Multiparametric fat–water separation method for fast chemical-shift imaging guidance of thermal therapies

Jonathan S. Lin

Department of Bioengineering, Rice University, 6100 Main Street, Houston, Texas 77005 and Department of Imaging Physics, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030

Ken-Pin Hwang

Department of Imaging Physics, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030 and Global Applied Science Laboratory, GE Healthcare Technologies, Waukesha, Wisconsin 53188

Edward F. Jackson, John D. Hazle, R. Jason Stafford,^{a)} and Brian A. Taylor

Department of Imaging Physics, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030

(Received 25 March 2013; revised 18 June 2013; accepted for publication 10 July 2013; published 10 September 2013)

Purpose: A k-means-based classification algorithm is investigated to assess suitability for rapidly separating and classifying fat/water spectral peaks from a fast chemical shift imaging technique for magnetic resonance temperature imaging. Algorithm testing is performed in simulated mathematical phantoms and agar gel phantoms containing mixed fat/water regions.

Methods: Proton resonance frequencies (PRFs), apparent spin-spin relaxation (T_2^*) times, and T_1 -weighted (T_1 -W) amplitude values were calculated for each voxel using a single-peak autoregressive moving average (ARMA) signal model. These parameters were then used as criteria for k-means sorting, with the results used to determine PRF ranges of each chemical species cluster for further classification. To detect the presence of secondary chemical species, spectral parameters were re-calculated when needed using a two-peak ARMA signal model during the subsequent classification steps. Mathematical phantom simulations involved the modulation of signal-to-noise ratios (SNR), maximum PRF shift (MPS) values, analysis window sizes, and frequency expansion factor sizes in order to characterize the algorithm performance across a variety of conditions. In agar, images were collected on a 1.5T clinical MR scanner using acquisition parameters close to simulation, and algorithm performance was assessed by comparing classification results to manually segmented maps of the fat/water regions.

Results: Performance was characterized quantitatively using the Dice Similarity Coefficient (DSC), sensitivity, and specificity. The simulated mathematical phantom experiments demonstrated good fat/water separation depending on conditions, specifically high SNR, moderate MPS value, small analysis window size, and low but nonzero frequency expansion factor size. Physical phantom results demonstrated good identification for both water (0.997 ± 0.001 , 0.999 ± 0.001 , and 0.986 ± 0.001 for DSC, sensitivity, and specificity, respectively) and fat (0.763 ± 0.006 , 0.980 ± 0.004 , and 0.941 ± 0.002 for DSC, sensitivity, and specificity, respectively). Temperature uncertainties, based on PRF uncertainties from a 5×5 -voxel ROI, were 0.342 and 0.351 °C for pure and mixed fat/water regions, respectively. Algorithm speed was tested using 25×25 -voxel and whole image ROIs containing both fat and water, resulting in average processing times per acquisition of 2.00 ± 0.07 s and 146 ± 1 s, respectively, using uncompiled MATLAB scripts running on a shared CPU server with eight Intel Xeon™ E5640 quad-core processors (2.66 GHz, 12 MB cache) and 12 GB RAM.

Conclusions: Results from both the mathematical and physical phantom suggest the k-means-based classification algorithm could be useful for rapid, dynamic imaging in an ROI for thermal interventions. Successful separation of fat/water information would aid in reducing errors from the non-temperature sensitive fat PRF, as well as potentially facilitate using fat as an internal reference for PRF shift thermometry when appropriate. Additionally, the T_1 -W or R_2^* signals may be used for monitoring temperature in surrounding adipose tissue. © 2013 American Association of Physicists in Medicine. [<http://dx.doi.org/10.1118/1.4819815>]

Key words: machine learning, fat–water separation, chemical shift imaging, thermometry, k-means

1. INTRODUCTION

The use of minimally invasive thermal therapies is increasing for the management of cancer.^{1–4} Image guidance for tumor localization, treatment monitoring, and verification is critical. The use of magnetic resonance (MR) guidance is attractive due to its multiple soft-tissue contrast mechanisms and fully adjustable imaging planes.^{5,6} In particular, magnetic resonance temperature imaging (MRTI) provides an effective and noninvasive means of monitoring heat-mediated therapies which can aid in real-time estimation of damage to tissue.⁷

Different MR parameters, including the diffusion coefficient, spin-lattice (T_1) and spin-spin (T_2) relaxation times, magnetization transfer, and proton density, have been used as the basis for different MRTI implementations.^{8,6} However, MRTI methods based on the proton resonance frequency (PRF) shift^{9,10} have emerged as the most frequently used, owing to PRF's linear relationship with temperature and its independence from tissue type.^{8,6,11}

PRF shift measurements are usually achieved by calculating the phase change of a gradient echo acquisition. Such acquisitions, however, are only optimized to a single tissue apparent spin-spin relaxation (T_2^*) time, are vulnerable to errors from echo-time offset¹² and are susceptible to contamination from temperature-insensitive fat peaks.¹³ Chemical shift imaging (CSI) techniques can also measure PRF shifts,^{14–18} and allow fat spectral peaks to serve as an internal reference when present, thereby turning a weakness of phase change methods into an advantage. Recently, a fast CSI technique was introduced^{19,20} that allows PRF measurement at high signal-to-noise ratios (SNR) with relatively high spatiotemporal resolution, making it an ideal approach for real-time temperature monitoring in interventional applications. Additionally, other spectral parameters obtained by this technique, such as T_2^* and peak T_1 -weighted (T_1 -W) amplitude values, have been shown to aid in verifying thermal damage.²¹

Methods for separating fat and water tissue during interventional applications would have to maintain the temporal resolution already achieved by this fast CSI method's acquisition and processing techniques. Using a reduced ROI could facilitate such rapid processing,²² provided that the ROI was still positioned to monitor local heating in the relevant area. Analysis could also be facilitated by fully using the technique's available information, including the three spectral parameters (PRF, T_2^* , and T_1 -W). Fat–water separation with this fast CSI technique therefore becomes a multiple parameter information problem, and such multiparameter tissue classification has already been performed for different tissues using different types of information.^{23–32} Within this field, one highly evolved grouping method for multiparameter data is k-means clustering,^{26,33,23,28} which could be ideal for this problem owing to its unsupervised nature and high efficiency relative to other clustering techniques.^{34,35} There has even been preliminary work on using k-means specifically for multispectral fat/water tissue classification,³⁶ of which this work is an extension.

In this study, we investigated a peak identification algorithm based on k-means clustering of multiparametric CSI

data for each species (PRF, R_2^* , and T_1 -W amplitudes) to differentiate and classify a two-peak fat/water system in both a simulated mathematical phantom and a physical phantom. Performance in the mathematical phantom, which contained regions of fat, water, and fat-water mixed together, was systematically analyzed over a range of simulated acquisition and processing conditions. Performance in the physical phantom, also made of fat, water, and mixed regions, was then analyzed on acquisitions from a 1.5T MR clinical scanner. In contrast to existing MR-related methods of fat–water tissue separation for diagnostic purposes that involve in-phase/out-of-phase techniques (i.e., Dixon),^{37,38} constrained reconstructions (i.e., IDEAL),³⁹ or region growing based on phase vectors,⁴⁰ our algorithm's end goal involves rapid and dynamic imaging in an ROI for thermal interventions.

2. MATERIALS AND METHODS

2.A. Mathematical phantom

A mathematical phantom (Fig. 1) was created for rigorous *in silico* testing of the k-means classification algorithm under highly controlled conditions, in order to provide an initial controlled assessment of algorithm performance under different imaging and processing conditions for algorithm optimization prior to implementation on acquired data. The mathematical phantom consisted of a 256×256 matrix containing sections of pure water (a 114-voxel radius circle), pure fat (circles with 1, 3, 7, 11, and 35-voxel radii), and mixed fat/water combined (circles with 1, 3, 7, 11, and 35-voxel radii). The fat and mixed regions were placed inside the water region at variable distances from the image center because simulated isotropic field shifts increased in strength with radial distance from that location. Note that fat/water concentrations in the mixed regions were fixed. All calculations were performed using uncompiled, in-house scripts written in MATLAB (MathWorks, Natick, MA).

FIG. 1. Illustration of spectral measurement observations taken from individual voxels of a single $N \times N$ block (highlighted square) inside mathematical phantom. The block is N voxels in length along each side, and contains N^2 voxels total. Each voxel possesses a set of PRF, T_2^* , and T_1 -W values, meaning N^2 observations are plotted in the feature space based on the voxels contained in that block. After these observations are analyzed, the highlighted square moves to an adjacent location in the grid, and the process is repeated. Note that although the observations are plotted using two spectral parameters for illustrative purposes, three spectral parameters (PRF, T_2^* , and T_1 -W) are collected.

TABLE I. Mathematical phantom parameter values. A.U. = arbitrary units.

MR parameter	Water	Fat
T1 (ms)	500	250
T2* (ms)	50	40
PRF (Hz)	0	224
Proton density (A.U.)	10000	2500

A damped exponential signal model, monoexponential or biexponential depending on the presence of water and/or fat, and with no initial phase/frequency offset, was synthesized with additive Gaussian noise in the real and imaginary components of the signal for each voxel, and then sampled similar to the echo-times of the multiple fast-gradient, refocused echo (MFGRE) acquisition. Table I contains parameter values used for water and fat in the mathematical phantom. Note that the water T1 value is comparable to that found in other tissues, including liver (~600 ms),⁴¹ kidney (~630–700 ms),^{42,41} and white matter (~650 ms).⁴³ The water:fat relative signal amplitude ratio was 4:1 to imitate recent literature.²⁰ Other simulated parameters included a flip angle of 17°, field strength of 1.5T, ETL = 16, TR = 70 ms, and initial TE = 2 ms. Echo spacing (ESP), the amount of time between consecutive echoes in a multiple-echo imaging sequence, was 3.3 ms. Spectral bandwidth (BW) was −2.37 to +2.37 ppm.

The k-means-based classification algorithm consisted of a preliminary training step, followed by a more thorough classification step, both of which used PRF, T2*, and T1-W amplitude values calculated using the previously described Stieglitz-McBride (SM) algorithm on an autoregressive moving average (ARMA) signal model.^{20,19} Briefly, the preliminary training step used the initial unclassified values to generate a database of PRF values labeled as fat and water. The classification step used the fat/water PRF database to label the contents of each remaining voxel not already used in that database, in addition to finding secondary chemical species in each voxel. The process is outlined in detail below.

The preliminary training step used square analysis windows, or blocks, to process the 256 × 256-voxel phantom in a regional N × N block fashion (Fig. 1), with N being the voxel length of each side. The PRF, normalized T2*, and normalized T1-W amplitude values for each N × N block were plotted in three dimensions using a single-peak (i.e., dominant peak) ARMA signal model. K-means was used to generate a single centroid (or geometric center) for the plot using all three estimated parameters, with the sum total of point-to-centroid distances being used to determine the need for further sorting. If the sum total of these distances demonstrated large variation in parameter values [i.e., greater than an empirically determined threshold of 10 arbitrary units (A.U.)], then k-means was used to generate two centroids for PRF-based sorting of data points into two clusters. The clusters were labeled as water or fat, with corresponding voxels labeled accordingly. When the sum total of point-to-centroid distances was below the threshold, data points remained unsorted and their voxels remained unclassified. After process-

ing the whole image in this manner by shifting the block to different nonoverlapping spatial positions in a raster dot scanning fashion, the algorithm tabulated overall PRF ranges for water and fat based on the above presumptions. The algorithm attempted to counter fat aliasing (the more common type of aliasing), when detected, by using absolute PRF values for training and classification, then subsequently labeling PRF clusters under the assumption that the normal relative positions of water and fat PRF spectral peaks would be reversed, with fat being higher.

Next, water and fat PRF ranges from the preliminary training step were increased by a frequency expansion factor to create a window to help account for B₀ field inhomogeneity across the field-of-view (FOV). Statistical classification of voxels was facilitated by comparing their PRF values with these expanded PRF ranges. Voxels left unclassified by the preliminary training step were reprocessed using a two-peak ARMA model to aid in characterizing mixed fat/water voxels with two PRF values. Any remaining voxels still unclassified after this two-peak processing were processed a second time with the single-peak ARMA model, with resulting PRF values used to classify voxel contents. Voxels still unclassified after these three classification attempts remained unclassified. A flow diagram of the algorithm is shown in Fig. 2.

Metrics used to characterize algorithm performance included the Dice Similarity Coefficient (DSC), sensitivity, and specificity. The DSC metric⁴⁴ calculates the fractional overlap of a true image A with a measured image B, where

$$DSC = \frac{2|A \cap B|}{A + B}.$$

Sensitivity was defined as the true positive rate (True Positive/All Positive), while specificity was defined as the true negative rate (True Negative/All Negative).³⁶

Several key parameters were modulated to quantitatively assess effects on algorithm performance. These parameters included SNR, field change, block size, and frequency expansion factor size. The SNR was changed uniformly for the fat, water, and mixed signals by using a scaling factor that determined the addition of noise. Field change effects were incorporated according to the complex exponential equation:

$$S(x, y, z) = S_0(x, y, z) \times e^{i2\pi \text{esp} * (z-1) * \sqrt{(x*\text{db})^2 + (y*\text{db})^2}},$$

where S₀ = signal of first echo, ESP = echo spacing (s), z = echo number, x = voxel distance along x-axis from image center, y = voxel distance along y-axis from image center, and db = isotropic variable affecting rate of magnetic field change per voxel in both x and y dimensions. Based on this equation, the magnetic field changes as a function of the radial distance from the image center, and the maximum PRF shift (MPS) at the image edges was used as the defining metric. The MPS values were modulated by altering the variable “db” and using positive/negative versions of the resulting magnitude. Applying these MPS values to the phantom allowed assessment of algorithm performance under conditions of varying field strength, even if such field change patterns as modeled here do not necessarily represent real-world phenomena. Simulations were also performed that applied a range of MPS values

FIG. 2. Flow chart describing the k-means-based classification algorithm.

uniformly across the FOV, meaning magnetic field changes (and the accompanying PRF shifts) did not vary with location but were equivalent for the entire phantom. Block sizes were modulated in factors of 2, where $N \times N$ is the window's voxel dimensions and $N = 8, 16, 32, 64$, and 128 . Frequency expansion factor values were modulated by increasing a variable “df” starting from 0 ppm until reaching 1.0 ppm. This df variable was added to the maximum PRF and subtracted from the minimum PRF of each chemical species, in order to expand the species' PRF range for voxel classification. For each set of parameter values, the k-means classification algorithm ran five times and standard deviations for DSC, sensitivity, and specificity were displayed as error bars when appropriate.

After an optimal set of parameter values was determined, algorithm performance was further assessed by two additional experiments: (1) varying the water-fat ratio in the mixed regions of the phantom and (2) varying the water T1 value over the entire phantom. For the first experiment, the water fraction in the mixed regions (where water fraction + fat fraction = 1) was gradually increased from 0.05 to 0.95. For the second experiment, the water T1 value was increased from 500 to 2500 ms, affecting the water signal in both pure water and mixed regions of the phantom.

2.B. Physical phantom

A multicomponent phantom was fabricated using a plastic cylinder filled with 1% agar (diameter 10 cm) and two smaller cylindrical wells (diameter 2.7 cm). One cylindrical well contained only fat (Pure Vegetable Oil, The Kroger Co., Cincinnati, OH), while the other contained a fat/water mixture consisting of roughly 50% mayonnaise (Canola Mayonnaise, Hain Celestial Group, Inc., Melville, NY) and 50%

lemon juice (Organic Pure Lemon Juice, Santa Cruz National, Inc., Chico, CA), before being combined with 1% agar.¹⁶ The phantom was immersed in water to reduce susceptibility effects near the phantom edges.

All images were acquired on a 1.5T clinical MR scanner (Excite HD, GEHT, Waukesha, WI) using a phased-array knee coil. MFGRE acquisition parameters were as follows: Flip Angle 17°, FOV 17 × 17 cm, TE₀ 1.98 ms (full echo), ESP 3.30 ms, TR 70 ms, BW ± 31.25 kHz, ETL 16, matrix 128 × 128, slice thickness 5 mm, acquisition time 10.5 s/image. Fourteen acquisitions with these parameters were obtained.

An ROI encompassing the entire cylinder was used to demarcate the area for CSI processing, and PRF, R2*, and ln(T1-W) values for each voxel within the ROI were tabulated. Data points were plotted based on all three parameters, and a single centroid was generated for these data points using k-means. If the sum of point-to-centroid distances exceeded a threshold (>5 A.U.), this demonstrated the likely presence of both fat and water, and caused sorting of the PRF values into two separate clusters with their corresponding voxels labeled as those species (Fig. 3, I–III). Voxels remained unclassified if the relevant metric was below the threshold.

The PRF classification range for each chemical species was equal to its PRF mean ± df (the frequency expansion factor), as this was empirically determined to be the best method for calculating PRF classification boundaries. Subsequent refinement steps then reanalyzed all voxels in the ROI (Fig. 3, IV). The PRF, T2*, and T1-W amplitude values for each voxel were recalculated using a two-peak model, and these two-peak PRF values were compared to fat/water PRF ranges to classify mixed-component voxels. For voxels still unclassified after this step, single-peak PRF, T2*, and

FIG. 3. Amplitude maps for water (a) and fat (b) during the initial classification step (I, II, III), and after the refinement steps (IV). For columns I and II, the right side of the image contains many unclassified voxels because the algorithm has not yet extracted spectral parameters from voxels in that portion of the image. For column III, the algorithm has finished extracting such parameters on a first-pass basis but has not yet analyzed voxels for two-peak signals, leaving those regions (i.e., the mixed-region well) unclassified relative to their less dominant chemical species. For column IV, voxels have been analyzed for multiple chemical species and the mixed-region well is correctly classified with respect to both water and fat.

T1-W amplitude values were recalculated for another attempt at PRF-based classification.

Performance metrics (DSC, sensitivity, specificity) were calculated by comparing algorithm-generated maps of the fat, water, and mixed regions of the phantom with manually segmented maps. Algorithm speed was tested using both 25×25 -voxel and whole image (256×256 -voxel) ROIs, each containing both fat and water, with processing performed on a shared CPU server with eight Intel Xeon™ E5640 quad-core processors (2.66 GHz, 12 MB cache) and 12 GB RAM. Results were compared with the speeds of several algorithms from the ISMRM fat-water toolbox (<http://ismrm.org/workshops/FatWater12/data.htm>), including implementations of iterative decomposition of water and fat with echo asymmetric and least-squares estimation (IDEAL).³⁹

3. RESULTS

3.A. Mathematical phantom

As SNR reached 20, water and fat DSC, sensitivity, and specificity values approached 1.0 asymptotically (Fig. 4). At

FIG. 4. Algorithm classification performance for water (both pure and mixed) and fat (both pure and mixed) inside the phantom across a range of SNR values. SNR varied from 5 to 20, $db = 1.70$ ppm, $N = 8$, df = half the difference between the fat and water PRF cluster boundaries.

SNR 10, each metric for fat and water exceeded 0.8, and at SNR 15, all metrics exceeded 0.95.

For field change simulations, all fat/water classification metrics reached their maximum at an MPS magnitude value of 1.70 ppm (Fig. 5) and then deteriorated for MPS magnitudes above that value. Due to these results, an MPS value of +1.70 ppm was applied to the mathematical phantom for other simulations to optimize algorithm performance when testing different parameters.

Positive and negative MPS values yielded the same results up to ± 2.13 ppm for water and ± 1.98 ppm for fat. Specifically, for MPS values below -2.13 ppm, water DSC and sensitivity suddenly decreased, while water specificity also decreased but at a slower rate. For MPS values below -1.98 ppm, all fat classification metrics decreased, although fat sensitivity decreased the slowest. For uniformly applied off-resonance PRF shifts over the entire FOV (Fig. 6), both water and fat metrics suffered the most at PRF shifts of -2.27 to -1.13 ppm and $+2.41$ to $+3.54$ ppm.

Erratic metric behavior occurred in Fig. 6 for the uniform off-resonance MPS simulations because of the disruption [Fig. 7(b)] and restoration [Fig. 7(d)] of relative spectral peak positions caused by fat and water aliasing. Such aliasing also explains the deterioration in Fig. 5 of algorithm performance for increasingly negative (but not increasingly positive) MPS values, since the fat spectral peak aliases more easily for negative MPS values because of its initial location, while positive MPS values do not cause aliasing for either the fat or water peak because neither peak starts close enough to the upper spectral bandwidth (BW) boundary.

For block size (N) simulations, the highest values for all fat/water metrics occurred at $N = 8$ (Fig. 8). For $N \geq 32$, the classification metrics decreased for both water and fat, but much more noticeably for fat. Water specificity decreased at $N = 32$ but then rebounded for $N = 64$ and $N = 128$.

Up to frequency expansion (df) values of 0.3 ppm, all classification metrics for both water and fat exceeded 0.95 (Fig. 9). Above df values of 0.3 ppm, most metrics experienced a large reduction, the exceptions being water DSC, water sensitivity, and fat sensitivity. These latter metrics decreased as well, but more slowly.

Based on these results, optimal algorithm performance was achieved at high SNR (20), a moderate MPS value

FIG. 5. Algorithm classification performance across a range of MPS values from -3.54 to $+3.54$ ppm. SNR = 20, $N = 8$, and df = half the difference between the fat and water PRF cluster boundaries. Spectral bandwidth = -2.37 to $+2.37$ ppm. Water classification deteriorates below -2.13 ppm, fat below -1.98 ppm.

($+1.70$ ppm), small analysis window size (8×8 voxels), and low but nonzero frequency expansion factor size (0.1 – 0.2 ppm).

For water fractions ≥ 0.1 , all water metrics stayed above 0.96. For water fractions ≤ 0.9 , fat DSC stayed above 0.86, while fat sensitivity and specificity stayed above 0.96. Overall, the algorithm suffered in performance near the extreme ends of the water fraction range, but performed well otherwise (Fig. 10).

Increasing water T1 values did not seem to greatly affect algorithm performance (Fig. 11). For water T1 values from 500 ms to 2500 ms, water metrics stayed above 0.98, and fat metrics stayed above 0.94. Fat DSC decreased the most noticeably as water T1 increased. Fat specificity decreased, as well, but more gradually.

3.B. Physical phantom

Algorithm classification metrics are in Table II. Mean water DSC, sensitivity, and specificity values all exceeded 0.98, while mean fat DSC, sensitivity, and specificity values spanned a greater range. Although fat DSC was below 0.77, both fat sensitivity and specificity exceeded 0.94.

Physical phantom metrics compare favorably with metrics from MPS simulations at $+0.85$ ppm, which is closest to the average PRF variation over the entire agar gel phantom (0.90

± 0.03 ppm). For those simulations, mean water DSC, sensitivity, and specificity were 0.998 ± 0.001 , 1 ± 0 , and 0.987 ± 0.001 , respectively, and mean fat DSC, sensitivity, and specificity were 0.941 ± 0.004 , 0.999 ± 0.001 , and 0.983 ± 0.001 , respectively. For both physical phantom analysis and mathematical phantom simulations, therefore, fat DSC had the lowest value. Also, simulation performed better than physical phantom analysis in all fat/water classification metrics, especially fat DSC where the performance gap is 0.178. However, the differences in results are otherwise small, in the range of 0.001–0.042, and the fat DSC performance gap may be influenced by differences in the size/number of fat and mixed wells between the physical and mathematical phantoms.

Parameter values from the SM algorithm (Table III) were averaged over 14 separate acquisitions from multiple ROIs (Fig. 12, left). Notable pure/mixed differences exist within tissue types for all parameter values. The fat–water PRF shift in pure and mixed regions was 3.51 and 3.53 ppm, respectively.

Physical phantom PRF uncertainties were calculated by processing the contents of 5×5 -voxel ROIs containing water and/or fat. Pure water and pure fat PRF uncertainties were 0.00160 ppm and 0.00255 ppm, respectively, giving a temperature uncertainty of 0.342°C . Mixed-region water and fat PRF uncertainties were 0.00192 ppm and 0.00242 ppm, respectively, resulting in a temperature uncertainty of 0.351°C .

FIG. 6. Algorithm classification performance across a range of uniform off-resonance MPS values from -3.54 to $+3.54$ ppm. SNR = 20, $N = 8$, and df = half the difference between the fat and water PRF cluster boundaries.

FIG. 7. Representative aliasing of spectral peaks. (a) Fat (left) and water (right) peaks at uniform off-resonance MPS value of 0 ppm. Same peaks shown at (b) -1.42 ppm, (c) -2.27 ppm, and (d) -2.83 ppm. Aliasing of the fat peak in (b) is followed by aliasing of the water peak in (d).

As can be seen in the parameter maps (Fig. 13), water parameter maps contained the main phantom body and the mixed well, while fat parameter maps contained the fat and mixed wells. Additional misclassified fat points were scattered throughout the phantom and focused around the well borders (likely due to border susceptibility effects that exacerbated field inhomogeneities).

Table IV compares processing times for our algorithm and several algorithms from the ISMRM fat-water toolbox. When analyzing 25×25 -voxel ROIs, our algorithm's speed exceeded that of the technique by Sharma,⁴⁵ while it was competitive with IDEAL implementations by Hernando⁴⁶ and Tsao.⁴⁷

4. DISCUSSION

Although previous investigators have looked at k-means clustering to separate fat and water tissues located in non-mixed, separate compartments based on T1-W amplitude values,⁴⁸ the present work incorporates all multiparametric information obtained from a fast CSI technique (PRF, T2*, and T1-W) to help perform fat–water tissue separation based on all three parameters in both mixed and nonmixed areas. These parameters already have the potential to serve useful therapy guidance functions, such as PRF's role in monitoring temperature, but recent work has also revealed that the change in temperature dependence for T2* and peak T1-W amplitudes correlates with protein denaturation, thereby allowing

those two parameters to serve as methods for verifying thermal damage.²¹ The fact that all three parameters can serve multiple functions, and that they are all simultaneously obtained during PRF thermometry with this fast CSI technique, renders the use of our algorithm particularly apt.

Separating fat and water tissues also helps disentangle their accompanying information, providing opportunities for the meaningful use of tissue-specific parameter values. In particular, because of the advantages of fat internal referencing for PRF shift thermometry in countering motion artifacts, susceptibility changes, and magnetic field changes,^{18,16,14} it would be worthwhile to see if the algorithm-generated fat PRF maps could also be used as an internal reference, specifically by extending them into nonfat regions using a least-squares fitted polynomial plane and using the estimated fat PRF values in the thermometry ROI for temperature calculation. For MRTI applications in which fat PRF values were not explicitly in the thermometry ROI or located near enough to be a good reference, such a strategy would ameliorate the above vulnerabilities and improve temperature calculations. Note that the method described descends from referenceless PRF shift techniques that use fitted polynomials to estimate background phase values in an image for complex phase difference temperature calculations.¹⁰

A potential weakness of the above method involves susceptibility changes, since extrapolating the fat signal to an area with different susceptibility could lead to inaccurate calculations of the fat-water PRF shift for that new area, especially

FIG. 8. Algorithm performance across a range of analysis window, or block, sizes from 8 to 128. SNR = 20, db = 1.70 ppm, and df = half the difference between the fat and water PRF cluster boundaries.

FIG. 9. Algorithm classification performance across a range of frequency expansion factor values from 0 to 1 ppm. SNR = 20, db = 1.70 ppm, and N = 8.

if the water signal were heavily influenced by the new susceptibility and the extrapolated fat signal were not. In such cases, the method could be extended to incorporate susceptibility changes and field inhomogeneities into the polynomial fitting process, which would allow the extrapolated fat signal to more accurately represent true fat signal behavior at these new locations, thus increasing the accuracy of thermometry based on the fat-water PRF difference. Gradual susceptibility gradients could probably be estimated and accounted for, although sharp susceptibility gradients would be more difficult.

Because k-means clustering does not require user input or prespecified data ranges for classification but rather attempts to learn such ranges on its own, relative and not absolute differences in data values matter most, and any chemical species (not simply water and fat) could theoretically be separated by this method, provided that the species possessed adequate separation in a feature space determined by one or more parameters.

Based on mathematical phantom results, regions with SNR ≥ 15 would possibly be sufficient for distinguishing fat/water with our algorithm, and regions of low SNR would cause the algorithm to fail, especially with respect to fat. Noticeable variation for fat DSC values is shown by the standard deviation bars at SNR 9–13 in Fig. 4. These values of SNR appear to straddle the limits of the algorithm's abilities with respect

to fat. Differences between iterations for other metrics were negligible and are therefore not visible as standard deviation bars (and such is the case for most metrics in Figs. 4–6 and 8–11).

The fact that the optimum MPS magnitude was 1.70 ppm is important because that is approximately half the frequency shift between water and fat. Such a value most likely caused fat/water PRF cluster ranges to expand so as to cover most intermediate PRF values between the two clusters while avoiding overlap. As MPS magnitudes increased above 1.70 ppm, fat/water PRF ranges expanded and probably did overlap (and/or underwent aliasing), and the training step of the algorithm, unable to accurately discern the two groups within this PRF distribution, probably placed boundaries for the fat/water PRF ranges incorrectly and thereby reduced classification accuracy, especially for fat.

Because only the relative position of PRF values determines the initial assignment of voxels as water or fat, the sign of MPS values (whether positive or negative) does not affect algorithm performance except insofar as it involves aliasing. For MRTI applications, such adaptability to sliding PRF values is beneficial, helping to counter uniform shifts in field strength as well as the change in water PRF that accompanies rising temperature. The uniform off-resonance MPS simulations, in particular, show how robust the algorithm is in such

FIG. 10. Algorithm classification performance over a range of water fraction values for the mixed regions of the phantom (where water fraction + fat fraction = 1). Regions consisting solely of pure water or fat signals were not altered. SNR = 20, N = 8, db = 1.70 ppm, df = half the difference between the fat and water PRF cluster boundaries.

FIG. 11. Algorithm classification performance over a range of water T1 values. SNR = 20, N = 8, db = 1.70 ppm, df = half the difference between the fat and water PRF cluster boundaries.

circumstances, as all metrics perform well for the majority of MPS values, save for regions of aliasing.

Aliasing consistently caused problems for the algorithm, despite the algorithm's aliasing-correction strategy of using absolute PRF values for training and classification. This strategy seems effective for negative MPS cases where fat's spectral peak is still close to the aliasing frequency [Fig. 7(b)], but not for negative MPS cases of greater magnitude where water's spectral peak has moved closer to the aliasing frequency than fat's [Fig. 7(c)]. In the latter case, the absolute PRF values for water's spectral peak would be higher, causing incorrect labeling of PRF clusters. Future work, perhaps incorporating nearest neighbor considerations, could improve this aliasing-correction scheme not to be vulnerable to such cases. Such use of nearest neighbors could also play a role in cleaning up voxels left unclassified by the algorithm. Alternatively, T1-W and R2* values could be used for preliminary clustering before PRF values helped label and applied aliasing rules to the clusters.

For the block size simulations, the drop in water specificity for N = 32 occurred because a block of that size covers a large amount of the large fat-only circular region in the lower-left portion of the mathematical phantom, without extending outside of that area. Within that block, field inhomogeneities and noise created enough signal variations to where the algorithm classified that block as containing both fat/water and therefore attempted double-cluster PRF sorting. Fat voxels with higher PRFs were therefore labeled as water, resulting in a reduction of water specificity. The ROIs in MRTI usually contain a mixture of water and fat-based tissues, however, which would help counter this problem, since any water-based tis-

sues would serve to establish accurately the water PRF range and prevent such systematic misclassifications.

As df changed from 0.0 to 0.1 ppm, almost all classification metrics for both water and fat increased in value, meaning the use of a nonzero frequency expansion factor was better than using none at all. Values of df used for the SNR, field inhomogeneity, and block size simulations were calculated by halving the PRF difference between fat/water cluster boundaries after the training step, meaning the df value varied with each iteration of each simulation. Values of df were between 0.19 and 0.25 ppm for when simulations performed best, and usually remained below 0.5 ppm. Such df values correspond with regions of high DSC, sensitivity, and specificity for both water and fat (Fig. 9).

Over a wide range of water fraction values for mixed regions, water metrics did not drop below 0.9, even at a water fraction of 0.05 (Fig. 10). As the water fraction increased, all water metrics increased slightly and seemed to reach a plateau at water fractions greater than 0.5. Fat metrics suffered worst at a water fraction of 0.95, but performed well for water fractions below that value. The drop in fat DSC at water fraction 0.5 is likely due to roughly equal T1-W values for water and fat in mixed regions, thereby making clustering more difficult than in cases of large T1-W differences between the two species where there is more separation.

Water T1 values appear to exert little influence on algorithm performance (Fig. 11). As water T1 increased from 500 to 2500 ms, water metrics decreased by a miniscule amount, with the largest reduction being less than 0.01. Fat metrics decreased by a larger amount, with the largest reduction being greater than 0.04. As water T1 increased from 500 to 1000 ms

TABLE II. Algorithm classification results for agar gel phantom images (n = 14).

	Water	Fat
DSC	0.997 ± 0.001	0.763 ± 0.006
Sensitivity	0.999 ± 0.001	0.980 ± 0.004
Specificity	0.986 ± 0.001	0.941 ± 0.002

TABLE III. Parameter values for different regions within agar gel phantom (n = 14).

	PRF (ppm)	T2* (ms)	T1-W amplitude (A.U.)
Pure water	-0.04 ± 0.01	49.6 ± 0.7	604 ± 3
Pure fat (vegetable oil)	3.47 ± 0.01	37.4 ± 0.4	590 ± 3
Mixed-region water	-0.10 ± 0.01	23.5 ± 0.3	477 ± 4
Mixed-region fat	3.43 ± 0.01	25.5 ± 0.8	189 ± 4

FIG. 12. Phantom in water bath (left), with square 5×5 -voxel ROIs showing origins of Table III spectral parameter values (left, middle, and right for mixed region, pure water, and pure fat, respectively). Perpendicular lines show origins of PRF profiles for y-axis (middle) and x-axis (right), which demonstrate influence of field inhomogeneities. In x-axis PRF profile, note the pure versus mixed-region PRF differences for water (above -0.1 ppm) and fat (below -1.2 ppm), and note that fat PRF is here shown in its uncorrected, aliased form.

(which is comparable to T1 values found in skeletal muscle, heart, cartilage, gray matter,⁴¹ and brown adipose tissue⁴²), the largest reductions in water and fat metrics were 0.0025 and roughly 0.01, respectively.

The spectral parameter values in Table III were affected by magnetic field inhomogeneities, caused by either field gradients or border susceptibility effects from the smaller mixed-region and fat wells. In other words, a voxel's location influenced the spectral parameters derived from it. Note, for example, the PRF gradient along the y-axis of the image (Fig. 12, middle), specifically the difference between pure water at either the top or bottom edge of the phantom (-0.5 ppm or -0.65 ppm, respectively) versus pure water at the central red square ROI (-0.04 ppm).

The fat-water PRF shift in pure and mixed regions was 3.51 and 3.53 ppm, respectively, meaning the individual species' PRFs experienced similar amounts of change (Fig. 12, right), and that field inhomogeneities were therefore the likeliest cause of such change. These values also corresponded well with the 3.49 ± 0.03 ppm fat-water PRF shift previously recorded in a similar fat-water phantom.²⁰

The decrease in mixed-region T2* values (relative to pure regions) may be explained by the different materials used to create the mixed-region well (mayonnaise and lemon juice/agar in the mixed well, instead of vegetable oil and agar in the pure fat and pure water regions, respectively). Note that such compositional differences also create differences in macroscopic susceptibility relative to the rest of the phantom.

FIG. 13. Algorithm-generated parameter maps for water (top row) and fat (bottom row) showing PRF values (left column), T2* times (middle column), and T1-W amplitude values (right column).

TABLE IV. Processing times for whole image and 25×25 -voxel ROIs using different fat–water separation techniques.

	Lin-Taylor (s)	Sharma (Ref. 45) (s)	Hernando (Ref. 46) (s)	Tsao (Ref. 47) (s)
Whole image (256×256 -voxel ROI)	146 ± 1	1420 ± 80	117 ± 2	34.5 ± 3.5
25×25 -voxel ROI	2.00 ± 0.07	3.92 ± 0.06	2.09 ± 0.08	1.53 ± 0.08

In addition to our algorithm's competitive speed performance (Table IV), it also incorporates a unique feature that differentiates it from other water–fat separation methods. Specifically, it incorporates a robust, accurate spectral parameter estimation technique, one which has previously demonstrated performance that matches the Cramer-Rao lower bound,²⁰ and which therefore facilitates the accuracy of PRF shift thermometry based on such data. This characteristic highlights the algorithm's unique suitability for MRTI interventional applications.

Certain thermal therapies have a focal heating pattern, one that causes significant heating in a target region but leaves surrounding areas undisturbed. In cases of such sharp spatial temperature gradients (i.e., a nonheated region that is adjacent to a heated one), the fat PRF would remain stable between both regions, helping the algorithm to identify it as a single chemical species. Water PRF would shift from one region to the next, but the amount of this shift, given water's temperature sensitivity coefficient as demonstrated in recent literature,⁴⁹ would not be large enough to (1) cause misidentification as two separate chemical species or (2) cause aliasing that would confuse its signal with fat's. Algorithm performance would therefore not be greatly affected in such cases.

A limitation of k-means clustering is its difficulty in dealing with spatially varying information. This fact could become relevant for MR acquisitions when susceptibility changes affect $T2^*$ values, when RF and surface coil effects alter $T1$ -W amplitude values, and when different distributions and combinations of fat/water exist across the image FOV. Restricting use of the algorithm to an ROI immediately surrounding the thermal treatment area minimizes this limitation. Another limitation of the study is that the potential impact of magnetic field gradients on $T2^*$ values was not modeled for the mathematical phantom simulations, which could affect the spread of $T2^*$ measurements collected and the success of subsequent clustering and classification. Future work could incorporate such information into its analysis.

5. CONCLUSION

We have demonstrated the feasibility of separating two chemical species measured from a fast chemical shift acquisition via a k-means clustering-based algorithm on PRF, $T2^*$, and $T1$ -W amplitude values. A simulated mathematical phantom of varying fat/water composition was used to test algorithm performance over a range of acquisition and processing parameter values, with results showing the possibility of good fat–water separation depending on conditions. Use of the algorithm on MR images of a multicomponent physical phantom, acquired under conditions similar to simulation as-

sumptions, yielded results that compared favorably with simulation predictions. Future work will include expanding the algorithm's fat PRF maps to serve as internal references for MRTI, improving the algorithm's aliasing-correction capabilities, and perhaps incorporating information from neighboring voxels when helpful.

ACKNOWLEDGMENTS

This research was supported in part by the National Cancer Institute (Cancer Center Support Grant CA016672 and Training Grant 5T32CA119930). J.S.L. acknowledges support from the Baylor College of Medicine Medical Scientist Training Program and the Cullen Trust for Higher Education Physician/Scientist Fellowship Program. The authors report no conflicts of interest.

^{a)}Author to whom correspondence should be addressed. Electronic mail: jstafford@mdanderson.org

¹A. H. Hawasli, W. Z. Ray, R. K. J. Murphy, R. G. Dacey, and E. C. Leuthardt, "Magnetic resonance imaging-guided focused laser interstitial thermal therapy for subinsular metastatic adenocarcinoma: Technical case report," *Neurosurgery* **70**, 332–337 (2012) (discussion 338).

²C. J. Diederich, "Thermal ablation and high-temperature thermal therapy: Overview of technology and clinical implementation," *Int. J. Hyperthermia* **21**, 745–753 (2005).

³A. Carpentier, R. J. McNichols, R. J. Stafford, J.-P. Guichard, D. Reizine, S. Delaloge, E. Vicaut, D. Payen, A. Gowda, and B. George, "Laser thermal therapy: Real-time MRI-guided and computer-controlled procedures for metastatic brain tumors," *Laser Surg. Med.* **43**, 943–950 (2011).

⁴C. Tempny, N. McDannold, K. Hynynen, and F. Jolesz, "Focused ultrasound surgery in oncology: Overview and principles," *Radiology* **259**, 39–56 (2011).

⁵R. Blanco Sequeiros, R. Ojala, J. Kariniemi, J. Perälä, J. Niinimäki, H. Reinikainen, and O. Tervonen, "MR-guided interventional procedures: A review," *Acta Radiol.* **46**, 576–586 (2005).

⁶B. Denis de Senneville, B. Quesson, and C. T. W. Moonen, "Magnetic resonance temperature imaging," *Int. J. Hyperthermia* **21**, 515–531 (2005).

⁷N. McDannold, R. King, F. Jolesz, and K. Hynynen, "Usefulness of MR imaging-derived thermometry and dosimetry in determining the threshold for tissue damage induced by thermal surgery in rabbits," *Radiology* **216**, 517–523 (2000).

⁸V. Rieke and K. Butts Pauly, "MR thermometry," *J. Magn. Reson. Imaging* **27**, 376–390 (2008).

⁹J. Hindman, "Proton resonance shift of water in the gas and liquid states," *J. Chem. Phys.* **44**, 4582–4592 (1966).

¹⁰V. Rieke, K. K. Vigen, G. Sommer, B. L. Daniel, J. M. Pauly, and K. Butts, "Referenceless PRF shift thermometry," *Magn. Reson. Med.* **51**, 1223–1231 (2004).

¹¹R. D. Peters, R. S. Hinks, and R. M. Henkelman, "Ex vivo tissue-type independence in proton-resonance frequency shift MR thermometry," *Magn. Reson. Med.* **40**, 454–459 (1998).

¹²A. H. Chung, K. Hynynen, V. Colucci, K. Oshio, H. E. Cline, and F. A. Jolesz, "Optimization of spoiled gradient-echo phase imaging for in vivo localization of a focused ultrasound beam," *Magn. Reson. Med.* **36**, 745–752 (1996).

¹³J. Poorter, "Noninvasive MRI thermometry with the proton resonance frequency method: Study of susceptibility effects," *Magn. Reson. Med.* **34**, 359–367 (1995).

- ¹⁴K. Kuroda, R. V. Mulkern, K. Oshio, L. P. Panych, T. Nakai, T. Moriya, S. Okuda, K. Hynynen, and F. Jolesz, "Temperature mapping using the water proton chemical shift: Self-referenced method with echo-planar spectroscopic imaging," *Magn. Reson. Med.* **43**, 220–225 (2000).
- ¹⁵K. Kuroda, Y. Suzuki, Y. Ishihara, and K. Okamoto, "Temperature mapping using water proton chemical shift obtained with 3D-MRSI: Feasibility in vivo," *Magn. Reson. Med.* **35**, 20–29 (1996).
- ¹⁶N. McDannold, K. Hynynen, K. Oshio, and R. V. Mulkern, "Temperature monitoring with line scan echo planar spectroscopic imaging," *Med. Phys.* **28**, 346–355 (2001).
- ¹⁷R. V. Mulkern, L. P. Panych, N. J. McDannold, F. A. Jolesz, and K. Hynynen, "Tissue temperature monitoring with multiple gradient-echo imaging sequences," *J. Magn. Reson. Imaging* **8**, 493–502 (1998).
- ¹⁸A. V. Shmatukha, P. R. Harvey, and C. J. G. Bakker, "Correction of proton resonance frequency shift temperature maps for magnetic field disturbances using fat signal," *J. Magn. Reson. Imaging* **25**, 579–587 (2007).
- ¹⁹B. A. Taylor, K.-P. Hwang, A. M. Elliott, A. Shetty, J. D. Hazle, and R. J. Stafford, "Dynamic chemical shift imaging for image-guided thermal therapy: Analysis of feasibility and potential," *Med. Phys.* **35**, 793–803 (2008).
- ²⁰B. A. Taylor, K.-P. Hwang, J. D. Hazle, and R. J. Stafford, "Autoregressive moving average modeling for spectral parameter estimation from a multi-gradient echo chemical shift acquisition," *Med. Phys.* **36**, 753–764 (2009).
- ²¹B. Taylor, A. Elliott, K. Hwang, J. D. Hazle, and R. J. Stafford, "Correlation between the temperature dependence of intrinsic MR parameters and thermal dose measured by a rapid chemical shift imaging technique," *NMR Biomed.* **24**, 1414–1421 (2011).
- ²²B. Quesson, J. A. de Zwart, and C. T. Moonen, "Magnetic resonance temperature imaging for guidance of thermotherapy," *J. Magn. Reson. Imaging* **12**, 525–533 (2000).
- ²³R. A. D. Carano, J. A. Lynch, J. Redei, S. Ostrowitzki, Y. Miaux, S. Zaim, D. L. White, C. G. Peterfy, and H. K. Genant, "Multispectral analysis of bone lesions in the hands of patients with rheumatoid arthritis," *Magn. Reson. Imaging* **22**, 505–514 (2004).
- ²⁴A. Lundervold and G. Stovrik, "Segmentation of brain parenchyma and cerebrospinal fluid in multispectral magnetic resonance images," *IEEE Trans. Med. Imaging* **14**, 339–349 (1995).
- ²⁵M. W. Vannier, R. L. Butterfield, D. L. Rickman, D. M. Jordan, W. A. Murphy, and P. R. Biondetti, "Multispectral magnetic resonance image analysis," *Crit. Rev. Biomed. Eng.* **15**, 117–144 (1987).
- ²⁶K. H. Barck, B. Willis, J. Ross, D. M. French, E. H. Filvaroff, and R. A. D. Carano, "Viable tumor tissue detection in murine metastatic breast cancer by whole-body MRI and multispectral analysis," *Magn. Reson. Med.* **62**, 1423–1430 (2009).
- ²⁷M. B. Merickel, J. W. Snell, T. R. Jackson, D. M. Skyba, and W. K. Katz, "Multispectral tissue identification in MR images," *Conf. Proc. IEEE Eng. Med. Biol. Soc.* **13**, 1323–1325 (1991).
- ²⁸R. A. D. Carano, A. L. Ross, J. Ross, S. P. Williams, H. Koeppen, R. H. Schwall, and N. Van Bruggen, "Quantification of tumor tissue populations by multispectral analysis," *Magn. Reson. Med.* **51**, 542–551 (2004).
- ²⁹R. A. D. Carano, K. Takano, K. G. Helmer, T. Tatlisumak, K. Irie, J. D. Petrucci, M. Fisher, and C. H. Sotak, "Determination of focal ischemic lesion volume in the rat brain using multispectral analysis," *J. Magn. Reson. Imaging* **8**, 1266–1278 (1998).
- ³⁰L. M. Fletcher, J. B. Barsotti, and J. P. Hornak, "A multispectral analysis of brain tissues," *Magn. Reson. Med.* **29**, 623–630 (1993).
- ³¹G. N. Abras and V. L. Ballarin, "A weighted k-means algorithm applied to brain tissue classification," *J. Comput. Sci. Technol.* **5**, 121–126 (2005).
- ³²G.-C. Lin, W.-J. Wang, C.-C. Kang, and C.-M. Wang, "Multispectral MR images segmentation based on fuzzy knowledge and modified seeded region growing," *Magn. Reson. Imaging* **30**, 230–246 (2012).
- ³³R. A. D. Carano, F. Li, K. Irie, K. G. Helmer, M. D. Silva, M. Fisher, and C. H. Sotak, "Multispectral analysis of the temporal evolution of cerebral ischemia in the rat brain," *J. Magn. Reson. Imaging* **12**, 842–858 (2000).
- ³⁴A. K. Jain, "Data clustering: 50 years beyond K-means," *Pattern Recogn. Lett.* **31**, 651–666 (2010).
- ³⁵A. K. Jain, M. N. Murty, and P. J. Flynn, "Data clustering: A review," *ACM Comput. Surv.* **31**, 264–323 (1999).
- ³⁶B. A. Taylor, Dynamic Chemical Shift Imaging for Image-Guided Thermal Therapy, UT Graduate School of Biomedical Sciences at Houston, Dissertations and Theses, Paper 57, 2010.
- ³⁷G. H. Glover and E. Schneider, "Three-point Dixon technique for true water/fat decomposition with B0 inhomogeneity correction," *Magn. Reson. Med.* **18**, 371–383 (1991).
- ³⁸G. H. Glover, "Multipoint Dixon technique for water and fat proton and susceptibility imaging," *J. Magn. Reson. Imaging* **1**, 521–530 (1991).
- ³⁹S. B. Reeder, A. R. Pineda, Z. Wen, A. Shimakawa, H. Yu, J. H. Brittain, G. E. Gold, C. H. Beaulieu, and N. J. Pelc, "Iterative decomposition of water and fat with echo asymmetry and least-squares estimation (IDEAL): Application with fast spin-echo imaging," *Magn. Reson. Med.* **54**, 636–644 (2005).
- ⁴⁰J. Ma, "Breath-hold water and fat imaging using a dual-echo two-point Dixon technique with an efficient and robust phase-correction algorithm," *Magn. Reson. Med.* **52**, 415–419 (2004).
- ⁴¹G. J. Stanisz, E. E. Odobina, J. Pun, M. Escaravage, S. J. Graham, M. J. Bronskill, and R. M. Henkelman, "T1, T2 relaxation and magnetization transfer in tissue at 3T," *Magn. Reson. Med.* **54**, 507–512 (2005).
- ⁴²H. H. Hu and K. S. Nayak, "Change in the proton T(1) of fat and water in mixture," *Magn. Reson. Med.* **63**, 494–501 (2010).
- ⁴³P. J. Wright, O. E. Mougin, J. J. Totman, A. M. Peters, M. J. Brookes, R. Coxon, P. E. Morris, M. Clemence, S. T. Francis, R. W. Bowtell, and P. A. Gowland, "Water proton T1 measurements in brain tissue at 7, 3, and 1.5 T using IR-EPI, IR-TSE, and MPRAGE: Results and optimization," *MAGMA (N.Y.)* **21**, 121–130 (2008).
- ⁴⁴L. R. Dice, "Measures of the amount of ecologic association between species," *Ecology* **26**, 297–302 (1945).
- ⁴⁵S. D. Sharma, H. H. Hu, and K. S. Nayak, "Accelerated water-fat imaging using restricted subspace field map estimation and compressed sensing," *Magn. Reson. Med.* **67**, 650–659 (2012).
- ⁴⁶D. Hernando, P. Kellman, J. P. Haldar, and Z.-P. Liang, "Robust water/fat separation in the presence of large field inhomogeneities using a graph cut algorithm," *Magn. Reson. Med.* **63**, 79–90 (2010).
- ⁴⁷J. Tsao and Y. Jiang, "Hierarchical IDEAL: Fast, robust, and multiresolution separation of multiple chemical species from multiple echo times," *Magn. Reson. Med.* **70**, 155–159 (2013).
- ⁴⁸L. F. Donnelly, K. J. O'Brien, B. J. Dardzinski, S. A. Poe, J. A. Bean, S. K. Holland, and S. R. Daniels, "Using a phantom to compare MR techniques for determining the ratio of intraabdominal to subcutaneous adipose tissue," *Am. J. Roentgenol.* **180**, 993–998 (2003).
- ⁴⁹B. A. Taylor, A. M. Elliott, K.-P. Hwang, A. Shetty, J. D. Hazle, and R. J. Stafford, "Measurement of temperature dependent changes in bone marrow using a rapid chemical shift imaging technique," *J. Magn. Reson. Imaging* **33**, 1128–1135 (2011).