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#### WASHINGTON UNIVERSITY IN ST. LOUIS

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New Techniques and Optimizations of Short Echo-time <sup>1</sup>H MRI with Applications in Murine Lung by Jinbang Guo

> A dissertation presented to The Graduate School of Washington University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

> > December 2016 St. Louis, Missouri

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# **List of Abbreviations**

ACR	Acute Cellular Rejection
ADC	Analog-to-Digital Converter
ANOVA	Analysis of Variance
CCSP	Clara Cell Secretory Protein
CMV	Cytomegalovirus
СТ	Computed Tomography
Dox	Doxycycline
DTA	Diphtheria Toxin A
EGFR	Epidermal Growth Factor Receptor
EPI	Echo Planar Imaging
FA	Flip Angle
FFT	Fast Fourier Transform
FID	Free Induction Decay
FIR	Finite Impulse Response
FOV	Field-of-View
FRC	Functional Residual Capacity
FWHM	Full Width at Half Maximum
GRE	Gradient-Recalled-Echo
H & E	Hematoxylin and Eosin
ID	Inner Diameter
IPF	Idiopathic Pulmonary Fibrosis
I/R	ischemia-reperfusion
IR	Inversion Recovery
LFA	Limited Flip Angle
LTx	Lung Transplantation
max	Maximum
min	Minimum
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
NMR	Nuclear Magnetic Resonance
OLTx	Orthotopic Vascularized Aerated Left Lung Transplantation
PBS	phosphate-buffered saline
PC	Personal Computer
PE	Phase Encoding
PET	Positron Emission Tomography
PGD	Primary Graft Dysfunction
POCS	Projection Onto Convex Subsets
POD	Postoperative Day

PSF	Point Spread Function
RF	Radiofrequency
rtTA	tetracycline-responsive transactivator
SD	Standard deviation
SE	Standard error
SG	Self-gating
SI	Signal Intensity
SNR	Signal-to-Noise Ratio
SPECT	Single Photon Emission Tomography
TE	Echo Time
TEM	Transverse Electromagnetic
TGF	Transforming Growth Factor
TR	Repetition Time
TTL	Transistor-Transistor Logic
TV	Tidal Volume
Tx	Transplantation
UTE	Ultra-short Echo Time
2D	Two-dimensional
3D	Three-dimensional

### **List of Notations**

$B_0$	External magnetic field
B <sub>1</sub>	RF field, perpendicular to $B_0$
С	Capacitance
FOV <sub>x</sub> , FOV <sub>y</sub> , FOV <sub>z</sub> ,	FOV along x, y, z axes respectively
$\vec{G}$	Magnetic field gradient
$G_x$ , $G_y$ , $G_z$	Components of magnetic field gradient along x, y, z axes
G <sub>x, R</sub>	Readout gradient
G <sub>y, PE</sub>	Phase encoding gradient
G <sub>z, SS</sub>	Slice select gradient
G <sub>SS</sub>	Slice select gradient
$\vec{k}$	k-Space coordinate, Fourier pair of image-space coordinate
$k_x, k_y, k_z$	k-Space coordinate, Fouirer pair of image-space coordinates x, y, z
L	Inductance
$\vec{M}$	Magnetization
$M_x, M_y, M_z$	Components of magnetization along x, y, z axes
$M_0$	Longitudinal magnetization at thermal equilibrium
R	Resistance
$\vec{r}$	Image-space coordinate
$s(\vec{k})$	MR signal as a function of k-space coordinates
S(t)	FID signal as a function of time
SW_h	Receiver bandwidth
t	time
$T_2^*$	Transverse magnetization relaxation time constant
$T_1$	Spin-lattice relaxation time
T <sub>acq</sub>	Acquisition time or sampling time
T <sub>acqshift</sub>	Delay of readout gradient relative to data acquisition
x, y, z	Orthogonal components of image-space coordinate $\vec{r}$
$\alpha_x, \alpha_y, \alpha_z$	k-Space expansion factors along $k_x$ , $k_y$ , $k_z$ axes respectively
γ	Gyromagnetic ratio
$ ho(ec{r})$	Proton density as a function of image-space coordinates
H <sub>2</sub> O	Water
$^{1}\mathrm{H}$	Proton
<sup>3</sup> He	Helium-3
O <sub>2</sub>	Oxygen
<sup>129</sup> Xe	Xenon-129

### Acknowledgments

First and foremost, I want to express my sincere gratitude to my advisor, Dr. Jason Woods, for his continuous mentoring, full support and knowledgeable guidance in my PhD study. From him, I learned a lot about MR physics and American culture. Under his valuable guidance and support, I was able to explore and work on different topics of research, as part of a great team. I appreciate the opportunities he provided to collaborate with different groups, which expanded my horizons in application of MRI to different fields of preclinical studies. I'm also grateful to him for reviewing and commenting my thesis and paper manuscripts.

I would like to thank Dr. Cleveland Zackary for his help to my research. He gave me a lot of good suggestions to my imaging experiments. He also spent a fair amount of time editing my paper manuscripts.

Many thanks to Dr. Wei Wang who was my schoolmate in undergraduate university and labmate at Washington University in St. Louis. I learned data analyzing methods and hyperpolarized gas diffusion MRI technique from her. She also give me a lot of help in common life.

I want to firstly thank two members of my 3-person committee, Dr. Mark Conradi and Dr. James Miller, for their supervision and constructive comments on my research, which guaranteed and improved the quality of my research. Special thanks are given to Dr. Mark Conradi who taught me how to make RF coil and helped me fix hardware problems. I also want to thank all members of my dissertation committee, Dr. Jason Woods, Dr. Mark Conradi, Dr. James Miller, Dr. Kater Murch, Dr. Alexander Barnes, and Dr. Francesc Ferrer, for their time and constructive comments to make this dissertation better.

Great thanks to Dr. Xingan Wang, who was in charge of performing lung transplantation surgery for us, both at Washington University in St. Louis and in Cincinnati Children's Hospital Medical Center. I appreciate the collaboration with Dr. Howard Huang and Dr. Anne Karina Perl in projects of MRI monitoring acute cellular rejection and chronic cellular rejection in lung transplantation respectively. I also appreciate the collaboration with Dr. William Hardie in project of MRI monitoring pulmonary fibrosis in a transgenic mouse model.

I would like to thank my fellow students, researchers, and lab technicians in the lab: Jenna Green, Kenneth Parks, Robert Thomen, Xuefeng Cao, Teckla Akinyi, Nara Higano, Dr. David Roach, and Dr. Laura Walkup, for their friendship and support.

Last but not least, my sincere gratitude goes to my family: my grandparents, my parents, and my wife for their accompanying and supporting me throughout the writing of this dissertation.

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Washington University in St. Louis December 2016

### Abstract

New Techniques and Optimizations of Short Echo-time <sup>1</sup>H MRI with Applications in Murine Lung by

> Jinbang Guo Doctor of Philosophy in Physics

Washington University in St. Louis, 2016 Professor Mark S. Conradi, Co-Chair Professor Jason C. Woods, Co-Chair

Although x-ray computed tomography (CT) is a gold standard for pulmonary imaging, it has high ionizing radiation, which puts patients at greater risk of cancer, particularly in a longitudinal study with cumulative doses. Magnetic resonance imaging (MRI) doesn't involve exposure to ionizing radiation and is especially useful for visualizing soft tissues and organs such as ligaments, cartilage, brain, and heart. Many efforts have been made to apply MRI to study lung function and structure of both humans and animals. However, lung is a unique organ and is very different from other solid organs like the heart and brain due to its complex air-tissue interleaved structure. The magnetic susceptibility differences at the air-tissue interfaces result in very short  $T_2^*$  (~ 1 ms) of lung parenchyma, which is even shorter in small-animal MRI (often at higher field) than in human MRI. Both low proton density and short  $T_2^*$  of lung parenchyma are challenges for pulmonary imaging via MRI because they lead to low signal-to-noise ratio (SNR) in images with traditional Cartesian methods that necessitate longer echo times ( $\geq$  1 ms).

This dissertation reports the work of optimizing pulmonary MRI techniques by minimizing the negative effects of low proton density and short  $T_2^*$  of murine lung parenchyma, and the application of these techniques to imaging murine lung. Specifically, echo time (TE) in the Cartesian sequence is minimized, by simultaneous slice select rephasing, phase encoding and

read dephasing gradients, in addition to partial Fourier imaging, to reduce signal loss due to  $T_2^*$ relaxation. Radial imaging techniques, often called ultra-short echo-time MRI or UTE MRI, with much shorter time between excitation and data acquisition, were also developed and optimized for pulmonary imaging. Offline reconstruction for UTE data was developed on a Linux system to regrid the non-Cartesian (radial in this dissertation) k-space data for fast Fourier transform. Slabselected UTE was created to fit the field-of-view (FOV) to the imaged lung without fold-in aliasing, which increases TE slightly compared to non-slab-selected UTE. To further reduce TE as well as fit the FOV to the lung without aliasing, UTE with ellipsoidal k-space coverage was developed, which increases resolution and decreases acquisition time. Taking into account  $T_2^*$ effects, point spread function (PSF) analysis was performed to determine the optimal acquisition time for maximal single-voxel SNR. Retrospective self-gating UTE was developed to avoid the use of a ventilator (which may cause lung injury) and to avoid possible prospective gating errors caused by abrupt body motion.

Cartesian gradient-recalled-echo imaging (GRE) was first applied to monitor acute cellular rejection in lung transplantation. By repeated imaging in the same animals, both parenchymal signal and lung compliance were measured and were able to detect rejection in the allograft lung. GRE was also used to monitor chronic cellular rejection in a transgenic mouse model after lung transplantation. In addition to parenchymal signal and lung compliance, the percentage of highdensity lung parenchyma was defined and measured to detect chronic rejection. This represents one of the first times quantitative pulmonary MRI has been performed.

For 3D radial UTE MRI, 2D golden means (1) were used to determine the direction of radial spokes in k-space, resulting in pseudo-random angular sampling of spherical k-space coverage. Ellipsoidal k-space coverage was generated by expanding spherical coverage to create an

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ellipsoid in k-space. UTE MRI with ellipsoidal k-space coverage was performed to image healthy mice and phantoms, showing reduced FOV and enhanced in-plane resolution compared to regular UTE. With this modified UTE,  $T_2^*$  of lung parenchyma was measured by an interleaved multi-TE strategy, and  $T_1$  of lung parenchyma was measured by a limited flip angle method (2). Retrospective self-gating UTE with ellipsoidal k-space coverage was utilized to monitor the progression of pulmonary fibrosis in a transforming growth factor (TGF)- $\alpha$ transgenic mouse model and compared with histology and pulmonary mechanics. Lung fibrosis progression was not only visualized by MRI images, but also quantified and tracked by the MRIderived lung function parameters like mean lung parenchyma signal, high-density lung volume percentage, and tidal volume. MRI-derived lung function parameters were strongly correlated with the findings of pulmonary mechanics and histology in measuring fibrotic burden.

This dissertation demonstrates new techniques and optimizations in GRE and UTE MRI that are employed to minimize TE and image murine lungs to assess lung function and structure and monitor the time course of lung diseases. Importantly, the ability to longitudinally image individual animals by these MRI techniques minimizes the number of animals required in preclinical studies and increases the statistical power of future experiments as each animal can serve at its own control.

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# Chapter 1 Introduction

### **1.1 Objectives**

The overall objective of this project was to develop and optimize short echo-time (TE) MRI techniques that were appropriate for pulmonary imaging and then apply these techniques to investigate the lung function of murine animals in various pulmonary disease models.

Specifically, the objectives were:

(1) To optimize the gradient-recalled echo (GRE) pulse sequence on a Varian 4.7 T scanner by minimizing echo time (TE). This sequence was used to monitor acute cellular rejection in mouse lung allografts.

(2) To minimize TE of GRE sequence on a Bruker 7 T scanner to image chronic cellular rejection in mouse lung allografts.

(3) To develop ultra-short echo-time (UTE) MRI and add "randomly uniform" k-space sampling.

(4) To develop offline reconstruction for UTE MRI based on the work of Zwart et al.

(5) To reduce field-of-view (FOV) and enhance resolution by UTE MRI with ellipsoidal k-space coverage.

(6) To apply UTE MRI with ellipsoidal k-space coverage to measure the  $T_2^*$  and  $T_1$  of healthy mouse lung at 7 T.

(7) To develop retrospective self-gating UTE MRI.

(8) To apply retrospective self-gating UTE MRI to monitor the progression of pulmonary fibrosis in a TGF- $\alpha$  transgenic mouse model.

### **1.2 Brief overview of pulmonary imaging techniques**

In the past decades a variety of imaging modalities have been developed, most of which are applicable in some way to pulmonary imaging. Unlike traditional lung function measurement approaches, e.g., spirometry and plethysmography, which measure the volume and flow of air during respiration under various breathing conditions, pulmonary imaging provides insight into lung function and lung structure. Common imaging modalities for clinical or preclinical pulmonary imaging include chest x-ray, x-ray computed tomography (CT), positron emission tomography (PET), single photon emission tomography (SPECT), optical imaging and magnetic resonance imaging (MRI), which can quantify different properties of lung function and structure. Chest x-ray is used for lung structure imaging to detect lung abnormalities and has less radiation exposure than CT, but can't provide 3D images or functional information. CT can visualize lung structure with high-resolution 3D images and can serve as a densitometer to quantify lung disease over time, but places the imaged subject at higher risk of lung injury and cancer due to the high radiation exposure. PET and SPECT use radioactive labeled tracers to pinpoint differences in tissues at the molecular level, yet have poor spatial resolution and ionizing radiation risk. In MRI, nuclear spins inside an imaged object in a constant external magnetic field  $B_0$  are excited by radiofrequency (RF) pulse and encoded by magnetic gradients, with the resulted magnetization detected by an RF coil for reconstructing images. Due to the multi-factor dependent MR signal, MRI can image lung structure and lung function with a variety of contrasts including  $T_1$ ,  $T_2$ ,  $T_2^*$ , susceptibility, and density. Compared to other imaging modalities, one of the most important advantages of MRI is its non-ionizing radiation property.

The earliest MRI is similar to the current radial ultra-short echo time (UTE) pulse sequence rather than other pulse sequences (3), which was inspired by the technology of CT. In the past several decades, various MRI techniques have been developed and pulsed field gradients have become the preferred way of performing MRI, largely due to the ease of image reconstruction. These sequences scan k-space in a Cartesian grid, and image reconstruction is relatively straightforward by the 2D Fourier transform.

Pulmonary MRI faces some additional challenges though, in its low proton density and short  $T_2^*$  (~ 0.8 ms at 3 T) of lung parenchyma, with the latter being caused by magnetic susceptibility differences at air-tissue interfaces. As a basic and simple sequence, GRE may be used for pulmonary imaging by minimizing its echo time (TE) to sub-ms to reduce signal decay caused by the short  $T_2^*$  of lung parenchyma (4). However, small animal MR scanners typically have higher field (e.g., 4.7-9.4 T) than clinical MR scanners, which further reduces the  $T_2^*$  of lung parenchyma, thus leading to more significant signal decay and lower signal-to-noise ratio (SNR). Therefore many pulmonary imaging studies (5-8) revisited the earliest MRI technique, radial imaging in k-space, to take advantage of its near-zero "echo-time", which is generally less than 0.1 ms. Ultra-short "echo-time" imaging is a term often used here to represent radial imaging, even though there is no traditional echo. In this dissertation, both GRE and UTE were used for pulmonary imaging to visualize the lung structure and measure lung function of mice.

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### **1.3 Lung disease models**

#### **1.3.1** Mouse model of orthotopic vascularized aerated lung transplantation

Lung transplantation (LTx) has gained widespread application to treat end-stage lung diseases in humans (9). Outcomes after LTx are significantly worse than those after transplantation of other solid organs, although improvements in surgical techniques, peri-operative management and refinements in immunosuppression have been made. Graft failure due to chronic cellular rejection is still the major barrier to long-term survival of transplant recipients (10-12). Although the pathogenesis of chronic lung allograft rejection is poorly understood, clinical studies have consistently identified acute cellular rejection (ACR) as a major risk factor (13-15).

Okazaki et al. developed a novel method of orthotopic vascularized aerated left LTx (OLTx) in the mouse utilizing cuff techniques for the anastomoses for pulmonary artery, pulmonary veins and bronchus (16). This technique facilitates hypothesis-driven studies of lung transplant immunobiology in a small animal model that physiologically approximates an aerated, vascularized human lung allograft.

#### Acute cellular rejection in lung Tx

Without immunosuppression, ACR progresses and the allograft become completely consolidated and atelectatic with diffuse hemorrhage and necrosis by post-operative day (POD) 28. Importantly, established ACR in this model is largely T cell mediated and can be ameliorated by treating recipients with T cell depleting antibodies (17). In detail, GK1.5 (anti-CD4 Ab) and YTS169.4 (anti-CD8a Ab) monoclonal anitbodies can be used to deplete CD4- and CD8-specific T cells for immunosuppression.

#### Chronic cellular rejection in lung Tx

Perl et al. recently showed that chronic Clara cell (upper-airway epithelial cell) depletion in the mouse lung results in persistent metaplasia, lack of normal re-epithelialization and peribronchiolar fibrosis, similar to chronic rejection in human lung allografts (18). In this mouse model, the expression of diphtheria toxin A (DT-A) gene initiated by doxycycline in a triple transgenic mouse (termed Scgb1a1/DT-A or DTA hereafter) causes Clara cell depletion and thus chronic injury, similar to viral and environmental exposures experienced by humans.

#### **1.3.2 Pulmonary fibrosis in TGF-α transgenic mice**

Pulmonary fibrosis remains a significant public health burden that contributes to morbidity and mortality and develops with a number of forms including cystic fibrosis, interstitial lung diseases such as idiopathic pulmonary fibrosis (IPF), and some systemic connective tissue diseases. It also occurs in response to many types of lung injuries like the ones induced by radiation or chemotherapeutic drugs (19-23). As a progressive process, pulmonary fibrosis is characterized pathologically by mesenchymal cell proliferation, expansion of the extracellular matrix, and extensive remodeling of the lung parenchyma and airways (19, 22). However, the molecular pathways and cellular mechanisms leading to fibrosis still remain poorly understood. For this purpose, animal models with rodents, particularly mice (24) are critical to understanding the disease. In this project, we used a mouse model of pulmonary fibrosis by overexpressing transforming growth factor (TGF)- $\alpha$  in the epithelium under control of doxycycline (Dox)regulatable Clara cell secretory protein promoter (25). Details of this transgenic mouse model are described in Chapter 10.

### **1.4 Dissertation overview**

An overview of this thesis is described below. Briefly, Chapters 2-7 cover the methods used in this project, while Chapters 8-10 illustrate the application of imaging techniques to lung disease models.

Chapter 2 describes the hardware, i.e., MR scanners and the accessory equipments used in this study. A custom-built double-tuned single-turn solenoid RF coil, a home-built small animal ventilator, and a home-built heating system are introduced for the Agilent (Varian) 4.7 T MR scanner; a home-built quadrature birdcage RF coil, a commercial isoflurane vaporizer for anesthesia and a commercial small animal monitoring system are also introduced for the Bruker 7 T scanner.

Chapter 3 depicts the principle of GRE sequence and the efforts made to minimize TE. Chapters 4-6 explain the details of UTE MRI techniques. Specifically, Chapter 4 recalls the basic UTE sequence, k-space sampling patterns, and the techniques for readout gradient calibration, and details the regridding method for image reconstruction. Chapter 5 introduces the variations of 3D UTE developed in this project, including slab-selected 3D UTE, radial UTE with ellipsoidal k-space coverage, and retrospective self-gating UTE. Chapter 6 discusses the optimization of UTE MRI including the correction of signal build-up and ringing at the beginning of each free induction decay (FID), and the optimization of signal-to-noise ratio (SNR) and resolution.

Chapter 7 begins with brief description of the experimental procedures on the Agilent (Varian) 4.7 T scanner and Bruker 7 T scanner respectively, which include animal anesthesia, animal ventilation, and MR imaging setup. It then ends with data analysis methods, i.e., the normalization of raw data, lung segmentation, MRI-derived lung function parameters, and statistical methods.

Chapter 8 presents the application of the GRE sequence to monitor acute cellular rejection and chronic cellular rejection after lung transplantation in two different disease models respectively. Chapter 9 shows the use of UTE MRI with ellipsoidal UTE k-space coverage in imaging healthy mice as well as the measurement of  $T_1$  and  $T_2$ \* of lung parenchyma. Chapter 10 gives the study of pulmonary fibrosis in a TGF- $\alpha$  transgenic mouse model by retrospective self-gating UTE with ellipsoidal k-space coverage. Measurements in histology, pulmonary mechanics, and biochemistry were also performed for comparison with MR imaging.

Chapter 11 concludes the dissertation by summarizing the accomplishments of this project, discussing the limitations of GRE and UTE <sup>1</sup>H MRI, and giving the directions for future study.

# Chapter 2 Methods: Hardware

# 2.1 Agilent (Varian) 4.7 T MR imaging system and related hardware

#### 2.1.1 MR scanner

An Agilent (Varian) 4.7 T MR scanner which employs a horizontal Oxford Instruments (Abingdon, Oxfordshire, UK) magnet with a 40-cm clear bore diameter was used for part of the experiments. The console is multinuclear and dual channel. The actively shielded Magnex gradient/shim coil assembly of 12 cm inner diameter produces a maximum gradient strength of 60 gauss/cm per axis with a rise time of ~  $300 \mu s$ .

#### 2.1.2 RF coil

Radiofrequency coils (RF coils) are the transmitters and receivers of radiofrequency (RF) signals used in MR system. The transmitters send out RF pulses to generate electromagnetic fields to flip the nuclear spins in the imaged object in an external magnetic field  $B_0$ , while the receivers detect the MR signal in the RF range of tens of MHz emitted by the flipped spins as they precess about  $B_0$  at high frequency (resonance frequency). Of note, to optimize the efficiency of energy transmission/reception, the transmitters and receivers have to be electrically resonant at the nuclear resonance frequency of the nucleus that is to be imaged in  $B_0$ .

Many kinds of RF coils have been invented in the past decades. Basically, they can be grouped into two different classes: volume coils and surface coils. Volume coils have a homogeneous RF excitation across a large volume. The common designs for this type of coils include birdcage coils (26), saddle coils, Helmholtz coils and TEM coils (27). Surface coils can provide a high

localized SNR for reception, but they are poor transmission coils because of the poor RF homogeneity.

A custom-built double-tuned RF coil was used for <sup>1</sup>H imaging in this work (**Figure 2.1**), which was previously used for <sup>3</sup>He imaging (28). This coil is a single-turn solenoid coil and allows <sup>3</sup>He and <sup>1</sup>H imaging on Varian 4.7 T scanner without the need to move the imaged object.



Figure 2.1: Custom-built double-tuned single-turn solenoid RF coil.

The schematic circuit of the coil is shown in **Figure 2.2**. The inductive coupling loop (on the right) is inductively coupled to the resonator, avoiding the electrical connection between the coaxial line to the transmitter/receiver and the resonator.



Figure 2.2: Circuit of an inductively coupled RF resonator, where inductor L<sub>1</sub> detects MR signal directly.

#### 2.1.3 Small animal ventilator

Respiratory motion is a big challenge for lung imaging because it introduces motion artifacts into images. To minimize motion artifacts, a ventilator or breath hold needs to be applied to keep the position of the imaged subject unchanged during imaging.

For pulmonary imaging of small animals, we used a home-built ventilator which was built by Dugas et al. (30) and further optimized by Wei Wang (29). In addition to the external triggering for synchronizing ventilation with imaging provided by most commercial MR-compatible small animal ventilators, the home-built ventilator also rendered us the precise control over the pressure and volume of delivered gas, and more importantly, the breathing cycle.

**Figure 2.3** shows the 3 main parts of the home-built ventilator: electronic controller, eletromechanical system, and breathing valve system. The electronic controller is the "brain" of the ventilator, and a set of timing parameters corresponding to each of the solenoid valves on the electromechanical system are available for setting up, which ultimately determines the respiratory cycle, respiratory waveform, and timing of delivered gas (air, oxygen, <sup>3</sup>He, etc.) and

exhalation. The electromechanical system mainly consists of a series of solenoid valves connected by corresponding gas supply tubes (vacuum and high pressure air). The solenoid valves determine when vacuum/air supply goes to the breathing valve system. The breathing valve system is made of a latex sheet sandwiched between two LEXAN® (GE polycarbonate) halves, with several passageways for <sup>3</sup>He, oxygen, and exhalation (29). Each passageway is pneumatically controlled by vacuum and high pressure air.



**Figure 2.3:** Small animal ventilator. The electronic controller determines the on/off timing of the solenoid valves on the electromechanical system which in turn controls the timing of the breathing valve system via vacuum and high pressure air.

More detailed mechanism and operating description of the home-built ventilator are referred to

Wei Wang's dissertation (29). A respiratory waveform of a balloon (to simulate lung) acquired

by our home-built ventilator is shown in Figure 2.4.



**Figure 2.4:** Respiratory waveform (yellow curve) of a balloon (to simulate lung) acquired by the home-built ventilator. Blue pulses are TTL signal generated by the ventilator to trigger MR scanner.

#### 2.1.4 Heating system

It is important to keep the anesthetized animals warm during imaging, which is accomplished by a heating system. We used a heating system consisting of a heating pad and a hot water recirculating pump. The heating pad is made of a wound thin tube with both ends connected to the hot water recirculating pump. To keep small animals warm, the heating pad (at 32-36 °C) is placed near the RF coil and underneath the plastic holder that holds the RF coil.

### 2.2 Bruker 7 T MR imaging system and related hardware

#### 2.2.1 MR scanner

The Bruker 7 T Biospec 70/30 NMR imaging spectrometer (Bruker BioSpin MRI GmbH,

Ettlingen, Germany) was used and has a 30 cm inner diameter (ID) with a 20.5 cm actively

shielded imaging gradient system (B-GA 20S with RT-shim, 200 mT/m) for conventional

imaging and a 12 cm ID high field gradient (B-GA 12S HP, 400 mT/m) insert for micro imaging. It is a broadband imaging spectrometer operating at a magnetic field of 7.0 Tesla with multinuclear and fast imaging (including EPI) capabilities. ParaVision 5.1 and ParaVision 6.0 were the software packages to control the scanner for data acquisition and carry out image reconstruction.

#### 2.2.2 RF coil

RF birdcage coils are widely used in MRI since they can generate a very homogenous RF magnetic field in the volume of interest (31). In addition, birdcage coils can be used for quadrature excitation and reception that provides better power efficiency in transmit mode and higher MR signal sensitivity in recieve mode than linear excitation and reception (32).

A home-built quadrature birdcage RF coil (**Figure 2.5**) with inner diameter of 35 mm was built by Randy Giaquinto at Cincinnati Children's Hospital Medical Center. This birdcage coil is designed for operating as both a transmitter and receiver with the support of decoupling circuitry, low noise preamplifiers and a transmit-receive switch.



Figure 2.5: Home-built quadrature bird-cage coil.
## 2.2.3 Isoflurane vaporizer

An E-Z Anesthesia isoflurane vaporizer (**Figure 2.6**) was used for anesthetization. The gas flow rate should be no larger than 6 cc/min to avoid overflow of isoflurane in the vaporizer. A Y-shaped connecting tube was added to the input of the vaporizer so that air and oxygen can be mixed with isoflurane simultaneously if necessary.



Figure 2.6: An E-Z anesthesia isoflurane vaporizer. Both flow rate and isoflurane concentration can be precisely controlled.

## 2.2.4 Small animal monitoring system

A commercial small animal MRI-compatible monitoring and gating system (Model 1025) from Small Animal Instruments (Stony Brook, NY, USA) is used, with part of the modules shown in **Figure 2.7**. It allows monitoring core temperature, breathing frequency, etc. The modules used for this thesis include a personal computer (PC), Control/Gating Module, Respiration Module, Fiber Optic Temperature Module, LG monitor, and Heater system.



**Figure 2.7:** Part modules of MR-compatible monitoring and gating system. Other modules including a PC, a Control/Gating Module, and a LG monitor are not shown here.

The relationships between different modules are shown in **Figure 2.8**. The Control/Gating Module is connected to a PC to provide operator control as well as display and storage of waveforms, computed gates, measured values and trends. The Respiration Module measures respiration using a small pneumatic sensor secured on the abdomen of small animals and then sends respiration signal to the Control/Gating Module for Gating calculation. The gating position in the respiratory cycle can be set on the PC by users. After gating calculation, the Control/Gating Module sends out a TTL signal to trigger scanner. The Fiber Optic Temperature Module is equipped with an fiber optic temperature probe, which extends into the magnet bore and is attached to the small animals, to measure the ambient temperature. Temperature signal is sent from Fiber Optic Temperature Module to the Control/Gating Module for real-time temperature display and controlling the Heater Module to keep the ambient temperature of small animals at the value set by users. The Heater system consists of a Fan Module located in the low fringe field of the magnet and a Heater Module with "smart circuitry" located near the bore of the magnet. The Heater Module is RF shielded and non-magnetic. The Fan Module is connected to the Heater Module by a air tube and supplies warm air to small animals via a second air tube. An LG monitor is connected to the PC to display the respiratory waveforms, gating signal, and temperature.





Respiratory waveforms, gating signal, and temperature generate by this small animal monitoring system are shown on the monitor in Figure 2.9.



Figure 2.9: Respiratory waveform (white curve), gating signal (white-red bars on the top), and temperature (orange curve) are displayed on a monitor.

# **Chapter 3 Methods: 2D Gradient Echo Imaging**

Gradient echo or gradient-recalled echo (GRE) sequences are a class of pulse sequences mainly used for fast scanning (33), which is widely used in 3D volume imaging and other applications that require acquisition speed. For example, it is particularly useful in cardiac and lung imaging that require breath-holding. GRE pulse sequences do not have 180° refocusing radiofrequency (RF) pulse which is used in spin echo pulse sequences to form an RF spin echo. It is the gradient reversal in the frequency-encoding direction that forms the echo. A small flip angle (typically less than 90°) of RF excitation pulse can be used in GRE sequences which maintains enough longitudinal magnetization even by short repetition time (TR), i.e., fast scanning. Because there is no 180° refocusing RF pulse to cancel the phase dispersion caused by the static field inhomogeneity, GRE sequences provide susceptibility or T<sub>2</sub>\*-weighted images. Although appropriate  $T_2^*$  weighting increases image contrast (e.g., brain imaging), excessive  $T_2^*$ weighting can cause significant signal loss as in lung imaging. In this thesis, we applied an optimized multi-slice 2D GRE sequence to image mouse lung in vivo. In this chapter, firstly, a brief description of the principle of a basic 2D GRE sequence is given. Secondly, the optimizing modifications of basic GRE sequence for minimizing echo-time (TE) to image mouse lung with short  $T_2^*$  is introduced. Finally, a multislice GRE sampling scheme for murine lung imaging is discussed.

## **3.1 Pulse sequence**

To better understand the principle of GRE pulse sequence, both image space (described by Cartesian coordinate system with x, y, and z axes) and k-space (spatial frequency space, described by Cartesian coordinate system with  $k_x$ ,  $k_y$ , and  $k_z$  axes) are described in the following

sections (34, 35). Image space and k-space comprise a Fourier pair. Any magnetic gradient can cause the traversal of k-space. The complete path of the traversal that a sequence takes through is often referred to as the k-space trajectory. The k-space trajectory can be described by equation

$$\vec{k}(t) = \frac{\gamma}{2\pi} \int_0^t \vec{G}(t') \, dt'$$
[3.1]

where  $\vec{G}(t')$  is magnetic field gradient as a function of time.

The basic 2D GRE pulse sequence is shown in **Figure 3.1** (a). An RF excitation pulse (typically with a sinc envelope corresponding to a boxcar frequency spectrum) is first applied during a constant z-gradient (slice select lobe of the slice select gradient) to selectively excite a specific slice of an imaged object. The location and thickness of the excited slice are determined by the frequency and bandwidth of the RF excitation pulse respectively. After the RF pulse, the rephase lobe of the slice select gradient opposite to the slice select lobe is used to make all the transverse magnetization components within the slice in phase, with a common accumulated phase value of zero. To perfectly rephase the spins in the slice, the area under resphase lobe must equal half of the area under the slice select lobe.



**Figure 3.1:** (a) Diagram of a basic 2D GRE imaging sequence, and (b) corresponding traversal of k-space (red lines) in one repetition. Note that signal acquisition is only performed on the last arrow.

After the slice select gradient, a phase encoding gradient is applied in the y direction to encode the density information in the y direction into k-space. The area under the phase encoding gradient determines how far the k-space trajectory moves from the  $k_y = 0$  along  $k_y$  and varies from TR to TR interval to uniformly sample the k-space along  $k_y$ . The area under the phase encoding gradient can be varied either by varying the phase encoding gradient strength with a constant duration, or by varying the gradient duration with a constant gradient strength. We would like to use the former phase encoding scheme rather than the latter, because the latter has longer TE and TR. There are typically 3 phase encoding orderings: sequential ordering, centric ordering, and reverse centric ordering. By sequential ordering, the phase encoding gradient strength is incremented or decreased by a fixed amount from TR to TR, starting from the negative maximum or positive maximum respectively so that the k-space is sampled in  $k_y$ direction from the most negative ( $k_{PE,min}$ ) or positive ( $k_{PE,max}$ ) point. The centric ordering samples  $k_y = 0$  first and then samples other points outward along  $k_y$ , while the reverse centric ordering samples the outermost points and then samples other points inward along  $k_y$ . Here we used the sequential ordering which is simpler and more common.

After phase encoding, a readout gradient is applied in the x direction to traverse k-space along  $k_x$ . The negative dephasing lobe of the readout gradient is applied before the positive rephasing or read lobe to create an echo in the middle of the read lobe. In k-space as seen in **Figure 3.1** (b), the k-space trajectory (red lines with arrow) moves to  $k_{R,min}$  while the dephasing lobe is on; then the k-space trajectory moves from  $k_{R,min}$  to  $k_{R,max}$  during the read lobe, with the echo formed at  $k_x = 0$ . This traversal path of k-space during read lobe is called a phase encoding line. Data are acquired simultaneously with the read lobe to encode the density information along x axis. This process is also called frequency encoding, which samples one k-space line in each TR interval and the position of line in k-space is determined by the phase encoding. The dash lines in **Figure 3.1** (b) show the entire k-space coverage.

## **3.2 Minimizing TE**

Generally, echo time (TE) of GRE sequence is defined as the duration from the center of sinc RF pulse to the time point when the gradient echo is formed. Because we were to image murine lung which has rather short  $T_2^*$  (5, 36), it is very helpful to minimize signal loss by minimizing the TE of GRE sequence. Below are several efforts made for this purpose.

**3.2.1 Simultaneous slice select rephasing, phase encoding and read dephasing** The superposition principle for the phase of spins states that the phase accumulation for any superposition of contributions to the z-component of the magnetic field is just the sum of the phases for each contribution (37). Thanks to the superposition principle, the rephase lobe of slice select gradient, the phase encoding gradient, and the dephase lobe of readout gradient can be

applied simultaneously. The modified pulse sequence based on the basic 2D GRE imaging sequence is shown in **Figure 3.2** (a) and the resulting TE is largely reduced.



**Figure 3.2:** (a) Diagram of a modified 2D GRE imaging sequence for simultaneous slice select rephasing, phase encoding and read dephasing, and (b) corresponding traversal of k-space (red lines) in one repetition. As a comparison to the traversal of k-space by a basic 2D GRE pulse sequence, the traversal of the modified 2D GRE imaging sequence is shown in **Figure 3.2** (b). The traversal of k-space in  $k_x$ - $k_y$  plane during phase encoding gradient is the vector sum of k-space traversals caused by each of the phase encoding gradient and the dephase lobe of the read gradient if they are applied non-simultaneously. The k-space traversal during read lobe of the readout gradient is the same as that of basic 2D GRE pulse sequence.

## **3.2.2 Partial Fourier imaging**

Assuming the image representing the proton density  $\rho(\vec{r})$  is a real function, there exists a complex conjugate symmetry relation for its Fourier counterpart, the MR signal, which can be expressed by equation (37)

$$s(-\vec{k}) = s^*(\vec{k}) \tag{3.2}$$

Equation [3.2] implies that partial k-space coverage is possible to reconstruct the images for proton density  $\rho(\vec{r})$  as long as at least half of the full k-space coverage in k<sub>x</sub>-k<sub>y</sub> plane (for 2D GRE sequence) is sampled.

In other words, only an asymmetric echo is required for data acquisition. The asymmetric echo can be achieved by decreasing the same amount of area under the dephase lobe and the read lobe of the readout gradient, as shown in **Figure 3.3** (a)



**Figure 3.3:** (a) Diagram for a modified 2D GRE imaging sequence for asymmetric gradient echo sampling, and (b) corresponding traversal of k-space (red lines) in one repetition.

Correspondingly in k-space, the right half plane of k-space is fully sampled and the left plane is partially sampled, as demonstrated in **Figure 3.3** (b). Assuming  $n_+$  and  $n_-$  points ( $n_+ \le n_-$ ) are

sampled along the phase encoding lines falling in the right and left half planes respectively. The echo position is defined as

$$p = \frac{n_{\perp}}{2n_{+}}$$
[3.3]

where  $p \in [0, 0.5]$ , with p = 0 corresponding to a half echo and p = 0.5 a full echo.

A new data set with  $2n_+$  points symmetrically distributed about the echo peak can be created by filling  $(n_+-n_-)$  points in front of the sampled asymmetric echo according to equation [3.2]. This method can be called direct conjugate synthesis. Then the new data set can be used to reconstruct images that well represent the proton density.

However, due to field inhomogeneity and object motion, the image is effectively complex and phase errors appear in the signal. To compensate for the phase errors, more than half of the k-space plane (i.e., p > 0) should be collected. Several phase-constrained reconstruction methods like Homodyne method (38) and POCS (projection onto convex subsets) method (39) were developed to correct the phase errors and create a new data set. Whereas, if the echo position p is no smaller than 0.2, the direct conjugate synthesis method can still avoid possible artifacts (oscillations along frequency encoding direction). In our experiments, p = 0.2 and the direct conjugate synthesis method for reconstruction were used.

## 3.3 Multislice imaging

To cover a 3D volume, the multi-slice 2D approach is utilized by applying a number of RF pulses within a TR interval. A different frequency offset is added to each RF pulse to excite a different slice. Within a TR, each RF pulse is accompanied by the same slice select gradient, phase encoding gradient and readout gradient, therefore the same phase encoding line is sampled

for all slices. In a series of TR intervals, all phase encoding lines for all slices are sampled. TR is not necessarily equal to the sum of the individual scanning time for all slices within a TR interval, for example, in gating acquisition.

Due to the imperfect RF pulses, the immediate neighborhood of an excited slice is also partly excited. In most cases, the  $T_1$  of imaged object is longer than the individual scanning time for each slice. Therefore the partly excited neighborhood doesn't have time to recover toward equilibrium and the signal in each slice is altered, resulting in artifacts. To avoid this problem, a small gap is left between adjacent slices.

In murine lung imaging for this thesis, scanning was respiratory gated and only one phase encoding line was sampled for all slices at end-inspiration or end-expiration in each respiratory cycle. In this case, the TR interval equals the respiratory period, which is usually around 500 ms. The  $T_2$  and  $T_2^*$  of soft tissues and lung are much less than 500 ms at 3 T or higher fields (5, 36, 40), so the transverse magnetization decays to zero by the end of each TR interval. The spoiling is therefore automatically accomplished and no RF spoiling and gradient spoiling are needed. The resulting images are both  $T_1$ - and  $T_2^*$ -weighted.

# Chapter 4 Methods: UTE MRI

## 4.1 Overview of UTE

In UTE sequences, echo time (TE) is defined as the duration from the center of RF pulse to the time point when data acquisition begins. With ultra-short TE (< 0.1 ms), UTE pulse sequences have the advantage of imaging tissues like lung with short  $T_2^*$  (41), which are otherwise undetectable with regular spin-echo or gradient-echo pulse sequences which have echo time typically longer than 1 ms. The earliest MRI pulse sequence was UTE in effect because of its simplicity (3), which was inspired by x-ray computed tomography or CT. In x-ray computed tomography (42), an x-ray beam is attenuated as it passes through the object to be imaged, and the x-ray beam intensity changes (original vs. transmitted) can be characterized by exponential decay using linear attenuation coefficients of the imaged object. After radial data are collected with x-ray beam rotating by a set of angles, images are created by projection reconstruction. Similarly in UTE MRI, the MR free-induction-decay (FID) signal and the object density are a Fourier pair and are radially encoded (radial sampling) by magnetic field gradients in the spatial frequency domain or k-space (34, 35). It is worth noting that other sampling patterns like spiral sampling are also very common in UTE MRI. The MR images can be reconstructed by applying a projection reconstruction algorithm to the non-Cartesian (radial, spiral etc.) data. Currently, the more common and faster image reconstruction method is to resample the non-Cartesian data onto Cartesian coordinates (regridding) and perform a fast Fourier transform.

# 4.2 UTE pulse sequences

The basic components for all MRI pulse sequences are RF excitation pulses and readout gradients to relate frequency and space. Unlike gradient-echo sequence, UTE sequences

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generally don't have phase encoding gradients, thus reducing echo-time. In addition to the ability to detect the signal of short- $T_2^*$  tissues, UTE sequences are also motion "resistant" due to the oversampling at lower space-frequencies or near k-space center. Basically, UTE sequences can be categorized into 2D and 3D UTE sequences.

### **4.2.1 2D UTE pulse sequences**

2D UTE can be realized by applying a slice select gradient simultaneously with an RF excitation pulse followed by a slice refocusing gradient, then readout gradients and data acquisition, as shown in **Figure 4.1**. A spoiler gradient is usually applied after data acquisition to spoil or destroy residual transverse magnetization. Although this pulse sequence may reduce echo time relative to a gradient-echo sequence, the slice refocusing gradient limited the potential for further reduction of echo time.



**Figure 4.1:** 2D UTE applied with slice selection. Spins are selectively excited by a sinc RF pulse combined with a slice selection gradient Gss. Readout gradients ( $G_x$ ,  $G_y$ ) are not necessarily trapezoidal, therefore the resulted trajectory can be radial, spiral, etc.

Pauly et al. first (43) reported a novel method for slice selection as shown in **Figure 4.2**. By this method, they were able to avoid the use of slice refocusing gradient by first collecting data with a positive slice select gradient and then collecting data with a negative slice select gradient. The same readout gradients were applied for both positive and negative slice select gradients. The excitation RF pulse is half of a sinc RF pulse and is used with same amplitude and duration for both positive and negative slice select gradients for both positive slice select gradients. Each of the two data acquisitions covers half of

excitation k-space. When the two acquisitions are added, the resulting signal is effectively inphase and is the same as the signal acquired by a single complete sinc RF excitation pulse. Data sampling can begin as soon as the RF pulse and slice select gradient are ramped down to zero. This method was subsequently further developed and widely applied to imaging short  $T_2$ \* tissues such as lung parenchyma (44), ligament and tendon (45), and cortical bone (46). However the limitations are non-trivial. RF power deposition increases as the reciprocal of pulse duration with a constant flip angle and is a particular problem for this method with multiple short RF pulses. Further, the slice profile is less sharply defined due to the truncation of RF excitation pulse relative to a sinc RF pulse.



**Figure 4.2:** A typical half-sinc RF pulse UTE. The spoiler gradient is not shown explicitly here for simplicity. For radial sampling, each center-out line sampled in k-space is called a radial spoke or projection. The sampling pattern in k-space consists of a series of radial spokes in different directions. The simplest sampling pattern is a set of projections with equal angular-space as shown in **Figure 4.3** (a). The Nyquist criterion requires that the sampling step in radial direction and the largest angular step must not be larger than the reciprocal of the maximum extent of the imaged object. To effectively spoil residual transverse magnetization, a pseudo-random distribution of radial spokes by random numbers in **Figure 4.3** (b) can be used. However,

preferred random radial spokes that are able to sample k-space with more uniformity are created by golden mean as shown in **Figure 4.3** (c). Spiral sampling patterns are also very common in UTE and have been thoroughly investigated by Liao et al. (47). A uniform spiral sampling pattern is shown in **Figure 4.3** (d).



**Figure 4.3:** Common UTE sampling patterns: (a) equal angular-space radial spokes, (b) random-angle radial spokes, (c) golden mean-ordered radial spokes, (d) uniform spirals.

## **4.2.2 3D UTE pulse sequences**

Contrary to the case of 2D UTE, 3D UTE is much easier to realize. The basic diagram of a UTE

pulse sequence is shown in Figure 4.4. After a RF excitation pulse is ramped down to zero, the

data acquisition begins immediately and simultaneously with readout gradients.



**Figure 4.4:** Diagram of 3D UTE pulse sequence. A hard RF pulse is applied to non-selectively excite all spins inside the RF coil. Combination of gradients  $G_x$ ,  $G_y$ , and  $G_z$  determines the sampling pattern of k-space (k-space trajectory). When  $G_x$ ,  $G_y$ , and  $G_z$  are trapezoidal or rectangular, the trajectory consists of a set of radial spokes. ADC (data acquisition) begins simultaneously with readout gradients and immediately after the RF pulse, thus resulting in a very short TE, typically less than 0.1 ms. The spoiler gradient is not shown explicitly here for simplicity.

Similar to the 2D radial UTE, the readout gradients for 3D radial UTE vary with both polar and azimuthal angles to uniformly encode 3D k-space. Two different radial sampling pattern — radial-spiral sampling from Subashi et al. (48) and 2D golden mean sampling modified from Chan et al. (1) — can be used. These sampling techniques define the location of the endpoints of individual spokes on a unit sphere.

The radial-spiral sampling pattern is defined by a series of continuous spiral paths slightly rotated about  $k_z$  axis on the surface of the unit sphere, resulting in a nearly uniform distribution in k-space. This scheme can be described by Eq. [4.1] as below.

$$\begin{cases} \alpha(i,j) = \frac{c\pi}{2} [j + (i-1)M] \\ k_z(i,j) = 1 - \frac{2[j+(i-1)M]}{N} \\ k_x(i,j) = \cos \alpha \sqrt{1 - k_z^2(i,j)} \\ k_x(i,j) = \sin \alpha \sqrt{1 - k_z^2(i,j)} \end{cases}$$

$$\tag{4.1}$$

where j (= 1,2, ..., M) references the j<sup>th</sup> spiral interleave, i (= 1,2, ..., N/M) references the i<sup>th</sup> point of a specific spiral interleave, *M* is the number of interleaves, and *N* is the total number of projections. The constant c in the expression of angle  $\alpha$  is to randomize the order of spokes in the

 $k_x$ - $k_y$  direction and is set to 203 in this study. A representative sampling pattern with only endpoints plotted is shown in **Figure 4.5**.



**Figure 4.5:** Radial-spiral sampling pattern, with end-points of radial spokes only plotted. M = 13 interleaves, N = 1001 projections, and c = 203 were used to generate this sampling pattern.

The 2D golden mean sampling pattern adopts generalized 2D golden means ( $\varphi_1 = 0.4656$  ...,  $\varphi_2 = 0.6823$  ...) to generate well distributed points on a unit square as shown in **Figure 4.6** (a) by taking the successive fractional parts of m $\varphi_1$  and m $\varphi_2$  (denoted by curly brackets) with m = 1,2,3, ..., etc. It is worth noting that the generalized 2D golden mean can be derived from the modified Fibonacci sequence given by Eq. [4.2] (1). However, the random-numbers based method tends to leave areas of sparsely sampled k-space **Figure 4.6** (b).

$$G_i = G_{i-1} + G_{i-3}$$
  
 $G_0 = 0,$   
 $G_1 = 1,$   
 $G_2 = 1$   
[4.2]



**Figure 4.6:** (a) 2D golden-means based points (N = 250) distribution on a unit square is more uniform than (b) random-numbers based points (N = 250) distribution.

The unit square is mapped to a unit sphere to obtain a uniform distribution of samples on the unit sphere. However, with 4-digit precision of 2D golden means, no more than 10000 different points on the unit sphere are generated, which limits the number of spokes available for use. Therefore, it is necessary to increase the precision of 2D golden means to more than 4 digits (8 digits were used in this study) for large numbers of spokes and high resolution. In this work, the mapping relations between the unit square and unit sphere are described by Eq. [4.3],

$$\begin{cases} \alpha = 2\pi \{m\phi_2\} \\ k_z = 2\{m\phi_1\} - 1 \\ k_x = \cos \alpha \sqrt{1 - k_z^2} \\ k_y = \sin \alpha \sqrt{1 - k_z^2} \end{cases}$$

$$[4.3]$$

where  $\{m\phi_1\}$  and  $\{m\phi_2\}$  mean taking the successive fractional parts of  $m\phi_1$  and  $m\phi_2$  respectively, with m = 1, 2, 3, ...

Eq. [4.3] also applies to the mapping between the unit square and unit sphere for random numbers, only if  $\{m\phi_1\}$  and  $\{m\phi_2\}$  are replaced by random numbers. The resulting unit spheres from 2D golden means and random numbers are demonstrated in **Figure 4.7** respectively, with the former much better distributed than the latter.



**Figure 4.7:** (a) 2D golden-means based points (N = 1000) distributed on a unit sphere, (b) random-numbers based points (N = 1000) distributed on a unit sphere.

# 4.3 Gradient calibration

MR images are reconstructed from the discrete samples of the imaged object's distribution in kspace (34, 35). An actual k-space trajectory usually unavoidably deviates from the ideal k-space trajectory specified by the spatial encoding gradients due to readout timing errors, imperfections of gradient amplifiers, and eddy currents induced by gradient pulses. The misregistered k-space sampling causes artifacts in images reconstructed by the ideal k-space trajectory (49-51). Modern MR scanners are able to reduce and compensate for induced eddy currents by using actively shielded gradients and electronic compensation measures (52-55). With these corrections applied, significant k-space trajectory deviations may still exist for fast gradient switching and/or ramping, and complicated non-Cartesian sampling patterns. During gradient switching or ramping, the gradient amplifier may be driven into a non-linear operating range, causing gradient waveform distortions. In addition, image errors caused by readout timing errors tend to get amplified due to high sampling rates.

However, the negative effects of k-space sampling misregistration can be corrected by using the actual k-space trajectories for image reconstruction. Several methods have been suggested to estimate the actual trajectory (51, 56-58). Here we used a novel k-space trajectory measuring technique described by Zhang et al. (59) to measure the actual k-space trajectory for UTE MRI. This technique determines the actual k-space trajectory using phase values of acquired MR signals from a number of excited slices. The correct k-space locus for each readout point is calculated from the corresponding phase difference between acquired MR signals of adjacent slices.

Neglecting spatially varying  $B_0$  field inhomogeneity and relaxation effects and assuming that slice thickness is infinitely thin, the acquired signal from an excited slice normal to the x axis at  $x = x_0$  is:

$$S_0(t) = e^{-jx_0k_x(t) - j\phi(t)} \int \rho(x_0, y, z) e^{-jyk_y(t) - jzk_z(t)} dydz$$
[4.4]

where  $\rho(x_0, y, z)$  is the spin density function over the excited slice at  $x = x_0$ ,  $\phi(t)$  is the accumulated phase shift induced by temporal B<sub>0</sub> field shifts, and  $k_x(t)$  is the k-space trajectory to be measured, which is explicitly encoded in the phase of the acquired data.  $k_y(t)$  and  $k_z(t)$  are the projections of the actual trajectory onto y and z axes respectively, if the readout gradients along y and z axes are nonzero. However, nonzero  $k_y(t)$  and  $k_z(t)$  will lead data sampling into regions where little signal is present due to the phase dispersion to spins caused by gradients along y and z axes. To accurately measure the trajectory  $k_x(t)$ , it is necessary to turn off both the gradients along both y and z axes, therefore significantly increasing SNR.

One way to extract the  $k_x(t)$  trajectory according to Eq. [4.4] is to selectively excite two close slices at  $x = x_1$  and  $x = x_2$  respectively and acquire data. Provided that the slice distance is determined accurately,  $k_x(t)$  can be calculated by the phase differences of acquired signals from the excited slices, explicitly described by Eq. [4.5].

$$k_{x}(t) = \frac{\angle S_{2}(t) - \angle S_{1}(t)}{x_{1} - x_{2}}$$
[4.5]

where slice locations  $x_1$  and  $x_2$  are determined by slice selection, i.e., the slice select gradient and RF excitation pulse;  $\angle$  denotes the phase of MR signal.

For radial UTE imaging, the readout gradient duration is kept constant and the readout gradients is determined by polar and/or azimuthal angles of the projection (spoke) direction, scaling factors and maximum gradient amplitudes along x, y, and z axes. The actual k-space trajectories  $k_{xm}(t)$ ,  $k_{ym}(t)$ , and  $k_{zm}(t)$  corresponding to maximum gradient amplitudes along x, y, and z axes are measured by the trajectory measuring technique described above; the actual k-space trajectories (radial spokes) in other directions are calculated based on the linear relationship between k-space trajectories and readout gradients. This significantly decreases the time for measuring trajectories while not decreasing the accuracy of measurement, considering the high linearity of most modern MR gradient systems.

The representative measured trajectory and ideal trajectory along an arbitrary direction are shown in **Figure 4.8** (a), and their ratio in **Figure 4.8** (b), both indicating significant differences between measured and ideal trajectories.



**Figure 4.8:** (a) Ideal and measured trajectories along an arbitrary direction demonstrate significant differences near the k-space center, and (b) ideal-to-measured trajectory ratio shows the ideal trajectory deviations from the measured trajectory more obviously near the k-space center.

Phantom images reconstructed with measured and ideal trajectories are shown in Figure 4.9 (a)

and (b) respectively, highlighting the image quality improved by measured trajectories.



**Figure 4.9:** (a) Images by ideal trajectory with severe blurring and artifacts, and (b) images by measured trajectory with many fewer artifacts.

## 4.4 Image reconstruction methods

In the standard 2D Fourier MR imaging method (Cartesian trajectories in k-space), voxel position with respect to the two orthogonal directions within the slice is encoded into the phase of the collected data at different instants of time (37). One direction involves phase encoding and the other frequency encoding, being independent of each other so that 2D Fourier inversion operator is separable in the two directions and can be implemented as two independent 1D Fourier inversions. This property enables the fast Fourier transform (FFT) in image reconstruction for the standard 2D Fourier MR imaging. However, in non-Cartesian imaging methods like 2D/3D spiral or radial sampling, all spatial coordinates are encoded at the same time and are dependent on each other. In this case, a 2D/3D Fourier inversion cannot be separated into a set of 1D Fourier inversions, therefore FFT is not applicable. One way to do imaging reconstruction on 2D/3D non-Cartesian data is to apply a filtered back-projection method, which is essentially 2D/3D inverse Fourier transform (37). However, this method is only useful for radial k-space coverage and does not apply to spiral sampling. In addition, the time to process large data by filtered back-projection can be rather long due to the unavailability of FFT. In order to take advantage of the high computational speed afforded by the fast Fourier transform, the data must lie on a Cartesian grid. Many algorithms have been developed for mapping non-Cartesian data onto a Cartesian grid. Such methods include the gradient-descent method (60), reconstruction using coordinate transformation (61), and interpolation methods (62-64). In this dissertation, we use regridding method with a convolution kernel (65) (referred as regridding method in the subsequent context) to implement image reconstruction due to its high efficiency and accuracy, which is applicable to any non-Cartesian data. The non-Cartesian data (mainly radially sampled data in this dissertation) are regridded onto Cartesian coordinates so

that faster Fourier transform similar to Cartesian imaging method can be applied to yield the final image.

#### 4.4.1 Regridding method used for image reconstruction

Data samples are weighted for sampling density and convolved with a finite kernel, then resampled onto a grid preparatory to a fast Fourier transform (65). Gridding for 3D k-space is given by Eq. [4.6]

$$M_{c}(u,v,w) = \{ [(M \bullet S \bullet W) \otimes C] \bullet R \} \otimes^{-1} C$$

$$[4.6]$$

where  $M_c$  is the gridded data, (u, v, w) are k-space coordinates, M is the MR signal as a function of k-space coordinates, S is the sampling function, W is the sampling density compensation function and is defined to be zero everywhere except at the sampling coordinates, C is the convolution function, R defines a Cartesian grid,  $\otimes$  and  $\otimes^{-1}$  are the convolution and deconvolution, respectively. Deapodization or roll-off correction is performed in the image domain and is equivalent to the deconvolution ( $\otimes^{-1}$ ) of the gridded points with the grid kernel C.

In image domain, the Fourier transform of Eq. [4.6] gives Eq. [4.7],

$$m_c(x, y) = \frac{[(m \otimes w) \bullet c] \otimes r}{c}$$
[4.7]

where lower case characters denote the Fourier representations of functions denoted by uppercase characters in Eq. [4.6], and  $S \bullet W = S$  is used. Image m(x, y, z) is spatially bounded within some field of view (FOV) of diameter  $\xi$ . If w(x, y, z) is a delta function within a radius  $\xi$  from the origin, then within the FOV Eq. [4.7] becomes

$$m_c(x, y) = \frac{[m \bullet c] \otimes r}{c}$$
[4.8]

which is ideally expected.

## 4.4.2 Sampling density compensation

One step that is of great significance is weighting the data to compensate for nonuniform sampling density before regridding. The compensation is necessary for accurate interpolation and minimizing reconstruction error due to uneven weighting of k-space. Pipe et al. (66) developed an iterative, numerical method to calculate the sampling density compensation function. To ensure that the weighting and convolution does not affect the image m, i.e., Eq. [4.8] is fulfilled, the Fourier transform of the weighting function W must satisfy Eq. [4.8],

$$w(x, y, z) = \begin{cases} 1, & x = y = z = 0\\ 0, & 0 < \sqrt{x^2 + y^2 + z^2} < \xi \end{cases}$$

$$\tag{4.9}$$

One way given by Pipe et al. (66) to produce a function w(x, y, z) satisfying Eq. [4.9] is to use a function  $\Psi(u, v, w)$  with its Fourier pair  $\psi(x, y, z)$  satisfying

$$\psi(x, y, z) = \begin{cases} >0, & \sqrt{x^2 + y^2 + z^2} < \xi \\ 0, & \sqrt{x^2 + y^2 + z^2} > \xi \end{cases}$$

$$\tag{4.10}$$

to solve

$$w \bullet \psi = \delta(x, y, z) \tag{4.11}$$

Eq. [4.11] can be expressed in k-space as

$$W \bullet (W \otimes \Psi) = W \tag{4.12}$$

The equivalent iterative form for Eq. [4.12] is

$$W_{i+1} = \frac{W_i}{W_i \otimes \Psi}$$
[4.13]

As the denominator approaches unity,  $W_{i+1} = W_{i+1}$ .

The iteration begins with  $W_0 = S$ .

To choose the optimal convolution kernel  $\Psi$  for calculating the sampling density compensation function W, both a direct method and a grid method have been developed (67).

In the direct method, Eq. [4.13] is used directly. The optimal conditioning profile  $\psi$  is constructed by convolving the region of the signal source with the region over which error is to be minimized. Both regions can be represented by the FOV, defined as

$$\phi(x, y, z) = \begin{cases} 1, & \sqrt{x^2 + y^2 + z^2} < \xi/2 \\ 0, & \sqrt{x^2 + y^2 + z^2} > \xi/2 \end{cases}$$
[4.14]

It can be proven that  $\psi = \phi \otimes \phi$  satisfies Eq. [4.10]

The convolution kernel  $\Psi_{direct}$  is the Fourier transform of  $\psi$ , and can be expressed as (68)

$$\Psi_{direct} = \left(\frac{\sin(\pi\xi \,|\,\vec{k}\,|) - \pi\xi \,|\,\vec{k}\,|\cos(\pi\xi \,|\,\vec{k}\,|)}{2\pi^2 \,|\,\vec{k}\,|^3}\right)^2 \tag{4.15}$$

In the grid method, the convolution in the denominator of Eq. [4.13] can be evaluated through a two-stage gridding process as described by

$$W \otimes C_{direct} \approx \{ [(W \otimes C_{grid}) \bullet III] \otimes C_{grid} \} \bullet S$$

$$[4.16]$$

where  $C_{grid}$  is the convolution kernel, and *III* is a temporary grid with a grid resolution of  $1/(2 \cdot \xi)$  to avoid aliasing, as  $c_{grid}$ , the Fourier pair of  $C_{grid}$ , has region of support within a sphere of diameter  $2 \cdot \xi$ .

The grid kernel is designed to have the same net effect as the direct kernel, which in image domain requires

$$c_{grid} = \sqrt{c_{direct}}$$
 [4.17]

where  $c_{grid}$  and  $c_{direct}$  are the Fourier transforms of kernels  $C_{grid}$  and  $C_{direct}$ , respectively.

However,  $C_{grid}$  cannot be fully supported in practical calculation. Truncation is applied to  $C_{grid}$  to leave only the main lobe (cutoff happens at  $|\vec{k}| = 0.97/\xi$ ).

The package for calculating sampling density compensation function described above is attributed to Zwart et al. (69). The MATLAB codes to apply this package to dealing with any 3D k-sampling pattern were developed and can be found in Appendix I. The Representative sampling compensation function along an arbitrary radial spoke is shown in **Figure 4.10**.



Figure 4.10: Sampling density compensation versus distance of sampled points from origin.

Phantom images reconstructed without sampling density compensation have severe blurring in

Figure 4.11 (a), compared to the ones reconstructed with sampling density compensation in





**Figure 4.11:** Axial, coronal, sagittal images of a resolution phantom (filled with deionized water) reconstructed (a) without sampling density compensation, and (b) with sampling density compensation.

### 4.4.3 Convolution kernel for regridding

A detailed description of the regridding algorithm is given by O'Sullivan (70), who shows that the optimal regridding method is convolution with an infinite sinc function, but this is computationally impractical. Jackson (65) compared different convolution functions including two-term cosine, three-term cosine, Gaussian, Kaiser-Bessel window, and prolate spheroidal wave function, with respect to the amplitude and placement of the aliasing side lobes after the image has been corrected for roll-off near the edges of image. The Kaiser-Bessel window is found preferable since it is easily computable and offers near-optimal performance. Kaiser-Bessel window is therefore used as gridding convolution kernel in this dissertation and is expressed as below

$$C(\vec{k}) = \frac{1}{U} I_0(\beta \sqrt{1 - (\frac{2\vec{k}}{U})^2}) \qquad \text{for } |\vec{k}| \le \frac{U}{2}$$
[4.18]

where  $I_0$  is the zero-order modified Bessel function of the first kind, U is the kernel width in grid units,  $\beta$  is the shape parameter, and k-space coordinates  $\vec{k}$  is also in grid units.

Instead of using the typically employed grid oversampling ratio of two, Beatty et al. derived a relationship between minimal grid oversampling ratio  $\alpha$  and shape parameter  $\beta$  as expressed in Eq. [4.19], to reduce computation memory and time for a given kernel width U while maintaining high accuracy.

$$\beta = \pi \sqrt{\frac{U^2}{\alpha^2} (\alpha - 0.5)^2 - 0.8}$$
[4.19]

where  $\alpha$  is the oversampling ratio, defined as the ratio of number of grids and with of image in pixels.

In actual imaging reconstruction, kernel width U = 5 (grid units) and oversampling ratio  $\alpha = 1.5$  are used.

To reduce the computation time further, a presampled kernel is used for gridding. The convolution kernel in Eq. [4.18] is finely sample over the  $|\vec{k}|^2$ , for example  $(\frac{U}{2})^2$  can be divided into 10000 equal segments, and any kernel value located inside the support region of kernel can be well estimated by looking up the presampled values and then using linear interpolation. The package for regridding using the method above is also attributed to Zwart et al. (69). The

MATLAB codes to apply this package to dealing with any 3D data were created and can be found in Appendix II.

# **Chapter 5 Methods: Variations of UTE MRI**

A uniform radial k-space sampling with spherical coverage for 3D UTE gives a spherical (fieldof-view) FOV (71, 72) and isotropic resolution. However, the imaged object (like most animals in medical research) usually has anisotropic dimensions, which requires a big spherical FOV covering the entire body to avoid fold-in artifacts. This forces us to choose between long acquisition time and relatively low resolution, or equivalently, leads to sampling redundancy and unnecessarily long scan time. To address this problem, Larson et al. (73) proposed a method of matching the fully sampled trajectories (often with varying angular density) to the desired anisotropic FOV shape, resulting in scan time reductions without aliasing artifacts introduced. However, this method is not easily combined with other view orderings like the golden-angle (74), which is an important acquisition scheme for dynamic imaging in need of a relatively similar angular distribution for any time interval.

We suggest several variations of UTE pulse sequence to avoid sampling redundancy or increase resolution without changing the scanning time. This chapter first introduces one variation of radial UTE, i.e., a slab selected UTE, which selectively excites a slab covering the region of interest by replacing the hard RF pulse, common for UTE, with a sinc RF pulse, and simultaneously adding slab selective gradients. Second, this chapter describes a novel technique that achieves both anisotropic (increased) resolution and ellipsoidal FOV that better matches the object being imaged, by expanding the fully sampled spherical k-space volume to an ellipsoidal k-space volume with varying spoke length and angular density for the same number of projections. In addition to the variations of UTE for avoiding sampling redundancy, finally, a retrospective self-gating UTE technique is introduced for *in vivo* lung imaging.

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## 5.1 Slab-selected 3D UTE

Half-sinc RF pulse UTE for slab selection is advantageous to reduce TE, but the half-sinc RF pulse is difficult to incorporate into the pulse sequence. For simplicity, a sinc RF pulse is used for slab selected UTE method in this dissertation which obtains TE smaller than 0.5 ms. Similar to the slice selected 2D UTE described in Chapter 4, slab selected 3D UTE can be realized by applying a slab select gradient simultaneously with a RF excitation pulse followed by a slice refocusing gradient, then readout gradients and data acquisition, as shown in **Figure 5.1**. A spoiler gradient is usually applied after data acquisition to spoil residual transverse magnetization. The increased thickness of slab in 3D UTE relative to that of slice in 2D UTE provides the potential to reduce TE further. It is worth noting that the FOV and slab thickness are set separately in the pulse sequence. The spherical FOV by uniform radial sampling with spherical k-space coverage should be set large enough to cover the entire selectively excited slab to avoid aliasing artifacts. Specifically, if the selected slab is located in the isocenter of magnet, the diameter of spherical FOV should be larger than the maximum dimension size of the slab, which is estimated by localizer imaging.



**Figure 5.1:** 3D UTE applied with slab selection. Spins are selectively excited by a sinc RF pulse combined with a slab selection gradient Gss. Readout gradients  $(G_x, G_y, G_z)$  are not necessarily trapezoidal, therefore the resulted trajectory can be radial, spiral, etc. ADC (analog-to-digital conversion) denotes data acquisition. Spoiler gradients are not shown in the figure for simplicity.

A representative axial image of mouse lung in **Figure 5.2**, which was acquired by slab-selected UTE, shows no fold-in aliasing artifacts, compared to the one in **Figure 5.2**, which was acquired by regular UTE. Both UTE acquisitions have the same imaging parameters except the addition of slab selection module. Obviously, slab-selected UTE demonstrates its advantage of fitting FOV to the imaged object tightly thus increasing resolution or decreasing scanning time for the same resolution.



**Figure 5.2:** Representative axial images of mouse lung acquired by UTE (a) with slab selection, and (b) without slab selection, respectively. Aliasing artifacts are obvious in (b).

# 5.2 Radial UTE with ellipsoidal k-space coverage

Unlike slab selected UTE, radial sampling UTE with ellipsoidal k-space coverage has the same advantage as regular UTE in reducing TE. First, this section shows how to expand the fully sampled spherical k-space volume to an ellipsoidal k-space volume, with varying spoke length and angular density for the same number of projections, to achieve both anisotropic (increased) resolution and ellipsoidal FOV that better matches the object being imaged. Secondly, the ellipsoidal k-space coverage is combined with golden mean sampling as described in subsection 4.2.2, to which the point spread function (PSF) analysis by numerical calculation is applied to demonstrate the ellipsoidal FOV and anisotropic resolution. FOV reduction and anisotropic resolution are also shown by increasing k-space expansions.

### 5.2.1 k-Space expansion

Larson et al. (73) have shown that a varying angular-density sampling in 2D radial UTE leads to a non-circular FOV. Similarly in 3D radial UTE, the uniform spherical k-space coverage can be deformed into any shape of k-space coverage by expansion or compression of a Nyquist-sampled sphere in arbitrary directions to give any shape of the FOV that fully covers the object. In this dissertation, an ellipsoidal k-space coverage was used, for simplicity in imaging.

The expansion of  $k_i$  by  $\alpha_i$  can be described as  $k_{i,ellipse} = \alpha_i k_{i,sphere}$ , where  $\alpha_i$  measures the ratio of expansion with i = x, y, z. For simplicity and without loss of generality, we study ellipsoidal k-space coverage from expansions in the  $k_x$  and  $k_y$  directions by factors  $\alpha_x$  and  $\alpha_y$ , respectively. In the case that  $\alpha_x, \alpha_y > 1, \alpha_x \neq \alpha_y$ , the spherical k-space coverage is reshaped into a tri-axial (scalene) ellipsoid, resulting in a FOV in shape of an altered tri-axial ellipsoid as well.

In the more specific case, if the spherical k-space coverage is isotropically expanded in the  $k_x$ - $k_y$  plane ( $\alpha_x = \alpha_y > 1$ ), the spherical k-space coverage is reshaped into an oblate ellipsoid of revolution with short axis parallel to the  $k_z$  axis, resulting in a FOV shape of a prolate ellipsoid of revolution with long axis parallel to the *z* axis, with care taken to ensure full coverage of the object by the ellipsoidal FOV. The FOV in the *x*-*y* plane decreases by a ratio of  $\alpha_x$  accordingly, and the resolution in the *x*-*y* plane is enhanced correspondingly by the same ratio. A  $k_z$ - $k_x$  view of the sampling patterns in **Figure 5.3** shows the evolution of an ellipsoidal k-space coverage from a spherical one when  $\alpha_x = \alpha_y = 2$ .



**Figure 5.3:** The  $k_z$ - $k_x$  view of a spherical k-space coverage (left) which is isotropically expanded by a factor of 2 in the  $k_x$ - $k_y$  plane into an ellipsoidal k-space coverage (right).

### **5.2.2 PSF analysis over FOV and resolution**

The FOV and resolution given by a particular k-space sampling pattern are characterized by the point spread function in image space. The PSF (the Fourier transform of the sampling pattern) is convolved with the imaged object and results in the output image. The central peak of the PSF with finite width introduces blurring, thus determining the true resolution of the resulting image, while the aliasing peaks outside the central peak determine the FOV by the distance between them.

PSFs of the golden means sampling pattern (combined with ellipsoidal k-space coverage) were calculated by gridding data of all ones in MATLAB (MathWorks, Inc., Natick, MA) by the regridding method described in chapter 4 for image reconstruction. 32 points were sampled on each of 12868 projections and 4 pairs of expansion factors ( $\alpha_x = \alpha_y = 1, 2, 4; \alpha_x = 2, \alpha_y = 4$ ) were used to investigate the influence of k-space expansion on FOV and resolution. All 4 k-space sampling patterns were rescaled by the same factor before gridding in order to obtain the same voxel size in image space and a grid oversampling ratio of 8 was used to show the full aliasing lobes.

The cross sections of the PSF of the sampling pattern at z = 0, y = 0, and x = 0 in Figure 5.4 demonstrate the influence of k-space expansion on FOV. The separation between main peak and
aliasing peak along x or y axis is FOV<sub>z</sub>, FOV<sub>z</sub>/2 and FOV<sub>z</sub>/4 for k-space expansion factors  $\alpha_x = \alpha_y = 1, 2, 4$  respectively. Similarly, the separations between main peak and aliasing peak are FOV<sub>z</sub>/2 and FOV<sub>z</sub>/4 along the x and y axes respectively for  $\alpha_x = 2, \alpha_y = 4$ .



**Figure 5.4:** PSF cross sections at z = 0 (Axial), y = 0 (Coronal), and x = 0 (Sagittal) for expansion factors (a)  $\alpha_x = \alpha_y = \alpha_z = 1$ , (b)  $\alpha_x = \alpha_y = 2$ ,  $\alpha_z = 1$ , (c)  $\alpha_x = \alpha_y = 4$ ,  $\alpha_z = 1$ , and (d)  $\alpha_x = 2$ ,  $\alpha_y = 4$ ,  $\alpha_z = 1$  respectively. Separation between the aliasing peaks (pointed by the white arrows) and the main peaks (bright regions in the center) equals the FOV. The separation between the main peak and aliasing peak along the *z* axis equals the FOV<sub>z</sub> for all expansion factors (with  $\alpha_z = 1$ ), while the separation equals FOV<sub>z</sub>/ $\alpha_x$  and FOV<sub>z</sub>/ $\alpha_y$  along the *x* and *y* axes respectively.

The PSF main peaks (measure of true resolution) along x, y or z axes are shown in Figure 5.5.

The full widths at half maximum (FWHM) of the PSFs along the z axis are almost equal for

different expansion factors (6.4 pixels, 6.4 pixels, 6.9 pixels and 6.6 pixels for  $\alpha_x = \alpha_y = 1, 2, 4$ ;

 $\alpha_x = 2$ ,  $\alpha_y = 4$  respectively). The FWHMs of the PSFs along *x* and *y* axes are 6.2, 3.3, and 1.8 pixels, for  $\alpha_x = \alpha_y = 1$ , 2, 4 respectively, while the FWHMs of the PSF for  $\alpha_x = 2$ ,  $\alpha_y = 4$  are 3.5 pixels and 1.7 pixels along *x* and *y* axes, respectively. The FWHMs of the PSF conclude that the resolution improvement in the expansion direction is proportional to the expansion factor while the resolution in the direction without k-space expansion is slightly reduced. In addition, resolution in other directions is improved to the extent between the non-expansion direction and the expansion direction (not shown here).



**Figure 5.5:** Normalized PSFs along (a) *z* axis, (b) *x* axis and (c) *y* axis. FWHMs along the *z* axis are almost equal: 6.4, 6.4, 6.9, and 6.6 pixels for  $(\alpha_x, \alpha_y) = (1, 1), (2, 2), (4, 4), \text{ and } (2, 4)$  respectively, with  $\alpha_z = 1$  in all cases. FWHMs along the *x* and *y* axes are 6.2, 3.3 and 1.8 pixels for  $(\alpha_x, \alpha_y) = (1, 1), (2, 2), \text{ and } (4, 4)$  respectively, while FWHMs are 3.5 and 1.7 pixels along the *x* and *y* axes respectively for  $(\alpha_x, \alpha_y) = (2, 4)$ .

#### 5.2.3 Imaging resolution phantom

To demonstrate the resolution enhancement by the ellipsoidal k-space coverage and verify the

PSF analysis of FOV and resolution, a commercial resolution phantom (High Precision Devices,

Inc., Boulder, CO) filled with deionized water was imaged by the golden means sampling with  $\alpha_x$ 

 $= \alpha_y = 1$  (spherical k-space coverage) and  $\alpha_x = \alpha_y = 3$  (ellipsoidal k-space coverage) respectively.

64 points were sampled on each of 51480 projections with TR = 10 ms, TE = 0.075 ms,  $FA = 5^{\circ}$ ,

receiver bandwidth = 100 kHz, FOV = 20 mm × 20 mm × 60 mm for  $\alpha_x = \alpha_y = 3$ , and 60 mm ×

60 mm × 60 mm for  $\alpha_x = \alpha_y = 1$ . The pore size ranges from 0.4 mm to 0.8 mm, while the inter-

pore spacing ranges from 0.8 mm to 1.6 mm.

The resolution phantom images by UTE with expansion factors  $a_x = a_y = 1$  and  $a_x = a_y = 3$  are shown in **Figure 5.6**; dashed lines depict the FOV. There is obvious blurring in the *x* and *y* directions in **Figure 5.6** (a) left and middle columns, relative to the counterparts in **Figure 5.6**. In addition to the blurring around the small structures in the expansion plane (*x*-*y* plane) in **Figure 5.6**, weak blurring was also seen in the non-expansion direction (*z* axis), because the structure blocks were not exactly parallel to the *x* axis. Signal intensity at both ends of the phantom is lower than in the middle because of the RF excitation inhomogeneity. The SNR in the bright region is 320.6 and 38.8 for  $a_x = a_y = 1$  and  $a_x = a_y = 3$  respectively, consistent with the corresponding voxel-volume ratio of 9. In conclusion, resolution phantom imaging confirms the PSF analysis in terms of the influence of k-space expansion on the FOV and resolution.



**Figure 5.6:** Representative axial (z = 0), coronal (y = 0) and sagittal (x = 0) slices of resolution phantom (filled with deionized water; pore diameter = 0.8-0.4 mm and inter-pore spacing = 1.6-0.8 mm from top to bottom) imaged by UTE with k-space expansion factors (a)  $\alpha_x = \alpha_y = \alpha_z = 1$ , and (b)  $\alpha_x = \alpha_y = 3$ ,  $\alpha_z = 1$ . Imaging parameters are TR = 10 ms, TE = 0.21 ms, FA = 5°, FOV = 20 mm × 20 mm × 60 mm (in *x*, *y*, and *z* directions) in (b), and matrix = 128 × 128 × 128. The dashed lines depict the FOV in (b), while the FOV = 60 mm × 60 mm × 60 mm is larger in each image in (a). Severe blurring appears in (a) rather than (b).

# **5.3 Retrospective self-gating imaging**

#### **5.3.1** Overview and theory

In in vivo imaging, motion due to respiration is one of the major difficulties in data acquisition. This problem is particularly pronounced in mice, which have respiratory rate 10-20 times higher than humans. To compensate for respiratory motion *in vivo*, MR acquisitions are usually synchronized with the respiratory cycle using either ventilator or prospective respiratory gating.

In many cases spontaneous breathing is advantageous to avoid ventilator-induced lung injury or for physiological measurement. Prospective gating errors result from abnormal respiratory waveforms caused by body motion. In addition, ventilator gating or respiratory gating disrupts the steady state. To overcome these problems, several self-gating (SG) methods have been developed that extract the motion data used for gating directly from the acquired MR data (75-81). Most SG methods are derived from the integral of the imaged object, that is, the signal sampled at the center of k-space, which is also used for retrospective gating technique in this dissertation.

In traditional Cartesian imaging, retrospective gating typically requires that specialized sequences be employed. However, the situation is different in center-out radial encoding because respiratory motion slightly alters the local field experienced by spins within the imaged animals, providing a detectable signal from the leading point of free induction decay (FID) which reflects the respiratory motion. It is also argued that signal variations caused by respiration are induced by moving structures, such as lung, liver, and abdomen, that change their positions inside sensitive imaging volume over time during each respiratory cycle (81). The signal amplitude of each FID (i.e., at k = 0) can be used for retrospective "self"-gating. Even though the phase of signal also reflects respiratory motion, it's not used for self-gating in this dissertation due to the

higher noise. Here we demonstrate this retrospective "self"-gating technique by imaging mouse lung. Sufficient angular oversampling of k-space is necessary to ensure the retrospective registered data fulfill the Nyquist criterion.

#### 5.3.2 In vivo imaging

Imaging was performed using a Bruker 7 T scanner on a free-breathing 7-week old C57BL/6J mouse. The mouse was anesthetized with isoflurane and placed supine inside a quadrature birdcage coil (length: 50 mm, inner diameter: 35 mm). In addition, a pressure sensor of a small animal monitoring system (SA Instruments, Inc.) was taped on the mouse stomach to monitor the respiratory motion.



**Figure 5.7:** Diagram of slab-selected UTE pulse sequence for self-gating imaging. Lobe A denotes the readout gradient. Lobe B denotes a gradient negating readout gradient and Lobe C a strong spoiler gradient for gradient spoiling. A linear phase increment of 117° x RF pulse number was added to each RF pulse for RF spoiling.

Spherical k-space coverage was used for 3D UTE MRI with 2D golden means determining the azimuthal and polar angles of the endpoint of each radial spoke (1). The RF excitation pulse was applied with a slab selection gradient to focus the field-of-view (FOV) on the lung, with data acquisition starting immediately when the readout gradient was ramped after a slab refocusing

gradient, as shown in **Figure 5.7**. A linear phase increment of 117° x RF pulse number added to each RF pulse was combined with a negating readout gradient and a strong spoiler gradient for spoiling (82).

Total acquisition time was 12 minutes with TR = 6 ms, TE = 0.63 ms, FA = 5°, FOV =  $(30 \text{ mm})^3$ , voxel =  $(0.23 \text{ mm})^3$ , 64 points along each projection and 2-fold angular oversampling (2 • 51480 projections). The respiratory rate of mouse was changed by adjusting isoflurane level to investigate the period of the FID signal amplitude and phase as a function of projection number.

FID signal amplitude as a function of projection number at 3 different monitored respiratory rates (~110/min, 80/min, and 60/min) is shown in Figure 5.8 (a), with peaks and troughs corresponding to expiration and inspiration respectively. FID phase as a function of projection number is shown in Figure 5.8 (b), with peaks and troughs corresponding to inspiration and expiration respectively. Percentage difference, which is defined as the peak-trough difference divided by the mean of peak and trough values, is estimated around 10% and 5% for FID signal amplitude and phase respectively, indicating FID signal amplitude is a better choice for data registration. The respiratory rates estimated from the amplitude and phase plots are 109/min, 75/min and 64/min respectively, consistent with external monitoring.

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**Figure 5.8:** (a) FID signal amplitude as a function of projection number at a series of respiratory rates (~110/min, 80/min, and 60/min, read from small animal monitoring system). Peaks and troughs correspond to expiration and inspiration respectively. (b) FID phase (phase of the leading FID point) as a function of projection number. Peaks and troughs correspond to inspiration and expiration respectively.

#### 5.3.3 Retrospective registration

FID signal amplitude as a function of projection number was smoothed by a moving-average

filter and the resulting smoothed first- and second-order derivatives were combined to extract data at expiration and inspiration respectively, as shown in Figure 5.9. The MATLAB codes for retrospective registration can be found in Appendix III. Direct thresholding the amplitude plot can mix the data at slopes into the data at expiration or inspiration, thus was not used.



Figure 5.9: First- and second-order derivatives were combined to extract projections for expiration and inspiration.

The projections are registered for end-expiration and end-inspiration respectively, as shown in



Figure 5.10.

**Figure 5.10:** Projections extracted for end-expiration and end-inspiration, marked by red and blue lines respectively. The retrospective self-gated radial data were resampled onto a Cartesian grid by convolving with a Kaiser-Bessel window (65) before Fourier transform (Gridding details are described in Chapter 4). Tidal volume was calculated by measuring the lung volume difference between the expiration and inspiration images. Lung parenchyma SNR was defined as the lung parenchyma signal divided by the standard deviation in the background.

The resulting coronal and sagittal images are shown in **Figure 5.11**. The red lines emphasize the diaphragm displacement between expiration and inspiration. Lung volume measured at

inspiration and expiration is 0.55 ml and 0.44 ml respectively, resulting in 0.11 ml of tidal volume, consistent with previous plethysmographic measurements in (non-anesthetized) C57BL/6J mice of 0.16 ml (range 0.13-0.21 ml) (83). The lung parenchyma SNR at inspiration (11.2) is lower than that at expiration (21.2) because of bulk density decrease, motion artifacts, and projection undersampling. The expiration/inspiration signal-intensity ratio in lung parenchyma is 1.2, consistent with the inspiration/expiration lung-volume ratio of 1.25, assuming similar  $T_2$ \*.



**Figure 5.11:** Example lung images reconstructed from data extracted at expiration and inspiration. TR = 6 ms, TE = 0.63 ms, and voxel =  $(0.23 \text{ mm})^3$ . The red lines demonstrate the diaphragm displacement between expiration and inspiration.

## **5.3.4 Miscellaneous**

Although the pulse sequence used for the demonstration of self-gating technique here is UTE with slab selection, UTE with ellipsoidal k-space coverage is preferrable for self-gating in imaging applications due to the shorter TE.

# **Chapter 6 Optimization of UTE MRI**

Although UTE MRI has the advantage of being able to detect short  $T_2^*$  materials, it still faces some challenges that must be overcome to optimize imaging. Non-linearities of magnetic field gradients lead to inaccurate theoretical k-space trajectories, which is corrected for by trajectory measurement (59) as described in Chapter 4. Additionally, the signal build-up (or transient response) due to digital filtering (84, 85) during data acquisition and  $T_2^*$  blurring further degrade image quality if not properly corrected for.

# 6.1 FID distortion

Most modern MR scanners are equipped with nearly perfect digital circuits for signal processing, which allows the use of fully digital transceivers (85). Implemented in receivers is an on-line digital decimation finite impulse response (FIR) audio filter, which has a nearly ideal frequency response, resulting in a very large constant group delay and significant ringing in the transient response. The group delay in FID signals caused by digital filters has been easily handled by most MR scanners, however, signal distortion at the leading FID points due to transient responses of FIR filter and/or hardware warm-up is not well solved, especially in Bruker and GE scanners, causing image-quality degradation.

Representative distorted FIDs in radial scan acquired at different receiver bandwidths (SW\_h) by the Bruker scanner are shown in **Figure 6.1**. The signal build-up and ringing in the leading points obviously deviates from the standard decayed FID signal. In addition, the number of build-up and ringing points increases with receiver bandwidth.



**Figure 6.1:** FIDs acquired at various receiver bandwidth ranging from 51 kHz to 208 kHz. Signal build-up and ringing appear at more than 10 leading points when  $SW_h = 125$  kHz and 208 kHz; while signal build-up and ringing appear at less than 10 leading points when  $SW_h = 51$  kHz, 70 kHz, and 90 kHz.

Therefore, one intuitive resolution to this problem is to shift data acquisition ahead of the encoding readout gradient (see **Figure 6.2**) and then cut off the leading to-be-distorted points. Extra data points (same as the number of cut-off points) are sampled to compensate for the data acquisition shift. In this dissertation, 20 points are shifted in data acquisition for imaging.



**Figure 6.2:** Pulse sequence diagram for 3D radial encoding. A hard RF pulse (~  $4.3 \mu$ s) is used for non-selective spin excitation and a linear phase increment (n x 117°) is added to RF pulse for RF spoiling. Following a short delay  $\delta$  (~  $8 \mu$ s), data acquisition starts before ramping the readout gradient. The readout gradient (lobe A) is delayed slightly by T<sub>acqshift</sub> with direction (and magnitude) being changed between repetitions. Gradient lobe B is used to negate the effects of lobe A on spins so that a constant large spoiler gradient C, combined with RF phase increment, effectively spoils the transverse magnetization after each data acquisition.

Phantom images acquired with and without the acquisition shift are shown in Figure 6.3. The

uncorrected distortion in the FID signal causes severe blurring around the defect, indicated by

red arrows in Figure 6.3.



**Figure 6.3:** Phantom (copper-sulfate doped water) images (a) with data acquisition shift, and (b) without data acquisition shift. The defect indicated by a red arrow in (a) is severely blurred in (b) due to the uncorrected distortion in the FID signal.

# **6.2 Short T<sub>2</sub>\* relaxation effects**

In MR imaging, the short  $T_2^*$  of lung can significantly decrease the actual resolution and SNR of images. If the readout-gradient encoded FID signal is weighted by short  $T_2^*$  relaxation effects, the resulting images can show severe blurring artifacts and low SNR at long acquisition times. To minimize the blurring caused by  $T_2^*$  decay, the acquisition time should be shortened as much as possible, which will also decrease the  $T_2^*$  effects on SNR. However, for a given number of samples per radial scan, the SNR is proportional to the square root of the acquisition time or readout duration (86). Therefore, with contrary effects of  $T_2^*$  decay and short acquisition time on SNR, there is an optimal acquisition time that maximizes SNR for materials of a given  $T_2^*$ .

#### 6.2.1 PSF analysis

The PSFs of the ellipsoidal k-space coverage (generated by 2D golden mean, with  $\alpha_x = \alpha_y = 2$ and 64 points sampled on each of 51480 projections) under influence of T<sub>2</sub>\* decay were calculated to investigate the dependence of single-voxel SNR and blurring on T<sub>2</sub>\* decay. For both PSF calculations and actual experiments, the minimum gradient ramp time of 0.12 ms was used. The dependence of single-voxel SNR on acquisition time T<sub>acq</sub> and T<sub>2</sub>\* is obtained from the PSF amplitude weighted by the square root of the readout duration, i.e., SNR  $\propto \sqrt{T_{acq}} \cdot PSF(0)$ . The resolution is characterized by the full width at half maximum (FWHM) of the PSF.

The normalized SNR as a function of acquisition time  $T_{acq}$  is given in **Figure 6.4** (a). By numerical calculation, we obtain the maximum at  $T_{acq} = 0.68 T_2^*$ , close to the result of spherical coverage (0.69  $T_2^*$ ) by Rahmer et al. (87). The FWHM as a function of acquisition time  $T_{acq}$  is shown in **Figure 6.4** (b), and increases with  $T_{acq}$  due to  $T_2^*$  blurring. FWHM along the *z* axis is twice the value along the *x* or *y* axis at all  $T_{acq}$ , consistent with  $\alpha_x = \alpha_y = 2$ ,  $\alpha_z = 1$ .



**Figure 6.4:** SNR and FWHM of PSF ( $T_2^* = 1$  ms) when  $\alpha_x = \alpha_y = 2$ ,  $\alpha_z = 1$ . (a) Normalized SNR as a function of sampling duration  $T_{acq}$  in units of  $T_2^*$ , which is proportional to  $\sqrt{T_{acq}} \bullet PSF(0)$ . (b) PSF linewidth (FWHM) as a function of  $T_{acq}/T_2^*$  along *x*, *y*, and *z* axes

#### 6.2.2 Phantom imaging

To verify the theoretical analysis of blurring caused by the  $T_2^*$  decay, a phantom made of 2-mmthick rubber rolled like a rug was imaged by UTE with a golden-mean sampling pattern and a kspace expansion factor of 2 at one short readout duration and one long readout duration respectively by changing the receiver bandwidth (500 kHz vs. 90 kHz) and readout-gradient strength. 100 points were sampled on each of 125664 projections with TR/TE (ms) = 10/0.075, FA = 20°, and FOV = 30 mm × 30 mm × 60 mm ( $\alpha_x = \alpha_y = 2$ ). Images of the rubber phantom were acquired by UTE with multiple TEs to measure  $T_2^*$  before carrying out the variable readout-duration imaging.

 $T_2^*$  of the rubber phantom was measured to be around 0.6 ms. The images acquired by UTE at two different readout durations ( $T_{acq} = 0.43 T_2^*$ , 1.96  $T_2^*$ ) when  $\alpha_x = \alpha_y = 2$ ,  $\alpha_z = 1$  are shown in Figure 5. Severe blurring was observed in **Figure 6.5** acquired at the long readout-duration which was caused by  $T_2^*$  decay, but no obvious blurring was observed in the image acquired at the short readout duration in **Figure 6.5**.



**Figure 6.5:** Axial slices of a rubber phantom imaged by golden means based UTE at (a)  $T_{acq} = 0.43 T_2^*$  and (b)  $T_{acq} = 1.96 T_2^*$  with slice thickness = 0.3 mm and in-plane resolution = 0.15 mm × 0.15 mm. No blurring was observed in images acquired at  $T_{acq} = 0.43 T_2^*$ , but severe blurring was observed in images acquired at  $T_{acq} = 1.96 T_2^*$ .

# Chapter 7 Experimental procedures and Data analysis

All imaging experiments in this dissertation were carried out either on an Agilent (Varian) 4.7 T scanner and related ventilation system, or on a Bruker 7 T scanner and related system. The experimental procedures for both scanners are described below respectively.

# 7.1 Procedures on the Agilent (Varian) 4.7 T scanner

#### 7.1.1 Anesthesia

Before imaging, the mice were anesthetized briefly by intraperitoneal injection of a mixture of 80-100 mg/kg ketamine and 5-10 mg/kg xylazine so that anesthesia time was enough for oral intubation with a 20-gauge angiocatheter and attaching mice to the home-built ventilator. Details for intubation are in Wei Wang's dissertation (29).

During imaging, a mixture of pure oxygen and 1.5%-2% isoflurane was supplied to the homebuilt ventilator to anesthetize the mice and reduce motion artifacts in images. The mixing of oxygen and isoflurane was completed in a commercial isoflurane vaporizer.

## 7.1.2 Ventilation

After intubation, the mice were placed supine inside a solenoidal coil and then connected to the breathing valve system of the home-built ventilator for imaging. During imaging, the mice were breathing passively, with breathing pattern controlled by the ventilator. Gas flow and airway pressure (at the breathing valve) were closely monitored by flow meters and an electronic pressure transducer (Honeywell, Model No. 24PCAFA6D), respectively. Airway pressure waveforms were monitored in real time by oscilloscopes in the control room before, during, and after imaging.

#### 7.1.3 MR imaging

A custom-built solenoid coil covered the entire lungs with high transverse homogeneity. A trigger signal from the ventilator allowed the scanner to begin the short (gated) data acquisition at peak inspiration ("high pressure") or end expiration ("low pressure"). Rapid <sup>1</sup>H scout imaging by a standard 2D multi-slice gradient-echo sequence was carried out at the very beginning of every study. The scout protocol acquired 3 slices in each of three cardinal planes: axial, coronal, and sagittal. The scout images were used to estimate the location of the imaged object to adjust size and location of field-of-view (FOV) to fit the imaged object in the subsequent normal scan. For normal imaging, a standard 2D multi-slice gradient-echo sequence with an radiofrequency (RF) excitation pulse with an appropriate flip angle was used, and multiple transverse/axial slices were acquired to cover the whole mouse lung. This respiratory-triggered scanning minimized motion artifacts and allowed multiple sets of images to be acquired (high and low pressures, multiple averages).

# 7.2 Procedures on the Bruker 7 T scanner

#### 7.2.1 Anesthesia

The mice were anesthetized with 2%-3% isoflurane (up to 5%) for imaging. Before imaging, the mice were settled down by being placed in a plastic box filled with 5% isoflurane for approximately 1 minute. If necessary, the mice were instead anesthetized by intraperitoneal injection of a mixture of 80-100 mg/kg ketamine and 5-10 mg/kg xylazine. The mixing of isoflurane with air and oxygen was also completed in a similar isoflurane vaporizer as mentioned in previous section, with the flow rate kept below 6 cc/min due to the limitation of the isoflurane vaporizer.

#### 7.2.2 Ventilation

After anesthetization the mice were placed supine for free breathing with the lung in the center of an RF coil during imaging. A small-animal monitoring system (SA Instruments, Inc., Stony Brook, NY) as described in subsection 2.2.4 was used to monitor the respiratory rate and body temperature and provided a TTL signal for respiratory gating at end-expiration/inspiration. A heating system controlled by the small-animal monitoring system kept the ambient temperature of mice around 36 °C for stable respiration.

#### 7.2.3 MR imaging

On the Bruker 7 T scanner, a localizer protocol (modified from a standard 2D multi-slice gradient-echo pulse sequence) was carried out at the beginning of each study to estimate the position of the imaged object to adjust the position and size of FOV to fit the imaged object. The localizer also provided location information to make sure the region of interest in the imaged object was located in the isocenter of magnet.

Before imaging the mice, a 0.1% Gd doped water phantom was imaged for shimming, and calculating basic frequency, reference power of RF transmitter, and receiver gain of RF receiver. For formal scans, both UTE and GRE sequences were available for proton imaging, and respiratory gating could be activated if necessary (usually for mouse imaging). Similar to the Agilent (Varian) 4.7 T scanner, multiple slices and multiple averages were available for selection. For UTE imaging, the position of FOV can not be easily changed by adjusting imaging parameters. The imaged object was physically adjusted to the desired position in the magnet. Additional attention was paid to ensure the FOV was large enough to cover entire imaged object to avoid aliasing artifacts.

# 7.3 Data analysis

#### 7.3.1 Raw data normalization

The receiver gain for MR imaging may differ between experiments on different days due to shimming differences. The receiver gain difference causes problems for longitudinal studies, but can be solved by normalizing the raw image data. In this dissertation, the raw image data were normalized by the average soft-tissue (heart, muscle, etc.) signal. As  $T_2^*$  of soft tissues is much longer than TE used in lung imaging, the lung parenchyma signal after normalization is only weighted by  $T_2^*$  of lung and  $T_1$  of both lung and soft tissues, and ultimately reflects the lung parenchyma density.

#### 7.3.2 Lung segmentation

A commercial graphic processing software package (Amira, FEI Company, Hillsboro, OR) was used to semi-manually segment the lung from other soft tissues, with the major bronchi and large blood vessels excluded. An example of lung segmentation was shown in **Figure 8.3**.

A segmentation mask with multiple slices corresponding to each slice of the images was created and saved as dicom or tiff file for further analysis in MATLAB (MathWorks, Inc., Natick, MA). The segmentation mask consisted a set of materials with different integer values (0, 1, 2, 3...) which represented segmented lung, soft tissues, background and other regions of interest respectively. The lung parenchyma could be easily segmented out in MATLAB by applying an equality formula to the segmentation mask and the pixel values for the lung parenchyma were saved into a vector.

#### 7.3.3 Lung function parameters

After segmentation, the lung parenchyma signal intensity was calculated. Lung volume was estimated by multiplying the total number of pixels in lung by pixel volume. Tidal volume was

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calculated by subtracting the functional residual capacity (FRC) of lung from the lung volume measured at end-tidal inspiration. Lung compliance was the tidal volume divided by the pressure difference between end-tidal expiration and end-tidal inspiration. High-density lung volume percentage was the percentage of pixels in the lung with signal intensity larger than a threshold.

### 7.3.4 Statistics

A significance level or α level of 0.05 was used for all Student's t tests in this dissertation. A linear mixed effects model (88) implemented by lme4 package in R language(89) was used to analyze the influence of time on MRI-derived lung function parameters in a longitudinal study of lung diseases, where different time points were not independent and a simple linear regression didn't not work.

# Chapter 8 Application of GRE in monitoring lung Tx

Lung transplantation (LTx) has gained widespread acceptance as a last-resort treatment of end-stage lung diseases (9). Despite improvements in surgical techniques, peri-operative management and refinements in immunosuppression, outcomes after LTx remain significantly worse compared to transplantation of other solid organs. Additionally, despite improvements in short-term outcomes, graft failure due to chronic rejection continues to limit long-term survival of lung recipients (10-12). Although the pathogenesis of chronic lung allograft rejection is poorly understood, clinical studies have consistently identified acute cellular rejection (ACR) as a major risk factor (13), even in mild stages of ACR (14, 15).

# 8.1 Mouse model of orthotopic vascularized aerated lung transplantation

Primary Graft Dysfunction (PGD), a form of acute lung injury characterized by impaired oxygenation and pulmonary edema, is mainly thought to be the result of inflammatory mechanisms induced by ischemia–reperfusion (I/R) injury at early time points following LTx. The incidence of PGD remains high. A recent study from Washington University School of Medicine indicated that 81% of LTx recipients developed PGD, with severe PGD observed in 20–25% of LTx recipients. In addition to its role in acute lung injury, PGD has also been linked to the development of bronchiolitis obliterans, the dominant morbidity factor following LTx. I/R injury is regulated by neutrophils and neutrophil-chemotactic factors such as IL-8, exacerbating PGD. Neutrophils accumulate in the airways preceding acute and chronic lung allograft rejection remain poorly understood.

In order to investigate and genetically dissect the role of neutrophils in lung allograft injury and tolerance, Okazaki et al. developed a novel method of orthotopic vascularized aerated left LTx (OLTx) in the mouse utilizing cuff techniques for the anastomoses of pulmonary artery, pulmonary vein and bronchus (16). This technique facilitates hypothesis-driven studies of lung transplant immunobiology in a small animal model that physiologically approximates an aerated, vascularized human lung allograft. Accordingly, investigators have utilized the model to study various aspects of lung transplant immunobiology including ischemia-reperfusion injury (90), immune cell trafficking and molecular mechanisms underlying the pathogenesis of ACR and bronchiolitis obliterans syndrome (91-93). Fully mismatched allografts (major histocompatibility complex) develop edema, impaired oxygenation, and perivascular and interstitial mononuclear infiltrates on histology by postoperative day (POD) 7 (93, 94). Without immunosuppression, ACR progresses and the allograft becomes completely consolidated and atelectatic with diffuse hemorrhage and necrosis by POD 28. Importantly, established ACR in this model is largely T cell mediated and can be ameliorated by treating recipients with T cell depleting antibodies (17). Most centers perform routine surveillance biopsies to facilitate early detection and treatment of ACR (95). However, invasive transbronchial biopsies are subject to sampling heterogeneity, grading variability, and potentially life-threatening complications (96-98). <sup>1</sup>H MRI is a noninvasive imaging modality that may serve as a useful clinical adjunct for detecting lung allograft rejection and assessing treatment response. The goal of this chapter is to use <sup>1</sup>H MRI methods available on most clinical scanners (short TE, but not UTE) and ventilation-synchronization to monitor lung allograft (both acute and chronic) rejection in the model of OLTx (16). It is worth noting that the surgical procedures for mouse lung transplantation from the work of Okazaki et

al. (16) consists of donor procedure and recipient procedure, and the cuff technique for the microscopic bronchial and vascular anastomoses is finally attributed to Krupnick et al. (99).

# 8.2 Acute allograft rejection

#### 8.2.1 Disease model

Established ACR in the OLTx model is largely T cell mediated and can be ameliorated by treating recipients with T cell depleting antibodies (17). In detail, GK1.5 (anti-CD4 Ab) and YTS169.4 (anti-CD8a Ab) monoclonal anitbodies can be used to deplete CD4- and CD8-specific T cells for immunosuppression.

#### 8.2.2 Animal groups and LTx

This prospective study was approved by the Washington University School of Medicine Animal Studies Committee; transplantation and imaging were performed at Washington University, with subsequent analysis performed in Cincinnati. The experimental plan is shown in **Figure 8.1**. Male Balb/c and C57 BL/6 mice (8-10 weeks old) were used as lung donors and recipients, respectively. OLTx were performed as previously described (16). Recipient mice were started on antibiotic water 24 hours before transplant and maintained on antibiotic water for the duration of the experiment. Nineteen Balb/c (H2<sup>d</sup>)  $\rightarrow$  BL/6 (H2<sup>b</sup>) allogeneic lung transplants were performed. All recipients were treated with buprenorphine for analgesia for 2 days post surgery. Eight graft recipients in the control cohort received intraperitoneal injection of 500 µg of hamster isotype control antibody (control-Ig) at POD 3. Eleven graft recipients in the experiment cohort received intraperitoneal injection of 250 µg of anti-CD4 (GK1.5) and 250 µg of anti-CD8 (YTS169.4) monoclonal antibody (Bio X Cell. West Lebanon, NH 03784-1671, USA), also at POD 3. Lung recipients were serially imaged at POD 3, 7 and 14. All surviving recipients were sacrificed at POD 14 for lung tissue histological analysis.



Figure 8.1: Experimental plan.

#### 8.2.3 Ventilation

Allograft recipients were anesthetized, intubated, and connected to our home-built ventilator as described in subsection 7.1. Each mouse was then ventilated with isoflurane at 120 breaths per minute and approximate tidal volume of 0.25 ml (at constant peak pressure of 15 cm H<sub>2</sub>O and positive end-expiratory pressure of 3 cm H<sub>2</sub>O). A schematic of the ventilation waveform (100) is shown in **Figure 8.2**. Each respiratory cycle consisted of a brief breath hold for imaging, an inhalation of  $O_2$ , a second brief breath hold, followed by further inhalation of pure N<sub>2</sub>, then a third brief breath hold for imaging, and finally passive exhalation. Approximately 25% of the total gas inhaled each breath was pure oxygen (for only 70 ms of each 500-ms breathing period), only slightly elevated from the normal oxygen content in air (101). No physiological changes were expected from this split-breath maneuver, compared to a simple breath of air. The actual respiratory waveform measured by pressure transducer slightly deviated from the schematic with the sharp angles smoothed.



Figure 8.2: Schematic of ventilation and MRI acquisition.

#### 8.2.4 MR imaging

MR imaging was performed on a Varian 4.7 T horizontal-bore magnet (40 cm magnet bore and 12 cm gradient bore; Agilent Technologies, Santa Clara, CA) with a custom-built solenoidal coil, and was gated at end-expiration or end-inspiration by the trigger signal from our home-built ventilator. A standard 2D multi-slice gradient-echo sequence with an appropriate flip angle pulse (flip angle= $20^{\circ}$ ) was used for <sup>1</sup>H MR imaging, for simplicity and potential translation. For each mouse, 9 transverse slices (thickness = 2.0 mm, gap = 0.2 mm) were acquired to cover the whole mouse lung with in-plane field of view of 30 mm × 30 mm, and spatial resolution of 0.3 mm × 0.3 mm (96 × 96). One line of k-space was scanned for each of the 9 slices, per breath, with echo time TE = 0.95 ms, repetition time TR = 2.46 ms, sweep width = 0.21 MHz, and readout duration = 0.46 ms; image sets were acquired with both 1 and 4 averages. Effective TR between

radiofrequency (RF) pulses in the same slice was 500 ms. Thus, the number of respiratory cycles during imaging was equal to the number of total phase encoding steps times the number of averages. One scan (1 average) took 48 seconds to acquire all slices, at a respiratory rate of 120 breaths/min.

#### 8.2.5 Lung tissue histology

Surviving lung allograft recipients were sacrificed at POD 14 for lung tissue histology. Heart and lung tissue were resected en bloc. Lung blocks were inflated and fixed in 10% neutral buffered formalin overnight. Left transplanted lungs (allograft) and right untransplanted lungs (native lung) were divided and submitted for paraffin embedding, sectioning, and hematoxylin and eosin (H & E) slide preparation.

#### 8.2.6 Data analysis

A commercial graphic processing software package (Amira, FEI Company, Hillsboro, OR) was used to semi-manually segment the lung from other soft tissues; the major bronchi and large blood vessels were excluded for analysis. The left lung (allograft) and right lung (native) were also separated from each other with particular care taken to separate the accessory lobe from the left lung as shown in **Figure 8.3**. Then the total number of pixels (for volume calculation) and parenchymal signal were measured in all slices.



**Figure 8.3:** Contiguous slices near the lung base to demonstrate segmentation of the left & right lungs by color. We note the challenging segmentation of the accessory lobe (arrows pointing to the accessory lobe of the right lung in the left hemi-thorax) from the left lung in both (a) and (b).

While RF field homogeneity was high in the transverse direction (perpendicular to the solenoidal axis), homogeneity varied in the slice direction as demonstrated in **Figure 8.4**. The raw image data were thus normalized by the average soft-tissue signal of each slice (excluding the lung). This method was found to be more robust than a reference to a single organ or region. Parenchymal signal was measured specifically in regions of interest near the lung periphery to avoid the effects of blood vessels. Since images were acquired over many heart cycles, perfusion effects were averaged (102). Compliance was obtained by dividing the change in lung volumes

at high and low pressures by the change in pressure measured at the breathing valve. Since allograft and native lung volumes can be measured separately, this allows the allograft and native-lung compliances to be measured separately, with total lung compliance being the sum of the two. The volume-percentage of high intensity signal (3x of mean value of native lung parenchyma at POD 3) in the allografts was also measured and compared to allograft compliance. In order to quantify the stability of ventilation over time, the 1-average and 4average image sets were analyzed separately.

Paired student t tests with an alpha level of 0.05 were used to compare lung parenchymal signal intensity and lung compliance between time points and between different groups. One tailed tests were used in cases where lung density was known to increase. Mean and standard deviation (SD) of lung parenchymal signal intensity and lung compliance were calculated.



**Figure 8.4:** MR signal variation (mean  $\pm$  SD) of a water bottle phantom along the axis of coil symmetry, and in concentric annuli in one slice.

#### 8.2.7 Results

Ventilation in our apparatus was precise and stable (3.8% average percentage difference in volume per mouse between 1 and 4 averages at the same peak pressure). Three mice of the total 19 died as a result of surgery, intubation, anesthesia, and mechanical ventilation. Two mice were not imaged at POD 3 and POD 7 because of scheduling difficulties, thus were not included in the data. Images were of high quality at both high and low volumes/pressures (**Figure 8.5**). Signal-to-noise ratio (SNR) in the parenchyma (away from airways & vessels) was about 6 for 4 averages in the native lung; in the normalized images this corresponded to about 5% of the average soft-tissue SNR. **Figure 8.5** presents representative axial images from a control-Ig treated mouse and an anti-CD4/anti-CD8 treated mouse at all 3 time points along with histology at POD 14. No obvious rejection (visually or quantitatively) was seen in the allograft at POD 3 in either group. At POD 7, little signal change was seen in most anti-CD4/anti-CD8 treated mice; parenchymal signal was still near normal in most anti-CD4/anti-CD8 treated mice at POD 14.



**Figure 8.5:** (a) Representative axial 1H MR images (TE = 0.954 ms, flip angle =  $20^{\circ}$ ) of a control-Ig treated mouse at POD 3, POD 7 and POD 14 (in-slice allograft [left lung] lung signal: 0.043, 0.077 and 0.701 respectively; allograft compliance: 0.010, 0.005 and 0.001 in cc/cm H2O respectively), and one corresponding histological slice (H & E, 200x) of the allograft at POD 14. (b) Corresponding 1H MR images and histological slice (H & E, 200x) of an anti-CD4/anti-CD8 treated mouse (allograft signal: 0.045, 0.049 and 0.042 respectively; allograft compliance: 0.015, 0.017 and 0.016 in cc/cm H2O respectively). Rejection is visually apparent in the control-Ig treated allograft at POD 14, though signal increases slightly at POD 7.

Parenchymal signal as percent of mean soft-tissue signal is shown in Figure 8.6 and **Table 8.1**. At POD 3 there is no statistically significant difference between the allograft and native lung (P > 0.05). Allograft parenchymal signal demonstrates large variations for both control-Ig treated and anti-CD4/anti-CD8 treated groups, reflecting both temporal change and different responses to treatment. For example, a suspected pneumonia in mouse 219 at POD 7 was resolved at POD 14; varying degrees of ACR were observed in most mice in the control-Ig treated group (all confirmed by histology). Because the true inter-mouse variation was so large, standard deviation of the measured values was concomitantly large. Mean parenchymal signal in the allograft for control-Ig treated and anti-CD4/anti-CD8 treated mice at POD 7 increased by 1109% (P = 0.06) and 290% (P = 0.13), respectively, over POD 3. Allograft mean parenchymal signal of control-Ig treated mice saw an increase by 42% at POD 14 over POD 7 (P = 0.12), but was near-constant for anti-CD4/anti-CD8 treated mice (7% decrease, P = 0.48). However, allograft mean parenchymal signal of control-Ig treated mice at POD 14 increased significantly compared to POD 3 (increase by 1615%, P < 0.05). As was expected, the right native lung parenchymal signal for all mice remained nearly constant (11% increase from POD3 to POD7 and 5% decrease from POD7 to POD 14, P > 0.05).



Parenchymal signal (@ high pressure/volume)

**Figure 8.6:** Mean parenchymal signal (mean  $\pm$  SD) of control-Ig treated and anti-CD4/anti-CD8 treated mice as percent of mean soft-tissue signal at POD 3, POD 7 and POD 14. We note that the anti-CD4/anti-CD8 treated allograft signal increases at POD 7 & POD 14, which we believe results from edema and/or atelectasis in the transplanted lung in mouse 217 at POD 7 and mouse 278 at POD 14. <sup>a</sup>P = 0.04 compared to POD 3, allograft, control-Ig. <sup>b</sup>P = 0.04 compared to POD 14, allograft, control-Ig.

		Signal, POD 3		Signal, POD 7		Signal, POD 14	
Group	Mouse #	Allograft	Native lung	Allograft	Native lung	Allograft	Native lung
Control-Ig treated	<u>170</u>	0.043	0.056	0.152	0.048	1.144	0.056
	219	0.050	0.056	0.170	0.049	0.047	0.060
	<u>336</u>	0.043	0.050	0.077	0.057	0.701	0.058
	<u>D</u>	0.054	0.048	1.259	0.055	1.262	0.055
	<u>196</u>	0.042	0.057	1.124	0.044	а	а
	mean	0.046	0.053	0.556	0.051	0.789 <sup>b</sup>	0.057 <sup>c</sup>
	<u>SD</u>	0.005	0.004	0.583	0.005	0.550	0.002
Anti-CD4/anti-CD8 treated	<u>171</u>	0.045	0.047	0.049	0.050	0.042	0.053
	<u>217</u>	0.045	0.056	1.300	0.055	0.058	0.054
	<u>218</u>	0.051	0.057	0.042	0.132	0.115	0.061
	<u>278</u>	0.051	0.052	0.054	0.051	1.320	0.062
	<u>279</u>	0.042	0.045	0.054	0.066	0.050	0.054
	<u>335</u>	0.053	0.056	0.039	0.044	0.062	0.062
	<u>337</u>	0.151	0.066	0.046	0.068	0.053	0.047
	<u>C</u>	0.044	0.044	0.050	0.048	0.044	0.050
	<u>199</u>	0.057	0.048	0.473	0.054	а	а
	mean	0.060	0.052	0.234	0.063	0.218	0.055
	<u>SD</u>	0.035	0.007	0.424	0.027	0.446	0.006

Table 8.1: Normalized Lung Parenchymal Signal.

Five control-Ig treated mice (170, 219, 336, D, 196) and nine anti-CD4/anti-CD8 treated mice (171, 217, 218, 278, 279, 335, 337, C, 199) at high pressure/ volume are presented here. Data are expressed as a fraction of the mean inslice soft-tissue signal (excluding lung).

<sup>a</sup>No data available.

 ${}^{b}P = 0.04$  compared to POD 3, allograft.

 $^{\circ}P = 0.04$  compared to POD 14, allograft.

Respiratory compliance is presented in **Figure 8.7** and **Table 8.2**. Allograft compliance of control-Ig treated mice at POD 14 and POD 7 decreases with statistical significance compared to POD 3 (by 77% [P < 0.001] and 69% [P = 0.01] respectively). There is no statistically significant change for native lung compliance (P > 0.05), but the mean compliance of native lung at POD 14 increases by 11%. Allograft compliance of anti-CD4/anti-CD8 treated mice remains constant (P >> 0.05), while there is slight increase (25%) in native lung compliance at POD 14. Allograft compliance at both POD 7 and POD 14 shows a statistically significant difference between the control-Ig and anti-CD4/anti-CD8 treated mice (P < 0.05).



## Compliance

**Figure 8.7:** Compliance (mean  $\pm$  SD) of control-Ig treated and anti-CD4/anti-CD8 treated mice at 3 timepoints. <sup>a</sup>*P* < 0.05 compared to POD 3, allograft, control-Ig. <sup>b</sup>*P* = 0.04 compared to POD 3, native lung, anti-CD4/anti-CD8. <sup>c</sup>*P* < 0.05 compared to allograft, anti-CD4/anti-CD8, at POD 7 and POD 14 respectively.
Group	Mouse #	Compliance, POD 3		Compliance, POD 7		Compliance, POD 14	
Gloup		Allograft	Native lung	Allograft	Native lung	Allograft	Native lung
treated	170	0.011	0.025	0.007	0.030	0.000	0.035
	219	0.016	0.033	0.002	0.028	0.007	0.025
	336	0.010	0.027	0.005	0.025	0.001	0.033
ol-Ig	D	0.012	0.025	0.003	0.019	0.002	0.028
ontro	196	0.015	0.024	0.002	0.030	a	а
Cc	mean	0.013	0.027	0.004 <sup>b</sup>	0.027	0.003 <sup>c</sup>	0.030
	<u>SD</u>	0.003	0.004	0.002	0.005	0.003	0.005
Anti-CD4/anti-CD8 treated	171	0.015	0.031	0.017	0.035	0.016	0.024
	217	0.011	0.024	0.000	0.024	0.009	0.025
	218	0.011	0.024	0.014	0.015	0.006	0.035
	278	0.009	0.022	0.010	0.021	0.001	0.031
	279	0.009	0.023	0.009	0.022	0.014	0.032
	335	0.011	0.018	0.013	0.019	0.012	0.029
	337	0.005	0.029	0.007	0.019	0.013	0.039
	С	0.006	0.021	0.016	0.034	0.005	0.023
	199	0.009	0.026	0.000	0.031	а	а
	mean	0.009	0.024	0.010 <sup>e</sup>	0.024	0.010 <sup>e</sup>	0.030 <sup>d</sup>
	<u>SD</u>	0.003	0.004	0.006	0.007	0.005	0.006

 Table 8.2: Image-determined Lung Compliance (cc/cm H<sub>2</sub>O).

Five control-Ig treated mice (170, 219, 336, D, 196) and nine anti-CD4/anti-CD8 treated mice (171, 217, 218, 278, 279, 335, 337, C, 199) are presented here.

<sup>a</sup>No data available.

 ${}^{b}P = 0.01$  compared to POD 3, allograft.

 $^{c}P < 0.001$  compared to POD 3, allograft.  $^{d}P = 0.04$  compared to POD 3, native lung.

<sup>e</sup>P < 0.05 compared to control-Ig treated mice, allograft, at POD 7 and POD 14 respectively.

Histological analysis demonstrated severe ACR in control-Ig treated mice; no evidence of ACR was seen in most anti-CD4/anti-CD8 treated mice or in the control (native) lungs (**Figure 8.8**).



**Figure 8.8:** An array of histological slides from 2 anti-CD4/anti-CD8 treated and 1 control-Ig treated mice demonstrating (a) some residual edema and cellular consolidation, (c) resolution of POD 7 edema, (e) near-complete consolidation & rejection, and (b, d, f) corresponding control, native lungs (H & E, 100x).

Quantification of the high-intensity volume of allografts showed negative correlation between volume percentage of high intensity signal and allograft compliance (**Figure 8.9**).



Figure 8.9: Negative correlation between the volume percentage of high-intensity signal in the allografts and allograft compliance.

#### 8.2.8 Discussion

We have demonstrated with MR imaging an ability to serially monitor lung ACR in individual mice. Importantly, ACR is dependent on T cells (94); accordingly, we observed sharp differences in MR measurements between recipients of lungs that received anti-CD4/anti-CD8 compared to control-Ig treated lung recipients. This opens the door to a much better understanding of the efficacy of the new treatment strategies through the ability to visualize and longitudinally monitor changes in individual lungs. For example, among the 9 anti-CD4/anti-CD8 treated mice, one mouse demonstrated signs of pneumonia at POD 14 (mouse 278), and one of the control-Ig

treated mice recovered from what appeared to be pneumonia or atelectasis at POD 7 (mouse 219). In the future we anticipate that serial MR monitoring after murine lung transplantation will allow for better understanding of individual immune responses to treatment and reduce the number of animals used in pre-clinical studies. Moreover, this technique may be useful as a clinical adjunct for detecting ACR and monitoring response to treatment.

Since the mean difference in total lung volume between 1-average and 4-average images for all mice at high pressure is small, we expect little variation due to ventilation of the mice. Variation of parenchymal signal due to between-slice RF inhomogeneity (**Figure 8.4**) was an initial concern, which was overcome by normalizing each slice separately. Time variation due to perfusion was averaged over many heart cycles. The fact that we see little difference (both visually and statistically) in mean parenchymal signal between control-Ig treated and anti-CD4/anti-CD8 treated native lungs at POD 3 is evidence that the ventilation and normalization schemes are robust. Very small longitudinal variation in native-lung signal also speaks to the robustness of the measurement technique.

Compliance is a standard physiological measurement for characterization of lung mechanics. Here we have introduced regional compliance, only possible because we can measure allograft and native lung volumes and volume changes separately (with pressure monitoring). Segmentation of the accessory lobe of the right lung (native) from the left lung (allograft) required expertise and care; we suspect this sometimes-invisible boundary contributed to some variation or error in the compliance measurement. Compliance of the native lung, however, was quite stable between time-points. It is also important to note that the total respiratory compliance here is the sum of the allograft and native lungs ( $\Delta V_I / \Delta P + \Delta V_R / \Delta P$ ), not the volume-average. A size-independent measure might be specific compliance, where each compliance is normalized

by the volume at high pressure. We have chosen to cite absolute compliance here, for clarity. In control-Ig treated mice, compliance of the allograft decreased with time, due to lung rejection; compliance of the native lung increased slightly from POD7 to POD14, due to left-lung shrinkage with rejection (103, 104). In anti-CD4/anti-CD8 treated mice, compliance of the allograft remained unchanged, as expected, though compliance of the native lung increased slightly. Total compliance at POD 3 (all mice combined) was  $0.036 \pm 0.006$  cc/cm H<sub>2</sub>O, which is relatively consistent with measurements via FlexiVent (0.022-0.033 cc/cm H<sub>2</sub>O) by other groups (105, 106). As expected, negative correlation was seen between allograft compliance and volume percentage of high-intensity signal in the allografts (Figure 8.9). We note that our approach to compliance is quite simple compared to that measured by the FlexiVent, (regarded as a standard for mouse physiology), with the advantage being that our measurements are within or close to the normal tidal-volume range, and the near-linear portion of the pressure-volume curve. This allows our technique to be easily repeated (not a terminal procedure, as the FlexiVent is often regarded), yet does not allow the full range of robust measurements or ventilation like the commercial device.

<sup>1</sup>H MRI signal density at zero echo time (and long effective TR) is a direct reflection of proton density in tissue. For the more practical case of nonzero TEs, as is the case here, the signal is significantly lowered in lung due to the effects of magnetic susceptibility gradients arising from the multiple interfaces between lung tissue and alveolar airspaces. Since we have normalized lung signal to the average in-slice soft-tissue signal (with much longer  $T_2^*$ , but roughly the same  $T_1$ ), we can estimate the expected signal of lung tissue in our imaging regime. The parenchymal density of the native lung in our study at peak pressure (15 cm H<sub>2</sub>O) is expected to be 33% of the average soft-tissue (mainly heart) density (near 1 g/cm<sup>3</sup>) (41, 107). Taking into account  $T_2^*$  =

0.46 ms of C57BL/6 mouse lung at 4.7 T (41, 108), the expected parenchymal signal of native lung is 4.2%, which is consistent with our result of 5.3% at POD 3. In future studies, we expect to minimize the effects of short  $T_2^*$  of lung by using techniques that allow shorter or near-zero TE (72, 90, 109-116), but note that such non-Cartesian techniques will increase total image-acquisition time, for similar image resolution.

It is notable that some control-Ig treated mice may have experienced slower lung rejection (mouse 170 & 336 from POD 3 to POD 7); serial imaging allows close monitoring of the rejection in individual animals by the parenchymal signal intensity increase and by the compliance decrease. This can improve accuracy and reduce the number of mice needed in a study, thus increasing rapidity and accuracy of information about an experimental treatment. When a mouse model of chronic lung rejection is fully developed, imaging can serve an important role in understanding spatial variation and time course of that rejection for more accurate biological targeting. By utilizing MRI instead of CT, we are preparing the technique for translation to imaging in humans, without ionizing radiation (particularly important in vulnerable populations, like children). The fact that we are able to easily measure lung signal and lung compliance in vivo shows the potential for translation to the clinic for longitudinal monitoring of the allograft.

The increased parenchymal signal intensity and decreased lung compliance are consistent with histological findings of severe ACR at POD 14 in each of the control-Ig treated mice. The histological analysis also demonstrates no ACR in the anti-CD4/anti-CD8 treated mice (**Figure 8.8**). These results are consistent with our analysis of MR images.

<sup>1</sup>H MRI has somewhat low SNR due to low proton density of lung parenchyma and short  $T_2^*$ . The main limitation of the technique's ability to monitor rejection, however, is that we cannot easily distinguish pulmonary edema or atelectasis that will resolve from consolidated pneumonia (e.g. resolution of abnormalities in mouse 217 at POD 14) demonstrated in **Figure 8.8**, yet we note that the longitudinal imaging allows repeated measures in the same animal. The anti-CD4/anti-CD8 allografts that demonstrated ephemeral increased parenchymal signal intensity did not show significant ACR, but the POD 14 histological results match the POD 14 imaging results, even in these more ambiguous cases. Residual edema and cellular consolidation are seen, for example, in mouse 278 allograft, no edema is seen in the 217 allograft and complete cellular consolidation is seen in mouse 170 allograft as shown. Some small segmentation errors in volume (< 5%) result from our inability to distinguish the accessory lobe of the native lung from the allograft. Also, this particular system did not allow UTE (which would lead to higher SNR), but the gradient echo sequence had the advantage of being reliable and robust.

We have demonstrated the ability to acquire quantitative <sup>1</sup>H MR images of murine lung at reasonable resolution and SNR in a longitudinal study of unilateral lung transplantation and ACR. By repeated imaging in the same animals, we were able to measure clear parenchymal signal increases in the rejecting lung and statistically-significant decreases in lung compliance, even in small groups (N = 5, 9). The same measurements in the native lungs were near-constant at all timepoints. Monitoring lung rejection in the mouse is straightforward using standard pulse sequences, without the need for hyperpolarization equipment; the techniques used here can be easily translated to clinical studies of lung-transplant recipients.

## 8.3 Chronic allograft rejection

#### 8.3.1 Disease model

A recent study has shown that chronic Clara cell depletion in the mouse lung can result in persistent metaplaisia, lack of normal reepithelialization and peribronchiolar fibrosis, similar to chronic rejection in human lung allografts (18). In this study, the expression of diphtheria toxin A (DT-A) gene initiated by doxycycline in a triple transgenic mouse (termed Scgb1a1/DT-A or DTA hereafter) can cause Clara cell depletion and thus chronic injury.

### 8.3.2 Animal groups and LTx

The lung transplantation plan is shown in **Figure 8.10**. Twelve mouse-lung allografts were performed, with 6 triple transgenic (DTA) and 6 non-triple transgenic (control) mice used as donors. Left donor lungs were transplanted into C57BL/6 recipients. Recipients were treated with MR1/anti-CD40L on post-operative day (POD) 0 and CTLA4\_Ig on POD 2 to suppress acute allograft rejection. On POD 30, doxycycline was fed for 48 hours to induce Clara cell depletion by activating DTA expression; this induces an upper-epithelial injury similar to a human epithelial injury due to environmental exposure or infection.





#### 8.3.3 MR imaging

MR imaging was performed on a Bruker 7 T MR scanner with data acquired at end expiration during free-breathing of mice on POD 28, POD 33, POD 35, and POD 42. A 2D gradient echo (GRE) sequence was used to acquire both low resolution and high resolution <sup>1</sup>H images with field of view = 24 mm × 24 mm, pixel size =  $0.25 \text{ mm} \times 0.25 \text{ mm}$  or  $0.16 \text{ mm} \times 0.16 \text{ mm}$ , slice thickness = 2.0 mm or 0.59 mm, echo time (TE) = 0.64 ms or 0.93 ms, flip angle =  $30^{\circ}$ , and effective TR = 0.5 second (breath period), with a home-built bird-cage coil.

#### **8.3.4 Data analysis**

A commercial software package (Amira, FEI Company, Hillsboro, OR) was used to semimanually segment lung from other soft tissues with major bronchi and large blood vessels excluded; the left lung and right lung were also separated from each other with particular care taken to separate the accessory lobe from the left lung via the fissure visible on high resolution images. Raw image data were normalized by the average soft-tissue signal of each slice (excluding the lung). Mean parenchymal signal intensity and percent of pixels above normalized signal intensity of 0.4 were measured and calculated.

#### 8.3.5 Results & discussion

The signal-to-noise ratio in right-lung parenchyma for <sup>1</sup>H MR images (TE = 0.64 ms) is around 6, allowing the quantification of longitudinal changes. Representative single-slice <sup>1</sup>H MR images of three Tx-DTA recipients and one Tx-control recipient are shown in **Figure 8.11** to demonstrate the variation of response that would not have been detected without imaging. For example, in **Figure 8.11** (b), regional rejection appeared even before scheduled injury was induced and remained through all time points; in **Figure 8.11** (c), severe rejection appeared at

scheduled injury and remained through POD 42; **Figure 8.11** (d), rejection on POD 33 was resolved on POD 35 and POD 42.



**Figure 8.11:** Demonstration of mouse-to-mouse variation detected via MRI, with corresponding histological images on POD 42. (a) Tx-control mouse. (b)(c)(d) Tx-DTA mice. We note that (b) regional rejection appeared in left lung even before injury was activated and remained constant through POD33 to POD 42; (c) severe rejection appeared on POD 33, and was observed unchanged on POD 35 and POD 42; (d) rejection appeared on POD 33, but resolved on POD 35 and POD 42.

The right lung mean normalized parenchymal signal intensity is shown in **Figure 8.12**; this is an approximate measure of proton density of tissue (attenuated by  $T_2^*$  relaxation). The parenchymal signal intensities in right lungs of Tx-DTA and Tx-control mice are consistent among mice and groups longitudinally.



Figure 8.12: Low and constant right lung mean parenchymal signal intensity (mean ± SD).

The lung parenchyma percentage of high density (corresponding to signal intensity [SI] >0.4) is shown in **Figure 8.13**; this percentage of Tx-DTA left lung is larger than that of Tx-control left lung at all time points, indicative of rejection in Tx-DTA mice; large standard deviations indicate large variation in rejection severity between individual mice, confirmed by left lung histology (**Figure 8.11**). Signal in the Tx-control left lungs is much larger than that of right lung because of cellular infiltration and/or inflammation.



**Figure 8.13:** Lung parenchyma percentage of high signal intensity (SI > 0.4) (mean  $\pm$  SD).

# Chapter 9 Imaging healthy mice with ellipsoidal UTE

In this chapter, the ellipsoidal UTE or UTE with ellipsoidal k-space coverage described in chapter 5, is applied to imaging the lungs of healthy mice in preparation for lung disease imaging. In <sup>1</sup>H MR lung imaging, the MR signal depends not only on proton density, but also on longitudinal relaxation time  $T_1$  and transversal relaxation time  $T_2^*$ , all of which relate to specific tissue characteristics. For example,  $T_1$  shortening is found in the lungs of patients with fibrosis and emphysema (117) and in the rat lungs at the fibrotic stage of lung injury (118).  $T_2^*$  shortening is found in mouse lungs with emphysema (41). Thus the potentials of  $T_1$  and  $T_2^*$  in characterizing lung diseases motivates the need to develop the techniques for measuring  $T_1$  and  $T_2^*$  in the healthy mouse lung and then apply these techniques to quantifying lung diseases in the future.

### 9.1 Imaging

#### 9.1.1 Animal preparation

22-23 week old (N = 14) and 17 week old (N = 4) healthy C57BL/6 mice were used. Animals were anesthetized by 1.5-2% isoflurane for free breathing and placed supine with the lung in the center of the coil during imaging. A small-animal monitoring system (SA Instruments, Inc.) was used to monitor the respiratory rate and body temperature and provided a TTL signal for respiratory gating at end-expiration.

#### 9.1.2 Imaging parameters

Mice were imaged by golden means sampling UTE with ellipsoidal k-space coverage ( $\alpha_x = \alpha_y = 2$ ); 64 points were sampled on each of 51480 projections with FOV = 30 mm × 30 mm × 60 mm, TR (ms) = 10, and TE (ms) = 0.075. A receiver bandwidth of 300 kHz was used for the near-optimal readout duration for maximum single-voxel SNR based on the initial T<sub>2</sub>\* measurement of two mice. For comparison, mice were also imaged by UTE with spherical k-space coverage, i.e.,  $\alpha_x = \alpha_y = 1$ .

#### 9.1.3 Pulse sequence

The pulse sequence of the radial center-out k-space acquisition used for 3D steady-state UTE imaging is shown in **Figure 9.1**. After RF excitation with a hard pulse of length of 4.3 µs, the radial readout gradient was ramped up to the predetermined direction and amplitude and remained constant until the end of data acquisition. Data acquisition began after the RF pulse with echo time TE. After data acquisition, an opposite-polarity readout gradient was used to negate effects of readout gradient on spins. Subsequently a large constant spoiler gradient was applied for every repetition. Each RF pulse with a linear phase increment of n•117° (n is RF pulse number) was combined with a large spoiler gradient to spoil residual transverse magnetization and maintain incoherent steady-state magnetization (82, 119).



**Figure 9.1:** Pulse sequence diagram for 3D radial encoding. A hard RF pulse (~  $4.3 \mu$ s) is used for non-selective spin excitation and a linear phase increment (n x 117°) is added to RF pulse for RF spoiling. After a delay TE from the middle of the RF pulse, data acquisition starts simultaneously with the readout gradient. The direction and magnitude of the readout gradient are changed between repetitions. Gradient lobe B is used to negate the effects of lobe A on spins so that a constant large spoiler gradient C, combined with RF phase increment, effectively spoils the transverse magnetization after each data acquisition.

# 9.2 <sup>1</sup>H images

Representative images of the mouse lung acquired by UTE with k-space expansion factors  $\alpha_x = \alpha_y = 1$  and  $\alpha_x = \alpha_y = 2$  are shown in **Figure 9.2**. The SNR in lung parenchyma is 33.0 and 123.6 for  $\alpha_x = \alpha_y = 2$  and  $\alpha_x = \alpha_y = 1$  respectively, consistent with the corresponding voxel-volume ratio of 4. With the same number of projections of 51480, UTE with  $\alpha_x = \alpha_y = 2$  provides higher resolution as is demonstrated by the sharper lung boundary (indicated by the red arrows in the axial and sagittal slices in **Figure 9.2**) and clearer blood vessels (red arrows in the coronal slices in **Figure 9.2**).



**Figure 9.2:** Axial, coronal and sagittal images acquired by UTE with (a) spherical k-space coverage, voxel size =  $(0.47 \text{ mm})^3$  and (b) ellipsoidal k-space coverage, voxel size =  $(0.23 \text{ mm})^2 \times 0.47$  mm, for the same number of projections. The higher resolution of ellipsoidal UTE in the axial plane is evident to the eye, as indicated by the red arrows.

## 9.3 Lung volumes

Seven 22-week old mice were also imaged by retrospective self-gating UTE as described in

Chapter 5 to measure the functional residual capacity (FRC) and tidal volume (TV). The only

modification here to the self-gated UTE is that the spherical k-space coverage was replaced with

ellipsoidal k-space coverage for minimal echo-time. The imaging parameters and pulse sequence

are exactly the same as in subsection 9.1.

The results of lung volume at expiration and inspiration are shown in Table 9.1. The lung

volumes at expiration and inspiration are  $0.55 \pm 0.08$  ml and  $0.75 \pm 0.11$  ml respectively,

resulting in tidal volume of  $0.20 \pm 0.03$  ml, which is consistent with previous plethysmographic

measurements in (non-anesthetized) C57BL/6J mice of 0.176 ml (range 0.143-0.228 ml) (83).

The inspiration-to-expiration lung volume ratio is  $1.37 \pm 0.02$ .

Mouse #	Mass (g)	FRC (ml)	FRC + TV(ml)	TV (ml)	(FRC+TV)/FRC
3	23.8	0.40	0.56	0.16	1.39
5	25.2	0.48	0.66	0.18	1.39
7	24.1	0.60	0.81	0.21	1.35
8	24.4	0.57	0.78	0.21	1.36
9	24.6	0.57	0.77	0.20	1.36
10	27.0	0.66	0.92	0.26	1.39
11	26.5	0.54	0.73	0.19	1.36
Mean $\pm$ SD	$25.1 \pm 1.2$	$0.55\pm0.08$	$0.75\pm0.11$	$0.20\pm0.03$	$1.37\pm0.02$

Table 9.1: Lung volume at expiration and inspiration of seven 22-week old mice.

### 9.4 Spin-lattice relaxation time, T<sub>1</sub>

#### 9.4.1 Overview of Inversion Recovery method

Many NMR and MRI methods have been developed to measure the spin-lattice relaxation time,

 $T_1$  (120), among which inversion-recovery (IR) method is most commonly used for its highly accurate  $T_1$  measurements (121). Based on the IR method, a fast inversion-recovery (122) method and a modified fast inversion-recovery method (123) were developed to reduce acquisition time for  $T_1$  mapping. The basic principles of the IR method and a timing diagram for a generic IR pulse sequence are not the focus of this work and are discussed in detail by Bernstein et al. (124). Even though IR methods are appropriate for measuring high density tissues (usually with long  $T_2^*$ ), they are not applicable to assessing the  $T_1$  of short  $T_2^*$  tissues like the lung due to the fast signal decay in a spin-echo signal acquisition (with long TE) after an IR module. In addition, IR methods require a relatively long TR (e.g., TR = 2-11s), extending scanning time for 3D imaging techniques.

#### 9.4.2 LFA method

An alternative approach to overcome the drawbacks of the long TE and long scanning time of IR methods is to use a limited flip angle (LFA) method. The LFA method was first developed in the field of nuclear magnetic resonance (NMR) spectroscopy to calculate  $T_1$  relaxation times from data acquired at different flip angles (125-127). Shortly thereafter, the LFA method was applied to MRI as well (128), with Wang et al. (2) further optimizing the precision in  $T_1$  estimation. The basic principles of LFA method are described below.

In a gradient-recalled echo (GRE) or UTE sequence, if an RF excitation pulse with fixed flip angle (FA) is repeatedly applied at a fixed interval TR (repetition time), and RF spoiling together with gradient spoiling destroys the residual transverse magnetization after data acquisition, the nuclear magnetization will be driven into an equilibrium cycle or a steady state. At steady state, both longitudinal magnetization and transverse magnetization depend on RF excitation pulse flip angle, T<sub>1</sub> relaxation time, and TR. A set of images can be acquired at various flip angles with the same TR and used to generate a computed T<sub>1</sub> map, potentially with less scanning time than conventional IR methods. The amplitude of the steady-state transverse magnetization as a function of the flip angle  $\theta$  is given by the equation (125, 126)

$$M(\theta) = \frac{M_0(1 - e^{-TR/T_1})\sin\theta}{(1 - e^{-TR/T_1}\cos\theta)}$$
[9.1]

where  $M_0$  is the longitudinal magnetization at thermal equilibrium, and  $\theta$  is the flip angle.

Rearranging equation (9.1) yields

$$\frac{M(\theta)}{\sin \theta} = e^{-TR/T_1} \frac{M(\theta)}{\tan \theta} + M_0 (1 - e^{-TR/T_1})$$

$$\tag{9.2}$$

which is the basis for a linear plot of  $M(\theta)/\sin\theta$  against  $M(\theta)/\tan\theta$  with a slope of  $e^{-TR/T_1}$ . It is worth noting that a knowledge of  $M_0$  is not needed for obtaining  $T_1$  by this method.

According to equation (9.2), by acquiring a set of images at various flip angles, a T<sub>1</sub> map can be calculated by doing linear regression if TR is known. The linear fitting may be a more accurate method for T<sub>1</sub> calculation, but it is time consuming, especially for 3D imaging. It is also possible to deduce T<sub>1</sub> that involves measurements at two flip angles only. If signal intensities  $I_1$  and  $I_2$  are measured for flip angles  $\theta_1$  and  $\theta_2$  respectively, T<sub>1</sub> can be calculated from equation

$$-\frac{TR}{T_1} = \ln\left[\frac{(I_1\sin\theta_2 - I_2\sin\theta_1)}{(I_1\sin\theta_2\cos\theta_1 - I_2\sin\theta_1\cos\theta_2)}\right]$$
[9.3]

In this thesis, the two-FA method instead of linear regression is used for simplicity. However, the LFA method is sensitive to FA especially when  $T_1$  is long. It is necessary to measure the actual FA in order to increase the precision in  $T_1$  estimation. A long  $T_1$  phantom is usually used for this purpose. If no dummy scans are applied, for the leading few scans (total scanning time  $\ll T_1$  so that the recovery of longitudinal magnetization is negligible) of UTE imaging experiments, magnetization doesn't reach steady state and the amplitude of FID signal decaying as a function of RF excitation pulse number *n* and FA is given by equation

$$S_n = S_0 \cos^n \theta \tag{9.4}$$

where  $S_n$  is the amplitude of the  $n^{th}$  FID and n = 0,1,2,3...

Rearranging equation (9.4) yields

$$\ln S_n / S_0 = n \ln \cos \theta \tag{9.5}$$

which is the basis for a linear plot of  $\ln S_n/S_0$  against *n* with a slope of  $\ln \cos \theta$ .

By doing a linear regression (linear least squares),  $\ln \cos \theta$  can be estimated from the slope and then the actual flip angle estimated.

There also exists a pair of optimal FAs for maximal precision (2), which can be calculated by the analytical formula (129)

$$\theta = \cos^{-1}\left(\frac{f^2 E_1 \pm (1 - E_1^2)\sqrt{1 - f^2}}{1 - E_1^2(1 - f^2)}\right)$$
[9.6]

where f = 0.71, and  $E_1 = e^{-TR/T_1}$ .

#### 9.4.3 Measuring T<sub>1</sub>

22-week old (N = 6) healthy C57BL/6 mice were imaged by UTE with golden-mean sampling pattern and ellipsoidal k-space coverage ( $\alpha_x = \alpha_y = 2$ ); 64 points were sampled on each of 51480 projections with FOV = 30 mm × 30 mm × 60 mm, TR (ms) = 10, and TE (ms) = 0.075. A receiver bandwidth of 300 kHz was used for the near-optimal readout duration that maximized single-voxel SNR. The T<sub>1</sub> of lung parenchyma was initially estimated around 1400 ms, therefore two optimal flip angles 2.85° and 16.33° were used for imaging respectively, according to equation [9.6].

A deionized water phantom ( $T_1 = 2 \sim 3$  s) was scanned by UTE without dummy scans and with TR = 8 ms for 11 repetitions to measure the actual flip angles. Representative linear regression results of logarithms of the FID amplitude as a function of FID number according to equation [9.5] are shown in **Figure 9.3**. The actual FAs calculated from the slope are 2.95° and 15.50° respectively.



**Figure 9.3:** Representative linear fitting of logarithm of FID amplitude to FID number for expected  $FA = 16.33^{\circ}$ . The measured FA from the slope is 15.50°.

With TR = 10 ms,  $\theta_1 = 2.95^\circ$  and  $\theta_2 = 15.50^\circ$ , a  $T_1$  map was calculated according to equation [9.3] on a pixel-by-pixel basis from images acquired at the two different FAs, e.g., in Figure 9.4 (a) & (b). The representative  $T_1$  map of one mouse is shown in Figure 9.4 (c). ROIs indicated by the blue mask in Figure 9.4 (d) were selected in lung parenchyma, with particular care taken to exclude blood vessels, to measure  $T_1$  of lung parenchyma. ROIs in the heart indicated by the red mask were also selected to measure  $T_1$  of heart tissue.



**Figure 9.4:** Representative axial slices of proton images acquired at different flip angles (a) FA = 2.95°, (b) FA = 15.50°, and the resulting (c) T<sub>1</sub> mapping in color and (d) segmenting mask for T<sub>1</sub> measurement. Voxel = 0.23 mm × 0.23 mm × 0.47 mm, expansion factor  $\alpha_x = \alpha_y = 2$ , and number of projections = 51480. In this case, T<sub>1</sub> of heart = 1824.0 ± 58.8 ms and T<sub>1</sub> of lung parenchyma = 1409.4 ± 78.8 ms.

 $T_1$  values of heart and lung are listed in **Table 9.2**. Lung  $T_1$  is 1409.4  $\pm$  78.8 ms, smaller than

heart T<sub>1</sub> (=  $1824.0 \pm 58.8$  ms).

Mouse #	Mass (g)	$T_1$ of heart (ms)	$T_1$ of lung (ms)
1	33.8	1748.0	1361.2
2	26.8	1834.7	1352.1
3	23.8	1878.5	1515.4
4	20.6	1853.1	1355.0
5	25.2	1868.0	1506.3
6	32.7	1862.0	1366.3
Mean ± SD	$27.1\pm5.2$	$1824.0\pm58.8$	$1409.4\pm78.8$

**Table 9.2:** Heart and lung  $T_1$  of six 22-week old mice.

### 9.5 Measuring T<sub>2</sub>\* of lung parenchyma

Due to alveolar-air susceptibility differences, the  $T_2^*$  of mouse lung is usually shorter than 1 ms, and even shorter (0.5 ms) at high fields like 4.7 T (41). Therefore, the ellipsoidal UTE was used to measure  $T_2^*$  of mouse lung at 7 T in this dissertation.

For measuring  $T_2^*$  of mouse lung, both 22-23 week old mice (N = 8) and 17 week old mice (N = 4) mice were imaged by ellipsoidal UTE with the same imaging parameters as used for proton imaging and  $T_1$  mapping, except that an interleaved multi-TE acquisition strategy was used. Mice were imaged at a series of TEs (0.075, 0.2, 0.4, 0.6, 0.9 and 1.3). The RF pulses were applied periodically (period = TR) as shown in **Figure 9.5** to reach a steady state, but the data were only sampled inside the gating window to minimize motion artifacts. Each k-space spoke was sampled for a consecutive series of TEs (interleaved multi-TE method).



**Figure 9.5:** Interleaved multi-TE acquisition used by UTE. Data were sampled on each spoke in k-space at 6 consecutive TEs, gated at end expiration. The RF pulse and gradient pulse were also applied outside the gating window so that longitudinal magnetization remained at steady state at all scanning times.

Representative axial images of mouse lung acquired at different TEs are shown in Figure 9.6 (a). The lung parenchyma signal decreases dramatically with TE, highlighting the importance of short echo times in imaging lung at 7 T. For  $T_2$ \* measurements, lung parenchyma was segmented from other soft tissues with particular care taken to exclude blood vessels (blue mask in Figure 9.6 (b)). Average signal intensity (SI) in the lung parenchyma was corrected by noise estimated by the average SI in air in each image (130).  $T_2$ \* was calculated by performing exponential fitting to the noise-corrected lung parenchyma SI versus the TEs with custom codes in MATLAB (MathWorks, Inc., Natick, MA).  $T_2$ \* mapping in Figure 9.6 (c) was generated by performing exponential fitting to the magnitude images versus the TEs pixel-by-pixel.



**Figure 9.6:** (a) Axial images of mouse lung at TE (ms) = 0.075, 0.2, 0.4, 0.6, 0.9 and 1.3 acquired by ellipsoidal k-space coverage and (b) corresponding segmenting mask for lung parenchyma and heart, and (c) representative  $T_2^*$  mapping generated by exponential fitting the magntude images versus TEs pixel-by-pixel.

An example of exponential fitting for a  $T_2^*$  measurement is shown in **Figure 9.7**. Images

acquired at TE = 0.075 ms were selected for lung parenchyma density analysis. Heart ROIs with

homogeneous intensity were also segmented out (orange mask in **Figure 9.6**) to normalize the lung parenchyma intensity.



**Figure 9.7:** Exponential fitting (nonlinear least squares) for  $T_2^*$  measurement.

The T<sub>2</sub>\* results of lung parenchyma measured from the UTE images are listed in **Table 9.3** and no significant differences (P = 0.91) are observed between 22-23 week-old mice (N = 8) and 17week-old mice (N = 4). The T<sub>2</sub>\* values are  $0.394 \pm 0.040$  ms and  $0.396 \pm 0.011$  ms for 22-23 week-old mice and 17-week-old mice respectively. Lung parenchyma signal intensity (SI) in the images at TE = 0.075 ms, normalized by heart signal intensity, was  $0.63 \pm 0.06$  and  $0.60 \pm 0.04$ respectively for 22-23 week-old and 17-week-old mice without statistical significance as well (P= 0.31). After correction by T<sub>2</sub>\* and T<sub>1</sub> of mouse lung and heart, the normalized lung signal is  $0.61 \pm 0.06$  and  $0.57 \pm 0.04$  for 22-23 week-old and 17-week-old mice respectively (P = 0.33),

similar to the lung density of 0.55 to 0.58 g/cm<sup>3</sup> previously observed for this strain (41).

Mouse #	Age (weeks)	Weight (g)	$T_2^*$ of lung (ms)	Normalized lung	Lung parenchyma SI
				parenchyma SI	post $T_1 \& T_2^*$ correction
1	23	33.8	0.425	0.59	0.56
3	22	23.8	0.399	0.64	0.61
4	22	20.6	0.393	0.65	0.63
5	22	25.2	0.372	0.70	0.68
6	22	32.7	0.443	0.56	0.52
12	23	30.3	0.330	0.62	0.62
13	23	27.5	0.354	0.72	0.70
14	23	28.2	0.438	0.58	0.55
Mean $\pm$ SD	$22.5\pm0.5$	$27.8\pm4.5$	$0.394\pm0.040$	$0.63\pm0.06$	$0.61\pm0.06$
15	17	26.4	0.409	0.55	0.52
16	17	27.2	0.385	0.58	0.56
17	17	26.1	0.401	0.66	0.63
18	17	27.1	0.390	0.60	0.57
$Mean \pm SD$	$17 \pm 0$	$26.7\pm0.5$	$0.396\pm0.011$	$0.60\pm0.04$	$0.57\pm0.04$

**Table 9.3:**  $T_2^*$  and corrected signal intensity (SI) of mouse lung parenchyma.

No data for mouse lung  $T_2^*$  was previously reported at 7 T. Olsson et al. found a  $T_2^*$  of 0.46 ± 0.05 ms in lung of 8-week-old C57BL/6 mice at 4.7 T (41), and Togao et al. reported a  $T_2^*$  of 0.91 ± 0.10 ms in 8-week-old mice at 3 T (8). Takahashi also reported a  $T_2^*$  of 1.00 ± 0.13 ms in 5-6 week-old mice at 3 T (5). The shorter  $T_2^*$  of 0.394 ± 0.040 ms in 22-23 week-old mice and 0.396 ± 0.011 ms in 17-week-old in this dissertation (**Table 9.3**), is due to the higher field inhomogeneity near the air-tissue interfaces inside the lung induced by stronger field and air-tissue susceptibility difference. The  $T_2^*$  of heart is much longer than the  $T_2^*$  of lung (40). To compare the lung-to-heart density ratio with CT data, the heart-normalized lung parenchyma SI was corrected by  $T_1$  and  $T_2^*$  to estimate normalized lung parenchyma density, and was 0.61 ± 0.06 and 0.57 ± 0.04 for 22-23 week old mice and 17 week old mice respectively. The mass density of mouse lung measured by  $\mu$ CT at end-expiration was 0.57 g/cm<sup>3</sup> as reported previously (41). Considering the mass density of soft tissue, 1.07 g/cm<sup>3</sup> (41, 131), the density ratio of

lung/heart is 0.53, close to the value estimated from MRI signal in this dissertation. Assuming the lung density is inversely proportional to lung volume, the normalized lung parenchyma density at end-expiration was divided by an inspiration-to-expiration volume ratio of 1.37 (**Table 9.1**) to estimate the normalized lung parenchyma density at end-tidal-inspiration, which was 0.44 and 0.42 for 22-23 week old mice and 17 week old mice respectively.

# Chapter 10 Application of retrospective self-gating UTE to imaging pulmonary fibrosis in TGF-α mice

Pulmonary fibrosis remains a significant public health burden that contributes to morbidity and mortality in a number of lung diseases including cystic fibrosis, interstitial lung diseases such as idiopathic pulmonary fibrosis (IPF), and some systemic connective tissue diseases. It also occurs in response to many types of lung injuries like the ones induced by radiation or chemotherapeutic drugs (19-23). As a progressive process, pulmonary fibrosis is characterized pathologically by mesenchymal cell proliferation, expansion of the extracellular matrix, and extensive remodeling of the lung parenchyma and airways (19, 22). Previous studies suggested that pulmonary fibrosis in diseases such as IPF is due to a chronic inflammatory process that initiates focal accumulation of extracellular matrix in the interstitium (132). However, the clinical measurements of inflammation fail to correlate with disease severity in IPF and initial therapies focusing on aggressive anti-inflammatory treatment have not improved lung function or survival (133). Although no therapies exist for pulmonary fibrosis (133, 134) currently, studies investigating the pathophysiology of lung fibrosis show that inflammation is not responsible for the fibrosis development (134). The molecular pathways and cellular mechanisms leading to fibrosis still remain poorly understood and deserve thorough investigation in the absence of inflammation to identify new therapeutic targets. For this purpose, animal models with rodents, particularly mice (24) are critical to lung fibrosis study. In this dissertation, we used a mouse model of pulmonary fibrosis that overexpresses transforming growth factor (TGF)- $\alpha$  in the epithelium under control of doxycycline (Dox)-regulatable Clara cell secretory protein promoter (25). The retrospective

self-gating UTE MRI, as described in Chapter 5, was utilized to longitudinally monitor *in vivo* the development of pulmonary fibrosis in mice.

### **10.1 Disease model**

The epidermal growth factor receptor (EGFR) is a ubiquitous, highly conserved 170-kDa membrane-spanning glycoprotein that is expressed by many cell types in the lung, including the epithelium, smooth muscle cells, endothelium, and fibroblasts (135). One of the ligands for the EGFR found in the lung is TGF- $\alpha$ . In the past decades, many experimental and clinical studies have implied or demonstrated the role for EGFR and TGF- $\alpha$  in the pathogenesis of pulmonary fibrosis (136-140). Several more studies were further designed showing that constitutive or conditional expression of TGF- $\alpha$  in the respiratory epithelium of transgenic mice caused peribronchial, perivascular, and pleural fibrosis in the absence of inflammation (25, 141, 142). In this dissertation, transgenic mice were designed in which the expression of TGF- $\alpha$  was placed under the control of a doxycycline-inducible gene control system directed by the 2.3-kb rat Clara cell secretory protein (CCSP) gene promoters (25).

Transgenic mouse lines were previously generated bearing the reverse tetracycline-responsive transactivator (rtTA) fusion protein under control of the 2.3-kb rat CCSP gene promoter (143), as illustrated in the top part of **Figure 10.1**. Separate transgenic mouse lines were generated by microinjection of a (TetO)<sub>7</sub>-cmv-TGF- $\alpha$  transgene. The (TetO)<sub>7</sub>-cmv-TGF- $\alpha$  transgene consists of seven copies of the tet operator DNA binding sequence linked to a minimal cytomegalovirus (CMV) promoter, the human TGF- $\alpha$  cDNA, and SV40 polyadenylation signal, as shown in the bottom part of **Figure 10.1**. Bitransgenic mice were generated by mating CCSP rtTA mice to (TetO)<sub>7</sub>-cmv-TGF- $\alpha$  mice. All mice were derived from the FVB/NJ inbred strain. Mice were housed under pathogen-free conditions and handled in accordance with protocols approved by

the Institutional Animal Care and Use Committee of the Cincinnati Children's Hospital Research Foundation. To induce TGF- $\alpha$  expression in the bitransgenic mice, Dox in food or drinking water was administered to animals. The schematic process of Dox regulating conditional expression of TGF- $\alpha$  is also shown in **Figure 10.1**.



**Figure 10.1:** Constructs used to generate Dox-regulatable transgene expression in the lung. The CCSP-rtTA transgene (top) consists of the 2.3-kb rat CCSP promoter, 1.0-kb rtTA coding sequence, and SV40 polyadenylation signal. The (TetO)7-cmv-TGF- $\alpha$  consists of seven copies of the tet operator, a CMV minimal promoter, the human TGF- $\alpha$  cDNA, and SV40 polyadenylation signal. The resulting transgenic mice were called TGF- $\alpha$  transgenic mice for simplicity in this study, in which pulmonary fibrosis can be induced by continuous administration of Dox.

# **10.2 Animal groups**

Only bitransgenic mice were used in this study and were divided into two cohorts. One cohort of

mice (N = 6) were fed Dox food for 8 weeks (referred to as Dox cohort in all subsequent

contexts), while the other cohort (N = 5) were fed normal food (referred to as Control cohort in

all subsequent context). To longitudinally monitor the progression of pulmonary fibrosis, both cohorts were imaged by MRI at the same time points: baseline (the day just before Dox treatment), 4 weeks and 8 weeks of Dox treatment (Dox week 4 and Dox week 8, respectively, in later text). After the final MRI session, mice were placed under a Flexi Vent system (SCIREQ, Montreal, PQ, Canada) for lung mechanics and then euthanized by an overdose of sodium pentobarbital (65 mg/ml, Fort Dodge Animal Health, Fort Dodge, IA) for histology and hydroxyproline assay.

### 10.3 MR imaging

All imaging experiments were performed on a Bruker 7 T (Bruker, Billerica, MA) with a homebuilt quadrature birdcage transmitter/receiver coil (inner diameter = 35 mm, and length = 50 mm). Animals were anesthetized by 1.5-2% isoflurane (in air) to maintain the respiratory rate at 80-120 breaths per minute and placed supine for free breathing with the lung in the center of the RF coil . A small-animal monitoring system (SA Instruments, Inc., Stony Brook, NY) was used to monitor the respiratory rate via a small pneumonic sensor secured on the animal abdomen, and maintain the body temperature at 34 °C.

All images were acquired by the self-gating UTE with an ellipsoidal k-space coverage, as previously described in section 5.3. 2D golden means were used to generate ellipsoidal k-space sampling pattern as described in section 5.2. MRI acquisition parameters included k-space expansion factors  $\alpha_x = \alpha_y = 2$ ,  $\alpha_z = 1$ , number of points per projection = 64, number of projections = 154440, receiver bandwidth = 200 kHz, TE = 0.108 ms, TR = 6 ms, FA = 6.3°, FOV = 30 mm × 30 mm × 60 mm, and voxel = 0.23 mm × 0.23 mm × 0.47 mm. Total acquisition time for each mouse was ~ 15.4 min, ignoring dummy scans. Approximately 30-40% and 4-8% of the total

projections were retrospectively registered for end-expiration and end-inspiration, respectively, depending on the respiratory rate.

### **10.4 Data analysis**

#### **10.4.1 Lung histology**

Lung histology was done as previously described (25, 143). Lungs were inflation fixed using 4% paraformaldehyde at 25 cm H<sub>2</sub>O pressure and then allowed to fix overnight at 4 °C, washed with phosphate-buffered saline (PBS), dehydrated through a graded series of ethanols, and processed for paraffin embedding. Paraffin embedded sections (5  $\mu$ m thick) were loaded onto polysine slides for hematoxylin and eosin (H & E) staining. Histomorphometric measurements of subpleural thickness were obtained from lung sections by using the measured distance function in MetaMorph (Molecular Devices, LLC., Sunnyvale, CA) as previously described (144). Five random measures per lung section were obtained for each animal by using a Leica DM2700 M bright-field microscope (Leica Microsystems, Buffalo Grove, IL). High-magnification images (×40) were captured with a 3CCD color video camera and analyzed with MetaMorph.

#### **10.4.2 Lung mechanics**

Lung mechanics were assessed on mice with a computerized Flexi Vent system (SCIREQ, Montreal, PQ, Canada), as previously described (145, 146). Mice were anesthetized intraperitoneally with 0.1 ml/10 g body weight PBS solution containing 178 mM (40 mg/ml) ketamine and 7.8 mM (2 mg/ml) xylazine. To determine dynamic lung compliance, mice were tracheostomized and ventilated with a tidal volume of 8 ml/kg at rate of 450 breaths/min and positive end-expiratory pressure of 2 cm H<sub>2</sub>O. To determined respiratory impedance, the ventilation mode was changed to forced oscillatory signal (0.5-19.6 Hz). The outputs of Flexi Vent measurement that characterize lung function are airway resistance, airway elastance, and lung compliance. Airway resistance quantitatively assesses the level of constriction in the lungs. Airway elastance captures the elastic rigidity or the stiffness of the lungs. Lung compliance captures the ease with which the lungs can be extended.

#### **10.4.3 Hydroxyproline assay**

Hydroxyproline is a major component of the protein collagen, which is found in few other proteins. That makes hydroxyproline content a critical indicator of collagen content in the lung or pulmonary fibrosis. A hydroxyproline assay was performed as previously described (25). Briefly, lung tissue was lyphophilized overnight, and 10 mg of dried tissue were incubated overnight in 500 µl of 6 N HCl. Five microliters of the samples and standards were applied to an ELISA plate. Fifty microliters of citric-acetate buffer (5% citric acid, 7.24% sodium acetate, 3.4% NaOH, and 1.2% glacial acetic acid, pH 6.0) and 100 µl of chloramines T solution (564 mg of chloramines T, 4 ml of H<sub>2</sub>O, 4 ml of n-propanol, and 32 ml of citrate-acetate buffer) were added and incubated for 20 min at room temperature. Then 100 µl of Ehrlich's solution (4.5 g 4-dimethylaminobenzaldehyde, 18.6 ml n-propanol, and 7.8 ml sulfuric acid) were added and incubated for 15 min at 100 °C. Reaction product was read at optical density of 525 nm light. Hydroxyproline (Sigma, St. Louis, MO) standard solutions of 0-800 µg/ml were used to construct the standard curve.

#### **10.4.4 Lung function parameters by MRI**

Lung parenchyma signal intensity, tidal volume, high-density lung parenchyma percentage (SI > 0.8) and functional residual capacity (FRC) were measured and calculated from MRI data by the method described in section 7.3.

#### **10.4.5 Statistics**

To examine the differences between the control and Dox cohorts, a two-way unpaired Student's t test was performed to compare the means of the MRI-derived lung function parameters at baseline and a one-way unpaired Student's t test was used to compare the means of the MRI-derived lung function parameters at Dox week 4 and Dox week 8. A one-way Student's t test was also applied to histology, lung mechanics, and hydroxyproline content. In addition, to examine the differences between two time points for MRI-derived lung function parameters, a paired Student's t test was also performed.

The R language (89) and lme4 package (88) were used to perform a linear mixed effects analysis of the relationship between MRI-derived lung function parameters and time. As a fixed effect, time was entered into the model. As random effects, intercepts for subjects (individual mice) and by-subject random slopes for the effect of time were entered into the model. P-values were obtained by likelihood ratio test (using ANOVA) of the full model with the effect in question against the model without the effect in question.

An  $\alpha$ -level of 0.05 was used for all tests, i.e., differences were considered significant when P < 0.05.

### **10.5 Results**

3D MR images covering entire mouse lungs were successfully acquired by self-gating UTE and are of high quality with minimal motion artifacts. **Figure 10.2** presents representative axial images at FRC from a control mouse and 2 Dox mice at all 3 time points along with the photomicrographs of histological slides at Dox week 8. Signal-to-noise ratio (SNR) in the lung parenchyma (away form airways & vessels) of control mice is about 18. Consistent with previous studies of the TGF- $\alpha$  model, histology can distinguish between fibrotic and non-fibrotic tissue and detect differences in fibrotic burden between control and Dox cohorts in this study (25), as seen in the photomicrographs in **Figure 10.2**. Histology qualitatively shows that the pleura of the control mouse is thinner than that of the Dox mice and is with thickness similar to the alveolar walls. No pulmonary fibrosis was visually seen at baseline in Dox cohort and at all 3 time points in control cohort, as displayed by low MRI signal throughout the lung parenchyma excluding the larger blood vessels. The volume-increased blood vessels indicated by the blue arrows showed that perivascular fibrosis began to appear at Dox week 4 and became severe at Dox week 8. High-signal structures of pulmonary fibrosis also appeared near the subpleural regions (indicated by a red arrow) at Dox week 4 and extended into the interstitium (indicated by a yellow arrow) at Dox week 8. The volume of high-signal structures for Dox cohort increased significantly at Dox week 8 as shown in **Figure 10.2** (A). The severity of fibrosis also varied significantly between Dox mice as seen by the volume of high-signal structures at Dox week 8 and also the histological slides in **Figure 10.2** (A) vs. (B). The MR signal differences visually demonstrated between Dox and control cohorts were consistent with the histological slides.



**Figure 10.2:** Representative MR images (TR = 6 ms, TE = 0.108 ms, FA =  $6.3^{\circ}$ ) of (A) one Dox mouse with severe fibrosis, (B) one Dox mouse with intermediate fibrosis, and (C) one control mouse, at baseline, Dox week 4 and Dox week 8, plus corresponding histological slides (H&E stain; original magnification, ×1) at Dox week 8. No pulmonary fibrosis was visually seen at baseline in the Dox cohort and at all 3 time points in the control cohort. Perivascular fibrosis appeared in both Dox mice at Dox week 4 and Dox week 8 (pointed by blue arrows) in (A) & (B). Fibrosis also appeared near the plerual region at Dox week 4 (red arrow) and extended into the interstitium at Dox week 8 (yellow arrow), as seen in (B). Apparent interstitial fibrosis also appeared in the Dox mouse in (A) at Dox week 8. Fibrosis severity varied among different mice as demonstrated in (A) & (B) at Dox week 8.

Besides the visual agreement, MRI-derived lung function parameters obtained at Dox week 8 are

also correlated with histological measurements, hydroxyproline content, and pulmonary

mechanics (Figure 10.3). Specifically in Figure 10.3 (I)-(L), tidal volume (TV) measured by

MRI and hydroxyproline content demonstrated the strongest correlation with R = -0.95; TV was

also strongly correlated with pulmonary mechanics parameters: airway resistance (R = -0.76),

and compliance (0.88); TV was negatively correlated with pleural thickness (R = -0.86). High-

density lung volume percentage was observed to positively correlate with hydroxyproline (R =
0.80), airway resistance (R = 0.50), and pleural thickness (R = 0.89), and negatively correlate with compliance (R = -0.58), as shown in **Figure 10.3** (E)-(H). Finally, mean lung-parenchyma signal increased with increasing hydroxyproline content, airway resistance, and pleural thickness (R = 0.70, 0.45, and 0.78, respectively), and increased with decreasing compliance (R = -0.50) as demonstrated by **Figure 10.3** (A)-(D). To sum up, all three MRI-derived quantities were correlated with histological measurements, hydroxyproline content, and pulmonary mechanics to different extents in quantifying fibrotic burden.



**Figure 10.3:** Correlations between MRI-derived parameters (mean lung-parenchyma signal, high-density lung volume percentage, TV) and biochemical measurements (hydroxyproline content), pulmonary mechanical measurements (airway resistance, compliance), and histological measurements (pleural thickness). Of all three MRI-derived parameters, TV demonstrated the strongest correlation with non-MRI measurements as seen in (I)-(L).

Group-wise comparisons by MRI-derived lung function parameters, and non-MRI measurements (pulmonary mechanics, pleural thickness and hydroxyproline content) are summarized in Figure 10.4, Figure 10.5,

Table 10.1 and Table 10.2. By unpaired student t test, differences between cohort means were all statistically significant for non-MRI measurements. Specifically, hydroxyproline content was most sensitive to distinguish between cohorts (P < 0.0000001). Airway Resistance, compliance, and pleural thickness were also able to distinguish between the Dox and control cohorts (P < 0.01).



**Figure 10.4:** Non-MRI measurements of fibrosis: (A) hydroxyproline content, (B) airway resistance, (C) compliance, and (D) pleural thickness for control (n = 5) and Dox (n = 6) cohorts. The bar graphs were created based on the mean and standard error (SE) of each parameter. All of these non-MRI measurements can significantly discriminate between control and Dox cohorts, with hydroxyproline content showing the largest significance.

Cohort	Mouse #	Hydroxyproline Content (µmoles/right lung)	Airway Resistance (cmH <sub>2</sub> O•s/ml)	Compliance (ml/cmH <sub>2</sub> O•kg)	Pleural Thickness (µm)	
Dox	467	3.61	0.92	0.02	69.85	
	468	3.44	1.19	0.01	77.68	
	469	3.72	0.60	0.02	26.78	
	476	3.90	0.71	0.02	96.46	
	478	3.79	1.03	0.01	115.51	
	479	3.29	0.73	0.02	49.06	
	Mean	3.63	0.86	0.02	72.56	
	SE	0.09	0.09	0.00	13.04	
Control	477	1.20	0.48	0.04	9.83	
	480	1.24	0.45	0.05	6.49	
	484	1.07	0.50	0.04	7.38	
	485	1.31	0.57	0.04	7.04	
	486	1.25	0.71	0.02	6.54	
	Mean	1.21	0.54	0.04	7.46	
	SE	0.04	0.05	0.00	0.62	

**Table 10.1:** Summary of hydroxyproline content, airway resistance, compliance, and pleural thickness of lung for individual mice at Dox week 8.

Differences between cohort means were also seen for MRI-derived lung function parameters. By Dox week 8, high-density lung volume percentage, mean lung-parenchyma signal, and TV were all able to discriminate the control and Dox cohorts (P = 0.006 for high-density lung volume percentage, P = 0.011 for mean lung-parenchyma signal, and P < 0.00001 for TV). By Dox week 4, all 3 MRI-derived parameters also discriminated between control and Dox cohorts (P = 0.033, 0.006, and 0.003 for mean lung-parenchyma signal, high-density lung volume percentage, and TV, respectively). At baseline, differences between cohort means for mean lung-parenchyma signal and high-density lung volume percentage were not significant, however the TV of the Dox cohort was significantly larger than that of the control cohort (P = 0.026).



**Figure 10.5:** Cross-sectional (between control and Dox mice) and longitudinal (between baseline, Dox week 4, and Dox week 8) monitoring of fibrotic burden by (A) mean lung-parenchyma signal, (B) high-density lung volume percentage, and (C) tidal volume (TV). The bar graphs represent mean  $\pm$  SE. Of all three MRI-derived parameters, high-density lung volume percentage and TV were better able to detect differences between time points, and between cohorts.

Comparisons between time points for MRI-derived lung function parameters by paired Student's t test are summarized in **Figure 10.5** and **Table 10.2**. Mean lung-parenchyma signal for the Dox cohort increased significantly from baseline to Dox week 4 (P = 0.009). High-density lung volume percentage for the Dox cohort also increased significantly over time (P = 0.008 between baseline and Dox week 4, P = 0.02 between Dox week 4 and Dox week 8). Contrarily, tidal volume (TV) for the Dox cohort decreased significantly over time (P = 0.003 between baseline and Dox week 4, P = 0.014 between Dox week 4 and Dox week 8), whereas TV for the control cohort increased significantly over time (P = 0.003 between baseline and Dox week 4, P = 0.022 between Dox week 8).

		Mean Lung-			Н	High-density			TV (ml)		
		Parenchyma Signal			Lung V	Lung Volume Percentage					
Cohort	Mouse #	Baseline	week 4	week 8	Baseline	week 4	week 8	Baseline	week 4	week 8	
Dox	467	0.59	0.68	0.61	6.71%	13.02%	22.75%	0.22	0.21	0.12	
	468	0.50	0.68	0.70	5.15%	16.34%	29.80%	0.22	0.13	0.10	
	469	0.58	0.66	0.63	5.98%	6.75%	19.91%	0.20	0.14	0.13	
	476	0.48	0.55	0.90	3.48%	13.06%	69.67%	0.25	0.20	0.06	
	478	0.51	0.62	0.79	4.45%	8.21%	44.22%	0.22	0.19	0.07	
	479	0.58	0.57	0.73	5.36%	20.72%	25.99%	0.20	0.13	0.11	
	Mean	0.54	0.63	0.73	5.19%	13.01%	35.39%	0.22	0.17	0.10	
	SE	0.02	0.02	0.04	0.46%	2.10%	7.69%	0.01	0.02	0.01	
Control	477	0.60	0.52	0.44	5.02%	3.33%	4.46%	0.19	0.23	0.35	
	480	0.46	0.56	0.63	4.15%	5.00%	7.87%	0.20	0.23	0.33	
	484	0.67	0.56	0.65	9.07%	3.92%	7.41%	0.18	0.22	0.32	
	485	0.55	0.42	0.55	4.82%	5.22%	4.67%	0.20	0.26	0.27	
	486	0.48	0.64	0.57	5.93%	9.00%	5.02%	0.20	0.22	0.22	
	Mean	0.55	0.54	0.57	5.80%	5.30%	5.89%	0.19	0.23	0.30	
	SE	0.04	0.04	0.04	0.87%	0.99%	0.72%	0.00	0.01	0.02	

**Table 10.2:** Summary of mean lung-parenchyma signal, high-density lung volume percentage, and tidal volume (TV) for individual mice.

The results of performing a linear mixed effects analysis over MRI-derived longitudinal lung function parameters to study the progression of pulmonary fibrotic burden are given in **Figure 10.6**. The tidal volume (TV) was the most sensitive measure of lung fibrotic progression, which decreased significantly at a rate of 0.015 ml/week ( $\chi^2(1) = 13.56$ , P = 0.0002) for the Dox cohort. However, for the control cohort, TV increased significantly at a rate of 0.013 ml/week ( $\chi^2(1) = 8.37$ , P = 0.004). Mean lung-parenchyma signal also increased significantly at a rate of 2.3% /week ( $\chi^2(1) = 6.44$ , P = 0.01) for the Dox cohort, but didn't change significantly over time for the control cohort. High-density lung volume percentage showed a progressive pattern similar to mean lung-parenchyma signal, with an increasing rate of 3.8%/week for the Dox cohort ( $\chi^2(1) = 7.60$ , P = 0.006).



**Figure 10.6:** Longitudinal analysis by linear mixed effects model over (A) mean lung-parenchyma signal, (B) highdensity lung volume percentage, and (C) tidal volume (TV). All 3 MRI-derived parameters for Dox mice changed significantly with time, with TV being the most robust. Contrary to Dox mice, TV for control mice increased significantly with time.

### **10.6 Discussion**

Self-gating UTE was successfully performed in mouse lung MR imaging, with minimal motion artifacts and high SNR in lung parenchyma as shown in **Figure 10.2**. Prospective respiratory gating requires either passive breathing of animals under control of ventilator or a pressure sensor tightly secured to the abdomen of animals, which may deviate the lung from its normal state (for example, TV and compliance), leading to measurement errors. The self-gating UTE MRI, however, can image the lung in its normal state, except that animals were anesthetized. The retrospective registering method using the combination of 1<sup>st</sup>-order and 2<sup>nd</sup>-order derivatives of the artificial respiratory waveform as a functions of time (which was generated from MR FIDs) was developed and used for the first time in this study. This registration method is superior to the traditional registration method (147) in minimizing motion artifacts in the sense that the former can separate the data only at end-expiratory waveform, inevitably mixes data at other respiratory phases into end-inspiratory or end-expiratory data. Because the images acquired at end-inspiration and end-expiration are the focus of interest of many studies that can provide good

measures of lung function such as FRC, TV, and mean lung parenchyma signal, our registration method is more robust in quantifying lung diseases in animal models. The fact that little difference (both visually and statistically) was seen in mean lung parenchyma signal between control and Dox cohorts at baseline (**Figure 10.4**) also speaks to the robustness of our self-gating UTE.

With self-gating UTE MRI, we have successfully demonstrated the ability to longitudinally monitor the progression of pulmonary fibrosis in a TGF- $\alpha$  mouse model. In this TGF- $\alpha$  mouse model of pulmonary fibrosis, pulmonary fibrogenesis was induced by continuous administration of Dox, leading to fibrosis progressing with time (25). This fibrosis progression was also visually seen in the MR images of the Dox cohort as compared to that of the control cohort, with collagen and extracellular matrix deposition (manifested as high signal in MR images) gradually appearing and increasing in the perivascular, subpleural, and interstitial regions (**Figure 10.2**). The high signal structure (indicated by red arrows) near the pleura in **Figure 10.2** (B) at Dox week 4 was not likely to be atelectasis caused by anesthetization, because it was not resolved and still at the same location at Dox week 8. The existence of pulmonary fibrosis in the Dox cohort was further verified by the photomicrographs of histological slides, especially the thickening of pleura.

The formation of pulmonary fibrosis was also quantitatively and statistically verified by the significant correlations between MRI-derived lung function parameters and pleural thickness, hydroxyproline content, and pulmonary mechanical parameters (**Figure 10.3**). However, the strength and direction of the correlations varied, depending on the tested parameters which reflect different aspects of lung properties. Among the MRI-derived parameters, the tidal volume (TV) was observed to have the strongest correlation with other non-MRI-derived parameters. In

particular, TV was negatively correlated with hydroxyproline content and positively correlated with compliance. This is reasonable, because TV is similar to compliance in the sense that they both reflect the ease of lung to inflate, while hydroxyproline content reflects the amount of collagen or fibrotic severity in the lung, the increase of which decreases the ease of lung to inflate (146). TV was negatively correlated with airway resistance and pleural thickness as well, because the increase of these two parameters in the TGF- $\alpha$  mouse model also results from the increase of fibrotic burden (146). However, contrary to TV, both high-density lung volume percentage and mean lung-parenchyma signal were observed to have reversed correlations with other non-MRI-derived parameters, because they both increased with fibrotic burden (i.e., collagen and extracellular matrix deposition) during fibrotic progression. It is worth noting that the correlation strength (R = 0.70) between mean lung-parenchyma signal and hydroxyproline content observed in this study was close to that (R = 0.87) found in a previous study in intratracheal-bleomycin induced lung fibrosis in C57BL/6 mice, which were also imaged by the UTE sequence (148).

In addition to the significant correlations between MRI-derived lung function parameters and non-MRI-derived parameters, each of these parameters can individually distinguish between the Dox and control cohorts at Dox week 8 by statistically significant differences (**Figure 10.4** & **Figure 10.5**), endorsing the reliability of this TGF- $\alpha$  mouse model in conditionally generating pulmonary fibrosis under control of Dox, and further strengthening the robustness of MRI methods for quantifying and monitoring pulmonary fibrosis. All MRI-derived parameters were even able to distinguish between the Dox cohort with much less fibrosis and the control cohort at Dox week 4, demonstrating the high sensitivity of MRI for quantifying pulmonary fibrosis. However, high-density lung volume percentage and TV were more sensitive than mean lung-

parenchyma signal in longitudinally monitoring pulmonary fibrosis, as the former two parameters distinguished between baseline and Dox week 4 as well as between Dox week 4 and Dox week 8 with larger significance, and mean lung-parenchyma signal did not discriminate between Dox week 4 and Dox week 8 with statistical significance (**Figure 10.5**). TV increased significantly between Dox week 4 and baseline, and between Dox week 8 and Dox week 4 for the control cohort, contrary to the trend for the Dox cohort, emphasizing the negative effects of fibrosis on lung ventilation.

Finally as measured by the linear mixed effects model, all three MRI-derived parameters were ready to quantify the rate of fibrosis progression, with high-density lung volume percentage and TV being the most robust measures of fibrosis progression according to statistical significance. In addition, the TV of the Dox cohort showed a trend opposite to that of the TV of the control cohort, consistent with the Student's t test, indicating that TV was most sensitive to detecting fibrosis progression.

### **10.7 Conclusions**

We have demonstrated, for the first time, the application of self-gating UTE MRI with ellipsoidal k-space coverage to monitor lung fibrosis in a TGF- $\alpha$  transgenic mouse model. MRI-derived lung function parameters were well correlated with pulmonary-mechanical, histological, and biochemical measurements in quantifying fibrotic burden. These MRI-derived parameters were also able to differentiate cohorts receiving different treatments (with/without Dox administration) and track fibrosis progression in individual animals. This is the first time that TV has been shown to correlate with pulmonary fibrosis with high robustness and sensitivity.

# Chapter 11 Conclusions

### **11.1 Accomplishments**

#### 11.1.1 GRE quantifying acute and chronic rejection in lung Tx model

We have successfully performed orthotopic vascularized aerated lung transplantation in mice (16), as well as demonstrated the ability to acquire <sup>1</sup>H MR images of the murine lung using GRE MRI with respiratory gating in longitudinal studies of both acute (ACR) and chronic cellular rejection in lung transplantation, despite the fact that ACR and chronic cellular rejection were induced by different mechanisms. By repeated imaging across several time points in the same animals, we were able to measure clear parenchymal signal increases as well as significant decreases of lung compliance in the rejecting lung of the ACR model. We also observed consistently higher high-signal lung volume percentage in the rejecting lungs of the chronic cellular rejection model than that in the controls. Notably, We have demonstrated the ability of respiratory-gated GRE MRI to longitudinally monitor both acute and chronic cellular rejections, which is readily translatable to clinical studies of lung transplantation.

#### **11.1.2 UTE with ellipsoidal k-space coverage**

We have devised a method for reducing the field of view (FOV) for a radially-sampled UTE MRI through deforming the spherical k-space coverage into an ellipsoidal k-space coverage by simple expansion in one plane. Point-spread function (PSF) analysis demonstrates the in-plane resolution enhancement and FOV reduction by the UTE with ellipsoidal k-space coverage, as well as the influence of  $T_2$ \* relaxation on single-voxel SNR and resolution. This modified UTE with ellipsoidal k-space coverage was successfully applied to imaging phantoms and the murine lungs, the results of which correlate with the PSF analysis. We also applied this modified UTE to measuring the  $T_1$  of lung parenchyma through a limited flip-angle method (2). An interleaved multi-TE method for UTE MRI was developed to measure  $T_2^*$  of the healthy mouse lungs at 7 T for the first time.

#### **11.1.3 Retrospective self-gating UTE**

Although retrospective self-gating UTE has gained wide-spread application, particularly in cardiac imaging to date, it has been under-utilized in pulmonary imaging. To compensate for the possible motion artifacts associated with traditional registration method (direct thresholding), we developed a novel method by combining the first- and second-order derivatives of the artificial respiratory waveform to extract data for expiration or inspiration phase, without the need to physically monitor the respiratory waveform. Here, the artificial respiratory waveform was created from the amplitude of FID data. Our registration method better minimizes motion artifacts by exclusively separating the data for end-expiration and end-inspiration, as compared to direct thresholding (147), which inevitably mixes in data from other respiratory phases.

We have demonstrated, for the first time, the application of self-gating UTE MRI with ellipsoidal k-space coverage to longitudinally monitor the progression of pulmonary fibrosis in a TGF- $\alpha$  transgenic mouse model (25). Lung function parameters such as mean lung-parenchyma signal, high-density lung volume percentage, and tidal volume were derived from MR images and correlated well with pulmonary mechanical, histological, and biochemical measurements in quantifying fibrotic burden. These MRI-derived parameters were also able to differentiate cohorts with and without pulmonary fibrosis and track fibrosis progression in individual animals. In addition, to our knowledge, tidal volume was, for the first time, found in this study to quantify pulmonary fibrosis with high robustness and sensitivity.

### **11.2 Limitations**

Even though GRE <sup>1</sup>H MRI is advantageous for its ease of use and short scanning time, it suffers from low SNR in pulmonary imaging, due to a low proton density in healthy lung parenchyma and intrinsically short  $T_2^*$ , which in turn provides an advantage in terms of high contrast between normal lung parenchyma and consolidated lung parenchyma caused by diseases such as pulmonary fibrosis, pulmonary edema, and allograft rejection. However, GRE MRI is not sensitive to low density changes, for example, at the early stages of lung disease.

UTE MRI, for the purpose of this dissertation, generally has TE shorter than 0.1 ms, compensating for  $T_2^*$  related signal decay and increasing the SNR in low-density lung parenchyma. However, its scanning time is longer than that of GRE for the same resolution and FOV. Additionally, because the online reconstruction cannot perfectly deal with the signal buildup and ringing at the beginning of each FID, reconstruction must be performed offline.

<sup>1</sup>H MRI may not be able to discriminate between atelectasis and high-density regions in lung caused by diseases like pulmonary edema in a cross-sectional study. Generally a longitudinal study can be used to discriminate atelectasis and disease-induced high-density regions because atelectasis is resoluble. Additional MR properties (not studied here) such as  $T_1$ ,  $T_2$ ,  $T_2$ \*, and apparent diffusion coefficient can also be measured in <sup>1</sup>H MRI and may provide more information to better characterize pulmonary diseases.

#### **11.3 Directions for future study**

We have demonstrated the application of retrospective self-gating UTE MRI, UTE MRI with ellipsoidal k-space coverage, and the combination of them to imaging phantoms, healthy mice, and mouse model of fibrosis. We hope to expand the application of our technique to quantifying other preclinical disease models such as the alveolar simplification in neonatal mice, and the

mouse lung with amyotrophic lateral sclerosis. Additionally, these UTE MRI methods may be combined with hyperpolarized gas MRI, using such nuclei as <sup>129</sup>Xe and <sup>3</sup>He, for pulmonary imaging, to fully utilize the imaging toolbox at the Center for Pulmonary Imaging Research for quantifying lung function.

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## **Appendix I**

```
%%Author: Jinbang Guo; sampling density compensation for 3D k-space
clear all;
NPro = 51480; %Number of projections.
beta = 4; % For psf calculation, the same as in grid3; ratio of maximum
expansion factor and current expansion factor; alpha x=[1 \ 2 \ 4 \ \ldots],
alpha y=[1 2 4...].
Matrix = 128; %Matrix size of final images.
NPoints = 90; %Number of points along each projection, = Matrix/2 + NPShift +
            %gradient ramp points.
fidpoints = 128; %Number of points in each FID ( = NPoints + numbe of zero-
                  %filling points).
NPShift = 20; %number of points shifted to compensate for signal build-up and
                %ringing at the beginning of FID.
path = '/home/jinbang/Recon'; % Directory of raw data.
fileNumber = 19; % file number for a specific scan
rampoints = NPoints - NPShift - Matrix/2; %Number of points sampled during
                                           %gradient ramp.
RespMode = 'measured'; %How was the trajetory generated? Theoretical, or
                      %Measured?
RealNPoints = NPoints - NPShift;
realpath = fullfile(path,num2str(fileNumber(i)));
trajfilename = strcat('traj ', RespMode);
DCFfilename = strcat('DCF ', RespMode, '.raw');
% load coordinates
fid = fopen(fullfile(realpath,trajfilename));
tmp = squeeze(fread(fid, inf, 'double'));
fclose(fid);
tmp = reshape(tmp,[3,NPoints(i),NPro(i)]);
crds = tmp(:,1:RealNPoints,:);%cut ending points along one spoke;
r = sqrt(crds(1,RealNPoints,:).^2 + crds(2,RealNPoints,:).^2 +
crds(3,RealNPoints,:).^2);
crds = crds./max(r(:))/2;
crds = crds./max(r(:))/2/beta; % Normalized k-space to [-0.5 0.5].
disp(['generating DCF for ',realpath]);
   SDC params:'
numIter = 25; %Number of iternations to iteratively calculate sampling
              %density compensation.
effMtx = (RealNPoints - rampoints(i)) * 2; %Matrix size of images.
osf = 2.1; %oversampling ratio of intermediate grid.
verbose = 1;
' start SDC calc'
DCF = sdc3 MAT(crds,numIter,effMtx,verbose,osf); %Call the sampling density
                                    %compensation package from Zwart et al.
DCF = single(DCF); % float32
' write output DCF'
tmp = DCF;
fid = fopen(fullfile(realpath,DCFfilename),'w');
```

%End

```
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```

## **Appendix II**

```
%%Author: Jinbang Guo; Regridding and image reconstruction.
function grid3 (NPoints, NPro, rampoints, fidpoints, NPShift, RespMode, path)
alpha=2; %gridding oversampling ratio.
RealNPoints = NPoints - NPShift; % actual number of points encoded;
trajfilename = strcat('traj_',RespMode);
DCFfilename = strcat('DCF ', RespMode, '.raw');
fidname = strcat('fid ',RespMode);
imagefilename = strcat('img ',RespMode,'.raw');
%Read and normalize k-space coordinates
fid = fopen(fullfile(path,trajfilename));
tmp = squeeze(fread(fid, inf, 'double'));
fclose(fid);
tmp = reshape(tmp,[3,NPoints,NPro]);
crds = tmp(:,1:RealNPoints,:);
r = sqrt(crds(1,RealNPoints,:).^2 + crds(2,RealNPoints,:).^2 +
crds(3,RealNPoints,:).^2);
crds = crds./max(max(max(r)))/2;
%Read sampling density compensation function.
' read pre-weights'
fid = fopen(fullfile(path, DCFfilename));
tmp = squeeze(fread(fid, inf, 'float32'));
fclose(fid);
DCF = reshape(tmp, [RealNPoints, NPro]);
%Read FID data.
' read k-space data'
fid = fopen(fullfile(path,fidname));
tmp = squeeze(fread(fid, inf, 'int32'));
fclose(fid);
tmp = reshape(tmp,[2,fidpoints,NPro]);
data = squeeze(tmp(:,(NPShift +1):NPoints,:));
clear tmp r;
%Regridding
' Grid3 params:'
effMtx = (RealNPoints - rampoints) * 2* alpha; % effective matrix size of
                                       %images after gridding oversampling.
% numThread = 8 % or 1 for no pthread lib exec
numThread = 1;
%Regridding package grid3 MAT from Zwart e tal. was used.
   start Grid3 calc'
gdata = grid3 MAT(data,crds,DCF,effMtx,numThread);
clear data crds DCF numThread;
%Make a rolloff kernel for rolloff correction or deapodization.
' make a rolloff kernel'
delta = [1.0, 0.0];
k not = [0.0, 0.0, 0.0];
```

```
DCF = [1.0];
numThread = 1;
rokern = grid3 MAT(delta', k not', DCF, effMtx, numThread);
clear delta k not DCF numThread;
%Faster Fourier Transform
' fft into image space'
% change to complex, fft, then shift
gdata = squeeze(gdata(1,:,:,:) + 1j*gdata(2,:,:,:));
gdata = fftn(gdata);
gdata = fftshift(gdata,1);
gdata = fftshift(gdata,2);
gdata = fftshift(gdata, 3);
% ROLLOFF
% change to complex, shift, then fft
rokern = squeeze(rokern(1,:,:,:) + 1j*rokern(2,:,:,:));
rokern = fftn(rokern);
rokern = fftshift(rokern,1);
rokern = fftshift(rokern,2);
rokern = fftshift(rokern,3);
rokern = abs(rokern);
1.1
    apply rolloff and crop'
gdata(rokern > 0) = gdata(rokern > 0) ./ rokern(rokern > 0);
gdata = single(abs(gdata)); % magnitude, float32
' write output file'
tmp = rot90(gdata, 2);
fid = fopen(fullfile(path,imagefilename),'w');
fwrite(fid,tmp,'float32');
fclose(fid);
end %end function grid3.
```

## **Appendix III**

```
%% Created by Jinbang Guo
%%This method combine first order and second order derivatives of the
%%magnitudeplot of the first point of each FID to extract data for
%%inspiration and expiration. The output traj and fid
%%inspiration/expiration files are used for image reconstruction.
%% Data acquisition shift (no readout gradient during acquisition) is 20 for
%%the following example. The 20th point of FID was used for creating
%%respiratory waveform.
path = '/home/jinbang/ReconMethod/20151023raw/144';
RespMode = 'expiration'; %Extracting data for expiration.
MagnitudedifflLLexp = -50; MagnitudedifflULexp = 50; Magnitudediff2ULexp = 2;
Slower and upper thresholds for the first- and second-order derivatives for
%extracting data at expiration
Magnitudediff1LLinsp = -200; Magnitudediff1ULinsp= 200; Magnitudediff2LLinsp
= 3;
%lower and upper thresholds for the first- and second-order derivatives for
%extracting data at inspiration. These thresholds may be adjusted to get
%correct data extraction;
NPro = 100000;
NCutPro=0; % cut leading projections.
NPoints = 90;
disparray = 40000:41000;
trajfilename = strcat('traj ', RespMode);
fidfilename = strcat('fid ', RespMode);
RealNPro = NPro - NCutPro;
fid = fopen(fullfile(path,'fid'));
kdata = fread(fid,[2,inf],'int32');
fclose(fid);
magnitude = sqrt(kdata(2,:).^2 + kdata(1,:).^2);
magnitude = reshape(magnitude, [128 NPro]);
magnitude = magnitude(:,NCutPro+1:NPro);
% Amplitude of each FID; data acquisition shift = 20, with the 20th point
used.
amplitude = squeeze(magnitude(20,:))';
%smooth plot of amplitude vs FID #;
amplitudesm1 = smooth(squeeze(magnitude(20,:)),29);
h = figure;
subplot(2,2,1);
plot(amplitude(disparray),'o','MarkerEdgeColor','b','MarkerFaceColor','w','Ma
rkerSize',5);
xlabel('FID number', 'FontSize', 10, 'FontWeight', 'bold', 'Color', 'k');
ylabel('Amplitude (a.u.)','FontSize',10,'FontWeight','bold','Color','k');
title('Amplitude vs FID number', 'FontSize', 15, 'FontWeight', 'bold',...
'Color','k');
hold on;
```

```
plot(amplitudesm1(disparray),'-k','linewidth',3);
legend('Amplitude','Smoothed curve');
hold off;
subplot(2, 2, 2);
amplitudediff1 = diff(amplitudesm1,1);%1st order derivative;
plot(amplitudediff1(disparray),'o','MarkerEdgeColor','b','MarkerFaceColor','w
', 'MarkerSize',5);
xlabel('FID number', 'FontSize',10, 'FontWeight', 'bold', 'Color', 'k');
ylabel('1st order derivative','FontSize',10,'FontWeight','bold','Color','k');
title('First order derivative of amplitude', 'FontSize', 15, 'FontWeight',...
'bold', 'Color', 'k');
hold on;
amplitudediff1sm = smooth(amplitudediff1,29);%smooth 1st order derivative;
plot(amplitudediff1sm(disparray),'-k','linewidth',3);
legend('1st order derivative','smoothed 1st order derivative');
hold off;
%2nd order derivative and smoothing;
subplot(2, 2, 3);
amplitudediff2 = diff(amplitudediff1sm,1);
amplitudediff2sm = smooth(amplitudediff2,29);
plot(amplitudediff2(disparray),'o','MarkerEdgeColor','b','MarkerFaceColor','w
', 'MarkerSize', 5);
xlabel('FID number', 'FontSize', 10, 'FontWeight', 'bold', 'Color', 'k');
ylabel('2nd order derivative','FontSize',10,'FontWeight','bold','Color','k');
title('Second order derivative of
amplitude', 'FontSize', 15, 'FontWeight', 'bold', 'Color', 'k');
hold on;
plot(amplitudediff2sm(disparray), '-k', 'linewidth', 3);
legend('2nd order derivative','smoothed 2nd order derivative');
hold off;
subplot(2,2,4);
amplitudediff2sm(RealNPro-1) = amplitudediff2sm(RealNPro-2);
amplitudediff2sm(RealNPro) = amplitudediff2sm(RealNPro-2);
amplitudediff1sm(RealNPro) = amplitudediff1sm(RealNPro-1);
amplitudesm2 = amplitudesm1;
plot(amplitudesm1(disparray),'o','MarkerEdgeColor','b','MarkerFaceColor','w',
'MarkerSize',5);
xlabel('FID number', 'FontSize', 10, 'FontWeight', 'bold', 'Color', 'k');
ylabel('Ampliutude and derivatives (a.u.)', 'FontSize', 10, 'FontWeight', ...
'bold', 'Color', 'k');
title('Projections selected for reconstruction', 'FontSize', 15, ...
'FontWeight', 'bold', 'Color', 'k');
hold on;
if strcmp(RespMode, 'expiration')
    disp('display data for expiration');
amplitudesm2(amplitudediff1sm>amplitudediff1LLexp&amplitudediff1sm<amplituded</pre>
iff1ULexp&amplitudediff2sm<amplitudediff2ULexp) =
max(amplitudesm1(:));%expiration
plot(amplitudesm2(disparray),'s','MarkerEdgeColor','k','MarkerFaceColor','w',
'MarkerSize',3);
```

```
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```

```
legend('smoothed plot of amplitude vs FID number', 'data selected for recon');
ylim([min(amplitudesm1(:)) max(amplitudesm1(:))]);
hold off;
saveas(h,fullfile(path,strcat(RespMode,'.tiff')),'tiffn');
else
    disp('display data for inspiration');
amplitudesm2(amplitudediff1sm>amplitudediff1LLinsp&amplitudediff1sm<amplitude
diff1ULinsp&amplitudediff2sm>amplitudediff2LLinsp) =
min(amplitudesm1(:));%inspiration
plot(amplitudesm2(disparray),'s','MarkerEdgeColor','k','MarkerFaceColor','w',
'MarkerSize',3);
legend('smoothed plot of amplitude vs FID number', 'data selected for recon');
ylim([min(amplitudesm1(:)) max(amplitudesm1(:))]);
hold off;
saveas(h,fullfile(path,strcat(RespMode,'.tiff')),'tiffn');
end
clear h disparray amplitude amplitudediff1 amplitudediff2 amplitude
amplitudesm1 amplitudesm2;
% select data @ end expiration
if strcmp(RespMode, 'expiration')
    disp('extracting data for expiration');
SelectVector =
amplitudediff1sm>amplitudediff1LLexp&amplitudediff1sm<amplitudediff1ULexp&amp
litudediff2sm<amplitudediff2ULexp;</pre>
%select data @ end inspiration
else
    disp('extracting data for inspiration');
    SelectVector =
amplitudediff1sm>amplitudediff1LLinsp&amplitudediff1sm<amplitudediff1ULinsp&a
mplitudediff2sm>amplitudediff2LLinsp;
end
%Extract FID data
kdata = reshape(kdata, [2 128 NPro]);
kdata = kdata(:,:,NCutPro+1:NPro);
kdata selected = kdata(:,:,SelectVector);
'Selected Number of Projections:'
SelectedNPro = size(kdata selected,3)
fid = fopen(fullfile(path, fidfilename), 'w');
fwrite(fid,kdata selected,'int32');
```

```
%Extract k-space coordinates
fid = fopen(fullfile(path,'traj'));
trajectory = reshape(fread(fid,[3,inf],'double'),[3 NPoints NPro]);
```

fclose(fid);

```
fclose(fid);
trajectory = trajectory(:,:,NCutPro+1:NPro);
trajectory_selected = trajectory(:,:,SelectVector);
fid = fopen(fullfile(path,trajfilename),'w');
fwrite(fid,trajectory_selected,'double');
fclose(fid);
clear all;
% end
```