Methods for Chemical Exchange Saturation Transfer Magnetic Resonance Imaging

by

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Submitted to the Harvard-MIT Division of Health Sciences and Technology
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Abstract

Chemical exchange saturation transfer (CEST) is a relatively new magnetic resonance imaging (MRI) acquisition technique that generates contrast dependent on tissue microenvironment, such as protein concentration and intracellular pH. CEST imaging has the potential to become an important biomarker in a wide range of disorders. As an indicator of tissue pH, CEST imaging may allow the identification of the ischemic penumbra in stroke, and predict chemo- and radiation therapy outcomes in cancer. As a marker of protein concentration, CEST may be able to delineate tumor margins without contrast enhancement, identify disease onset in Alzheimer’s disease, and monitor cartilage repair therapies.

Despite several promising pilot studies, CEST imaging has had limited clinical application due to two main technical challenges. First, CEST imaging is extremely sensitive to magnetic field inhomogeneity. Images suffer from large susceptibility artifacts unless specialized B₀ inhomogeneity correction methods are employed that tremendously increase scan time. Second, the CEST contrast cannot be separated from the intrinsic macromolecular magnetization transfer (MT) asymmetry and brain images reflect the MT properties of white and gray matter rather than the desired protein and pH contrast.

We have developed a novel CEST imaging acquisition scheme, dubbed saturation with frequency alternating RF irradiation (SAFARI), designed to be insensitive to B₀ inhomogeneity and MT asymmetry. Studies in healthy volunteers demonstrate that SAFARI is robust in the presence of B₀ inhomogeneity and eliminates the need for specialized B₀ correction, thereby reducing scan time. In addition, results show that SAFARI removes the confounding MT asymmetry. We applied SAFARI imaging towards the study of the saturation transfer contrast in patients with high grade glioma. Results show that the contrast in brain tumors, which was previously attributed to an increase in the CEST signal from amide protons due to an elevated protein concentration, is instead the result of the loss of MT asymmetry found in the normal brain. Therefore, our work has lead to a new understanding of the different sources of signal in saturation transfer images of the brain with important implications for the design and analysis of future CEST studies of brain tumors.

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Chapter 1: Introduction

1.1 Overview

Chemical exchange saturation transfer (CEST) imaging (1-3) is an emerging magnetic resonance imaging (MRI) technique that holds great promise for in-vivo imaging of tissue microenvironment. CEST is a type of off-resonance saturation transfer imaging method that is sensitive to protons from endogenous molecules and proteins that exchange with water. It can be used both as a marker of endogenous molecular and protein concentration (4, 5) and, because the chemical exchange rate is sensitive to hydrogen ion concentration, as a marker of intracellular pH (6). In addition, exogenous CEST contrast agents are being developed for molecular imaging (7).

The utility of the CEST contrast has been explored in a wide range of disorders. In ischemic stroke imaging (8-15), CEST correlates with pH (10, 14) and may help differentiate the ischemic penumbra from regions with benign oligemia and predict progression to infarction (8, 9). In musculoskeletal imaging, the CEST signal correlates with the concentration of glycosaminoglycans in cartilage, which decreases proportionally to the severity of disease in osteoarthritis (16-19). CEST imaging of glycosaminoglycans could also have applications for the non-invasive assessment of cartilage repair therapies (18). In cancer imaging, CEST has shown promise as an indicator of tumor grade (20-22). It can delineate active tumor from edema and from necrotic tumor cores with greater specificity than conventional MR images without gadolinium enhancement (20, 23-26).

Despite these promising pilot studies, CEST imaging has had limited clinical applications due to two main technical challenges. First, CEST imaging is extremely sensitive to magnetic field inhomogeneity. Second, the CEST contrast cannot be separated from the intrinsic macromolecular magnetization transfer (MT) asymmetry, and therefore, CEST images tend to reflect the MT properties of white and gray matter rather than the proposed protein and pH contrast. Within this thesis, I describe my efforts towards developing robust methods for CEST imaging with reduced dependence on B₀ inhomogeneity and MT asymmetry. I apply these techniques towards the study of the different contributions to the saturation transfer contrast in the healthy human brain and in brain tumors.

1.2 Background

1.2.1 Off-resonance saturation transfer MR imaging

Magnetic resonance imaging has revolutionized our ability to obtain images of the living human body, highlighting both anatomy and function. Compared to other imaging modalities, MRI offers a large range of different contrast mechanisms that can improve the visualization of different tissue types and physiological events. Standard MRI contrast is sensitive to the
magnetic properties of tissue, such as proton density or relaxation time ($T_1$, $T_2$, $T_2^*$). However, it can also be manipulated to obtain images with contrast sensitive to physiological parameters such as arterial blood flow, capillary blood flow, blood oxygenation, white matter fiber tracts, iron content, and many more.

Off-resonance saturation transfer imaging is an MRI technique that can significantly increase the contrast between different tissue types based on the chemical and/or molecular composition of the tissue. Protons in different molecules experience a different range of magnetic environments and shielding effects from the surrounding electrons. As a result, their Larmor frequencies vary slightly from one type of molecule to another. In particular, protons from different brain metabolites have a narrow range of resonance frequencies that differ by about 1-3 ppm from the water resonance frequency. Protons on the backbone of proteins have a frequency that differs by about 3.5ppm and protons from large macromolecules and membranes have a very broad range of resonance frequencies that extend out to several tens of ppm. These different frequency distributions allow the selective excitation of specific molecular protons without exciting those from water, through the delivery of an off-resonance radiofrequency (RF) pulse. The subsequent interaction between these protons and those from water protons surrounding the molecules transfers the saturated magnetization to the water protons. As a result, the overall MR signal is lower than it would have been without the transfer of saturation, giving an image contrast directly dependent on the concentration of the targeted molecule.

Because protons from metabolites, small molecules, peptides and small mobile proteins have a different frequency distribution and exchange magnetization with water through different mechanisms than protons on large proteins, macromolecules and membranes, there are two different general types of off-resonance saturation transfer contrast, known as magnetization transfer (MT) imaging and chemical exchange saturation transfer (CEST) imaging. Applications of CEST and MT include the design of new types of MR contrast agents that can be switched on and off, detection of brain metabolites, detection of proteins, imaging of myelin content and monitoring brain pH.

### 1.2.1.1 Magnetization Transfer Imaging

Magnetization Transfer (MT) imaging is a method to detect large macromolecules and membranes based on the magnetization transfer from semi-solid protons on these molecules, and potential water temporarily bound them, to free water. Selective saturation of the broad macromolecular proton pool over many exchange cycles leads to a significant decrease in the free water signal because saturation is transferred to water by magnetization exchange (Figure 1-1). Several mechanisms contribute to the magnetization transfer including intramolecular dipolar cross-relaxation, and cross-relaxation by nuclear Overhauser effect (NOE) between the non-labile protons and the exchangeable protons of the macromolecular phase followed by fast proton exchange with water (4, 27). The resulting signal change is primarily a function of the macromolecular proton concentration and exchange rate, as well as the relaxation times of water.
Magnetization transfer imaging is now widely used in clinical applications (28). Because MT imaging reduces the signal intensity of normal tissue, it is commonly used for tissue suppression in applications such as MR angiography (29, 30) and contrast enhanced imaging (31, 32). Another category of applications of MT is to characterize tissue based on their macromolecular composition. In the brain, MT is of particular interest for detecting macromolecules from the myelin sheath surrounding axons, thereby providing a biomarker of demyelination. MT imaging has been applied to investigate pathological changes in a wide range of central nervous system disorders including cancer (33-35), multiple sclerosis (36-38), Alzheimer’s disease (39-42), Parkinson’s disease (43-45), Huntington’s disease (46-48), etc.

1.2.1.2 Chemical Exchange Saturation Transfer Imaging

Chemical Exchange Saturation Transfer (CEST) imaging (1-3, 49) is a method to detect low-concentration solutes based on the chemical exchange of mobile protons with water. Selective saturation of the exchangeable group over many exchange cycles leads to a significant decrease in the water signal because saturation is transferred to water by chemical exchange (Figure 1-2). CEST provides a sensitivity enhancement mechanism, allowing detection of metabolites present in the micro- to milli- Molar concentration ranges. The resulting signal change is a function of the mobile proton concentration and exchange rate (6, 50). Therefore, this technique is of interest both for detecting particular molecules or types of molecules and also as a measure of pH, since the exchange rate of protons is pH sensitive (51).
RF

Figure 1-2: CEST enhancement mechanism. When a radiofrequency (RF) saturation pulse is applied to the exchangeable solute pool (shown in red), the magnetization from the solute protons is nulled. The saturated protons then move to the larger water pool (shown in blue) via chemical exchange, while unsaturated protons from water move to the solute pool, replenishing the magnetization. If the duration of the RF pulse is long compared to the exchange time, the water signal decreases significantly due to an accumulation of saturation transfer.

Both endogenous and exogenous molecules can be used as CEST agents. Among the stronger in-vivo contributions to CEST contrast are hydroxyl protons at approximately 1 ppm above water, that have been used to assess glycogen (52) and glycosaminoglycans (16) in tissues where these molecules dominate, rapidly exchanging amine protons of amino acids and other molecules between 2-3 ppm above water, and the more slowly exchanging amides on the backbone of proteins, at approximately 3.5 ppm. Because amide protons have a larger chemical shift and their slow exchange permits saturation at power levels more compatible with human applications, amide proton transfer (APT) imaging (4, 53) is particularly attractive for pH measurement with endogenous CEST contrast.

1.2.2 Theory

1.2.2.1 Two site exchange theory for MT modeling

Magnetization transfer processes are commonly described by a two-pool exchange model (Figure 1-3). A small pool (m) represents the semi-solid macromolecular protons and a large pool (w) represents bulk water proton.
Figure 1-3: Two-pool exchange model for MT modeling. The small pool represents semisolid macromolecular protons and the large pool represents bulk water protons. $T_1$ and $T_2$ are the longitudinal and transverse relaxation rates. $M_0$ is the equilibrium magnetization. $\omega_{0m}$ is the resonance frequency of the MT line and $\omega_{0w}$ is the resonance frequency of the water line. $k_{mw}$ and $k_{wm}$ are the exchange rates of magnetization from pool m to pool w and vice versa.

The MR signal for the two exchanging pools can be modeled by modified Bloch equations with exchange terms, known as the Bloch-McConnell equations (54-56):

\[
\frac{d}{dt} \begin{bmatrix}
M_{0m} \\
M_{1m} \\
M_{2m}
\end{bmatrix} = \begin{bmatrix}
-\frac{1}{T_{2w}} & -(\omega_{m} - \omega) & 0 & 0 & 0 & 0 \\
\omega_{m} - \omega & -\frac{1}{T_{2w}} & -\omega_1 & 0 & 0 & 0 \\
0 & \omega_1 & -\frac{1}{T_{2w}} & -RM_{0m} & 0 & 0 \\
0 & 0 & 0 & -\frac{1}{T_{2w}} & -(\omega_{m} - \omega) & 0 \\
0 & 0 & 0 & \omega_1 - \omega & -\frac{1}{T_{2w}} & -\omega_1 \\
0 & 0 & 0 & R M_{0w} & 0 & \omega_1 - \frac{1}{T_{2w}} & -RM_{0w}
\end{bmatrix} \begin{bmatrix}
M_{0w} \\
M_{1w} \\
M_{2w}
\end{bmatrix}
\]

$\omega$ and $\omega_1$ are the frequency offset and amplitude of the off-resonance RF pulse, respectively. Explicit MT exchange between the transverse components can be ignored, since the very short $T_2$ of the macromolecular pool will destroy any transverse coherence between the two pools. The exchange between the pools is characterized by a fundamental rate constant $R$ such that:

\[ k_{mw} = RM_{0w} \] \[ k_{wm} = RM_{0m} \]

Therefore, the system under equilibrium obeys the relationship:

\[ k_{mw} M_{0m} = k_{wm} M_{0w} = RM_{0w} M_{0m} \]
As done previously (54, 55, 57, 58) a six-parameter model that describes the steady state longitudinal magnetization of the free water pool can be derived. The key steps and results are outlined below.

In the steady state, all six time derivatives must be equal to zero. This allows solving the equations for the transverse components of the magnetization and produces coupled equations for the longitudinal components:

\[ 0 = -R_{1w}(M_{zw,ss} - M_{0w}) - RM_{0m}M_{zw,ss} + RM_{0w}M_{zm,ss} - \frac{\omega^2 w T^2_{2w}}{1 + (\Delta \omega_w T^2_{2w})^2} M_{zw,ss} \]  

\[ 0 = -R_{1m}(M_{zw,ss} - M_{0m}) - RM_{0w}M_{zw,ss} + RM_{0m}M_{zm,ss} - \frac{\omega^2 m T^2_{2m}}{1 + (\Delta \omega_m T^2_{2m})^2} M_{zm,ss} \]

with \( \Delta \omega_w = \omega_{0w} - \omega \) and \( \Delta \omega_m = \omega_{0m} - \omega \). Defining,

\[ R_{rfw} = \frac{\omega^2 w T^2_{2w}}{1 + (\Delta \omega_w T^2_{2w})^2} \]

\[ R_{rfm} = \frac{\omega^2 m T^2_{2m}}{1 + (\Delta \omega_m T^2_{2m})^2} \]

and solving for \( M_{zw,ss} \) yields:

\[ M_{zw,ss} = \frac{R_{1m}\left(\frac{RM_{0m}}{R_{1w}}\right) + R_{rfm} + R_{1m} + R}{M_{0w}\left(\frac{RM_{0m}}{R_{1w}}\right)(R_{rfm} + R_{1m} + R) + \left[1 + \left(\frac{\omega^2}{R_{1w} T^2_{2w}}\right)\left(\frac{1}{R_{1w} T^2_{2w}}\right)\right](R_{rfm} + R_{1m} + R)} \]

Eq. [1.8] can be further simplified to a six parameter model by realizing that \( (\Delta \omega_w T^2_{2w})^2 \gg 1 \) for all significantly non zero points:

\[ \frac{M_{zw,ss}}{M_{0w}} = \frac{R_{1m}\left(\frac{RM_{0m}}{R_{1w}}\right) + R_{rfm} + R_{1m} + R}{\left(\frac{RM_{0m}}{R_{1w}}\right)(R_{rfm} + R_{1m} + R) + \left[1 + \left(\frac{\omega^2}{R_{1w} T^2_{2w}}\right)\left(\frac{1}{R_{1w} T^2_{2w}}\right)\right](R_{rfm} + R_{1m} + R)} \]

It has been shown (55, 58) that the Lorentzian line shape \( R_{rfm} \) is not adequate to describe the absorption of the semisolid macromolecular pool. Instead, \( R_{rfm} \) is replaced by a super-Lorentzian line, which is better suited to biological tissues:

\[ R_{rfm} = \omega^2 \pi g_m(\Delta \omega_m) \]

\[ g_m(\Delta \omega_m) = \int_0^{\pi/2} \sin \theta \cdot \sqrt{\frac{2}{\pi}} \cdot \frac{T_{2m}}{3 \cos^2 \theta - 1} \cdot \exp\left[-2 \left(\frac{\Delta \omega_m T_{2m}}{3 \cos^2 \theta - 1}\right)^2\right] d\theta \]

A time dependent solution for the longitudinal magnetization can also be derived, assuming the transverse magnetization has reached the steady state. In this case, Eq. [1.1] reduces to two coupled equations (59-61):

\[ \frac{dM_{zw}}{dt} = -R_{1w}(M_{zw} - M_{0w}) - RM_{0m}M_{zw} + RM_{0w}M_{zm} - R_{rfw}M_{zw} \]

\[ \frac{dM_{zm}}{dt} = -R_{1m}(M_{zm} - M_{0m}) - RM_{0w}M_{zm} + RM_{0m}M_{zw} - R_{rfm}M_{zm} \]
The solution has the form:

$$M_{zw} = c_1 \exp(m_1T_{sat}) + c_2 \exp(m_2T_{sat}) + M_{zw,ss}$$  \[1.14\]

where $M_{zw,ss}$ is the steady state solution derived in Eq. [1.9] and $T_{sat}$ is the duration of the RF saturation pulse. In practice, it has been shown that the second exponential term is negligible and Eq. [1.14] simplifies to a monoexponential decay (60):

$$M_{zw} = (M_{0w} - M_{ss})\exp(m_1T_{sat}) + M_{zw,ss}$$  \[1.15\]

with

$$m_1 = \frac{\alpha_w + \alpha_m \pm \sqrt{\left(\alpha_w - \alpha_m\right)^2 + 4R^2M_{0m}}}{2}$$  \[1.16\]

$$\alpha_{w,m} = -\left(R_{1(w,m)} + RM_{0(m,w)} + R_{rf(w,m)}\right)$$  \[1.17\]

The magnitude of the MT effect is described by the magnetization transfer ratio (MTR):

$$MTR = 1 - \frac{S_{sat}}{S_0}$$  \[1.18\]

where $S_{sat} (=M_{zw})$ is the water signal intensity measured with RF saturation and $S_0$ is measured without RF saturation. The amplitude of the measured MTR is a function of the intrinsic MR parameters of the water and macromolecular proton pools as well as the specifics of the RF saturation pulse, which include the offset frequency $\omega$ and irradiation power $\omega_1$. In general, MTR increases with higher saturation power and lower offset frequency, as can be seen in Figure 1-4. For clinical applications MTR is typically measured at a single off-resonance offset frequency several kilohertz from the water line. However, the off-resonance RF pulse can also be swept across a range of frequencies in order to generate a spectrum of the MT effect on water, known as a z-spectrum (62, 63). This spectrum can then be fit to derive the 6 quantitative MT parameters in Eq. [1.9] and determine whether changes in MTR are the result of changes to the macromolecular properties such as MT rate, concentration and mobility, or simply changes in water $T_1$ and $T_2$. 

\[18\]
Figure 1-4: z-spectrum as a function of RF power at 3T. Simulation of a z-spectrum for conventional MT in which water saturation is measured as a function of irradiation frequency. When the applied RF frequency is close to the water resonance frequency, direct water saturation, also known as spillover, occurs. When the applied RF frequency is away from the water resonance, magnetization transfer from the macromolecular proton pool decreases the water signal. Due to its very broad range of resonance frequencies, characterized by a short $T_2$ and super-Lorentzian line shape, the MT effect can be detected up to several hundreds of ppm from the water resonance at 3T. The simulated model parameters corresponding to healthy white matter were $R_{1m}=1s$, $R_{M0m}/R_{1w}=3.8$, $1/(R_{1w}T_{2w})=60$, $R=50Hz$, $T_{2m}=10\mu s$, $\omega_{0m}=-2.3ppm$ (54).
Because the center of the super-Lorentzian macromolecular MT line is shifted towards the aliphatic spectral region compared to the water resonance, the MT z-spectrum is slightly asymmetric with respect to water. The asymmetry in the MT spectrum is defined as the difference in MTR on either side of the water line:

\[ MTR_{\text{asym}}(\omega) = MTR(\omega^+) - MTR(\omega^-) = \frac{S_{\text{sat}}(\omega^-) - S_{\text{sat}}(\omega^+)}{S_0} \]  

where \( \omega \) is the frequency offset of the RF saturation. It has been shown that for a given frequency offset \( \omega \) there is a characteristic saturation power \( \omega_{1c} \) that maximizes the amplitude of the MT asymmetry (54). The characteristic saturation power is given by:

\[ \omega_{1c} = \sqrt{\frac{-A(B\Delta C - B\Delta C)}{C^2(B\Delta C - B\Delta C) - C^2(B\Delta C)}} \]  

where

\[ A = R_{1m} \left( \frac{R_{M0m}}{R_{1w}} \right) + R_{1m} + R \]  

\[ B_{\pm} = \pi g_m \left( \pm \Delta \omega_w + \omega_{0m} \right) \]  

\[ C_{\pm} = \left( 1 + \frac{R_{M0m}}{R_{1w}} \right) \pi g_m \left( \pm \Delta \omega_w + \omega_{0m} \right) + \left( R_{1m} + R \right) \left( \frac{1}{\Delta \omega_w} \right)^2 \left( \frac{1}{R_{1w} T_{2w}} \right) \]

The characteristic saturation power \( \omega_{1c} \) and the corresponding maximal MT asymmetry are shown in Figure 1-5. At 3T, the asymmetry peaks at the frequency offset \( \omega = 2.8 \text{ppm} \) with an amplitude of -3.5%. At high frequency, offsets it levels off at amplitude of -2.0%. As the frequency offset increases, higher powers are needed to maximize the MT asymmetry.

![Figure 1-5 Simulation of the characteristic saturation power that maximizes MT asymmetry (a) and the corresponding maximal MT asymmetry (b) at 3T. The simulated model parameters corresponding to healthy white matter were \( R_{1m} = 1 \text{s}, R_{M0m}/R_{1w} = 3.8, 1/(R_{1w} T_{2w}) = 60, R = 50 \text{Hz}, T_{2m} = 10 \mu \text{s}, \omega_{0m} = -2.3 \text{ppm} \). Adapted from (54).](image-url)
The amplitude of the MT ratio and MT asymmetry both depend on MR sequence parameters including RF irradiation power level, RF irradiation duration and RF frequency offset. These parameters must be chosen appropriately to enhance (or minimize) the MT effects.

1.2.2.2 Two site exchange theory for CEST modeling

Chemical exchange saturation transfer processes are also described by a two-pool exchange model (Figure 1-6). A small pool (s) represents the exchangeable solute protons and a large pool (w) represents bulk water protons.

![Diagram of two-pool exchange model](image)

**Figure 1-6:** Two-pool exchange model for CEST modeling. The small pool represents exchangeable protons on small solute molecules and the large pool represents bulk water protons. $T_1$ and $T_2$ are the longitudinal and transverse relaxation rates. $M_0$ is the equilibrium magnetization. $\omega_{0s}$ is the resonance frequency of the exchangeable proton of interest and $\omega_{0w}$ is the resonance frequency of the water line. $k_{sw}$ and $k_{ws}$ are the exchange rates of magnetization from pool s to pool w and vice versa.
The MR signal for the two exchanging pools is also modeled by modified Bloch equations including exchange terms (56, 64):

\[
\begin{bmatrix}
\frac{1}{T_{2w}} - k_{ws} & -(\omega_0 - \omega) & 0 & k_{sw} & 0 & 0 \\
\omega_0 - \omega & \frac{1}{T_{2w}} - k_{ws} & -\omega_1 & 0 & k_{sw} & 0 \\
0 & \omega_1 & \frac{1}{T_{2w}} - k_{sw} & 0 & 0 & k_{sw} \\
0 & k_{ws} & 0 & \omega_0 - \omega & \frac{1}{T_{2s}} - k_{ws} & -\omega_1 \\
0 & 0 & k_{ws} & 0 & \omega_1 & \frac{1}{T_{2s}} - k_{sw} \\
0 & 0 & 0 & \omega_0 - \omega & \frac{1}{T_{2s}} - k_{sw} & -\omega_1
\end{bmatrix}
\begin{bmatrix}
M_{sw} \\
M_{wy} \\
M_{sz} \\
M_{wz} \\
M_{wm} \\
M_{zm}
\end{bmatrix}
= 
\begin{bmatrix}
0 \\
0 \\
0 \\
0 \\
0 \\
0
\end{bmatrix}
\]

\[\omega \text{ and } \omega_1 \text{ are the frequency offset and amplitude of the off-resonance RF pulse, respectively. Under equilibrium, the system obeys the relationship } k_{sw} M_{0s} = k_{ws} M_{0w}. \text{ The main difference between the Bloch equations with chemical exchange compared to magnetization transfer is the inclusion of the transverse exchange terms due to the longer } T_{2s}.\]

We can compare the appearance of the z-spectrum from macromolecular MT with that of a typical CEST agent. Macromolecular MT that arises from the interaction of protons from semi-solid macromolecules with water can be detected over a very wide range of frequency offsets up to tens of ppm at 3T (Figure 1-4). The MT effect is symmetric but slightly shifted with respect to the water line. In contrast, CEST from protons on endogenous mobile proteins and molecules is detected over a much smaller frequency range less than 6ppm from water. The CEST effect is not symmetric but occurs at individual frequencies, as seen in Figure 1-7 for amide protons at 3.5ppm. In a two pool-model, the z-spectrum for a CEST agent exhibits both direct water saturation and a CEST peak centered at the exchangeable proton resonance frequency. Similarly to MT, the amplitude of the CEST effect is described by the proton transfer ratio (PTR). The PTR can be quantified by an asymmetry analysis where the signal acquired with saturation on resonance with pool s is subtracted from a control image with saturation applied symmetrically on the opposite side of the water resonance.

\[PTR = MTR_{\text{asym}}(\omega_0) = \frac{S_{\text{sat}}(-\omega_0) - S_{\text{sat}}(+\omega_0)}{S_0} \]

This removes the effect of direct water saturation yielding the CEST peak (Figure 1-7).
Several useful analytical solutions to Eq. [1.24] can be derived under special assumptions (64-69). In the weak saturation approximation (2, 64), it is assumed that the RF irradiation is applied on resonance with the solute pool s while leaving the water pool w unperturbed (no direct water saturation). Therefore, \((\omega_{0s} - \omega) = 0\), \((\omega_{0w} - \omega) \to \infty\) and the Bloch equations can be simplified to four equations with exact steady state analytical solutions. The steady state longitudinal magnetization for the water and the solute pools under the weak saturation approximation are (2, 64):

\[
\frac{M_{zs,ss}}{M_{0s}} = 1 - \frac{M_{0w}}{M_{0w}} \cdot \frac{\alpha \cdot k_{sw}}{R_{1w} + k_{ws}}
\]

where the saturation efficiency \(\alpha\) is given by

\[
\alpha = \frac{\omega_{0s}^2}{1 + \left(\frac{1}{T_{2s}} + k_{rw} \cdot \frac{T_{1s}}{\gamma_{1w} + k_{ws}}\right) \left(\frac{1}{T_{1s}} + k_{rw} \cdot \frac{T_{1s}}{\gamma_{1w} + k_{ws}}\right)}
\]

Time dependent solutions can also be derived from Eq. [1.24] in the weak saturation approximation by separating the saturation process of pool s and transfer to pool w into two separate steps (2, 64). Assuming that pool s approaches the steady state \(M_{zs,ss}\) instantly, the dynamics for pool w can be described by a single equation:

\[
\frac{dM_{zw}}{dt} = -(1/T_{1w} + k_{ws})(M_{zw} - M_{0w}) + k_{sw}(M_{zs,ss} - M_{0s})
\]

with the solution:
\[ M_{zw} = M_{0w} - \frac{k_{sw} \alpha M_{0w}}{R_{1w} + k_{ws}} \left[ 1 - \exp \left( -(R_{1w} + k_{ws})T_{sat} \right) \right] \]  

\[ [1.30] \]

where \( T_{sat} \) is the applied RF saturation duration. Since direct water saturation is assumed to be zero in the weak saturation approximation, the proton transfer ratio simplifies to:

\[ PTR_{weak} = 1 - \frac{M_{zw}}{M_{0w}} \]

\[ = \frac{M_{0w}}{M_{0w}} \frac{k_{sw} \alpha}{R_{1w} + k_{ws}} \left[ 1 - \exp \left( -(R_{1w} + k_{ws})T_{sat} \right) \right] \]  

\[ [1.31] \]

While Eq. [1.31] provides a concise description of the CEST signal amplitude, the assumption of no direct water saturation is seldom met experimentally. A different analytical solution can be derived under the assumption of strong saturation pulse, which provides an effective way to account for direct water saturation (67, 68). In order to derive an analytical solution, the Bloch McConnell equations are simplified by decomposing the magnetization to a new basis set along the effective field orientation. This corresponds to a transformation to a doubly tilted single rotating frame. The steady state analytical solution under the strong pulse approximation is then given by (68):

\[ \frac{M_{zw,ss}(\omega)}{M_{0w}} = \cos \theta_w \cdot \frac{R_{1w} R_{2s} \cos \theta_w + R_{1s} k_{ws} \cos \theta_s \cos(\theta_w - \theta_s)}{R_{zw} R_{2s} - k_{ws} k_{sw} \cos^2(\theta_w - \theta_s)} \]  

\[ [1.32] \]

in which

\[ R_{zw} = (R_{1w} + k_{ws}) \cos^2 \theta_w + (R_{2w} + k_{ws}) \sin^2 \theta_w \]  

\[ [1.33] \]

\[ R_{zs} = (R_{1s} + k_{sw}) \cos^2 \theta_s + (R_{2s} + k_{sw}) \sin^2 \theta_s \]  

\[ [1.34] \]

\[ \theta_i = \tan^{-1} \left( \frac{\omega_i}{\omega_{0i} - \omega} \right), \quad i = w, s \]  

\[ [1.35] \]

When the RF irradiation is applied on resonance with the solute pool, \( \theta_s = \pi/2 \). In this case, Eq. [1.32] can be simplified to:

\[ \frac{M_{zw,ss}(\omega)}{M_{0w}} = \frac{R_{1w} \cos^2 \theta_w}{R_{zw} \left( \cos^2(\theta_w) - k_{ws} k_{sw} \sin^2(\theta_w) \right)} \]  

\[ [1.36] \]

In the strong saturation approximation, direct water effects must be removed by asymmetry analysis to quantify the CEST signal amplitude. When the RF irradiation is applied at the control frequency \(-\omega_s, \theta_s = \theta_w/2\) and \( \theta_w = \tan^{-1} \left( -\omega_i / (\omega_{0w} - \omega_s) \right) \). Therefore the CEST signal amplitude is:
\[ P_{TR\text{strong}} = MTR_{asym}(\theta_w) \]
\[ = \frac{R_{1w}r_{sz} \cos^2 \theta_w + R_{1w}k_{sw} \cos \theta_w \cos^2 \left(\frac{\theta_w}{2}\right)}{R_{sz}r_{sz} - k_{sw}k_{sw} \cos^2 \left(\frac{\theta_w}{2}\right)} - \frac{R_{1w} \cos^2 \theta_w}{R_{zw} - \frac{k_{sw}k_{sw}}{R_{sz} + k_{sw}} \sin^2 \left(\frac{\theta_w}{2}\right)} \]

where

\[ r_{sz} = (R_{sz} + k_{sw}) \cos^2 \left(\frac{\theta_w}{2}\right) + (R_{sz} + k_{sw}) \sin^2 \left(\frac{\theta_w}{2}\right) \]

The two steady state solutions for the weak saturation approximation and the strong saturation approximation can be combined to derive an analytical solution that is valid for an arbitrary RF power (69):

\[ P_{TR\text{general}} = \alpha \cdot \left[ \frac{R_{1w}r_{sz} \cos^2 \theta_w + R_{1w}k_{sw} \cos \theta_w \cos^2 \left(\frac{\theta_w}{2}\right)}{R_{sz}r_{sz} - k_{sw}k_{sw} \cos^2 \left(\frac{\theta_w}{2}\right)} - \frac{R_{1w} \cos^2 \theta_w}{R_{zw} - \frac{k_{sw}k_{sw}}{R_{sz} + k_{sw}} \sin^2 \left(\frac{\theta_w}{2}\right)} \right] \]

A comparison of the weak saturation approximation, the strong saturation approximation and the general saturation solution is shown in Figure 1-8. At low RF irradiation power, direct water saturation is low and the weak saturation approximation gives a good estimate of the CEST effect. In this power range, the CEST effect increases with power as the saturation efficiency increases. At high RF irradiation power, direct water saturation becomes significant. The weak saturation approximation fails to describe the CEST effect in this regime, as it does not account for spillover effects. In this case, the strong saturation approximation gives a good estimate of the CEST effect. At high power, the CEST effect decreases with increasing power due to increasing spillover effects that reduce the proton transfer ratio.

Figure 1-8: Comparison of the analytical solutions of the two-pool Bloch equations for the weak RF saturation approximation, the strong RF saturation approximation and the general RF saturation approximation at 3T as a function of RF irradiation power. The simulated model parameters were \( T_{1w}=1.5s, T_{2w}=60ms, T_{1s}=0.77s, T_{2s}=0.33ms, M_{0s}=M_{0w}/2000, k_{sw}=30Hz, \omega_{0s}=3.5ppm, T_{sat}=15s \).
The overall CEST contrast is a function of both the solute proton concentration \( (M_{0s}/M_{0w}) \) and the chemical exchange rate \( (k_{sw}) \) of the solute proton with water (Figure 1-9). The exchange rate is particularly important because it depends on the pH. For a given set of exchange parameters there is an optimum RF power that maximizes the CEST contrast, as seen in Figure 1-9. The optimum RF power increases with increasing proton exchange rate and solute pool resonance frequency. When designing CEST contrast agents, the sensitivity enhancement can be maximized with a large exchange rate \( k_{sw} \), a high concentration of solute protons and a large chemical shift from the water resonance. For endogenous CEST contrast, however, these parameters are fixed and depend on the physiology and pathological state of the tissue. The CEST contrast is then a function of the applied saturation scheme. Enough saturation power must be applied to maximize the saturation efficiency \( \alpha \) of the solute protons while minimizing the amount of direct water saturation. In addition, the saturation duration must be long enough to establish a large proton transfer ratio.

![Simulation of the two-pool Bloch equations as a function of RF irradiation power.](image)

**Figure 1-9** Simulation of the two-pool Bloch equations as a function of RF irradiation power. a) Effect of the proton exchange rate on the optimal saturation power and CEST signal. b) Effect of the exchangeable proton concentration on the CEST signal. Model parameters were \( T_{1w}=1.5s, T_{2w}=60ms, T_{1s}=0.77s, T_{2s}=0.33ms, M_{0s}=M_{0w}/2000, k_{sw}=30Hz, \omega_{0s}=3.5ppm, T_{sat}=15s. \)

### 1.2.2.3 Three site exchange theory for MT and CEST

For *in-vivo* applications, exchange of protons from both CEST and MT must be taken into account. This is accomplished by a three-pool model with exchange processes between bulk water (pool w) and the solute exchangeable protons (pool s) and the macromolecular protons (pool m). The exchange between pool s and pool m is assumed to be negligible.
Figure 1-10: Three-pool exchange model for in-vivo CEST modeling with MT. The small pools represent exchangeable protons on small solute molecules and broad macromolecular protons and the large pool represents bulk water protons. $T_1$ and $T_2$ are the longitudinal and transverse relaxation rates. $M_0$ is the equilibrium magnetization. $\omega_{0s}$ is the resonance frequency of the exchangeable proton, $\omega_{0m}$ is the resonance frequency of the macromolecular protons and $\omega_{0w}$ is the resonance frequency of the water line. $k_{sw}$ and $k_{ws}$ are the exchange rates of magnetization from pool s to pool w and vice versa. $k_{mw}$ and $k_{wm}$ are the exchange rates of magnetization from pool m to pool w and vice versa.

The combined three pool Bloch-McConnell equations for CEST in the presence of super-Lorentzian macromolecular MT are (70-72):

\[
\frac{d}{dt} \begin{bmatrix} M_s \\ M_r \\ M_m \\ M_{x} \\ M_{y} \\ M_{z} \\ M_{xm} \\ M_{ym} \\ M_{zm} \end{bmatrix} = \begin{bmatrix} \frac{-1}{T_{2w}} - k_{sw} & 0 & -\omega_{0w} & 0 & 0 & 0 & 0 & 0 & 0 \\ \omega_{0s} - \omega & \frac{-1}{T_{2w}} - k_{sw} & 0 & k_{sw} & 0 & 0 & 0 & 0 & 0 \\ 0 & \omega_{0s} & \frac{-1}{T_{2w}} - k_{sw} & 0 & 0 & 0 & 0 & 0 & 0 \\ k_{sw} & 0 & 0 & \frac{-1}{T_{2w}} - k_{sw} & 0 & 0 & 0 & 0 & 0 \\ 0 & k_{sw} & 0 & \omega_{0s} - \omega & \frac{-1}{T_{2w}} - k_{sw} & 0 & 0 & 0 & 0 \\ 0 & 0 & k_{sw} & 0 & 0 & \omega_{0s} & \frac{-1}{T_{2w}} - k_{sw} & 0 & 0 \\ 0 & 0 & k_{sw} & 0 & 0 & 0 & 0 & -k_{sw} - \pi \omega \chi_e (\Delta \omega) & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -k_{sw} - \pi \omega \chi_e (\Delta \omega) \end{bmatrix} \begin{bmatrix} M_s \\ M_r \\ M_m \\ M_{x} \\ M_{y} \\ M_{z} \\ M_{xm} \\ M_{ym} \\ M_{zm} \end{bmatrix} \]

Again, explicit MT exchange between the transverse components of the magnetization has been ignored. This allows us to solve the equations for $M_{xm}$ and $M_{ym}$, assuming the transverse magnetization of the macromolecular pool has reached a steady state, and replace the Lorentzian lineshape by a super-Lorentzian, as described in Section 1.2.2.1. Therefore, no explicit equations for the transverse magnetization of pool m are present in Eq. [1.40].
There are no algebraic solutions of Eq. [1.40]. However, given that it has the form:

\[
\frac{dM}{dt} = A \cdot M + B \tag{1.41}
\]

The analytical solution, which is valid for time-independent \( \omega_1 \), is given by:

\[
M = \left( M_0 + \frac{B}{A} \right) \exp(At) - \frac{B}{A} \tag{1.42}
\]

Because of the broad frequency distribution of the MT effect, it is difficult to obtain pure CEST images with no MT interference when both effects are present. When off-resonance saturation pulses are applied at the resonance frequency of exchangeable protons, the resulting saturation transfer will be a combination of the CEST effect from the target protons and MT effects from the broad macromolecular protons. The approach typically used in the literature to remove the MT effect from CEST images is again to define a MT ratio asymmetry (MTR\(_{\text{asym}}\)) parameter by subtracting the MTR obtained at the resonance frequency (\( \omega_s \)) of the target proton from that of a control frequency acquired on the opposite side of the water line.

\[
MTR_{\text{asym}}(\omega_s) = MTR(\omega_s) - MTR(-\omega_s) = \frac{S_{\text{sat}}(-\omega_s) - S_{\text{sat}}(\omega_s)}{S_0} \tag{1.43}
\]

If the conventional MT effect were symmetric with respect to the water line, this analysis would remove macromolecular contributions to the image and yield a positive MTR\(_{\text{asym}}\) reflecting only the CEST effect. In the brain, however, it has been shown that the MT effect is shifted from the water line with a center frequency in the aliphatic spectral range (54, 73). Moreover, the MT asymmetry tends to peak in the same spectral range as where most endogenous exchangeable proton resonances are located (e.g., Figure 1-5). Consequently, in a three-pool model, the MTR\(_{\text{asym}}\) parameter is no longer a pure measure of the proton transfer ratio but instead is a combination of the intrinsic asymmetry of the macromolecular MT effect, referred to as MTR\(_{\text{asym,}}\), and the CEST effect, referred to as PTR:

\[
MTR_{\text{asym}}(\omega_s) = MTR_{\text{asym,}}(\omega_s) + PTR(\omega_s) \tag{1.44}
\]

MTR\(_{\text{asym}}\) has negative amplitude (Figure 1-5) and PTR has positive amplitude (Figure 1-7). The sum of the two effects, often results in MTR\(_{\text{asym}}\) values with negative or near zero amplitudes at the typical RF saturation power levels employed for endogenous CEST imaging. When comparing MTR\(_{\text{asym}}\) in pathological lesions, the change in PTR in the lesion compared to the normal brain is sometimes assessed under the assumption that MTR\(_{\text{asym}}\) is unaffected. This assumption, however, remains to be verified.
1.2.3 Measurements techniques: Pulse sequences for saturation transfer imaging

A general pulse sequence scheme for off-resonance saturation transfer imaging consists of an RF irradiation preparation in order to establish the saturation transfer contrast followed by a conventional fast imaging readout such as echo-planar imaging (EPI). In this section, we review some of the different RF schemes that can be employed to generate saturation transfer contrast.

1.2.3.1 Continuous Wave Saturation

Saturation transfer was originally performed with a long off-resonance continuous wave (CW) RF irradiation pulse. In this scheme, a constant RF pulse of amplitude $B_1$ is applied for a long saturation duration $T_{sat}$ before each excitation (Figure 1-11). $T_{sat}$ is typically on the order of several seconds.

![Figure 1-11: Illustration of a CEST MRI pulse sequence with CW saturation. The pulse sequence consists of two periods: a saturation period of amplitude $\omega_1$ and duration $T_{sat}$ and a conventional image acquisition period. EPI image readout is illustrated above, but can be replaced by other fast acquisition schemes.](image)

The CW saturation scheme has several advantages. First, the saturation preparation depends only on $B_1$ and $T_{sat}$, making the optimization of the pulse sequence straightforward based on the
equations described in Section 1.2.2. Second, steady state saturation and maximal proton transfer enhancement can easily be achieved using long saturation durations. Third, long $T_{sat}$ produces a narrow saturation bandwidth resulting in narrow peaks on the z-spectrum.

However, CW saturation also has several drawbacks. Long RF irradiation cannot be generated on most clinical scanners due to hardware limitations. In addition, the typical applied RF power and long irradiation times can exceed the specific absorption rate (SAR) safety limits and cannot be used for in-vivo imaging. Therefore, for human imaging applications, CW RF irradiation must be reduced to shorter irradiation times at low power or alternate saturation schemes, such as pulsed methods, must be used.

1.2.3.2 Pulsed Saturation

Pulsed saturation schemes consisting of a train of short RF pulses repeated over several seconds can be used to generate the saturation transfer contrast instead of CW saturation. Compared to CW saturation, pulsed saturation schemes are more complicated to optimize as they depend on a number of parameters such as pulse shape, flip angle (FA), pulse width ($pw$), inter-pulse delay ($\tau_d$) or pulse repetition time ($TR_{RF}$) and total duration ($T_{sat}$). In addition, because $\omega_1$ is no longer time-independent, the analytical solutions to the Bloch-McConnell equations described in Section 1.2.2.2 cannot be used and instead the equations must be numerically integrated, which is time consuming. In general, it has been shown that the equivalent RF power that maximizes the pulsed-CEST contrast is approximately equal to the optimal CW power (74-76) for protons in the slow exchange regime. The equivalent CW power for an RF pulse train is given by:

$$B_{CW} = \sqrt{\frac{1}{TR_{RF}} \int_0^{TR_{RF}} B_1^2 dt}$$  \[1.45\]

where $B_1$ is the pulse shape. The optimal power for protons with exchange rates less than 100Hz is about 0.8-1µT (74, 75, 77). Several studies in creatine phantoms have also shown that Gaussian-shaped pulses provided better frequency selectivity and higher CEST contrast than sinc and rectangular pulses (78, 79).
Figure 1-12: Illustration of a CEST MRI pulse sequence with pulsed saturation. The pulse sequence consists of two periods: a saturation period of duration $T_{sat}$ and a conventional image acquisition period. The saturation period consists of a train of RF pulses. The pulse shape, flip angle, pulse width (pw) and pulse repetition time ($TR_{RF}$) can be optimized for maximal CEST contrast. Crusher gradients can be used during the inter-pulse delay period to suppress residual transverse magnetization and prevent unwanted echo formation. EPI image readout is illustrated above, but can be replaced by other fast acquisition schemes.

Many attempts have been made at further optimizing the other RF pulse train parameters (72, 74-76, 78-80), but no consensus on the optimum RF pulse train has been reached. For example, simulations at 9.4T for a creatine-agar phantom, with exchange rates on the order of 85Hz, report optimum parameters for an RF train of 400 Gaussian pulses to be $FA=180^\circ$, $TR_{RF}=24.0\text{ms}$, and $pw=12.0\text{ms}$ (75). Another study of creatine at 4.7T reports optimum parameters for a 3-second train of Gaussian pulses with 50% duty cycle to be $FA=257^\circ$ and $pw=20\text{ms}$ (79). At 3T, the optimum parameters for creatine imaging with a pulse train of 10 Gaussian pulses are reported to be $pw=100\text{ms}$, $TR_{RF}=100\text{ms}$ and $B_{CW}=1\mu\text{T}$ (78). Finally, it has been shown that, although the optimal flip angle is about $180^\circ$, it increases with increasing proton exchange rates (in the range 10-100Hz) and chemical shift of the exchangeable pool (79). For $180^\circ$ pulses, the optimal pulse width decreases with increasing exchange rate and chemical shift (79).
1.2.3.3 Spin-locking for chemical exchange contrast

Another strategy that has been proposed to achieve saturation transfer contrast is via a spin-locking experiment. In this approach, the water magnetization is first flipped away from the z-axis and then spin-locked by an off-resonance $B_1$ RF pulse. While the spin-lock field is on, transverse magnetization decays with a relaxation time $T_{1p}$ rather than $T_2$.

![Illustration of a CEST MRI pulse sequence with spin locking preparation.](image)

**Figure 1-13:** Illustration of a CEST MRI pulse sequence with spin locking preparation. The pulse sequence consists of two periods: a spin locking period where the magnetization is first flipped by a hard pulse and then locked by a spin lock pulse of amplitude $\omega_1$ and duration TSL before being flipped back to the z-axis, and a conventional image acquisition period. EPI image readout is illustrated above, but can be replaced by other fast acquisition schemes.

For a two-pool exchange model with chemical exchange between a solute pool $s$ and a water pool $w$, the Bloch-McConnell equations can be solved to find the relaxation rate $R_{1p}=1/T_{1p}$ (81):

$$R_{1p} = R_1 \cos^2 \theta + (R_2 + R_{ex}) \sin^2 \theta$$  \[1.46\]

$$R_{ex} = \frac{\sin^2 \theta \cdot M_0 s M_0 w (\omega_{os} - \omega_{ow})^2 k_{ex}}{[\omega_{os}^2 + \omega_1^2] [\omega_{ow}^2 + \omega_1^2] [M_0 s \omega_{os} + M_0 w \omega_{ow} - \omega]^2 + \omega_1^2 + k_{ex}^2}$$  \[1.47\]

where $R_1$ and $R_2$ are the longitudinal and transverse relaxation, $M_{0s,w}$ is the population of each pool, $\omega_{0s,w}$ is the resonance frequency of each pool, $\omega_1$ is the resonance frequency of the applied...
RF pulse, $\omega_1$ is the amplitude of the applied RF pulse, $k_{ex} = k_{sw} + k_{ws}$ is the exchange rate between the two pools, $\theta$ is the angle of the effective spin-locking field:

$$\theta = \arctan(\omega_1 / (\omega_{0w} - \omega))$$

For chemical exchange of endogenous protons, the population of the solute pool is typically much smaller than the population of the water pool ($M_{0s} < M_{0w}$) and $R_{ex}$ simplifies to (81):

$$R_{ex} = M_{0s} \frac{(\omega_{0s} - \omega_{0w})^2 k_{ex}}{(\omega_{0s} - \omega)^2 + \omega_1^2 + k_{ex}^2}$$

where $M_{0w}$ has been normalized to 1. Note that $R_{ex}$ is maximal when the spin locking pulse is applied at the resonance frequency of the solute proton ($\omega = \omega_{0s}$).

Spin-locking experiments can be performed by varying the RF frequency offset $\omega$, similar to a CEST z-spectrum. The longitudinal magnetization at the end of the spin-locking preparation is given by (82, 83):

$$SLR(\omega) = \frac{M(\omega)}{M_0} = \left(1 - \frac{R_1 \cos \theta}{R_{1p}}\right) \cdot \exp(-R_{1p} \cdot TSL) + \frac{R_1 \cos \theta}{R_{1p}}$$

where $TSL$ is the length of the spin locking RF pulse.

The spin locking ratio asymmetry is defined as:

$$SLR_{asym} = SLR(-\omega) - SLR(+\omega)$$

Note the similarity between Eq. [1.51] describing the spin-lock chemical exchange contrast and Eq. [1.43] describing the CEST contrast. The two experiments are very similar and differ only by the application of the spin-lock preparation pulse $\theta$ before and after the RF saturation. In the CEST experiment, when off-resonance saturation is turned on, the magnetization undergoes oscillations as it approaches the steady state because of residual transverse magnetization in the effective frame. In the spin-lock experiment, the oscillations are suppressed because the transverse magnetization in the effective frame is zero. After the transient when off-resonance saturation is turned on, these two experiments approach the same steady state. Therefore, if the off-resonance power is turned on adiabatically in the CEST experiment, the spin-lock and CEST saturation analysis become identical.

### 1.2.4 Applications of CEST imaging as a marker of tissue pH

In normal tissue, intra and extracellular pH are highly regulated by the CO$_2$ / HCO$_3$- buffering system and by cellular ion transporters. Small dynamic alterations in tissue pH occur during normal physiological processes, such as neuronal signaling and apoptosis. In pathology, however, the acid-base homeostasis can be significantly altered. Alterations in pH balance have been reported in a wide range of pathological conditions such as renal failure, chronic obstructive pulmonary disease, ischemic heart disease, stroke, and cancer with potentially dramatic consequences. For instance, in stroke and myocardial infarction, pH is severely decreased leading to cell death and brain and cardiac injury, respectively. In cancer, alterations in pH promote tumor growth and metastasis. Because proton chemical exchange depends strongly...
on pH, CEST provides a new approach for MRI based pH measurements. Compared to pH measurements with phosphorous MR spectroscopy, CEST imaging has higher spatial resolution, higher temporal resolution and does not require specialized hardware.

1.2.4.1 Proton exchange rates vs pH

The seminal paper on CEST imaging by Ward et al. (1) demonstrated the pH dependence of the CEST effect in various phantoms. In 5,6-dihydrouracil phantoms, the amplitude of the CEST peak at 2.67 ppm increased with pH from 5.0 to 7.0 while in barbituric acid phantoms, the amplitude of the CEST peak at 5.00 ppm decreased with pH increasing from 7.0 to 8.0. These results first underscored the potential for CEST imaging to map the distribution of pH in biological systems.

The pH dependence of the CEST effect is the result of the pH dependence of the chemical exchange rate (6) of labile protons. Theoretically, the proton exchange rate in aqueous solution is given by (51, 84):

\[
k_{sw} = k_a[H^+] + k_b[OH^-] + k_o = k_a10^{-pH} + k_b10^{pH-pK_w} + k_o
\]

where \(k_a\) is the acid catalyzed exchange rate, \(k_b\) is the base catalyzed exchange rate, and \(k_o\) denotes spontaneous exchange. Most endogenous exchangeable protons are predominantly base-catalyzed under physiological conditions (6.5<pH<7.5), as seen in Figure 1-14 (51). Therefore, the pH dependence of proton exchange rate in-vivo reduces to:

\[
k_{sw} = k_b10^{pH-pK_w}
\]

where \(pK_w=15.4\) at \(37^\circ C\) (85) is the negative logarithm of the dissociation constant of water. This means that higher pH increases proton exchange rates. Given a series of exchange rate measurements at known pH, \(k_b\) can be derived for specific exchangeable resonances.

Figure 1-14: Exchange rate constant \(k_{sw}\) for labile protons of polypeptide chains in H\(_2\)O solution at 25\(^\circ\)C. The individual curves are identified with the proton Reprinted from (86) with permission from Elsevier.
In order to generate significant CEST contrast, the exchangeable protons must be rapidly and selectively saturated, must exchange quickly with water and must remain on the water long enough to allow for large signal enhancement. Therefore, the proton chemical exchange rate \( k_{sw} \) should be as high as possible while remaining slow on the NMR time scale, i.e. \( |\omega_0 - \omega_{0w}| \gg k_{sw} \).

Endogenous molecules and proteins contain several types of exchangeable protons with a wide range of exchange rates, as seen in Figure 1-14. At physiological conditions, amine protons and hydroxyl protons from small molecules and amino acid side have fast chemical exchange rates on the order of 1,000 to 30,000 Hz (27, 87). Amine protons resonate primarily between 2 to 3 ppm from the water resonance and hydroxyl protons between 0.5 to 2 ppm, putting them in the intermediate to fast exchange regime at clinical MRI field strengths (51). Amide protons on the backbone of peptides and proteins have much slower exchange rates, typically less than 100Hz (88-93). The amide resonance is centered at 3.5 ppm, which puts them in the slow exchange regime. Therefore, CEST imaging of amide protons, known as amide proton transfer (APT) (53), is a good candidate for an endogenous marker of intracellular pH.

1.2.4.2 Review of endogenous CEST pH weighted measurements

In 2003, Zhou et al (53) proposed using the CEST signal from amide protons on the backbone of endogenous proteins and peptides as a marker of tissue pH. They reported a decrease of 1.90% in the saturation transfer signal at 3.5ppm measured in the normal rat brain compared to the postmortem brain. The corresponding amide proton exchange rates were 28.6±7.4Hz and 10.1±2.6Hz, measured by WEX spectroscopy and the pH values were 7.11±0.13 at normocapnia and 6.66±0.10 postmortem measured by phosphorus spectroscopy. Given these measurements the following amide proton transfer ratio (APTR) pH calibration was derived (53):

\[
APTR = \frac{k_{sw}[\text{amide}]}{2[H_2O]R_{lw}} \left[ 1 - e^{-R_{lw}t_{sat}} \right] \]

\[
= k_{b} \times 10^{pH-pKw} \frac{[\text{amide}]}{2[H_2O]R_{lw}} \left[ 1 - e^{-R_{lw}t_{sat}} \right] \]

\[1.54\]

where \( R_{lw}=0.714 \text{Hz} \) at 4.7T is the longitudinal relaxation rate and \( T_{sat}=4s \) is the saturation time. \( pH = 15.4 \) is the pH value of water. The computed amide proton concentration was 71.9mM. Eq. [1.54] is derived from Eq. [1.31] assuming that amide protons are fully saturated \((\alpha=1)\) and \( k_{we} << R_{lw} \). In order to calibrate the parameters in Eq. [1.54] several other assumptions are made including that there is no back exchange from water to amide and that the amide proton concentration, water content of tissue and water longitudinal relaxation rate remain unchanged with ischemia. If these assumptions are met, APTR is then a simple function of pH. In the same paper, pH imaging of permanent ischemia was performed in a rat model following middle cerebral artery occlusion (MCAO). The infarct in the caudate nucleus was detectable on the MTRasym(3.5ppm) image and the quantitative pH image, in good agreement with the diffusion-weighted image. These changes were seen before any changes were detectable on the T2-
weighted image, demonstrating the potential for CEST imaging of amide protons to detect acute pH changes with possible applications to clinical stroke imaging.

Follow up APT studies in MCAO rat models have also shown that saturation transfer contrast at the amide proton frequency decreases in the ischemic lesion (8-15), and that it correlates with pH (10, 14) and lactate content (15). In addition, it has been proposed that CEST imaging could help identify regions within the diffusion-perfusion mismatch area that correspond to the ischemic penumbra and distinguish it from benign oligemia (8, 9). Several studies have shown a mismatch in the lesion area detected with saturation transfer imaging compared to perfusion and diffusion weighted images: pH alterations on the MTR$_{sym}$(3.5ppm) image were always larger or equal to the diffusion abnormality but smaller than the perfusion abnormality (8, 9, 12). In addition, the lesion detected by saturation transfer imaging in the acute phase coincided with the final infarct area seen on T2-weighted imaging at 24 hours post MCAO (8). Another study with shorter MCAO time reported that in the recovery period post-occlusion the saturation transfer signal remained decreased in tissue that ultimately progressed to infarction, while perfusion and diffusion signals recovered (11). Therefore, saturation transfer imaging could potentially help differentiate the ischemic penumbra, characterized by hypoperfusion and decreased pH, from regions with benign oligemia, characterized by hypoperfusion without pH abnormalities, in acute stroke imaging (8). As a result, CEST imaging could become an important marker to predict progression to infarction in clinical stroke imaging.

The first CEST images of human stroke patients were published recently (25). Even though patients were scanned in the subacute phase, an average of 4 days after the stroke onset, the saturation transfer signal was still decreased in the infarct area compared to the contralateral normal appearing brain tissue. An example of saturation transfer imaging at the amide proton frequency in clinical stroke patients can be seen in Figure 1-15.
1.2.4.3 Limitations

Despite the potential for CEST imaging to measure pH, most studies have focused on measuring pH-weighted saturation transfer images rather than quantitative pH maps. Several needs must be addressed before quantitative pH imaging becomes feasible, including accurately measuring proton exchange rates and distinguishing changes in proton exchange from other effects.

Separating changes in proton exchange rates from changes in other MR parameters is one of the major challenges to obtaining accurate pH measurements in-vivo. The signal measured in vivo during a CEST experiment is a combination of the CEST signal and the MT signal, given by Eq. [1.44]:

\[ MTR_{asym}(\omega_s) = MTR'_{asym}(\omega_s) + PTR(\omega_s) \]

The proton transfer ratio is pH dependent, as seen in equation [1.54] while \( MTR'_{asym} \) is usually assumed to be pH independent. A typical saturation transfer experiment acquires \( MTR_{asym} \) maps, which are pH-weighted, but are not suitable to obtain quantitative pH measurements since the PTR cannot be extracted. Therefore, the first step in quantifying pH in-vivo will be the development of acquisition techniques that can separate PTR from \( MTR'_{asym} \) in order to obtain pure CEST images. Once the CEST effect can be separated from the macromolecular MT effect.
and PTR can be accurately measured, the contribution to changes in PTR from changes in exchange rates and other parameters will have to be evaluated. PTR is a function of multiple parameters other than the proton exchange rate including the concentration of exchangeable protons, water content, water relaxivity and RF irradiation duration and power. While it has previously been assumed that these parameters remain unchanged during ischemia, the second step in accurately quantifying pH, will be to separate changes in the PTR related to the proton exchange rate from these other effects. This will require the careful modeling of the CEST signal intensity using the equations presented in Section 1.2.2.

Third, in order to calibrate the relationship between the PTR and pH for human imaging, the proton exchange rate $k_{sw}$ must be measured. This can be achieved in-vitro by water exchange (WEX) spectroscopy where the exchange rate can be estimated from the mixing-time evolution of the exchangeable proton peak using a two site exchange model (4, 64):

$$S_s(t) = \frac{k_{sw}S_{0s}}{k_{sw} + R_{s} - R_{sw}} \left[ e^{-R_{sw}t} - e^{(k_{sw} + R_{s})t} \right]$$

where $S_s$ is the exchangeable proton peak amplitude, $R_1$ is the longitudinal relaxation rate, and $t_m$ is the mixing time. The WEX approach, however, is time consuming and not suitable for measurement of fast exchange rates because of signal loss due to line broadening. Therefore, for in-vivo human imaging a different approach is necessary. Alternative CEST based measurements of the proton exchange rates have been developed where the exchange rates are measured through the water resonance by exploiting the effect of exchange rate on signal intensity as a function of saturation time or saturation power. These methods include the quantification of exchange as a function of saturation time (QUEST) and saturation power (QUESP) (94) techniques, the measurement of the optimal RF power with numerical fitting of the corresponding exchange rate (77), omega plots (95) and QUEST with ratiometric analysis (QUESTRA) (96). While these methods work well in phantoms, they cannot be applied in-vivo because of the presence of additional saturation transfer from broad macromolecular MT effects. Again, acquisition methods that separate the CEST signal from the MT signal will be needed for measuring endogenous proton exchange rates and quantifying pH.

Finally, the validity of the model for the pH dependence on exchange rates will have to be addressed. While the fast base catalysis model (Eq. [1.53]) is valid for exchangeable protons from small molecules with no secondary structure in solution, proton exchange in-vivo is much more complicated. In-vivo the exchange rates are modulated by a number of factors including pH, temperature, the concentration of exchange catalysts such as phosphate and bicarbonate, and the presence of negative and positive charges on proteins and membranes (27, 97). Moreover, exchangeable protons from amino acid side chains and backbone amide protons inside proteins may have their exchange modulated by hydrogen bonding, which stabilizes amide protons and decreases exchange rates by several orders of magnitude, and also by the protein primary structure and secondary structure, which may limit water accessibility to many residues (27, 88, 90, 98, 99). As a result, exchange rates of amide protons in proteins can be several orders of magnitude slower than in unstructured polypeptides. Given these additional factors, the exponential dependence of exchange rates on pH given in Eq. [1.53] may not hold for in-vivo imaging and alternate models may have to be considered.
1.2.5 Applications of CEST imaging as a marker of protein and metabolite concentration

As previously discussed in Section 1.2.2, the magnitude of the CEST effect described by the proton transfer ratio is proportional to the concentration of exchangeable protons. This can be exploited for the design of contrast agents that can be turned on and off by the application of the RF irradiation or to detect physiological and pathological changes in cellular density, and in protein and metabolite concentration via endogenous exchangeable protons. Endogenous CEST imaging of altered molecular and protein concentration may become a useful diagnostic marker in a wide range of body, musculoskeletal and brain imaging applications.

1.2.5.1 Review of CEST concentration measurements of small molecules and metabolites

Several studies have demonstrated the feasibility of in-vivo CEST imaging of small molecules and metabolites in various tissues. In body imaging applications, it has been shown that glycogen concentration in the liver can be assessed via hydroxyl proton exchange at 0.5-1.5ppm (52). Glycogen is the body's primary storage form of glucose, which is accessed to replenish blood sugar levels as needed. Therefore, CEST imaging of glycogen could have applications for the study of metabolism in conditions such as insulin resistance, type 2 diabetes and obesity. In musculoskeletal imaging applications, the concentration of glycosaminoglycans in cartilage has been assessed via hydroxyl proton exchange at 0.9-1.9ppm in knee articular cartilage (e.g. Figure 1-16) and intravertebral discs (16-19). Glycosaminoglycans are a major component of cartilage, where they act as a lubricant. The glycosaminoglycan content of cartilage decreases in osteoarthritis and is proportional to the severity of the disease, therefore CEST imaging of glycosaminoglycans could have applications for the study and diagnosis of the early stages of osteoarthritis and for the assessment of cartilage repair therapies (18).
In brain imaging application, several brain metabolites have been the subject of saturation transfer studies in healthy volunteers. CEST imaging of altered metabolite concentration could find application in a number of neurologic and psychiatric disorders. Hydroxyl proton exchange at 0.6ppm has been evaluated as a marker for the concentration of myo-inositol in the brain (100). The chemical exchange rate of the myo-inositol exchangeable hydroxyl proton was estimated to be 600Hz (100). Myo-inositol is one of the most abundant brain metabolites. It is located primarily in glial cells and its concentration is altered in systemic diseases such as type 2 diabetes (101) and systemic lupus erythematosus (102), in CNS disorders such as Alzheimer's disease (103) and in psychiatric disorders such as bipolar disorder (104). Amine proton exchange at 3ppm has been evaluated as a marker for the concentration of glutamate in the brain (105, 106). The chemical exchange rate of the glutamate exchangeable amine proton was estimated to be 5,500±500Hz (105). Glutamate is the primary excitatory neurotransmitter in the brain and is involved in almost all aspects of normal and pathological brain activity. Excessive glutamate signaling and its accumulation in cells is thought to be a direct cause of cell death and brain injury in a number of disorders such as stroke and multiple sclerosis (for reviews see (107, 108)). The amine proton of creatine at 1.9ppm has also been evaluated as a CEST marker in phantom studies. It was shown that CEST contrast could be generated from free creatine with no contamination from phosphocreatine at physiologic conditions (109).
Finally, a CEST reporter gene that encodes an artificial lysine-rich protein resulting in large concentration of exchangeable amide protons (110) has been designed for the study of gene expression and cell tracking applications. In that study, glioma cells were genetically modified to express the gene for the lysine rich protein, thereby generating an increased amide CEST signal compared to wild-type glioma cells, allowing differentiating between the genetically modified and wild-type implanted cells (Figure 1-17).

![Figure 1-17: CEST imaging of a reporter gene that encodes an artificial lysine rich protein yields a large saturation transfer signal from amide protons at 3.5ppm. a) anatomical image, b) MTR asym map overlaid on the anatomical image distinguishes the protein-expressing xenografts from control xenografts. Note that the anatomical image shows no contrast enhancement, thereby illustrating how the CEST signal can be turned on and off by the application of the RF irradiation pulse. Reprinted by permission from MacMillan Publishers Ltd: Nature Biotechnology (110), copyright 2007.](image)

1.2.5.2 Review of CEST concentration measurements of mobile proteins and peptides

Amide protons are found primarily on the backbone of proteins and peptides. Therefore, the CEST signal from amide protons is thought to be a marker of the protein content inside cells, although it is not yet clear which specific proteins in which compartments contribute to the signal. Nevertheless, this property of CEST imaging has been exploited for the study of tumors, where it is presumed that an increase in the protein content in tumors compared to healthy tissue leads to an increase in the saturation transfer signal (5).

Initial studies on implanted 9L gliosarcoma tumors and human glioblastoma tumors in rat brain showed that MTR asym is elevated in tumors compared to the normal brain (5, 111). In addition, the tumor boundaries were better delineated on the saturation transfer images than on conventional T1-weighted, T2-weighted and diffusion weighted MR images where the tumors appeared diffuse. When taking the difference between MTR asym in the tumor and in the contralateral brain, ΔMTR asym peaked at about 3 ppm and remained somewhat elevated at the largest measured offset of 6 ppm. In contrast, ΔMTR asym between the peritumoral tissue and the contralateral brain tissue was zero. Together, these results indicate that saturation transfer
imaging may be able to differentiate between cancerous regions and edema without the use of injectable contrast agents.

Follow up studies in human brain tumors confirmed the initial animal results. MTR\textsubscript{sym} was elevated in tumors compared to contralateral brain (23) with the highest CEST signal measured in the most solid region of the tumors (20) (e.g. Figure 1-18). The images successfully delineated active tumor from edema and from necrotic tumor core (Figure 1-19), with borders similar to the gadolinium enhanced scan (20, 23-26). In addition, the MTR\textsubscript{sym} images appear to correlate with tumor grade, as only high grade tumors were hyperintense while low grade tumors were indistinguishable from the normal brain (20).

Figure 1-18: CEST imaging of brain tumor patients. a) z-spectra, b) MTR\textsubscript{sym} and c) \( \Delta \)MTR\textsubscript{sym} measured in brain tumor patients (n=8) with RF saturation power \( B_1 = 2 \mu T \). The MTR\textsubscript{sym}(3.5ppm) value for the CNAWM is approximately zero for 2\( \mu T \). The \( \Delta \)MTR\textsubscript{sym} spectrum is maximized at offsets of about 3-4ppm with respect to the water resonance. (d-g) Conventional and CEST MR images of a patient with lung cancer metastasis. The tumor (red arrow) is hyperintense on the saturation transfer images (APT\textsubscript{w}). Reprinted from (25) with permission from Wiley.
Figure 1-19: CEST imaging of a patient with high grade recurrent astrocytoma (grade III), acquired four months after treatment. a) T2-weighted image shows the recurrence of glioma with heterogeneous hyperintensity in the left parietal lobe. b) T1-weighted images show a heterogeneously hypointense lesion. The exact location of the tumor core is not clear. c) Post-contrast T1-weighted image reveals a gadolinium-enhancing tumor core (red arrow) and a necrotic area (pink arrow). The recurrent tumor is associated with surrounding edema and mass effect. d) Saturation transfer images show that there is a clear increase in $\text{MTR}_{\text{asym}}(3.5\text{ppm})$ in the tumor core, identified by the Gd-enhanced T1-weighted image. The regions of necrosis (pink arrow) and edema (orange arrow) are almost isointense on $\text{MTR}_{\text{asym}}(3.5\text{ppm})$ images. Reprinted from (24) with permission from Elsevier.

CEST imaging of brain tumors has also shown potential to differentiate between tumor recurrence and radiation necrosis (112, 113), which otherwise can appear identical on radiological images. MTR$_{\text{asym}}$ signal was decreased in regions undergoing radiation necrosis but was increased in regions with active tumor (Figure 1-20). In contrast, radiation necrosis, 9L gliosarcoma tumors and glioma tumors were all hyperintense on conventional T2-weighted imaging and Gd-enhanced imaging. Therefore, CEST imaging may have the potential to provide an early imaging biomarker to predict treatment response to radiation therapy.
Finally, more recently, CEST imaging has also been applied to the study of extracranial tumors. In orthotopic models of lung cancer in mice, $\text{MTR}_{\text{asym}}$ was higher in Lewis lung carcinoma than in human lung adenocarcinoma (22). Lewis lung carcinoma is a highly malignant cancer with a more aggressive progression in mice than human lung adenocarcinoma (114, 115). In human prostate cancer, $\text{MTR}_{\text{asym}}$ was elevated in tumors compared to benign regions, with good correlation to histology (21). $\text{MTR}_{\text{asym}}$ was higher in peripheral zone tumors than in transition zone tumors and in stage T3 tumors than stage T2 tumors. In human breast cancer, CEST imaging was performed before and after one cycle of neoadjuvant chemotherapy to evaluate the potential of CEST imaging as a marker of treatment response (116). It was found that the APTR decreased in a patient that went on to be a complete responder at the end of treatment while it increased in a patient who went on to have progressive disease. Together, these results suggest that CEST imaging may have the potential to improve the localization of tumors, and predict tumor stage, aggressiveness and treatment response in a wide variety of cancers.
1.2.5.3 Limitations

The major limitation in performing CEST imaging of metabolites, small molecules and proteins is related to the lack of specificity of off-resonance saturation transfer imaging. This includes contamination to the CEST signal of interest from broad macromolecular MT asymmetry but also from other exchangeable protons.

In cancer, it has been assumed that the saturation transfer hyperintensities seen at 3.5ppm in brain tumors are caused by an increase signal from amide protons due to an increase in protein concentration. However, other effects can contribute to the change in the amplitude of the saturation transfer signal. As discussed in Sections 1.2.2.3 and 1.2.4.3, the total saturation transfer signal \( MTR_{\text{asym}} \) measured in-vivo is a combination of the intrinsic \( MTR'_{\text{asym}} \) of the broad macromolecular resonance and on the \( MTR_{\text{asym}} \) from the exchangeable protons of interest (PTR), given by Eq. \[1.44\]:

\[
MTR_{\text{asym}}(\omega) = MTR'_{\text{asym}}(\omega) + PTR(\omega)
\]

The change in the measured saturation transfer signal between lesions and normal appearing tissue is thus the combination of the change in \( MTR'_{\text{asym}} \) and in PTR:

\[
\Delta MTR_{\text{asym}}(\omega) = \Delta MTR'_{\text{asym}}(\omega) + \Delta PTR(\omega)
\] \[1.56\]

In previous studies in brain tumors, it has been assumed that the increased saturation transfer signal is the result of an increase in PTR due to a higher protein content in tumors, while \( \Delta MTR'_{\text{asym}} \) is assumed to be zero. However, this assumption has not been verified and an increase in \( \Delta MTR'_{\text{asym}} \), corresponding to a reduction in the amplitude of the negative MT asymmetry, would also result in increased saturation transfer signal. In addition, changes in PTR can be due to a number of factors including increased protein concentration but also increased intracellular pH in the tumor and changes in the water proton pool parameters such as longitudinal relaxation rate and water concentration. Therefore, acquisition methods that can separate the MT asymmetry from the CEST signal and improved quantification of the different parameters contributing to the CEST signal are needed to assess and understand the cause of the saturation transfer signal change in cancer.

1.2.6 Technical challenges

CEST imaging suffers from too many sources of errors in its current form for clinical translation. The RF irradiation needed to saturate amide protons also induces direct water saturation and macromolecular magnetization transfer. These effects both decrease the water signal, along with the CEST effect, thereby confounding the measurement. The standard method employed in the literature to separate the CEST signal from direct water saturation and MT contrast is MT ratio asymmetry (MTR\(_{\text{asym}}\)) analysis, where the CEST image acquired with RF irradiation at the exchangeable proton frequency \( \omega \) is subtracted from a control image acquired at the opposite frequency, as described in Section 1.2.2. Asymmetry analysis, however, introduces further sources of errors. First, because asymmetry analysis relies on the water frequency being centered, it leads to severe artifacts in the presence of magnetic field inhomogeneity that occurs in-vivo. Second, because the center of symmetry of the macromolecular MT line is shifted with respect to the water line (54, 73, 117), MT does not completely cancel in the asymmetry subtraction. Instead, it produces a negative asymmetry that cancels the CEST signal leading to additional errors in the CEST measurement. Third, saturation
peaks attributed to aliphatic protons are present in a frequency range from approximately -1 ppm to -5 ppm. They also add a negative contribution to the asymmetry, further canceling the CEST signal (4, 16, 50, 51, 53, 82, 118-122).

Errors induced by B₀ inhomogeneity can be corrected before asymmetry analysis. This is typically accomplished by acquiring a z-spectrum and recentering the water line before performing the asymmetry subtraction. Because the z-spectrum involves acquiring many images with a broad range of RF irradiation frequencies (20-40 offsets), this method increases scan time beyond what is suitable for clinical application. Several alternative B₀ correction methods have been developed to reduce scan time. These include variations on the z-spectrum method (20, 24, 123), multiple offset acquisitions with additional B₀ mapping (124, 125), and fitting of an analytical model (126). These approaches all have relatively long scan times and do not address errors induced by MT asymmetry. Recently, sophisticated alternative acquisition methods, such as frequency labeled exchange transfer (FLEX) (127), two frequency RF irradiation (128), CEST phase mapping using a length and offset varied saturation scheme (LOVARS) (129) or chemical exchange rotation transfer (CERT) (130, 131) have been proposed to separate the amide proton signal from MT asymmetry.

1.3 Goals and organization

My goals for this thesis were to develop methods for fast CEST imaging, with improved robustness to magnetic field inhomogeneity and MT asymmetry, and apply them to assess the different contributions to CEST contrast in brain tumors. In this chapter, I have reviewed the theoretical aspects of off-resonance saturation transfer imaging, along with its applications and challenges. In Chapter 2, I introduce a new CEST acquisition, dubbed Saturation with Frequency Alternating RF irradiation (SAFARI) and demonstrate its utility in removing errors due to B₀ inhomogeneity and MT asymmetry in healthy volunteers. In Chapter 3, I propose a continuous wave (CW) variant of the SAFARI acquisition and apply it to obtain quantitative measurements of the amide proton exchange rate and concentration in the healthy brain. In Chapter 4, I describe work in which the different contributions to the off-resonance saturation transfer contrast in brain tumors was assessed with CEST and SAFARI imaging. Finally, in Chapter 5, I describe next steps that must be taken to further the understanding of CEST imaging in cancer and conclude by discussing promising applications for SAFARI imaging in other diseases.
Chapter 2: Amide proton transfer imaging with improved robustness to magnetic field inhomogeneity and magnetization transfer asymmetry using Saturation with Frequency Alternating RF Irradiation (SAFARI)

Abstract

Amide proton transfer (APT) imaging has shown promise as an indicator of tissue pH and as a marker for brain tumors. Sources of error in APT measurements include direct water saturation, and magnetization transfer (MT) from membranes and macromolecules. These are typically suppressed by post-processing asymmetry analysis. However, this approach is strongly dependent on $B_0$ homogeneity and can introduce additional errors due to intrinsic MT asymmetry, aliphatic proton features opposite the amide peak, and radiation damping-induced asymmetry. Although several methods exist to correct for $B_0$ inhomogeneity, they tremendously increase scan times and do not address errors induced by asymmetry of the z-spectrum. In this chapter, a novel saturation scheme - saturation with frequency alternating RF irradiation (SAFARI) - is proposed in combination with a new magnetization transfer ratio (MTR) parameter designed to generate APT images insensitive to direct water saturation and MT, even in the presence of $B_0$ inhomogeneity. The feasibility of the SAFARI technique is demonstrated in phantoms and in the human brain. Experimental results show that SAFARI successfully removes direct water saturation and MT contamination from APT images. It is insensitive to $B_0$ offsets up to 180Hz without using additional $B_0$ correction, thereby dramatically reducing scanning time.

This chapter was previously published as:
2.1 Introduction

Chemical Exchange Saturation Transfer (CEST) imaging (1, 2, 49) is a method to detect molecules with exchangeable protons by saturating the exchangeable resonance and then observing the effects of the saturation transferred to water by exchange. This technique is of interest both for detecting particular molecules or types of molecules and also as a measure of pH, since the exchange rate of protons is often pH sensitive (6, 50). Among the stronger in-vivo contributions to CEST contrast are hydroxyl protons at approximately 1 ppm above water, that have been used to assess glycogen (52) and glycosaminoglycans (16) in tissues where these molecules dominate, rapidly exchanging amine protons of amino acids and other molecules between 2-3 ppm above water, and the more slowly exchanging amides on the backbone of proteins, at approximately 3.5 ppm. Because amide protons have a larger chemical shift and their slow exchange permits saturation at power levels more compatible with human applications, amide proton transfer (APT) imaging (4, 53) is particularly attractive for pH measurement with endogenous CEST contrast.

At pH values occurring in-vivo, the chemical exchange of amide protons is base-catalyzed (84), therefore APT contrast decreases with decreasing intracellular pH. This has been exploited for the study of acute cerebral ischemia (8-12, 53, 64). It has been shown that the APT contrast correlates with pH (10, 53) and may help distinguish benign oligemia from the ischemic penumbra (8). As a result, APT imaging could become an important marker to predict progression to infarction in stroke.

In addition to being sensitive to pH, APT contrast is also related to the protein content inside cells, although it is not yet clear which specific proteins in which compartments contribute to the signal. Nevertheless, this property of APT imaging has been exploited for the study of tumors (5, 20, 23, 111, 132, 133), multiple sclerosis (134, 135) and to design a CEST reporter gene to map a specific gene expression pattern (110). In cancer imaging, APT imaging appears to correlate with tumor grade (20) and may distinguish active tumor regions from edema and normal-appearing white matter (23), as well as from necrotic tumor cores (24, 111). APT imaging has also shown potential to differentiate between tumor recurrence and radiation necrosis (133), which otherwise appear identical on radiological images.

Although, APT imaging has emerged as a new source of MRI contrast with several promising clinical applications, including stroke and cancer imaging, it has so far been limited to research use due to the difficulty of measuring the APT effect in-vivo without errors. The RF irradiation needed to generate APT contrast also induces direct water saturation and magnetization transfer (MT) from semisolid protons in membranes and macromolecules. These effects both decrease the signal of the APT-weighted image, confounding the APT measurement. The standard method to correct for direct water saturation and MT is MT ratio asymmetry (MTR_{asym}) analysis, where the APT image is subtracted from a control image acquired with RF irradiation at the frequency opposite the amide protons with respect to the water line. This method, however, is dependent on the water frequency being centered and leads to severe artifacts when B_0 is not homogeneous, as occurs in-vivo.
Errors induced by B₀ inhomogeneity can be corrected to generate APT maps free of B₀ artifacts. This is typically accomplished by acquiring a z-spectrum and recentering the water line before performing the asymmetry subtraction. Because the z-spectrum involves acquiring many images with a range of RF irradiation frequencies (20-40 offsets), this method increases scan time beyond what is suitable for clinical application. Several alternative B₀ correction methods have been developed to reduce scan time. These include variations on the z-spectrum method (20, 24, 123), multiple offset acquisitions with additional B₀ mapping (124, 125), and fitting of an analytical model (126). Yet, even with B₀ correction asymmetry analysis introduces additional errors in the APT measurement due to asymmetries in the z-spectrum.

Several sources of asymmetry of the z-spectrum – other than amide protons – contribute to the MTRₘₐₜ measurement, introducing errors in the quantification of the amide proton signal. First, aliphatic protons that have resonances in the range from -1.3ppm to -4.8ppm (4, 53) can decrease the signal of the control image by saturation transfer. At low RF power, this tends to cancel out the amide proton signal in the MTRₘₐₜ calculation (136). Second, probe detuning can cause radiation damping-induced asymmetry of the z-spectrum (137) introducing additional errors in MTRₘₐₜ. Third, the center of symmetry of the MT line is shifted with respect to the water line (54, 73, 117) and, therefore, does not completely cancel in the asymmetry subtraction. Again, this will cancel out the amide proton signal leading to MTRₘₐₜ values near zero or negative. Recently, an approximate analytical model (138), and sophisticated alternative acquisition methods (127, 139) have been proposed to separate the amide proton signal from MT.

In this chapter, we propose a novel APT acquisition method using saturation with frequency alternating RF irradiation (SAFARI) along with a new magnetization transfer ratio (MTRₘₐₜ) to remove direct water saturation and MT from APT images without additional B₀ correction. This technique was first tested in phantoms including a control relaxation phantom without chemical exchange in order to establish that SAFARI does not introduce contrast based on T₂ rather than CEST, and a bovine serum albumin protein phantom to test the sensitivity of SAFARI to amide proton exchange and aliphatic proton contamination. The phantoms were also imaged with increasing RF frequency offsets to test the range at which MTRₘₐₜ remains insensitive to B₀ shifts while still saturating amide protons. The performance of APT-SAFARI in the presence of CEST and MT was then evaluated in a set of experiments performed in humans. High Signal-to-Noise Ratio (SNR) APT images of the brain were acquired to compare MTRₘₜ and MTRₘₐₜ maps without B₀ correction. Lower SNR APT images were also acquired at 51 frequency offsets, akin to a z-spectrum, in order to compare both the B₀ and MT sensitivity of MTRₘₜ with B₀ correction and MTRₘₐₜ without B₀ correction. Finally, the sensitivity of MTRₘₐₜ to chemical exchange was studied by varying the average saturation power, similar to the “quantification of exchange as a function of saturation power” (QUESP) technique (94).
2.2 Theory

The key to optimizing APT contrast is achieving full amide proton saturation while minimizing the effects of direct water saturation and MT. We propose a novel scheme to produce amide proton transfer contrast based on saturation with frequency alternating RF irradiation (SAFARI). The APT-SAFARI technique relies on the non-linearity of the saturation process: once the amide proton line has been fully saturated, increasing the RF irradiation power should not affect the saturation significantly. Therefore, if the applied RF power is sufficient to produce complete amide proton saturation, the APT effect becomes independent of power. The broad macromolecular line and the water line, however, are not fully saturated. As a result, the effect of direct water saturation and MT vary mostly linearly with power. The APT-SAFARI acquisition and post-processing method exploits this different power dependence of the amide proton line compared to the water line to cancel out the effects of MT and direct water saturation, even in the presence of B₀ inhomogeneity and MT asymmetry, as described below.

The SAFARI image is acquired with RF irradiation applied with equal power at both the control (-3.5ppm) and label (+3.5ppm) frequencies. This is achieved within a pulsed-RF irradiation module by alternating every other saturation pulse between the two frequencies. If the total RF power applied at 3.5ppm is sufficient, the amide proton line will be fully saturated. Therefore, the signal in the SAFARI image can be modeled as a combination of the chemical exchange saturation transfer (CEST), as well as signal loss due to direct water saturation (W) and magnetization transfer (MT) from both off-resonance frequencies:

$$S_{\text{sat}}(\text{SAFARI}) = S_0 - CEST - P \cdot W(\omega_s + \delta_{B0}) - P \cdot W(-\omega_s + \delta_{B0})$$

$$- P \cdot MT(\omega_s + \delta_{MT} + \delta_{B0}) - P \cdot MT(-\omega_s + \delta_{MT} + \delta_{B0})$$

[2.1]

where $S_0$ is the unsaturated reference signal intensity, $\delta_{B0}$ is the B₀ inhomogeneity, $\delta_{MT}$ is the center of MT asymmetry, $\omega_s$=3.5ppm is the amide proton frequency and P represents the RF irradiation power. In the presence of B₀ inhomogeneity, such as susceptibility effects in-vivo, the water line is shifted and direct water saturation is no longer symmetric around 0ppm, taken into account by the $\delta_{B0}$ variable. The CEST effect, however, is independent of $\delta_{B0}$ as long as the RF saturation bandwidth is broad enough to maintain full amide proton saturation despite the shift of the line. The center of MT symmetry is known to be intrinsically shifted with respect to the water line, and is represented here by $\delta_{MT}$. The SAFARI image, therefore, contains the individual asymmetric effects of direct water saturation and MT at both the positive and negative saturation frequencies.

In addition, we acquire the standard CEST image with RF irradiation of the amide proton line only. This is achieved by applying all of the RF pulses of the saturation module at the label frequency only. As a result, the total RF power deposited on the amide proton line is doubled compared to the SAFARI image. Consequently, the MT and direct water saturation effects double, while the APT effect does not change:
Similarly, the signal in the control image with all RF pulses applied at the control frequency only can be modeled as:

\[ S_{sat}(-\omega_s) = S_0 - 2P \ast W(\omega_s + \delta_B) - 2P \ast MT(\omega_s + \delta_{MT} + \delta_B) \]  \[ \text{[2.3]} \]

The signal amplitudes resulting from the three different saturation preparations of the APT-SAFARI scan are illustrated in Figure 2-1.

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**Figure 2-1:** Schematic illustration of the APT-SAFARI experiment. The spectral line represents the free water signal. W, MT and CEST are the signal loss attributed to direct water saturation, conventional magnetization transfer and amide proton transfer, respectively. The plus and minus subscripts indicate the effects induced by RF saturation applied at the amide proton frequency \((\omega_s=+3.5\text{ppm})\) and the opposite frequency \((-\omega_s)\), respectively. For clarity, W is depicted to be symmetric around the water line, while MT is not. Because of the MT asymmetry, the standard two-offset CEST difference \(MTR_{asym} = [S_{sat}(-\omega_s) - S_{sat}(+\omega_s)]/S_0\) is not equal to the CEST effect. The SAFARI image with half the saturation power applied simultaneously at the control and label frequencies contains the contributions of W and MT at both frequencies, as well as signal decrease due to APT. Therefore, the difference \(MTR_{SAFARI} = [S_{sat}(-\omega_s) + S_{sat}(+\omega_s) - 2S_{sat}(SAFARI)]/S_0\) isolates the CEST effect without contamination from \(B_0\) inhomogeneity and asymmetric MT.

The common approach for separating CEST contrast from other effects is asymmetry analysis, where the reference image is subtracted from the CEST image. The contrast generated by standard asymmetry analysis is given by \((\text{[2.3]} - \text{[2.2]})\):
\[ MTR_{asym} = MTR(\omega_p) - MTR(-\omega_p) \]
\[ = \left[1 - S_{sat}(\omega_p)/S_0\right] - \left[1 - S_{sat}(-\omega_p)/S_0\right] \]
\[ \propto S_{sat}(-\omega_p) - S_{sat}(\omega_p) \]
\[ = CEST + 2P*\Delta MT + 2P*\Delta W \]

MTR\textsubscript{asym} fails to isolate the CEST contrast because direct water saturation and MT are not symmetric around 0ppm. Instead of the standard asymmetry analysis, we employ a three-way subtraction where the SAFARI image is doubled and subtracted from the sum of the positive and negative frequency images ([2.2]+[2.3]-2[2.1]):
\[ MTR_{SAFARI} = 2* MTR(SAFARI) - MTR(+\omega_p) - MTR(-\omega_p) \]
\[ = 2*\left[1 - S_{sat}(SAFARI)/S_0\right] - \left[1 - S_{sat}(\omega_p)/S_0\right] - \left[1 - S_{sat}(-\omega_p)/S_0\right] \]
\[ \propto S_{sat}(\omega_p) + S_{sat}(-\omega_p) - 2S_{sat}(SAFARI) \]
\[ = CEST \]

As a result, both MT and direct water saturation cancel out exactly, even in the presence of B\textsubscript{0} inhomogeneity and MT asymmetry.

2.3 Methods

2.3.1 APT Pulse sequence design

The APT-SAFARI imaging pulse sequence is shown in Figure 2-2. Four images are acquired for each APT-SAFARI scan: one label image \( S_{sat}(\omega_+) \) with all saturation pulses applied at a single frequency \( \omega_+ \) (Figure 2-2a), one SAFARI image \( S_{sat}(SAFARI) \) with saturation pulses alternating between \( \omega_+ \) and \( \omega_- \) (Figure 2-2b), one control image \( S_{sat}(\omega_-) \) with all saturation pulses applied at a single frequency \( \omega_- \) (Figure 2-2c), and another SAFARI image \( S_{sat}(SAFARI') \) with saturation pulses alternating between \( \omega_- \) and \( \omega_+ \) (Figure 2-2d). Note that the first and third images constitute a standard CEST experiment. It was necessary to acquire two SAFARI images with reversed ordering of the pulses to ensure that identical RF powers were deposited on the positive and negative frequencies.

For all four images, the off-resonance pulsed-RF irradiation consisted of a 3 second train of Blackman shaped inversion pulses with pulse width (pw) = 9 ms repeated every TR\textsubscript{RF} = 15 ms while incrementing the change in phase shift linearly by 117° for spoiling of transverse magnetization (140). The corresponding continuous wave (CW) RF power is given by
\[ B_{cw} = \frac{1}{\gamma TR_{RF}} \int_{0}^{\text{pw}} B_1(t)dt = 0.78\mu T \] (74), where \( B_1(t) \) is the Blackman waveform given by the MATLAB (Mathworks, Natick MA) “blackman” function. These pulse parameters were optimized to saturate the amide protons at 3.5ppm even in the presence of B\textsubscript{0} shifts, while within the constraint of not producing strong direct water saturation (80). Crusher gradients were turned on between every RF pulse to destroy remaining transverse magnetization. The saturation module was followed by a single-shot echo-planar acquisition described below.
Figure 2-2: Pulse sequence for APT-SAFARI imaging with a pulsed off-resonance saturation module followed by a single-slice EPI readout. A 3 second train of Blackman inversion pulses (pw=9ms, TRRF=15ms, flip angle = 180°, average RF power = 0.78µT) was used. Each scan consists of acquiring four images: a) Standard CEST image with saturation at the labile proton frequency (ω+ = 3.5ppm for amide protons). b) SAFARI image with saturation alternating between the label and control frequencies. c) Standard CEST control image with saturation at the control frequency (ω- = -3.5ppm for amide protons). d) SAFARI image with saturation alternating between the control and label frequencies.
2.3.2 Phantom preparation

Control phantoms were prepared with eight different concentrations of MnCl$_2$ in water. Each solution was divided amongst four 15mm-diameter centrifuge tubes. The tubes were arrayed in a single 4 by 8 Styrofoam holder for imaging. T$_1$ and T$_2$ relaxation times have been measured previously (141). T$_1$ values range from 2000ms to 400ms and T$_2$ values range from 280 ms to 40ms. A protein phantom was prepared by dissolving 5g of bovine serum albumin (BSA, Sigma-Aldrich) in 50ml phosphate saline buffer at pH=5.5. The BSA solution was transferred to a 29.1mm-diameter tube for imaging.

2.3.3 Imaging

All studies were performed on a 3T GE SIGNA EXCITE MR system using the body coil for transmit. A standard eight-channel receive-only head array was used for signal reception for both phantom and human imaging. A single shot spin-echo echo-planar imaging (EPI) readout was used for all control phantom and human studies with TR = 4s, TE between 63 and 64 ms, FOV = 24cm, matrix size = 96x96, and slice thickness = 12mm. The BSA phantom was imaged with the same parameters, except for a FOV = 15cm and TE = 17.8 ms.

The control phantoms were imaged with the long axis of the tubes along the direction of the magnetic field. A modified z-spectrum was acquired to test the robustness of MTRSAFARI against B$_0$ inhomogeneity. Shifts in the water line due to B$_0$ offsets were mimicked by introducing an offset f to the off-resonance irradiation frequency. Instead of acquiring APT scans with off-resonance irradiation at the amide proton frequency $\omega_a/2\pi = 450$Hz, the applied frequencies were $\omega_+ = 2\pi f + \omega_a$ and $\omega_- = 2\pi f - \omega_a$, where the frequency offset f was swept from -900 Hz to +900Hz in steps of 30 Hz. Therefore, the frequency shift f simulates an offset of the water line due to a B$_0$ shift of -f. The modified z-spectrum acquisition resulted in a total of 61 APT scans (244 images). Note that for the single frequency images the data set constitutes a standard z-spectrum acquired out of order. For the SAFARI images, however, it differs from a z-spectrum because the offset f leads to RF irradiation at frequencies with increasing asymmetry around the water line, rather than symmetric frequencies with increasing offsets. Total scan time was 15 minutes for each phantom.

The BSA phantom was imaged with the long axis of the tube along the direction of the magnetic field. The amide proton resonance of BSA is located at 350Hz (see Figure 2-4b), therefore, the modified z-spectrum was acquired with applied frequencies $\omega_+ = 2\pi( f +350$Hz) and $\omega_- = 2\pi( f - 350$Hz), where the frequency offset f was swept from -350 Hz to +350Hz in steps of 14 Hz. The modified z-spectrum acquisition resulted in a total of 51 APT scans (204 images) in just under 14 minutes. Because the BSA amide resonance is 100Hz closer to the water resonance than the amide proton resonance in the brain, the experiment was also repeated with a modified RF irradiation module with pw = 12 ms repeated every TR$_{RF}$ = 20 ms to provide a more selective saturation bandwidth and reduce direct water saturation.

Human imaging was performed following a protocol approved by the institutional review board. Four healthy volunteers (2 men, 2 women; age range 23-40 years, mean age 30 years)
were scanned after giving written informed consent. For each volunteer, a single slice was located at the level of the ventricles. One unsaturated $S_0$ image was acquired for reference with 12 averages. One high SNR APT-SAFAIRi scan with 12 time-interleaved repetitions of the sequence (48 images total) was acquired with off-resonance irradiation at the amide proton frequency. Scan time was 3 minutes. In order to evaluate the robustness of MTRSAFARI against $B_0$ inhomogeneity, 3 high SNR APT-SAFAIRi scans were acquired with frequency offsets $f = 100, 150, \text{ and } 200\text{Hz}$, corresponding to RF irradiation at the frequency pairs $+550\text{Hz} / -350\text{Hz}$, $+600\text{Hz} / -300\text{Hz}$, and $+650\text{Hz} / -250\text{Hz}$, respectively. Scan time for the high SNR scans with frequency offsets was 3 minutes each for a total of 9 minutes. In addition, a lower SNR (no scan repetitions) modified z-spectrum was acquired similar to the phantom protocol. 51 APT-SAFAIRi scans (204 images) were acquired with the frequency offset $f$ swept from $-450\text{Hz}$ to $+450\text{Hz}$ in steps of 18Hz, thereby simulating $B_0$ shifts of the water line. Total scan time was just under 14 minutes.

The sensitivity of APT-SAFAIRi imaging to chemical exchange was characterized by studying the effect of saturation power on the image intensity, similarly to the QUESP technique (94). Power was modulated by changing the delay time between saturation pulses while keeping the total saturation time constant. Due to RF duty cycle limitations on the scanner, the minimum $TR_{RF}$ allowed for a 9ms pulse was 15ms. Therefore, $TR_{RF}$ could only be increased, thereby decreasing power. Seven APT-SAFAIRi scans were acquired at the amide proton frequency ($f = 0\text{Hz}$) with $TR_{RF} = 20, 30, 45, 60, 80, 100 \text{ and } 150\text{ ms}$ corresponding to a CW RF power $B_{cw} = 0.58, 0.39, 0.26, 0.19, 0.12, \text{ and } 0.08\mu\text{T}$, respectively. As $TR_{RF}$ increases from 15ms to 150ms, the number of saturation pulses applied in the 3 second saturation module decreases from 200 to 20, thereby reducing the average saturation power. Each scan was repeated 6 times to increase SNR, resulting in a total acquisition time of 11 minutes.

### 2.3.4 Image analysis

Image analysis was performed in MATLAB 7.6 (Mathworks, Natick MA). All images were normalized voxel-by-voxel by the unsaturated reference image $S_0$. For the high SNR APT-SAFAIRi scans, quantitative maps of $MTR_{asym}$ and $MTR_{SAFARI}$ were calculated voxel-by-voxel according to:

\[
MTR_{asym} = \frac{S_{sat}(\omega_-)}{S_0} - \frac{S_{sat}(\omega_+)}{S_0}
\]

\[
MTR_{SAFARI} = \frac{S_{sat}(\omega_-)}{S_0} + \frac{S_{sat}(\omega_+)}{S_0} - \left[ \frac{S_{sat}(SAFARI)}{S_0} + \frac{S_{sat}(SAFARI')}{S_0} \right]
\]

where $\omega_- = 2\pi f - \omega_s$ and $\omega_+ = 2\pi f + \omega_s$. $MTR_{asym}$ and $MTR_{SAFARI}$ were each averaged across the entire slice excluding the skull and ventricles. Results are presented as the mean MTR for each volunteer with standard deviations across the slice. In addition, the error in the APT measurement due to the offset $f$ was calculated voxel-by-voxel by error = $[MTR(f) - MTR(f = 0)] / MTR(f = 0)$, and averaged across all volunteers.

For the lower SNR modified z-spectrum scans, $B_0$ correction of the single frequency images was performed following a previously published method (53) in order to remove unintentional $B_0$ inhomogeneity shifts across the FOV before calculating $MTR_{asym}$. The single frequency images $S_{sat}(\omega_0)$ and $S_{sat}(\omega_+)$ were reordered to generate a standard z-spectrum. The z-spectrum was fit voxel-by-voxel to a $30^{th}$ order polynomial. The resulting polynomial was evaluated with a
frequency resolution of 1Hz. The frequency with the minimum fit intensity was assumed to be the water peak. A map of the water frequency deviation from 0 was generated. To correct for \( B_0 \) inhomogeneity across the FOV, the z-spectrum was interpolated by a piecewise cubic spline to a resolution of 1Hz and shifted by the water frequency deviation such that the water resonance occurred at 0Hz for all voxels. After \( B_0 \) correction, the z-spectrum was resampled back to its original resolution (30Hz steps for control phantom data, 14Hz steps for BSA phantom data, 18Hz steps for human data). The resulting corrected images were used to calculate MTR\(_{\text{asym}}\) according to Eq. [2.6]. MTR\(_{\text{SAFARI}}\) was calculated from the original data without \( B_0 \) correction according to Eq. [2.7]. The control phantom MTR maps were then averaged for each of the eight solutions across the four tubes with identical relaxivities. The human MTR maps were averaged across the entire brain slice, excluding the skull and ventricles. Results are presented as the mean MTR across all volunteers with standard errors.

For the APT-SAFARI scans as a function of saturation power, the MTR parameters were calculated voxel-by-voxel according to Eqs. [2.6] and [2.7] with \( f = 0\)Hz. MTR\(_{\text{asym}}\) and MTR\(_{\text{SAFARI}}\) were each averaged across the entire slice excluding the skull and ventricles. Results are presented as the mean MTR across all volunteers with standard errors.

### 2.4 Results

The control phantom study confirms that MTR\(_{\text{SAFARI}}\) does not generate erroneous contrast in the absence of CEST agent. Figure 2-3 illustrates the effect of frequency shifts on APT imaging in the control phantom without chemical exchange. For clarity, only three of the eight different MnCl\(_2\) solutions are shown, including the longest and shortest \( T_2 \) solutions. It can be seen that when the off-resonance saturation is applied at the amide proton frequency (\( f = 0\)Hz), both MTR\(_{\text{SAFARI}}\) and MTR\(_{\text{asym}}\) are zero regardless of \( T_2 \), as expected. Moreover, MTR\(_{\text{SAFARI}}\) remains below 0.5\% for frequency offsets of at least \( \pm 120\)Hz for all relaxation times. Increasing \( T_2 \) increases the stability of MTR\(_{\text{SAFARI}}\), with the longest \( T_2 \) solution remaining at MTR\(_{\text{SAFARI}} < 0.5\%\) for up to \( \pm 180\) Hz. Therefore, \( T_2 \) does not affect the amplitude of MTR\(_{\text{SAFARI}}\) as long as water saturation remains small. Instead, it modulates the frequency offset at which direct water saturation becomes significant and can no longer be removed by MTR\(_{\text{SAFARI}}\). MTR\(_{\text{asym}}\), in contrast, deviates from zero as the frequency offset increases. |MTR\(_{\text{asym}}| exceeds 1\% before the smallest offset of \( \pm 30\)Hz. It reaches a value greater than 3\%, which is on the order of the expected in-vivo APT effect, at a frequency offset of only \( \pm 60\) Hz, except for the longest \( T_2 \) solution, which remains below 3\% up to \( \pm 90\)Hz. In addition, the rate of change of MTR\(_{\text{asym}}\) increases with decreasing \( T_2 \), which induces additional errors in the APT measurement.
Figure 2-3: Modified z-spectra in the control phantom (no CEST agent) demonstrating the effect of RF saturation frequency shifts – similarly to a $B_0$ offset – on the APT measurements. Results are shown for three MnCl$_2$ solutions. a) $\text{MTR}_{\text{SAFARI}}$ is consistent with zero and independent of frequency offset and relaxivity up to $\pm 180$Hz. b) $\text{MTR}_{\text{asym}}$ varies quickly with frequency offset and relaxivity parameters.

A BSA protein phantom was imaged to test the sensitivity of SAFARI to amide and aliphatic proton features and to study the robustness of SAFARI against frequency offsets in the presence of chemical exchange. The standard z-spectrum shown in Figure 2-4b reveals the amide proton peak at 350Hz or 2.75ppm, consistent with previous measurements (136). Even the more selective saturation preparation ($\text{pw} = 12$ms, $\text{TR}_{\text{RF}} = 20$ms, data shown in Figure 2-4) has a fairly broad saturation bandwidth, as can be seen by the width of the water and amide peaks. The water peak has a full width at half maximum of 320Hz. Therefore, when the RF preparation is applied at the amide proton frequency, these should remain mostly fully saturated even if $B_0$ offsets shift the line by up to $\pm 160$Hz. Note, also, that neither of the two RF preparations used produced noticeable saturation transfer from aliphatic protons and no peak was detected in the aliphatic region of the spectrum. Prior work (136) with BSA has shown a more pronounced aliphatic peak when much lower power is applied. Our data cannot exclude some level of aliphatic contribution to the SAFARI signal, however, the major contribution to the $\text{MTR}_{\text{SAFARI}}$ measurement is expected to be from amide protons alone. The $\text{MTR}_{\text{SAFARI}}$ spectrum is shown in Figure 2-4a. On resonance with the amide protons ($f = 0$ Hz), the baseline SAFARI signal is 2.0% $\pm$ 0.3%. In the presence of frequency offsets $f$, $\text{MTR}_{\text{SAFARI}}$ remains within 0.5% of baseline in the range of offsets from -140Hz to 100Hz. Therefore, the SAFARI strategy can successfully saturate amide protons even when $B_0$ offsets shift the line and remains insensitive to $B_0$ shifts of at least 100Hz. These phantom results are expected to provide a lower bound for the performance of SAFARI in-vivo. Since the resonance of in-vivo amide protons is centered around 450Hz instead of 350Hz, the robustness of SAFARI to $B_0$ shifts should increase, as will be seen below.
Figure 2-4: APT imaging of the BSA phantom. Data plotted for saturation with RF parameters $\text{pw} = 12\text{ms}$ and $\text{TR}_{RF} = 20\text{ms}$. a) Mean $\text{MTR}_{\text{SAFARI}}$ in the BSA phantom as a function of frequency offset $f$. Error bars represent the standard deviation across the phantom. $\text{MTR}_{\text{SAFARI}}$ has an nearly constant amplitude for frequency offsets of at least ±100Hz. b) The z-spectrum of the BSA phantom shows the amide proton peak at 350Hz and no aliphatic proton features. The vertical line is located at 350Hz. Error bars have been omitted for clarity.

Figure 2-5 compares a representative high SNR in-vivo $\text{MTR}_{\text{SAFARI}}$ map at $f = 0\text{Hz}$ with the corresponding uncorrected $\text{MTR}_{\text{asym}}$ map and the water frequency map derived from the lower SNR z-spectrum. The water frequency map (Figure 2-5a) shows large positive $B_0$ shifts at the front of the brain, negative $B_0$ shifts on the left side of the brain and small positive or zero shifts elsewhere. As shown in Figure 2-3, positive $B_0$ shifts ($f < 0$) lead to increased $\text{MTR}_{\text{asym}}$ values, while negative $B_0$ shifts ($f > 0$) lead to decreased $\text{MTR}_{\text{asym}}$ values. However, the $\text{MTR}_{\text{asym}}$ map (Figure 2-5) displays mostly negative values throughout the brain (except at the front of the brain), even in regions with zero or small positive $B_0$ shifts where $\text{MTR}_{\text{asym}}$ should be increased, indicating that the contrast is dominated by intrinsic MT asymmetry rather than APT or small $B_0$ shifts. In comparison, the $\text{MTR}_{\text{SAFARI}}$ map (Figure 2-5c) values are positive suggesting that the proposed APT-SAFARI method can successfully separate the amide proton transfer signal from the MT signal. $\text{MTR}_{\text{SAFARI}}$ also performs much better in the presence of $B_0$ inhomogeneity than $\text{MTR}_{\text{asym}}$. Comparing Figure 2-5a and b shows that $\text{MTR}_{\text{asym}}$ closely follows the $B_0$ inhomogeneity distribution. Elevated $\text{MTR}_{\text{asym}}$ values are found at the front of the brain and decreased $\text{MTR}_{\text{asym}}$ values on the left side of the brain, corresponding to susceptibility artifacts near air-tissue interfaces seen on the water frequency map. In contrast, the $\text{MTR}_{\text{SAFARI}}$ map is homogeneous throughout the brain except for a small gray-white matter contrast. There are no apparent erroneous $\text{MTR}_{\text{SAFARI}}$ values induced by off-resonance effects indicating that $\text{MTR}_{\text{SAFARI}}$ is insensitive to the range of $B_0$ inhomogeneity found in the brain.
Figure 2-5: APT imaging results in a representative human volunteer. a) B₀ map calculated from the low SNR z-spectrum images by the B₀ correction algorithm. b) MTR₁ asym map and c) MTRSAFARI map calculated from the high SNR APT images without B₀ correction. The uncorrected MTR₁ asym map shows large spatial intensity variation over the brain, which corresponds to the water frequency shift map. The uncorrected MTRSAFARI map shows a remarkable improvement in homogeneity over the brain.

The effect of B₀ inhomogeneity was further investigated by acquiring APT-SAFARI scans with asymmetric saturation around the water line. Figure 2-6 shows quantitative MTR maps for RF frequency offsets f = 0, 100, 150 and 200 Hz. In addition, mean values of MTR₁ asym and MTRSAFARI for each volunteer are shown in Table 2-1. In the presence of a 100Hz offset simulating a B₀ shift of -100Hz across the entire field of view, MTR₁ asym decreases on average by 300% compared to baseline and completely fails at detecting the APT effect. Instead it is dominated by asymmetric direct water saturation yielding large negative MTR₁ asym values. At offsets above 100Hz, the errors in MTR₁ asym increase even more reaching over 700% at f = 200Hz. In contrast, MTRSAFARI performs much better and mean values remain within one standard deviation of the f = 0Hz MTRSAFARI map for offsets f = 100Hz and f = 150Hz. An average change of ~155% is seen in the map at f = 200Hz, roughly half the error seen in the MTR₁ asym map at f = 100Hz.

Table 2-1: Average values of high SNR MTR maps across the healthy human brain (n=4) without B₀ correction

<table>
<thead>
<tr>
<th>f</th>
<th>MTR₁ asym [%]</th>
<th>f</th>
<th>MTRSAFARI [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>f = 0Hz</td>
<td>f = 100Hz</td>
<td>f = 150Hz</td>
</tr>
<tr>
<td>1</td>
<td>-0.3 ± 2.3</td>
<td>-8.6±2.9</td>
<td>-14.4±3.7</td>
</tr>
<tr>
<td>2</td>
<td>0.7 ± 2.1</td>
<td>-7.6±2.7</td>
<td>-12.6±3.7</td>
</tr>
<tr>
<td>3</td>
<td>-0.5 ± 1.5</td>
<td>-7.9±2.5</td>
<td>-15.0±4.1</td>
</tr>
<tr>
<td>4</td>
<td>-0.04 ± 1.9</td>
<td>-9.1±2.6</td>
<td>-14.8±3.3</td>
</tr>
<tr>
<td>mean ± SD</td>
<td>0.1± 0.5</td>
<td>-8.3±0.7</td>
<td>-14.3±1.1</td>
</tr>
<tr>
<td>mean error ± SD [%]</td>
<td>0</td>
<td>-290±150</td>
<td>-510±270</td>
</tr>
</tbody>
</table>
Figure 2-6: Comparison of MTR\textsubscript{asym} and MTR\textsubscript{SAFARI} maps calculated from high SNR APT images as a function of RF frequency offset. The frequency offset f mimics shifts of the water line due to $B_0$ shifts of $-f$. a) When displayed with the same contrast, MTR\textsubscript{asym} deviates from baseline becoming more and more negative as f increases, while MTR\textsubscript{SAFARI} remains invariant. b) The same MTR\textsubscript{SAFARI} images from (a) displayed with more contrast to better show the quality of the MTR\textsubscript{SAFARI} images and the deviation with frequency offset.

The same trends seen in the high SNR images (Figure 2-5 and Figure 2-6) are observed on the \textit{in-vivo} modified z-spectrum illustrated in Figure 2-7. At f = 0Hz, MTR\textsubscript{SAFARI} = 2.0% ± 0.3% is in good agreement with the high SNR measurements in Table 2-1. MTR\textsubscript{asym} = -3.8% ± 0.2%, however, is quite different from the values derived from the high SNR maps without $B_0$ correction, because the large positive MTR\textsubscript{asym} values due to $B_0$ inhomogeneity above the sinuses have now been removed. Again, we see that MTR\textsubscript{SAFARI} is positive and MTR\textsubscript{asym} is negative. In addition, while MTR\textsubscript{asym} (Figure 2-7b) varies quickly with the RF frequency offset, MTR\textsubscript{SAFARI} (Figure 2-7a) gives a much flatter response up to f = ±200Hz. The error in MTR\textsubscript{SAFARI} remains under 50% of baseline for a frequency range f = -216Hz to f = 162 Hz. In contrast, the error in MTR\textsubscript{asym} is less than 50% only in the range f = ±18 Hz. At offsets above ±200Hz, both parameters increase steeply and peak at f = ± 450 Hz when the saturation is directly on resonance with the water line (saturation at +900/0Hz and 0/-900Hz).
Figure 2-7: APT modified z-spectra averaged across the entire brain slice in four healthy volunteers. Error bars represent the standard error of the mean over the 4 volunteers. a) MTRSAFARI as a function of induced frequency offset shows the APT measurement remains fairly stable up to \( f \leq \pm 200\text{Hz} \). At \( |f| > 250\text{Hz} \), MTRSAFARI increases steeply as direct water saturation becomes significant. b) MTRasym – calculated after \( B_0 \) correction - as a function of induced frequency offset varies quickly with \( f \) because it is dominated by direct water saturation. The negative value of MTRasym at \( f = 0\text{Hz} \) is due to asymmetry in the conventional MT effects.

The power dependence of MTRasym and MTRSAFARI is shown in Figure 2-8. At the highest power (\( \text{TR}_{RF}=15\text{ms}, B_{CW}=0.78\mu\text{T} \)), MTRasym = -0.3% \( \pm \) 0.3% is negative, as seen previously. Again, the value of MTRasym calculated here without \( B_0 \) correction does not match the value calculated from the modified z-spectrum data with \( B_0 \) correction, but is in good agreement with the uncorrected high SNR measurements in Table 2-1. This again, indicates that without \( B_0 \) correction, MTRasym fails at quantifying the APT effect. As TRRF increases beyond the chemical exchange time, the APT contrast should decrease due to inefficient amide proton saturation. This was observed experimentally by increasing TRRF up to 150ms. MTRasym initially decreases, then reaches a minimum at -3.4% when TRRF=80ms (\( B_{CW}=0.15\mu\text{T} \)), and starts increasing. Because MTRasym is the sum of the MT effect (negative) and the APT effect (positive), as TRRF increases and the APT effect decreases, MTRasym becomes more negative. The increase in MTRasym when TRRF exceeds 80ms could be explained by the fact that the MT asymmetry is a function of saturation power (54). As the power decreases, the MT asymmetry lessens, and therefore MTRasym becomes less negative. MTRSAFARI has an initial value of +2.5% \( \pm \) 0.1 % and appears to decrease exponentially towards zero as the power decreases. Note that if amide protons are fully saturated, MTRSAFARI should in principle remain constant as TRRF increases, until the amide proton saturation begins to decrease. Simulations (69) suggest that a minimum CW RF saturation power of \( B_{CW}=0.5\mu\text{T} \) is required to fully saturate amide protons. Therefore, the two shortest TRRF used here should produce full saturation and have similar MTRSAFARI measurements. Although, MTRSAFARI (\( \text{TR}_{RF}=15\text{ms} \)) and MTRSAFARI (\( \text{TR}_{RF}=20\text{ms} \)) are within one standard deviation of each other no clear plateau was reached at low TRRF, indicating that higher RF power may be needed to fully saturate amide protons. However, the saturation level achieved at
TR<sub>RF</sub> = 15ms was sufficient for the APT-SAFARI strategy to be successful as illustrated in the other results. As TR<sub>RF</sub> becomes larger and amide proton saturation decreases, the assumption that the CEST effect is independent of power no longer holds. Therefore, the CEST effect will tend to cancel out in the MTR<sub>SAFARI</sub> calculation similar to direct water saturation and MT.

**Figure 2-8:** APT results in four healthy volunteers as a function of the saturation module RF pulse repetition time. Error bars represent the standard error of the mean over the 4 volunteers. The data point at TR<sub>RF</sub> =15ms was derived from the high SNR images in Figure 2-4 recalculated with only 6 averages to match the SNR of the images acquired as a function of TR<sub>RF</sub>. a) MTR<sub>asym</sub> – without B<sub>0</sub> correction – is initially negative due to MT asymmetry. As TR<sub>RF</sub> increases, MTR<sub>asym</sub> initially becomes more negative and then reaches a minimum around a TR<sub>RF</sub> of 80 ms. This reflects amide proton exchange and MT asymmetry effects. b) MTR<sub>SAFARI</sub> is initially positive and decreases approximately exponentially towards 0 as TR<sub>RF</sub> increases.

### 2.5 Discussion

We have introduced a novel acquisition technique – saturation with frequency alternating RF irradiation or SAFARI - combined with a new MTR subtraction to measure the amide proton transfer effect in-vivo. The SAFARI technique was designed to be insensitive to B<sub>0</sub> inhomogeneity and MT asymmetry. We have shown by comparing uncorrected MTR<sub>asym</sub> and MTR<sub>SAFARI</sub> phantom and in-vivo results, that MTR<sub>SAFARI</sub> can reliably measure the APT effect even in the presence of B<sub>0</sub> shifts and MT. Both phantom and human studies indicate that MTR<sub>SAFARI</sub> can measure the APT signal accurately for frequency offsets up to about ±180Hz at 3T. This frequency range is well above the range of susceptibility offsets that were observed in the brain (see Figure 2-5a), removing the need for additional B<sub>0</sub> correction of APT images. MTR<sub>asym</sub>, in contrast, relies on symmetric subtraction around the water line to cancel direct water saturation. This approach is intrinsically dependent on the B<sub>0</sub> homogeneity as was seen in both modified z-spectra data and quantiative brain maps. Unless specialized B<sub>0</sub> correction is used, standard MTR<sub>asym</sub> images closely reflect the field inhomogeneity rather than the APT effect, as
was observed in Figure 2-5. In addition, even after B\textsubscript{0} correction, MTR\textsubscript{sym} maps are negative even though the APT effect should yield positive MTR\textsubscript{sym} values. This is caused by intrinsic asymmetry in the z-spectrum, with the negative offset frequencies having lower intensities than the positive frequencies. This asymmetry is a well-known effect of conventional MT from semisolid protons (54, 73, 117) and has been observed in other APT experiments (4, 5, 10, 23, 74, 111). Not only does MTR\textsubscript{SAFARI} largely remove the need for B\textsubscript{0} correction, it also appears to eliminate MT contamination from the APT maps. Because the APT-SAFARI scan only acquires four images, instead of dozens needed to produce a full z-spectrum for B\textsubscript{0} correction, scan times can be dramatically reduced. Therefore, the SAFARI approach could help make APT, and more generally CEST, a clinically viable research tool.

In this work, we have applied SAFARI imaging to acquire CEST images of amide protons on the backbone of endogenous proteins. In principle, the SAFARI technique is not limited to amide proton exchange. SAFARI could also be used to acquire CEST images of other exchangeable protons as long as the two main assumptions are met. Recall that for SAFARI to be successful, saturation of the water line must be linear with power, i.e. direct water saturation must be small, and the exchangeable protons must be almost fully saturated. These assumptions impose limits on the design of the RF saturation module. The pulse shape, width and repetition rate must be optimized to maximize saturation of exchangeable protons while minimizing direct water saturation. For the case of amide proton exchange, we found that a 9ms Blackman-shape pulse with TR\textsubscript{RF} = 15ms produced a saturation bandwidth broad enough to saturate the amide protons at 3.5ppm even in the presence of B\textsubscript{0} shifts, while within the constraint that the bandwidth not be so broad as to produce strong direct water saturation. Given these parameters, direct water saturation remains insignificant for frequency offsets up to f = 180 Hz, as we’ve seen from the z-spectra data. At this frequency offset, the off-resonance saturation is applied at a frequency v = ω\textsubscript{R}/2π = 180Hz from the water line. Therefore, the saturation module used here can be applied for SAFARI imaging of exchangeable protons with chemical shifts greater than 270Hz (2.1ppm at 3 Tesla). For protons with smaller chemical shifts, different pulse parameters must be employed to provide a more selective saturation.

The second assumption also imposes a limit on the maximal chemical exchange rate of the protons of interest. If the exchange time is so fast that saturation of the amide protons is not achieved then the SAFARI approach will not be successful. This places requirements on the amplitude and repetition rate of the RF pulse train. For the pulse parameters used here, simulations (80) suggest that SAFARI will only detect exchangeable protons with exchange rates smaller than 200Hz. For faster exchanging protons, higher RF power and potentially simultaneous dual frequency irradiation with pulsed or continuous wave RF would be possible options to pursue.

The experiments described were optimized for the frequency and expected exchange rates of amide protons, but the possibility of other contributions to the SAFARI signal in-vivo cannot be excluded. Nearby amine protons (1) would likely be affected by the saturation pulses we employed, but their typically very high exchange rates would likely ensure saturation was not achieved and therefore the contribution to SAFARI in our experiments should be small. Lines on the other side of water may also contribute to SAFARI. In particular, a number of lines which exchange with water through spin exchange rather than chemical exchange have been reported,
for example see (4, 16, 50, 53, 118). A positive contribution of these lines to our SAFARI images, and a negative contribution to asymmetry analysis should be expected.

2.6 Conclusion

We have developed a new method for measuring amide proton transfer contrast without errors caused by direct water saturation and magnetization transfer. This feasibility study demonstrates that $\text{MTR}_{\text{SAFARI}}$ is much more robust in the presence of $B_0$ inhomogeneity than $\text{MTR}_{\text{asym}}$ and minimizes the need for specialized $B_0$ correction. In addition, the confounding MT asymmetry is removed from $\text{MTR}_{\text{SAFARI}}$ maps, allowing for accurate quantification of the CEST effect. Further evaluation is needed to assess whether APT-SAFARI imaging will permit improved characterization of brain pathology in clinical applications.
Chapter 3: Continuous Wave SAFARI imaging for quantification of amide proton exchange parameters in healthy volunteers

Abstract

Chemical exchange saturation transfer (CEST) imaging has the potential to measure pH in-vivo based on the amide proton exchange rate. In this chapter, we present a CEST imaging sequence with continuous wave saturation preparation relying on a 3-way subtraction between label frequency, control frequency, and simultaneous dual frequency RF irradiation to remove errors due to $B_0$ inhomogeneity and macromolecular magnetization transfer (MT) asymmetry. We demonstrate this approach yields amide proton transfer (APT) images free of susceptibility artifacts and MT asymmetry, without any additional $B_0$ correction. This allows clear and robust measurement of the amide proton peak in the $z$-spectrum acquired at 3T enabling accurate quantification of amide exchange rate, relaxation time and concentration in the healthy human brain as a function of saturation power. The exchange rate was measured to be 45Hz, consistent with previous animal experiments.

Results reported in this chapter were also presented at the International Society for Magnetic Resonance in Medicine Annual Meeting as:

Scheidegger R, Vinogradov E, Dai W and Alsop DC. Amide proton transfer imaging with continuous wave dual frequency saturation can detect the amide proton peak in the $z$-spectrum acquired at 3T. Proc ISMRM 2011: p 2769

3.1 Introduction

Chemical exchange saturation transfer (CEST) (1) of amide protons offers a new method for tissue characterization based on protein concentration and pH (6, 53). In stroke, CEST hypointensities are correlated with decreased pH (8, 11, 15, 25, 142). Measuring pH could help distinguish benign oligemia from the ischemic penumbra and predict patient outcome. In brain tumors, it is generally assumed that the CEST hypertintensities are caused by increased protein concentration (5, 20, 23-25, 111), however, viable alternatives include an increased intracellular pH, decreased magnetization transfer (MT) from membranes and macromolecules or combination thereof. Quantifying the amide proton concentration and exchange rates would provide valuable insights into the lesions’ pathology and may help predict chemotherapeutic outcomes based on tumor pH.

Quantifying the amide proton parameters in lesions and healthy tissue remains difficult due to multiple sources of error in CEST images. First, CEST is extremely dependent on B0 homogeneity, making it prone to severe susceptibility artifacts. Second, measurements are contaminated by macromolecular MT asymmetry. Because direct water saturation and macromolecular MT have a larger effect on the water signal than saturation transfer from amide protons, in-vivo z-spectra acquired at 3T typically show no features from amide protons. Even after asymmetry analysis, the macromolecular MT asymmetry competes against the CEST signal, leading to saturation transfer images at the amide proton frequency with negative or near zero values. While McMahon et al (94) proposed a technique, quantification of exchange as a function of saturation power (QUESP), for measurement of amide proton parameters, these measurements cannot be obtained in-vivo because the intrinsic MT asymmetry cancels out the CEST signal.

We recently proposed a new acquisition and post-processing method based on a pulsed saturation with frequency alternating radiofrequency irradiation (SAFARI) designed to correct for direct water saturation and intrinsic MT asymmetry in APT measurements (143). In this chapter, we introduce the analogous continuous wave (CW) saturation scheme and show it similarly decreases the dependence of APT on B0 inhomogeneity and MT asymmetry compared to standard MTR asymmetry analysis. In addition, we demonstrate the ability of MTRSAFARI to identify the amide proton peak in the z-spectrum at 3T. Because the CW scheme allows easily modulation of the saturation power, we can now apply QUESP in combination with SAFARI to measure the amide proton transverse relaxivity ($T_2s$), exchange rate ($k_{sw}$) and concentration ($M_0s$) in the healthy human brain.

3.2 Theory

The SAFARI scheme has been described in detail previously (143). Briefly, SAFARI exploits nonlinearity in the saturation process to subtract out magnetization transfer (MT) and direct water saturation effects from CEST images. The key to the SAFARI technique is the acquisition of an image with RF irradiation applied simultaneously at both the amide proton ($\omega_0 = +3.5$ppm) and control ($-\omega_0$) frequencies. On-resonance irradiation of the amide protons will fully saturate the line at relatively low power, while the macromolecular and water lines require
larger powers to reach saturation. As a result, there is a range of RF powers for which the amide proton saturation is independent of power, while direct water saturation and MT vary approximately linearly with power. If the standard CEST images are acquired with double the power compared to the dual frequency image, the MT and direct water saturation effects at each frequency double, while the CEST effect does not change. Subtracting two-times the dual frequency image from the sum of label frequency and control frequency images will yield the CEST signal, while both MT and direct water saturation cancel out exactly, even in the presence of B₀ inhomogeneity and MT asymmetry.

3.3 Methods

3.3.1 APT Pulse sequence design

The CW-SAFARI imaging pulse sequence is shown in Figure 3-1. Four images are acquired for each CW-SAFARI scan: one label image $S_{sat}(\omega_+)$ with CW irradiation applied at a single frequency $\omega_+$ (Figure 3-1a), one SAFARI image $S_{sat}$(SAFARI) with on resonance CW irradiation with amplitude modulation (Figure 3-1b), one control image $S_{sat}(\omega_-)$ with CW irradiation applied at a single frequency $\omega_-$ (Figure 3-1c), and another SAFARI image $S_{sat}$(SAFARI') identical to b) (Figure 3-1d). Note that the first and third images constitute a standard CEST experiment with CW RF preparation.

For all four images, the off-resonance RF irradiation consisted of a 250ms CW-RF irradiation [25ms of half Blackman window shaped rise – CW saturation at power $B_1$ for 200ms–25ms half Blackman window shaped ramp down]. Dual frequency preparation was achieved by CW saturation on resonance with amplitude modulation given by $B_1(t) = \sqrt{2} B_1 \sin(\omega_0 t)$. This generates a frequency response with components at $+\omega_0$ and $-\omega_0$. Crusher gradients were turned on at the end of the RF saturation pulse to spoil any remaining transverse magnetization. The saturation module was followed by a single-shot spin-echo echo-planar imaging (EPI) acquisition.
Figure 3-1: Pulse sequence for SAFARI imaging with CW off-resonance saturation followed by EPI readout. Each scan consists of acquiring four images: a) standard CEST image with CW saturation at the labile proton frequency ($\omega_*$ = 3.5ppm for amide protons). b) SAFARI image with dual frequency saturation achieved with on-resonance irradiation with amplitude modulation. c) standard CEST control image with saturation at the control frequency ($\omega_*$ = -3.5ppm for amide protons). d) a second SAFARI image.

3.3.2 Phantom Preparation

Control phantoms were prepared with eight different concentrations of MnCl$_2$ in water. Each solution was divided amongst four 15mm-diameter centrifuge tubes. The tubes were arrayed in a single 4 by 8 Styrofoam holder for imaging. $T_1$ and $T_2$ relaxation times have been measured previously (141). $T_1$ values range from 2000ms to 400ms and $T_2$ values range from 280 ms to 40ms. All results are presented for the phantoms with $T_1$=600ms and $T_2$=65ms.
A CEST phantom was prepared at a concentration of 3% w/v by dissolving 450mg of poly-L-lysine (PLL, Sigma-Aldrich) in 15ml phosphate saline buffer at pH=4.5. The PLL solution was transferred to a 29.1mm-diameter tube for imaging.

3.3.3 Imaging

All studies were performed on a 3T GE SIGNA EXCITE MR system using the body coil for RF transmission. A standard eight-channel receive-only head array was used for signal reception.

A single shot spin-echo EPI readout was used for all control phantom and human studies with TR = 2s, TE=20.2 ms, FOV = 24cm, matrix size = 96x96, and slice thickness = 8mm. The PLL phantom was imaged with the same parameters, except for a FOV = 10cm.

3.3.3.1 Phantom studies

The control phantom was imaged with the long axis of the tube along the direction of the magnetic field. To evaluate the robustness of the pulse sequence against B₀ inhomogeneities as a function of power, a modified z-spectrum was acquired at different power levels: shifts in the water line due to B₀ offsets were mimicked by introducing an offset f to the off-resonance irradiation frequency. Instead of acquiring CEST scans with off-resonance irradiation at the amide proton frequency \( \omega_s/2\pi = 450\text{Hz} \), the applied frequencies were \( \omega_+ = 2\pi f + \omega_s \) and \( \omega_- = 2\pi f - \omega_s \), where the frequency offset f was swept from -450 Hz to +450Hz in steps of 30 Hz. Therefore, the frequency shift f simulates an offset of the water line due to a B₀ shift of -f. The modified z-spectrum acquisition resulted in a total of 31 SAFARI scans (124 images). Note that for the single frequency images the data set constitutes a standard z-spectrum acquired out of order. For the SAFARI images, however, it differs from a z-spectrum because the offset f leads to RF irradiation at frequencies with increasing asymmetry around the water line, rather than symmetric frequencies with increasing offsets. Total scan time was 4 minutes for each power level. The modified z-spectrum was acquired at powers of B₁=1, 2, 3, 4, and 5μT. To further evaluate the effect of power on the MTRSAFARI parameter in the control phantom, SAFARI scans were acquired at 3.5ppm (f=0) with B₁=0, 0.25, 0.5, 1, 1.5, 1.75, 2.0, 2.25, 2.5, 2.75, 3, 3.25, 3.5, 3.75, 4.0, 4.25, 4.5, 5.0, 5.5, and 6.0 μT. For averaging, forty-eight time-interleaved images (12 label, 12 control and 24 dual frequency images) were acquired at each power in 1.5min. Total acquisition time was 28.5min.

The PLL phantom was imaged with the long axis of the tube along the direction of the magnetic field. To compare the performance of MTR\textsubscript{asym} and MTR\textsubscript{SAFARI} for amide proton transfer imaging, a standard z-spectrum was acquired. SAFARI z-spectra were acquired at 28 frequency offsets pairs up to ±1.5 kHz (0 to ±550 in 25Hz steps, ±600, ±650, ±700, ±900, ±1500). For averaging, forty-eight time-interleaved images (12 label, 12 control and 24 dual frequency images) were acquired at each frequency offset in 1.5min. Total acquisition time was 42min for each z-spectrum. Z-spectra were acquired with B₁ = 1 μT and 2 μT. For QUESP, SAFARI scans were acquired at 2.7ppm with B₁ = 0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.5,
3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0 µT with 12 averages. Total acquisition time was 28.5 min for the QUESP data set. In addition, one unsaturated S₀ image was acquired for reference (NEX=12).

### 3.3.3.2 Human studies

All volunteers were scanned following a protocol approved by our institutional review board. Three healthy men (mean age 36.6 years) were scanned after giving written informed consent. For each volunteer, a single slice was located at the level of the ventricles. One unsaturated S₀ image was acquired for reference with 12 averages. To identify the amide proton peak in-vivo, a SAFARI z-spectrum was acquired at B₁=1µT at 13 frequency offset pairs up to ±1.5kHz (±200 to ±650 in 50Hz steps, ±700, ±900, ±1500). For averaging, forty-eight time-interleaved images (12 label, 12 control and 24 dual frequency images) were acquired at each frequency offset pair in 1.5 min. Total acquisition time was 19.5 min for the z-spectrum. In addition, single frequency images were acquired between -200 and 200Hz in steps of 25Hz to map the center of the water line for B₀ correction. For QUESP, SAFARI scans were acquired at eight saturation powers B₁=0, 0.25, 0.5, 1.0, 1.5, 2, 3 and 4 µT with 12 averages. Total acquisition time for the QUESP data set was 12 min.

### 3.3.4 Analysis

Image analysis was performed in MATLAB 7.6 (Mathworks, Natick MA). All images were normalized voxel-by-voxel by the unsaturated reference image S₀.

For the modified z-spectrum scans in the control phantom and the standard z-spectrum scans in the PLL phantom and healthy volunteers, B₀ correction of the single frequency images was performed following the water saturation shift referencing (WASSR) method (123). For the modified z-spectrum in the control phantom, the single frequency images S_{sat}(ω_-) and S_{sat}(ω_+) were first reordered to generate a standard z-spectrum. Z-spectra were fit voxel-by-voxel to a 3rd order polynomial in the frequency range ±200 Hz only. The resulting polynomial was evaluated with a frequency resolution of 1 Hz. The frequency with the minimum fit intensity was assumed to be the center of the water peak. Each z-spectrum was interpolated by a piecewise cubic hermite interpolating polynomial to a resolution of 1 Hz and shifted to center the water frequency at 0 Hz in all voxels. After B₀ correction, the z-spectra were resampled back to their original frequency resolution.

For the modified z-spectrum scans, the corrected images were used to calculate MTR_{asym} according to Eq. [3-1]. MTR_{SAFARI} was calculated from the original data without B₀ correction according to Eq. [3-2]:

\[
MTR_{asym} = \frac{S_{sat}(\omega_-)/S_0 - S_{sat}(\omega_+)/S_0}{S_{sat}(\omega_-)/S_0 - [S_{sat}(SAFARI)/S_0 + S_{sat}(SAFARI')/S_0]} \quad [3-1]
\]

\[
MTR_{SAFARI} = \frac{S_{sat}(\omega_-)/S_0 + S_{sat}(\omega_+)/S_0 - [S_{sat}(SAFARI)/S_0 + S_{sat}(SAFARI')/S_0]}{S_{sat}(\omega_-)/S_0} \quad [3-2]
\]

where \(\omega_- = 2\pi f - \omega_0\) and \(\omega_+ = 2\pi f + \omega_0\). For the standard z-spectrum scans, the corrected images were used to calculate MTR_{asym} according to Eq. [3-3]. MTR_{SAFARI} was calculated from the original data without B₀ correction according to Eq. [3-4]:

70
\[ MTR_{asym} = S_{sat}(-\omega_s)/S_0 - S_{sat}(+\omega_s)/S_0 \]  \[ MTR_{SAFARI} = S_{sat}(-\omega_s)/S_0 + S_{sat}(+\omega_s)/S_0 - \left[ S_{sat}(SAFARI)/S_0 + S_{sat}(SAFARI')/S_0 \right] \]

where \( \omega_s \) is the amide proton frequency. For human results, \( MTR_{asym} \) and \( MTR_{SAFARI} \) were each averaged across a region of interest in the occipital white matter. Results are presented as the mean MTR with standard errors across all volunteers.

For QUESP data, quantitative maps of \( MTR_{asym} \) and \( MTR_{SAFARI} \) were calculated voxel-by-voxel with no \( B_0 \) correction according to Eqs. [3-3] and [3-4]. For the PLL data, amide proton exchange was quantified by fitting the \( MTR_{asym} \) spectra to the Bloch equations with a two-pool exchange model, using the standard iterative least square fitting algorithm in MATLAB. The resulting fit parameters were used to simulate the \( MTR_{SAFARI} \) spectrum. The correlation between the acquired \( MTR_{SAFARI} \) data and the simulation was evaluated by the coefficient of determination \( R^2 \). For human imaging, \( MTR_{asym} \) and \( MTR_{SAFARI} \) were each averaged across a region of interest in the occipital white matter. Results are presented as the mean MTR with standard errors across all volunteers. To quantify amide proton parameters, the \( MTR_{SAFARI} \) contrast was modeled using the Bloch equations for a two-pool exchange model. Because the equations must be numerically integrated to simulate the SAFARI sequence, iterative fitting procedures could not be used in any reasonable amount of time. Instead, numerical simulations of the SAFARI signal were calculated for the following grid of parameters: amide proton content \( M_{0s}=1/[10\ 25\ 50\ 75\ 100\ 125\ 150\ 175\ 200\ 300\ 500\ 1000\ 5000\ 2000] \) \( M_{0w} \), longitudinal relaxation times \( T_{1w}=1.5s \), \( T_{1s}=0.77s \), transverse relaxation times \( T_{2w}=60ms \), \( T_{2s}=[0.5\ 1\ 2\ 3\ 4\ 5\ 10\ 20\ 30\ 35\ 60]ms \) and chemical exchange rate from the amide group to free water \( k_{sw}=[10\ 15\ 20\ 25\ 30\ 35\ 40\ 45\ 50\ 60\ 75\ 90\ 100\ 150\ 200\ 300\ 400\ 500\ 1000]Hz \). In-vivo amide proton parameters were then estimated by finding the best fit between the experiment and simulation grid.

### 3.4 Results

#### 3.4.1 Control phantom results

The control phantom results demonstrate the robustness of the CW-SAFARI acquisition against \( B_0 \) shifts, compared to standard asymmetry analysis. A control phantom with no CEST or MT was used to assess the effect of \( B_0 \) shifts and saturation power on the MTR parameters. In the absence of proton exchange or MT, both \( MTR_{asym} \) and \( MTR_{SAFARI} \) should be zero. As expected, \( MTR_{asym} \) was found to be zero at \( f=0Hz \) (Figure 3-2). \( MTR_{SAFARI} \) was also zero in the absence of frequency shift, but only at low powers of 2\( \mu T \) and below. At powers higher than 2\( \mu T \), direct water saturation became significant and \( MTR_{SAFARI} \) increased, even in the absence of exchangeable protons. In the presence of \( B_0 \) shifts (\( f \neq 0 \)) \( MTR_{SAFARI} \) was much more robust than \( MTR_{asym} \). \( MTR_{asym} \) deviated quickly from zero as the frequency shift \( f \) increased (Figure 3-2), making this measurement extremely dependent on \( B_0 \) inhomogeneities and prone to susceptibility artifacts. In addition, the rate of change of \( MTR_{asym} \) increased with increasing \( B_1 \) power, which induces additional errors in the measurement. In contrast, \( MTR_{SAFARI} \) remained constant in the presence of frequency shifts up to \( \pm 240Hz \) at 1\( \mu T \) and \( \pm 150Hz \) at 2\( \mu T \). Based on these results, in-vivo z-spectra were acquired with a power of 1\( \mu T \).
3.4.2 PLL phantom results

A PLL phantom was imaged to test the sensitivity of SAFARI to amide proton exchange and validate the SAFARI measurement against the standard CEST acquisition with asymmetry analysis. The standard z-spectrum with $B_0$ correction, shown in Figure 3-3, detected the amide proton peak at 350Hz (2.75ppm). Compared to the pulsed-SAFARI sequence described in Chapter 2, the CW-SAFARI acquisition had a much narrower saturation bandwidth, as seen on the z-spectrum, leading to sharper CEST peaks. The peak was also slightly narrower with saturation power $B_1=1\mu T$ than at $B_1=2\mu T$. The amplitude of the PLL peak measured by MTR$_{asym}$ was $4.45\pm0.38\%$ at $1\mu T$ and $5.33\pm0.39\%$ at $2\mu T$. The amplitude of the PLL peak measured by MTR$_{SAFARI}$ was $3.31\pm0.67\%$ at $1\mu T$ and $5.08\pm0.44\%$ at $2\mu T$. Therefore, MTR$_{SAFARI}$ gave comparable results to a standard CEST acquisition with asymmetry analysis in the absence of MT and $B_0$ inhomogeneity.

The PLL phantom was also used to demonstrate the feasibility of using QUESTP to fit the amide proton parameters. The QUESTP method relies on fitting MTR$_{asym}$ measurements as a function of saturation power to estimate exchange parameters. It has been shown to yield accurate results in phantoms with no MT but fails in-vivo because of the presence of the negative MT asymmetry (94). Therefore, in the PLL phantom with no MT, the MTR$_{asym}$ QUESTP data can be fit directly. MTR$_{asym}$ as a function of RF power is shown in Figure 3-3d. The QUESTP fit to a two-pool model yielded the amide proton parameters $T_{2s}=8.2ms$, $k_{sw}=98.9ms$, $M_{0w}=1/311.3M_{0w}$ with excellent correlation to the data ($R^2=0.984$). The exchange rate was lower than measured previously at pH=7.3 (93), as expected.

The measured amide exchange parameters were used to simulate the predicted SAFARI signal. The simulations are overlaid on top of the PLL phantom data in Figure 3-3e and Figure 3-3f for MTR$_{SAFARI}$ as a function of power and the MTR$_{SAFARI}$ spectrum, respectively. There was
excellent agreement between the simulations and the SAFARI acquisition as a function of power ($R^2=0.971$) and frequency offset ($R^2=0.948$). Because the QUESP measurement accurately predicts the MTRSAFARI data, SAFARI rather than MTR$_{asym}$ can be employed for QUESP measurements in-vivo removing the need to model MT asymmetry.

![Graphs](image)

Figure 3-3: CEST and SAFARI imaging in a PLL phantom. Top row: a) $z$-spectra with $B_0$ correction. b) MTR$_{asym}$ spectra and c) MTR$_{SAFARI}$ spectra as a function of RF power. In the PLL phantom without MT and with corrected $B_0$ inhomogeneity, SAFARI and standard CEST acquisitions give similar results when the RF power is high enough for complete amide proton saturation. Bottom row: QUESP results. d) MTR$_{asym}$ as a function of RF power. e) MTR$_{SAFARI}$ as a function of RF power along with the simulation calculated from the best fit parameters. f) MTR$_{SAFARI}$ spectrum along with the simulation calculated from the best fit parameters.

### 3.4.3 Human results

For in-vivo imaging of the human brain, MTR$_{asym}$ and MTR$_{SAFARI}$ spectra yield dramatically different results. The standard $z$-spectrum in a white matter ROI, with $B_0$ correction, showed no sign of the amide proton peak at 3.5ppm (Figure 3-4a). In addition, the corresponding MTR$_{asym}$ spectrum was negative and also showed no evidence of the amide proton peak (Figure 3-4b). The amplitude of MTR$_{asym}$ at 3.5ppm was $-1.72\pm0.04\%$, indicating a primary contribution from the negative macromolecular MT asymmetry rather than the CEST effect. In contrast, MTR$_{SAFARI}$ spectrum was positive and exhibited a significant amide proton peak centered at 3.5ppm. The
amplitude of the SAFARI signal at 3.5 ppm was 1.91±0.12%, indicating that MT asymmetry was successfully removed. The amide peak was about ~1.6 ppm wide, in good agreement with the distribution of amide chemical shifts found in proteins (144).

Figure 3-4: z-spectroscopy with CW-SAFARI in a white matter ROI in healthy volunteers. a) standard z-spectrum with B₀ correction. b) Corrected MTRₐₚₚ signal. c) MTRSAFARI spectrum identifies the amide proton peak at 3.5 ppm.

SAFARI images as a function of power were acquired for QUESP measurement of amide exchange rates. Sample QUESP maps, showing the power dependence of MTRₐₚₚ and MTRSAFARI in a healthy volunteer, are shown in Figure 3-5. At low power, MTRₐₚₚ was negative due to the primary contribution of MT asymmetry that dominates over the CEST effect. As the power increased, the CEST signal increased and MTRₐₚₚ maps became positive. In addition, large susceptibility artifacts were present throughout the brain when no special B₀ correction methods are used. In contrast, MTRSAFARI was positive demonstrating that MT had been successfully removed by the CW-SAFARI saturation and subtraction scheme. The susceptibility artifacts seen on the MTRₐₚₚ map had also been removed and the MTRSAFARI maps were much more homogeneous across the brain, except for a small white matter-gray matter contrast. At low power, MTRSAFARI was small due to incomplete amide proton saturation. As the power increased MTRSAFARI increased and leveled off once amide protons were fully saturated.
Figure 3-5: Example of CEST imaging as a function of power in a healthy volunteer. Top row: uncorrected MTR$_{\text{asym}}$ exhibit large signal variations due to $B_0$ inhomogeneity across the brain and contamination from macromolecular MT asymmetry. Bottom row: MTR$_{\text{SAFARI}}$ corrects for $B_0$ inhomogeneity and MT asymmetry.

QUESP MTR$_{\text{SAFARI}}$ images were acquired in the control phantom for calibration and in the human brain. The QUESP curves are shown in Figure 3-6. In the control phantom with no exchangeable protons MTR$_{\text{SAFARI}}$ should be zero. However, as the saturation power increased, direct water saturation became significant and the SAFARI strategy failed, as indicated by increasing MTR$_{\text{SAFARI}}$ at high power, despite the lack of exchangeable protons. T-tests indicated that MTR$_{\text{SAFARI}}$ in the phantom was not significantly different from zero for RF powers up to 1$\mu$T and MTR$_{\text{SAFARI}}$ remained under 0.5% up to 2$\mu$T. At higher powers, direct water saturation (and thus MT $\text{in-vivo}$) became too large to be corrected by the SAFARI strategy. Because the QUESP technique relies on a two-pool model which does not take macromolecular MT into account, only data points with RF power less than to 2$\mu$T were used in the QUESP fit of $\text{in-vivo}$ data to ensure that MT was successfully removed. The $\text{in-vivo}$ MTR$_{\text{SAFARI}}$ QUESP data is shown in Figure 3-6a along with the best fit. There was excellent agreement between the data and the fit for RF power up to 2$\mu$T ($R^2=0.996$). The fitted amide parameters were $T_2=2\text{ms}$, $k_{sw}=45\text{Hz}$ and $M_{0s}=1/175M_{0w}$. 


3.5 Discussion:

In this chapter, we have described a CW dual frequency saturation scheme for the acquisition of CEST images. Compared to the pulsed-SAFARI technique described in Chapter 2, the CW sequence has the advantage that the signal depends only on the saturation time and RF power, as opposed to the pulse width, interpulse delay, flip angle, and saturation time of the pulsed sequence. This simplifies sequence optimization, modeling and interpretation of results and facilitates comparison with other results obtained using CW methods. While hardware restrictions limited the irradiation to 250 ms, the short saturation duration minimizes T1 effects that can complicate quantification. As a result, we note that the white matter-gray matter contrast measured in the brain images in this chapter is reversed compared to the images in Chapter 2.

The phantom and in-vivo results demonstrate that the CW-SAFARI scheme can successfully correct for B0 inhomogeneity and MT asymmetry at low to moderate RF saturation power. We have shown that SAFARI is insensitive to frequency shifts up to ±240Hz at B1=1μT, removing the need for B0 correction across the brain. When f = 240Hz, the RF irradiation is applied at an offset of 210Hz from the water line. Therefore, for the SAFARI strategy to successfully remove direct water saturation at 1μT, the RF irradiation must be applied at offsets greater than 210Hz from the water line. The amide proton line is centered at 350Hz in the PLL phantom and at 450Hz in-vivo, allowing for enough spectral separation for SAFARI to remove direct water saturation from amide proton transfer images. In addition, assuming the MT line is centered at -1.5ppm (73), it is 255Hz from the control frequency of -3.5ppm and should also be successfully removed by the SAFARI strategy. We note that as the RF power increases beyond 2μT, SAFARI starts to generate erroneous contrast. This can be seen in Figure 3-6b, where MTRSAFARI is non zero despite the lack of exchangeable protons in the control phantom. The SAFARI scheme relies on the assumption that direct water saturation is small and thus increases linearly with power. As the saturation power increases this assumption no longer holds. Therefore, at high
power the SAFARI scheme is no longer successful at removing direct water saturation and MT asymmetry and should not be used.

By using a CW RF irradiation rather than the previously proposed pulsed irradiation, the saturation bandwidth decreases, which enables the clear identification of the amide proton peak in the human brain at 3T. Through the measurement of the amide proton peak, CW SAFARI enables the quantification of protein concentration and amide exchange rates \textit{in-vivo}. The measured exchange rate of 45Hz is fairly consistent with previous reports (4, 53) The measured $T_{2s}$ of 2ms is shorter than the $T_{2s}$ in the tens of milliseconds typically measured in protein solutions, suggesting the \textit{in-vivo} amide proton signal may have a possible contribution from bound proteins. The amide peak width is on the order of 300Hz, consistent with a short $T_{2s}$. It should be noted that our model has assumed amide protons have a single resonance frequency at 3.5ppm from the water line. However, it is known that the amide proton chemical shift is a function of protein structure (144), and this model is therefore an oversimplification. The $\text{MTR}_{\text{SAFARI}}$ spectrum is also consistent with the peak being a composite of individual amide protons with a range of chemical shifts of 2ppm (144). Further studies are needed to evaluate how such a model would impact the $T_{2s}$ measurement.

### 3.6 Conclusion

In this Chapter, we have introduced a CW version of the SAFARI CEST acquisition scheme. We demonstrated that similarly to the original pulsed method, the CW-SAFARI can accurately remove errors due to $B_0$ inhomogeneity and MT asymmetry from CEST images of the human brain. As a result, the amide proton peak can be clearly measured on the z-spectrum, enabling the quantification of exchange parameters using the QUESP method.
Chapter 4: Contributors to contrast between glioma and brain tissue in chemical exchange saturation transfer sensitive imaging at 3 Tesla

Abstract

Off-resonance saturation transfer images have shown intriguing differences in intensity in glioma compared to normal brain tissues. Interpretation of these differences is complicated, however, by the presence of multiple sources of exchanging magnetization including amide, amine, and hydroxyl protons, asymmetric magnetization transfer contrast (MTC) from macromolecules, and various protons with resonances in the aliphatic spectral region. We report a study targeted at separating these components and identifying their relative contributions to contrast in glioma. Off-resonance z-spectra at several saturation powers and durations were obtained from 6 healthy controls and 6 patients with high grade glioma. Results indicate that broad macromolecular MTC in normal brain tissue is responsible for the majority of contrast with glioma. Amide exchange could be detected with lower saturation power than has previously been reported in glioma, but it was a weak signal source with no detectable contrast from normal brain tissue. At higher saturation powers, amine proton exchange was a major contributor to the observed signal but showed no significant difference from normal brain. Robust acquisition strategies that effectively isolate the contributions of broad macromolecular MTC asymmetry from amine exchange were demonstrated that may provide improved contrast between glioma and normal tissue.

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Scheidegger R, Wong ET, Alsop DC. Contributors to contrast between glioma and brain tissue in chemical exchange saturation transfer sensitive imaging at 3 Tesla. Magn Reson Med
4.1 Introduction

Off-resonance saturation transfer imaging methods, such as magnetization transfer (MT) imaging (145, 146) and chemical exchange saturation transfer (CEST) imaging (1-3), have been used increasingly for the study of brain tumors. Saturation transfer imaging at the amide proton frequency, known as amide proton transfer (APT) (4, 53) imaging, is thought to generate MRI contrast related to pH and the protein content inside cells. It has emerged as a potentially important tool for localizing tumors both in animal models (5, 111) and humans (21, 23, 24, 26), and for grading (20) brain tumors. It has also shown promise at evaluating tumor treatment response, as it may distinguish tumor recurrence from radiation necrosis (112, 113), which otherwise can appear similar on magnetic resonance images. Though the origin of the saturation transfer signal in tumors has not been fully explained, it has been attributed to increased mobile protein concentrations in tumors (5, 20, 23, 24, 113).

Despite the initial success of brain tumor imaging with saturation transfer at the amide proton frequency, isolating the contribution of amide proton concentration to the contrast remains difficult. The off-resonance RF irradiation used to generate the APT signal also induces direct water saturation (DS) and broad macromolecular magnetization transfer contrast (MTC). These effects are typically removed by magnetization transfer ratio asymmetry (MTR\textsubscript{asym}) analysis, where an image acquired with saturation at the amide proton frequency is subtracted from a control image acquired with RF saturation on the opposite side of the water line. MTR\textsubscript{asym} analysis, however, introduces further sources of errors. The intrinsic macromolecular MTC effect is asymmetric with respect to the water line (54, 73, 117). Therefore, it produces a negative asymmetry that cancels the amide proton signal. In addition, saturation peaks attributed to aliphatic protons are present in a frequency range from approximately -1 ppm to -5 ppm. They also add a negative contribution to the asymmetry, further canceling the amide proton signal (4, 16, 50, 51, 53, 82, 118-122). Note that aliphatic protons are believed to exchange magnetization through nuclear Overhauser enhancement (NOE) (51, 53), rather than chemical exchange. As a result of these two confounds, MTR\textsubscript{asym} values at the amide proton frequency are negative in normal tissue when saturation powers less than 2 \(\mu\)T are employed. In order to account for these multiple sources of saturation transfer, the MTR\textsubscript{asym} parameter can be broken up into two components (53):

\[
\text{MTR}_{\text{asym}}(\omega) = \text{MTR'}_{\text{asym}}(\omega) + \text{APTR}(\omega)
\]

where APTR is the proton transfer ratio from amide protons and MTR'\textsubscript{asym} incorporates all other sources of saturation transfer and errors.

One approach proposed to remove the undesired contributions of MTR'\textsubscript{asym} to MTR\textsubscript{asym} is to subtract the asymmetry of a control region from the diseased region (5, 25, 111):

\[
\Delta\text{MTR}_{\text{asym}}(\omega) = \text{MTR}_{\text{asym}}(\omega, \text{disease}) - \text{MTR}_{\text{asym}}(\omega, \text{control}) = \Delta\text{MTR'}_{\text{asym}}(\omega) + \Delta\text{APTR}(\omega)
\]

If MTR'\textsubscript{asym}, which includes the NOE from aliphatic peaks and macromolecular MTC asymmetry, is unchanged between regions (\(\Delta\text{MTR'}_{\text{asym}} = 0\)), the subtraction will eliminate the negative contribution to the asymmetry and yield the APT effect. The assumption of constant MTR'\textsubscript{asym} in cancer has not been verified, however, and may be suspect.
Recently new methods have been proposed to quantify the APT effect and separate it from broad macromolecular MTC and/or NOE. These methods fall into two categories. The first class of approaches employs a standard z-spectrum acquisition followed by fitting of individual peaks in the amide and aliphatic regions of the z-spectrum without employing asymmetry assumptions (121, 122, 147). The second involves specialized acquisition schemes, such as frequency labeled exchange transfer (FLEX) (127), saturation with frequency alternating RF irradiation (SAFARI) (143), two frequency RF irradiation (128), CEST phase mapping using a length and offset varied saturation scheme (LOVARS) (129) or chemical exchange rotation transfer (CERT) (130, 131).

In this chapter, we used a combination of imaging methods including standard z-spectroscopy as a function of irradiation power, low-power z-spectroscopy with fitting of the amide and aliphatic peaks and SAFARI imaging to assess the individual contributions of saturation transfer from amide protons, aliphatic protons, amine protons, and broad macromolecular MTC in human glioma at 3 Tesla.

4.2 Methods

4.2.1 Subjects

A total of 12 subjects were recruited for this study. There were six healthy volunteers (4 men, 2 women; median age 43 [range 31-52] years) and six subjects with malignant gliomas (4 men, 2 women; median age 54 [range 48-65] years). Tumor locations and pathological findings are described in Table 4-1. For all patients, diagnosis and grading of disease was confirmed in the Brain Tumor Clinic prior to our examination. Five of six subjects had histologically confirmed glioblastoma at initial diagnosis while one had histological diagnosis of malignant astrocytoma with features suggestive of secondary glioblastoma. All were scanned following a protocol approved by our institutional review board and after giving written informed consent. Four of six subjects had their scans performed at the time of tumor recurrence while two were scanned at initial diagnosis. Patients with recurrent disease had been treated with a combination of surgical resection or biopsy, radiation, cytotoxic chemotherapy, targeted therapy and/or NovoTTF-100A device (148-150), prior to enrollment in this study. These patients were enrolled in the study after the diagnosis of disease or progression was made clinically and scanned an average of 21 days after their last clinical MRI scan.
Table 4-1: Pathology report and clinical management of the 6 glioma patients scanned.

<table>
<thead>
<tr>
<th>Number</th>
<th>Sex</th>
<th>Age</th>
<th>Pathology</th>
<th>Lesion Location</th>
<th>Prior treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>48</td>
<td>Glioblastoma, WHO Grade 4</td>
<td>Left parieto-occipital, left temporal lobes</td>
<td>Biopsy, temozolomide chemo-irradiation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Additional treatments: adjuvant monthly temozolomide(^a) (1 cycle), dose-dense temozolomide(^b) (1 cycle), bevacizumab (5 cycles), lomustine (1 cycle), daily etoposide (1 cycle), irinotecan (1 cycle), and NovoTTF-100A (2 cycles)</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>53</td>
<td>Glioblastoma, WHO Grade 4</td>
<td>Left occipital mass with recurrence in left parietal lobe</td>
<td>Gross total resection, temozolomide chemo-irradiation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Additional chemotherapy: adjuvant monthly temozolomide(^a) (12 cycles)</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>57</td>
<td>Glioblastoma, WHO Grade 4</td>
<td>Bi-frontal mass with extension across the genu of the corpus callosum</td>
<td>Biopsy</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>65</td>
<td>Glioblastoma, WHO Grade 4</td>
<td>Right parietal mass with recurrence in right frontal lobe</td>
<td>Gross total resection, temozolomide chemo-irradiation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Additional chemotherapy: adjuvant monthly temozolomide(^a) (1 cycle) and bevacizumab (1 cycle)</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>54</td>
<td>Malignant astrocytoma (probable secondary glioblastoma)</td>
<td>Right thalamus</td>
<td>Biopsy, ventriculostomy, proton beam radiation therapy</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>49</td>
<td>Glioblastoma, WHO Grade 4</td>
<td>Right frontal lobe, centrum semiovale</td>
<td>Partial resection</td>
</tr>
</tbody>
</table>

\(^a\) Monthly temozolomide at a dose of 200 mg/m\(^2\)/day x 5 days in 28-day cycle.
\(^b\) Dose-dense temozolomide at a dose of 150 mg/m\(^2\)/day, 7 days on and 7 days off, in 28-day cycle.

4.2.2 Imaging

All studies were performed on a 3 Tesla GE SIGNA HDxt (GE Healthcare, Waukesha WI) whole-body MR scanner. The body coil was used for excitation and a standard eight-channel receive-only head array was used for signal reception.

In the glioma patients, conventional \(T_2\) weighted fluid attenuated inversion recovery (FLAIR: TE/TI/TR = 130/2250/10000 ms, FOV=24cm, slice thickness=5mm, slice spacing = 1.5mm, matrix=320x224, scan time approximately 3.5min) and 3D pulsed continuous arterial spin labeling (151, 152) perfusion images (ASL: labeling duration = 3.5 s, post labeling delay = 1.5s. Stack of spirals RARE readout: 7 spiral interleaves, TE/TR = 10/6952 ms, FOV = 24cm, resolution = 3.35 x 3.35 x 4mm, NEX = 3, scan time approximately 6 min) were acquired to localize the tumor. A single slice passing through a region with intense ASL signal, FLAIR
hyperintensity, and with signs of contrast enhancement on the prior clinical scan was selected. Preference was given to slice locations away from regions of greater $B_0$ nonuniformity. In the healthy volunteers, a single axial slice passing through the superior part of the lateral ventricles was selected.

The off-resonance saturation transfer imaging sequence consisted of continuous wave (CW) RF irradiation [20ms of half Blackman window shaped rise – CW saturation at power $B_1$ for $T_{sat}$ – 20ms half Blackman window shaped ramp down] followed by a single shot spin-echo EPI acquisition [$TR = 2000$ ms, $TE$ ranging from 15.8 to 17.4 ms, FOV = 24 cm, matrix = 96 x 96, slice thickness = 8 mm]. One unsaturated $S_0$ image was acquired for reference (NEX=12). Z-spectra were acquired at 32 frequency offsets pairs up to ±5 kHz (0, ±25, ±50, ±100, ±150, ±175, ±200, ±250, ±275, ±300, ±325, ±350, ±375, ±400, ±425, ±475, ±500, ±525, ±550, ±600, ±700, ±900, ±1200, ±1500, ±2000, ±3000, ±4000, ±5000 Hz). Z-spectra were first acquired with $B_1 = 0.5 \mu T$ (NEX = 4) and 1.5 $\mu T$ (NEX = 1) and saturation time $T_{sat} = 200$ ms. This 240 ms CW pulse was the longest permitted by the RF amplifier. The chosen power levels are typical of prior studies focused on saturation transfer from the slow exchanging amide protons. Next, Z-spectra were acquired with $B_1 = 3 \mu T$ (NEX = 1) and 6 $\mu T$ (NEX = 1) and $T_{sat} = 100$ ms. These power levels are more optimal for study of rapidly exchanging lines from amine and hydroxyl protons. The shorter $T_{sat}$ reduces deposited power, the $T_{rho}$ shortening effects of DS (82) and broad macromolecular MTC, and the contribution from MTC asymmetry (82). Total scan time was approximately 30 min.

To further characterize saturation transfer effects in glioma patients, APT-SAFARI images (143) were acquired. The APT-SAFARI imaging sequence consisted of a 3 second pulsed-RF irradiation [Blackman window shaped inversion pulses: pulse width = 9 ms, interpulse delay = 6 ms] followed by a single shot EPI acquisition [$TR = 4000$ ms, $TE$ ranging from 15.8 to 17.4 ms, FOV = 24 cm, matrix = 96 x 96, slice thickness = 8 mm]. One unsaturated $S_0$ image was acquired for reference followed by twenty-four images for the SAFARI scan: 6 at RF offset = +3.5 ppm, 6 at RF offset = -3.5 ppm and 12 with alternating frequency preparations, interleaved in time. Total scan time was 1.5 minutes.

### 4.2.3 Image analysis

Image analysis was performed in MATLAB 7.11 (Mathworks, Natick MA). All images were realigned to the first $S_0$ image using the motion correction algorithm in the SPM8 software package (Wellcome Trust Centre for Neuroimaging, UCL, London). Then, for each acquisition type, images were normalized voxel-by-voxel by the corresponding unsaturated reference image $S_0$.

For the z-spectrum scans, $B_0$ correction was performed following the water saturation shift referencing (WASSR) method (123): The $B_1 = 0.5 \mu T$ z-spectrum was fit voxel-by-voxel to a Lorentzian line in the frequency range ±250 Hz only. The fit function was given by (122):

\[ y = 1 - A \frac{LW^2}{LW^2 + 4(f_0 - x)^2} + B \]  

[4.3]

where LW is the Lorentzian linewidth, $f_0$ is the center frequency, and A and B are scaling constants. The resulting lineshape was evaluated with a frequency resolution of 1 Hz. The
frequency with the minimum fit intensity was assumed to be the center of the water peak. Following the WASSR method, the $B_0$ map derived from the 0.5μT z-spectrum was used to correct all of the z-spectra acquired at higher $B_1$ power. Each z-spectrum was interpolated by a piecewise cubic hermite interpolating polynomial to a resolution of 1 Hz and shifted to center the water frequency at 0 Hz in all voxels. After $B_0$ correction, the z-spectra were resampled back to their original frequency resolution.

The resulting $B_0$-corrected images were used to perform standard asymmetry analysis given by:

$$MTR_{\text{asym}} = \frac{S_{\text{sat}}(\omega_-)}{S_0} - \frac{S_{\text{sat}}(\omega_+)}{S_0}$$  \[4.4\]

Asymmetry maps at the amide proton frequency were generated by averaging $MTR_{\text{asym}}$ across the 425-475 Hz frequency range (referred to as $MTR_{\text{asym}}$ (3.5 ppm)). Similarly, asymmetry maps at the amine proton frequency were generated by averaging $MTR_{\text{asym}}$ across the 200-350 Hz frequency range (referred to as $MTR_{\text{asym}}$ (2 ppm)) Broad macromolecular MTC asymmetry maps were generated by averaging $MTR_{\text{asym}}$ across the 1.5-4 kHz frequency range (referred to as $MTR_{\text{asym}}$ (20 ppm)). Conventional MTC maps were also generated by averaging $MTR=1-S_{\text{sat}}/S_0$ across the -1.5 to -4kHz frequency range and across the +1.5 to +4kHz frequency range, referred to as $MTR$ (-20 ppm) and $MTR$ (+20 ppm), respectively.

Following recently published methods (121, 122), amide and aliphatic proton saturation transfer parameters were also isolated by fitting the low power z-spectrum. Several modifications were made to these high field methods to account for the lower SNR and decreased separation between the amide and aliphatic lines and the water line at 3T. The z-spectrum was constructed voxel-by-voxel from the 0.5 μT acquisition and averaged across specific regions of interest (ROIs) (see below for ROI selection). The resulting mean z-spectrum was interpolated by a piecewise cubic hermite polynomial except at data points in the amide (300 to 600 Hz) and aliphatic (-275 to -700 Hz) frequency ranges. The interpolated z-spectrum was then evaluated at the omitted points in order to calculate the difference between the acquired mean z-spectrum and the interpolated z-spectrum. Amide and aliphatic peak integral values were generated by summing the differences multiplied by the step size (25 Hz) across the amide frequency range (300 to 600 Hz) and the aliphatic frequency range (-275 to -700 Hz), respectively. Units are given in Hz.

For the APT-SAFARI scans, quantitative maps of $MTR_{\text{SAFARI}}$ were calculated voxel-by-voxel as described previously (143):

$$MTR_{\text{SAFARI}} = \frac{S_{\text{sat}}(-3.5 \text{ppm})/S_0 + S_{\text{sat}}(+3.5 \text{ppm})/S_0}{S_0} - \left[\frac{S_{\text{sat}}(\text{SAFARI})/S_0 + S_{\text{sat}}(\text{SAFARI'})/S_0}{S_0}\right]$$  \[4.5\]

where $S_{\text{sat}}$(SAFARI) is the signal after alternating frequency irradiation and $S_{\text{sat}}$(SAFARI') is a similar image but with the order of positive and negative frequency reverse to minimize any timing related systematic errors.

In each glioma patient, three ROIs were selected: two in the tumor and one in the normal appearing brain on the contralateral side. Regions were hand drawn on the $S_0$ images, using the $T_2$-FLAIR and ASL perfusion from the research scan and $T_1$ pre and post Gd from the previously acquired clinical scan as guides. Tumor ROIs were drawn in the region with maximum perfusion hyperintensity and in a region with prior contrast enhancement on clinical scans but no current perfusion hyperintensity. The contralateral ROI was drawn symmetric to the tumor ROIs in the
opposite hemisphere, except for one frontal tumor with bilateral extent. For that tumor a region in the occipital lobe was selected as a control. To compare parameters across regions in all patients, statistical analysis was performed by one-way analysis of variance (ANOVA) with Tukey-Kramer test for multiple comparisons. All across subject data are reported as mean ± standard error.

In healthy volunteers, two regions were selected corresponding to white matter and gray matter. The white matter ROI was located laterally in the occipital white matter. The gray matter ROI was placed in the occipital cortex along the midline. Note that due to the relatively low resolution of the echoplanar images, the gray matter ROI may be partially contaminated by both white matter and cerebrospinal fluid. To compare parameters across regions in all healthy volunteers, statistical analysis was performed by paired t-test with Bonferroni correction for multiple comparisons. Data are reported as mean ± standard error.

4.3 Results

All participants completed the entire study and image quality was acceptable for all studies.

4.3.1 Normal Subject Results

Z-spectra from the healthy volunteers showed the expected features of off-resonance saturation transfer spectroscopy. Broad macromolecular MTC caused increasing attenuation of the image intensity with increasing power and greater attenuation in white matter than gray matter (Figure 4-1a). At 0.5 μT, both white and gray matter z-spectra showed a narrow saturation peak at +3.5 ppm consistent with the APT effect. A broader peak on the opposite side of the spectrum that approximately spans the frequency range from -2.2 ppm to -5.5 ppm (Figure 4-1c) was also observed. This broader peak had previously been attributed to NOE transfer of magnetization from aliphatic protons (4, 16, 50, 51, 53, 82, 118-122). The amide peak appeared somewhat larger in gray matter than white matter. However, the peak integrals showed no statistically significant tissue contrast in the amide or aliphatic regions (Table 4-2). At saturation power higher than 0.5 μT, direct water saturation and broad macromolecular magnetization transfer increased along with higher line broadening of the amide and aliphatic peaks, which were no longer identifiable.
Figure 4-1: a) The z-spectrum derived from occipital white matter (WM) and gray matter (GM) ROIs in healthy volunteers (N = 6) as a function of saturation power. b) MTR\textsubscript{asym} shows a large negative asymmetry, which is more prominent in white matter than gray matter. The amide peak at +3.5ppm is not observed, but a CEST peak from faster exchanging protons, centered in the 2-3 ppm range, can be seen at high power. c) A close up of the amide and aliphatic regions of the z-spectrum acquired at 0.5 \mu T shows saturation peaks centered at +3.5 ppm and approximately -3.7 ppm respectively. The amplitude of the amide and aliphatic peaks were quantified by the difference between the z-spectrum fit (dotted lines) and the acquired data (solid lines) integrated over the frequency ranges delimited by the black boxes. Corresponding line shapes and amplitudes for d) the amide and e) the aliphatic spectral regions. The peaks represent the remainder after subtracting the interpolated fit to the z-spectrum excluding these points from the original 0.5 \mu T z-spectrum data. In subfigures a)-c) error bars are only shown for a subset of acquired data points for clarity.

The MTR\textsubscript{asym} curves (Figure 4-1b) revealed several features. First, there was a large negative asymmetry due to the aliphatic lines and broad macromolecular MTC asymmetry effects. At low RF power (B\textsubscript{l} \leq 1.5 \mu T) this asymmetry peaked close to the water line (offsets < 10 ppm) but remained prominent up to several tens of ppm. The negative asymmetry was maximal at a saturation power of 1.5 \mu T and was greater in white matter than in gray matter. Second, there was a broad positive peak that was most prominent at high saturation power (B\textsubscript{l} \geq 3 \mu T). The peak was centered approximately at 1.6 ppm at 3 \mu T and 2.7 ppm at 6 \mu T. It was much broader and more power dependent than expected for APT, suggesting a faster exchanging component such as amine and hydroxyl protons. The shape, amplitude and frequency of this broad peak were in very good agreement with the saturation transfer peak reported from amine proton
solutions (109) and in-vivo studies of amine exchange (82, 105). Therefore, it can most likely be attributed to fast exchanging amine protons from brain metabolites, other small molecules, and protein and peptide side chains, although an additional contribution from hydroxyl protons cannot be excluded. Third, the APT peak at +3.5 ppm was not seen on the MTR_{asym} curves at any power level as its amplitude was much smaller than that of the negative macromolecular MTC asymmetry and aliphatic proton effects.

Table 4-2: Amide and aliphatic peak integrals derived from the 0.5 μT z-spectrum in healthy volunteers (N = 6) with standard errors. All values were statistically different from zero (P < 0.02). No differences were found between white matter and gray matter regions.

<table>
<thead>
<tr>
<th></th>
<th>Amide peak integral [Hz]</th>
<th>Aliphatic peak integral [Hz]</th>
</tr>
</thead>
<tbody>
<tr>
<td>WM</td>
<td>0.31 ± 0.03</td>
<td>1.01 ± 0.15</td>
</tr>
<tr>
<td>GM</td>
<td>0.56 ± 0.17</td>
<td>0.96 ± 0.09</td>
</tr>
</tbody>
</table>

Table 4-3: Saturation transfer parameters derived from white matter (WM) and gray matter (GM) regions with standard errors across N=6 healthy volunteers. (†) represent statistically significant differences between WM and GM.

<table>
<thead>
<tr>
<th>MTR_{asym} (2ppm) [%]</th>
<th>0.5 μT</th>
<th>1.5 μT</th>
<th>3 μT</th>
<th>6 μT</th>
</tr>
</thead>
<tbody>
<tr>
<td>WM</td>
<td>-0.72 ± 0.02 †</td>
<td>0.65 ± 0.07</td>
<td>2.23 ± 0.08</td>
<td>2.20 ± 0.07</td>
</tr>
<tr>
<td>GM</td>
<td>-0.45 ± 0.04 †</td>
<td>0.96 ± 0.12</td>
<td>2.34 ± 0.11</td>
<td>2.41 ± 0.08</td>
</tr>
<tr>
<td>MTR_{asym} (3.5ppm) [%]</td>
<td>-1.36 ± 0.04 † †</td>
<td>-1.26 ± 0.06 †</td>
<td>0.81 ± 0.04</td>
<td>2.14 ± 0.05</td>
</tr>
<tr>
<td>WM</td>
<td>-0.86 ± 0.05 † † †</td>
<td>-0.35 ± 0.14 †</td>
<td>1.12 ± 0.10</td>
<td>2.31 ± 0.12</td>
</tr>
<tr>
<td>GM</td>
<td>-0.24 ± 0.05 †</td>
<td>-1.08 ± 0.02 † †</td>
<td>-0.87 ± 0.04 † †</td>
<td>-0.41 ± 0.02</td>
</tr>
<tr>
<td>MTR_{asym} (20ppm) [%]</td>
<td>-0.08 ± 0.05 †</td>
<td>-0.62 ± 0.06 † †</td>
<td>-0.52 ± 0.05 † †</td>
<td>-0.15 ± 0.06</td>
</tr>
<tr>
<td>WM</td>
<td>0.48 ± 0.01 †</td>
<td>8.05 ± 0.22 † †</td>
<td>13.20 ± 0.25 † †</td>
<td>26.49 ± 0.39 † †</td>
</tr>
<tr>
<td>GM</td>
<td>0.36 ± 0.04 †</td>
<td>4.92 ± 0.16 † †</td>
<td>8.41 ± 0.25 † †</td>
<td>19.41 ± 0.29 † †</td>
</tr>
<tr>
<td>MTR(+20ppm) [%]</td>
<td>0.72 ± 0.01 †</td>
<td>9.13 ± 0.22 † †</td>
<td>14.07 ± 0.28 † †</td>
<td>26.91 ± 0.39 † †</td>
</tr>
<tr>
<td>WM</td>
<td>0.44 ± 0.04 †</td>
<td>5.55 ± 0.17 † †</td>
<td>8.93 ± 0.27 † †</td>
<td>19.56 ± 0.33 † †</td>
</tr>
<tr>
<td>GM</td>
<td>0.24 ± 0.05 †</td>
<td>5.06 ± 0.16 † †</td>
<td>8.41 ± 0.25 † †</td>
<td>19.41 ± 0.29 † †</td>
</tr>
</tbody>
</table>

† P < 0.04  † † P < 0.008

Quantitative maps and regional averages (Table 4-3) mirrored the qualitative impression of the z-spectra. MTR_{asym}(3.5 ppm, 1.5 μT) maps were negative, suggesting a primary contribution to the signal from broad macromolecular MTC asymmetry. They showed significant tissue contrast with white matter being more negative than gray matter (P = 0.002). Correspondingly, MTR_{asym}(20 ppm, 1.5 μT) maps showed signal amplitude and contrast very similar to the MTR_{asym}(3.5 ppm, 1.5 μT) map despite being acquired at frequency offsets far away from the amide resonance and other mobile protons. As the power increased from 1.5 μT to 6 μT, broad macromolecular MTC increased while the amplitude of the MTC asymmetry decreased. Note that at 3 μT and 6 μT, the saturation time had been reduced to 100 ms instead of 200 ms.
MTR_{asym}(2 \text{ ppm}, 6 \mu\text{T}) maps were positive, suggesting that the primary contribution to the asymmetry at high power is from saturation transfer of fast exchanging protons rather than broad MTC asymmetry. MTR_{asym}(2 \text{ ppm}, 6 \mu\text{T}) maps did not show significant tissue contrast.

4.3.2 Glioma results

Z-spectra in the glioma patients (Figure 4-2) were qualitatively similar to those in the normal subjects. At 0.5 \mu\text{T}, saturation peaks corresponding to amide and aliphatic protons could be seen in the tumor and contralateral regions, similar to those observed in healthy volunteers. The amide peak in the tumor regions appeared increased compared to the contralateral region, while the aliphatic peak appeared decreased. However, the peak integrals did not reveal any statistically significant difference between regions (Table 4-4). No statistically significant differences were detected between the peak integral values measured in the contralateral region and those from healthy volunteers.

![Figure 4-2: a) The z-spectrum derived from the ASL hyperintensity tumor region and the contralateral region in glioma patients (N = 6) as a function of saturation power. For simplicity, the contrast enhancing ROI is not shown as both tumor ROIs overlap on this scale. b) A close up of the amide and aliphatic regions of the z-spectrum acquired at 0.5 \mu\text{T} shows saturation peaks centered at +3.5 ppm and approximately -3.5 ppm respectively. Line shapes and amplitudes for c) the amide and d) the aliphatic spectral regions represent the remainder after subtracting the interpolated fit to the z-spectrum excluding these points from the original 0.5 \mu\text{T} z-spectrum data. In panels a) and b) error bars are only shown for a subset of acquired data points for clarity.](image-url)
At higher saturation power, the amide and aliphatic peaks were no longer identifiable, as was observed in healthy volunteers, due to increasing DS, broad macromolecular MTC and line broadening of the amide and aliphatic proton peaks. There was, however, a large decrease in the overall MTC saturation in the tumor regions compared to the contralateral region, which became more prominent as the power increased.

Table 4-4: Peak integrals derived from the 0.5 µT z-spectrum and SAFARI parameters in glioma patients (N=6) with standard errors. No statistically significant differences were detected.

<table>
<thead>
<tr>
<th></th>
<th>Amide peak integral [Hz]</th>
<th>Aliphatic peak integral [Hz]</th>
<th>MTR_{SAFARI} [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASL hyperintensity</td>
<td>0.48 ± 0.14</td>
<td>0.64 ± 0.14</td>
<td>2.59 ± 0.17</td>
</tr>
<tr>
<td>Gd enhancement</td>
<td>0.55 ± 0.11</td>
<td>0.83 ± 0.13</td>
<td>2.46 ± 0.11</td>
</tr>
<tr>
<td>Contralateral</td>
<td>0.31 ± 0.13</td>
<td>0.83 ± 0.11</td>
<td>2.35 ± 0.06</td>
</tr>
</tbody>
</table>

Table 4-5: Saturation transfer parameters derived from tumor regions (ASL hyperintensity and Gd enhancement) and from contralateral normal appearing brain with standard errors across N=6 glioma patients. (†) represent statistically significant differences between tumor and contralateral regions. No statistically significant differences were found between the two tumor regions.

<table>
<thead>
<tr>
<th></th>
<th>0.5 µT</th>
<th>1.5µT</th>
<th>3µT</th>
<th>6µT</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTR_{asym (2ppm)} [%]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASL hyperintensity</td>
<td>-0.21 ± 0.08 †</td>
<td>1.18 ± 0.18 †</td>
<td>2.16 ± 0.20</td>
<td>2.53 ± 0.18</td>
</tr>
<tr>
<td>Gd enhancement</td>
<td>-0.27 ± 0.07 †</td>
<td>1.07 ± 0.11</td>
<td>2.00 ± 0.10</td>
<td>2.57 ± 0.15</td>
</tr>
<tr>
<td>Contralateral</td>
<td>-0.50 ±0.03</td>
<td>0.64 ± 0.07</td>
<td>2.07 ± 0.08</td>
<td>2.29 ± 0.12</td>
</tr>
<tr>
<td>MTR_{asym (3.5ppm)} [%]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASL hyperintensity</td>
<td>-0.47 ± 0.06 ††</td>
<td>0.32 ± 0.22 ††</td>
<td>1.24 ± 0.13 †</td>
<td>2.31 ± 0.15</td>
</tr>
<tr>
<td>Gd enhancement</td>
<td>-0.52 ± 0.09 ††</td>
<td>0.02 ± 0.24 ††</td>
<td>1.06 ± 0.07</td>
<td>2.23 ± 0.19</td>
</tr>
<tr>
<td>Contralateral</td>
<td>-0.95 ± 0.08</td>
<td>-0.83 ± 0.13</td>
<td>0.76 ± 0.07</td>
<td>2.13 ± 0.04</td>
</tr>
<tr>
<td>MTR_{asym (20ppm)} [%]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASL hyperintensity</td>
<td>-0.06 ± 0.02 †</td>
<td>-0.32 ± 0.06 †</td>
<td>-0.37 ± 0.06 †</td>
<td>-0.07 ± 0.08</td>
</tr>
<tr>
<td>Gd enhancement</td>
<td>-0.07 ± 0.03</td>
<td>-0.35 ± 0.08 †</td>
<td>-0.46 ± 0.05 †</td>
<td>-0.06 ± 0.11</td>
</tr>
<tr>
<td>Contralateral</td>
<td>-0.16 ± 0.03</td>
<td>-0.74 ± 0.04</td>
<td>-0.68 ± 0.04</td>
<td>-0.29 ± 0.03</td>
</tr>
<tr>
<td>MTR_{(+20ppm)} [%]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASL hyperintensity</td>
<td>0.15 ± 0.04</td>
<td>3.96 ± 0.77</td>
<td>6.30 ± 0.89 †</td>
<td>14.32 ± 1.28 †</td>
</tr>
<tr>
<td>Gd enhancement</td>
<td>0.11 ± 0.04 †</td>
<td>4.19 ± 0.85</td>
<td>6.61 ± 1.13 †</td>
<td>14.82 ± 1.61 †</td>
</tr>
<tr>
<td>Contralateral</td>
<td>0.28 ± 0.03</td>
<td>6.05 ± 0.35</td>
<td>10.20 ± 0.43</td>
<td>21.50 ± 0.68</td>
</tr>
<tr>
<td>MTR_{(-20ppm)} [%]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASL hyperintensity</td>
<td>0.21 ± 0.05 ††</td>
<td>4.28 ± 0.81</td>
<td>6.67 ± 0.90 †</td>
<td>14.39 ± 1.31 †</td>
</tr>
<tr>
<td>Gd enhancement</td>
<td>0.18 ± 0.04 ††</td>
<td>4.53 ± 0.88</td>
<td>7.07 ± 1.14 †</td>
<td>14.88 ± 1.62 †</td>
</tr>
<tr>
<td>Contralateral</td>
<td>0.44 ± 0.03</td>
<td>6.79 ± 0.37</td>
<td>10.87 ± 0.44</td>
<td>21.79 ± 0.68</td>
</tr>
</tbody>
</table>

† Regions statistically different from contralateral P < 0.03
†† Regions statistically different from contralateral P < 0.003

Clear differences between tumor and contralateral tissue were apparent in the MTR_{asym} curves (Figure 4-3). At low RF power (B1 ≤ 1.5 µT), MTR_{asym} was much more negative in the normal appearing brain than in the tumor. The difference between MTR_{asym} in the tumor and
contralateral regions, $\Delta MTR_{\text{asym}}$, was maximal at 1.5 $\mu$T and peaked approximately at 3.5 ppm. $\Delta MTR_{\text{asym}}$ remained prominent at frequency offsets greater than 10 ppm. At high RF power ($B_1 \geq 3 \mu$T) this difference faded, especially at low frequency offsets, as the positive broad $MTR_{\text{asym}}$ line attributed to amine exchange became more dominant. There was minimal difference between the amine line shape and magnitude in tumors and contralateral tissue.

**Figure 4-3:** $MTR_{\text{asym}}$ vs. saturation power in glioma (N=6). a) $B_1 = 0.5 \mu$T, b) $B_1 = 1.5 \mu$T, c) $B_1 = 3.0 \mu$T, d) $B_1 = 6.0 \mu$T. The insets show $\Delta MTR_{\text{asym}}$ between the tumor and contralateral region. Error bars are only shown for a subset of acquired data points for clarity.
Quantitative maps (Figure 4-4, Figure 4-5) and regional averages (Table 4-5) confirmed the qualitative impression of the z-spectra. All glioma regions exhibited an elevated $MTR_{asym}(3.5$ ppm, $1.5$ μT), as had been observed in other studies of human high grade brain tumors performed with similar saturation power (25, 26). While the normal appearing contralateral brain had negative $MTR_{asym}(3.5$ ppm, $1.5$ μT), tumors had positive values. Similarly, $MTR_{asym}(20$ ppm, $1.5$ μT) was less negative and $MTR(+20$ ppm) and $MTR(-20$ ppm) were decreased in all tumors compared to the contralateral brain. In contrast, $MTR_{SAFARI}$ showed no significant differences (Table 4-4). At higher power, $MTR_{asym}(2$ ppm, $6$ μT) also showed no significant differences between tumors and normal appearing brain. Suggestive small hyperintensities within the tumors on $MTR_{SAFARI}$ maps and $MTR_{asym}(2$ ppm, $6$ μT) maps were observable in a few of the patients, e.g. Figure 4-4, but further studies would be required to establish the significance of such image features.

Figure 4-4: Saturation transfer maps compared with several other types of standard MR images for a patient with an untreated glioblastoma.
4.4 Discussion

In this chapter, we have obtained saturation transfer measurement at a wide range of frequency offsets in order to assess the individual contributions of saturation transfer from conventional broad macromolecular magnetization transfer contrast and mobile protons such as amide protons, aliphatic protons and amine protons in human glioma tumors at 3T.

4.4.1 Saturation transfer from amide protons, aliphatic protons and broad macromolecular protons in glioma

Our primary finding is that contrast between glioma and normal brain tissue is dominated by broad macromolecular magnetization transfer asymmetry, rather than chemical exchange from mobile protons. The contrast in glioma can be modeled in terms of Eq. [4.2]:

\[ \Delta \text{MTR}_{\text{asym}} = \text{MTR}_{\text{asym}} \text{ (glioma)} - \text{MTR}_{\text{asym}} \text{(contralateral)} \]

\[ = \Delta \text{MTR}^{' \text{asym}} + \Delta \text{APTR} \]
At $B_1$ power less than 2 μT, our study shows a statistically significant increase in $\text{MTR}_{\text{asym}}(3.5 \text{ ppm})$ in the glioma tumors compared to the contralateral brain, as was observed previously (5, 20, 23-26, 111-113) with longer saturation times. However, our results show that most of the signal amplitude and contrast in the $\text{MTR}_{\text{asym}}(3.5 \text{ppm})$ images can be accounted for by the signal amplitude of $\text{MTR}_{\text{asym}}(20 \text{ ppm})$ (e.g., Figure 4-4). Given that $\text{MTR}_{\text{asym}}(20 \text{ppm})$, $\text{MTR}(+20 \text{ppm})$ and $\text{MTR}(-20 \text{ppm})$ were all statistically increased in brain tumors, $\Delta \text{MTR}'_{\text{asym}}$ is a major source of contrast and cannot be assumed to be zero. In contrast, the low power z-spectrum acquired in the tumor region (Figure 4-2b) exhibited a slightly larger amide peak compared to the contralateral brain, but the change in the amide peak integral was not statistically significant (Table 4-4), meaning that $\Delta \text{APT} \sim 0$. Taken together, these results indicate that broad macromolecular magnetization transfer asymmetry, and not APT, is the dominant signal contributor to saturation transfer asymmetry at 1.5 μT. These results also underscore that $\Delta \text{MTR}_{\text{asym}}$ analysis is not sufficient to accurately quantify the amide proton signal change in tumors ($\Delta \text{APT}$) since $\Delta \text{MTR}'_{\text{asym}}$ is also a major contributor to the contrast.

Transfer from protons in the aliphatic spectral region appears to make a major contribution to negative z-spectrum asymmetry at lower powers. This finding confirms recent other reports characterizing the aliphatic lines (121, 122). Since the nature and characteristics of these lines are not well understood, we cannot exclude the possibility that our empirical baseline fitting approach does not appropriately model and quantify these lines. It is clear, however, that they make simple asymmetry analysis inaccurate at low powers because of their overlap with the amide and amine lines, contributing a negative component to $\text{MTR}'_{\text{asym}}(3.5 \text{ppm})$ at low power levels.

Our previously proposed SAFARI technique (143) was highly successful at eliminating magnetic field inhomogeneity errors in this clinical population, but it produced relatively little contrast between tumors and normal tissue. Since SAFARI is mostly sensitive to saturable lines, amine and broad MTC contributions should be minimized. The symmetric nature of the saturation should combine the amide and the aliphatic protons contributions. Therefore, similarly to Eq. [4.1], we can express the SAFARI contrast as:

$$\text{MTR}_{\text{SAFARI}}(3.5 \text{ppm}) = \text{APT} + \text{AliphaticPTR}$$

Since the SAFARI acquisition employs a 3s saturation duration, the APT effect should be much larger than in the z-spectra acquisitions and be near steady-state. Based on the measurements of the amide peak in this chapter and extrapolation to longer saturation duration using Eq. [4.7], the amide contribution to SAFARI is likely about 1.9% (see below for more details on the estimates). The roughly 2.5% SAFARI signal observed in the brain is fairly consistent with this estimate but slightly larger, due to the additional positive component of aliphatic contribution to SAFARI. The expected amplitude of the aliphatic contribution to SAFARI with long saturation is difficult to estimate because the physical properties of the aliphatic protons, including T2, are poorly constrained.

The absence of tumor contrast on the longer saturation time SAFARI images is consistent with the absence of contrast with either the aliphatic or amide line individually as measured at shorter saturation time (Table 4-4). We note that an increase in the APT effect concurrent with a decrease in the aliphatic PTR could provide an alternate explanation for the lack of SAFARI
tumor contrast. To the extent that the aliphatic signal is relatively stable with pathology, the amide contrast in SAFARI could still potentially be a useful diagnostic marker in other disease models.

4.4.2 Chemical exchange saturation transfer of amine protons in glioma

Amine protons from brain metabolites, small molecules, free amino acid and protein and peptide side chains is also a source of endogenous CEST contrast (1, 2, 82, 105, 109). In addition to broad macromolecular MTC asymmetry, amine exchange appears to be a major contribution to MTR asymmetric from 2-5 ppm at the powers above 1.5 µT used here. Since several previous studies have been performed with similar high powers (23-26) they were likely also measuring a significant amine signal. The combination of this positive amine signal with the negative asymmetry of macromolecular MTC leads to complex CEST signal magnitudes and signs. For example, reports of minimal tissue signal at 2 µT (25) can readily be explained by the cancellation of the negative asymmetry in normal tissue by its amine contribution. Despite its major contribution to the signal, amine exchange did not exhibit significant contrast between tumor and brain tissue. However, several individual patients exhibited hyperintensities in the tumor regions, therefore, it remains uncertain whether amine protons can contribute to the diagnostic value of off-resonance saturation imaging beyond the dominant purely broad macromolecular MT contrast previously reported.

4.4.3 Comparison with previous studies

Many studies have identified promising clinical applications of saturation transfer imaging at 3.5ppm as an imaging biomarker for identifying (5, 20, 21, 23, 24, 26, 111), and monitoring treatment response (112, 113) in brain tumors. While the saturation transfer contrast in brain tumors was originally thought to be caused by increases in the APT effect, our results offer an alternate interpretation based on a primary contribution to the contrast caused by the loss of broad macromolecular MTC and MTC asymmetry normally found in the healthy brain. However, care must be taken when comparing our results with other studies as it has been shown here and elsewhere that different saturation schemes, and specifically saturation durations, will change the relative contributions of saturation transfer effects from amide, amine, aliphatic and broad macromolecular protons.

Since this study has employed shorter saturation durations than previous published reports of saturation transfer in brain tumor patients, we can attempt a comparison with the literature by extrapolating our results to longer saturations. In Figure 4-2c, we found a non-significantly higher APTR(3.5ppm) = 0.30±0.05% in tumors than the APTR(3.5ppm) = 0.23±0.04% in the contralateral brain acquired with B1=0.5 µT and Tsat = 200 ms. APTR can be modeled as follows (3, 53, 64, 122):

\[
APTR = x_s \cdot \alpha \cdot k_{sw} \cdot T_{1w} \cdot (1 - \exp(-T_{sat}/T_{1w}))
\]

\[
\alpha \approx \frac{(\gamma B_1)^2}{(\gamma B_1)^2 + k_{sw}^2}
\]

where \(\alpha\) is the saturation efficiency, \(k_{sw}\) is the exchange rate from solute to water, \(T_{1w}\) is the longitudinal relaxation time of water and \(x_s\) is the concentration of amide protons relative to...
water protons. If we assume for simplicity that the difference in APTR between brain tumors and normal brain is due only to a difference in amide concentration $x_s$, while $k_{sw}$ and $T_{1w}$ are unchanged, we can estimate the steady state values for APTR at long saturation time (APTRss). Given $T_{1w}=1.5s$ and $k_{sw}=30Hz$, we find APTRss~1.9% in contralateral brain and APTRss~2.5% in glioma, giving a maximum $\Delta$APTR~0.6%, independent of saturation power for $B1 \geq 1\mu T$. These estimates are in very good agreement with recent steady state reports of APTR~1.5-2.5% in the normal rat brain (121) and APTR~1.4-2.9% in the normal human brain (122). Prior reports of saturation transfer contrast in brain tumor patients (23-26) typically employed $500 ms \text{ saturation at 3T}$. For $T_{sat} = 500ms$, we estimate APTR~0.54% in contralateral brain and APTR~0.71% in tumors, giving $\Delta$APTR~0.17%. Since these studies typically report $\Delta$MTR$_{asym}$ values on the order of 2% it is likely that the contrast previously reported in brain tumors was also primarily the result of changes in MTR$_{asym}$ rather than APTR, as measured in this study.

Since the saturation transfer contrast between tumors and normal tissue has previously been ascribed to an increase in free protein concentration in malignant cells solely based on an assumed major contribution from amide proton exchange (5, 20, 23, 24, 113), this understanding of contrast is brought into question. Our results show that increases in MTR$_{asym}$ (3.5 ppm, 1.5 $\mu$T) in tumors coincide with decreased saturation transfer from broad macromolecular MTC and loss of MTC asymmetry compared to the normal brain (Table 4-5). The existence of similar contrast (Figure 4-4, Figure 4-5) in the unsubtracted broad macromolecular magnetization transfer ratio at both positive and negative frequencies distant from the exchanging lines of mobile protons suggests that differences in MTR, rather than in its fractional asymmetry, are most responsible for the contrast in brain tumors. Previous studies have also shown that broad macromolecular MTC is altered in glioma (35, 153-156) with higher MTR in normal brain than in brain tumors and higher MTR in high grade than low grade glioma (155), which was shown to correlate with tumor nuclear density.

### 4.4.4 Technical Considerations

This study demonstrates that a low power (0.5 $\mu$T, $T_{sat} = 200 ms$) z-spectrum acquisition can successfully identify the amide and aliphatic peaks at 3T, similar to recent higher field results (121, 122). However, our results highlight the difficulties of imaging the amide CEST signal. Due to the decreased SNR at 3T compared to higher field strengths, voxel-by-voxel fitting could not be performed reliably without significant noise contamination. Instead, fitting was performed after averaging the z-spectrum over each ROI. Note that our pulse sequence had a short CW saturation of 200 ms and that a longer saturation pulse increasing the amide saturation should be beneficial and could improve the statistical significance of the result.

This study also demonstrates how choice of frequency, power, and duration could be used to separate contrast from different sources. To the extent that MTR or MTC asymmetry is a useful diagnostic marker, this contrast can be obtained without CEST contamination at large frequency offsets. In contrast, the amine component can be emphasized at low frequency offsets by high power irradiation for short duration (82). This maximizes the fast exchanging amine contribution while minimizing MTC asymmetry and saturation related $T_1$ shortening. Both these imaging methods demonstrated the capability to create high quality, robust images from clinical patients.
in quite reasonable scan times. Further research will be needed to fully characterize the
diagnostic value of the methods in glioma and other diseases.

4.5 Conclusion

Tumor contrast from off-resonance saturation transfer images usually attributed to amide
proton transfer and protein concentration is instead the result of MTC and MTC asymmetry
differences. While the amide peak is detectable, it is a weak signal that requires careful
optimization and analysis to avoid contamination from other sources. A previously reported
strategy for measuring the amide signal, SAFARI, demonstrated excellent image quality but
showed no contrast in glioma. Amine exchange sensitive imaging showed promise for producing
relatively robust and high signal measures of exchanging protons with minimal contamination
from other sources when shorter saturations with higher powers are used.
Chapter 5: Summary and Future Work

5.1 Summary

In this thesis, we have developed a novel MRI pulse sequence - saturation with frequency alternating RF irradiation (SAFARI) - in combination with a new magnetization transfer ratio parameter designed to generate chemical exchange saturation transfer (CEST) images of amide protons free of errors induced by direct water saturation and magnetization transfer asymmetry. In addition, we have shown how the choice of frequency, power, and duration in continuous wave saturation preparations could be used to separate contrast from different sources in order to maximize the chemical exchange signal from amine protons while minimizing magnetization transfer asymmetry. We demonstrated the use of these techniques for the measurement of exchange parameters in healthy volunteers and applied them towards a study of saturation transfer imaging in patients with recurrent brain tumors. Our results show that the saturation transfer contrast in brain tumors, which was previously attributed to an increase in the CEST signal from amide protons due to an elevated protein concentration, is instead the result of the loss of MT asymmetry found in the normal brain.

5.2 Contributions

5.2.1 CEST imaging insensitive to $B_0$ inhomogeneity

CEST imaging is notoriously prone to artifacts due to $B_0$ inhomogeneity and requires long acquisition times to correct these artifacts, making it unsuitable for clinical translation in its current form. Our new SAFARI technique was designed to be insensitive to $B_0$ inhomogeneity. In Chapters 2 and 3, we have shown that SAFARI can reliably measure the CEST effect from amide protons even in the presence of $B_0$ inhomogeneity. Our results indicate that $\text{MTR}_{\text{SAFARI}}$ can measure the amide signal accurately in the presence of $B_0$ shifts up to about ±180Hz for pulsed SAFARI and 150Hz for CW-SAFARI at 3T. Because this frequency range is well above the range of susceptibility offsets that were observed in the brain, SAFARI removes the need for additional $B_0$ correction of CEST images. Therefore, using SAFARI, robust CEST images can be generated from a 4-image acquisition instead of the full z-spectrum acquisition, typically needed to correct standard $\text{MTR}_{\text{asym}}$ images. This dramatically reduces scan times and post-processing manipulation of images, which will allow the introduction of CEST as a clinical imaging tool.

5.2.2 CEST imaging insensitive to MT asymmetry:

Another major challenge in the acquisition of CEST images in the brain has been the presence of the intrinsic macromolecular MT asymmetry (54, 73, 117), which contaminates the images. Because of this negative background, typical brain saturation transfer images have a signal amplitude that is near zero or negative (4, 5, 10, 23, 74, 111), indicating that the CEST effect is being canceled by the MT asymmetry. Not only does $\text{MTR}_{\text{SAFARI}}$ largely remove the
need for $B_0$ correction, it also eliminates MT contamination. In addition to SAFARI, we have shown that a high power CW CEST acquisition was highly successful at generating amine exchange contrast with reduced MT asymmetry. Therefore, amine CEST images can also be obtained with fewer errors caused by MT asymmetry. As a result, the \textit{in-vivo} SAFARI signal of amide proton or CEST signal of amine proton exchange can be modeled by a two-pool exchange model, rather than the three-pool model that would be needed to account for MT. This greatly simplifies the modeling and quantification of exchange parameters \textit{in-vivo}.

### 5.2.3 Pulse sequence development

In Chapters 2 and 3, we have introduced two different pulse sequences to perform SAFARI imaging. Chapter 2 focused on a pulsed version, where dual frequency preparation was achieved by alternating the frequency of the RF pulses between the control and label frequency. Chapter 3 focused on a CW version, where dual frequency preparation was achieved by on-resonance CW saturation with sinusoidal amplitude modulation. We demonstrated that both methods can successfully remove errors due to $B_0$ inhomogeneity and MT asymmetry from CEST images of the human brain.

The pulsed SAFARI version has the advantage that long saturation times can be employed. This allows the achievement of steady state conditions and maximizes the saturation efficiency of exchangeable protons. However, the effective RF power of the saturation train is a complicated function of RF pulse width, interpulse delay, flip angle and saturation time, which makes optimization of the pulse sequence for different applications complicated. In comparison, the CW sequence was constrained to a 250 ms long RF irradiation due to hardware limitations on our scanner. As a result, the saturation efficiency is much smaller than with pulsed-SAFARI, which reduces the amplitude of the CEST effect. However, the CW version has the advantage that the signal depends only on the saturation time and RF power. This simplifies sequence optimization, modeling and interpretation of results. It also allows for the straightforward modulation of RF power, which can be tuned to optimize the contrast from different exchangeable protons. In addition, the short saturation duration minimizes $T_1$ effects that can complicate quantification.

### 5.2.4 Translation to clinical scanners

One of the goals of this thesis was to optimize CEST imaging methods for \textit{in-vivo} imaging on clinical scanners. Many of the initial CEST papers employed saturation schemes that were optimized to maximize the CEST contrast but were only applicable on animal or research scanners. Several second long CW saturation, which maximizes the saturation efficiency, was often used in animal studies. But hardware limitations on clinical scanners do not allow for CW irradiation longer than a few hundreds of milliseconds. Even pulsed saturation schemes often employed high RF duty cycles that are limited on clinical scanners or require the use of multiple transmitters not available on all manufacturers. The pulse sequences presented in this thesis were optimized keeping in mind the restrictions limiting clinical scanners. For the pulsed-SAFARI sequence, the RF duty cycle was optimized with the constraint of remaining less than 60%. For the CW-SAFARI version, the RF irradiation was kept under 250ms. In addition, the RF duration
was reduced as the power was increased in order to remain below SAR limits for *in-vivo* imaging. Therefore, we anticipate that the methods developed in this thesis could be translated to any 3T clinical scanner with no further optimization.

In addition, we note that the SAFARI sequence consists of a saturation module followed by a standard EPI acquisition. No specialized image reconstruction methods are required. Therefore, the SAFARI saturation module could easily be placed in front of any preferred readout scheme for translation to other scanner manufacturers and other body applications. Currently, SAFARI post-processing has been performed off-line. However, image processing consists of a simple image subtraction rather than the more complicated z-spectrum fitting and $B_0$ correction methods needed to analyze standard CEST images. Therefore, SAFARI post-processing could easily be performed online for clinical imaging applications.

### 5.2.5 SAFARI imaging of amide proton transfer

We have focused on optimizing SAFARI to image endogenous amide proton transfer. The pulse shape, width and repetition rate of the pulsed-SAFARI RF train was selected to maximize saturation of amide protons while minimizing direct water saturation. For the case of amide proton exchange, we found that a 9ms Blackman-shape pulse with $T_{RF} = 15$ms produced a saturation bandwidth broad enough to saturate the amide protons at 3.5ppm even in the presence of $B_0$ shifts, while within the constraint that the bandwidth not be so broad as to produce strong direct water saturation. This pulse train has an equivalent CW power of 0.78μT, ideally suited to maximize the saturation efficiency of amide protons, as described in Section 1.2.3. For the CW SAFARI acquisition, calibrations have shown that powers less than 2μT must be employed to effectively remove direct water saturation and MT. Again this is ideal for saturation of slow exchanging amide protons. By using a CW RF irradiation of the amide protons rather than the pulsed irradiation, the saturation bandwidth decreases, which enables the clear identification of the amide proton peak in the human brain at 3T and the quantification of protein concentration and amide exchange rates *in-vivo*. Therefore, SAFARI imaging of amide proton exchange may have important applications for quantitative pH measurements in stroke and cancer imaging that could be explored in future studies.

### 5.2.6 SAFARI imaging of other endogenous exchangeable protons and exogenous CEST contrast agents:

It may also be interesting to apply SAFARI for CEST imaging of other exchangeable protons such as endogenous amines and hydroxyl protons in order to obtain contrast related to small molecules and brain metabolites. In order to successfully employ SAFARI for imaging of amine or hydroxyl protons, two assumptions must be met: saturation of the water line must be linear with power, i.e. direct water saturation must be small, and the exchangeable protons must be mostly fully saturated. In Chapter 2, we have shown that for the first assumption to hold, the RF offset of the SAFARI saturation must be at least 2.1ppm from the water line. For the second assumption to hold, the exchange rate must be smaller than 200Hz.
At physiological conditions, amine protons and hydroxyl protons from small molecules and amino acid side have fast chemical exchange rates on the order of 1,000 to 30,000 Hz \((27, 87)\). Amine protons resonate primarily between 2 to 3 ppm from the water resonance and hydroxyl protons between 0.5 to 2 ppm. Therefore, the SAFARI sequence with the pulse train parameters described in Chapter 2 cannot be used to generate CEST contrast from amine and hydroxyl protons. Since these protons have smaller chemical shifts than amide protons, different pulse parameters must be employed to provide a more selective saturation. However, because the RF duty cycle is limited to 60% on our scanner, the interpulse delay must increase with increasing pulse width. As a result, it is unlikely that enough power could be applied to saturate the fast exchanging protons.

In Chapter 3, we have described a CW version of the SAFARI strategy. Because the power can be easily increased, beyond the capabilities of the pulsed sequence, CW-SAFARI may be more appropriate for imaging of fast exchanging protons. In this case, full saturation of amine and hydroxyl protons can be achieved with appropriately high RF power. However, results in Chapter 3 have shown that as the power increases beyond 2\(\mu\)T, direct water saturation becomes significant and SAFARI fails because the first assumption is not met. This effect will be amplified for amine and hydroxyl protons, which have resonance frequencies closer to the water line. Therefore, CW-SAFARI also cannot be employed for the measurement of endogenous amine and hydroxyl exchange.

While this thesis has focused on the measurement of endogenous chemical exchange saturation transfer contrast, there is a broad literature on the development of exogenous CEST contrast agents using lanthanide-based chelates \((7, 157, 158)\). These agents can have exchangeable protons with resonances several tens to hundreds of ppm from water and exchange rates of a few thousand Hertz. Because their resonance frequency is much higher than endogenous protons, higher RF powers can be employed with minimal direct water saturation. Hence, CW-SAFARI may be ideal for imaging such compounds.

### 5.2.7 Understanding contributions to the saturation transfer signal in the healthy human brain

The field of endogenous amide proton transfer (APT) imaging has grown quickly over the last ten years \((2, 3, 53)\) and more and more applications of APT are being explored. However, there is still a lack of understanding of the different contributions to the saturation transfer signal \textit{in-vivo} that contaminate the APT signal and many published results may have been based on erroneous assumptions. In this thesis we have made a concerted attempt to develop and apply methods to separate MT asymmetry from amide, aliphatic and amine effects for \textit{in-vivo} imaging rather than focusing on the contrast properties of the combination. As a result, this has lead to a new understanding of the different sources of signal in saturation transfer images of the brain. It is important to understand which forms of contrast dominate a particular acquisition so observed differences in pathology can be best understood in relation to their physiology and so acquisitions that best highlight the contrast can be optimized.
The CEST signals from individual exchangeable protons on specific molecules are easily identifiable in phantom studies of single molecules. In these simple models, it can be shown that specific exchangeable protons, such as the amine protons from creatine or glutamate, can be selectively saturated by tuning the applied RF frequency and power to the resonant frequency and exchange rates of these protons (100, 105, 109). In previous work, it has often been assumed that such RF optimization can also be employed in-vivo to selectively generate CEST contrast from specific protons. The data presented in this thesis, however, challenge this assumption. Signal contributions in-vivo are much more complicated than in simple phantoms, as a multitude of different molecules and proteins contain exchangeable protons with a broad range of resonance frequencies and exchange rates. In addition, the CEST peaks broaden as the RF saturation power increases, increasing the overlap between different exchangeable protons on the z-spectrum. Therefore, for in-vivo imaging, exchangeable protons from many different sources will contribute to the CEST contrast at a given frequency offset and power.

In the early days of CEST imaging, it was recognized that the saturation transfer signal in-vivo at 3.5ppm was a combination of amide proton transfer and macromolecular magnetization transfer, leading to negative MTRasym values in the normal brain. However, other sources of saturation transfer such as hydroxyl protons, amine protons and aliphatic protons had mostly been ignored. Typical published z-spectra showed featureless broad saturation effects due to direct water saturation and MT but failed to capture any narrow CEST peaks from endogenous protons. The corresponding MTRasym spectra typically showed a very broad peak centered around ~2ppm and a negative asymmetry at frequency offsets larger than about 3-4ppm as seen in Figure 1-15 and Figure 1-18. The amplitude of this broad peak typically increased with saturation power, which was attributed to an increase in amide proton saturation, even though the amide peak at 3.5ppm was not distinguishable from the broader feature.

The results in this thesis have helped shed new light on the different contributions to the in-vivo saturation transfer signal. We have shown that the amide proton peak can be seen on the z-spectrum at 3T, when a low saturation power is applied. This is in agreement with recent high field results (121, 122). The steady state amplitude of the amide peak was estimated to be about 2%, as was measured with our SAFARI acquisition. We note that the power level needed to measure the amide peak was much lower than had been typically employed for APT imaging. For saturation power of 1.5μT and higher, direct water saturation and broad macromolecular magnetization transfer increased along with higher line broadening of the amide peak, which could no longer be identified, explaining why previous studies have failed to identify the amide proton peak in-vivo. Another feature that was apparent from the low power acquisition was the presence of saturation transfer from NOE of aliphatic protons centered roughly at -3.5ppm. The amplitude of the aliphatic peak was about twice that of the amide peak. Therefore, aliphatic protons will tend to cancel out the APT effect and add to the negative component of macromolecular MT when asymmetry analysis is used. As a result, the amide peak is not detectable on MTRasym spectra.

We also found that amine exchange is a major contribution to MTRasym from 2-5 ppm at RF powers above 1.5 μT. This explains the broad MTRasym peak that has typically been measured in previous studies. Because amine protons resonate primarily around 2ppm, it had not been recognized that they would introduce significant contamination to the amide signal at 3.5ppm. In
In conclusion, the in-vivo saturation transfer at 3.5ppm is a combination of direct water saturation, macromolecular magnetization transfer, amine proton exchange and amide proton exchange. When asymmetry analysis is used to attempt to isolate the APT effect, direct water saturation contributions are removed but magnetization transfer asymmetry and aliphatic protons effects are added to the saturation transfer signal. For applications where the measurement of the APT effect is of interest, it can be maximized by a low power long RF saturation. While the amide peak is then detectable, it is one of the weaker signal contributions and requires careful optimization and analysis to avoid contamination from other sources. In contrast, the amine component of the saturation transfer signal can be emphasized by high power irradiation for short duration. This maximizes the fast exchanging amine contribution while minimizing MTC asymmetry.

5.2.8 Understanding contributions to the saturation transfer contrast in brain tumors

Many studies have measured the saturation transfer signal in brain tumors. It has been shown that MTR$_{asym}$(3.5ppm) is elevated in tumors compared to the normal brain (5, 20, 23-26, 111-113), allowing the identification and delineation of brain tumors (5, 20, 21, 23, 24, 26, 111) and monitoring of treatment response (112, 113). The contrast in brain tumors was originally attributed to increases in the APT effect, due to an increase in free protein concentration in malignant cells (5, 20, 23, 24, 113). The results in this thesis, however, offer an alternate interpretation.

Our primary finding is that contrast between glioma and normal brain tissue is dominated by broad macromolecular magnetization transfer asymmetry, rather than chemical exchange from mobile protons. Similar to previously published results (5, 20, 23-26, 111-113), the results in Chapter 4 show an increase in MTR$_{asym}$(3.5 ppm) in glioma compared to the contralateral brain. Direct measures of the amide proton peak and aliphatic proton peak, however, did not reveal any significant increase in the APT effect in tumors. Measures of the amine proton transfer effect also revealed no contrast in tumors. Instead, we found that the intrinsic macromolecular
magnetization transfer ratio and magnetization transfer asymmetry were increased in brain tumors. Therefore, our results offer a new understanding of the saturation transfer contrast in brain tumors, where broad macromolecular magnetization transfer asymmetry, and not APT, is the dominant signal contributor. While MTR\textsubscript{asym}(3.5 ppm) may become a useful imaging biomarker of brain tumors, it is unlikely that it specifically reflects changes in free protein concentration in tumors.

Our findings in glioma have several implications for the design and analysis of future saturation transfer studies of brain tumors. Initial studies of the CEST contrast in tumors noted that the total saturation transfer signal MTR\textsubscript{asym} measured in-vivo is the sum of the intrinsic asymmetry (MTR'\textsubscript{asym}) of the broad macromolecular resonance and the amide proton transfer ratio (APTR). However, it was commonly assumed that the difference in MTR\textsubscript{asym} between tumor and normal brain, \( \Delta \text{MTR}\textsubscript{asym} \), was predominantly a measure of the APT effect in the tumor, i.e.:

\[ \Delta \text{MTR}\textsubscript{asym} = \Delta \text{APTR} \]

implying,

\[ \Delta \text{MTR}'\textsubscript{asym} = 0 \]

This relationship has been used in many papers to assess what was thought to be the CEST contrast from amide protons in tumors. Our results show, however, that this assumption is not accurate and \( \Delta \text{MTR}'\textsubscript{asym} \) is not only significantly different in glioma than in the normal brain but it dominates over any other source of contrast. In fact, changes in magnetization transfer contrast had previously been observed in CEST studies (111) and in magnetization transfer studies (35, 153-156) of glioma. As a result:

\[ \Delta \text{MTR}\textsubscript{asym} = \Delta \text{APTR} + \Delta \text{MTR}'\textsubscript{asym} \]

Therefore, our results underscore the failure of \( \Delta \text{MTR}\textsubscript{asym} \) analysis to isolate the amide proton transfer effect from the macromolecular magnetization transfer effect. If \( \Delta \text{APTR} \) and protein concentration measurements are quantities of interest in brain tumors, alternate acquisition and analysis methods must be developed to measure them more accurately, such as the ones presented in this thesis. In contrast, if the macromolecular MT asymmetry is a quantity of interest, pulse sequences that maximize the MTC signal should be used instead. Further studies will be needed to assess the efficacy of these different methods to localize and predict treatment response in brain tumors.

5.3 Future Work

This thesis has contributed to the CEST literature by furthering our understanding of the different signal contribution to the saturation transfer contrast in the human brain. The results presented here have raised questions regarding the interpretation of previously published CEST results that should be addressed in future work. In addition, by providing new acquisitions techniques that allow for faster and more robust CEST imaging, the methods developed in this thesis may find applications in a range of other clinical applications reviewed below.
5.3.1 Imaging brain tumors and other cancers

The SAFARI technique developed in this thesis was highly successful at eliminating magnetic field inhomogeneity and MT asymmetry errors in brain tumor patients, but it produced relatively little contrast between tumors and normal tissue in most patients. Amine proton exchange with CW saturation at high power was also found to produce robust images with high signal-to-noise ratio, but also showed little contrast in tumors. Still, several individual patients, particularly those with newly diagnosed untreated tumors, exhibited hyperintensities on both MTRSAFARI and MTRasym (2 ppm, 6 μT) in the tumor regions. Therefore, it remains unclear whether CEST may contribute slightly to the contrast in untreated glioblastoma tumors. Additional studies with larger sample sizes will be needed to assess whether there is a difference in the contribution from CEST of amine and amide protons between recurrent versus untreated tumors.

Recently, saturation transfer imaging has been evaluated as a marker of breast cancer (116) and prostate cancer (21). In human prostate cancer, for instance, MTRasym was elevated in histology-confirmed cancerous tissue and appeared to correlate with tumor grade (21). While our data has shown that the saturation transfer contrast in brain tumors is caused by a loss of the magnetization transfer asymmetry from the normal brain, it is unclear what the primary contributors to contrast in extracranial tumors may be. High magnetization transfer ratio values are found in many organs such as the brain, liver, spleen, kidney, cartilage and muscle (159). But, while it is well known that macromolecular magnetization transfer is asymmetric in the brain (54, 73) and spinal cord (160), the presence of MT asymmetry has not yet been measured outside of the nervous system. Therefore, several additional studies are needed to assess the sources of saturation transfer contrast in body tumors. First, we must determine whether MT asymmetry also exists outside of the brain. Second, studies similar to the one presented in Chapter 4 will be needed to identify the major sources of saturation transfer contrast in extracranial cancers and assess whether magnetization transfer asymmetry, chemical exchange from mobile protons or both are responsible for the contrast measured previously.

While our data have shown the CEST signal of mobile protons is not a major contributor to contrast in brain tumors at baseline, the question remains whether it may be sensitive to treatment response. Particularly, tumor pH has been shown to play an important role in tumor growth, aggressiveness and response to therapy, as described in recent reviews (161-165). Briefly, the combination of poor perfusion and high metabolic rates found in tumors generate an acidic extracellular pH (pHe) environment (166, 167), while the intracellular pH (pHi) of brain tumors tends to be weakly elevated (166, 168-170). This creates a reversed pH gradient compared to normal tissue, which increases as the tumor progresses. In the preclinical stage, the high pHi drives cell proliferation (171-173) and exacerbates the decrease in pHe by promoting aerobic glycolysis (164, 174-176). The resulting low pHe, along with other factors, triggers angiogenesis required for the tumor to grow beyond the preclinical stage (177-179). As the tumor progresses, low pHe is associated with genetic instability, loss of cell-cell contacts, increased cell motility and degradation of the extracellular matrix, thereby facilitating invasion and metastasis (180-184). Tumor pH also governs the response of cancer cells to therapy. High pHi inhibits the apoptosis of cancer cells, imparting a physiological drug resistance. In addition, low pHe reduces sensitivity to radiation therapy (185, 186) while pHe, pHi and the pH gradient...
all modulate the efficacy of specific chemotherapeutic drugs (187-190). Because the pH of brain tumors is only slightly elevated compared to the normal brain, the studies presented in this thesis did not detect any CEST contribution to the contrast of recurrent brain tumors. Therefore, it is unlikely that CEST imaging will be able to predict the best treatment to pursue based on baseline tumor pH.

Tumor pH can vary dramatically after therapy, however, and CEST imaging may play a role as an early marker of treatment response. It has been shown that tumor intracellular pH significantly changes with therapy (191). Several preclinical and clinical studies have shown that tumor intracellular pH is affected by radiation treatment. pH was found to decrease immediately following irradiation (192) followed by a reversal at about 12 hours and subsequent increase anywhere from 0.2 to 0.7 pH units 1 to 2 days after radiation (193-199). Several of these studies found that the increased pH correlated with increased necrosis, decrease tumor volume and cell survival. Changes in tumor pH have also been observed following various chemotherapy regimens. For example, tumor pH increased by about 0.2 pH units 48 to 72 hours after treatment by 5-fluorouracil in RIF-1 tumors (200, 201). In Friend leukemia cell tumors in mice, pH increased by 0.2 pH units at 6 hours and 0.4 pH units at 20 hours after injection of tumor necrosis factor (TNF) (202) and by 0.4 pH units after 7 days of daily interferon injections (203). Finally, in patient studies, increases in tumor pH following chemotherapy have been observed in various musculoskeletal cancers (204). Therefore, tumor pH may provide an early indicator of tumor sensitivity to radiation and chemo-therapies.

Additional studies are needed to assess whether CEST and SAFARI imaging can detect treatment induced pH changes in different tumors. Recently, CEST imaging was performed in breast cancer patients before and after one cycle of neoadjuvant chemotherapy (116). It was found that the APTR decreased in a patient that went on to be a complete responder while it increased in a patient who went on to have progressive disease. Using the methods presented in Chapter 4, saturation transfer imaging studies of tumors undergoing therapy should be performed to determine whether the amide proton transfer signal is altered following treatment. Then, the quantitative methods developed in Chapter 3 could be applied to assess the change in exchange rate and concentration of amide protons, in order to confirm the pH dependence of the CEST signal. If successful, CEST imaging could become an early marker for the effectiveness of anti-cancer therapy and may facilitate more personalized designs of treatment protocols.

5.3.2 Imaging brain function and neuronal signaling

Blood oxygen level dependent (BOLD) functional MRI has rapidly become the most widely used technique to map brain activity. The success of BOLD fMRI has highlighted, however, how little is known about the coupling between neuronal and glial activity and vascular response, and what metabolic and functional purpose it serves. As a result, the spatial specificity of blood flow regulation and the local vascular distribution bias the localization of functional activation. Therefore, metrics of brain cellular functional activity other than BOLD fMRI are needed to help probe neurovascular coupling physiology. One possible candidate for such measurement is the pH shift that occurs inside neurons and supporting glial cells during neuroactivation.
Brain pH is highly regulated and normal intracellular brain pH is estimated to be between 7.0 and 7.15 pH units (205-209). In the normal healthy brain, the acid-base behavior of glial cells and neurons is governed primarily by local neuronal activity. Experiments in cell cultures and animals show an intracellular acidification on the order of 0.1 pH units in mammalian neurons concurrent with an intracellular alkalization of astrocytes and oligodendrocytes on the order of 0.3 pH units during neuronal activity (210). Therefore, on a voxel scale we might expect an increase in intracellular pH with brain activation. Attempts at measuring physiologic pH alterations in the human brain during functional activation using phosphorous MR spectroscopy, however, have yielded conflicting results. While some studies report pH increases up to 0.22 pH units during visual stimulation in healthy volunteers, others found no statistically significant pH changes (211-214).

We may perform additional studies to investigate the potential of amide proton exchange with SAFARI and amine proton exchange techniques developed in this thesis to detect pH alterations in the healthy human brain during functional activation. Because pH changes occur directly inside activated neurons and supporting glial cells, pH fMRI has the potential to provide a direct measure of cellular functional activity. The successful mapping of functional neuroactivation using pH weighted MRI would enable the detailed study of neurovascular coupling and potentially improved spatial and temporal specificity of fMRI studies.

5.3.3 Imaging brain pH and the ischemic penumbra in stroke

Ischemic stroke is caused by the thrombosis of arteries in the brain. The resulting shortage in blood supply leads to a decrease in the concentration of oxygen and glucose available for metabolism and an accumulation of acidic waste products. Ischemia will quickly induce cell death unless the blood flow can be reestablished in a timely manner. Recombinant tissue plasminogen activator (tPA) is administered in acute stroke patient to dissolve the clot. However, because the injection of tPA can cause hemorrhage, there is a narrow window during which it must be administered, within 3 to 4.5 hours of symptom onset (215, 216). This minimizes the risks for adverse events post thrombolytic therapy. However, individual patients may benefit from tPA therapy beyond the initial 4.5 hours, despite the increase risk of hemorrhage, if it can be determine that the affected brain tissue is still salvageable and would otherwise progress to infarction (217-219). Therefore, one of the major challenges in clinical acute stroke imaging is the identification of salvageable brain tissue, known as the ischemic penumbra.

CEST imaging offers the potential to identify the ischemic penumbra based on the lesion’s pH. Standard MRI measurements often used for this purpose, such as the diffusion-perfusion mismatch (220) remain controversial since it remains uncertain whether they can accurately identify and delineate the penumbra (221, 222). Thus, more accurate methods are needed to help guide treatment. Because ischemic damage is in part caused by pH changes (223), CEST imaging of pH may provide a marker of tissue viability in stroke imaging. In the healthy brain, intracellular pH is tightly regulated. During stroke, the decreased blood flow and perfusion slows the removal of metabolic acid resulting in acidification of the intracellular environment. Once the pH decreases past 6.4 pH units, pH-related cellular damage is triggered (223). Acidic intracellular pH elicits the production of free radicals contributing to tissue damage (224-228). Acidic pH also deregulates the calcium balance of the cells, which can activate apoptosis (229,
Therefore, the measurement of intracellular pH could predict the potential for cell death after stroke.

Extensive preclinical studies of CEST imaging in animal stroke models have been performed. The saturation transfer contrast at 3.5 ppm decreases in the ischemic lesion (8-15) correlating with pH (10, 14). CEST had also been shown to help identify regions within the diffusion-perfusion mismatch area that correspond to the ischemic penumbra and distinguish it from benign oligemia (8, 9). The methods developed in this thesis could improve upon these initial preclinical studies by enabling the quantitative measurement of pH. In addition, the faster acquisition time of the SAFARI method compared to a standard z-spectrum acquisition is crucial for the translation of CEST imaging to acute stroke patients. Therefore, SAFARI imaging could play a role in the early and accurate identification of potentially salvageable versus irreversibly infarcted brain tissue, thereby helping in the identification of patients who may still benefit from late recanalization or neuroprotective treatment.

### 5.3.4 Imaging neurodegenerative diseases

Another class of diseases where CEST and SAFARI imaging may become useful are neurodegenerative disorders. A broad variety of neurodegenerative diseases are characterized by the accumulation of protein (or peptide) aggregates (231). The most common neurodegenerative disorder, Alzheimer's disease, is initiated by the deposition of β-amyloid (Aβ) peptide into extracellular plaques (232, 233). Other hallmarks of the disease, such as neurofibrillary tangles and cell and synapse loss, are thought to be secondary to Aβ aggregation (234). Many other neurodegenerative diseases are also caused by abnormal protein aggregation. Parkinson's disease is characterized by the deposition of α-synuclein into cytoplasmic inclusions known as Lewy bodies in dopaminergic neurons (235). Huntington's disease and other polyglutamine diseases are caused by an increased number of the CAG trinucleotide repeats, resulting in a polyglutamine tract in the abnormal protein (196, 236). Longer expansions correlate with earlier onset and more severe disease. The expanded polyglutamine form neuronal intranuclear inclusions that correlate with cell toxicity (196, 237). Other groups of disorders such as prion diseases (238, 239) and tauopathies (240) are also caused by the accumulation of abnormal proteins, namely prion protein into plaques and tau protein into cytoplasmic tangles, respectively. Because CEST imaging is sensitive to protein concentration, it is possible that it may be able to detect these protein accumulations in at risk populations before clinical symptoms become evident, enabling early intervention in a wide range of neurodegenerative disorders. However, once the abnormal proteins start to aggregate, they become insoluble. Therefore, it is not entirely clear whether they would actually result in an increased concentration of exchangeable amide protons, resulting in increased CEST signals. In-vitro and in-vivo studies will be needed to assess whether the different protein aggregates (i.e. plaques, tangles, Lewy bodies, etc) responsible for neurodegenerative disorders increase the CEST signal. In this case, CEST imaging may be able to successfully identify early stages of disease in patients at risk for neurodegenerative disorders and could become an important early biomarker of neurodegenerative diseases.
5.3.5 Conclusion

In this thesis, we have introduced new methods for fast and robust chemical exchange saturation transfer MR imaging. We have demonstrated how these techniques can be used to improve the understanding of off-resonance saturation transfer imaging in the healthy brain and in brain tumor patients. Because our methods have improved temporal resolution compared to standard CEST imaging, reduced errors due to $B_0$ inhomogeneity and MT asymmetry, and simplified post-processing analysis, they may pave the way for the application of CEST imaging in a wide range of clinical disorders such as cancer, stroke and neurodegenerative diseases.
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