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Normalized Magnetization Ratio (NOMAR) Filtering for Creation of Tissue Selective Contrast Maps

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Abstract

An MRI segmentation technique based on collecting two additional saturation transfer images is proposed as an aid for improved detection of CEST agents. In this approach, the additional images are acquired at saturation frequencies of -12.5 and -50 ppm. Use of the ratio of these images allows differentiation of voxels with low MT contrast (such as fat, CSF, edema or blood) from target tissue voxels using a global threshold determined by histogram analysis. We demonstrate that this technique can reduce artifacts, *in vitro*, in a phantom containing tubes with CEST contrast agent embedded in either cross-linked BSA or buffer, and *in vivo* for detecting DIACEST liposomes injected into mice.

Introduction

Recently, Chemical Exchange Saturation Transfer (CEST) has emerged as a promising MRI contrast mechanism(1–5). Small quantities of contrast agents can be detected by continuously transferring the saturated magnetization from exchangeable protons to a much larger number of water molecules during a prolonged RF irradiation. CEST contrast agents have advantages over conventional T1 or T2 agents including the capabilities of switching the contrast on/off(6,7) and performing so-called multicolor imaging(6,8–11) in which exchangeable protons at multiple frequencies are studied. To date, most of these applications have only been demonstrated *in vitro* and the development of technologies to improve *in vivo* applications is still needed.

CEST contrast is generated through the application of frequency-selective saturation pulses to exchangeable protons and observing water signal loss upon saturation transfer. The contrast is determined by MR parameters such as the saturation time (t_{sat}), saturation field strength (ω_1), agent concentration, agent properties such as the exchange rates and intrinsic relaxation rates of their protons, and factors affecting exchange rates such as pH and temperature(12–14). One of the challenges is that signal loss is also produced through competing mechanisms such as direct saturation (DS) of the water line(15) and conventional magnetization transfer (MT)(16,17) with the amount of such additional contrast varying

with ω_1 and the tissue relaxation times (T_1 and T_2) and composition. MT contrast is described using the Magnetization Transfer Ratio (MTR) defined by:

$$\text{MTR} = 1 - S^{\Delta\omega} / S_0, \quad [1]$$

In which $\Delta\omega$ is the frequency difference of the protons of interest with respect to the water protons, $S^{\Delta\omega}$ is the signal acquired with RF irradiation at offset $\Delta\omega$, and S_0 is the signal without RF irradiation. In order to separate out CEST contrast from other sources of signal loss, asymmetry analysis with respect to the water frequency is widely used, with the magnetization transfer ratio asymmetry (MTR_{asym}) defined as $\text{MTR}_{\text{asym}} = (S^{-\Delta\omega} - S^{+\Delta\omega}) / S_0$. CEST contrast is then defined as MTR_{asym} assuming that DS and MT are symmetric with respect to the water resonance. However this assumption doesn't really hold *in vivo* (18). Moreover, CEST agents may reside in multiple types of tissue, where in addition to DS and MT, the amount of fat or endogenous CEST contrast (e.g., from, urea in the bladder (19), endogenous peptides and proteins in tumors (20), or glycogen in liver (21)) may be appreciable and produce additional frequency dependent asymmetries in signal loss. In addition to using alternative imaging schemes to improve CEST quantification in the presence of MT and endogenous CEST contrast (22,23), appropriate tissue segmentation techniques can, as we will show, also be useful for accurately interpreting CEST maps by segmenting potentially interfering compartments from the targeted tissues in these maps.

To compliment strategies for filtering out unwanted compartments in the z-spectra that are typically collected for *in vivo* CEST imaging (24,25), we propose an MT based MRI segmentation technique for selectively filtering CEST contrast maps based on two resonance frequency offsets $\Delta\omega_1$ and $\Delta\omega_2$ to calculate the Normalized Magnetization Ratio (NOMAR), defined by

$$\text{NOMAR}(\Delta\omega_1 / \Delta\omega_2) = (1 - \text{MTR}(\Delta\omega_1)) / (1 - \text{MTR}(\Delta\omega_2)) \quad [2]$$

MT is chosen because it has been shown to be capable of classifying tissues (16) including the separation of CSF containing pixels from brain parenchyma in MS studies (26) and removal of background tissue from contrast enhanced blood signal (27). Using NOMAR to improve *in vivo* CEST imaging offers the advantage that this parameter can be determined using standard CEST acquisition schemes. Here, the additional frequencies can be easily added with only a minor increase of the acquisition time. Furthermore, it is anticipated that NOMAR can effectively separate out tissue signals from unwanted signals such as those from fat and fluids. As an example, we demonstrate here how the NOMAR segmentation technique can be applied to improve *in vivo* DIACEST Liposome (DL) contrast maps.

Theory

MT has been extensively described previously (26,28,29), with one of the commonly used models being the two-pool Super-Lorentzian model (29). The analytical solution for $\text{MTR}(\Delta\omega)$ at steady state is given by (29,30):

$$\text{MTR}(\Delta\omega) = 1 - \frac{M_z^W(\omega_1, \Delta\omega)}{M_0^W} = 1 - \frac{R_B \left[\frac{R_f}{R_W} \right] + R_{fB} + R_B + R}{\frac{R_f}{R_W} (R_{fB} + R_B) + \left(1 + \left(\frac{\omega_1}{2\pi\Delta\omega} \right)^2 \left[\frac{1}{R_W T_{2W}} \right] \right) (R_{fB} + R_B + R)}, \quad [3]$$

where M_z^W is the magnetization of the bulk water pool, R is the exchange rate between the two pools, R_B and R_W are the R_1 relaxation rates for the macromolecule-bound water pool (pool B) and bulk water (pool W), T_{2W} is the T_2 relaxation time of free water, and $\omega_1/2\pi$ is

the RF pulse amplitude (Hz) for irradiation at offset $\Delta\omega$ from water. The bound and free pool sizes are related through $f = M_0^B/M_0^W$. R_{fB} is the rate of saturation of pool B and given by:

$$R_{fB} = \omega_1^2 \pi g_B(2\pi\Delta\omega), \quad [4]$$

where $g_B(2\pi\Delta\omega)$ is the absorption line shape for the spins in pool B, which is Lorentzian for the water pool and super-Lorentzian for the semi-solid pool upon application of a continuous wave (CW) pulse(28).

The choice of the first frequency ($\Delta\omega_1$) for NOMAR($\Delta\omega_1/\Delta\omega_2$) is determined by the MT characteristics of the tissue type that one intends to differentiate from the target tissues, while the second frequency ($\Delta\omega_2$, or the reference offset) is chosen to normalize the NOMAR($\Delta\omega_1/\Delta\omega_2$) to reduce the possible impact of B1 field inhomogeneity. A saturation frequency that produces a maximal MT contrast and minimal DS effect can be considered as

an optimized solution. Considering $(\frac{\omega_1}{2\pi \cdot \Delta\omega})^2 \frac{1}{R_{1w} \cdot T_{2w}}$ represents the DS contrast in Eq. [3], an appropriate saturation offset ($\Delta\omega_{obs}$) can be estimated by setting the term

$[1 + (\frac{\omega_1}{2\pi \cdot \Delta\omega})^2 \frac{1}{R_{1w} \cdot T_{2w}}] \approx 1$ in Eq [3]. In practice, an expression of (A+B) can be approximated as A if the condition $A \gg B$ is satisfied. Using this criterion, we assume that when:

$$(\frac{\omega_1}{2\pi \cdot \Delta\omega})^2 \frac{1}{R_{1w} \cdot T_{2w}} \approx 1/5, \quad [5]$$

or

$$\Delta\omega_{obs} \approx 5 \times \frac{\omega_1}{2\pi} \sqrt{\frac{1}{R_{1w} T_{2w}}}, \quad [6]$$

the DS is negligible. The saturation offset $\Delta\omega_{obs}$ can be determined using Eq [6] for a particular application. Negative offsets are always chosen to avoid the potential influence of endogenous CEST that is not taken into account in our equation.

In contrast to water protons, fat protons do not chemically exchange magnetization with other protons, and therefore display negligible MT contrast(31,32). The frequency dependent saturation profile (z-spectra) of fat tissue can be directly derived from Eq. [3] by assigning fat to pool A and removing all the exchange terms. The saturation absorption lineshape of fat protons is considered super-Lorentzian and defined by Eq. [4].

Methods

Simulations

All simulations were performed using MATLAB (Mathworks, Natick, MA). Based on Eqs. [3], [4], the saturation frequency-dependent MT effects of representative tissues including cerebral spinal fluid (CSF), blood, fat, muscle, white matter (WM) and gray matter (GM) were simulated at three field strengths (1.5T, 3T and 9.4T) using a 3 second CW pulse with a B1 field strength $\omega_1/2\pi = 150$ Hz. These tissues were selected for the simulation based on model parameters in the literature (30,33–40), which are listed in Table 1. The simulation for fat was only conducted for 1.5T due to the lack of relaxation times at other field

strengths. To test whether the use of reference offset at -50ppm can reduce the impact of B_1 inhomogeneity, we considered up to $\pm 30\%$ error of a nominal $B_1 = 3.6\text{ }\mu\text{T}$ ($\omega_1/2\pi = 150\text{ Hz}$). The MT effects of WM and CSF at -12.5ppm and -50ppm were calculated using the parameters for 1.5T listed in Table 1, with the saturation power set to either B_1 ($\omega_1/2\pi = 150\text{ Hz}$) or B_1' ($B_1' = B_1 + \text{error}$). Both MTR(-12.5ppm) contrast and NOMAR ($-12.5\text{ppm}/-50\text{ppm}$) contrast between WM and CSF were calculated. The relative error in MTR and NOMAR was then calculated using $|1 - \text{contrast}(B_1')/\text{contrast}(B_1)| * 100\%$ and plotted with respect to the variation of B_1 ($\omega_1/2\pi$) with respect to a nominal $\omega_1/2\pi$ of 150 Hz .

***In vitro* phantom preparation**

A phantom was prepared consisting of ten glass capillaries with inner diameter of $1.1\text{--}1.2\text{ mm}$. Solutions containing either 0 or 10 mM free L-arginine (Larg, Sigma, MO) and 0%, 5%, 10%, 15% and 20% bovine serum albumin (BSA, Sigma, MO) in 0.01M phosphate – buffered saline buffer were prepared in 1.5mL Eppendorf tubes and titrated to $\text{pH}=7.3$. These samples were all loaded into the glass capillary tubes and then heated in a 80°C water bath for 10 minutes in order to crosslink the BSA(41).

***In vivo* Animal Studies**

The Larg filled version of DLs was prepared as described previously(10). Their size and concentration were measured using dynamic light scattering (Nanosizer, 90ZS, Malvern Instruments) and fluorescence (Victor V, Perkin Elmer) with a size determined to be approximately 100nm . All animal experiments were approved by our institutional animal care and use committee. Eight-week old C57BL/6.SJL mice bearing the CD45.1 alloantigen were purchased from Jackson Labs (Bar Harbor, Maine) and bred in-house. The mice were injected with a cancer cell vaccine composed of irradiated ($10,000\text{ Rad}$) B16 melanoma and irradiated ($5,000\text{ Rad}$) B78-H1 GM-CSF-expressing bystander melanoma cells(42) to produce an immunoresponsive enlargement of the popliteal lymph nodes 1 week prior to DL injection.

MRI

All *in vitro* MRI datasets were acquired at 310 K on a 9.4 T Bruker scanner using a 15 mm sawtooth resonator. CEST imaging was conducted as described previously(9) through collection of two sets of saturation images using a continuous wave (CW) RF saturation pulse: a water saturation shift referencing (WASSR) set for B_0 mapping (24) and a CEST set for characterizing contrast. For the WASSR images, the saturation parameters were: block-shaped RF pulse, $t_{\text{sat}}=200\text{ ms}$, $\omega_1/2\pi = 21.3\text{ Hz}$ ($B_1 = 0.5\text{ }\mu\text{T}$), $\text{TR}=1.5\text{ sec}$ with saturation offset incremented from -1 to $+1\text{ ppm}$ with respect to water in 0.1 ppm steps. For the CEST images we used a block-shaped RF pulse, $t_{\text{sat}}=4\text{ sec}$, $\omega_1/2\pi = 150\text{ Hz}$ ($B_1 = 3.6\text{ }\mu\text{T}$), $\text{TR}=6.0\text{ sec}$, with offset incremented from -4 to $+4\text{ ppm}$ (0.2 ppm steps). In addition, we acquired a third set of MT weighted images using: block shape RF pulse, $\omega_1/2\pi = 50, 100, 150$ and 200 Hz , $t_{\text{sat}}=3\text{ sec}$ with the saturation frequency swept from -100 ppm (-40 kHz) to 100 ppm (40 kHz) in 5 ppm (2 kHz) steps. The MR imaging were acquired using fast spin echo RARE sequence(43) with parameters set to: effective $\text{TE}=40\text{ ms}$, RARE factor=16, acquisition bandwidth=50 kHz, slice thickness=1 mm, acquisition matrix size=128×64, field of view (FOV)=13×13 mm, and 2 averages. An unsaturated image (S_0) was also acquired with same parameters except B_1 field strength set to zero.

In vivo images were collected 24 hours after three mice were intradermally injected with $40\text{ }\mu\text{L}$ of $\sim 30\text{ nM}$ DL solutions in their right hind footpad. The images were acquired on a 9.4 T Bruker scanner using a 25 mm sawtooth resonator and the same imaging scheme as *in vitro* with the addition of a frequency-selective fat suppression pulse (3.4 ms hermite pulse, offset= -3.5 ppm) and the following acquisition parameters: $\text{TR}=5.0\text{ sec}$, effective $\text{TE}=21.6$

ms, RARE factor=8, $t_{\text{sat}}=3$ sec, $\omega_1/2\pi=150$ Hz, slice thickness=0.7 mm, acquisition matrix size=128×64, FOV=20×20 mm, and 2 averages. The saturation offset was incremented ± 2 ppm (0.1 ppm steps) with respect to water for B_0 mapping. The CEST contrast was characterized using a z-spectra acquisition, in which the offset was set to ± 4 ppm (0.2 ppm steps), with three additional images acquired at -5 ppm, -12.5 ppm and -50 ppm.

All data were processed using custom-written MATLAB scripts. Images were first filtered by SNR > 40 to remove low SNR pixels, with the noise determined by the standard deviation of a noise ROI (often the black regions at upper right corner) of the S_0 image. ROI masks were drawn manually based on the T2w images with the mean intensities used for plotting NOMAR and MTR_{asym} . For simplicity, we use NOMAR instead of $\text{NOMAR}(-12.5 \text{ ppm}/-50 \text{ ppm})$ for the remainder of this study. The CEST contrast was quantified by calculating MTR_{asym} . NOMAR filtering was conducted using the following procedure. First, a NOMAR map was generated using MR images with saturation offsets of -12.5 ppm and -50 ppm ($\omega_1/2\pi=150$ Hz). Next, a pixel count vs NOMAR histogram was generated and fit to a mixed Gaussian distribution using the expectation-maximization (EM) algorithm in the MATLAB function 'fit_mix_gaussian.m'. For the *in vivo* images, we assumed three independent Gaussian functions (three compartments: low, moderate, and high water content) over the image, with higher NOMAR in high fluid compartments. While our fitting was not restricted, the means of the three compartments were consistent and in the range of mean NOMAR <0.5 (low), $0.5 < \text{mean} < 0.7$ (moderate) and mean >0.7 (high). Note that this depends on $\omega_1/2\pi$ and the type of transmit coil used, so for each particular experimental setup the optimum ratios must be determined separately. The global threshold (a fixed threshold for all pixels in a given image) was initially determined by finding the point in the histogram where the high water content compartment crossed the moderate water content compartment for one mouse and kept at NOMAR <0.7 for the other mice. All pixels possessing a higher ratio than this value were removed from the subsequent images to selectively display CEST contrast in the tissues with relatively high MT effect (low NOMAR).

RESULTS

Simulations

The simulations clearly showed that tissue containing high fluid content such as CSF, blood or fat demonstrated distinguishable saturation contrast patterns compared to regular tissues such as muscle, white matter (WM) and gray matter (GM) at all three field strengths (1.5T, 3T, 9.4T; Figs. 1A–C) simulated. To test whether NOMAR filtering can effectively segment tissues using the simulated data, we first estimated the two frequencies used in NOMAR. Using Eqs [6], the saturation offset $\Delta\omega_{\text{obs}}$ was determined to be -12.5 ppm (-5.0 kHz) with $T_{1W}=1.66$ sec, $T_{2W}=37.2$ msec at 9.4T (34) and $\omega_1/2\pi=150$ Hz using white matter (WM) as the target tissue. $\omega_1/2\pi$ was chosen based on our previous study which showed that 150 Hz is a good field strength for observing CEST contrast at 9.4T(10). As shown in Fig. 1, when the saturation offset is -12.5 ppm (-5 kHz), the MT contrast divides to either $S/S_0 > 0.85$ (CSF, blood) or $S/S_0 < 0.7$ (WM, GM, muscle) at all three field strengths analyzed in the simulation. Fig 1D shows that using a reference offset $\Delta\omega_2 = -50$ ppm can reduce the impact of B1 inhomogeneity on the contrast between WM and CSF. For example, this simulation shows that the relative error of $\text{MTR}(-12.5 \text{ ppm})$ could be reduced by 2% compared to that of $\text{NOMAR}(-12.5 \text{ ppm}/-50 \text{ ppm})$ when there is a 20% change in $\omega_1/2\pi$. As shown in Fig. 1E, $\text{NOMAR}(-12.5 \text{ ppm}/-50 \text{ ppm})$ allows the separation of CSF, blood from muscle, WM, and GM tissues at all 3 magnetic field strengths analyzed in the simulation. Fat tissue, on the other hand, has only negligible MT contrast and shares the same NOMAR characteristics as fluid as is displayed in Figs 1A and 1E.

Phantom experiments

S/S_0 was determined as a function of frequency for $S(-12.5 \text{ ppm})/S_0 = 1.0, 0.65, 0.51, 0.42$ and 0.37 ppm for cross-linked BSA concentrations of 0, 5%, 10%, 15%, and 20% w/v (I assume), respectively. The S/S_0 values are close to the simulations shown in Fig. 1C. The use of NOMAR with $\Delta\omega_1 = -12.5 \text{ ppm}$ and $\Delta\omega_2 = -50 \text{ ppm}$, as shown in Fig. 2B, allows separation of samples with different concentrations of BSA with the presence of relatively low concentrations (i.e. 10 mM) of Larg not significantly altering the segmentation. The NOMAR values to separate these compartments were manually determined using the histogram (Fig 2C) to be $\text{NOMAR}=0.8$ or 0.67 for separating out the 0% BSA and 5% BSA samples respectively which display similar $S(-12.5 \text{ ppm})/S_0$ to blood, fat or CSF. Figure 2D shows that the NOMAR filter can be used to discriminate weak CEST contrast in samples containing moderate to high macromolecule concentrations from the high CEST contrast in samples containing high water concentrations using the NOMAR thresholds determined in Fig. 2C. Based on the measurements shown in Fig. 2, the samples were found to be appropriate for mimicking low (15–20% BSA), moderate (10% BSA), and high (0–5% BSA) water content tissues. The peak of the Larg CEST contrast is 1.8ppm downfield from water. At this offset the CEST contrast of 10 mM Larg gradually decreases ($\text{MTR}_{\text{asym}} = 9.4\%, 5.6\%, 3.1\%, 2.2\%$ and 1.2%) when the concentration of embedded BSA was increased from 0 to 20%. This is due to the proximity of the Larg resonance to the water resonance, which is broadened at the higher BSA concentrations, with the signal loss not being a result of NOMAR-filtering.

In vivo application of NOMAR based segmentation

We then applied NOMAR-filtering to select tissues *in vivo*. A representative axial T_2w image is shown in Fig. 3A, with popliteal lymph nodes (DLs will migrate to these nodes after footpad s.c. injection) near the top of each leg. For this animal, visual inspection revealed a bright region on the left (ROI2) due to edema, later confirmed by physical examination. Fig. 3B displays the frequency dependence of S/S_0 for 4 ROIs consisting of lymphatic tissue (ROI1), edema (ROI2), muscle tissue (ROI3), and residual unsuppressed fat (ROI4). Based on the mean S/S_0 plot for these ROIs, it is apparent that the proposed NOMAR filter is capable of discriminating edema (ROI2) or fat (ROI4) from muscle and lymph node. In order to validate the definition of the NOMAR filter using the -12.5 ppm frequency rather than frequencies already collected for the CEST z-spectra acquisition (i.e. $<4 \text{ ppm}$), histogram analysis was performed for the following frequencies: -3 ppm , -5 ppm (which was acquired *ad hoc*), and -12.5 ppm on all pixels within the ROIs in the image (Fig. 3C). Figures 3C and 3E show that collecting the additional -12.5 ppm and -50 ppm frequency images allows a better segmentation which not only can separate ROI1 (lymph nodes) and ROI3 (muscle) from ROI2 (edema) and ROI4 (fat) but also separate ROI1 from ROI3. In addition, selection of -12.5 ppm results in $\sim 25\text{--}50\%$ better signal strength for ROI1 and ROI3 compared to -5 ppm (Fig. 3B). Both fat and edema pixels were grouped together as one compartment and fit using a single Gaussian. As shown in Fig. 3F, using a threshold of $\text{NOMAR} < 0.7$ (based on the mixed-Gaussian fitting in Fig. 3E) allows the removal of most of the pixels in ROI2 and ROI4. In addition, most of the muscle tissue can be segmented out by using a threshold of $\text{NOMAR} < 0.5$.

An example of how to apply this segmentation to CEST contrast maps on mice injected with DLs is shown in Fig. 4. Figs. 4A,B show the T_2w and NOMAR maps for a mouse injected with DLs, with the injected leg on the left of the image. As shown in Figs. 4C,D,E,F, there are additional hot spots (pointed out by the yellow arrows) that are not expected based on the histological and SPECT studies performed previously(10) as well as pixels with larger CEST contrast on the right of the highlighted lymph node (blue arrow). These hot spots were segmented out from the resulting CEST maps (Figs. 4D&F) after applying a NOMAR

filter, even in the presence of large B_0 field variations as measured to be $> 1000\text{Hz}$ (2.5 ppm) using the WASSR B_0 mapping method (Fig. 4G). The mean CEST contrast after B_0 correction in the lymph node areas marked by the green and blue arrows are shown in Fig. 4H, indicating a large difference in the right edge of the node. Application of a threshold $\text{NOMAR} < 0.70$ removed the contrast in pixels marked by the yellow and blue arrows (Fig. 4E). The disproportionally high CEST contrast in these pixels is likely due to the presence of edema in these pixels, based on our previous histological studies(10).

DISCUSSION

We have presented a simple method to filter CEST contrast maps based on NOMAR, which is directly related to the amount of MT contrast. To select an appropriate saturation offset for tissue thresholding, we used simulations and confirmed these with measurements on a BSA phantom (Fig. 2) at 9.4T. Variation in B_1 can influence measurement of MT contrast, however the use of a normalized magnetization ratio $\text{NOMAR}(-12.5\text{ppm}/-50\text{ppm})$ allows partial cancellation of this influence due to both the numerator and denominator being affected(44). Such an approach allows creation of tissue selective contrast maps to highlight contrast within targeted tissues and remove predominantly fluid (e.g. CSF and blood in brain imaging, or blood and edema in body imaging) and fat voxels. This is particularly useful for CEST imaging as fat and edema will be highlighted (over a certain range of saturation frequencies) on MTR_{asym} maps without containing the CEST agents of interest in a study. In principle, this segmentation technique can easily be tailored for other types of MRI contrast agents.

The proposed segmentation method is expected to improve quantitative interpretation of CEST contrast maps. For example, 5% CEST contrast is produced by 10 mM Larg in 5% cross-linked BSA sample (Fig. 2D), which is almost twice the amount produced in 15% cross-linked BSA. This is expected, because T_1 and T_2 relaxation times are shortened as the concentration of the semi-solid pool increases and cross terms due to exchange between the CEST pool and semi-solid macromolecule pool will also increase. This will result in a reduction of the magnitude of CEST contrast(45). The NOMAR filtering method can determine if compartments containing CEST agents have high, moderate or low concentrations of semi-solid pools, with the higher the concentration of semi-solid protons the lower the CEST contrast for the same concentration and exchange rate.

In our *in vivo* experiments, DLs spontaneously migrate along the lymphatic draining system to lymph nodes after injection. For the Larg DLs injected, image segmentation and tissue classification was necessary to remove: 1) the bladder, which always displays CEST contrast due to the presence of urea(19), 2) edema, which was found to produce a broad asymmetry from 1–3 ppm possibly due to extravasation of a macromolecule, 3) fat tissue, which was not completely removed using a single fat-suppression pulse and produced an asymmetry with respect to water at -3.5 ppm due to the chemical shift separation with water and can contaminate CEST images(46). As an immune response might be triggered by the injections, we were particularly interested in using NOMAR-based tissue selection to separate CEST contrast in the target tissues (i.e. lymph nodes) from those in edema, which can result from an immune response. Tissue selection reduces the possibility of the three issues listed above being misinterpreted as containing Larg DLs in the contrast maps through the NOMAR-filtration. We derived the final filter settings based on visual inspection of the images for regions containing fat or fluids, drawing ROIs, and then setting the threshold to 0.7 to remove all the pixels in these known regions. In addition, NOMAR is expected to be insensitive to small to moderate B_0 inhomogeneities (i.e. B_0 shifts $< 1\text{ppm}$) because the observing saturation offset is set at -12.5 ppm which is far enough from the water resonance to allow separation of compartments possessing low MT contrast using histogram analysis.

However, it should be noted that the proposed segmentation will filter out the whole voxel based on the MT contrast for the majority of the voxel, and may lead to misinterpretation if the voxel contains a mixture of several types of tissues. In some cases, NOMAR segmentation may result in an underestimation of pixels containing CEST contrast agents because the filtered edematous tissues may contain contrast agents. The segmentation is also incapable of removing endogenous CEST contrast (e.g. APT(20) and GlycoCEST(21)) within the target tissues, which may be removed by comparing the CEST contrast of a tissue with that prior to the injection of contrast agents or that of the contralateral tissue(10). Despite these drawbacks, the present NOMAR segmentation method is still expected to be useful for a broad array of *in vivo* applications.

CONCLUSION

We have proposed a new NOMAR-filter based segmentation technique for creating tissue selective images. This can be accomplished through the collection of two additional saturation images and performing histogram analysis on the resulting image. This technique can easily be implemented with a CEST acquisition to improve the *in vivo* detection of CEST agents.

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Figure 1.

(A–C) Simulation of Magnetization Transfer Contrast (MTC) for different tissues at 1.5T (A), 3.0T (B), and 9.4T (C) using the MT parameters and relaxation rates given in Table 1. (D) Relative error in tissue contrast between WM and CSF as a function of B1 error, calculated using NOMAR and MTR, respectively. (E) NOMAR values calculated for 12.5 ppm and –50 ppm.

Figure 2.

In vitro demonstration of NOMAR filtering for tubes containing multiple concentrations of cross-linked BSA. **(A)** Frequency dependency of S/S_0 for cross-linked BSA samples. **(B)** T2w image (top panel) and the corresponding NOMAR map (bottom panel) of 0%, 5%, 10%, 15%, and 20% cross-linked BSA with (top row) or without (bottom row) inclusion of 10 mM L-arginine. **(C)** histogram of NOMAR values for cross-linked BSA samples, with arrows pointing to 0.8 and 0.67 thresholds. **(D)** MTR_{asy} maps before (top) and after NOMAR filtering with the threshold set at NOMAR < 0.80 (middle) or NOMAR < 0.67 (bottom).

Figure 3.

In vivo frequency and threshold determination for NOMAR filter. (A) T2w image displaying location of ROIs 1–4. (B) Z-spectra for the 4 ROIs. (C) Histograms of pixels in the 4 ROIs based on three frequencies marked by lines in B: –3 ppm (left), –5 ppm (center), and –12.5 ppm (right). (D) NOMAR map. (E) Histogram for the ratio of NOMAR in all pixels in (A) with two thresholds set using mixed-Gaussian fitting. Red arrow marks NOMAR=0.7 and green arrow marks NOMAR=0.50. (F) Filtered T2w images containing only pixels with high (top panel), moderate-low (middle panel), or low (bottom panel) NOMAR.

Figure 4.

Demonstration of NOMAR-based segmentation for CEST contrast maps of DLs to filter out fluids and residual fat. (A) T2w image. (B) NOMAR map, (C,D) MTR_{asymp} maps before (C) and after (D) NOMAR filtering. All pixels shown have SNR > 40 in the S₀ image. (E,F) Corresponding overlay images before (E) and after (F) NOMAR filtering, with the CEST contrast windowed using CNR > 7.2. This highlights only those pixels with significant CEST contrast (i.e., lymph nodes). The CEST contrast in the left popliteal lymph node (green arrow) was separated from other sources of asymmetries (yellow and blue arrows) using this filter. (G, H) WASSR B₀ map (G) and MTR_{asymp} (H) plots of two ROIs outlined by the green and blue arrows in (E).

Table 1

Parameters used for theoretical simulations.

Tissue	$f(\%)$	$R(s^{-1})$	T_{2B} (μs)	1.5T		3.0T		9.4T	
				T_{1w} (sec)	T_{2w} (ms)	T_{1w} (sec)	T_{2w} (ms)	T_{1w} (sec)	T_{2w} (ms)
Muscle	7.4	66	8.7	1.0	35	1.4	50	1.2(37)	21(37)
WM	13.9	23	10.0	0.9	72	1.1	69	1.7(31)	37(31)
GM	5.0	40	9.1	1.1	95	1.8	99	2.1(31)	41(31)
Blood	2.8	35	280	1.4	327	1.9	275	2.4(32)	65(31)
CSF	0.01(27)	0.001(27)	N/A	2.26(27)	2.25(27)	4.3(35)	1.5(34)	4.3(33)	111(33)
Fat	0	0	N/A	0.8(36)	200(36)	N/A	N/A	N/A	N/A

Note: All values were taken from Stanisiz et al., (33) unless otherwise noted.