Measurement of APT using a combined CERT-AREX approach with varying duty cycles

Zhongliang Zu a,b,* , Hua Li a,b , Junzhong Xu a,b,c , Xiao-Yong Zhang a,b , Moritz Zaiss d , Ke Li a,b , Mark D. Does a,b,e , John C. Gore a,b,c,e,f , Daniel F. Gochberg a,b,c

a Vanderbilt University Institute of Imaging Science, Nashville, TN, United States
b Department of Radiology and Radiological Sciences, Vanderbilt University, Nashville, TN, United States
c Department of Physics and Astronomy, Vanderbilt University, Nashville, TN, United States
d Department of Medical Physics in Radiology, German Cancer Research Center, Germany
e Department of Biomedical Engineering, Vanderbilt University, Nashville, TN, United States
f Department of Radiology and Radiological Sciences, Vanderbilt University, Nashville, TN, United States

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The goal is to develop an imaging method where contrast reflects amide-water magnetization exchange, with minimal signal contributions from other sources. Conventional chemical exchange saturation transfer (CEST) imaging of amides (often called amide proton transfer, or APT, and quantified by the metric MTR asym) is confounded by several factors unrelated to amides, such as aliphatic protons, water relaxation, and macromolecular magnetization transfer. In this work, we examined the effects of combining our previous chemical exchange rotation (CERT) approach with the non-linear AREX method while using different duty cycles (DC) for the label and reference scans. The dependencies of this approach, named AREX double,vdc, on tissue parameters, including $T_1$, $T_2$, semi-solid component concentration ($f_{ss}$), relayed nuclear Overhauser enhancement ($r$NOE), and nearby amines, were studied through numerical simulations and control sample experiments at 9.4 T and 1.1 T. Simulations and experiments show that AREX double,vdc is sensitive to amide-water exchange effects, but is relatively insensitive to $T_1$, $T_2$, $f_{ss}$, nearby amine, and distant aliphatic protons, while the conventional metric MTR asym, as well as several other APT imaging methods, are significantly affected by at least some of these confounding factors.

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1. Introduction

Non-invasive magnetic resonance imaging techniques that are sensitive to changes in protein level are of considerable interest for detecting and characterizing pathological changes in many disorders. However, although cells contain an abundance of proteins/peptides, these molecules do not provide signals that are easily measurable in practical magnetic resonance spectroscopy (MRS) experiments, and they have been rarely examined in vivo or in cells [1–3]. Amide proton transfer (APT) imaging has developed as an alternative, sensitive approach to imaging the protein content [4–6]. APT is a variation of chemical exchange saturation transfer (CEST) imaging that can detect the effects of backbone amide protons associated with endogenous mobile, cytosolic proteins and peptides in biological tissue. However, there are inherent and critical technical obstacles to the in vivo application and interpretation of APT. APT imaging indirectly detects amide protons by observing the water signal changes caused by chemical exchange, and in practice depends on multiple tissue parameters.

APT images are acquired after a pre-saturating RF pulse, and the residual water signals are typically compared for two or more different irradiation offset frequencies. Commonly, an asymmetry analysis (comparing images obtained from offset frequencies symmetric about the water resonance) is used to quantify the APT contrast. Although this works well in phantom studies, there are several factors in vivo that confound this approach, including effects of tissue relaxation, semi-solid magnetization transfer (MT), MT asymmetry, relayed nuclear Overhauser enhancements ($r$NOE) from aliphatic protons, and contributions from other nearby exchanging protons such as amines [7–11]. The relative magnitudes of these depend on the irradiation power level. A three-point method (APT’), in which the measured signals obtained by irradiating the amide resonance and the average of two nearby frequencies are subtracted, has been suggested as one way to avoid some effects, such as MT asymmetry and rNOE contributions [12]. Similarly, an APT imaging method using saturation with frequency

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alternating RF irradiations (SAFARI) has also been introduced to reduce such effects [13]. All these methods, however, acquire data at multiple frequencies and hence implicitly assume relations concerning the composition and frequency dependence of the tissue. These assumptions are often not warranted in vivo. MTRdouble, a metric based on chemical exchange rotation transfer (CERT), takes a different approach by subtracting pulsed-CEST signals at two different irradiation frequencies (as in CERT methods and the MTRdouble metric [8,16]). Here, DC is defined by the previously introduced AREX metric [15]. Here we introduce a new metric, AREXdouble,vdc, which combines the use of a single frequency offset (as in CERT methods and the MTRdouble metric [8,16]) with the inverse subtraction approach (as in AREX [14]). In this way, we minimize contributions from MT asymmetry and rNOE effects (via single frequency acquisition) and DS and semi-solid MT effects (via inverse subtraction). Furthermore, the single frequency CERT approach has been shown [8] to filter (via exchange rate) the contributions from nearby fast exchanging amines, and hence we expect minimal contributions from amines in the new AREXdouble,vdc metric. In addition, we borrow the T1 normalization approach of AREX, and thus avoid this confounding effect. Finally, we also vary the duty cycles (vdc) in the π and 2π pulse trains, which greatly magnifies the resulting CERT signal compared to the previous single duty cycle (DC) MTRdouble approach.

Here, DC is defined as the ratio of the pulse duration to the pulse repetition time (PTR) (Fig. 1a). In sum, we address the three major deficiencies of our previous CERT metric. In this work, we study the specificity of conventional (MTRasym) and unconventional (APT*, MTRdouble,vdc, AREX with asymmetric analysis, and the new AREXdouble,vdc) APT imaging methods. We use numerical simulations and control phantom experiments to demonstrate the increased specificity of AREXdouble,vdc (in comparison to other APT methods) to changes in amide concentration and exchange rate.

### 2. Theory

#### 2.1. Asymmetric analysis and symmetric analysis

Conventionally, an asymmetry analysis has been used to process CEST Z-spectra in order to remove the DS and semi-solid MT effects [17]:

$$\text{MTR}_{\text{asym}} = \frac{S_+ - S_-}{S_0}$$  \hspace{1cm} (1)

Here \(S_+\) and \(S_-\) are the signals acquired with a downfield irradiation pulse (label) and the symmetrically opposite side of the water peak (reference). \(S_0\) is the signal acquired with no irradiation. For in vivo studies with MT asymmetry, rNOEs, and nearby amines, the MTRasym is influenced by multiple effects. To reduce the influence on MTRasym from MT asymmetry and rNOE effects, two methods have been developed that avoid acquisitions at negative offsets. One method is APT* which was defined to be [12],

$$\text{APT}^* = \frac{|S(4 \text{ ppm}) + S(3 \text{ ppm})|}{2S_0}$$  \hspace{1cm} (2)

Another approach is CERT, which attempts to address the problem of MT asymmetry and rNOE effects by making all the measurements at a single frequency. It produces a metric MTRdouble defined to be [8]

$$\text{MTR}_{\text{double}} = \frac{S_{2\pi} - 5n}{S_0} B_{\text{avg power}}$$  \hspace{1cm} (3)

where \(S_{2\pi}\) and \(S_m\) are the signals acquired with irradiation flip angles at \(\pi\) and \(2\pi\), but at the same frequency offset and average irradiation power (\(B_{\text{avg power}}\)). Distinct from conventional MTRasym analysis, these two APT imaging methods acquire both label and reference signals on one side of water, and thus avoid contamination from MT asymmetry and rNOE effects on the other side of water.

In this paper, we make two modifications to our previous CERT work. First, we vary the DC of the π and 2π pulse trains. This change is motivated by Fig. 1b, which shows that the separation in the labeling efficiencies (\(\eta\)) between the two pulse trains (which dictates the resulting exchange mediated contrast) is maximized when the π pulses are

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**Table 1**

List of sample parameters used in Fig. 5.

<table>
<thead>
<tr>
<th>(T_1) (s)</th>
<th>(T_2) (ms)</th>
<th>(f_i)</th>
<th>(k_{20}) (s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>a 0.71, 1.03, 1.27</td>
<td>53, 69, 94</td>
<td>0.0015, 0.0014, 0.0014</td>
<td>59, 50, 46</td>
</tr>
<tr>
<td>b 2.88, 2.72, 2.56</td>
<td>2040, 1873, 1725</td>
<td>0.0009, 0.0009, 0.0015</td>
<td>23, 38, 47,</td>
</tr>
<tr>
<td>2.88, 2.54</td>
<td>2044, 1654</td>
<td>0.0019, 0.0018</td>
<td>49, 62</td>
</tr>
</tbody>
</table>

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**Fig. 1.** (a) Diagram of the AREXdouble,vdc and MTRdouble,asym sequences, which have label and reference scans that vary the flip angle of the pulse trains while maintaining the same average irradiation power. Unlike in our previous work, the DC varies, with the train of π pulses at 70% dc and the 2π pulses at 30% dc. (b) Simulated amide labeling efficiency (\(\eta\)) vs. DC for π and 2π pulse trains at a \(B_{\text{avg power}}\) of 1 mT.

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applied at a high DC (70% in this work) and the 2\(\pi\) pulses are applied at a low DC (30% in this work). This change gives a new metric, MTR_{\text{double,vdc}}, which is identical to MTR_{\text{double}}, but with varying duty cycles for the two pulse trains.

### 2.2. Direct subtraction and inverse subtraction

Our second modification is based on the recent work by Zaiss [14,15] et al., who has shown that inverse subtraction of label and reference signals in the AREX metric is more effective at eliminating confounding DS and semi-solid MT signal contributions than is conventional linear signal subtraction. In addition, the AREX metric also corrects for \(R_1\) effects and is defined by the following equation when the reference signal is obtained by the asymmetric analysis.

\[
\text{AREX} = S_0 R_{\text{obs}} \left( \frac{1}{\tau - \frac{1}{\tau}} \right)_{\text{avg power}}
\]

where \(R_{\text{obs}}\) is the apparent longitudinal relaxation rate of water. Here we introduce AREX_{\text{double,vdc}}

\[
\text{AREX}_{\text{double,vdc}} = S_0 R_{\text{obs}} \left( \frac{1}{\tau - \frac{1}{\tau}} \right)_{\text{avg power}}
\]

The AREX_{\text{double,vdc}} is based on two pulsed-CEST signals with two different irradiation flip angles, varying duty cycles, but constant irradiation frequency offset and average power.

Results from numerical simulations below will demonstrate that (at lower powers)

\[
\text{AREX}_{\text{double,vdc}}(3.5 \text{ ppm}) \approx f_s k_{\text{sw}} (n_\pi - n_{2\pi})
\]

where \(f_s\) and \(k_{\text{sw}}\) are the amide proton concentration and exchange rate, respectively, and \(n_\pi\) and \(n_{2\pi}\) are label efficiencies of the \(S_{2\pi}\) (3.5 ppm) reference and \(S_\pi\) (3.5 ppm) label scans, respectively, for a two-pool model in ideal conditions (no semi-solid MT and DS effects). This result is consistent with the labeling efficiency interpretation of Zaiss et al. [14], but without the rNOE and MT asymmetry confounding factors.

### 3. Method

#### 3.1. Phantom preparation

A series of creatine samples served as phantoms to test different methods. Creatine amine-water exchange rates under the titrated pH (6.2–6.6) are close to the amide-water exchange rates in the physiological range [19,20], and can be used to mimic amide-water exchange. To study the sensitivity of different APT contrasts to relaxation, three samples were made of 50 mM creatine with 0.05 mM MnCl\(_2\), 0.075 mM MnCl\(_2\), and 0.1 mM MnCl\(_2\), pH was titrated to 6.4 for these three samples at room temperature. We expect the APT contrast to be proportional to the product of amide concentration (\(f_s\)) and exchange rate (\(k_{\text{sw}}\)), and to test this sensitivity, five samples were made with creatine concentration and pH of (50 mM, 5.9), (25 mM, 6.4), (50 mM, 6.4), (75 mM, 6.4), and (50 mM, 6.6) at room temperature. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

To study the influence of rNOEs or nearby amines, egg white and glutamate phantoms were prepared and measured at room temperature. Egg white consists of about 90% water into which is dissolved 10% proteins (including albumins, mucoproteins, and globulins), and contains almost no fat, and carbohydrate content is <1%, and thus is a good model for proteins in tissues. We have reported that boiled egg white has significant APT at 3.5 ppm and rNOE at 3.5 ppm [16]. The protein phantom was prepared by boiled chicken egg white. It was also reported that glutamate is the main amine proton component for metabolites [21]. The glutamate phantom was prepared by adding 30 mM glutamate in 1× phosphate buffered saline (PBS), and the solute pH was titrated to 7.0 using NaOH and HCl.

#### 3.2. Animal preparation

Three Sprague Dawley rats (250–300 g) bearing 9 L tumors were immobilized and anesthetized with a 2%/98% isoflurane/oxygen mixture. Respiration was monitored to be stable, and a rectal temperature of 37 °C was maintained constant throughout the experiments using a warm-air feedback system (SA Instruments, Stony Brook, NY). All experiments were measured in 1 h after onset of isoflurane. For brain tumor induction, each rat was injected with \(1 \times 10^5\) 9L glioblastoma cells in the right brain hemisphere, and was then imaged after 2 to 3 weeks. All procedures were approved by the Institutional Animal Care and Usage Committee at Vanderbilt University.

#### 3.3. MRI and experiment

MR data were acquired using a Varian DirectDrive™ spectrometer interfaced with a horizontal 9.4 T magnet and a 38-mm Litz RF coil (Doty Scientific Inc. Columbia, SC). The CERT sequences for AREX_{\text{double,vdc}} (Fig. 1a) are made up of two pulsed-CEST sequences with the same average power, but different irradiation flip angles \(\pi\) or \(2\pi\), respectively. The pulsed-CEST sequences used a series of Gaussian RF irradiation pulses followed by acquisition. Crusher gradients (with alternating sign) were applied between each irradiation pulse to spoil residual transverse magnetization. The continuous wave (CW)-CEST sequence contains a long rectangular RF pulse followed by acquisition. A total of 5 s irradiation for both pulsed- or CW-CEST was performed before acquisition to ensure the CEST system reached steady state. All images of rat brains were acquired using a single-shot Spin-echo Echo Planar Imaging (SE-EPI) with matrix size 64 × 64, field of view 30 × 30 mm, and number of acquisitions = 1. All measurements on samples were obtained by a free induction decay (FID) acquisition.

For all experiments on phantoms and animals, two pulsed-CEST sequences were implemented with the same \(B_{\text{avg power}}\), of 1.0 \(\mu\Tilde{\text{T}}\). The first sequence used an irradiation flip angle of \(\pi\) and DC of 70%. The second used \(2\pi\) and 30%. This leads to a FID of 18.3 ms and pulse duration of 12.8 ms for \(\pi\) pulses, and a FID of 56 ms and pulse duration of 16.8 ms.
for 2π pulses, respectively. (The RF amplitude was changed in order to maintain a constant $B_{\text{avg, power}}$, following the relations published previously [18].) A CW-CEST sequence with irradiation $B_1$ of 1.0 μT was also performed. Z-spectra were acquired with RF offsets from $-2000 \text{ Hz}$ to 2000 Hz ($-5 \text{ ppm}$ to 5 ppm at 9.4 T) with an interval of 50 Hz (0.125 ppm at 9.4 T). Control scans were performed to acquire $S_0$, by setting the RF offset to 100,000 Hz. The two pulsed-CEST signals were used to calculate $\text{MTR}_{\text{double,vdc}}$ and $\text{AREX}_{\text{double,vdc}}$. CW-CEST signals were used to calculate $\text{MTR}_{\text{symm. APT}}$, and $\text{AREX}$. $T_1$ and semi-solid component concentration ($f_{\text{ss}}$) were obtained using a selective inversion recovery (SIR) method with inversion time of 4, 5, 6, 7, 8, 10, 15, 20, 50, 100, 400, 800, 1000, 3000, 5000, and 8000 ms [22,23]. $T_2$ was obtained using five echo times of 30, 50, 70, 110, and 110 ms.

For fitting of $f_1$ and $k_{sw}$ in the creatine phantoms, CW-CEST sequences with irradiation powers of 0.5, 1.0, and 2.0 μT and RF offsets from $-2000 \text{ Hz}$ to 2000 Hz were performed ($-5 \text{ ppm}$ to 5 ppm at 9.4 T) with a step size of 50 Hz (0.125 ppm at 9.4 T). The acquired Z-spectra were numerically fit to a two-pool (solute and water) model. $k_{sw}$, $T_2$, and solute transverse relaxation rate ($R_2$) were fit with the creatine resonance assumed at 1.9 ppm from water, water $T_1$ assumed (0.125 ppm at 9.4 T). Control scans were performed to acquire $S_0$ by setting the RF offset to 100,000 Hz. The two pulsed-CEST signals integrated the differential equations through the adiabatic condition $\lambda = \gamma |B_{\text{eff}}|/\psi$, where $B_{\text{eff}}$ is the effective irradiation field and $\psi$ is the angle between $B_{\text{eff}}$ and $B_1$. The $\text{AREX}_{\text{double,vdc}}$ metric defined by Eq. (5) implicitly assumes that DS is a function of $B_{\text{avg, power}}$, which is true when the adiabatic metric is $\approx 1$ and no Rabi precession occurs.

Each three-pool model simulation included seven coupled Bloch equations which can be written as

$$\frac{dM}{dt} = A \cdot M + M_0,$$

where $A$ is a $7 \times 7$ matrix. For one-pool or two-pool model simulations, the parameters related to non-simulated pools were set to 0. The water and amide pool each have three coupled equations representing their $x$, $y$, and $z$ components. The semi-solid component pool has a single coupled equation representing the $z$ component, with an additional term for saturation effects [26]. A super-Lorentzian absorption lineshape (which best fits biological tissue [27–29]) was used for the semi-solid component pool in all simulations of the phantom. All numerical calculations of the CW or pulsed-CEST signals integrated the differential equations through the pulse sequence using the ordinary differential equation (ODE) solver in Matlab. Spoiling was modeled by nulling the transverse components of the magnetization between the irradiation pulse and acquisition. All simulations were performed with static field strength of 9.4 T except where noted.

3.5. Data analysis and statistics

Region of interests (ROIs) for tumors and contralateral normal tissues were outlined from each tumor rat brain based on the $T_1$ map. Student’s $t$-tests were employed to evaluate the signal differences, which were considered to be statistically significant when $P < 0.05$.

4. Results

Fig. 2 shows the simulated $\text{AREX}_{\text{double,vdc}}$, defined in Eq. (5), and our approximation based on differences in solute saturation (which

\begin{align*}
\text{AREX}_{\text{double,vdc}} &= \frac{\Delta M_{\text{power}}}{M_0} \\
\text{Eq. (5)}
\end{align*}

Fig. 2. Numerical simulations of Eqs. (5) and (6) with a variety of $B_{\text{avg, power}}$. Note that the two curves match well at relatively low $B_{\text{avg, power}}$. At 1 μT, which is used in this paper, the $\text{AREX}_{\text{static,vdc}}$ in Eq. (6) (2.26%) is 90.4% of that in Eq. (5) (2.50%).
implicitly assumes no net dependence on direct RF effects on macromolecular or water protons), defined in Eq. (6), under a variety of $B_{\text{avg power}}$. Note in Fig. 2 that the two curves match well at relatively low $B_{\text{avg power}}$, e.g. for $B_{\text{avg power}} \leq 1 \mu T$, the AREX$_{\text{double,vdc}}$ in Eq. (6) is $\geq 90.4\%$ of that in Eq. (5). At relatively high $B_{\text{avg power}}$, the curves separate, which may be due to Eq. (6) ignoring direct water and macromolecular saturation, or from additional terms not captured by this simplified phenomenological functional form.

Fig. 3 shows simulated MTR$_{\text{asym}}$, APT*, MTR$_{\text{double,vdc}}$, AREX, and AREX$_{\text{double,vdc}}$ as a function of T$_1$ (a), T$_2$ (b), $f_m$ for symmetric semi-solid component (c), $f_m$ for asymmetric semi-solid component (d), $f_s$ (e), and $k_{\text{sw}}$ (f), respectively. It was found that AREX$_{\text{double,vdc}}$ depends linearly on $f_s$ and $k_{\text{sw}}$ but not on other tissue parameters, while MTR$_{\text{asym}}$, APT*, and MTR$_{\text{double,vdc}}$ depend on several other (non-amide) tissue parameters. Specifically, MTR$_{\text{asym}}$ and AREX (which acquires data on both sides of water) have strong dependencies on the asymmetric semi-solid component, as seen in Fig. 3d. MTR$_{\text{asym}}$, MTR$_{\text{double,vdc}}$ and APT* (which assume effects add linearly with no correction for T$_1$ effects) have dependencies of T$_1$, T$_2$, and the symmetric semi-solid component.

Fig. 4 shows the simulated difference of two labeling efficiencies (for reference and label scans) under pulsed-irradiation. ($\eta_1 - \eta_2$) is plotted vs. the solute relaxation times T$_{1s}$ and T$_{2s}$, and $k_{\text{sw}}$ (limited to the slow to intermediate exchange regime compared with $B_{\text{avg power}}$). It was found that the subtraction of these two labeling efficiencies is roughly independent of the tissue parameters (though not the sequence parameters $B_{\text{avg power}}$ and DC) in our simulation range. Therefore, ($\eta_1 - \eta_2$) can be determined through numerical simulations with known sequence parameters, but with unknown tissue parameters. Hence, AREX$_{\text{double,vdc}}$ results can be interpreted in terms of the pure amide-water exchange effect $f_s k_{\text{sw}}$, which can then be accurately obtained using Eq. (6) for a wide range of tissue states.

Fig. 5(a–b) shows the experimental MTR$_{\text{asym}}$, APT*, MTR$_{\text{double,vdc}}$, AREX, AREX$_{\text{double,vdc}}$, and AREX$_{\text{double,vdc}}$ defined in Eq. (6) on creatine phantoms as a function of (T$_1$, T$_2$) (a) and $f_{\text{sksw}}$ (b). Similar to simulations in Fig. 3, AREX$_{\text{double,vdc}}$ depends linearly on $f_{\text{sksw}}$, but not on T$_1$ and T$_2$, while MTR$_{\text{asym}}$ and MTR$_{\text{double}}$ depend on all tissue parameters. The big differences between the simulated and experimental results in Fig. 3 and Fig. 5 are caused by limits in the ability to make phantoms that vary only one sample parameter at a time. MTR$_{\text{asym}}$, AREX, and AREX$_{\text{double,vdc}}$ spectra of egg white and glutamate phantoms are shown in Fig. 5c and d to show the influences from rNOE and nearby amines. While AREX showed a rough independence from T$_1$ and T$_2$ in...
a), acquisitions at multiple frequencies and limited exchange-rate filtering make it susceptible to influence from rNOE effects and amine proton contributions. These effects can be seen in the sloping negative AREX baseline in Fig. 5c in the egg white phantom (indicating the significant contamination from rNOE effect on the other side of water), and in the broad AREX peak from glutamate in Fig. 5d. MTRasym shows similar influences, but AREXdouble,vdc is largely immune from rNOE and amine signal contributions. In sum, Fig. 3 and Fig. 5 show that AREXdouble,vdc is more specific to amide-water exchange effects (at least, in these phantoms) than are MTRasym, APT*, MTRdouble,vdc, and AREX.

Fig. 6 shows the experimental CW-CEST Z-spectra (a), MTRasym spectra (b), APT* spectra (c), AREX spectra (d), pulsed-CEST Z-spectra (e), MTRdouble,vdc (f), and AREXdouble,vdc Spectra (g) from three rat brains bearing 9 L tumors. The AREXdouble,vdc at 3.5 ppm was measured to be 0.021 ± 0.0069 in tumor and 0.023 ± 0.004 in contralateral normal tissue, as will be discussed below. Note in (b) and (d) that the amide peak (arrow) in conventional MTRasym and AREX resides on top of a broad negative base line that originates from the MT asymmetry and rNOE effects. Also note in (f) and (g) that using CERT based metrics avoids this negative, sloping baseline, and hence isolates the amide peak from

Fig. 4. Simulated subtraction of two labeling efficiencies in the reference and label scan under pulsed-irradiation vs. $T_1$, (a), $T_2$, (b), $k_{sw}$ (c). Note that $\eta_1 - \eta_2$ is roughly independent of tissue parameters.

Fig. 5. Experimental MTRasym, APT*, MTRdouble,vdc, AREX, MTRdouble,vc, and AREXdouble,vdc of creatine phantoms as a function of measured ($T_1$, $T_2$) (a) and fitted $f_{k_{sw}}$ (b), $f_s$, and $k_{sw}$ in (a) were relatively constant, with fitted values of (0.0015, 0.0014, 0.0015) and (59, 50, 46 s$^{-1}$), respectively, for the three phantoms with varying MnCl2 concentrations. In (b), $f_{k_{sw}}$ values were (0.0217, 0.0347, 0.0689, 0.0921, 0.1109 s$^{-1}$) for five phantoms with varying pH and creatine concentration. Detailed parameters are listed in Table 1. Only AREX and AREXdouble,vdc show the desired linearity and $f_s$ and $k_{sw}$ while being independent of $T_1$ and $T_2$. However, only AREXdouble,vdc avoids contamination by rNOE effects in egg white (c) and amine contributions in glutamate (d), cases where AREX is non-specific similar to conventional MTRasym (which is also plotted in (c) and (d) for comparison).
other factors. (The signal at around 2 ppm is from amine sites in medium exchange regime. Based on the width of this peak, it has little influence on APT signal at 3.5 ppm). Fig. 7 show the images of $T_1$ (a), $T_2$ (b), $F_m$ (c), $MTR_{sym}$ (d), $APT^*$ (e), $AREX$ (f), $MTR_{double,vdc}$ (g), and $AREX_{double,vdc}$ (h) from a representative rat brain.

Fig. 8 shows the simulated $MTR_{sym}$, $MTR_{double,vdc}$, and $AREX_{double,vdc}$ vs. $B_0$ shift (a) and $B_{avg}$ power error (b), respectively. It was found that although $AREX_{double,vdc}$ has a little more deviation than $MTR_{sym}$ under $B_{avg}$ power error (18.58% for $AREX_{double,vdc}$ and 5.66% for $MTR_{sym}$ with $-10\%$ error), $AREX_{double,vdc}$ is much more robust to a $B_0$ shift than $MTR_{sym}$ (10.2% for $AREX_{double,vdc}$ and 54.76% for $MTR_{sym}$ with $-50\,\text{Hz}\,B_0$ shift). However, this $MTR_{sym}$ sensitivity to $B_0$ may be largely corrected at the time-cost of adding an additional WASSR scan (which we did not do in this work).

5. Discussion

In this paper, we extended our previous CERT work by incorporating inverse subtraction of label and reference scans, correcting for $T_1$ effects, and varying the duty cycle between the label and reference scans. We continue to use only a single irradiation frequency for both label and reference scans, in which irradiation by a train of $\pi$ pulses is used as the label scan and irradiation by a train of $2\pi$ pulses is used as the reference scan. The same average irradiation power is applied so that fast nearby amine (e.g. glutamate), DS, and semi-solid MT effects are the same in both the reference and label scans, and an inverse subtraction and $T_1$ correction are used to fully remove the influences of other factors while specifically isolating amide-water exchange effects.
AREX\textsubscript{double,vdc} is proportional to amide concentration ($f_1$) and amide-water exchange rate ($k_{aw}$) under the conditions simulated in this study (relatively slow $k_{aw}$), and this metric can be used to measure specifically protein/peptide concentration and pH, if one of these quantities is known by other means and the proportionality constant $\eta_1 - \eta_2$ (see Eq. (6)) is also known. Because $\eta_1 - \eta_2$ is roughly independent of tissue parameters when $B_{\text{avg}}$ power is on the same scale as $k_{aw}$ and $1/T_2^*$, this quantity can be determined through numerical simulations. ($T_2^*$ has only rarely been measured, so it is unclear if this restriction may in fact be problematic for CERT measures, e.g. if $T_2^*$ is exceptionally short, on the order of milliseconds.) In addition, a “pure” CEST effect in a simple two-pool model (solute and water) should have no net DS effects and be equal to the product of solute concentration, solute-water exchange rate, and the labeling efficiency\cite{14}. The rough equality (as indicated by Fig. 2) of the two definitions of $\text{AREX}_{\text{double,vdc}}$ in Eq. (5) and Eq. (6) indicates that $\text{AREX}_{\text{double,vdc}}$ (as defined by Eq. (5)) can successfully remove the confounding factors in simulated biological tissue, and obtain pure a CEST effect, at least at low powers.

The choice of the acquisition parameters $B_{\text{avg}}$ power and DC is non-trivial, and in the current work we did not optimize these (which in general would depend on field strength, chemical shift, and exchange rate) but instead made reasonable choices. The $B_{\text{avg}}$ power of 1 $\mu$T is a balance between being roughly equal or faster than $k_{aw}$ (since solute rotation has to be faster than exchange) while still avoiding extensive DS,
semi-solid MT effects, or direct water rotation. (Direct water rotation occurs when the pulse is no longer adiabatic from the water proton’s perspective, and Appendix B examines this issue.) The choice of 1 μT irradiation was driven by the requirements of the AREX_{double,vdc} method, and is nonetheless a reasonable choice for the other simulated and measured APT metrics, which are typically performed in the range 0.5 to 2.0 μT. However, note that the choice of irradiation power may affect the degree of signal contamination by confounding factors in non-trivial ways. The choices of 70% DC for the label pulse train and 30% for the reference pulse train were motivated by balancing maximizing the degree of signal contamination by confounding factors in non-trivial ways. The choices of 70% DC for the label pulse train and 30% for the reference pulse train were motivated by balancing maximizing \( \eta \pi - \eta 2\pi \) while again maintaining the adiabaticity of the pulses effect on the water magnetization. (Appendix plots A and B provided the numerical guidance on these choices.)

A similar work combined the three-point analysis method (APT\*) with inverse subtraction [15,30]. This method is similar to AREX_{double,vdc} except that it uses two signals acquired near the amide resonance (3 and 4 ppm) for the reference instead of acquiring at the amide resonance (3.5 ppm) at a different irradiation flip angle. As such it depends on the linewidth of the amide dip and also is easily influenced by nearby amines. The specificity of this method needs further evaluation through both simulations and phantom studies. Another method applying variable-delay multiple-pulse saturation (VDMP) was also developed to remove confounding factors, which also uses different DC [31,32]. However, the underlying mechanism for these two methods are quite different: the VDMP produces the same direct saturation and MT effects through different delay times, while our proposed method produces the same direct saturation and MT effects through using the same average irradiation power. The lack of contrast between tumor and normal tissue at 3.5 ppm in Fig. 6g (\( P = 0.38 \)) and Fig. 7h support the previous results [11,30,33] that tumor/contralateral MTR_{asym} contrast in a simple tumor model may be driven by \( T_1 \), semi-solid MT, and rNOE, and not amide differences. Note that there have been differences in the CEST literature [6,30] concerning the relative protein (and hence amide) concentration in tumors. Also, while amide AREX_{double,vdc} would be expected to be sensitive to changes in intracellular pH [20], these changes are much smaller than the well-known extracellular pH changes due to tumor acidosis [34–36]. The different contrast for the MTR_{double,vdc} and AREX_{double,vdc} in tumors may be due to their sensitivities (or insensitivities) to \( T_1 \) and semi-solid MT. The specificity in more realistic tumor models is an open question. The sensitivity of different APT methods is optimized with different sequence parameters, so it is difficult to compare their sensitivities with the parameters used in this paper.

On small animal system, \( B_0 \) is homogeneous and thus water saturation shift referencing (WASSR) was not applied [37]. (Also, the relative robustness of AREX_{double,vdc} under \( B_0 \) inhomogeneities, as indicated by Fig. 8, makes correction rarely necessary.) Under other \( B_{avg} \) power, DC may be changed to satisfy the adiabatic condition according to Eq. (2) in our previous publication [18].

6. Conclusion

In this work, simulations at 9.4 T using 1 μT irradiation show that conventionally measured APT contrast is influenced by other sample parameters and is not specific to amide-water exchange effects. Our proposed AREX_{double,vdc} is insensitive to \( T_1 \), \( T_2 \), \( T_{sym} \), MT asymmetry, and nearby amines, within the range of our simulated regime and, hence, is a good candidate for specific measures of amide-water exchange effects. The simulated effects are supported by experimental results on simple creatine, egg white, and glutamate phantoms. Results on a 9 L tumor model suggest limited baseline contributions to AREX_{double,vdc} and are consistent with a previous study indicating little contribution from amide exchange to APT contrast in this animal model.

Appendix A. Labeling efficiency

Labeling efficiency has been defined previously [17] to be the reduction in the ratio of the steady state solute spin magnetization under CW irradiation to the equilibrium solute spin magnetization. Here, we define the labeling efficiency under pulsed-irradiation to be the reduction in the ratio of the average of the steady state solute spin magnetization over a pulse repetition time (duration between two adjacent irradiations) to the equilibrium solute spin magnetization in a two pool model (solute and water), ignoring DS. Fig. A1 shows the labeling efficiencies for reference and label scans under pulsed-irradiation vs. sequence parameters \( B_{avg} \) power and DC. While \( B_{avg} \) power = 1 μT was chosen due to exchange rate, semi-solid MT, and adiabaticity constraints, the DC were chosen to maximize \( \eta_0 - \eta_{2\pi} \). In this paper, we use DC of 70% for π pulse and DC of 30% for 2π pulse irradiation for all phantom and animal experiments. Using these parameters, \( \eta_0 - \eta_{2\pi} \) is around 0.566. Fig. A1Simulated labeling efficiency vs. \( B_{avg} \) power and DC.
Appendix B. Adiabatic condition

In a previous paper, we have shown that the adiabatic condition should be satisfied to remove the DS effect [8]. The adiabatic condition can be quantified by $\lambda$. When $\lambda \gg 1$, the adiabatic condition is satisfied. Fig. B1 plots the minimum $\lambda$ during a Gaussian irradiation pulse. It decreases at higher $B_{\text{avg}}$ power and lower DC. Specifically, the low $\lambda$ values of the 2n pulse at 30% DC and high powers drives the deviation between Eq. (5) and Eq. (6) plotted in Fig. 2. In addition, $\lambda$ decreases at lower static field strength [8]. In our simulations at 9.4 T and 7 T, the adiabatic condition is easily satisfied, but is likely to create significant power and DC constraints at 3 T. Fig. B1 Minimum $\lambda$ during a Gaussian pulse as a function of applied $B_{\text{avg}}$ power and DC at 3.5 ppm at 9.4 T, 7 T, and 3 T for $\pi$ (a) and 2n (b) pulse irradiation.

References