils in three distinct magnetic field regions, formed using varying numbers of layers of magnetic material.

to implement flow gate operations which will only yield signal when the sequence of local magnetic environments experienced by the flowing liquid is synchronized with the sequence of pulses. Such a system of logic could allow discrimination between multiple flow pathways on a single chip by giving each a unique magnetic signature along the flow dimension and tuning the pulses to select only the pathway of interest. Such a method may be viewed as the flow analog of a bar code; we therefore refer to this methodology as the NMR “bar code”.

While the NMR “bar code” technique is ultimately intended to be implemented as a microfabricated component of an integrated microfluidic platform, techniques for patterning permanent magnetic materials require extremely specialized and expensive equipment. Therefore, we set out to demonstrate this technique on a larger scale as a series of proof-of-principle experiments using a home-built flow phantom. A diagram of the phantom is shown in Figure 5.2. Water traveled through a 150 μm inner diameter, 360 μm polyether ether ketone (PEEK) capillary which was wound to form three distinct microfluidic regions, each approximately a close-packed three-turn helix of 6 mm major diameter. The coiled capillary was cast in a thin layer of epoxy resin to prevent changes to the flow pathway. The resulting chip consisted of three regions of looped capillary, and was sandwiched between thin layers of magnetic material in order to create distinct, localized magnetic fields in each region. High-density thin film laminates consisting of Mylar sandwiched between a pair of 1 μm layers of cobalt-doped gamma-maghemite, $\text{Co-}\gamma\text{-Fe}_2\text{O}_3$, salvaged from double-sided, high-density 3.5” floppy diskettes, proved to be ideal thin-layer magnetic materials for creating small homogeneous fields. By varying the number of layers of magnetic material, each region was given a distinct magnetic environment. The number of layers on each side of the three regions is indicated in Figure 5.2, and was chosen to be non-linear with respect to the order in which fluid flow traversed the phantom. The phantom was connected with the flow system using commercially available connectors from IDEX Health & Science. Water flowed through the system at a constant pressure of 13.5 psi, driven by a nitrogen gas ballast system and monitored by a proportional control setup flow meter.

In selecting the correct magnetic material for the various encoding regions, there were two criteria to be considered: field strength and field homogeneity. Frequency selection could be made much easier by utilizing magnetic materials which generate very large magnetic fields, such as the various samarium cobalt (SmCo) or neodymium-iron-boron (NdFeB) alloys. This would allow for the generation of very large magnetic field (and, therefore, frequency) differences between the various regions, making selective excitation of the various regions much easier, similar to the manner in which a strong gradient gives rise to better spatial resolution in standard MRI experiments. However, strong permanent magnets would also give rise to very inhomogeneous, broad signals in each region and could generate a range of frequencies greater than the bandwidth of the NMR receiver. For this reason, we employed magnetic materials much weaker than SmCo or NdFeB. Because the proof-of-principle experiments were conducted at high magnetic fields, it would have been possible to generate sufficient frequency differences between the various regions by using paramagnetic or diamagnetic materials. However, the use of these materials is limited by the skin-depth effect, in which AC fields are relegated to the outer surface of an electrical conductor and do not penetrate into thicker materials. For good electrical conductors, the penetration depth δ_{skin} is given by:

$$\delta_{skin} = \sqrt{\frac{2\rho}{\omega_0\mu_r\mu_0}} \quad (5.1)$$

Here, ρ is the electrical resistivity of the material and μ_r is its relative permeability. At a frequency of 300 MHz, the NMR frequency used in our proof-of-principle experiments, paramagnetic and diamagnetic materials with large enough magnetic susceptibilities to achieve good frequency resolution will generally screen RF pulses when these materials are thicker than 1 μm . Thinner films may be constructed using microfabrication techniques; however, they require specialized and expensive equipment. The skin-depth effect is mitigated at lower frequencies; however, this also decreases the magnetization of paramagnetic and diamagnetic materials, leading to worse frequency resolution.

Ferrites are an ideal material for our application due to their combination of relatively low ρ , relatively high (but not too high) μ_r , and the availability of previously-manufactured thin films produced for magnetic recording applications. Moreover, as ferromagnets, these materials have a magnetization which saturates at relatively low magnetic fields (approximately 1 T), allowing them to be used at fields lower than those employed in our proof-of-principle experiments. There are numerous ways to implement different field strengths in the various regions using ferrites. One method would be to create a ferrite powder, mix it in a non-magnetic epoxy, and spin coat it on a sample. Different magnetic fields could be created from layers of uniform thickness by using different concentrations of ferrite in each region. Another possibility is to vary the distance to the magnetic material in each region. A final option is to vary the volume (or, equivalently, the thickness for regions of equal area) in each region. We elected to use this method in our experiments since it lends itself to use in microfabricated permanent magnet arrays, as discussed in Section 5.7.

In comparison to future microfabricated work which is considered in Section 5.7, the

construction of the phantom was rather unsophisticated. We started by laying down a layer of double-sided Scotch tape on a table. We attached a layer of masking tape with the sticky side up. The capillary tubing was then stuck to the masking tape in the desired pattern. Teflon tape was attached to a block of steel about the same size as the flow phantom. The capillary tubing was then coated liberally in two-part epoxy. The heavy steel block was then laid on the capillary tubing with the Teflon tape face down. This pushed excess epoxy off of the capillary tubing, leaving it coated in only a thin layer. As the epoxy hardened, it dissolved the glue on the masking tape. Once the epoxy had hardened, we simply removed the masking tape and Teflon tape. The capillary tube was then rigidly held in a thin layer of epoxy. A first layer of magnetic material was created by cutting a disk 1" in diameter from a 3.5" floppy diskette. Additional layers were cut and taped to the first layer (using double-sided Scotch tape) to form three regions, each subtending 120° consisting of 1, 2, or 3 layers of floppy disk ferrite. These ferrite phantoms were attached to both sides of the capillary phantom in order to achieve better magnetic field homogeneity. Additional layers of plastic were utilized to hold the flow connectors.

5.3 Experimental Setup

All remote detection experiments were performed with a 7.05 T (301 MHz proton precession frequency) Oxford Instruments wide bore magnet and a Varian imaging console. Excitation and storage pulses were applied with a 40 mm Varian volume imaging probe, while magnetic field gradients (for the selective imaging experiments described in Section 5.4) were applied using a Varian three-axis gradient system producing gradient strengths of up to 100 G cm^{-1} on all three axes. The homebuilt remote detection probe contained a 12-turn, 300 μm inner diameter copper solenoid detection coil encased in a cell filled with FC-43 Fluorinert, which has a magnetic susceptibility similar to that of copper and decreases inhomogeneous broadening due to susceptibility mismatches at the boundaries between the various construction materials. The flow phantom was mounted to the end of the detection probe and positioned such that both the phantom and the detection coil were within the homogeneous sweet spot region of the magnet. Shimming coils were employed to maximize the homogeneity in the encoding coil. During signal acquisition, the optimal shim values for encoding were augmented with previously calibrated magnetic field gradients in order to optimize the sweet spot for the detection region.

All data were processed using MATLAB. The direct (FID) dimension was apodized and Fourier transformed for all experiments. For the frequency-selective imaging experiments described in Section 5.4, the resulting spectra were integrated across the line-width of the water resonance, yielding complex-valued data points for the indirect phase-encoded dimensions of the experiment. These dimensions were then apodized by Gaussian functions centered at the origin of k-space, zero-filled by a factor of 2, and transformed by a multidimensional Fourier transform to obtain complex images across the TOF dimension. Summation of these images yielded an image representing the entire volume of encoded fluid. An additional summation was carried out across the TOF dimension in all of the sequential excitation experiments

described in Section 5.5 to obtain a full image of the phantom. The two-loop sequential excitation experiments also included an array across the inter-region travel delay. Integration across the direct spectral dimension yielded signal intensities at each point in this array. In the three-loop sequential excitation experiment, the two inter-region travel delays were fixed, and the spectra are shown without integration.

5.4 Frequency-Selective Imaging

All experiments were carried out within the framework of a remotely detected acquisition, in which encoding and detection steps are separated in time and space. The pulse sequence for the selective imaging experiment, shown in Figure 5.3, is similar to sequences described in a previous work [6]. Frequency-selective excitation was accomplished with a 90° eSNOB pulse of 1 kHz bandwidth [93], which was tuned to excite one of the individually addressable flow regions. Two-dimensional phase encoding gradients were then applied, and were compensated to correct for unwanted velocity effects [127]. Refocusing of transverse magnetization occurred via a 180° spin echo pulse, and a final 90° pulse was applied after the proper echo delay in order to store the encoded magnetization along the $\hat{z}$ for transport to the detection coil, allowing the signal to decay with the long longitudinal relaxation time T_1 in place of the much shorter transverse relaxation time T_2^* . The labeling thus persists for a time period of T_1 . Stroboscopic detection was carried out as the encoded fluid traveled through the microcoil, yielding additional TOF information. Phase cycling was employed to store each component of the magnetization and to filter out any signal from spins outside of the encoding region. The acquisition time for each FID was correlated to the amount of time each fluid packet spent in the detection coil, which was approximately 60 ms. A total of 50 TOF acquisitions were collected during the course of remote acquisition. The entire encoding and detection sequence was repeated for each k-space point of the image. In this case, we acquired 31×31 transverse images with a field of view of 3.88 cm and a resolution of $125 \mu\text{m}$ in the $\hat{x}$ and $\hat{y}$ dimensions.

By applying the RD-MRI sequence with a narrow band frequency selective eSNOB excitation pulse shown in Figure 5.3, we were able to selectively image fluid residing in each of the three regions at the time of encoding. A representative selection of the TOF images and the corresponding sum images are shown in Figure 5.4. As shown, each region may be selectively imaged simply by varying the frequency of the excitation pulse. The resonance frequencies of each region were determined either by running an array of travel curves or low-resolution images. The spectral offset for each additional pair of layers (above and below the flow phantom) was determined to be approximately 2.5 kHz. The bandwidth of each region was determined to be approximately 1 kHz. The bandwidth and shape of the pulse may be further optimized to minimize excitation overlap between regions of similar frequency. In the images of loop C, the signal outside of the loop is from the inlet capillary, which extended outside of the chip and did not have a controlled magnetic environment. The frequency of this signal could be easily shifted via shimming or removed with an alternate phantom design, but its presence here does not interfere with our initial experiments.

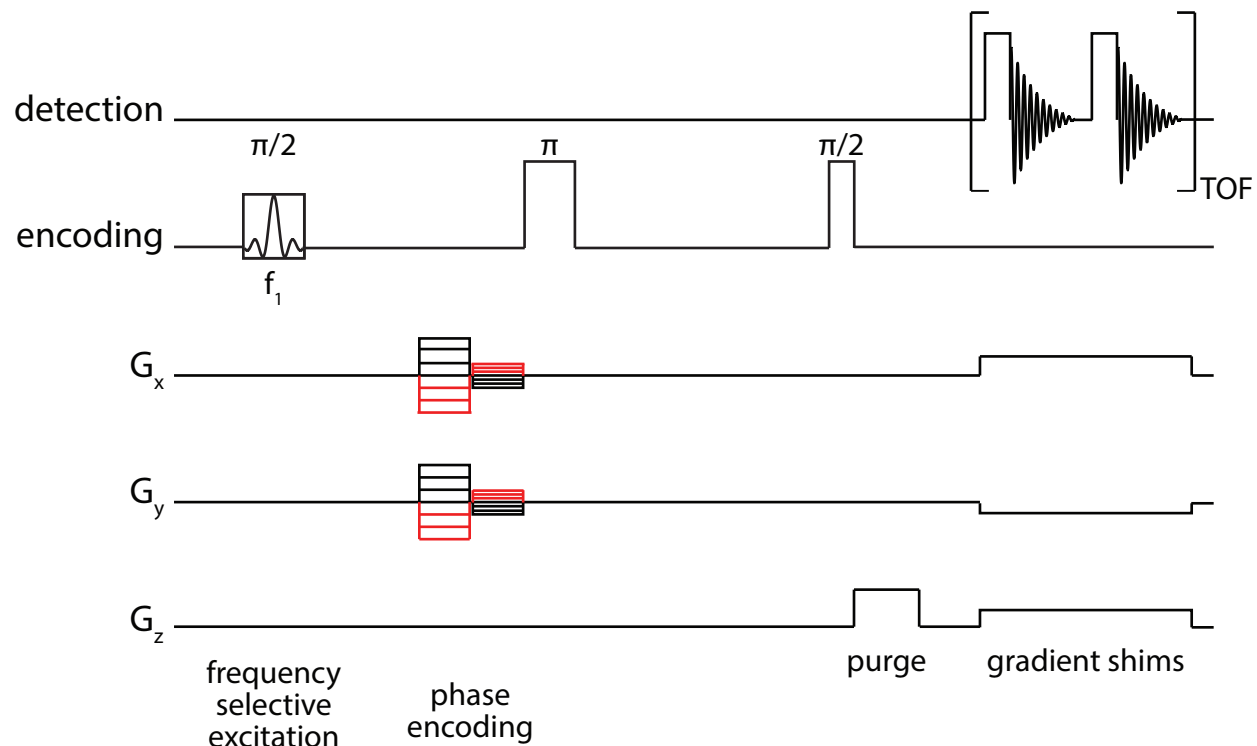


Figure 5.3: Frequency-Selective Imaging Sequence. A frequency-selective 90° excitation excites water in one of the flow regions. Two-dimensional phase encoding gradients are applied and refocusing of transverse magnetization occurs via a 180° spin-echo pulse. A final 90° pulse stores the encoded magnetization along $\hat{z}$ for transport to the detection coil. Finally, stroboscopic pulses are applied with the detection coil to detect the encoded magnetization.

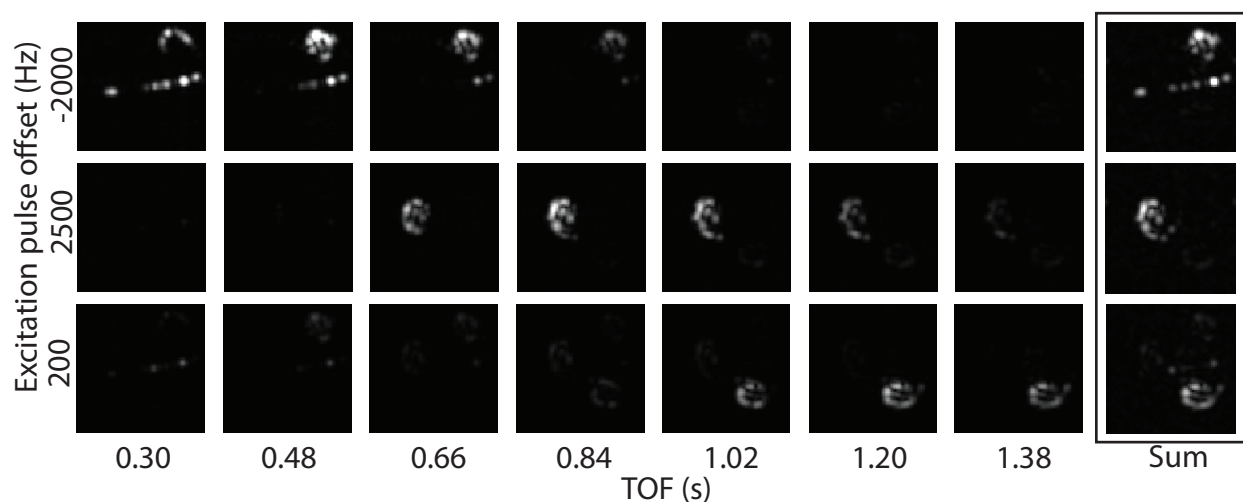


Figure 5.4: Frequency-Selective Image of the Flow Phantom. TOF and sum images illustrating the ability to selectively image each of the three flow regions by varying the excitation pulse offset frequency.

5.5 Sequential Excitation

Frequency-selective excitation allows for experiments which use the magnetic environment of the flow pathway as a means of spatial selectivity. To demonstrate this concept in our phantom, we applied a series of frequency-selective pulses with delays corresponding to the estimated inter-region travel time. As shown above, the three regions of this phantom can be individually addressed, allowing us to perform selective two- and three-loop “gating” operations. As shown in the pulse sequences in Figure 5.5, each excitation consists of a broadband 90° pulse followed by a frequency-selective 90° eSNOB pulse. The net effect of these pulses (and the subsequent crush gradient along $\hat{z}$ during the delay) is to ensure that the only surviving magnetization is from spins which were in the correct region during each of the corresponding frequency-selective pulses.

The pulse sequences for the two- and three-loop sequential excitation experiments, shown in Figure 5.5, replaced the encoding portion of the frequency-selective imaging sequence with a series of pulses separated by time delays to allow for fluid travel between regions. Each excitation consisted of a broadband 90° pulse followed immediately by a frequency-selective 90° eSNOB pulse of 1kHz bandwidth. As a result, only magnetization at the selected frequency (and thus, the selected region) was returned to $\hat{z}$. All remaining transverse magnetization was dephased by a gradient along $\hat{z}$ during the inter-region travel delay. The frequency-selective pulses and receiver stroboscopic pulses were phase-cycled (in 4- and 8-step cycles for the two- and three-loop experiments, respectively) to yield a net positive signal for spins that survived all of the excitation steps. Detection was carried out using the method described above.

Data from the three possible pairs (AB, BC, and AC) are also shown in Figure 5.5. For each, the timing of the inter-region travel delay has been incremented, and all other dimensions (TOF and spectral) have been summed to give a signal magnitude for each point in the travel time array. Each plot shows data from an experiment with the pulses applied in the order of flow (solid line) and one in the opposite order (dashed line). The resulting curves demonstrate the ability to selectively obtain signal based on the flow pathway, while the arrayed delay gives a quantitative measure of the travel time between regions. The signal maxima for these experiments are found at $\tau_{AB} = 0.35$ s, $\tau_{BC} = 0.35$ s, and $\tau_{AC} = 0.70$ s. A selective three-loop experiment may also be performed, as shown at the bottom of Figure 5.5. Here, the inter-region travel delays are fixed to give maximum signal ($\tau_{AB} = 0.35$ s, $\tau_{BC} = 0.35$ s) and the spectra are shown before integration. Signal can only be observed when the selective pulse frequencies follow the order of flow (ABC); the five other permutations do not yield any detectable signal.

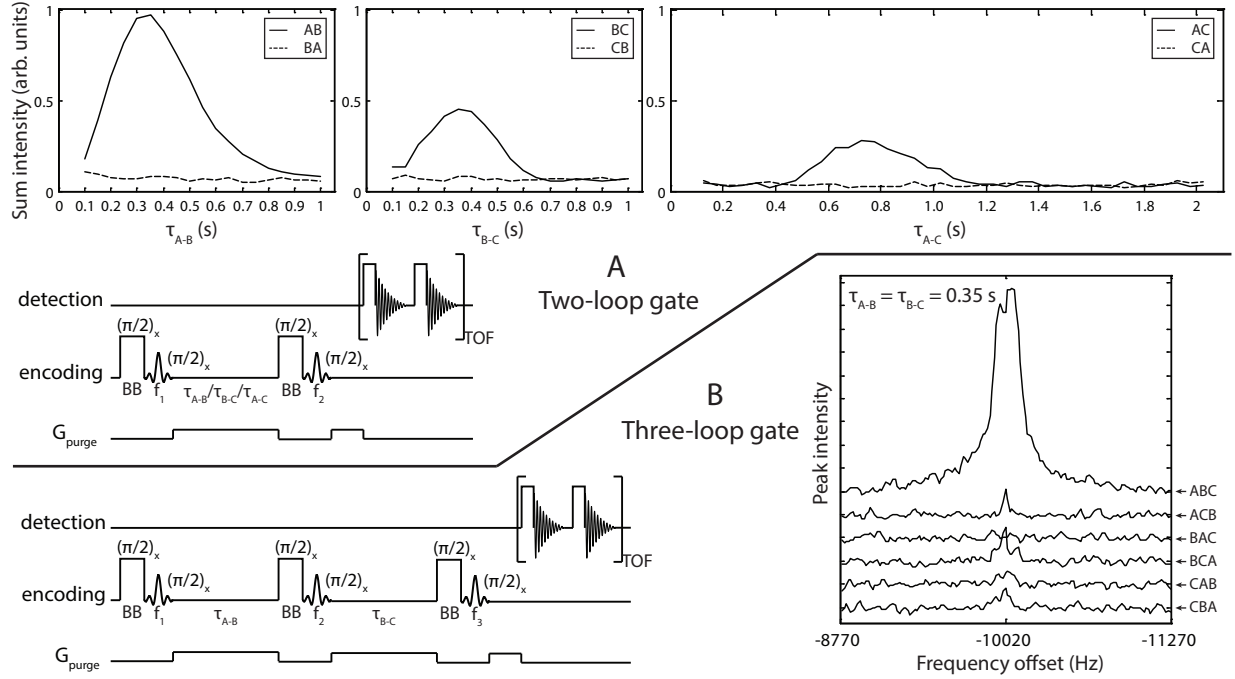


Figure 5.5: Sequential Excitation Imaging Sequence. A) Two-loop sequential excitation. Sequential excitation which aligns with flow direction (i.e. AB, BC, AC) yields measurable travel curves with maximum intensity at $\tau_{AB} = 0.35$ s, $\tau_{BC} = 0.35$ s, and $\tau_{AC} = 0.70$ s. When excitation ordering is inverted (i.e. BA, CB, CA), no signal is obtained. The two-loop gate for sequential excitation is shown below. B) Three-loop sequential excitation. Remotely-detected spectra (integrated across the time-of-flight dimension) are shown for all possible permutations of three-loop sequential excitation (i.e. ABC, ACB, BAC, BCA, CAB, CBA), with two-loop travel times set at $\tau_{AB} = 0.35$ s and $\tau_{BC} = 0.35$ s. Signal can only be observed when the selective pulse frequencies follow the order of flow (ABC). The three-loop gate for sequential excitation is shown at bottom left.

5.6 Imaging Optimization Simulations

We developed this technique with the ultimate goal of allowing micro-scale NMR and MRI experiments which do not require large and non-portable imaging hardware. In addition to the proof-of-principle experiments presented above, we conducted a computational analysis of the limitations of our technique. The hysteresis curve of a 4 mm x 5 mm piece of the floppy diskette material described above was obtained using a SQUID magnetometer capable of measuring the magnetization of the floppy diskette material at magnetic fields up to 7 T. The resulting magnetization curve was imported into the Magnetic Fields, No Currents module of the COMSOL Multiphysics software. The phantom described above was modeled to test agreement of the simulations with our experimental results. In general, the NMR frequencies and linewidths obtained in the simulation agreed with experimental data to within 5%. To determine the potential one-dimensional imaging resolution, we modeled the frequency response of a 1 pL voxel of water, which is of the order of magnitude of the smallest volume of water that we expect to be able to detect in a single-shot NMR experiment using a microcoil detector. As the ultimate goal of this research is to allow for the development of portable NMR and MRI experiments, we conducted these simulations at a field of 1.5 T, which is easily reached by high-resolution NMR magnets made from small Halbach arrays of permanent magnetic materials [36, 37, 38]. We simulated a line of these voxels surrounded by Pyrex glass, which was taken to be a representative construction material for a microfluidic device. We placed the voxels near thin magnetic films of $\text{Co-}\gamma\text{-Fe}_2\text{O}_3$ of varying thicknesses and optimized the frequency response by varying the geometry of the voxel, extent of the film, film thickness, and spacing of the voxels. We modified these values until the water signals barely began to overlap one another. Here, this is taken to mean that the tails in the NMR signals from each neighboring region did not overlap to greater than 10% of the peak height.

We were able to resolve the water signals from the various regions when using cubic voxels 10 μm on a side, with thin magnetic films placed 3 μm above and below each region. The thin films were rectangular prisms 28 μm on a side and ranged in thickness from 120 nm to 140 nm thick. The regions were separated by a center-to-center distance of 34 μm . To suppress frequency tailing behavior in the two outside regions, we placed additional thin magnetic films on the ends of the simulation. This geometry is shown in Figure 5.6a. The spatial distribution of NMR resonances frequencies is shown in Figure 5.6b. The resultant NMR signals for each water voxel are shown in Figure 5.6c. Here, we see that the various regions display a center-to-center frequency difference of approximately 800 Hz and a FWHM linewidth of approximately 300 Hz. We note the presence of extensive tailing in the low-intensity portion of the NMR signal, which limits the spatial resolution using this geometry. We conclude that this technique is able to achieve one-dimensional spatial resolutions of 34 μm . We note that this resolution should be taken as an upper bound on the achievable resolution, as it may be possible to get better results by using different thin film geometries or different magnetic materials. We defer an exhaustive analysis of these considerations to future work.

We do not expect substantial broadening of the NMR signal due to a mismatch in the magnetic susceptibilities of water and Pyrex glass at these fields. As such, we have not conducted an extensive study of the susceptibility-induced broadening in our simulations. In the event that such broadening degrades the NMR signal to an unacceptable extent, it may be possible to utilize alternative materials which have a magnetic susceptibility closer to that of water or another solvent. Such an approach has been employed at higher magnetic fields and includes the production of susceptibility-matched NMR tube inserts [85] as well as NMR tubes made out of susceptibility-matched glasses [164]. Such techniques might be adapted to a microfluidic device which operates according to the principles described in this work. We also note that such techniques may allow for additional improvement in the lineshapes of the NMR signals attained in the various imaging regions, and further improve the attainable imaging resolution. We once again defer an analysis of these effects to future optimization studies.

5.7 Future Microfabrication Experiments

While we have successfully demonstrated the NMR “bar code” concept through proof-of-principle experiments with a relatively large-scale flow phantom, the true utility of this method will not be fully apparent until it can be applied at the length scales which are typically employed in microfluidics experiments, on the order of a few microns to tens of micron. In the preceding Section 5.6, we considered the spatial resolutions which could be achieved using patterned arrays of permanent magnetic materials. We determined that the method presented in our proof-of-principle experiments could easily achieve spatial resolutions on the order of a few tens of microns using the permanent magnetic materials employed in our proof-of-principle experiments. However, we have not yet considered how these materials could be fabricated to enable such spatial resolutions.

The use of permanent magnetic materials necessitates specialized microfabrication techniques. Unlike many non-magnetic metals, permanent magnetic materials cannot be deposited using electroplating techniques in a standard atmospheric environment or even using chemical vapor deposition (CVD) techniques in a medium vacuum environment; instead, permanent magnetic materials are generally deposited on a substrate using physical vapor deposition (PVD) techniques which require high vacuum or UHV instrumentation, such as RF-magnetron sputtering [87]. These methods generally require long vacuum pumping steps. Moreover, permanent magnetic materials generally cannot be patterned using wet or dry chemical etching techniques; they can only be etched using high-energy high vacuum or UHV instrumentation, such as ion beam milling [83]. These techniques once again require vacuum pumping steps. For these reasons, our proposed method for patterning the permanent magnetic material will attempt to avoid multiple deposition or etching steps in an effort to make efficient use of manufacturing time.

The basic procedure consists of selectively etching pits of varying depths into a silicon wafer. The pits can then be filled with a permanent magnetic material using PVD methods

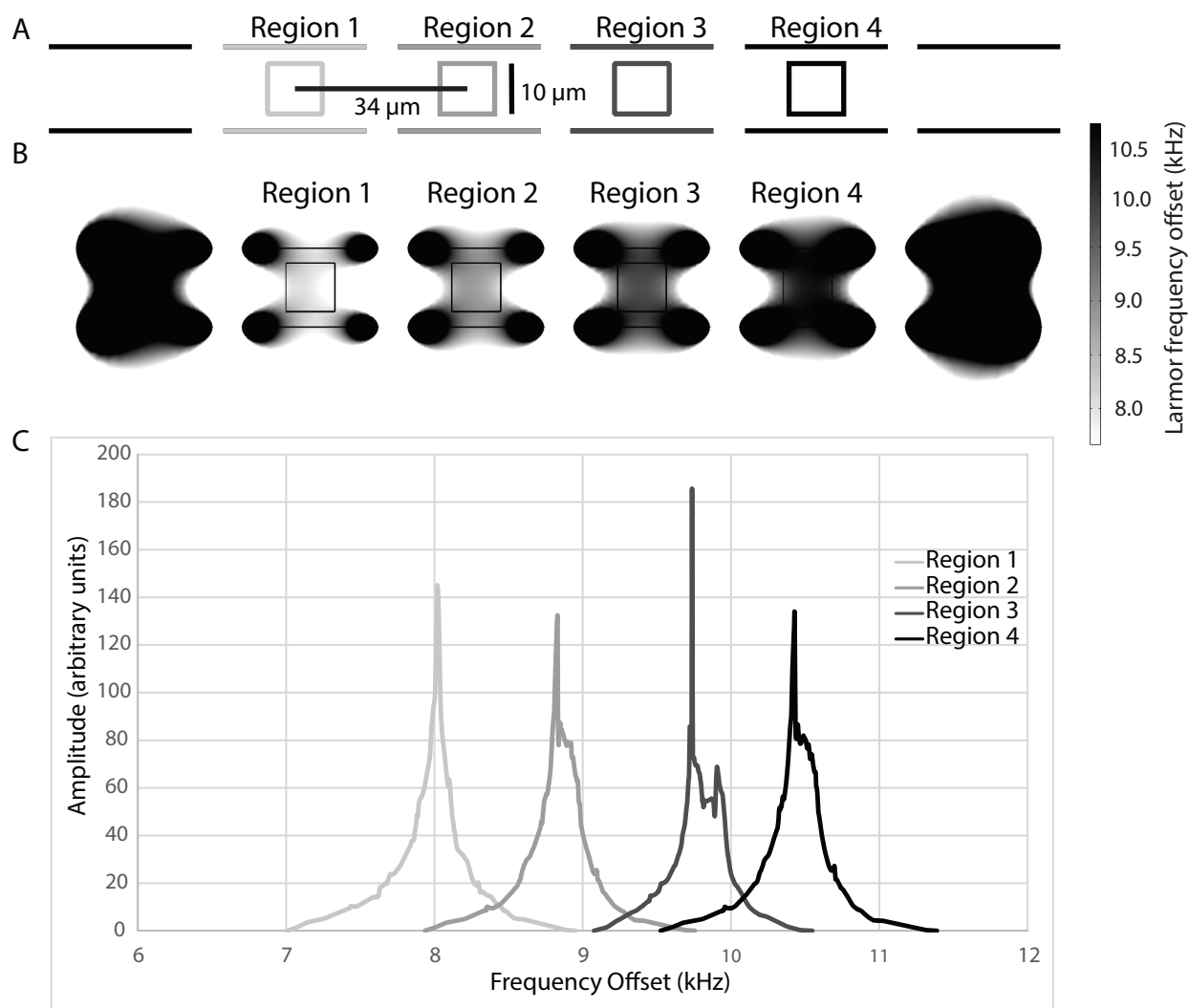


Figure 5.6: Imaging Optimization Simulation. A) Cross-section of the one-dimensional array simulation. Regions of distinct magnetic field are created using thin magnetic films of varying thickness. A 1 pL volume of water is placed between two of these films. Additional films are placed to the sides of the outer regions to suppress tailing in the frequency distribution of these regions. B) Spatial distribution of NMR resonance frequencies generated by this geometry. C) NMR signals of each region.

to form the various magnetic field regions. To achieve different magnetic film thicknesses in each region, we use a shadow masking technique. The proposed fabrication procedure is shown in Figure 5.7.

We use a silicon wafer as the mold for our magnetic field regions because silicon wafers can be patterned quickly using very simple wet chemical procedures which require very little time to carry out. Patterning of the silicon wafer proceeds as follows. A layer of positive photoresist such as Shipley S-1818, is spin coated onto the Si wafer (1500 rpm for 30 seconds for S-1818) and baked (1.5 minutes at 120 °C for S-1818) to form a thin layer (approximately 2 μm for S-1818) which is resistant to chemical attack. The photoresist-coated Si wafer is then pushed into contact with a photomask using a stepper alignment system. The photomask is opaque to UV light at 365 nm, except in the areas in which the Si wafer is to be etched. The photoresist-coated Si wafer is exposed to the 365 nm UV light for a short period of time (a few seconds for S-1818), which depolymerizes the photoresist and makes it susceptible to chemical attack in the exposed areas. The wafer is then submerged in a developing solution (CD-2000 developer for S-1818) which dissolves the depolymerized photoresist, leaving bare Si in the desired areas. The wafer is then submerged in potassium hydroxide; the Si is etched by a chemical reaction with KOH. The wafer is then washed in water to remove any remnant KOH and dried under nitrogen gas. A second round of photolithography and wet chemical etching, with a second photomask, is used to etch more Si. At the end of this round of photolithography and etching, the first area of Si is etched to a greater depth than the second. After another round of photolithography and etching, we have three etched regions, each etched to a different depth. This procedure is continued until the desired number of regions have been formed on the Si wafer. After all regions have been formed, the photoresist is removed by immersing the Si wafer in acetone. The wafer is washed in acetone, isopropanol, and water. It is then cleaned using either the RCA procedure (discussed in Section 3.4.2) or Piranha solution (discussed in Section 3.5.1), washed with water, dried under N_2 gas, and baked to 150 °C for a few minutes to remove any remaining water.

The permanent magnetic material is then deposited using a combination of RF-magnetron sputtering and shadow masking. The wafer is placed in the sputtering chamber and vacuum pumped to a pressure less than 10^{-6} Torr. A thin metallic adhesion layer, such as 10 nm of chromium, is deposited on the Si wafer to promote adhesion of the magnetic layer. The chamber is heated to approximately 450 °C and a magnetic field is turned on to promote alignment of the magnetic domains during deposition. A shadow mask, consisting of a substrate (Si, glass, etc.) with holes etched at the sites of all of the pits, is aligned with the Si wafer to be coated. The permanent magnetic material is then sputtered and forms a layer of magnetic material on the Si wafer. This continues until a film the thickness of the first pit has been deposited. At this point, sputtering ceases and a new shadow mask is aligned with the wafer. This shadow mask contains holes at all of the pits except the one whose desired thickness has been attained. Another round of sputtering is employed until a film the thickness of the second pit has been deposited. This continues until all of the pits have been filled with the permanent magnetic material. The final shadow mask is removed, the wafer is allowed to cool, the magnetic field is turned off, and another 10 nm layer of chromium (or other metallic adhesion layer) is deposited over the permanent magnetic material. The

coated wafer is then removed from the sputtering chamber and transferred to a low-pressure chemical vapor deposition (LPCVD) chamber. The chamber is vacuum pumped to a pressure below 1 Torr and heated to 500 °C. A mixture of tetraethoxysilane, oxygen, and argon gases are flowed into the chamber; the $\text{Si}(\text{OEt})_4$ and O_2 react and bombard the surface to form a layer of silicon dioxide glass a few microns thick. The chamber is cooled and the glass-coated wafer is removed.

Two such wafers are photolithographically patterned and the glass is etched to a depth of a few microns using hydrofluoric acid. The photoresist is removed, leaving one half of the glass channels on each wafer. The two wafers are then aligned and bonded under pressure at approximately 550 °C, forming sealed channels with magnetic thin films on top and bottom. In this fabrication method, all high temperature fabrication steps must employ slow heating and cooling in order to avoid delamination of the various layers due to their differing coefficients of thermal expansion.

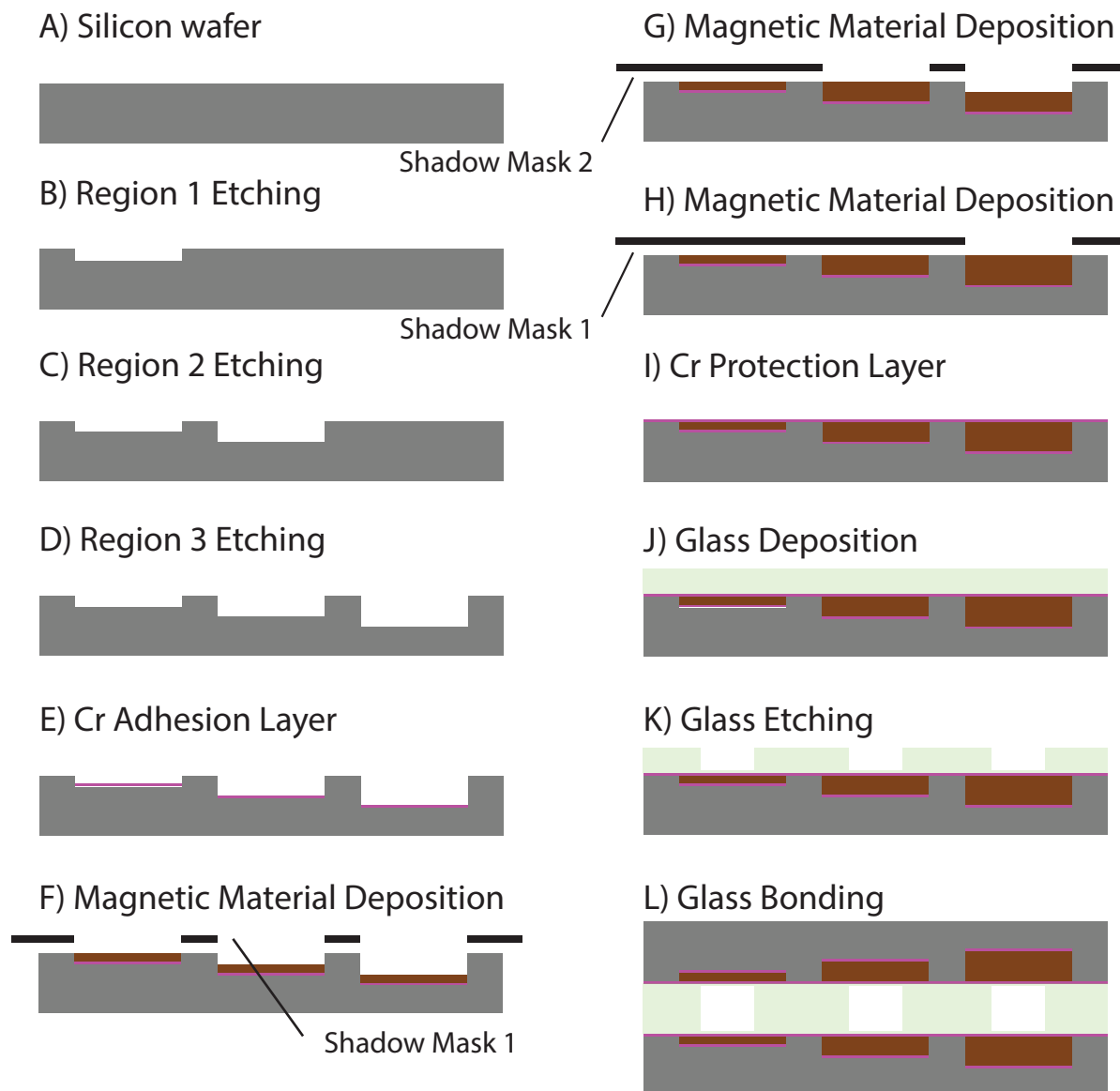


Figure 5.7: NMR "Bar Code" Microfabrication Procedure. The fabrication procedure employs photolithography and etching of a Si wafer, RF-magnetron sputtering of a magnetic thin film, CVD of glass, etching of glass, and thermal bonding to produce channels with magnetic thin films above and below.

Chapter 6

Prospects for Microfluidic NMR

In the previous chapters, we have considered a number of techniques which might allow for the development of integrated microfluidic devices using magnetic resonance techniques, with particular emphasis on the use of hyperpolarized ^{129}Xe -based molecular sensors to provide high-sensitivity detection of chemicals. Here, we examine the next steps to be taken in the development of the devices presented throughout this thesis.

In Chapter 3, we examined the development of two generations of microfluidic devices designed to produce hyperpolarized ^{129}Xe gas. Although we have proven the principle of producing a combined ^{129}Xe polarizer and optical detector in a first-generation device, this device showed disappointingly low ^{129}Xe polarizations, ^{129}Xe relaxation times, and device lifetimes. We are currently conducting experiments on a second-generation device which should achieve greatly improved ^{129}Xe polarizations, ^{129}Xe relaxation times, and device lifetimes; these improvements should increase the SNR of a microscale ^{129}Xe NMR experiment by more than two orders of magnitude and allow for very high detection sensitivities. Extensive optimization of the device's operating conditions (such as Xe/N₂ mixture, operating pressure, and operating temperature) will be undertaken to potentially provide a further increase in SNR. We are also performing experiments to demonstrate the device's use for imaging purposes.

It is also worth pursuing research to decrease the amount of electrical power drawn by the microfabricated ^{129}Xe polarizer. If the device is able to run on a battery instead of requiring wall power, it allows for the possibility of conducting experiments in remote locations. Both the first-generation and second-generation devices described in this thesis require approximately 5 W of power, making battery-powered operation presently infeasible. However, the vast majority of the power draw is due to the use of resistive heaters, which have the disadvantage of heating the entire silicon-glass device instead of just the ^{87}Rb in the pump and probe chambers. Heating in a more efficient manner, such as by irradiating the ^{87}Rb with an off-resonance infrared laser, would greatly reduce the power requirements. Assuming the use of an 80 mW SEOP laser with 50% electrical efficiency, a 1 mW magnetometer laser with 50% electrical efficiency, a 100 mW heating laser with 50% electrical efficiency, and

pulse and imaging coils operating with 1 A (10 W) pulses with a 1% duty cycle, we estimate that such a system would require less than 0.5 W of power for its operation. This would allow the device to operate for approximately 12 h using two lithium ion AA batteries or nearly 24 h using a single nickel metal hydride D battery. Thus, additional steps should be taken to allow operation of the microfabricated ^{129}Xe polarizer without the need for large attendant power supplies.

Finally, a microfluidic device based on a microfabricated ^{129}Xe polarizer should not require large vacuum and gas-handling instrumentation for its operation. The vast majority of the vacuum and gas-handling equipment used in our experiments was included for experimental purposes and can likely be eliminated in a fully-developed device. For instance, the system is connected to a large turbomolecular pump which is only used to evacuate a device when it has just been connected to the gas handling system. A microfabricated ^{129}Xe polarizer used as part of a microfluidic platform should be hermetically sealed from the environment and operated in a closed loop. In this case, it is only necessary to evacuate the device once, which can be achieved by fabricating under UHV conditions. The gas handling system allowed for the generation of arbitrary Xe/N_2 gas mixtures for use in optimization experiments; a fully-developed future device would not require such a system. The most difficult barrier to miniaturization is the use of vacuum valves; at present, no technology exists to allow on-chip valving at pressures ranging from UHV to just above atmospheric pressure. However, personal correspondence with Erik Jensen in the laboratory of Professor Richard Mathies leads us to believe that microfluidic valves built from Pyrex and Teflon which meet our operating requirements will soon be available. We expect that future generations of the microfabricated ^{129}Xe polarizer will implement this or similar technologies to allow complete miniaturization of all of the gas handling components used in a microfabricated ^{129}Xe polarizer.

These developments are necessary for this technology to find adoption in microfluidic NMR experiments; in addition, further experimental demonstrations must be demonstrated to establish the device's utility in the scenarios in which it is most likely to be used. In most applications which we envision, the hyperpolarized ^{129}Xe produced by the device will not be maintained in the gas phase; rather, it will need to be dissolved in water or another solvent. To maintain the SNR increase of 500 attained by implementing *in situ* detection with a ^{87}Rb magnetometer utilizing the Fermi-contact enhancement, it is further necessary to extract encoded ^{129}Xe gas from the solution in which it has been dissolved and flow it into the magnetometer chamber. These requirements present some major difficulties which do not arise in aqueous ^{129}Xe experiments utilizing large ^{129}Xe polarizers and high-field magnets.

For those applications which require the dissolution of hyperpolarized ^{129}Xe into water or another liquid, we note the prevalence of commercial systems for dissolving gases into water, an example of which was discussed in Section 4.3.3. Such systems generally use some form of hydrophobic interaction to allow for one-way passage of gas into water. In order to enable miniaturized liquid-state experiments such as the microfluidic XBMS arrays envisioned in Chapter 4, additional work must be performed to emulate such systems on a small scale. Fortunately, there is reason to be hopeful in this regard, given recent work demonstrating

microfluidic gas dissolution using porous interfaces [39]. We also note the development of techniques for creating porous membranes with controllable properties in microfluidics, including superhydrophobicity [185]. Personal correspondence with the authors leads us to believe that these methods are adaptable to our goal of dissolving ^{129}Xe in water, performing magnetic resonance experiments, and then extracting the ^{129}Xe to be detected using the *in situ* ^{87}Rb magnetometer utilizing the Fermi-contact enhancement.

Of course, our dissolution method will not be perfect. As our hyperpolarized ^{129}Xe gas mixture passes into the water (or other solvent) after SEOP, it will carry along ^{87}Rb from the pumping chamber. Given the extremely high reactivity of alkali metals, this might lead to contamination of a sample under analysis. We do not anticipate that this will be particularly problematic to the operation of the device. Assume that we're mixing the hyperpolarized ^{129}Xe gas mixture into water. In the first-generation polarizer, we used a Xe partial pressure of 200 Torr. If we're mixing into water at 25 °C, this means that the number density of gaseous Xe is $4.93 \times 10^{18} \text{ mm}^{-3}$. At a typical total gas flow rate of $5 \mu\text{L s}^{-1}$, this means that 2.46×10^{19} atoms of Xe pass by a given point in a period of 1 s. To get good mixing of Xe into the water, we will need a large excess of Xe atoms in comparison to water molecules. Assume that we use an extremely low liquid flow rate of 10 nL s^{-1} , or $0.01 \mu\text{L s}^{-1}$. The number density of liquid water is approximately $3.35 \times 10^{19} \text{ mm}^{-3}$. At a water flow rate of $0.01 \mu\text{L s}^{-1}$, this means that water molecules flow at a rate of $3.35 \times 10^{18} \text{ s}^{-1}$. We have a two order of magnitude excess of Xe atoms to water molecules, so these parameters should be sufficient for good mixing; they get better at higher liquid flow rates. We will most likely never need to flow more slowly than 10 nL s^{-1} in a typical microfluidic application; thus, we expect that our alkali metal is never likely to encounter a situation in which gas-liquid mixing is problematic.

The highest ^{87}Rb number densities employed in the first-generation device are approximately 10^{14} cm^{-3} , or 10^{11} mm^{-3} . At a gas flow rate of $5 \mu\text{L s}^{-1}$, this means that ^{87}Rb atoms flow at a rate of $5 \times 10^{11} \text{ s}^{-1}$. This is six orders of magnitude smaller than the water flow rate, in the absolute worst mixing scenario that a microfabricated ^{129}Xe polarizer is likely to encounter. Put another way, as a sample of water flows by and is mixed with Xe, it carries with it a quantity of ^{129}Rb six orders of magnitude smaller than the amount of water present. Of course, any ^{87}Rb atom that encounters water will oxidize quickly. The point is that this amount is so small in comparison to the amount of water present that it will have a negligible effect on the water. Due to the much higher density of liquids than gases, this is true for any liquid sample we might imagine. Thus, we don't have to worry about using only samples which don't react with ^{87}Rb . The effect is so small that it can be neglected in most cases we can imagine. In cases where even this small reactivity is not acceptable, it may be possible to employ chemical getters to render the 87 inert prior to its interaction with water (or another solvent).

While getting hyperpolarized ^{129}Xe into a liquid medium should be relatively easy, getting it out for *in situ* detection with a ^{87}Rb magnetometer is much more difficult. We have previously calculated (see Section 3.5.4) that even a small leak of oxygen is devastating to the ^{87}Rb metal used in the pump and probe chambers; this is also true of water and other

common solvents. Therefore, it is necessary to remove as much water (or other solvent) as possible during extraction of the ^{129}Xe . We note that such extractions have been performed on a large scale and allow signal enhancement by further compression of the ^{129}Xe (which increases the number density and the NMR signal) [187]; the authors demonstrated a SNR enhancement of a factor of 3, but calculate that efficient extraction and compression should allow SNR enhancements by a factor of 10^4 . While the high field, large-scale experiment is relatively straightforward, the application to low fields and small scales is much more challenging. For solvents with very low vapor pressures, extraction into an *in situ* ^{87}Rb magnetometer should not be a problem. Unfortunately, many useful solvents have very high vapor pressures, which creates a major separation problem. For instance, water has a vapor pressure of approximately 20 Torr at standard conditions. If we utilize a separation method based on the hydrophobic effect, no liquid water will flow into the ^{87}Rb magnetometer chamber; however, 20 Torr of water will still remain in the gas phase and will not be separated from the polarized gas mixture. This water vapor will quickly oxidize the ^{87}Rb and render the magnetometer useless.

One method of solving this problem might be to cool the gas/water mixture during separation in an attempt to decrease the water vapor pressure. At 0°C , the vapor pressure of water is approximately 5 Torr, which is not much of an improvement. In order to decrease the vapor pressure of water by a significant amount, it is necessary to employ strong cooling to temperatures well below the freezing point of water. One method of doing so might be the use of a Peltier thermoelectric cooler [130]. These devices can be made very small, allowing only local cooling of the water-gas mixture, and require very little electrical power to operate. However, they are only able to achieve a temperature differential of 80°C . Cooling from room temperature to -55°C only decreases the water vapor pressure to 4.26×10^{-2} Torr [115], requiring the use of additional methods to remove the remnant water vapor. Another alternative might be the use of liquid nitrogen as a means of cooling the device to very cold temperatures. In order to maintain Xe in the gas phase, it would be necessary to remain at a temperature above the boiling point of Xe (approximately 165 K). At this temperature, water has a vapor pressure of 2.22×10^{-5} Torr, an improvement of more than three orders of magnitude. Even in this case, it is still necessary to employ additional methods to remove the remnant water vapor, though the requirement is much lower. Unfortunately, it is very difficult to provide localized cooling with liquid N_2 ; moreover, this technique would require cryogenic hardware, complicating the design of a future microfabricated device.

The most likely means of removing water vapor from the gas mixture would utilize Peltier coolers to significantly reduce the vapor pressure. In a second stage, the remnant water vapor could then be removed by a combination of chemical gettering and the use of lithographically-patterned porous membranes with controllable properties [185]. For instance, charged porous membranes could be created to allow capture of a large amount of water molecules. Personal correspondence with the authors leads us to believe that these devices can be adapted to our goal of extracting ^{129}Xe within a microfluidic device. This technique is currently being developed for microfluidic extraction of ^{129}Xe from water vapor by Muller Gomes in the Pines laboratory.

If the aforementioned improvements to the miniaturized ^{129}Xe polarizer can be made, it will serve as an obvious replacement for large-scale polarizers in the microfluidic molecular sensing experiments discussed in Chapter 4. Of course, additional improvements must also be made to the microfluidic XBMS array. The remainder of the proof-of-principle microfluidic XBMS experiments using a macromolecular host with a large “payload” of XBMSs (Section 4.4.5) are being performed by Clancy Slack and Chris Vassiliou in the Pines laboratory. Assuming that these experiments allow microfluidic detection using a XBMS, the next step is to demonstrate the use of spectroscopic imaging to detect multiple chemical components in a complex mixed fluid sample. HyperCEST (discussed in Section 4.2) should be employed to provide a large increase in the SNR. It is also well worth experimenting with changes to the gas mixture in order to increase the SNR. Equally important is a fundamental rethinking of the manner in which the XBMSs detect an NMR signal.

Current research using XBMSs invariably relies on a change in the ^{129}Xe chemical shift upon the binding of a chemical species of interest. This is a useful method of detection at high magnetic fields; however, the chemical shift is linearly dependent on the applied magnetic field and vanishes rapidly as we move to NMR hardware which generate smaller and smaller magnetic fields. At the Earth’s field or lower (the magnetic fields at which a microfluidic NMR device is likely to operate), the chemical shift has all but ceased to exist. Even at 1.5 T (the magnetic field produced by the strongest currently-available permanent magnet arrays), the chemical shift is a very weak effect. Thus, alternative methods of detecting a binding event should be investigated. Muller Gomes in the Pines laboratory has recently demonstrated unpublished results showing a XBMS which relies on relaxation effects rather than chemical shifts as its sensing mechanism. The sensor has a second chemical moiety (in addition to the targeting moiety) - a 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) agent capable of chelating paramagnetic metals which are rich in unpaired electrons, such as gadolinium (Gd). Upon binding of a target molecule, the molecular structure of the XBMS changes slightly, moving the Gd to a slightly different position with respect to the CryA cage. Since the interaction between ^{129}Xe in CryA and Gd in DOTA scales with distance as r^{-6} , even a slight perturbation in the XBMS molecular structure can have a significant impact on the ^{129}Xe relaxation time. Thus, relaxation may represent another way of determining chemical binding to a XBMS. Though we have only seen these results at high magnetic fields, we expect the response to persist at the low magnetic fields likely to be employed in a microfabricated XBMS device.

Finally, we turn our attention to the gradient-free flow labeling technique presented in Chapter 5. The utility of this method has been demonstrated using a water-based microfluidic analog; it should be extended to actual microfluidic devices and to ^{129}Xe experiments. Beyond this, there remains a significant amount of technological development to be performed. The construction of the “bar code” should be adapted to conform with microfabrication standards, perhaps in the manner described in Section 5.7. Additional work should be performed to allow further improvement of the achievable spatial resolutions; this improvement may come from computer simulation to determine more optimal permanent magnet geometries, the use of different permanent magnetic materials, or the implementation of susceptibility-matched materials during construction of the microfluidic device.

Taken together, the techniques presented throughout this thesis offer the first steps toward the development of fully integrated microfluidic devices capable of detecting the presence or absence of arbitrary chemicals with very high sensitivity in a number of environments which are not amenable to the use of laboratory-scale equipment. The techniques described are currently in a relatively low state of development. However, we have explored engineering principles which should allow for the rapid development of microfluidic NMR devices capable of much more sensitive measurements than the experiments presented in this thesis. While the past development of microfluidic NMR has lagged in comparison to the miniaturization of other analytic techniques, its future is bright. As a detection modality, NMR offers a number of advantages over other methods, particularly its ability to analyze opaque or dirty samples without the use of covalent labeling techniques which are common in optical devices. The biggest disadvantage of NMR experiments, the low sensitivity and concomitant need for large and expensive superconducting magnets, has been partially overcome by employing hyperpolarization and magneto-optical detection methods; we can expect future developments to further alleviate this problem. When fully developed, we expect that devices based on the proof-of-concept experiments presented in this thesis may serve, for instance, as a means of quality control in chemical reactors (whether conventional or microfluidic) by continuously monitoring the output of the reactor for chemical impurities. Given the liberation from superconducting magnets and the marriage of magnetism and optics, the future of microfluidic NMR looks bright.

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