QUANTITATIVE APPROACHES IN MRI
WITH CLINICAL APPLICATIONS

DISSERTATION

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ABSTRACT

Magnetic Resonance Imaging (MRI) has been established as a primary imaging module in clinical practices because of its high spatial resolution and richness in soft tissue contrast. However, the mainstream clinical application of MRI is currently dominated by the qualitative reading approach. Advanced quantitative approaches, such as Dynamic Contrast Enhanced MRI (DCE-MRI) or MR relaxometry, provide more objective information about tissue component and function, which can be essential to improve the accuracy and efficiency of disease diagnosis, treatment plan design, and drug effect monitoring. Several studies had been conducted to explore techniques that can be used to improve various quantitative MRI approaches in clinical-related context. The results were summarized in this text.

Pharmacokinetic analysis of DCE-MRI data is an important method to assess pathophysiological permeability changes in cancerous tissue with abnormal angiogenesis. A modified two-compartment pharmacokinetic model was proposed, based on more realistic model assumptions. Pharmacokinetic parameters derived with this model were demonstrated to have better tissue specificity in distinguishing metastatic tumor from normal tissue in liver.

MRI also showed a great potential in quantitative assessment of iron overload diseases because proton relaxation behaviors are highly sensitive to the paramagnetic
tissue iron components. The feasibility of using several relaxometry-based or signal-intensity-ratio-based quantitative MRI approaches as liver iron content (LIC) biomarkers was assessed in a subgroup of 7 patients with sickle cell disease by statistically comparing MRI LIC measurements with the liver biopsy ‘golden standard’. Three relaxometry-based approaches (using the transverse relaxation rate $R_2$ or $R_2^*$) were demonstrated to have insignificant difference with liver biopsy, and identified as feasible LIC biomarkers at clinical-relevant 1.5 Tesla.

A technical obstacle in applying the MRI tissue iron biomarkers to high and ultrahigh field systems is the accelerated dephasing caused by macroscopic background field ($B_0$) inhomogeneity. A postprocessing strategy that can correct for nonlinear (quadratic) $B_0$ inhomogeneity was proposed and applied to phantom and volunteer data to demonstrate its superiority over no correction or linear correction methods. The proposed quadratic correction strategy can also generate an index of reliability of the correction results.

In clinical-relevant topics, computer simulation is a powerful complementary technique when the experimental design is compromised due to ethic consideration or funding limit. In order to explore subtle details in the tissue-iron contrast mechanism, a high speed Monte Carlo algorithm was proposed to enable simulation on nano scale. The prototype algorithm was implemented in IDL. Preliminary testing results showed a promising improvement in computing speed.
Dedicated to the earthquake victims in my home province
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CHAPTER 1

INTRODUCTION

Magnetic Resonance Imaging (MRI) is a noninvasive imaging technique that detects Zeeman split of energy levels of non-zero-spin nucleus placed in an external magnetic field (1-3). MRI is usually conducted with $^1$H nuclear (also referred to as ‘proton’ of ‘spin’) in water due to its rich abundance in nature, which ensures high signal-to-noise ratio (SNR) that allows formation of high resolution images. Modern ultrahigh fielded (above 7 Tesla) system can achieve 200 μm in-plane resolution in a head-size field-of-view (FOV) (4), thus enabling visualization of fine anatomic structures like vascular network or white matter fiber bundles. In MR, image contrast is determined by many physical properties including spin abundance, transverse and longitudinal relaxations, and electromagnetic interactions between tissue and its environment. Various superb soft tissue contrasts can be obtained by fine tuning these parameters, providing a wealth of information about the structural, functional, physiological or pathophysiological conditions of the subject from different aspects.

Because of all these advantages, MRI has been chosen as a primary diagnostic tool in many clinical applications, especially in those with pathological changes in soft tissues. A report prepared by major manufacturers of MR scanners in 2003 gives the
estimates of over 20,000 installations and over 200 million patients examined worldwide. Although there is no newer statistics available, both numbers are expected to have increased significantly during the past five years.

MRI is essentially a quantitative approach. The physics principles are well understood (5, 6). Mathematical tools have been developed to describe the temporal behaviors of MR signal under various experimental settings (7-10). Many advanced sequences have been elaborated to quantify functional or physiological properties like diffusion, perfusion, or brain activity (11-15). However, their application in the clinical field has been severely lagged behind lab development. Traditional qualitative image reading by radiologists is still the mainstream in MRI clinical application. Compared with the traditional method, new quantitative approaches are more informative, more objective, and less prone to individual bias. Their extensive involvement in clinical practices will definitely help to improve the scope and efficiency of radiology service.

A series of researches have been conducted at the Ohio State University Imaging Core Lab to explore various aspects in clinical application of quantitative MRI approaches. The results are summarized in this dissertation. A brief introduction to the MRI technique in general and the quantitative approaches involved in our works (namely, the dynamic contrast enhanced MRI and MR relaxometry) is given in Chapter 2 as background information.

Chapter 3 describes the results from a theoretical modeling work in which Brix’s two-compartment model for pharmacokinetic analysis of dynamic contrast enhanced
MRI (DCE-MRI) data was improved by incorporating a more realistic model assumption. The modified model was applied to a data set acquired in a group of patients with colorectal cancer with liver metastasis, and demonstrated to have better tissue specificity in differentiating tumor from normal liver tissue.

Chapter 4 checks the feasibility of using quantitative MRI approaches as biomarkers to monitor liver iron concentration (LIC) in iron overload diseases. Three approaches based on MRI relaxometric measurement of transverse relaxation rates ($R_2$ and $R_2^*$) were demonstrated to generate LIC measurements that are not significantly different from those obtained with standard liver biopsy, and identified as qualified biomarkers.

Compared with the $R_2$ MRI LIC biomarkers, the $R_2^*$ biomarker is more prone to macroscopic background field inhomogeneity. In Chapter 5, a postprocessing technique correcting for quadratic $B_0$ inhomogeneity was proposed as a possible remedy. Test results in phantom and volunteers demonstrated the novel method not only corrects for $R_2^*$ overestimation due to macroscopic $B_0$ inhomogeneity, but also generates an estimate of the reliability of this correction, thus extending the applicability of the $R_2^*$ biomarker to higher magnet strength and wider areas in clinical application.

In Chapter 6, a high-speed Monte Carlo algorithm called the ‘field profile algorithm’ was proposed for computational simulation of $R_2$ decay. Preliminary tests were conducted on a prototype implementation, and the results were examined. This algorithm is expected to provide complementary information about the relaxation properties of heterogeneous biological systems when direct measurement is impossible or
impractical, thus facilitating model development and protocol optimization for clinical applications.

Finally, a brief summary of the prospect of clinical related quantitative MRI approaches was given in Chapter 7.
2.1 Basics of MRI

2.1.1 From Spin to Magnetization

Quantum systems like electrons or nucleus possess an intrinsic angular momentum that has no classical analogue (2). This quantum property was named spin and characterized by the spin operator $\vec{S}$. Quantum theory states that the magnitude of the spin angular momentum can only take values according to the relation:

$$S = \hbar \sqrt{s(s+1)}$$  \[2.1\]

where $S$ is the angular momentum magnitude, $\hbar$ is the reduced Planck’s constant, and $s$ is the principle spin quantum number that can only take non-negative integer or half-integer values. Accordingly, projection of the spin angular momentum along any axis can only take $(2s + 1)$ discrete values (2):

$$S_z = \hbar s_z, \quad s_z = -s, -s+1, \ldots, s-1, s$$  \[2.2\]

Experiment shows that the $^1$H nucleus used in most clinical MRI applications has $s = 1/2$, so the spin angular momentum component measured along any direction has only two
possible values $\frac{\hbar}{2}$ and $-\frac{\hbar}{2}$, which are usually referred to as ‘spin up’ and ‘spin down’, separately. In the absence of external magnetic field, there is no energy difference between ‘up’ and ‘down’ spins. In an ensemble of identical spin-1/2 nuclei, the ratio between ‘up’ and ‘down’ spins is 1:1.

When an external static magnetic field $B_0$ is applied along the z-axis, there is an interaction between the applied magnetic field and spin angular momentum that causes a split in the nuclear energy level (a simplest example of the Zeeman Effect):

$$E = -\gamma s_z \hbar B_0$$  \hspace{1cm} [2.3]  

where $\gamma$ is the gyromagnetic ratio, $\gamma = 2\pi \times 42.6$ MHz/Tesla for $^1$H. Eq. 2.3 shows that the ‘up’ spins ($s_z = 1/2$, with their spin parallel to the external field) have lower energy than the ‘down’ spins ($s_z = -1/2$, with their spin anti-parallel to the external field), with an energy difference of:

$$\Delta E = \gamma \hbar B_0 = \hbar \omega_0$$  \hspace{1cm} [2.4]  

where $\omega_0 = \gamma B_0$ is the resonance frequency. This split in energy levels changes the thermal equilibrium distribution of spins, as there are more spins stay on the ‘up’ state with lower energy. The new equilibrium is governed by the Boltzmann distribution:

$$\frac{n_\downarrow}{n_\uparrow} = \frac{\exp(-\hbar \omega_0 / 2kT)}{\exp(\hbar \omega_0 / 2kT)}$$  \hspace{1cm} [2.5]  

where $n_\downarrow$ and $n_\uparrow$ are the number of spins in the ‘down’ and ‘up’ state, separately. $k$ is the Boltzmann constant, and $T$ is temperature in Kelvin.

Because there is a magnetic moment associated with the spin angular momentum:
\[ \dot{\vec{m}} = \gamma \vec{S} \]  

Imbalance in thermal equilibrium of nuclear magnetic moments adds up to form a macroscopic magnetization \( \vec{M} \) parallel to \( \vec{B}_0 \).

### 2.1.2 Excitation and Signal Detection

When the external magnetic field is disturbed and no longer aligned to \( \vec{M} \), the magnetization senses a torque exerted by the perpendicular component of the external field, and starts to rotate. Dynamic behavior of magnetization in external magnetic field is described by the Bloch equation:

\[ \frac{d\vec{M}}{dt} = \gamma \vec{M} \times \vec{B} \]  

where the external field \( \vec{B} \) is not necessarily along the z axis.

It can be demonstrated with Eq. 2.7 that a magnetic field \( \vec{B}_1 \) rotating at resonance frequency \( \omega_0 \) in the x-y transverse plane (applied through a circularly polarized radio frequency pulse) can ‘tilt’ the magnetization \( \vec{M} \) down to the transverse plane in a rotating reference frame that moves with \( \vec{B}_1 \). In the laboratory frame, the transverse component of \( \vec{M} \) precesses with \( \omega_0 \) in the x-y plane. Precession of magnetization induces voltage in receiver coils that can be detected by the resonant circuit (2, 3). The magnetization ‘tilting’ process is called excitation because it creates the detectable MR signal. The excitation radio frequency (RF) pulse is characterized by its ability to tip \( \vec{M} \) away from \( \vec{B}_0 \), i.e., its flip angle (FA) which is proportional to the total power deposited by this RF pulse.
2.1.3 Spatial Encoding

The MR signal detected by the resonant circuit does not carry any spatial information because the inductive voltage is a collective effect of all the spins inside the imaging volume. In order to get an image for a subject, spins from different locations must be differentiated by some encoding technique.

Spins can be encoded with different resonance frequencies by imposing a magnetic gradient along a direction in space. Lauterbur took advantage of this concept to get 1-D projections of a phantom object from nuclear magnetic resonance (NMR) measurements conducted with magnetic gradients along different directions. A 2-D image of the phantom can be reconstructed with projection reconstruction algorithms like those used in computed tomography (CT) (1). However, Lauterbur’s method (called NMR zeugmatography) was soon found to be suboptimal due to low SNR and hardware (mainly gradient) limitations. A new spatial encoding strategy called Fourier imaging replaced NMR zeugmatography, and dominated MR scanner design and production through today.

In Fourier imaging, the magnetic gradient applied during MR signal acquisition (the frequency encoding gradient or the readout gradient) is used to encode a line in the Fourier phase domain rather than a 1-D projection in the spatial domain. Other lines in the phase domain (k-space) are obtained by applying another gradient (the phasing encoding gradient) in an orthogonal direction prior to acquisition to get a phase shift in the corresponding direction. By cycling through a range of different phase encoding
gradient steps, a rectangular sampling grid of the k-space gets filled, whose inverse Fourier transformation generates a 2-D images of the subject.

Information on the third dimension can be obtained either by a second phase encoding (3D imaging) or by a selective excitation technique called slice selection (2D imaging). In slice selection, a linear gradient along the third dimension is applied during excitation with an RF pulse with narrow bandwidth (usually a truncated sinc pulse). The narrow bandwidth of the excitation pulse ensures that only spins in a thin layer can absorb photons and get excited.

2.2 MR Relaxometry

2.2.1 Relaxation

Excited magnetization has a tendency of returning to its equilibrium state parallel to the external field. This returning process is termed as relaxation.

The term longitudinal ($T_1$) relaxation refers to the recovery of the magnetization component parallel to the external field. During the longitudinal relaxation process, spins dissipate extra energy (absorbed from photons in the excitation RF pulse) to their environment through spin-lattice interaction. It is usually assumed that this process is governed by an exponential time constant $T_1$, which can be measured by inversion recovery or saturation recovery sequences.

Dissipation of the transverse component of the magnetization is termed as the transverse ($T_2$) relaxation, which is governed by dipole-dipole interactions between spins. Spins are always experiencing fluctuating dipole fields generated by the random motions of neighboring protons. During this stochastic process, they gradually collect different
phases (dephasing) and lose coherence. Attenuation of MR signal accompanies loss of phase memory because magnetic moments of dephasing spins cancel out with each other. Like the longitudinal relaxation process, the transverse relaxation process is also assumed to follow an exponential decay characterized by the transverse relaxation time constant $T_2$.

While $T_2$ relaxation is inherently a stochastic process rooted from the random Brownian motion of water molecules, additional dephasing could happen as a result of spatial inhomogeneity in the determinative $\vec{B}_0$ field. The new, shortened relaxation time is usually called apparent transverse relaxation time and denoted as $T_2^*$. Because the source of the additional dephasing is totally determinative, its effect can be removed by a 180° RF pulse applied in the middle of excitation and signal readout. Phase memories accumulated during the first half of the echo time $TE$ are reversed by this 180° pulse and get compensated (refocused) during the second half of $TE$. As a result, the ‘true’ transverse relaxation time $T_2$ needs to be measured with a spin echo (SE) sequence with the 180° refocusing pulse, while the apparent transverse relaxation time $T_2^*$ is measured with a gradient echo (GRE) sequence without any refocusing.

2.2.2. $T_2/T_2^*$ Relaxometry

Relaxometry is the study of measuring characteristic relaxation parameters with NMR or MRI. The following discussions are concentrated on $T_2/T_2^*$ relaxometry because they are most relevant to the studies discussed in later chapters.

Classical sequences, such as SE, GRE mentioned above, and Carr-Purcell-Meiboom-Gill (CPMG) sequences (8-10) can be used in either $T_2$ or $T_2^*$ relaxometry.
Some advanced sequences have been designed for more efficient acquisition of relaxation parameters. The Gradient Echo Sampling of Spin Echo (GESSE) and Gradient Echo Sampling of Free Induction Decay and Echo (GESFIDE) sequences use readout gradient trains to get multiple samplings of free induction decay (FID) in both dephasing and refocusing stages of a spin echo signal (Fig. 2.1, Fig. 2.2) in one pass, enabling simultaneous measurement of $T_2$ and $T_2^*$. 

![Timing diagram of GESSE sequence](image)

**Figure. 2.1: Timing diagram of GESSE sequence**
Both T₂ and T₂* relaxometry can be used to study the intrinsic heterogeneity of magnetic properties on the cellular or subcellular level. Compared with T₂ relaxometry, T₂* relaxometry is more sensitive to small changes in the paramagnetic environment because it mainly detects the static dephasing effect. This property has been used to facilitate various applications in many quantitative MR approaches, such as quantification of tissue iron (16, 17), perfusion evaluation with Dynamic Susceptibility Contrast imaging (DSC-MRI) (18), brain function assessment with Blood-Oxygen-Level Dependent functional MRI (BOLD-fMRI) (19), and quantitative monitoring of superparamagnetic iron-oxide particle contrast agents (20). T₂ relaxometry, on the other hand, is less prone to macroscopic B₀ inhomogeneity that is not related to the intrinsic heterogeneity within the imaging voxel. T₂ relaxometry is primarily used for tissue iron
quantification, whose clinical importance has been demonstrated in iron overload disease, multiple sclerosis, and normal aging.

### 2.3 Dynamic Contrast Enhance MRI

The family of Gd-based contrast agents is currently the primary MR contrast agents approved for routine clinical uses. Dipole-dipole interaction with the paramagnetic Gd$^{3+}$ ions predominantly affects the longitudinal ($T_1$) relaxation of water protons, leading to a decrease in the longitudinal relaxation time $T_1$ and a corresponding increase in the longitudinal relaxation rate $R_1 = 1/T_1$ (21). Experiments have established a linear relationship between contrast agent concentration and $R_1$ change (see Chapter 3 for more details). This property can be used to noninvasively monitor *in vivo* distribution of Gd-based contrast agents.

Gd-based contrast agents are delivered by intravenous injection. After injection, the contrast agents are confined in the circulation system because the Gd$^{3+}$ ion is chelated to a large organic molecule like DTPA and BOPTA to reduce its toxicity and improve its pharmacokinetic characteristics. However, when deficiencies develop on the capillary epithelium as a result of tumor or Alzheimer’s disease (22), contrast agent molecules leak into the interstitial space between cells, and causes a local increasing in $R_1$. By monitoring the time course of this leaking process, information about local perfusion and microvasculature can be obtained.

The contrast agent leaking process is a passive diffusion against concentration gradient so its kinetic behavior can be well described by a set of ordinary differential equations (ODE’s). Each ODE in the set describes the kinetics of a homogeneous
compartment that exchanges contrast agent with neighboring compartments through its borders. While the single compartment model is too simple to describe contrast agent kinetics in tumor, multi-compartment models with too many compartments suffer from over fitting due to large number of fitting parameters. The two compartment model, with various forms, has been accepted as a good balance point between mathematical simplification and complicated reality by the research community.

Pharmacokinetic parameters determined from variations of two compartment model can be interpreted as contrast transfer rate constants between the cellular interstitial space and blood plasma. They provide direct, intuitive measures for pathophysiological properties of local microvasculature, which is the target of anti-angiogenic agents. Thus, pharmacokinetic analysis of DCE-MRI has been extensively used in drug response assessment of anti-angiogenic prodrugs.
CHAPTER 3

IMPROVING THE PHARMACOKINETIC PARAMETER MEASUREMENT IN DCE-MRI BY USE OF THE ARTERIAL INPUT FUNCTION: THEORY AND CLINICAL APPLICATION

3.1 Overview

Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI), with its capability of revealing microvasculature and perfusion in soft tissues, has been established as an important non-invasive tool in many types of tumor (23-27). Since the very beginning of this technique, various methods have been proposed to describe and assess the contrast enhancement pattern, from the simplest qualitative description (28, 29) to more sophisticated empirical parameters (30) and model-based pharmacokinetic analysis (31-35). Among those approaches, qualitative description is subjective to radiologist’s experience and bias. Empirical parameters, such as area under the curve (AUC), are dependent on many details in acquisition platform and protocol, thus being infeasible for inter-study comparison. Model-based pharmacokinetic parameters are independent of individual bias, and more robust with different hardware settings or protocol designs. Moreover, some pharmacokinetic parameters can be interpreted, by definition, as exchange rates of contrast agent (CA) molecules between functionally
separated compartments. Thus, changes in those parameters can be arguably associated with underlying physiological or pathophysiological changes in a way more direct than pure statistical correlation. As a result, model-based pharmacokinetic analysis has become a preferable quantitative assessment method for DCE-MRI in oncological applications, especially antiangiogenic drug assessment (36, 37).

In DCE-MRI pharmacokinetic analysis, changes in tissue CA concentration over time are modeled as result of first-order exchange of CA molecules between compartments. This basic concept has been utilized by several groups to develop pharmacokinetic models describing DCE-MRI data acquired in multiple sclerosis, brain tumor and breast tumor studies in the early nineties (38-41). Tofts and collaborators reviewed those early approaches (42) and pointed out that the general mathematical form of the pharmacokinetic model is independent of the CA exchange mechanism (i.e. if the CA exchange process is governed by blood flow, or permeability, or a mixed case). The generalized kinetic model, in the standard nomenclature proposed by Tofts et al. (43), can be expressed as a differential equation:

\[
\frac{dC_t}{dt} = K^{\text{trans}} \left( C_p - \frac{C_t}{v_e} \right)
\]

where \( C_t \) is the CA concentration in tissue, \( C_p \) is the CA concentration in blood plasma, \( K^{\text{trans}} \) is the transfer constant, and \( v_e \) is the fractional volume of extracellular extravascular space (EES). When \( C_p \) is known, \( C_t \) can be solved as a convolution between \( C_p \) and an exponential kernel. However, in reality, \( C_p \) and \( C_t \) can only be measured indirectly from T1-weighted MR signal intensities in artery and tissue. Study
with Wistar rats shows that the relaxivity of Gd-EOB-DTPA and Gd-DTPA can be distinctively different in different tissues (44). Even when the tissue T1 relaxivity is comparable with that of plasma, the absolute values of T1 and T2 relaxation rates still vary (45). As a result, $K^{\text{trans}}$ estimated with Eq. 3.1 absorbs a constant factor determined by those quantities in blood plasma and tissue of interest, thus interfering with accurate estimation of $\nu_e$ and inter-tissue comparison of pharmacokinetic analysis.

This problem is solved by a model proposed by Brix et al. (46), in which $C_p$ is also treated as an unknown variable and solved simultaneously with $C_t$ from a set of two ordinary differential equations (ODE). In Brix’s model, tissue signal-intensity-time course is described by three parameters: the amplitude $A$, the exchange rate constant $k_{ep}$, and the elimination rate constant $k_{el}$, none of which is dependent on the form of $C_p$. However, although this model has been proved to provide a good fit to tissue DCE-MRI data, it predicts a monoexponential decay in blood plasma, which is seldom supported by experimental observations (47, 48).

Here we present a theoretical analysis of the pharmacokinetic equations, suggesting that this inconsistency is due to an inadequate assumption used by the Brix model. More general solutions are derived, and a limiting case that better describes MR signal from artery (the arterial input function, AIF) without sacrificing fitting quality in the tissue is discussed in detail. This limiting case is applied to a liver metastasis data set to demonstrate that the capability of utilizing pharmacokinetic parameters to differentiate normal and tumor tissues in MR image can be improved by making more realistic assumptions and considering more general solutions of the pharmacokinetic equations.
3.2 Theory

3.2.1 Mapping Tissue CA Concentration to MR Signal Intensity

In this chapter, we will restrict our discussion to Gd-chelate based CAs, currently the primary and available choices for clinical use. Presence of the large magnetic moments of paramagnetic Gd$^{3+}$ ions in aqueous solution causes fluctuation in local magnetic field. While such fluctuation enhances both longitudinal (T1) and transverse (T2) relaxation rates of water protons, the latter effect is negligible due to the low CA concentration and short echo time used in clinical applications. In the frequency range of modern clinical MR systems (63.9 to 127.8 MHz), Gd-enhanced T1 relaxation is entirely determined by dipole-dipole interaction between paramagnetic ions and neighboring water protons, whose behaviour is described by the Solomon-Bloembergen equations (49, 50). The Solomon-Bloembergen equations predict a linear relationship between Gd$^{3+}$ concentration and longitudinal relaxation rate $R_1$, which has been experimentally validated in aqueous solution (for free Gd$^{3+}$ ion and its DTPA chelate) (51) and blood plasma (for various Gd$^{3+}$ chelates) (52, 53). *Ex vivo* studies also revealed similar linearity in blood and other soft tissues (44, 54, 55):

$$R_{1CA} = R_{10} + R_1C_T$$  \[3.2\]

where subscripts CA and 0 denote relaxation rates measured in the presence and absence of CA, separately. $C_T$ is the tissue CA concentration. And $R_1$ is the longitudinal relaxivity for the CA in the tissue.
For the T1-weighted SPGR sequence used in this study, when TR is sufficiently short, the MR signal can be well approximated by a first-order Taylor expansion. Hence the MR signal intensity measured in the presence of CA in the tissue of interest:

\[ S_{Ca}(t) = S_0 [1 + FC_T(t)] \]  

where \( S_0 \) is the pre-contrast signal intensity and \( F \) is a constant factor determined by tissue extracellular space, TR, \( R1_0 \), and \( R1 \). From Eq. 3.3, it can be easily derived that the relative signal enhancement is proportional to tissue CA concentration:

\[ \frac{S_{Ca}(t)-S_0}{S_0} = FC_T(t) \]  

Therefore, kinetic equations of CA concentrations can be converted to equations of relative signal enhancements, quantities that are directly measurable from DCE-MRI acquisitions.

### 3.2.2 Pharmacokinetic Modeling

The open two-compartment model (Fig. 3.1) is the most commonly used pharmacokinetic model for CA exchange between tissue of interest and a blood plasma pool. The CA is assumed to have zero-order infusion kinetic, first-order elimination, and first-order transfer between a plasma and a peripheral (EES) compartment. Simple as these assumptions are, the open two-compartment model is able to fit real DCE-MRI data surprisingly well, thus being widely accepted by the research community in favor to more complex multi-compartment models (56).
Figure. 3.1: Schematic diagrams for: (a). our model, (b). Brix’s model, and (c). Larsson’s model. The relative importance of different CA transfer rate constants is represented by the thickness of the corresponding arrows.

Assuming that CA transfer is instant and no mass gets trapped in the transfer processes, the kinetic behavior of CA can be expressed by the following equations:

\[
\begin{cases}
    \frac{dC_p}{dt} = -(k_{pe} + k_{el})C_p + \frac{V_e}{V_p} k_{ep} C_e + \frac{K_{in}}{V_p}, & 0 \leq t \leq \tau \\
    \frac{dC_e}{dt} = \frac{V_p}{V_e} k_{pe} C_p - k_{ep} C_e \\
\end{cases}
\]  \[3.5\]

and

\[
\begin{cases}
    \frac{dC_p}{dt} = -(k_{pe} + k_{el})C_p + \frac{V_e}{V_p} k_{ep} C_e \\
    \frac{dC_e}{dt} = \frac{V_p}{V_e} k_{pe} C_p - k_{ep} C_e , & t > \tau \\
\end{cases}
\]  \[3.5\]

Here \( C_p \) and \( C_e \) are the CA concentrations in the plasma and peripheral (usually interpreted as EES in the tissue, hence the name) compartments, separately. \( V_p \) and \( V_e \)
are the corresponding volumes of these compartments. $k_{el}$ is the CA elimination rate constant, $k_{ep}$ is the one-way CA transfer rate constant from the peripheral (EES) compartment to the plasma compartment, $k_{pe}$ is the one-way CA transfer rate constant in the opposite direction, $K_{in}$ is the CA infusion rate constant, and $\tau$ is CA infusion duration. Brix assumed that the exchange terms are negligible compared to infusion and elimination terms in the plasma equation, and there is no net CA transfer between compartments when $C_p = C_e$. Based on these assumptions, he derived his solutions (57) for the initial conditions $C_p(0) = C_e(0) = 0$:

\[
\begin{align*}
C_p &= \frac{K_{in}}{V_p k_{el}} \left[ \exp(k_{el}^B t') - 1 \right] \exp(-k_{el}^B t) \\
C_e &= \frac{K_{in}}{V_p} \left[ a \left[ \exp(k_{el}^B t') - 1 \right] \exp(-k_{el}^B t) - b \left[ \exp(k_{ep}^B t') - 1 \right] \exp(-k_{ep}^B t') \right]
\end{align*}
\]

in which $a = \frac{k_{ep}^B}{k_{el}^B (k_{ep}^B - k_{el}^B)}$, $b = \frac{1}{(k_{ep}^B - k_{el}^B)}$, $t' = t$ for $t \leq \tau$, and $t' = \tau$ for $t > \tau$. The superscript B implies that the rate constants are obtained under Brix’s assumption.

From a mathematical point of view, Brix’s assumption dramatically simplifies the kinetic system, decouples the two ODE’s so that they can be solved successively. While the post-infusion monoexponential decay in plasma CA concentration is thought to be a sufficient description to Brix’s DCE-MRI brain data, in which CA infusion takes 4 min, plasma CA concentration in most research and clinical acquisitions with faster injection rates are better described by a biexponential decline (58). To resolve this inconsistency, we need to consider the general solutions of Eqs. 3.5, which can be obtained from the basic ODE theories.
\[
C_p = \frac{K_{in}}{V_p(\lambda_1 - \lambda_2)} \left\{ \begin{array}{l}
\frac{\lambda_1 + k_{ep}}{-\lambda_1} [\exp(-\lambda_1 t') - 1] \exp(\lambda_1 t) \\
- \frac{\lambda_2 + k_{ep}}{-\lambda_2} [\exp(-\lambda_2 t') - 1] \exp(\lambda_2 t)
\end{array} \right\}
\]

\[
C_e = \frac{K_{in} k_{pe}}{V_p(\lambda_1 - \lambda_2)} \left\{ \begin{array}{l}
\frac{1}{-\lambda_1} [\exp(-\lambda_1 t') - 1] \exp(\lambda_1 t) \\
- \frac{1}{-\lambda_2} [\exp(-\lambda_2 t') - 1] \exp(\lambda_2 t)
\end{array} \right\}
\] [3.7]

where \( \lambda_1 \) and \( \lambda_2 \) are eigenvalues of the ODE parametric matrix:

\[
\lambda_{1,2} = - \frac{(k_{el} + k_{ep} + k_{pe}) \pm \sqrt{(k_{el} + k_{ep} + k_{pe})^2 - 4k_{el}k_{ep}}}{2}
\] [3.8]

By substituting Eq. 3.4 into Eqs. 3.7, general expressions for the MR signal-time courses measured in AIF and tissue ROIs can be obtained:

\[
\frac{S_{CA}^{\text{AIF}} - S_0^{\text{AIF}}}{S_0^{\text{AIF}}} = \frac{B}{(\lambda_1 - \lambda_2)} \left\{ \begin{array}{l}
\frac{\lambda_1 + k_{ep}}{-\lambda_1} [\exp(-\lambda_1 t') - 1] \exp(\lambda_1 t) \\
- \frac{\lambda_2 + k_{ep}}{-\lambda_2} [\exp(-\lambda_2 t') - 1] \exp(\lambda_2 t)
\end{array} \right\}
\]

\[
\frac{S_{CA}^{\text{T}} - S_0^{\text{T}}}{S_0^{\text{T}}} = \frac{A}{\lambda_1(\lambda_1 - \lambda_2)} \left\{ \begin{array}{l}
\lambda_2 [\exp(-\lambda_2 t') - 1] \exp(\lambda_2 t) \\
- \lambda_1 [\exp(-\lambda_2 t') - 1] \exp(\lambda_2 t)
\end{array} \right\}
\] [3.9]

in which superscripts AIF and T denote signals measured in AIF and tissue ROIs, separately. \( B = F^{\text{AIF}} K_{in} / V_p \) and \( A = F^{\text{T}} K_{in} k_{pe} / (-\lambda_2 V_e) \) are amplitude parameters for AIF and tissue ROIs.

Despite the difference between the plasma part in the Brix’s solutions and the general ones, the general tissue solution in Eqs. 3.9 has exactly the same mathematical form as the Brix’s solution, except for that \( k_{el}^B \) and \( k_{ep}^B \) are now replaced by \(-\lambda_1\) and \(-\lambda_2\).
We will see in the Discussion section that this similarity is more than just coincidence. Thus, estimates of $A$, $\lambda_1$, and $\lambda_2$ can be obtained by fitting the tissue MR signal-time course to Eqs. 3.9 with the same fitting technique used in the Brix’s method, but the three rate constants cannot be resolved from the two eigenvalues without the aid of an arterial input function (AIF).

3.2.3 AIF-aided Eigenvalue Decomposition in a Limiting Case

Compared with CA exchange happening within the MR dynamic scan time scale ($10^0$–$10^1$ min), renal clearance of Gd$^{3+}$ based CAs is a relatively slow process. Weinmann et al have measured the mean half life of Gd-DTPA in the elimination phase in healthy volunteers as $(1.58 \pm 0.13)$ h (59). To simplify the problem, we consider the limiting case $k_{ep}, k_{pe} \gg k_{el}$. Take Taylor expansion of the squared root function, and ignore all the $O(k_{el}^2)$ terms. The two eigenvalues can be simplified as:

$$
\begin{align*}
\lambda_1 &\approx -2k_{ep}k_{el}/(k_{ep} + k_{pe}) \\
\lambda_2 &\approx -(k_{ep} + k_{pe})
\end{align*}
$$

[3.10]

And the plasma solution becomes:

$$
\frac{S_{CA}^{AIF} - S_0^{AIF}}{S_0^{AIF}} = B(\lambda_1 + k_{ep})\left\{ \frac{\exp(-\lambda_1t) - 1}{-\lambda_1(\lambda_1 - \lambda_2)} \right\} \\
- B(\lambda_2 + k_{ep})\left\{ \frac{\exp(-\lambda_2t) - 1}{-\lambda_2(\lambda_1 - \lambda_2)} \right\}
$$

[3.11]

Based on Eqs. 3.10 and 3.11, we propose the following algorithm for decomposing the three rate constants $k_{ep}$, $k_{pe}$, and $k_{el}$ from the two eigenvalues $\lambda_1$ and $\lambda_2$ with the aid from AIF:
1) Fit the tissue signal-time course to Eqs. 3.9 to obtain estimates of \( A \), \( \lambda_1 \), and \( \lambda_2 \);

2) Calculate two terms \( \frac{\exp(-\lambda_1 t') - 1}{\lambda_1(\lambda_1 - \lambda_2)} \) and \( \frac{\exp(-\lambda_2 t') - 1}{\lambda_2(\lambda_1 - \lambda_2)} \) with \( \lambda_1 \) and \( \lambda_2 \);

3) Perform multiple linear regression on the AIF with respect to the exponential terms to get estimates for the coefficients \( B(\lambda_1 + k_{ep}) \) and \( B(\lambda_2 + k_{ep}) \);

4) Take the ratio between the multiple regression coefficients:
   \[ \alpha = -\frac{(\lambda_1 + k_{ep})}{(\lambda_2 + k_{ep})} ; \]

5) Decompose the rate constants from \( \alpha \), \( \lambda_1 \) and \( \lambda_2 \):
   \[ k_{ep} = \frac{-\lambda_1 - \alpha \lambda_2}{1 + \alpha} , \quad k_{pc} = -\lambda_2 - k_{ep} , \quad k_{el} = -\frac{k_{ep} + k_{pc}}{2k_{ep}} \lambda_1 \]  \[ \text{[3.12]} \]
   When taking ratio of the multiple regression coefficients, the AIF amplitude \( B \) automatically cancels with itself so it does not interfere with rate constant estimation.

6) Apply physical restrictions:
   Since \( k_{ep} \) and \( k_{pc} \) are uni-directional CA transfer rate constants, they cannot be negative. So the following adjustment will be applied to the AIF-decomposed rate constants:
   - If \( k_{ep} < 0 \), set \( k_{ep} = 0 \), \( k_{pc} = -\lambda_2 \);
   - If \( k_{pc} < 0 \), set \( k_{ep} = -\lambda_2 \), \( k_{pc} = 0 \).
3.3 Method

3.3.1 DCE-MRI Imaging

Forty-five DCE-MRI data sets were acquired from ten patients with advanced colorectal cancer with liver metastases within nine weeks during treatment with an anti-angiogenesis agent. Informed consent was given by the patients prior to the study. The study was compliant with the HIPPA specifications.

MRI scans were performed on three 1.5T clinical MRI systems (two Symphony, Siemens, Erlangen, Germany and one Signa, GE, Milwaukee, WI). On the GE scanner, a T1-weighted spoiled gradient echo (SPGR) sequence (PSD filename: FSPGR) was used for DCE-MRI acquisition in the coronal/oblique plane with TR/TE/FA = 5.4ms/1.3ms/16° and 450mm FOV. On the two Siemens scanners, a saturation recovery turbo FLASH sequence (PSD filename: TFL2D1) was used with TR/TE/FA/TI = 1000ms/2.4ms/16°/340ms and 500mm FOV. Other scan parameters are: 128 x 128 or 256 x 256 matrices (acquisition matrices 128 x 100 or 256 x 192), 4 to 5 slices with 8mm slice thickness and 2mm gap, 80 or 120 time points, time resolution 5 or 6s. 0.1mmol/kg body weight Gd chelate (Magnevist, Bayer Healthcare AG) was intravenously injected after five pre-contrast data points with a power injector at a constant infusion rate of 2ml/s for 5 to 12 seconds.

3.3.2 Data Analysis

All DCE-MRI data were analyzed with an inhouse-developed software written in IDL (ITT, Boulder, CO). AIF, tumor, and normal liver ROIs were defined and verified by experienced radiologists. Since the contrasts of pre-Gd images are poor, ROI
definition was based on peak-enhancement images. Tumor ROIs were defined 2~4 mm (1~2 voxels) smaller than identified to accommodate for organ deformation. Central necrotic regions were not excluded in tumor ROIs (Fig. 3.2). MR signal fluctuations observed in those ROIs are primarily caused by respiratory and bowel motions, which contain both rigid-body displacement and elastic deformation of organs. The rigid-body motion (typical range of displacement is up to 2 cm) was partly compensated by manually adjusting ROI positions on each time point, but the limited number of slices hindered correction for elastic deformation by 3D flexible registration. In order to minimize estimation errors from mis-registration, an ROI-based technique was chosen in favor to a voxel-by-voxel analysis. Averaging signal intensities across the whole ROI also increases signal-to-noise ratio, thus reducing potential errors from fluctuations in pre-contrast $S_0$ measurement.

Since the time resolution of this DCE-MRI study is not high enough to resolve blood supplies from hepatic artery and portal vein, we can approximate the course of CA fed to liver by the AIF from the aorta. If the time resolution gets faster to around 1s, the dual-blood-supply nature of the liver needs to be considered, and a more sophisticated model should be used to analyze such data, which is beyond the scope of this discussion.

A subset of thirty AIFs was randomly picked to assess the validity of Brix’s solutions 3.6 for these liver data. The wash-out phase of the AIFs was truncated and fitted to a monoexponential function predicted by Brix’s model. Randomness of the residues was tested by the Box-Ljung test to detect systematic deviation from the model.

Pharmacokinetic parameters derived from ROI-averaged data can no longer be interpreted as representing local CA transfer properties within voxels. Instead, they are
now more empirical quantities that represent the overall microvasculature level and permeability over the whole ROI. It should be emphasized that those quantities are different from the corresponding mean values obtained by voxel-wise fit of the same ROI, as the latter are averaged local effects but the former are global by nature.

Figure 3.2: (a). Tumor (1) and AIF (2) ROI Definitions; (b). Our model generates the same fit as Brix’s in tumor data; (c). In AIF data, our biexponential plasma equation (solid line) is better than Brix’s monoexponential form (dashed line).
The following model-based and empirical parameters were determined on the full data set: a). the transfer constant $K_{\text{trans}}$ from Larsson’s model Eq. 3.1; b). the pharmacokinetic parameters $A$ and $k_{ep}^A$ from the deduced Brix’s model Eq. 3.6; c). the AIF-decomposed rate constants $k_{ep}$ and $k_{pe}$ determined by Eqs. 3.12; and d). the empirical parameter AUC, which is measured within 60 seconds after CA arrival and normalized to pre-contrast signal intensity. Tissue specificity of those parameters and their combinations were compared graphically with scatter plots and histograms, and quantitatively with two-tailed student’s t-test with unequal variance between normal liver and tumors. The Bonferroni method was used for multiple tests. Treatment effect was not considered in the analysis because the pro-drug was determined to be a failure, without any biological effect in imaging and other confirmatory tests.

A hot topic in MR research is to investigate the feasibility of utilizing MR-generated quantitative parameters to construct tissue-type classifiers, which can be used to facilitate automatic and semi-automatic analysis of MRI data. To assess the performances of classifiers based on the parameters discussed in this chapter, we performed discriminant analysis in Matlab (The Mathworks Inc., Natick, MA, USA) with a training set of 60 data points and a testing set of 30. False positive rate and false negative rate in the testing set were calculated. Bootstrap technique was used to compensate for the relatively small sample size, with 2,000 resamples.
3.4 Results

The Box-Ljung test generates small p values (p<0.05) for the monoexponential model in 24 of the 30 data sets tested, suggesting that monoexponential CA wash-out predicted by the Brix model is a poor description of the observed data (Fig. 3.2). Detailed inspection of the residual plot suggests that there is a non-linear trend left in the residues of the monoexponential fit, which can be largely removed by adopting a biexponential model (Fig. 3.3).

Among all 45 observations, the $k_{ep}/k_{ep}^B$ ratio ranges between 4% and 64%, with mean 35%, median 36%, 1st quartile 29%, and 3rd quartile 43%. The observation that the AIF-decomposed $k_{ep}$ only occupies a small portion of $k_{ep}^B$ further supports our statement that $k_{pe}$ should not be simply neglected from the model.

In order to assess the tissue specificity of pharmacokinetic parameters, we use scatter plots to compare the data dispersion pattern of the AIF-decomposed $k_{ep} - k_{pe}$ pair with that of the Brix’s $A - k_{ep}^B$ pair (Fig. 3.4), whose validity has been demonstrated by Hoffmann et al (60). There is a heavier overlay between normal and tumor data clouds in the Brix’s $A - k_{ep}^B$ pair plot, while the AIF-decomposed $k_{ep} - k_{pe}$ pairs from different tissue types are clearly separated by the $k_{ep} = k_{pe}$ line, suggesting that both $A$ and $k_{ep}^B$ have poorer tissue specificity than the AIF-decomposed rate constants in liver. This observation is verified by the $k_{pe}/k_{ep}$ ratio histogram, in which tumor and normal data points form distinct peaks separated by a valley at $k_{pe}/k_{ep} = 1$. Visual inspection of histograms of $k_{pe}/k_{ep}$ ratio, $K^{trans}$, and AUC (Fig. 3.5) generates the impression that
The $k_{pe}/k_{ep}$ ratio and $K^{\text{trans}}$ have similar good tissue specificity, but AUC is a poor indicator of tissue types. This impression is quantitatively validated by results of paired student’s t-test (Table 3.1). With an overall significance level of 0.1 (i.e., each single test has an individual significance level of 0.014), only the observed values of $K^{\text{trans}}$, $k_{ep}$, and $k_{pe}/k_{ep}$ ratio are significantly different between normal tissue and liver tumor.

![Figure 3.3: Residual plots from a sample AIF fitted to monoexponential (a) and biexponential (b) functions.](image-url)
Figure 3.4: Scatter plot of the Brix ($A$, $k_{ep}^B$) pair (a) and the AIF-decomposed ($k_{ep}$, $k_{pe}$) pair (b). Data clouds from tumor and normal tissues are separated by the $k_{ep} = k_{pe}$ line (dashed line) in the ($k_{ep}$, $k_{pe}$) pair plot.
Figure 3.5: Histogram of $k_{pe}/k_{ep}$ ratio (a), $K^{trans}$ (b) and AUC (c). Both the $k_{pe}/k_{ep}$ ratio and $K^{trans}$ have good tissue specificity, but AUC is not very tissue specific.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>p</th>
<th>Significance</th>
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<tr>
<td>$A$</td>
<td>0.31</td>
<td>No</td>
</tr>
<tr>
<td>$k_{ep}$</td>
<td>0.09</td>
<td>No</td>
</tr>
<tr>
<td>$K^{trans}$</td>
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<td>Yes</td>
</tr>
<tr>
<td>$k_{ce}$</td>
<td>3.1e-14</td>
<td>Yes</td>
</tr>
<tr>
<td>$k_{pe}$</td>
<td>0.84</td>
<td>No</td>
</tr>
<tr>
<td>$k_{pe}/k_{ep}$</td>
<td>6.9e-4</td>
<td>Yes</td>
</tr>
<tr>
<td>AUC</td>
<td>0.06</td>
<td>No</td>
</tr>
</tbody>
</table>

Table 3.1: Multiple student’s t-test (Bonferroni method, with an overall significance level of 0.1) results
Figure. 3.6: False positive rate (a) and false negative rate (b) for the performances of nine classifiers constructed with the parameters discussed in this chapter: seven single-parameter classifiers using AUC, $A$, $K^{\text{trans}}$, $k_{ep}^B$, $k_{ep}$, $k_{pe}$, and $k_{pe}/k_{ep}$ ratio, separately, and two dual-parameter classifiers using the $(A, k_{ep}^B)$ pair (denoted by ‘Brix’ in the plots) and the AIF-decomposed $(k_{ep}, k_{pe})$ pair (denoted by ‘AIF’ in the plots). Error bars represent the corresponding standard error of the rates.
Performances of seven single-parameter classifiers and two dual-parameter classifiers are investigated in terms of their false positive rates and false negative rates (Fig. 3.6). Among the nine classifiers, the classifier based on the AIF-decomposed $k_{ep} - k_{pe}$ pair has the best overall performance, with low values for both false positive and false negative rates (false positive rate $0.16\pm 0.10$, false negative rate $0\pm 0.01$). The next best classifier is the $K^{trans}$ classifier (false positive rate $0.26\pm 0.12$, false negative rate $0.12\pm 0.08$). While the single-parameter classifiers based on $k_{pe}/k_{ep}$ ratio and $k_{ep}$ have small false positive or negative rate, the other rate constant is large, thus limiting their application into few cases in which a specific type of misclassification is considered tolerable. All other classifiers, including those based on only $k_{ep}$ or $k_{pe}$, have comparably poor performances. This observation suggests that it is the relative importance of $k_{ep}$ and $k_{pe}$, but not their specific values, that is the most characteristic and distinctive feature of tissue.

3.5 Discussions and Conclusions

3.5.1 Relationship between the Brix’s Solutions and the General Solutions

The general solutions are a more complete description for the two-compartment system kinetic behavior than the deduced Brix’s solutions. Indeed, Brix’s solutions can be demonstrated to be another limiting case of Eqs. 3.7: when $k_{ep}B$, $k_{el}B >> k_{pe}B$, the eigenvalues can be approximated by $\lambda_1 \approx -k_{el}B$ and $\lambda_2 \approx -k_{ep}B$, and Eqs. 3.7 deduce to Eqs. 3.6.
Whether the $k_{ep}^B$, $k_{el}^B \gg k_{pe}^B$ or the $k_{ep}^B$, $k_{pe} \gg k_{el}$ limiting case is more appropriate is dependent on the physiology or pathology of the tissue of interest. In normal brain tissues with intact blood brain barrier (BBB), CA like Gd-DTPA molecules are confined in the circulation system by the BBB. CA exchange rate between blood plasma and tissue EES is extremely low. The $k_{ep}^B$, $k_{el}^B \gg k_{pe}^B$ limiting case for the Brix’s solutions is probably appropriate. In metastasis with defective capillary bed, or in normal liver parenchyma (whose sinusoid epithelium is leaky by nature), CA transfer can be hundreds of times faster than renal clearance. In these tissues the $k_{ep}^B$, $k_{pe} \gg k_{el}$ limiting case is more likely to be appropriate.

An important assumption in the Brix’s model is the relationship between the two one-way CA transfer rate constants:

$$k_{pe}^B V_p = k_{ep}^B V_e$$

[3.13]

with which $k_{pe}^B$ is expressed as a function of $k_{ep}^B$ and thus gets eliminated from the final solutions. Assumption 3.13 guarantees there is no net CA transfer between compartments when $C_p = C_e$, whose validity is obvious when both compartments are homogeneous and CA mixing within compartments is instant. However, in biological tissues, the major mechanism for CA mixing in the peripheral (EES) compartment is the relatively slow diffusion. It is not unusual for infused CA to take minutes to reach the central region of hepatic lesions. This time scale is at least one-order higher than the temporal resolution of a DCE-MRI scan. Thus, even when the mean CA concentrations measured from blood plasma and tissue ROIs are the same, the local concentrations near
the CA exchange surface, which determine the direction of net CA transfer, could still be imbalanced. For this consideration, the authors decided to abandon assumption 3.13 in the general solutions 3.7 and 3.9. Rate constants $k_{pe}$ and $k_{ep}$ are treated as independent rather than associated parameters, and their ratio can be utilized to characterize the local CA net transfer.

3.5.2 AIF-aided Eigenvalue Decomposition in a Limiting Case

The algorithm proposed above is definitely not the only way to find estimates for the three rate constants. Other methods can be constructed based on specific statistical assumptions. For example, if it is assumed that dynamic MR signals from AIF and tissue have the same error standard deviation, one can construct an overall likelihood function as the product of AIF likelihood and tissue likelihood, and find maximum likelihood estimators for those rate constants by differentiation. However, the authors prefer the proposed two-step algorithm over any overall estimator from a practical point of view. The arguments for our preference are as the following: 1). If the plasma solution in Eqs. 3.9 is treated equally to the tissue peripheral (EES) solution, the plasma amplitude $B$ must be fitted with other parameters as well. This parameter is generally not considered to be of primary interest in research and clinical practices. Including it into the fitting parameter set is not only unnecessary, but also harmful because an increase in fitting parameter number enhances the possibility of over-fitting and reduces the reliability of fitting results. 2). Experimental observations suggest that the AIF signals usually have larger error standard deviation than signals from tissue ROIs because of blood flow, more serious local motion and partial volume effect in the artery. Constructing an overall
estimator thus may overemphasize fluctuations from the plasma compartment. 3). Even if the differences in error standard deviations are not taken into account, the accuracy of plasma and tissue data are still not the same: ideally, the plasma data should be measured from capillary blood that is in direct contact with tissue EES. Due to limitations in MR spatial resolution and sensitivity, it can only be approximated by AIF measured from major arteries (the aorta if possible). The two-step nature of the proposed algorithm ensures the best fit to the more accurate tissue data, rather than compromising fitting quality with the biased AIF.

3.5.3 Tissue Specificity of dynamic MR Parameters

The observed differences in tissue specificity among pharmacokinetic and empirical DCE-MR parameters are not surprising. AUC, when integrated up to infinity, should be proportional to the mean duration that a CA molecule gets trapped within the peripheral (EES) compartment. However, in real measurements AUC integration has to be truncated at some finite time point so this parameter is only an incomplete reflection of tissue permeability with limited value for tissue type differentiation. The limiting condition for Brix’s solutions does not hold for the high CA exchange level in liver. $k_{ep}^n$ measured in this way is actually a combined effect of CA transfer in both directions. Thus, the information about the relative importance of one-way CA transfers gets lost, and pharmacokinetic parameter estimates suffer from reduced tissue specificity.

We propose a possible mechanism for the high sensitivity of the AIF-decomposed $k_{ep} - k_{pe}$ pair and $k_{pe}/k_{ep}$ ratio as follows: development of necrosis in advanced
metastases increases the EES to accessible capillary volume ratio, thus effectively enhancing net CA flow from blood plasma to peripheral (EES) compartment. Such a change is most sensitively detected by monitoring the relative importance of inter-compartmental one-way CA transfer rates, which is characterized by the $k_{ep} - k_{pe}$ pair or $k_{pe}/k_{ep}$ ratio.

Although the classifier performance results are promising, caution is especially needed in non-stationary organs in vivo. Mis-registration can cause severe errors on margins in a single voxel-by-voxel analysis. In organs with only slight motion, a practical approach of 9-voxel ‘hotspot’ assessment can be used. In abdominal imaging, however, accuracy of voxel-by-voxel analysis is challenged by physiological and patient motions. An additional step of motion correction and registration must be taken before any voxel-wise assessment. Fast DCE-MRI techniques, such as spiral DCE-MRI (61), would be beneficial for abdominal applications because they allow researchers to acquire more slices within a given temporal resolution, which can be used to improve registration quality.

In conclusion, our data show that dynamic MR measurements from liver metastases can be better described by the general solutions of the two-compartment pharmacokinetic equations than by the deduced Brix’s model. Pharmacokinetic parameter estimates obtained from a limiting case of general solutions have comparable tissue specificity to Larsson’s transfer rate $K^{\text{trans}}$. Both are superior to pharmacokinetic parameters determined by the original Brix’s model and the empirical AUC, as indicated
by visual inspection and statistical analysis. The classifier constructed with the AIF-decomposed \( k_{ep} - k_{pe} \) pair was demonstrated to have better performance in discriminating liver metastasis from normal tissue than all other classifiers investigated, including the widely-used Brix’s \( A - k_{ep}^B \) classifier. In clinical practices, controlling the false negative rate of a classifier is more important, because misclassifying tumor as normal tissue generally leads to more disastrous result than vice versa. Thus the near-zero false negative rate of the \( k_{ep} - k_{pe} \) classifier is a very desirable feature for clinical applications. Future possible applications include, but are not limited to, automatic or semi-automatic segmentation of MRI images, and automated expert system that can provide probability information to characterize likelihood of malignancy and improve objective assessment of biological effects during and after therapies.
CHAPTER 4

QUANTITATIVE MRI AS HEPATIC IRON CONTENT BIOMARKER

4.1 Overview

Iron overload is a condition that happens when iron uptake exceeds excretion for an extended time period. The excess iron builds up in liver, spleen, heart, and brain in the form of ferritin and hemosiderin. When the iron binding capacity of plasma transferrin gets saturated by the elevated iron level, free iron is released to plasma and rapidly taken by these iron storage organs. Free iron catalyzes the formation of highly cytotoxic oxygen free radicals that cause tissue damage and fibrosis in various organs (62). Fatal cardiac complications may develop if the excess iron is not removed.

Liver iron concentration (LIC) has been established as a reliable indicator of whole body iron loading (63). Currently, the standard clinical procedure for LIC determination is atomic absorption spectrophotometry analysis of liver biopsy samples. Due to the invasiveness and inconvenience of liver biopsy, a noninvasive, widely available alternative LIC quantification approach is highly desirable. MRI has been demonstrated to be a promising candidate because iron deposition changes the magnetic property of tissue, accelerating proton transverse relaxation behaviors that can be quantitatively measured by relaxometry. Several MRI LIC quantification methods have
been proposed, based on various quantities such as the transverse relaxation rate $R_2$ (64-67), the apparent transverse relaxation rate $R_2^*$ (68), and signal intensity ratios (SIR) (69-72) between liver and other reference tissues. The St. Pierre $R_2$ method has been approved by FDA and available for public service (73-75).

In this chapter, the feasibility of using quantitative MRI approaches as biomarkers in LIC evaluation was explored in a subset of 7 patients from a randomized, open label, phase II clinical trial for an oral iron chelating agent developed by a major pharmaceutical company. LIC was measured by liver biopsy and five MRI approaches (Wood $R_2$, Wood $R_2^*$, Gandon, Jensen, and St. Pierre) at multiple time points during a one year treatment period. The biopsy results, whose status as an LIC biomarker has already been well established, were taken as a ‘golden standard’. The MRI approaches would be identified as qualified LIC biomarkers if their measurement results were not significantly different from this ‘golden standard’ in statistical comparison.

4.2 Materials and Methods

4.2.1 Trial Design

The clinical trial was initially designed as a multi-center, randomized, open-label, phase II trial in pediatric and adult patients with sickle cell disease (SCD, in which iron overload is common in severe cases due to transfusion dependent anemia) to evaluate the safety and efficacy of a new oral iron chelating agent versus deferoxamine (DFO, currently the treatment-of-choice for iron overloading diseases) in the SCD patient population. Enrolled patients were randomized 2:1 to receive the new oral iron chelate (with a daily dose of 20-30 mg/kg body weight) and DFO for one year.
A subset of 7 patients were enrolled in an investigatory substudy, accepting multiple LIC assessments by five MRI approaches (Wood R2, Wood R2*, Gandon, Jensen, and St. Pierre R2), superconducting quantum interference device (SQUID), and liver biopsy. The schedule of the assessments is summarized in Table 4.1:

<table>
<thead>
<tr>
<th>Method</th>
<th>Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver MRI</td>
<td>baseline, 24 weeks, 52 weeks</td>
</tr>
<tr>
<td>SQUID</td>
<td>baseline, 24 weeks, 52 weeks</td>
</tr>
<tr>
<td>Liver biopsy</td>
<td>baseline, 52 weeks</td>
</tr>
</tbody>
</table>

**Table 4.1: Study Schedule**

Only one MRI center (Children’s Hospital in Los Angeles, CHLA) was included in this study due to early closing of enrollment. MRI images were analyzed in the Ohio State University Imaging Core Lab with an inhouse-developed software written in IDL (ITT, Boulder, CO) except for the St. Pierre R2 images, which were analyzed by the Ferriscan group (Resonance Health, Perth, Australia) due to contract issues. All MRI analysis results were collected by the OSU Imaging Core Lab and reported to the pharmaceutical company. SQUID and biopsy data were acquired by other central labs and directly reported to the pharmaceutical company.
4.2.2 MRI Acquisition

All MRI data were acquired on a GE CVI 1.5T whole body scanner (GE Medical Systems, Milwaukee, MI). The major scanning parameters of the five investigatory sequences were tabulated in Table 4.2:

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Type</th>
<th>Scan time</th>
<th>TE (ms)</th>
<th>TR (ms)</th>
<th>FA (°)</th>
<th>BW (Hz)</th>
<th>FOV(cm)</th>
<th>Slice thickness (mm)</th>
<th># slices</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wood R2</td>
<td>(SE)</td>
<td>0:14</td>
<td>3,3.5,5,8,12,18,30</td>
<td>300</td>
<td>120</td>
<td>31.25</td>
<td>49</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Wood R2*</td>
<td>(FGE)</td>
<td>0:15 min full</td>
<td>25</td>
<td>20</td>
<td>83.33</td>
<td>48</td>
<td>10</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Gandon</td>
<td>(GE)</td>
<td>0:14</td>
<td>4,9,14,21</td>
<td>120</td>
<td>20</td>
<td>12.5</td>
<td>42</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Jensen</td>
<td>(SE)</td>
<td>3:20</td>
<td>25</td>
<td>684</td>
<td>90</td>
<td>15.63</td>
<td>36</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>St.Pierre</td>
<td>(SE)</td>
<td>6:30</td>
<td>6,9,12,15,18</td>
<td>2500</td>
<td>90</td>
<td>31.25</td>
<td>40</td>
<td>5</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 4.2: Scanning parameters for the five liver iron sequences

The Wood R2 sequence is a modification of a standard spin echo (SE) sequence. In patient scan, this sequence is repeated seven times with different echo time TE’s. The patients are required to hold their breath during every repeat. When this requirement is beyond the patient’s physical capability, the scan is run at Nex = 3 with free breathing. Four axial slices are acquired with the largest coronal span of the liver, and away from the dome of the diaphragm. This sequence is acquired with a Torso PA coil.

The Wood R2* sequence is a generalization of a single echo gradient echo (GRE) sequence, with sixteen TE’s acquired at a single slice location. In patient scan, this sequence is repeated four times, at different anatomical locations corresponding to the four slices of the Wood R2 sequence. The patients are required to hold their breath...
during every repeat. When this requirement is beyond the patient’s physical capability, the scan is run at $\text{Nex} = 3$ with free breathing. This sequence is acquired with a Torso PA coil.

The Gandon R2* SIR sequence is a single echo GRE sequence acquired with the scanner’s body coil. In patient scan, this sequence is repeated four times with different TE’s. The patients are required to hold their breath during every repeat. When this requirement is beyond the patient’s physical capability, the scan is run at $\text{Nex} = 3$ with free breathing.

The Jensen R2 SIR sequence is a modification of a SE sequence acquired with the scanner’s body coil. No breath holding is required for this sequence.

The St. Pierre R2 sequence is a standard SE sequence acquired with a Torso PA coil. In patient scan, this sequence is repeated five times with different TE’s, with a total scanning time of about 35 min. No breath holding is required for this sequence. The scanning protocol is flow-charted in Fig. 4.1.
Figure 4.1: Flow chart of liver St. Pierre R2 scanning protocol
4.2.3 Data Analysis

The St. Pierre images were analyzed by the Ferriscan group. The other four approaches were implemented in IDL (ITT, Boulder, CO) with the following algorithms:

**Wood R2:**

1. Resize the interpolated images back to acquisition matrix size if necessary.
2. Liver Region of Interest’s (ROI’s) are defined on all four slices by an experienced radiologist. The ROI’s should cover the whole liver but exclude large blood vessels and partial-volume voxels on liver periphery (Fig. 4.2).

![Figure 4.2: Wood R2 ROI definition](image)

3. Calculate R₂ values for each voxel within the ROI’s by fitting the signal intensities at different echo times to a monoexponential-plus-constant model:

\[
S(T_E) = S_0 e^{-T_E R_2} + C
\]

[4.1]

where \(C\) is a constant representing thermal noise level.
4. All $R_2$ values from four ROI’s are averaged to get a global estimation for liver $R_2$:

$$\bar{R}_2 = \frac{\sum_{i=1}^{4} \sum_{j=1}^{N_i} R_{2,ij}}{\sum_{i=1}^{4} N_i}$$  \[4.2\]

where $N_i$ is the number of voxels in the ROI on the $i^{th}$ slice, $R_{2,ij}$ is the $R_2$ value of the $j^{th}$ pixel on the $i^{th}$ slice.

5. The global estimation of $R_2$ is then converted to LIC in mg [Fe]/g dry tissue by a calibration curve (76):

$$[Fe] = \left(\frac{\bar{R}_2}{3.18^{1.642}} - 21.49\right) / 40.64$$  \[4.3\]

**Wood R2***:

1. Resize the interpolated images back to acquisition matrix size if necessary.

2. Liver ROI’s are defined on all four slices by an experienced radiologist. The ROI’s should cover the whole liver but exclude large blood vessels and partial-volume voxels on liver periphery (Fig. 4.3).

3. Calculate $R_2^*$ values for each voxel within the ROI’s by fitting the signal intensities at different echo times to a monoexponential-plus-constant model:

$$S(T_e) = S_0 e^{-T_e R_2^*} + C$$  \[4.4\]

where $C$ is a constant representing thermal noise level.
4. All $R_2^*$ values from four ROI’s are averaged to get a global estimation for liver $R_2^*$:

$$\overline{R}_2^* = \frac{\sum_{i=1}^{4} \sum_{j=1}^{N_i} R_2^*}{\sum_{i=1}^{4} N_i}$$  \hspace{1cm} [4.5]

where $N_i$ is the number of voxels in the ROI on the $i^{th}$ slice, $R_2^*\_ij$ is the $R_2^*$ value of the $j^{th}$ pixel on the $i^{th}$ slice.

5. The global estimation of $R_2^*$ is converted to LIC in mg[Fe]/g dry tissue by a calibration curve (77):

$$[Fe] = \overline{R}_2^* \times 0.0246 - 0.5286$$  \hspace{1cm} [4.6]
Gandon $R2^*$ SIR:

1. Three liver ROI’s and two muscle ROI’s are defined by an experienced radiologist on one slice. All five ROI’s are circular, with their areas to be ~1cm$^2$ (Fig. 4.4).

![Figure 4.4: Gandon R2* SIR ROI definition](image)

2. SIR is calculated by:

$$SIR_{\text{Gandon}} = \frac{\bar{S}_{\text{Liver}}}{\bar{S}_{\text{Muscle}}}$$  \hspace{1cm} [4.7]

for each TE, where $\bar{S}_{\text{Liver}}$ and $\bar{S}_{\text{Muscle}}$ are the mean signal intensities averaged over all liver and muscle ROI’s, separately.

3. Estimations of LIC (in $\mu$mol [Fe]/g dry tissue) are calculated separately from the 4 sets of images with different TE’s at 4, 9, 14, and 21 ms:

$$[Fe]_4 = 360 - 230 \times SIR_4$$
\[ [Fe]_9 = 180 - 100 \times SIR_9 \]
\[ [Fe]_{14} = 140 - 87 \times SIR_{14} \]
\[ [Fe]_{21} = 100 - 62 \times SIR_{21} \] \[4.8\]

4. The most appropriate LIC estimation is picked up from those four by the following rule (78, 79):

If \([Fe]_{21} < 40 \ \mu\text{mol [Fe]/g dry tissue},\)

then method fail;

If \(40 < [Fe]_{21} < 75 \ \mu\text{mol [Fe]/g dry tissue},\)

then \([Fe] = [Fe]_{21};\)

If \([Fe]_{21} > 75 \ \mu\text{mol [Fe]/g dry tissue},\)

and \(0 < [Fe]_{14} < 120 \ \mu\text{mol [Fe]/g dry tissue},\)

and \([Fe]_9 < 120 \ \mu\text{mol [Fe]/g dry tissue},\)

then \([Fe] = [Fe]_{14};\)

If \([Fe]_{14} > 120 \ \mu\text{mol [Fe]/g dry tissue},\)

and \(120 \ \mu\text{mol [Fe]/g dry tissue} < [Fe]_9 < 160 \ \mu\text{mol [Fe]/g dry tissue},\)

and \([Fe]_4 < 150 \ \mu\text{mol [Fe]/g dry tissue},\)

then \([Fe] = [Fe]_9;\)

If \([Fe]_9 > 160 \ \mu\text{mol [Fe]/g dry tissue},\)

and \(50 \ \mu\text{mol [Fe]/g dry tissue} < [Fe]_4 < 280 \ \mu\text{mol [Fe]/g dry tissue},\)

then \([Fe] = [Fe]_4;\)

If \([Fe]_4 > 280 \ \mu\text{mol [Fe]/g dry tissue,}\)

method fail.
5. Convert LIC into mg [Fe]/g dry tissue by multiplying a conversion factor 56/1000.

**Jensen R2 SIR:**

1. One liver ROI and one muscle ROI are defined by an experienced radiologist on one slice. Both ROI’s are rectangular, with their areas to be ~1cm² (Fig. 4.5).

![Figure 4.5: Jensen R2 SIR ROI definition](image)

2. SIR is calculated by:

\[
SIR_{\text{Jensen}} = \frac{S_{\text{Liver}}}{S_{\text{Muscle}}} \quad [4.9]
\]

3. Estimation of LIC in μmol [Fe]/g dry tissue is obtained by numerically solving the following equation (80, 81):
\[
(SIR \times T_R) \times \left[1 - e^{-T_e \left(\frac{1}{T_{10L}} + k[F_e]\right)}\right] = K_0 \times e^{-T_2 \left(\frac{1}{T_{20L}} + k[F_e]\right)}
\]  

[4.10]

where \(T_{10L} = 521\) ms, \(T_{20L} = 42.5\) ms are standard longitudinal/transverse relaxation times \(T_1/T_2\) for liver at 1.5 Tesla. \(K_0 = 2130.8\) and \(k = 2.3290 \times 10^{-4}\) are deduced parameters. \(TR\) and \(TE\) are the repetition time and echo time used: \(TR = 684\) ms, \(TE = 25\) ms.

4. Convert LIC into mg [Fe]/g dry tissue by multiplying a conversion factor 56/1000.

### 4.2.4 Quality Check with a Standard Phantom

Standard phantoms were built for quality control and inter-site cross-checking purposes (Fig. 4.6 a). 13 plastic bottles containing 0 to 24 mM MnCl\(_2\) solutions were fixed in a plexiglass container (Fig. 4.6 b), which was filled with 0.25 mM MnCl\(_2\) solution. All MnCl\(_2\) solutions were doped with 0.03% NaN\(_3\) to prevent bacteria and fungi growth.

Ten phantom scans were acquired during two years, whose dates and contents are summarized in Table 4.3. Coronal images were acquired with the same scanning parameters as in patient scan for all five investigatory approaches. \(R_2/R_2^*\) values were assessed for Wood R2, Wood R2*, and St. Pierre R2 sequences with monoexponential-plus-constant model. SIR values between MnCl\(_2\) solutions and water control were calculated as an index of stability for Jensen SIR and four Gandon SIR (with different TE’s) sequences.
Figure 4.6: (a). Phantom construction; (b). MnCl₂ concentrations (view from the top)

<table>
<thead>
<tr>
<th>Date</th>
<th>JW R2</th>
<th>JW R2*</th>
<th>St. Pierre R2</th>
<th>Jensen SIR</th>
<th>Gandon 4</th>
<th>Gandon 9</th>
<th>Gandon 14</th>
<th>Gandon 21</th>
</tr>
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<tbody>
<tr>
<td>6/6/2004(I)</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
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<td>6/6/2004(II)</td>
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<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
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<tr>
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<td>10/23/2004</td>
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</tr>
</tbody>
</table>

Table 4.3: Phantom scan information
4.3 Results

4.3.1 Quality Check with Phantom

Because there is only one MRI site included in this substudy, there is no need for inter-site cross-checking. To evaluate quality control of this study, temporal stability of the five investigatory approaches were checked with model selection techniques, in which the phantom data were fitted to a constant-effect model:

\[ Y_{it} = \alpha + \varepsilon_{it}, \quad \varepsilon_{it} \sim N(0, \sigma^2) \quad [4.11] \]

(where \( Y_{it} \)'s represent measurement quantities, \( \alpha \) is the constant effect, \( i \) denotes different scans, and \( t \) denotes different measurements acquired within one scan) and an analysis of variance (ANOVA) model:

\[ Y_{it} = \alpha_i + \varepsilon_{it}, \quad \varepsilon_{it} \sim N(0, \sigma^2) \quad [4.12] \]

(In the ANOVA model it is assumed that each scan has a different effect) and calculate the mean square errors for both models (denote them as \( msE_{\text{const}} \) and \( msE_{\text{ANOVA}} \), separately). It can be shown that the ratio \( msE_{\text{const}} / msE_{\text{ANOVA}} \) follows an \( F_{\text{df const}, \text{df ANOVA}} \) distribution. Thus, a test for the null hypothesis \( H_0: \{ \text{The ANOVA model is the same as the constant effect model} \} \) versus the alternative hypothesis \( H_A: \{ \text{The ANOVA model is better than the constant effect model} \} \) at significance level \( \alpha \) is:

\[ \frac{msE_{\text{const}}}{msE_{\text{ANOVA}}} > F_{\text{df const}, \text{df ANOVA}, \alpha} \quad [4.13] \]

Further modifications are needed before the general procedure can be applied to the current phantom data set. First of all, not all the measurement quantities have replicate observations within each scan. For example, the SIR values are not calculated on a pixel-
by-pixel basis. For each bottle in each scan, there is only one SIR measurement for a specific protocol. However, it is still possible to get an estimate of the mean square error of the SIR value by error propagation (see below). In this case, the constant model is fitted with the mean value of the measurement quantity, which changes the form of the test statistics. Considering there might be different numbers of replicate observations for each scan and that the number of replicates might be unknown, one can get a more general, but stronger condition:

\[
\frac{msE_{\text{const,mean}}}{msE_{\text{ANOVA}}} > F_{m-1, \text{df ANOVA}, \alpha}
\]  
[4.14]

where \( m \) is the number of different scans and \( msE_{\text{const,mean}} \) is the mean square error of fitting the mean values to a constant effect model, which equals to the variance of the measurement quantity mean values among different scans.

For the SIR values, there is no replicate in an individual scan. So \( msE_{\text{ANOVA}} \) cannot be calculated by model fitting. One way of estimating this quantity is to utilize error propagation: we measure the standard deviations of \( S_{\text{MnCl}_2} \) and \( S_{\text{Water}} \), \( \delta S_{\text{MnCl}_2} \) and \( \delta S_{\text{Water}} \), and construct an estimate of the standard deviation of the SIR by:

\[
\delta S_{\text{IR}} = \delta\left(\frac{S_{\text{MnCl}_2}}{S_{\text{Water}}}\right) = \frac{S_{\text{MnCl}_2}}{S_{\text{Water}}} \sqrt{\left(\frac{\delta S_{\text{MnCl}_2}}{S_{\text{MnCl}_2}}\right)^2 + \left(\frac{\delta S_{\text{Water}}}{S_{\text{Water}}}\right)^2}
\]  
[4.15]

\( \delta S_{\text{IR}} \), \( \delta R_2 \), and \( \delta R_2^* \) are evaluated in two representative scans, one from the middle stage of the study (2/23/2005) and one from the late stage (8/22/2005). Mean square errors for the corresponding measurement quantities are estimated by:
\[ msE = \frac{(n_1 - 1)(\delta Y_1)^2 + (n_2 - 1)(\delta Y_2)^2}{n_1 + n_2 - 2} \]  

where \( n_1 \) and \( n_2 \) are the numbers of replicates in the corresponding scans, \( \delta Y_1 \) and \( \delta Y_2 \) are the standard deviations from the corresponding scans. \( Y \) can be \( SIR \), \( R_2 \), or \( R_2^* \).

The Bonferroni method was used for multiple hypotheses testing, because the hypothesis \( H_0 \) needs to be tested for every bottle with a given approach.

Multiple hypotheses testing results are tabulated in Table 4.4 to Table 4.11. Even with an overall significance level as low as at most 0.13 (for every single investigatory MRI approach; corresponds to an individual significance level 0.01 for each bottle), the ANOVA model is not significantly better than a constant effect model for all investigatory MRI approaches. These results support a statement that all five approaches are temporally stable during a two-year time span that covers the whole duration of the substudy.
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Table 4.4: Wood R2 phantom result summary
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Table 4.5: Wood R2+ phantom result summary
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|                |       | msE  |       |       |       |       |       |       |       |       |       |       |       |       |
| msE_mean (=Var) |       | 0.5  | 36.6  | 4.2   | 3.4  | 10.0  | 27.7  | 15.5  | 30.0  | 433.0 | 6010.3 | 2453.6 | 941.6 | 1511.1 |
| msE            |       | 0.7  | 32.6  | 15.1  | 14.2 | 26.6  | 52.9  | 60.3  | 356.8 | 16318.1 | 8008.5 | 15497.2 | 11638.2 | 143391.1 |
| msE_mean/msE   |       | 0.7  | 1.1   | 0.3   | 0.2  | 0.4   | 0.5   | 0.3   | 0.1   | 0.0   | 0.1   | 0.0   | 0.0   | 0.0   |

m = 9 def=465 F(8,465, 0.01)=2.5496

Table 4.6: St. Pierre R2 phantom result summary
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| msE_mean | 0.003 | 0.0005 | 0.0002 | 0.0001 | 0.00006 | 0.00003 | 0.00001 | 0.00001 | 0.00001 | 0.00001 | 0.00001 | 0.00001 |
| msE      | 0.008 | 0.003 | 0.001 | 0.001 | 0.002 | 0.0008 | 0.0003 | 0.0003 | 0.0002 | 0.0002 | 0.0002 | 0.0003 |
| msE_mean/msE | 0.4 | 0.2 | 0.1 | 0.1 | 0.03 | 0.04 | 0.03 | 0.02 | 0.03 | 0.04 | 0.06 | 0.04 |

m = 10  dof=427  F(9,427,0.01)=2.449

Table 4.7: Jensen R2 SIR phantom result summary
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Table 4.8: Gannon R2+ SIR (TE = 4 ms) phantom result summary.
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\[
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\text{msE} = 0.001 \\
\text{msE}_{\text{mean}}/\text{msE} = 0.4 \\
m = 10 \\
df=406 \\
F(9,406,0.01) = 2.4512
\]

Table 4.9: Gandon R2* SIR (TE = 9 ms) phantom result summary
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Table 4.10: Gandon R2* SIR (TE = 14 ms) phantom result summary
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<th>1.5mM</th>
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<th>2.5mM</th>
<th>3.5mM</th>
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<td>0.74</td>
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<td>0.06</td>
<td>0.04</td>
<td>0.03</td>
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<td>0.02</td>
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<td>0.17</td>
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<td>0.04</td>
<td>0.02</td>
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<td>0.17</td>
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<td>0.03</td>
<td>0.02</td>
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<td>0.57</td>
<td>0.40</td>
<td>0.17</td>
<td>0.06</td>
<td>0.05</td>
<td>0.02</td>
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<td>0.02</td>
<td>0.02</td>
<td>0.04</td>
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<td>0.60</td>
<td>0.41</td>
<td>0.18</td>
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<td>0.02</td>
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<td>0.03</td>
</tr>
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<td>0.76</td>
<td>0.57</td>
<td>0.38</td>
<td>0.16</td>
<td>0.09</td>
<td>0.05</td>
<td>0.02</td>
<td>0.03</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.03</td>
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<td>0.83</td>
<td>0.61</td>
<td>0.41</td>
<td>0.19</td>
<td>0.08</td>
<td>0.04</td>
<td>0.02</td>
<td>0.03</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.03</td>
</tr>
</tbody>
</table>

| msE_mean (=Var) | 0 | 0.001 | 0.0003 | 0.0001 | 0.0001 | 0.0001 | 0.0004 | 0.0003 | 0.0001 | 0.0002 | 0.0001 | 0.00003 | 0.00001 |
| msE | 0.001 | 0.0005 | 0.0003 | 0.0004 | 0.0003 | 0.0002 | 0.0001 | 0.0002 | 0.0001 | 0.00001 | 0.0002 | 0.00003 | 0.00005 |
| msE_mean/msE | 0.8  | 0.6    | 0.4    | 0.2    | 0.5    | 0.2    | 0.3    | 0.3    | 0.15    | 0.05    | 0.01    | 0.02 |

m = 10  \quad dof=392  \quad F(9,392,0.01)=2.4527

Table 4.11: Gandon R2* SIR (TE = 21 ms) phantom result summary
4.3.2 Patient Data

LIC values measured with liver biopsy, SQUID, and the five investigatory MRI approaches are tabulated in Table 4.12 to Table 4.18:

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>BSL Biopsy</th>
<th>24-wk Biopsy</th>
<th>52-wk Biopsy</th>
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</thead>
<tbody>
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<td>0514_00003</td>
<td>28.3</td>
<td>--</td>
<td>27.0</td>
</tr>
<tr>
<td>0514_00004</td>
<td>11.7</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>0514_00007</td>
<td>17.3</td>
<td>--</td>
<td>7.7</td>
</tr>
<tr>
<td>0514_00011</td>
<td>20.3</td>
<td>--</td>
<td>20.0</td>
</tr>
<tr>
<td>0514_00014</td>
<td>14.6</td>
<td>--</td>
<td>12.9</td>
</tr>
<tr>
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<td>20.6</td>
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<td>16.0</td>
</tr>
<tr>
<td>0514_00019</td>
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Table 4.12: Liver Biopsy Results (All values are in units of mg [Fe]/g dry tissue)

<table>
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</tr>
<tr>
<td>0514_00007</td>
<td>9.7</td>
<td>9.3</td>
<td>7.2</td>
</tr>
<tr>
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<td>9.6</td>
<td>11.4</td>
<td>10.8</td>
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<td>0514_00014</td>
<td>8.4</td>
<td>8.1</td>
<td>10.2</td>
</tr>
<tr>
<td>0514_00015</td>
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<td>10.2</td>
</tr>
<tr>
<td>0514_00019</td>
<td>10.2</td>
<td>11.1</td>
<td>13.3</td>
</tr>
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</table>

Table 4.13: SQUID Results (All values are in units of mg [Fe]/g dry tissue)
<table>
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<th>24-wk Wood R2</th>
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</tr>
</thead>
<tbody>
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<td>0514_00003</td>
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</tr>
<tr>
<td>0514_00004</td>
<td>8.8</td>
<td>--</td>
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</tr>
<tr>
<td>0514_00007</td>
<td>16.3</td>
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<td>26.0</td>
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<td>14.0</td>
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<tr>
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<td>18.1</td>
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<tr>
<td>0514_00019</td>
<td>21.0</td>
<td>27.1</td>
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Table 4.14: Wood R2 MRI Results (All values are in units of mg [Fe]/g dry tissue)

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<td>--</td>
</tr>
<tr>
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<td>16.5</td>
<td>14.8</td>
<td>10.9</td>
</tr>
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<td>23.3</td>
<td>22.2</td>
<td>25.4</td>
</tr>
<tr>
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<td>11.4</td>
<td>13.2</td>
<td>15.8</td>
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<td>0514_00015</td>
<td>31.4</td>
<td>23.8</td>
<td>15.4</td>
</tr>
<tr>
<td>0514_00019</td>
<td>19.5</td>
<td>23.4</td>
<td>26.6</td>
</tr>
</tbody>
</table>

Table 4.15: Wood R2* MRI Results (All values are in units of mg [Fe]/g dry tissue)
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<tbody>
<tr>
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<td>&gt;15.7</td>
<td>&gt;15.7</td>
</tr>
<tr>
<td>0514_00004</td>
<td>14.0</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>0514_00007</td>
<td>&gt;15.7</td>
<td>&gt;15.7</td>
<td>14.6</td>
</tr>
<tr>
<td>0514_00011</td>
<td>&gt;15.7</td>
<td>&gt;15.7</td>
<td>&gt;15.7</td>
</tr>
<tr>
<td>0514_00014</td>
<td>&gt;15.7</td>
<td>&gt;15.7</td>
<td>&gt;15.7</td>
</tr>
<tr>
<td>0514_00015</td>
<td>&gt;15.7</td>
<td>&gt;15.7</td>
<td>&gt;15.7</td>
</tr>
<tr>
<td>0514_00019</td>
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<td>&gt;15.7</td>
<td>&gt;15.7</td>
</tr>
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</table>

Table 4.16: Gandon SIR MRI Results (All values are in units of mg [Fe]/g dry tissue)

<table>
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<th>52-wk Jensen</th>
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<td>0514_00004</td>
<td>17.4</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>0514_00007</td>
<td>20.9</td>
<td>20.7</td>
<td>18.6</td>
</tr>
<tr>
<td>0514_00011</td>
<td>25.0</td>
<td>22.3</td>
<td>26.3</td>
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<tr>
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Table 4.17: Jensen SIR MRI Results (All values are in units of mg [Fe]/g dry tissue)
<table>
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<th>52-wk St Pierre</th>
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</thead>
<tbody>
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<tr>
<td>0514_00004</td>
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<td>--</td>
</tr>
<tr>
<td>0514_00007</td>
<td>18.6</td>
<td>16.2</td>
<td>10.8</td>
</tr>
<tr>
<td>0514_00011</td>
<td>25.8</td>
<td>25.9</td>
<td>23.2</td>
</tr>
<tr>
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<td>15.8</td>
</tr>
<tr>
<td>0514_00019</td>
<td>22.7</td>
<td>21.2</td>
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</tr>
</tbody>
</table>

Table 4.18: St. Pierre R2 MRI Results (All values are in units of mg [Fe]/g dry tissue)

Follow-up data were not collected for one patient (ID 0514_00004) due to an early withdraw from the study after the baseline scan. Baseline liver biopsy is lacking for patient 0514_00019. St. Pierre R2 MRI result in the 52-week follow-up for this patient is also missing because the data was determined to be non analyzable by the Ferriscan group, and a rescan was not requested by the pharmaceutical company.

Because it is unrealistic to expect for a complete removal of all the excess iron within one year in patients with heavy iron overload (LIC > 10 mg [Fe]/g dry tissue), an annual decrease in LIC of at least 3 mg [Fe]/g dry tissue was selected as a target. This endpoint ensures iron burden to be lowered to a safe level within a few years for most patients, and was agreed upon with health authorities in the United States and Europe. Patient responses based on this endpoint selection are tabulated in Table 4.19, where the symbol ↓ denotes an annual decrease of at least 3 mg [Fe]/g dry tissue, ↑ denotes an annual increase of at least 3 mg [Fe]/g dry tissue, and – denotes an annual change smaller than this target.
<table>
<thead>
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<th>Biopsy</th>
<th>SQUID</th>
<th>Wood R2</th>
<th>Wood R2*</th>
<th>Gandon SIR</th>
<th>Jensen SIR</th>
<th>St.Pierre R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0514_00003</td>
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<td>↓</td>
<td>↑</td>
<td>NA</td>
<td>--</td>
<td>↓</td>
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<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<td>↓</td>
<td>↓</td>
<td>NA</td>
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<td>↓</td>
</tr>
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<td>--</td>
<td>↑</td>
<td>NA</td>
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<td>NA</td>
</tr>
</tbody>
</table>

**Table 4.19: One-year patient response summary**

The Gandon SIR MRI approach was originally proposed for a healthier subject population, with a maximum measurable LIC limit at 15.7 mg [Fe]/g dry tissue. This limit has been demonstrated to be too low for the patient population involved in this sub-study. Only 2 of 19 scans generate measurable results. This approach was thus excluded from further analysis. Its feasibility of being used as an LIC biomarker should be assessed in a different subject population with lower iron burdens.

All other MRI approaches and SQUID show good agreements with liver biopsy assay in patient response assessments (3/5 for SQUID, 4/5 for Wood R2, 3/5 for Wood R2*, 3/5 for Jensen SIR, and 4/5 for St. Pierre R2). However, while the MRI approaches generate LIC measurements comparable to those obtained in liver biopsy assay, LIC values measured by SQUID are severely underestimated (6% to 80% underestimation, with a median of 43%), as has been previously reported (82).

Feasibility of using noninvasive MRI approaches as LIC biomarkers can be more quantitatively assessed by statistical analysis. In the following ANOVA analysis, LIC
measurements in mg [Fe]/g dry tissue are taken as experimental responses. Time of study (i.e. baseline, 24-week and 52-week follow ups) and LIC measurement approaches (Wood R2, Wood R2*, Jensen SIR, and St. Pierre R2) are identified as treatment factors, and different patients (who may accept different medical agents) are identified as a blocking factor. Because the experiment setting forms a single-replicate complete block design with empty cells (83), there are not enough degrees of freedom for an analysis incorporating all possible interactions between treatments and blocking factors. We assume that all block-treatment interactions are negligible. Moreover, the phantom data shows that all MRI approaches are temporally stable during the entire study, so it is safe to assume there is no time-approach interaction. Thus, the corresponding model for this experiment is:

\[ y_{hij} = \mu + \theta_h + \alpha_i + \beta_j + \epsilon_{hij} \]  \hspace{1cm} [4.17]

where \( y_{hij} \) is the LIC measurement on the \( h \)th patient at the \( i \)th time with the \( j \)th approach. \( \mu \) is an arbitrary constant, \( \theta_h \) is the blocking effect of the \( h \)th patient, \( \alpha_i \) is the effect of the \( i \)th time of study, \( \beta_j \) is the effect of the \( j \)th approach. \( \epsilon_{hij} \sim N(0, \sigma^2) \) is the random effect characterizes all other random factors that are not included in this model. \( \epsilon_{hij} \)'s are mutually independent with each other.

Three-way ANOVA table for model [4.17] is reproduced in Table 4.20:
Mean square value for the blocking factor (patient) is 19.27 times larger than error mean square. This observation suggests that blocking helps to reduce the size of the error mean square. With an overall significance level $\alpha$ of at most 0.05, individual significance levels for testing the hypotheses {Main effect of study time is negligible} and {Main effect of approach is negligible} are both $\alpha^* = 0.025$. The p values from Table 4.20 show that both hypotheses will be rejected with an overall significance level of at most 0.05, i.e, both study time and MRI approaches have non-negligible effects on LIC measurement.

In assessing the feasibility of using MRI approaches as LIC biomarkers, the question of primary concern is whether any of the MRI approaches generates statistically different results than others. Another question of interest is if there is any overall change in LIC level after treatment. To address these two questions, pair wise comparisons between the main effects of study time (abbreviated as BSL/F24/F52 for baseline, 24-week and 52-week follow-ups, averaged over patients and approaches) and MRI approaches (averaged over patients and study times) were performed using Tukey's honestly significant difference criterion. For each pair wise comparison, individual
simultaneous confidence level is set at 97.5% so the overall confidence level is at least 95%.

The corresponding simultaneous confidence intervals are tabulated in Table 4.21 (main effects in study times) and Table 4.22 (main effects in MRI approaches). The difference between the main effects of two MRI approaches (or two study times) is considered significant when the confidence interval of the corresponding contrast does not contain 0 (cells marked out by yellow).

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Contrast Estimate</th>
<th>Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wood R2 - Wood R2*</td>
<td>0.0211</td>
<td>[-4.0612, 4.1033]</td>
</tr>
<tr>
<td>Wood R2 - Jensen</td>
<td>-4.3947</td>
<td>[-8.4770, -0.3125]</td>
</tr>
<tr>
<td>Wood R2 - St. Pierre</td>
<td>-0.6130</td>
<td>[-4.7572, 3.5311]</td>
</tr>
<tr>
<td>Wood R2 - Biopsy</td>
<td>0.3639</td>
<td>[-4.3881, 5.1158]</td>
</tr>
<tr>
<td>Wood R2* - Jensen</td>
<td>-4.4158</td>
<td>[-8.4981, -0.3335]</td>
</tr>
<tr>
<td>Wood R2* - St. Pierre</td>
<td>-0.6341</td>
<td>[-4.7782, 3.5101]</td>
</tr>
<tr>
<td>Wood R2* - Biopsy</td>
<td>0.3428</td>
<td>[-4.4091, 5.0948]</td>
</tr>
<tr>
<td>Jensen - St. Pierre</td>
<td>3.7817</td>
<td>[-0.3624, 7.9259]</td>
</tr>
<tr>
<td>Jensen - Biopsy</td>
<td>4.7586</td>
<td>[0.0067, 9.5105]</td>
</tr>
<tr>
<td>St. Pierre - Biopsy</td>
<td>0.9769</td>
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</table>

Table 4.21: 97.5% simultaneous confidence intervals for MRI approach main effects

<table>
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<tr>
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<th>Confidence Interval</th>
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</thead>
<tbody>
<tr>
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<tr>
<td>BSL – F52</td>
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<tr>
<td>F24 – F52</td>
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</tr>
</tbody>
</table>

Table 4.22: 97.5% simultaneous confidence intervals for study time main effects
Table 4.21 shows that LIC measurements (averaged over patients and study times) made with Jensen’s SIR approach are significantly higher than those made by Wood R2, Wood R2*, and liver biopsy. **No significant difference can be detected between Wood R2, Wood R2*, St. Pierre R2, and liver biopsy approaches.**

Table 4.22 shows that LIC measurements (averaged over patients and MRI approaches) at the 52-week follow-up are significantly lower than those measured at the baseline. No significant change can be detected between baseline and the 24-week follow up, or between the two follow up scans.

### 4.4 Discussions and Conclusions

Feasibility of using the three relaxometric MRI approaches (Wood R2, Wood R2*, and St. Pierre R2) as noninvasive LIC biomarkers has been justified by quantitative ANOVA analysis and qualitative treatment response assessment through comparison with the ‘golden standard’ liver biopsy in 7 SCD patient with severe liver iron overload at 1.5 Tesla. Although the Jensen SIR approach and SQUID show some agreement with biopsy in treatment response assessment, they are not identified as feasible LIC biomarkers due to significant overestimation or underestimation of LIC. Feasibility of the Gandon SIR approach cannot be assessed with the current data set, due to the low detection limit of the approach.

Percent changes in LIC relative to baseline for the Jensen SIR approach are significantly smaller than those for the three relaxometric approaches (only 24% of those for Wood R2, 29% of those for Wood R2*, 16% of those for St. Pierre R2, all are median
values), suggesting that the Jensen SIR approach is less sensitive than the relaxometric approaches when iron concentrations are high. This is because hepatic $R_2$ values measured in this patient population are in the range of 100 to 300 Hz, which corresponds to $T_2$’s between 3.3 to 10 ms. SE signals from liver are almost completely lost at $TE = 25$ ms used by the Jensen SIR approach. Thus, the measured liver-to-muscle SIR reflects more of MR thermal noise than of the true proton relaxation behavior in liver. This problem is inherent for any SIR based MRI approach: the sensitivity can only be guaranteed for $T_2$ values close to the echo time used. If $T_2$ is too short compared with $TE$, signal loss happens before detection; if $T_2$ is too long, then there is not enough contrast to differentiate small differences in relaxation behavior induced by local iron deposit. Conversely, relaxometric approaches based on $R_2$ or $R_2^*$ avoid this problem by monitoring the transverse relaxation process for an extended time period rather than taking a snapshot, thus getting a better dynamic range in LIC detection.

The major drawback of relaxometric approaches is the acquisition time. Multiple acquisitions are required for accurate evaluation of $R_2$ or $R_2^*$. In standard SE or GRE sequences, the total acquisition time is proportional to the number of different $TE$’s acquired, making acquisition of high-resolution images intolerably long. The St. Pierre R2 sequence, for example, has an acquisition time of about 35 min. It is impossible to ask the patients to hold breath for such a long time, so errors from respiratory motion are inevitable in high resolution images. The Wood R2 and R2* approaches sacrifice image resolution to accelerate acquisition. Although this strategy works for liver, it cannot be transplanted to other iron-rich organs such as brain or heart, in which fine anatomic structures are more abundant and more important. Moreover, larger voxels in low-
resolution images are more vulnerable to macroscopic background field inhomogeneity. This problem would become more severe if the relaxometric MRI LIC biomarkers were to be used at high or ultrahigh field scanners. A postprocessing technique dealing with this issue will be discussed in the next chapter.

Another solution that accelerates acquisition without sacrificing spacing resolution is to implement special, relaxometry-oriented fast imaging sequences, such as the Gradient Echo Sampling of Spin Echo (GESSE) (84, 85) or Gradient Echo Sampling of Free Induction Decay and Echo (GESFIDE) (86, 87) sequences. However, these fast imaging sequences are primarily developed for research purposes and are usually not commercially available. Because customer-build sequences are poorly supported, sometimes even completely forbidden, on scanners dedicated for routine clinical services, clinical application of these advanced sequences are highly restricted. A promising candidate in this category is the modified echo planar imaging (EPI) sequence introduced in the next chapter, which has been integrated in Philips Achieva scanners (Philips, Cleveland, OH) and open for public use.

In conclusion, we have demonstrated that LIC measurements generated by MRI approaches based on relaxometric measurement of $R_2$ or $R_2^*$ are not significantly different from those obtained by the standard liver biopsy assay in a set of 7 SCD patients with heavy liver iron overload at 1.5 Tesla. These relaxometric MRI approaches are thus identified as feasible LIC biomarkers that can be used in the patient population with heavy liver iron overload to complement or replace the invasive liver biopsy to improve diagnosis, prognosis, and patient care. SIR-based MRI approaches and SQUID also
show some agreement with liver biopsy in qualitative treatment effect assessment, but are less informative than the relaxometric MRI approaches due to severe overestimation or underestimation of LIC values.
CHAPTER 5

POSTPROCESSING CORRECTION FOR DISTORTIONS IN $T_2^*$ DECAY

CAUSED BY NONLINEAR (QUADRATIC) CROSS-SLICE $B_0$

INHOMOGENEITY

5.1 Overview

Over the past few years, the MRI community have witnessed the evolution of high ($\geq 3$ Tesla) and ultra-high ($\geq 7$ Tesla) field whole-body scanners from lab prototypes into mature commercial products that are increasingly more involved in clinical studies and practices. Because the apparent transverse relaxation rate $R_2^*$ has been demonstrated to be proportional to the main field strength up to 7 Tesla (88), quantifying tissue iron content with $R_2^*$ at higher field has the advantage of being able to reveal more subtle changes in iron load, which can be beneficial for early detection of iron overload diseases. However, the main magnetic field $B_0$ is also less homogeneous at higher field due to engineering difficulties and in vivo susceptibility differences on tissue/environment or tissue/tissue interfaces. Higher level of $B_0$ inhomogeneity causes additional dephasing in larger regions in the field of view, whose effect needs to be corrected for quantification purposes.
As a compromise between acquisition time, signal-to-noise ratio (SNR) and spatial resolution, 2D gradient echo (GRE) sequences are usually used for \textit{in vivo} $R_2^*$ measurement. The number of slices is usually limited, with a slice thickness several times larger than the in-plane resolution. For a typical voxel like this, the slice selection direction is particularly sensitive to the cross-slice $B_0$ inhomogeneity effect. Several compensation methods have been proposed based on this concept, such as manipulation of the slice selection gradient (89) or RF pulse (90). These compensation methods require customer-made modifications on the source code level of this system, which cannot be authorized by the manufacturers. Clinical use of such un-authorized low-level modifications is highly restricted because of regulatory considerations, so postprocessing correction methods based on commercially available sequences are more favorable in clinical applications.

As a first-order approximation, Fernandez-Seara and Wehrli proposed an iterative postprocessing technique to correct for the sinc modulation generated by a linear $B_0$ gradient across slice thickness (91). Their method was later simplified by Dahnke and Schaeffter (92), combined with a fast $T_2^*$ estimation approach by numerical integration (93). Variations of the sinc-correction method have been implemented in some commercial postprocessing software packages and start to be used in clinical studies (94). More complex signal behaviors due to the nonlinear components in background $B_0$ inhomogeneity are expected in the presence of higher-order $B_0$ inhomogeneity, especially in high and ultrahigh field MRI, although no formal derivation was given in literature so far.
In this chapter, an analytical expression for the $T_2^*$ decay modulation was derived for a quadratic cross-slice $B_0$ inhomogeneity. Correction based on this expression was applied to phantom and volunteer data, and compared with the monoexponential model and the linear sinc correction method. The monoexponential-plus-constant model, which was used in the Wood’s $R_2$ and $R_2^*$ method for hepatic iron quantification at 1.5 Tesla, was also included in the comparison as a reference.

5.2 Theory

During the transverse relaxation process, individual proton spins within an ensemble experience slightly different local magnetic fields, thus acquiring dispersing phases that cancel out with each other. Variations in local magnetic fields can be divided into three categories by their spatial scale: 1). Macroscopic field inhomogeneity on a spatial scale larger than an image voxel; 2). Diffusion-related, microscopic field fluctuation on molecular scale; and 3). Mesoscopic field variation that varies significantly within a voxel but still can be approximated as being constant for individual protons (95). The inherently stochastic microscopic field fluctuation is associated with irreversible $T_2$ decay. Both the macroscopic and mesoscopic field inhomogeneities are temporally constant for a given spatial location, and contribute to the reversible $T_2^*$ component. The mesoscopic field variation is usually considered to be a result of sub-voxel tissue heterogeneity in the distribution of paramagnetic substances such as deoxyhemoglobin or ferritin, which is of primary interest in clinical applications. The main magnetic field is well controlled in modern commercial MR scanners (< 0.1 ppm/cm with an empty bore) so the major source of macroscopic field inhomogeneity is the susceptibility-induced
fields generated in and around a heterogeneous subject. Because dephasing associated with macroscopic susceptibility-induced fields does not contain any information about physiological or pathophysiological changes in local distribution of paramagnetic substances of interest, it is considered to be non-irrelevant and subject to correction in the context of quantitative clinical MRI.

For an asymmetric voxel with larger slice thickness, the cross-slice $B_0$ inhomogeneity effect dominates any in-plane dephasing when the in-plane resolution is high enough. Under the second order approximation that the nonlinear component in the background field inhomogeneity can be well approximated by a quadratic term across a voxel with slice thickness $2z_0$:

$$
\delta B_0(z) = az^2 + bz, \quad \text{for } z \in [-z_0, z_0]
$$

[5.1]

where $z$ is the spatial coordinate along the slice selection direction. Assume the spins within this voxel have an ideal monoexponential $T_2^*$ decay in the absence of any macroscopic field inhomogeneity, the magnetization at echo time $TE$ and location $z$ can be expressed as:

$$
M(z, TE) = M_0(z) \exp(-R_2^*TE) \exp[-i\gamma TE(a z^2 + bz)]
$$

[5.2]

where $M_0(z)$ is proportional to local spin density at $z$, $R_2^*$ is the apparent transverse relaxation rate constant of this voxel, $TE$ is the echo time, and $\gamma$ is the gyromagnetic ratio.

For an ideal rectangular slice profile between $[-z_0, z_0]$, the MR signal in the frequency domain is the Fourier transform of magnetization $M(z, TE)$:
\[ \tilde{A}(k_z, TE) = F(M(z, TE)) \]

\[ = \left[ \left( C \left( \left( z_0 + \frac{b}{2a} \right) \frac{1}{\sqrt{\gamma TE |a|}} \right) + C \left( \left( z_0 - \frac{b}{2a} \right) \frac{1}{\sqrt{\gamma TE |a|}} \right) \right) \right] \]

\[ \mp i \left[ \left( S \left( \left( z_0 + \frac{b}{2a} \right) \frac{1}{\sqrt{\gamma TE |a|}} \right) + S \left( \left( z_0 - \frac{b}{2a} \right) \frac{1}{\sqrt{\gamma TE |a|}} \right) \right) \right] \]

\[ \times \frac{A_0}{\sqrt{\gamma TE |a|}} \exp(-R_z^2TE) \exp\left[i \left( 2\pi k_z - \gamma TE b \right)^2 \right] \]

where \( A_0 \) is the un-modulated signal in the absence of macroscopic field inhomogeneity, C(x) and S(x) are the Fresnel integrals:

\[ C(x) = \int_0^x \cos(t^2)dt \]

\[ S(x) = \int_0^x \sin(t^2)dt \]

The minus and plus signs are for the positive and negative values of \( a \), respectively. Taking the limit of \( a \to 0 \) on Eq. 5.3 generates the sinc modulation function for linear \( B_0 \) inhomogeneity.

In 2D imaging, there is no frequency domain encoding on the slice selection direction. Only the signals on the central plane (\( k_z = 0 \)) are acquired. The free induction decay (FID) signal on a magnitude image can be expressed as:

\[ \text{FID (TE)} = \left| \tilde{A}(k_z = 0, TE) \right| \]

\[ = \left\{ \left( C \left( \left( z_0 + \frac{b}{2a} \right) \frac{1}{\sqrt{\gamma TE |a|}} \right) + C \left( \left( z_0 - \frac{b}{2a} \right) \frac{1}{\sqrt{\gamma TE |a|}} \right) \right) \right\}^2 \]

\[ + \left\{ \left( S \left( \left( z_0 + \frac{b}{2a} \right) \frac{1}{\sqrt{\gamma TE |a|}} \right) + S \left( \left( z_0 - \frac{b}{2a} \right) \frac{1}{\sqrt{\gamma TE |a|}} \right) \right) \right\}^2 \]

\[ \times \frac{A_0}{\sqrt{\gamma TE |a|}} \exp(-R_z^2TE) \]

\[ = A_0 \exp(-R_z^2TE) \times f^{quad}(a, b, z_0, TE) \]
where $f_{\text{quad}}^{\text{quad}}(a, b, z_0, TE)$ denotes the quadratic correction factor.

Eq. 5.6 describes a large family of functions with various signal behaviors. A few simulated samples were demonstrated in Fig. 5.1, where the simulations were performed with different $(a, b)$ combinations, $z_0 = 1$ mm, $R_2^* = 40$ Hz, and TE ranging from 1.5 ms to 49.5 ms. Monoexponential decay only happens when the cross-slice background field inhomogeneity is weak (solid line). Deviations from the ideal monoexponential decay

Figure 5.1: Simulated FID curves shows Eq. 5.6 describes a large family of functions with various signal behaviors

Eq. 5.6 describes a large family of functions with various signal behaviors. A few simulated samples were demonstrated in Fig. 5.1, where the simulations were performed with different $(a, b)$ combinations, $z_0 = 1$ mm, $R_2^* = 40$ Hz, and TE ranging from 1.5 ms to 49.5 ms. Monoexponential decay only happens when the cross-slice background field inhomogeneity is weak (solid line). Deviations from the ideal monoexponential decay
become more and more significant with increasing \( B_0 \) inhomogeneity. When the linear term is predominantly larger than the quadratic term, a sinc-modulated decay is observed (dotted line). However, when the quadratic term is non-negligible, a fluctuating signal behavior that cannot be explained by the linear sinc correction model may appear (dash-dot line).

The majority of the functions described by Eq. 5.6 are derivable (except for the \( a = 0 \) limiting case, where Eq. 5.6 deduces to the sinc modulated decay). In a small-scale exploratory study, we got the impression that nonlinear least-square regression generates more robust fitting results for Eq. 5.6 than the two-step fitting strategy adopted in the sinc correction method. As a result, a nonlinear least-square regression routine using Gauss-Newton algorithm was used for the following studies, with a slightly reformatted form of Eq. 5.6 as the fitting function:

\[
FID(TE) = \sqrt{C(m\sqrt{TE}) + C(n\sqrt{TE}) + S(m\sqrt{TE}) + S(n\sqrt{TE})^2} \times \frac{S_0}{\sqrt{TE}} \exp(-R_2^* TE)
\]

\[5.7\]

where \( m = \left( z_0 + \frac{b}{2a} \right) \sqrt{\gamma |a|} \), \( n = \left( z_0 - \frac{b}{2a} \right) \sqrt{\gamma |a|} \), \( S_0 = \frac{A_0}{\sqrt{\gamma |a|}} \), and \( R_2^* \) are the fitting parameters.

5.3 Materials and Methods

5.3.1 Phantom Study

A phantom was constructed with ten 15 ml centrifuge tubes and one 50 ml centrifuge tube, filled with \( \text{MnCl}_2 \) solutions with various concentrations ranging from
0.25 mM to 1.5 mM (Fig. 5.2). The centrifuge tubes were bundled together with tape and wax, and fixed inside a PVC pipe by wax blocks. Space between the centrifuge tubes and the PVC pipe was filled with 0.25 mM MnCl₂ solution doped with 0.125 M NaCl to attenuate dielectric resonance artifacts.

MRI scanning was conducted on a Philips Achiva 7T whole body scanner (Philips, Cleveland, OH) with a T/R head coil (Nova, Wilmington, MA). The cylindrical phantom was placed in the scanner with its long axis perpendicular to the main magnetic field. \( T_2^*/R_2^* \) measurement was conducted with a modified 2D GRE echo planar imaging (EPI) sequence (Fig. 5.3). In this sequence, the phase encoding gradient blips are knocked out so the echo train represents sampling of one line in k-space at different echo times, rather than different lines in k-space. The sequence is repeated n (the number of phasing encoding steps) times, with a varying phase encoding gradient applied immediately after the excitation pulse, to cover the whole k-space. A data set of 17 coronal slices with 64 × 59 matrix (reconstructed to 128 × 128 on a 120 mm FOV) was acquired at 59 echo times within 2 min 2 s. Slice thickness was 4 mm with 1 mm gap in between. The 59 echoes were acquired on both positive and negative lobes of the readout gradient train, and ranged from 1.5 ms to 44.5 ms, with \( \Delta TE = 0.74 \) ms. The repetition time TR was set to be 2000 ms, and the flip angle (FA) was set to be 90°.

To measure the cross-slice \( B_0 \) inhomogeneity, a high resolution \( B_0 \) map was acquired with a ‘\( B_0 \) Map’ sequence, which is essentially a 3D dual echo GRE sequence with low flip angle and short echo times. Phases accumulated between the two echoes were extracted from the phase images, and calibrated to shifts in local resonance.
Figure 5.2: Phantom construction. Signal loss due to B1 inhomogeneity (the ‘dielectric resonance’ effect) can be observed in the dark crescent-shaped region in the left side of the image.
Figure 5.3: The 2D GRE $T_2^*/R_2^*$ measurement sequence (a) modified from a 2D GRE EPI sequence (b)
frequencies in Hz. For this study, the combination of TR/TE1/TE2/FA = 9.1 ms/4.0 ms/5.0 ms/10° was used, and the reconstructed B₀ map ranged from -500 Hz to 500 Hz. The B₀ map was acquired with 1 mm × 1 mm in-plane resolution on the same 120 mm FOV, and reconstructed to the same matrix size with the modified EPI data. 256 slices with 0.5 mm slice thickness were acquired to cover the same expansion along the cylinder axis, so that the cross-slice background field inhomogeneity in each modified EPI slice can be described by eight local resonance frequency measurements in B₀ map. The acquisition duration was 8 min 45 s.

5.3.2 Volunteer Study

Modified 2D GRE EPI data and high resolution B₀ maps of axial brain were acquired on three healthy volunteers (all Asian males, 32 ± 4 years old) on a Philips Achiva 7T whole body scanner (Philips, Cleveland, OH) with a 16-channel head coil (Nova, Wilmington, MA). Both sequences have a higher in-plane resolution of 224 × 128 matrix (reconstructed to 256 × 256) on a 220 mm × 180 mm FOV, for better identification of small anatomical structures. The modified EPI sequence covers 20 slices with slice thickness 3 mm and no gap, while the B₀ map contains 120 0.5 mm – thick slices covering the same region of brain. Thus, each modified EPI slice has 6 B₀ measurements along its thickness, which provides enough data points for linear and quadratic polynomial fittings. An echo train of 31 TE’s ranging from 1.7 ms to 40.3 ms was acquired, with a ΔTE of 1.28 ms. TR/FA for the modified EPI sequence was 1988 ms/90°. TR/TE1/TE2/FA for the B₀ map sequence was 9.1 ms/4.0 ms/5.0 ms/10°. The
scan durations for the modified EPI and $B_0$ map sequences were 4 min 14 s and 4 min 49 s, separately.

Repeat scans with the same scanning parameters were acquired 3 days after the initial scan. The repeat data were used for stability assessment.

5.3.3 Data Analysis

Both the $B_0$ map and the modified EPI data set were extracted from the Philips PAR-REC files, and postprocessed with an inhouse-developed software written in Matlab (Mathworks, Natick, MA). The sinc correction method was implemented as described in the literature (96), while all other three models were implemented with a nonlinear least-square regression routine using Gauss-Newton algorithm (function \texttt{nlinfit} in Matlab). Eight (or six) $B_0$ measurements along the slice selection direction were fitted to a linear or quadratic polynomial, and the resulting coefficients were taken to calculate initial estimates for the fitting parameters in sinc and quadratic correction methods, separately. Performance of the four models was compared by visual inspection and statistical analysis of the $R_2^*$ parametric maps.

5.4 Results

5.4.1 Phantom Study

$\text{MnCl}_2$ $R_2^*$ values at different concentrations were measured on circular ROIs defined in the centrifuge tubes from a slice at the center of the phantom and a slice near the top. The top slice has the strongest cross-slice $B_0$ inhomogeneity due to the air-water interface nearby, while the central slice has almost no cross-slice variation in $B_0$. Thus,
performance of the four models can be assessed by comparing the $R_2^*$ measurements from the same tubes in the top and central slices. Paired two-tail student’s t-test was used to check if there is a significant difference between $R_2^*$ values measured in the central slice and those from the top. Bonferroni method was used for multiple tests, with an overall significance level of 0.05, which corresponds to an individual significance level of 0.0125 for each method. The results were tabulated in Table 5.1.

As indicated by the p value, $R_2^*$ values calculated with the monoexponential model are significantly biased in the presence of macroscopic $B_0$ inhomogeneity. While the $R_2^*$ differences are considered statistically insignificant for the monoexponential-plus-constant model, this statement is rather marginal because of the small p. Both sinc and quadratic corrections have large p values, suggesting the corrected $R_2^*$ values are more consistent between areas with different $B_0$ inhomogeneity levels.

<table>
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<tr>
<th>Method</th>
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</tr>
<tr>
<td>Exp + Const</td>
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</tr>
<tr>
<td>Sinc</td>
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<tr>
<td>Quad</td>
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</tr>
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</table>

Table 5.1: Significance of $R_2^*$ measurement differences between the top and the central slices, assessed by multiple paired-student’s t-test

A more intuitive idea about model performance can be obtained by inspecting the $R_2^*$ parametric maps of the central (Fig. 5.4) and top (Fig. 5.5) slices. In the central slice
with no significant $B_0$ fluctuation along the slice selection direction, all four models work well. While some residual macroscopic $B_0$ inhomogeneity effects got corrected by both sinc and quadratic corrections near the edges of wax blocks (black arrow in Fig. 5.4), both correction methods also overcorrected some other areas (white arrows in Fig 5.4), creating artificial “bands” (quadratic) or “spots” (sinc) in which $R_2^*$ was underestimated. $R_2^*$ measurements with monoexponential and monoexponential-plus-constant models are severely biased in the top slice due to macroscopic $B_0$ inhomogeneity. The sinc correction method successfully recovered $R_2^*$ in some voxels but failed in others, suggesting that it is less robust than the quadratic correction method, which generated the best overall correction effect, completely removed artificial variations in $R_2^*$ in the center of the phantom (white arrow in Fig. 5.5), and recovered the 0.25 mM tube on the left (black arrow in Fig 5.5) that is non-identifiable in all other three $R_2^*$ maps.

Relaxation theory predicts that in aqueous solution of MnCl$_2$ with low concentration, the apparent transverse relaxation rate $R_2^*$ has a linear relationship with Mn$^{2+}$ concentration:

$$R_{2,Mn}^* = R_{2,0}^* + C[Mn^{2+}]$$  \[5.8\]

where $R_{2,Mn}^*$ and $R_{2,0}^*$ are the apparent transverse relaxation rates of MnCl$_2$ solution and pure water, separately. $[Mn^{2+}]$ is manganese ion concentration, and $C$ is a slope coefficient.
Figure 5.4: $R_2^*$ parametric maps calculated with monoexponential model (Exp), monoexponential-plus-constant model (Exp + Const), sinc correction for linear macroscopic $B_0$ inhomogeneity (Sinc) and quadratic macroscopic $B_0$ inhomogeneity correction (Quad) on a slice at the center of the phantom.
Figure 5.5: $R_2^*$ parametric maps calculated with monoexponential model (Exp), monoexponential-plus-constant model (Exp + Const), sinc correction for linear macroscopic $B_0$ inhomogeneity (Sinc) and quadratic macroscopic $B_0$ inhomogeneity correction (Quad) on a slice near the top of the phantom
Figure 5.6: The linear relationships between manganese ion concentrations and $R_2^*$ values measured with monoexponential model (Exp), monoexponential-plus-constant model (Exp + Const), sinc correction for linear macroscopic $B_0$ inhomogeneity (Sinc) and quadratic macroscopic $B_0$ inhomogeneity correction (Quad) were assessed by linear regression in the top (blue) and central (red) slices.
R$_2^*$ values calculated with the four methods were plotted versus manganese concentrations in Fig. 5.6. Slope coefficients $C$ were calculated by linear regression and listed in Table 5.2 for both top and central slices, with the corresponding R$^2$ values from regression, and their relative differences (RD):

$$RD = \frac{|C_{\text{center}} - C_{\text{top}}|}{(C_{\text{center}} + C_{\text{top}})/2} \quad [5.9]$$

where $C_{\text{center}}$ and $C_{\text{top}}$ are the slope coefficients calculated from the central and top slices, separately.

<table>
<thead>
<tr>
<th>Method</th>
<th>Top</th>
<th>Center</th>
<th>Slope RD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope (Hz / mM)</td>
<td>R-square</td>
<td>Slope (Hz / mM)</td>
</tr>
<tr>
<td>Exp</td>
<td>99</td>
<td>0.67</td>
<td>154</td>
</tr>
<tr>
<td>Exp + Const</td>
<td>117</td>
<td>0.70</td>
<td>174</td>
</tr>
<tr>
<td>Sinc</td>
<td>89</td>
<td>0.70</td>
<td>141</td>
</tr>
<tr>
<td>Quad</td>
<td>148</td>
<td>0.98</td>
<td>162</td>
</tr>
</tbody>
</table>

Table 5.2: Slope coefficients and R$^2$ values for the [Mn$^{2+}$]-R$_2^*$ linear relationship in the top and the central slices of the phantom

As demonstrated by the R$^2$ values and slope RDs, the linear relationship between manganese concentrations and R$_2^*$ relaxation rates can only be recovered by the quadratic correction method. With all other three methods, B$_0$ inhomogeneity-induced signal loss causes both overestimation and spatial variation in R$_2^*$ calculation, leading to the large R$^2$ values and slope RDs.
Because monoexponential $T_2^*$ decay is expected for homogeneous aqueous solutions in the absence of $B_0$ inhomogeneity, the slope coefficient measured with monoexponential model in the central slice can be taken as a ‘golden standard’ for comparison. Mean slope coefficient calculated with quadratic correction (155 Hz/mM) is in well agreement with central slice monoexponential slope (154 Hz/mM). Moreover, the intercepts calculated from the central and top slices are 7 Hz and -7 Hz for the quadratic correction, which are in agreement with the proton transverse relaxation rate in pure water (~1 Hz) given the fact that $R_2^*$ measurement errors are in the range of ~10 Hz.

5.4.2 Volunteer Study

The following ROIs were defined on the morphological images in the modified EPI data set by experienced radiology investigators (Fig. 5.7): red nucleus (RN), substantia nigra (SN), globus pallidus (GP), putamen (P), caudate (C), and three white matter ROIs from different regions of the brain (WM1 to WM3, similar to those from literature (97)). Cortical gray matter ROIs were not defined due to the difficulty in identifying pure gray matter voxels whose signal was not contaminated by cerebrospinal fluid (CSF), blood or white matter partial volume effect. CSF ROIs were not defined because the TE range (1.7 ms to 40.3 ms) used in this study was too low for accurate determination of CSF $T_2^*/R_2^*$.

$R_2^*$ parametric maps of the corresponding slices are displayed in Fig. 5.8 to Fig. 5.11. $R_2^*$ overestimation caused by susceptibility-induced fields near the air spaces in the sinuses and inner ears was partially corrected by the quadratic correction method, with some overcorrected areas on the edge (white arrow in Fig. 5.8). Overcorrected regions
are more scattered with the sinc correction method, causing loss of anatomical structures in the $R_2^*$ map. The monoexponential-plus-constant model was demonstrated to be the worst among the four, showing no correction in regions with strong $B_0$ inhomogeneity while creating lots of $R_2^*$ underestimations in white matter.

Figure 5.7: ROI definitions for the volunteer study
Figure 5.8: $R_2^*$ parametric maps calculated with monoexponential model (Exp), monoexponential-plus-constant model (Exp + Const), sinc correction for linear macroscopic $B_0$ inhomogeneity (Sinc) and quadratic macroscopic $B_0$ inhomogeneity correction (Quad) on an axial slice across the red nucleus and substantia nigra.
Figure 5.9: $R_2^*$ parametric maps calculated with monoexponential model (Exp), monoexponential-plus-constant model (Exp + Const), sinc correction for linear macroscopic $B_0$ inhomogeneity (Sinc) and quadratic macroscopic $B_0$ inhomogeneity correction (Quad) on an axial slice across the globus pallidus and putamen.
Figure 5.10: $R_2^*$ parametric maps calculated with monoexponential model (Exp), monoexponential-plus-constant model (Exp + Const), sinc correction for linear macroscopic $B_0$ inhomogeneity (Sinc) and quadratic macroscopic $B_0$ inhomogeneity correction (Quad) on an axial slice across caudate, WM2 and WM3 ROIs
Figure 5.11: $R_2^*$ parametric maps calculated with monoexponential model (Exp), monoexponential-plus-constant model (Exp + Const), sinc correction for linear macroscopic $B_0$ inhomogeneity (Sinc) and quadratic macroscopic $B_0$ inhomogeneity correction (Quad) on an axial slice across the WM1 ROI
In vivo stability of the four models was checked with the following rationale: stability of a $R_2^*$ measurement method implies: 1). $R_2^*$ values measured with this method should be time independent; and 2). Variances of the $R_2^*$ measurements with this method should be time independent. To check that mean $R_2^*$ measurements are time independent, we normalize the $R_2^*$ difference by:

$$
\Delta \tilde{R}_2^* = \frac{R_{2,\text{rep}}^* - R_{2,\text{ini}}^*}{\sqrt{\sigma_{\text{rep}}^2 + \sigma_{\text{ini}}^2}}
$$

[5.9]

where $\Delta \tilde{R}_2^*$ is the normalized $R_2^*$ difference, $R_{2,\text{rep}}^*$ and $R_{2,\text{ini}}^*$ are the $R_2^*$ measurements from the repeat and initial scans, separately, with $\sigma_{\text{rep}}^2$ and $\sigma_{\text{ini}}^2$ being the corresponding standard deviations. The normalization process removes any tissue heterogeneity effect or intersubject variation. Normality of the normalized $R_2^*$ differences can be checked with the normal quantile-quantile (Q-Q) plot (Fig. 5.12).

Most of the data points are distributed along a straight line in the Q-Q plots except for WM1 of subject 2, which has been identified as an outlier in three of the four methods (with $\Delta \tilde{R}_2^*$ for monoexponential model to be 4.6. sinc correction 3.1, quadratic correction 1.9). Because this region of the brain is rich in axial veins, the excessive variation is most likely to be caused by different white matter / venous blood partial volume effects in initial and repeat scans. This data point was excluded from further statistical tests and comparison with literature values.
Figure 5.12: Q-Q Plots used to check the normality of normalized $R_2^*$ differences

Time independence of mean $R_2^*$ measurements was checked by one sample student’s t-test on $\Delta R_2^*$, with the null hypothesis that $\Delta R_2^*$ are random samples from a normal distribution with mean 0 and unknown variance. Bonferroni method was used for multiple tests, with an overall significance level of 0.05, which corresponds to an individual significance level of 0.0125 for each method. The results are listed in Table 5.3:
<table>
<thead>
<tr>
<th>Method</th>
<th>p</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp</td>
<td>0.05</td>
<td>No</td>
</tr>
<tr>
<td>Exp + Const</td>
<td>0.70</td>
<td>No</td>
</tr>
<tr>
<td>Sinc</td>
<td>0.58</td>
<td>No</td>
</tr>
<tr>
<td>Quad</td>
<td>0.43</td>
<td>No</td>
</tr>
</tbody>
</table>

Table 5.3: Results of multiple one-sample student’s t-tests checking the time independence of mean $R_2^*$ values measured by the four methods

The null hypothesis cannot be rejected for all four methods, suggesting that there is no significant time dependence in mean $R_2^*$ measurements.

Similarly, time independence of $R_2^*$ variances can be checked on the standard deviation difference:

$$\Delta \sigma = \sigma_{rep} - \sigma_{ini}$$  \hspace{1cm}  [5.10]

whose normality was demonstrated by the Q-Q plots in Fig. 5.13. Results of multiple one sample student’s t-tests were tabulated in Table 5.4. Bonferroni method was used for multiple tests, with an overall significance level of 0.05, which corresponds to an individual significance level of 0.0125 for each method. Again, the null hypothesis that $\Delta \sigma$ are random samples from a normal distribution with mean 0 and unknown variance cannot be rejected for all four methods. Thus, we can make the conclusion that all four methods are demonstrated to be temporally stable for \textit{in vivo} studies in human brain.
Figure 5.13: Q-Q Plots used to check the normality of standard deviation differences

<table>
<thead>
<tr>
<th>Method</th>
<th>p</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp</td>
<td>0.45</td>
<td>No</td>
</tr>
<tr>
<td>Exp + Const</td>
<td>0.58</td>
<td>No</td>
</tr>
<tr>
<td>Sinc</td>
<td>0.24</td>
<td>No</td>
</tr>
<tr>
<td>Quad</td>
<td>0.28</td>
<td>No</td>
</tr>
</tbody>
</table>

Table 5.4: Results of multiple one-sample student’s t-tests checking the time independence of variances of $R_2^*$ measurements by the four methods
Average $T_2^*$ values were measured in the deep gray matter structure and white matter ROIs, tabulated in Table 5.5, and compared with literature values as a confirmation of the validity of our implementations:

<table>
<thead>
<tr>
<th>Type</th>
<th>Exp (ms)</th>
<th>Exp + Const (ms)</th>
<th>Sinc (ms)</th>
<th>Quad (ms)</th>
<th>Li, 06</th>
<th>Pfeuffer, 04 (Monkey)</th>
<th>Peters, 07</th>
</tr>
</thead>
<tbody>
<tr>
<td>RN</td>
<td>13.5 ± 0.5</td>
<td>16.5 ± 0.9</td>
<td>20.3 ± 3.9</td>
<td>19.7 ± 1.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SN</td>
<td>11.6 ± 0.6</td>
<td>12.9 ± 0.9</td>
<td>20.2 ± 5.3</td>
<td>16.6 ± 1.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP</td>
<td>12.8 ± 0.5</td>
<td>13.8 ± 0.8</td>
<td>15.2 ± 1.6</td>
<td>15.9 ± 1.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>19.6 ± 1.2</td>
<td>24.7 ± 2.6</td>
<td>23.0 ± 2.2</td>
<td>25.6 ± 2.5</td>
<td>16.1 ± 1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>25.5 ± 1.5</td>
<td>28.0 ± 2.7</td>
<td>27.2 ± 2.2</td>
<td>30.0 ± 2.4</td>
<td>19.9 ± 2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WM3</td>
<td>25.4 ± 0.4</td>
<td>32.7 ± 1.9</td>
<td>25.0 ± 0.5</td>
<td>29.9 ± 2.0</td>
<td>29 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WM2</td>
<td>24.3 ± 0.4</td>
<td>27.1 ± 1.8</td>
<td>29.3 ± 2.6</td>
<td>26.2 ± 0.8</td>
<td>31 ± 2</td>
<td>28.3/29.1/24.4</td>
<td>26.8 ± 1.2</td>
</tr>
<tr>
<td>WM1</td>
<td>32.0 ± 0.5</td>
<td>30.1 ± 2.0</td>
<td>33.1 ± 1.4</td>
<td>32.5 ± 0.7</td>
<td>35 ± 2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5.5: Mean $T_2^*$ measured in deep gray matter structure and white matter ROIs, compared with literature values from Li (98), Pfeuffer (99), and Peters (100)

Our $T_2^*$ measurements in white matter are in good accordance with previously published data. However, our putamen and caudate $T_2^*$ values are significantly larger than those obtained by Peter et al (101). Because the white matter $T_2^*$ measurement does not show a similar trend, we argue that the observed $T_2^*$ differences in deep gray matter structures are not caused by an erroneous implementation of the model, but more likely to be a result of different iron deposition levels in subjects from different age groups. It has been known that iron deposition in deep gray matter structures increases with age (102), causing a decrease in the corresponding $T_2^*$ measurements. The subjects involved in our study are primarily young people, with a mean age of 32 ± 4 years. While the subject
group in Peters’ study includes some old people (mean age 37 ± 11 years), it is expected that our T2* measurements should be larger than theirs in the deep gray matter structures.

The white matter is heterogeneous in transverse relaxation properties. T2* measurements from three different regions of the brain (WM1 to WM3) have a coefficient of variation (CV) of 15% for the monoexponential model, 9% for the monoexponential-plus-constant model, 14% for the sinc correction method, and 11% for the quadratic correction method. These values are in agreement with data reported by Li et al. (CV of 10% for three similar regions, 17% for nine fiber bundles) (103). Under the current spatial resolution, whether the observed heterogeneity is due to fiber orientation or venous blood partial volume effect cannot be determined.

5.5 Discussions and Conclusions

In the presence of macroscopic magnetic field inhomogeneity, spatial variation of the B0 field induces a broadening of the resonance frequency distribution in the spin ensemble across the voxel, which corresponds to an additional dephasing term in the time domain. This extra dephasing term was treated as a part of T2* decay in the mononexponential and monoexponential-plus-constant models, thus causing overestimation in the R2* relaxation rate. The linear sinc and quadratic correction methods make efforts to estimate the macroscopic dephasing factor, so as to achieve better estimations for T2*/R2*. However, if this estimation is severely biased, the correction models would erroneously assign more weight to the macroscopic dephasing factor and generate an overcorrected T2*/R2*. 
An axial slice in the low brain was studied in detail for a better understanding of the \textit{in vivo} overcorrection mechanism (Fig. 5.14). Macroscopic $B_0$ inhomogeneity across the slice was visualized as a map of maximum changes in resonance frequency (Fig. 5.14 a). The quadratic correction method successfully recovers $R_2^*$ in areas with $|\Delta f_0| < 80$ Hz, but ends up with failure in regions with higher macroscopic $B_0$ inhomogeneity. Failure for correction is probably due to lack of enough data points, because a complete loss of the observed FID signal happens within 10 ms for $|\Delta f_0| \sim 100$ Hz. Overcorrection happens near the edges of the high-$B_0$-inhomogeneity regions above inner ears (black arrows in Fig. 5.14 d). In these regions, the measured background field fluctuations are low ($|\Delta f_0| < 30$ Hz), but the elevated relaxation rates in monoexponential $R_2^*$ map suggest a dephasing term not associated with cross-slice macroscopic $B_0$ inhomogeneity.

When overcorrection happens, the cross-slice background field inhomogeneity gets severely overestimated. This overestimation is reflected on the model parameters $m$ and $n$, which are proportional to the $B_0$ gradients on the upper and lower surfaces of the slices. Thus, the ratio between the final and initial values of $m$ and $n$ is expected to be a good indicator of overcorrection. We set a threshold for the absolute final-to-initial parameter ratios at 5, because small change in model parameters can be a result of slice profile imperfection or $B_0$ map measurement error. Moreover, major increases in model parameters do not reflect any significant change in the shape of the quadratic correction factor when the background inhomogeneity is too weak (no dephasing at all) or too strong (signal completely lost before the first echo). Computer simulation shows that for the TE range and slice thickness used in this study, a 5-fold increase in model parameters only causes significant change in the quadratic correction factor shape for $m, n$ values.
Figure 5.14: (a). $|\Delta f_0|$ map for an axial slice in low brain; (b). $R_2^*$ parametric maps calculated with monoexponential model; (c). Overcorrection mask generated with the quadratic model parameters m and n showing voxels whose $R_2^*$ values are unreliable; (d). $R_2^*$ parametric maps calculated with quadratic macroscopic $B_0$ inhomogeneity correction method
between 1 s^{-1/2} to 20 s^{-1/2}. Thus, an overcorrection mask can be created by thresholding the initial values and final-to-initial ratios of \( m \) and \( n \) with the following criterion:

\[
\begin{align*}
\left( m_{\text{ini}}, n_{\text{ini}} \right) & \in \left[ 1s^{-1/2}, 20s^{-1/2} \right] \\
\frac{m_{\text{final}}}{m_{\text{ini}}} & > 5, \quad \frac{n_{\text{final}}}{n_{\text{ini}}} > 5 \\
\end{align*}
\]

There is a good correspondence between the mask configuration (Fig. 5.14 c) and the shape of the overcorrected regions in R\(_2^*\) map (Fig. 5.14 d). Positive values in this mask mark out voxels in which the corrected R\(_2^*\) values are not reliable indexes of tissue relaxation property because of nonexponential dephasing effects other than that caused by cross-slice B\(_0\) inhomogeneity. In the overcorrected regions in vicinity of air-tissue interfaces in inner ears, this is most likely to be a result of intravoxel dephasing caused by the in-plane macroscopic B\(_0\) inhomogeneity. However, in regions with a large component of venous blood or iron deposition, intravoxel signal cancellation could also happen as a result of mesoscopic, subvoxel tissue heterogeneity, as demonstrated by Sedalcik et al (104). Although the mesoscopic effect is relevant in context of quantitative clinical MRI but the macroscopic effect is not, their effects are intervened with each other and cannot be separated under the current spatial resolution.

A similar overcorrection mask can be created for the sinc correction model, with the initial value and final-to-initial ratio of the background gradient strength (Fig. 5.15 a). However, the configuration of this mask has a poor correspondence with the overcorrected regions in the R\(_2^*\) map, making it of less value in monitoring the reliability of the fitting results. The overcorrected white matter regions are much broader (Fig. 5.15 b) than in higher slices (Fig 5.10), causing complete loss of anatomical structures in
medulla and vermis. Because subvoxel tissue heterogeneity is not supposed to be very
different between different slices, the observed overcorrections are most likely to be a
result of in-plane macroscopic $B_0$ inhomogeneity.

![Overcorrection mask](image1)

**Figure 5.15:** (a). Overcorrection mask generated with background field gradient
strength in the sinc correction model; (b). $R_2^*$ parametric maps calculated with sinc
correction for linear macroscopic $B_0$ inhomogeneity

With the sinc correction method, overcorrection happens in voxels with relatively
strong cross-slice background field inhomogeneity, where quadratic correction works
without any problem. This observation suggests that the sinc correction method is less
robust than the quadratic correction. This is probably due to the two-step nature of the
sinc correction algorithm, in which minimum search of an energy function is conducted
on a 1D parameter space. When the fitting procedure starts the search far away from the
desired global minimum, it will get trapped at the first local minimum in between. Conversely, the quadratic correction method conducted its search for minimum on a multi-dimensional parametric plane, on which local minimums are more sparsely distributed. So the chance that the fitting procedure may get trapped in a local minimum is decreased, and the correction becomes more robust. However, robustness does not come without any price: searching for a global minimum in a high-dimensional parameter space with a complex energy function contour is an inefficient process. Convergence is usually slow. In our implementation, the quadratic correction method is more than 10 times slower than the sinc correction method. Fitting speed might be improved by problem-specific optimizations, such as adopting a more efficient searching strategy, or an energy function with better contour configuration.

In conclusion, the superiority of the quadratic correction method over the other three models, including the linear sinc correction, has been clearly demonstrated by visual inspection and statistical analysis in phantom and volunteer data. It is the only method that can completely recover the linear relationship between Mn$^{2+}$ concentration and R$_2^*$ in the presence of macroscopic background field inhomogeneity. The quadratic R$_2^*$ maps also reveal more anatomical structures in the volunteer study. Moreover, the initial and final estimates of the model parameters from the quadratic correction model can be used to construct an overcorrection mask that provides a rough assessment on the reliability of the corrected R$_2^*$ values. This model provides a practical method to recover FID signal dephasing caused by air-tissue susceptibility difference in lower brain or upper liver, with the capability of qualitatively assessing detection limit under the spatial resolution used.
CHAPTER 6

EXPLORING TRANSVERSE RELAXATION MECHANISM BY SIMULATION AT THE NANO SCALE: INTRODUCTION OF A HIGH SPEED MONTE CARLO ALGORITHM

6.1 Overview

Change in the transverse ($T_2$) relaxation process in iron-rich tissue is believed to be governed by local mesoscopic variations in the background $B_0$ field induced by paramagnetic ferritin/hemosiderin and deoxyhemoglobin molecules. Spatial distributions of these paramagnetic substances are highly nonuniform on cellular and subcellular level. Deoxyhemoglobin molecules are predominantly confined in erythrocytes in venous blood. Ferritin and hemosiderin form clusters of various sizes (105) in iron storage cells which, on cellular level, are distributed in heterogeneous patterns in tissue matrices (106). In external magnetic field, these unevenly-distributed paramagnetic substances induce local magnetic fields that vary on a spatial scale comparable to the characteristic distance of water molecule self diffusion during the echo time $TE$. Due to the stochastic nature of the diffusion process, phases accumulated before the refocusing pulse cannot be completely recovered when an echo is formed at $TE$. The residual phase memories add up to cause an extra dephasing that leads to an increase in the observed $R_2$. 

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Physicists and biophysicists have proposed many theoretical models to describe this process and give quantitative predictions for $T_2/R_2$ changes (107-110). However, none of those models had been widely accepted for real world applications, probably because all of them rely on many assumptions and simplifications whose validity can hardly be checked in biological systems. So far, results from Monte Carlo simulations are still considered to be a ‘golden standard’ when direct experimental measurements are difficult or even impossible (111-114).

In Monte Carlo simulation, the mesoscopic field inhomogeneity induced dephasing process can be mimicked: spatially inhomogeneous magnetic field distribution is numerically calculated from the shape, size, susceptibility, and spatial distribution of paramagnetic substances (the magnetic field ‘perturbers’), whose size may vary from cell size to molecular level. A large ensemble of spins or idealized ‘spin packages’ are then put into this inhomogeneous field and allowed to diffuse freely. Their phases accumulated through the diffusion process are separately recorded and summed up. And the relaxation rate can be obtained by averaging over the whole ensemble. In principle, Monte Carlo simulation result can be made infinitely close to the real value, as long as spin diffusion through inhomogeneous field is a correct description of relaxation mechanism.

Most previous works on Monte Carlo simulation of field inhomogeneity induced dephasing were conducted with field perturbers of cell size or larger (115, 116). Venous capillaries and iron-rich cells, such as Kupffer cells in liver or oligodendrocytes in brain grey matter, are modeled as homogeneous paramagnetic cylinders or spheres without any internal structure. However, an experimental study suggests that iron distribution on the
subcellular scale may play a critical role in determining transverse relaxation rate. Wood et al. measured relaxation properties of horse spleen ferritin in free and aggregated form (attached to 0.4 μm diameter phospholipid liposomes). $T_2$ relaxivity was reported to be 6-fold stronger in the aggregated liposomal-ferritin (117). To take this aggregation effect into account, subcellular structures must be modeled. The simulation must be carried out at higher spatial resolution.

Unfortunately, the traditional Monte Carlo algorithm is $O(n^5)$. Thus a 100-fold increase in spatial resolution will be amplified to a $10^{10}$-fold increase in computing time, which makes sub-cellular simulation a forbidding task. Several techniques have been proposed to control the computing time. Kenan et al. assume periodical distribution of perturbers (118). Ghugre et al. calculate the inhomogeneous field distribution in a lower spatial resolution and interpolate it for phase accumulation calculation during spin diffusion (119). Jensen et al. constructed a theoretical model for general dephasing process and managed to associate relaxation parameters with a model parameter which can be directly calculated from inhomogeneous field distribution to avoid simulating diffusion processes (120). However, these techniques are either biased or too specific for general uses.

In this chapter, a novel high-speed Monte Carlo algorithm (the ‘field map algorithm’) based on 1D ‘jumping’ on the field profile was described and prototyped as an effort to solve the simulation time problem.
6.2 Methods

6.2.1 Classical Monte Carlo

The classical way of simulating field-inhomogeneity-induced dephasing by Monte Carlo method involves the following steps:

1. Generate a ‘universe’ with randomly distributed magnetic field perturbers;
2. Calculate the field distribution $B(\vec{r})$ in the ‘universe’;
3. Randomly assign an initial position $\vec{x}(0)$ to an idealized ‘spin package’. Simulate its diffusion process $\vec{x}(t)$ as a 3-D random walk process;
4. Calculate the phase accumulated by the ‘spin package’ at time $TE$:

$$\varphi = -\int_0^{TE} \sigma(t)B(\vec{x}(t))dt$$

[6.1]

where $\sigma(t)$ is a coefficient determined by the MR sequence used (for example, $\sigma(t) = 1$, $t \in [0,TE/2]$ ; $\sigma(t) = -1$, $t \in (TE/2,TE]$ for spin echo (SE), $\sigma(t) = 1$ for Gradient echo (GRE), etc.).

5. Repeat step 1-4. Average over ‘spin packages’ and ‘universes’.

This simple, straightforward scheme has been used by many previous researches [121-123] and proved to be successful. However, all those studies are performed on cell-size ($\phi \sim 10\,\mu m$) perturbers such as oligodendrocytes [124] or capillaries [125], while in this study, we are interested in nanoscale ($\phi \sim 100\,nm$) perturbers. To ensure necessary precision in approximating diffusion process as a 3-D random walk, spatial step size of the random walk must be smaller than the perturber size. But the ‘universe’ size $L$ is
restricted by $L > \sqrt{6DTE}$, where $D$ is the self diffusion constant of water molecules, and cannot be scaled down with perturber size. Thus, computational complexity for generating the ‘universe’ is $O(n^3)$. Computational complexities of other steps for the classical scheme can be similarly calculated, as listed below:

1. Generate the ‘universe’: $O(n^3)$
2. Calculate the field distribution: $O(n^6)$
3. Random walk: $O(n^2)$ (Because the temporal resolution $\Delta t$ of the 3-D random walk is associated with the spatial resolution $\Delta x$ through $\Delta t = \frac{(\Delta x)^2}{6D}$)
4. Calculate phase accumulation: $O(n^2)$

So the overall computational complexity of the classical scheme is $O(n^6)$. i.e, our $10^2$-fold decrease in perturber size will lead to a $10^{12}$-fold increase in computing time, which makes any simulation practically impossible.

The classical scheme can be improved by only calculating field strengths along the diffusion route. The modified scheme can be described as:

1’. Generate the ‘universe’: $O(n^3)$
2’. Random walk; calculate the field strengths along the diffusion route: $O(n^5)$
3’. Calculate phase accumulation: $O(n^2)$
Such a modification helps to decrease the overall computational complexity from $O(n^6)$ to $O(n^5)$ but is still not manipulable. To address this problem, we propose the following algorithm:

### 6.2.2 Field Profile Algorithm

Inspection of the traditional Monte Carlo algorithm reveals that most of the computational resources are occupied by repeatedly calculating the magnetic field from the positions of spin packages and perturbers in 3-D space. Those positions are only intermediate variables involved in the calculation of $B_s(\vec{r})$, but not directly related to the simulation of phase accumulation. If phase accumulation can be calculated without the need of knowing the physical diffusion route, simulation of field-inhomogeneity-induced dephasing will be dramatically simplified: this is the rationale behind the field profile algorithm, which is designed to eliminate those unnecessary position calculations.

Consider a ‘universe’ containing randomly (not necessarily uniformly) distributed impenetrable perturbers (not necessarily of the same shape, size, and susceptibility). By Maxwell’s equations, magnitude of magnetic field in the space outside the perturbers is continuous. So for a ‘spin package’ which is experiencing a local magnetic field $B(\vec{r}(t))$ at time $t$ and $B(\vec{r}(t + \Delta t))$ at time $t + \Delta t$, there are only three possibilities:

\[
\begin{align*}
B(\vec{r}(t + \Delta t)) &> B(\vec{r}(t)) \\
B(\vec{r}(t + \Delta t)) &= B(\vec{r}(t)) \\
B(\vec{r}(t + \Delta t)) &< B(\vec{r}(t))
\end{align*}
\]
No matter what physical positions \( \bar{r}(t) \) and \( \bar{r}(t + \Delta t) \) it has. Thus, **this ‘spin package’s 3-D diffusion in physical space is equivalent to a 1-D ‘jumping’ on the field profile of the ‘universe’**.

The field profile of the ‘universe’ can be discretized into a histogram with \( n \) bins (Fig. 6.1, 6.2 a). Height of each bin represents the volume of space in which the magnitude of magnetic field is in the corresponding range. Consider a ‘spin package’ in bin \( i \) at time \( t \). At time \( t + \Delta t \), this ‘spin package’ may stay within the same bin, diffuse to the neighbor bins \( i + 1 \) or \( i - 1 \), or ‘jump’ to bins farther away (Such ‘jumps’ are possible only when the ‘spin package’ is in proximity of a perturber). For general discussion, we can define the probability for a ‘spin package’ in bin \( i \) at time \( t \) to ‘jump’ to bin \( j \) at time \( t + \Delta t \) as \( p_{ij} \). \( p_{ij} \) is time invariant because the field profile of the ‘universe’ does not change with time. Thus, the \( n \)-by-\( n \) matrix:

\[
P = \begin{pmatrix}
  p_{11} & p_{12} & \cdots & p_{1n} \\
p_{21} & p_{22} & \cdots & p_{2n} \\
  \vdots & \vdots & \ddots & \vdots \\
p_{n1} & p_{n2} & \cdots & p_{nn}
\end{pmatrix}
\]  

[6.3]

sufficiently determines the diffusion process on the field profile (Fig. 6.2 b).
Figure 6.1: Discretized field profile for a hypothesized ‘universe’. ‘Jumping’ probabilities for the third bin are displayed
Figure 6.2: Sample field profile (a) and P Matrix (b) calculated from a simulation with uniformly distributed spherical perturbers.
Hence we have the following algorithm (Fig. 6.3):

1. Generate a ‘universe’ of randomly distributed perturbers.
2. Calculate the field distribution \( B(\vec{r}) \)
3. Generate the field profile by plotting \( B(\vec{r}) \) histogram.
4. Calculating the probability matrix \( P \) by counting pairs of neighbor grids in the ‘universe’.
5. Set \( T_d = 0, \phi = 0 \).
6. Assign a random start location to a ‘spin package’. The probability of assigning the start location to be in bin \( i \) is \( p_i = N_i / \sum_j N_j \), where \( N_j \) is the height of bin \( j \).
7. Mimic the diffusion process as random ‘jumps’ between bins. The probability of jumping from bin \( i \) to bin \( j \) within one step is \( p_{ij} \). Phase accumulated within such a step is \( \Delta \varphi = -\Delta t \frac{B_i + B_j}{2} \), where \( \Delta t \) is the temporal step size of the diffusion process, and \( B_i \) is the magnetic field strength in bin \( i \).
8. \( T_d = T_d + \Delta T_d, \phi = \phi + \Delta \varphi \).
9. Repeat steps 6-8, until \( T_d \geq TE \).
10. Repeat steps 1-9. Average over ‘spin packages’.
Figure 6.3: Field profile algorithm flow chart. This algorithm is composed of two independent steps: 1). Calculating the field profile and the P matrix; and 2). 1-D ‘jump’ of spin packages.
6.2.3 Prototype Testing

The field profile algorithm was implemented in IDL (ITT, Boulder, CO) on a Windows PC (64bit 1.74GHz processor and 1GB RAM) for prototype testing. As an example, the dependence of $T_2$ on water self diffusion constant $D$ was calculated for $D$ values ranging from 0.50 to 2.00 $\mu m^2/\mu s$, with 1,000 spin packages, spatial resolution 33 nm, maximal TE 50 ms, and universe size $100^3$. Other parameters used in this simulation include: perturber volume fraction $f = 0.174\%$, $B_0 = 1.5$ Tesla, and susceptibility difference $\Delta \chi = 2.2 \times 10^{-4}$ (cgs unit). 500 bins were used for the field profile.

6.3 Preliminary Results

The ‘universe’ size for the field profile algorithm can be controlled considerably smaller than the gigantic one used for the classical scheme, as both the field profile and the $P$ matrix can be computed from a much smaller ‘universe’. Because the only requirement on the ‘universe’ is that it should carry a representative field distribution, the restriction $L > \sqrt{6DTE}$ no longer applies to field profile calculation. Thus, step 1) in Fig. 6.3 is $O(1)$ with respect to spatial resolution. Computational complexity of this algorithm is determined by step 2) as $O(n^2)$.

It takes 6 to 12 hours to accomplish a simulation of 1,000 spin packages per parameter set. Conversely, a similar calculation with traditional Monte Carlo algorithm took more than 48 hours before the calculation was finally aborted by the researcher. Simulated $T_2$ values follow a linear relationship with $D$ as predicted by the outer sphere theory (Fig. 6.4).
6.4 Discussions and Conclusions

Our example study demonstrated the superior speed of the field profile algorithm. The speed of the algorithm can be further improved by using faster languages such as C or FORTRAN, and by adopting parallel computing concepts. We estimate that with all these improvements, simulations for 1,000 spin packages with spatial resolution in \( \sim 10 \) nm range can be accomplished within an hour.

An additional advantage of the field profile algorithm is its flexibility. There is no restriction on the shape, size, susceptibility, and distribution of the perturbers. When
interactions between perturbers are not negligible, the field distribution $B(\mathbf{r})$ can still be calculated as the numerical solution of the Maxwell’s equations.

In conclusion, this newly introduced field profile algorithm provides researchers a powerful tool to investigate transverse relaxation mechanisms on subcellular (~0.1 to 1 μm) to nano (~10 nm) scale, which was not previously achievable by traditional simulation techniques.

6.5. Future Works

Further tests for the proposed field profile algorithm have been planned, by comparing cellular-scale simulation results to literature values given in (126) and nano-scale simulation results to experimental measurements in phantoms.

Both $R_2$ and $R_2^*$ will be simulated and measured as well, because simulation has demonstrated that $\Delta R_2$ and $\Delta R_2^*$ have different relationship with cluster sizes that can be used to judge the validity of the proposed algorithm.

Clusters of paramagnetic substances can be created by assembling horse spleen ferritin into phospholipid liposomes as described in (127), or by attaching superparamagnetic iron oxide (SPIO) particles to MACS beads (with diameters ranging from 0.1 μm to 1 μm) with the following protocol:

1. Put $2.0 \times 10^6$ biotinylated ProActive polystyrene microspheres (Bangs Lab, Fishers, IN) into a 4 ml conical tube with 10 μL dH$_2$O;
2. Add varying amount of streptavidin conjugated MACS beads to the polystyrene microsphere suspension. The total staining volume is adjusted to 700 μL;
3. Incubate the mixture at 4°C for 30 min;
4. Wash the suspension twice with 2 × the staining volume;
5. Resuspend pellet in 200 μL of 1% agar;
6. Pipette the agar suspension into a 200 μL Eppendorf tube incubated in a water bath at 37°C. Care must be taken to avoid generation of air bubbles;
7. Let the agar gel to cool down slowly while gently shaking, to ensure homogeneity of sample preparation.

Phantoms can only be constructed in small sizes, due to the expensive prices of ferritin and SPIO. The postprocessing correction method proposed in Chapter 5 will be used to handle the possible problem of macroscopic B₀ inhomogeneity.

After the validity of the algorithm gets verified by experiments and simulations, it is planned to be applied, with more realistic perturber distributions that resembles the true subcellular structures in tissues, to explore the relationship between ferritin aggregation level and the observed R₂/R₂* relaxation rates.
CHAPTER 7

SUMMARY

From the series of studies conducted in various aspects of quantitative MRI but all closely related to specific clinical concerns, it is clear that the term ‘clinical related quantitative MRI techniques’ covers a large scope of different topics, whose research areas occupy the whole spectrum from theoretical modeling to everyday clinical practice, from \textit{in silico} to \textit{in vivo}, and from platform development to postprocessing correction. The huge number of unsolved problems and unfilled needs in this area creates a countless number of interdisciplinary niches in which almost all branches of modern science can fit themselves in, and keeps providing enormous opportunities as well as challenges to radiologists, MR physicists, engineers, and researchers from other background in the foreseeable future.
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