Evaluation of a similarity anisotropic diffusion denoising approach for improving in vivo CEST-MRI tumor pH imaging

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ABSTRACT

Purpose: Chemical Exchange Saturation Transfer (CEST)-MRI provides new approaches for investigating tumor microenvironment, including tumor acidosis that plays a key-role in tumor progression and resistance to therapy. Following iopamidol injection, the detection of the contrast agent inside the tumor tissue allows measurements of tumor extracellular pH. However, accurate tumor pH quantifications are hampered by the low contrast efficiency of the CEST technique and by the low signal-to-noise ratio of the acquired CEST images, hence in a reduced detectability of the injected agent. This work aims to investigate a novel denoising method for improving both tumor pH quantification and accuracy of CEST-MRI pH imaging.

Theory and Methods: An hybrid denoising approach was investigated for CEST-MRI pH imaging based on the combination of the non-local mean filter and the anisotropic diffusion tensor method. The denoising approach was tested in simulated and in vitro data and compared with previously reported methods for CEST imaging and with established denoising approaches. Finally, it was validated with in vivo data to improve the accuracy in tumor pH maps.

Results: The proposed method outperforms current denoising methods in CEST contrast quantification and detection of the administered contrast agent at several increasing noise levels with simulated data. In addition, it achieved a better pH quantification in in vitro data and demonstrated a marked improvement in contrast detection and a substantial improvement in tumor pH accuracy in in vivo data.

Conclusion: The proposed approach effectively reduces the noise in CEST images and increases the sensitivity detection in CEST-MRI pH imaging.

Keywords: Anisotropic Diffusion, CEST, Denoising, MRI, pH imaging, iopamidol

1. INTRODUCTION

Chemical exchange saturation transfer (CEST) imaging is a novel MRI-based contrast technique that allows to detect tumor metabolites such as glucose, glutamate and lactate (1-4) and to characterize several aspects of tumor microenvironment, including intracellular (5-9) and extracellular pH (10-15) or enzymatic activity (16,17). Among these applications, tumor pH imaging using iopamidol has attracted a wide interest in the medical field since it provided, for the first time, accurate and highly spatially resolved measurements of tumor acidosis and it can monitor treatment response to anticancer therapies (18,19). Moreover, owing to the FDA approval of iopamidol, clinical translatability has already been demonstrated in patients (20,21).

Despite the wide potential applicability of CEST-MRI pH imaging, clinical exploitation is still limited by reduced contrast capability and long acquisition times. In fact, the low sensitivity of the CEST approach results in low contrast enhancements, commonly in the range 1-10%, hence affecting the detection of exogenously administered contrast agents (22-25). On the other hand, the sampling of several frequency offsets (the so-called Z-spectrum) to accurately assess the CEST contrast, combined with the long (few seconds) saturation scheme needed for efficient saturation labeling, yields overall acquisition times as long as several minutes (26), partly reduced by efficient acquisition schemes (27-30). Consequently, CEST-MRI images inherently suffer of low signal-to-noise ratio (SNR), in particular for images acquired close to the water signal (due to direct saturation effects) and improvement by signal averaging is usually hampered by excessive elongation of the acquisition times at the cost of reduced spatial resolution in in vivo applications (31).

Several denoising methods have been proposed in the medical imaging field to improve the quality of the images by exploiting several properties of the acquired data, such as pattern redundancy or sparseness (32). Methods based on Partial Differential Equations (PDE) (33-38) are powerful smoothing tools preserving significant image features. The anisotropic nonlinear diffusion filter is based on PDE by applying a non-homogeneous process that reduces diffusivity using a diffusion function introduced by Perona and Malik (35) or by a diffusion tensor defined by Weickert (38). These approaches reduce noise in homogeneous areas while preserving natural discontinuities of the image. Neighborhood filters are another common method for preserving edges, under the assumption that all pixels belonging to the same object have a similar gray level value. Buades et al. (39) proposed a Non Local Mean (NLM) filter that restores voxel in the image according to a weighted average of the other similar voxels inside a search window in the image, allowing to obtain clean edges and sharp boundaries with efficient smoothing process. Moreover, Dabov et al. (40) proposed the Block Matching 3D (BM3D) filter, which looks for similar regions in the whole image and these regions

are filtered in a 3D transform domain with a selected threshold. Then, by aggregating all the estimated blocks using a weighted average the final denoised image is obtained. Other approaches that exploit unsupervised methods without requiring the tuning of any parameter, such as Random Markov Field and Bayesian approaches have also been investigated (41-43). In MRI, these filters have been applied in several techniques, for improving image quality, structural details, classification and accuracy in parameters estimation (44-49).

To date, only few and simple denoising approaches have been applied to CEST-MRI images, including cubic smoothing splines that are well designed to preserve the shape of the Z-spectrum (50) and Gaussian filtering (51) following the administration of contrast agents. Recently, more advanced approaches, such as Principal Component Analysis (PCA) and NLM filters have been proposed but so far only applied to denoise endogenous CEST images with a considerable increase in image quality (52,53).

In this paper, we investigate a hybrid denoising approach for CEST-MRI pH imaging based on the combination of the NLM filter and the anisotropic diffusion tensor method (54). This novel denoising approach was tested in both simulated data corrupted with noise and in vitro data and compared with previously reported methods for CEST imaging or with established smoothing approaches and 2D image filters. In addition, the proposed method was validated with in vivo data by assessing its capability to improve both the CEST contrast quantification and the accuracy of tumor pH calculation.

2. THEORY

Non-Local Mean Coherence Enhancing Diffusion (NLmCED) Filter

The anisotropic diffusion proposed by Perona and Malik (35) is an analogy between image processing and the diffusion of heat that homogenizes the temperature of materials, by applying a homogenous process to prevent diffusion to happen over edges. However, this method is very sensitive to signal variations in the directions of the structures, therefore Weickert (38) proposed the diffusion tensor D which summarizes the predominant directions of the gradient in a determined neighborhood of voxels, defined by:

$$ID = div(D.\nabla I) \qquad (1)$$

The construction of this model is based on the choice of the eigenvectors of the structure tensor J_ρ (eq.2) defining the gradient local directions and the associated diffusion functions (eq.2) presenting the intensity of the actions, called coherence enhancing diffusion (CED) (55) that allows to preserve small structures and strengthens tubular structures in 3D images.

$$J_\rho = K_\rho * \left(\nabla I_\sigma . \nabla I_\sigma^T\right) \qquad (2)$$

Where the first term ∇I_σ is the gradient of the smoothed image at scale noise σ and K_ρ is a Gaussian kernel with standard deviation ρ.

In addition, the NLM filter proposed by Buades (39) is another powerful filter that eliminates noise in the image while preserving the contours. It is based on the natural redundancy of the images. It exploits the repetitive nature of structures unlike conventional denoising algorithms that typically operate in a local neighborhood. The restored intensity $\hat{I}(x_i)$ of the voxel x_i for NLM filter, is computed as a weighted average of the voxels intensities $I(x_j)$ in the search window V_i (56).

$$\hat{I}(x_i) = \sum_{x_j \in \Omega^3} w(x_i, x_j) I(x_j) \qquad (3)$$

The combination NLmCED between NLM filter and the anisotropic diffusion tensor method has been presented in detail in a previous work and applied to both T_1 and T_2-weighted MR images (54). The weights $w(x_i,x_j)$ have been modified from its original definition to the equation (eq. 4) where it guarantees the similarity of the intensity of patches and respects the different forms of structure in the image :

$$w(x_i, x_j) = (1/Z(x_j)) \exp\left(-\left(d_I^2(x_i, x_j) + d_{ID}^2(x_i, x_j)/h\right)^2\right) \qquad (4)$$

Where the first term $d_I^2(x_i, x_j)$ and $d_{ID}^2(x_i, x_j)$ represent the original Gaussian weighted Euclidian distance between the intensity patches for in the noisy image I and the reconstructed image ID by the anisotropic diffusion tensor (eq. 1), respectively.

The index $Z(x_j)$ is a normalizing constant and h is a smoothing parameter to control the decreasing of the exponential function, depending on the estimation of the noise level in the original image as described in the work of Coupe (57). To ensure the robustness of this hybrid algorithm for CEST imaging, we proposed to use the CED functions (55) that were previously applied for denoising only CT images, based on a hyperbolic tangent function and on an edge indicator C_{edge} (58) defined by the following expression:

$$\lambda_1 = \alpha$$
$$\lambda_2 = \begin{cases} \alpha & if\ K = 0 \\ \left|\tanh\left(C_{edge}/K\right)\right| & ,\ else \end{cases} \qquad (5)$$
$$\lambda_3 = \begin{cases} \alpha & if\ K = 0 \\ \alpha + (1-\alpha).\exp(-C/K) & ,\ else \end{cases}$$

Where α is parameter $\alpha \in (0,1)$ that keeps the tensor D (38) uniformly positive definite and C is the CED contrast parameter. *K* is a measure of coherence that acts as a diffusion barrier between homogenous area and the edges in the image, based on the eigenvalues μ_i ($i = 1...3$) of the tensor and it is defined as follow:

$$K = (\mu_1 - \mu_2)^2 + (\mu_1 - \mu_3)^2 + (\mu_2 - \mu_3)^2 \qquad (6)$$

For $K \gg C$, the diffusion is along the two directions v_3 and v_2 and if *K* tends to 0 the diffusion seems to be isotropic and doesn't exceed α value. In all the reported results we set the parameters C = 1 and $\alpha = 0.001$ and the scale noise σ estimated by using the adaptive MAD estimator for Rician noise (57). In addition, the number of iterations and the standard deviation ρ parameter have been optimized for the specific noise level in CEST-MRI images (Supporting Information Figure S1).
The flowchart of the proposed algorithm NLmCED is summarized in Figure 1.

3. METHODS

3.1. Denoising methods

To validate the efficiency of our proposed model, we compared it with established denoising approaches such as Gaussian, Smoothing Cubic Splines and BM3D. The Gaussian filter was applied to each frequency offset image with a kernel of 7x7 pixels as used in (59). The Smoothing Cubic Splines was applied to interpolate Z-spectra voxels by voxels, by using a regularization factor equal to 0.99 for avoiding excessive smoothing of the data (60). Moreover, in the comparison we included also the BM3D filter, since this is an established filter that has been applied successfully for denoising images in different areas. We propose to set the parameters of BM3D denoising method on the original method in order to find similar neighborhoods (40). In addition, this method depends highly on the parameter σ_{BM3D} that controls the filtering strength which corresponds to the standard deviation of the noise level in the data. To select the optimal value of σ_{BM3D}, we calculated the dependencies of PSNR values as a function of the noise levels (Supporting Information Figure S2).

3.2. Simulations

To evaluate the performance of the denoising methods quantitatively, synthetic CEST data (ground truth) were generated at different iopamidol concentrations (2.5, 5, 10, 20 and 30 mM) and pH values (6, 6.4, 6.7, 7 and 7.4) to simulate Z-spectra with different shapes and peak intensities at physiological conditions for a B_0 field of 7 T. The z-spectra were generated by using the Bloch-McConnell equations modified for the exchange term with five pools (bulk water, two hydroxyl proton pools and the two amide proton pools indicated by A, B, C, D and E, respectively) by using the online available

Matlab-based code for CEST data simulation (61). We used a continuous-wave (CW) saturation scheme (3μT x 5s) with 201 frequency offsets sampled from -10 to 10 ppm with a step size of 0.1 ppm. All the other parameters used for the simulation are reported in Supporting Information Table S1.

To provide more realistic simulation data, three different simulated datasets have been generated with increased complexity, both in terms of number of proton pools (iopamidol, water and the semisolid component) and with different shapes. A full detailed description for each dataset can be found in the Supporting Information. Briefly, dataset #1 includes iopamidol + water with a chess box design and was exploited for parameter optimization; dataset #2 includes iopamidol + water with circular shapes for evaluation of denoising robustness at several noise realizations. Datasets #3 is a more realistic phantom including irregular shapes (as taken from in vivo tumor xenograft ROIs, Supporting Information Figure S3) incorporating varying ssMT amplitudes (0.02-0.10) as found in vivo (62) with unnormalized Z-spectra (Supporting Information Figure S4).

The molecule iopamidol has been exploited for creating synthetic data since it has two proton pools at 4.2 and 5.5 ppm with different pH responsiveness. Therefore, Z-spectra obtained from this molecule show two visible downward peaks that, upon the addition of noise, limit the capability to discriminate the contribution from each pool, hence resulting in a more stringent test for the investigated denoising approaches. From the Z-spectra the corresponding CEST contrast parametric saturation transfer (ST) maps were calculated according to the asymmetry analysis (eq.7) and only a CEST contrast greater than 1% for both pools (to avoid CEST artefacts from instrumental noise) was considered to show the presence of the iopamidol molecule.

$$ST = \left(SI_{-W} - SI_{+W}\right) \Big/ SI_{-W} \qquad (7)$$

Where SI_{+w} and SI_{-w} are the measured signal intensity with RF saturation at $+w$ and $-w$, respectively.

Secondly, the ratio between the two CEST contrasts is exploited for calculating the ratiometric value from which pH values can be calculated (11). Therefore, a third parameter (the calculated pH) can be exploited as an additional estimate to assess the robustness of the investigated denoising approaches, since the correct denoising of both the two ST parametric maps is mandatory for obtaining accurate pH values.

To compare the spatial similarity between the ground truth images and the denoised ones, the Peak Signal to Noise Ratio PSNR (63) was used as an indicator of quality between the ground truth *Iref*

and the denoised data $\hat{I}$:

$$PSNR = 10\log_{10}\left(\max((Iref))^2 \big/ MSE(Iref, \hat{I})\right) \qquad (8)$$

A second metric, the structural similarity index SSIM (64,65) which combines the similarity comparisons of indexes between the ground truth and the denoised parametric images, was used as a qualitative measurement of noise removal. A sliding window with size (8x8) was used to estimate the SSIM index locally between x and y of the ground truth *Iref* and the denoised data $\hat{I}$, respectively:

$$SSIM(x,y) = ((2\mu_x\mu_y + c_1)(2\sigma_{xy} + c_2))\big/((\mu_x^2 + \mu_y^2 + c_1)(\sigma_x^2 + \sigma_y^2 + c_2)) \qquad (9)$$

Where u_x and u_y are the mean; σ_x and σ_y are the standard noise variance; σ_{xy} is the covariance; $c_1 = (k_1L)^2$ and $c_2 = (k_2L)^2$ are variables to stabilize the division when the denominator is very small with $k_1 = 0.01$, $k_2 = 0.03$ and $L = 255$. The overall similarity index is given by the average value of all the local estimations.

3.3. In vitro data

In vitro data was acquired on a Bruker Avance 7T MRI scanner (Bruker BioSpin MRI GmbH, Ettlingen, Germany) using a iopamidol containing 50 mL falcon tube. A first phantom (#1) was prepared by dissolving iopamidol in a phosphate buffer solution (PBS) at several concentrations (2.5, 5, 10, 20 mM; pH 6.7) and titrated at several pH values (6.3, 6.7, 7.0, 7.4; 30 mM) and filling eight smalls (300 µL) plastic Eppendorf (Supporting Information Figure S5a). A second phantom (#2) with decreasing iopamidol concentrations was prepared and detailed in the Supporting Information (Supporting Information Figure S5b). Z-spectra were acquired with a modified RARE sequence with the following acquisition parameters: 181 frequency offsets were sampled from -10 to 10 ppm with a step size of 0.1 ppm by applying a saturation pulse with a power of 3 µT and duration of 5 s.

3.4. In vivo data

Detailed descriptions of the human prostate carcinoma cell line PC-3 and of the animal model are provided in the Supporting Information. Animal manipulation and experimental procedures were carried out in accordance with the European Community guidelines (Directive 2010/63) and under the approval of the Italian Ministry of Health.

Before the MRI setup, mice were intramuscularly anesthetized by injecting a mixture of tiletamine/zolazepam (Zoletil 100; Virbac, Milan, Italy) 20 mg/kg and xylazine (Rompun; Bayer, Milan, Italy) 5 mg/kg and a 27-gauge catheter was introduced into the tail vein for the injection of 4 g I/kg b.w. iopamidol (kindly provided by Bracco Imaging SpA, Colleretto Giacosa, Italy). The breath

rate was monitored by an air pillow placed below the animal (SA Instruments, Stony Brook, NY, USA) for the whole MRI experiment. MR imaging was performed on a Bruker Avance 7 T MRI scanner (Bruker BioSpin MRI GmbH, Germany) equipped with a micro 2.5 MICWB 30 mm quadrature (1H) imaging probe. Anatomical T_{2w} images were acquired with a Fast Spin Echo (FSE) sequence (TR: 4000 ms; TE: 4.4 ms; NEX: 2; FOV: 3 cm; MTX: 256x256) and the same geometry was used for the following CEST experiments. CEST images were acquired before and after iopamidol injection with a single shot FSE sequence (TR: 6000 ms; TE: 3.9: NEX: 1) with centric encoding preceded by a continuous-wave saturation pulse (power: 3 μT, duration: 5 s) on 1 central tumor slice (FOV: 3 cm; MTX: 96×96; in-plane resolution: 312.5 μm; slice thickness: 1.5 mm).

3.5 Statistical analysis

Statistical analysis was performed by using the GraphPad Prism 6 software (GraphPad Inc., San Diego, CA, USA). All the data are shown as mean and standard deviations. A one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison post-hoc test was used to test for statistically significant differences among several denoising methods. Differences were considered significant at $P<0.05$.

4. RESULTS

4.1. Results with synthetic data

To test whether the proposed denoising algorithm can provide more accurate quantifications of both CEST contrasts and pH values in comparison to other methods, several datasets with synthetic Z-spectra were generated by simulating iopamidol at several concentrations, pH values in the physiological range and amplitudes of the semisolid component. The corresponding noisy Z-spectra were obtained by adding different levels of Rician noise (1-7%) as described in Section 2.3.

For our algorithm, the choice of the number of iterations plays an important role in the noise reduction process. Supporting Information Figure S1 illustrates the quality assessment obtained by the PSNR metric for the ST parametric map at 4.2 ppm while varying the iterations number. We observed that when increasing the level of noise, a higher number of iterations is needed to achieve the highest PSNR. We therefore used these number of iterations according to the observed level of noise in the input images.

Figure 2 demonstrates the denoising of a single representative Z-spectrum taken from dataset #1 using our proposed method compared to Gaussian filter, Smoothing Cubic Splines and BM3D filter along with the synthetic and the noisy Z-spectrum with 3% of Rician noise. Both the BM3D and the NLmCED methods provided denoised Z-spectra that are very close and almost overlapping to the

simulated data (Figure 2c-d).

To compare the different denoising approaches, synthetic Z-spectra patches (10x10 pixels) with random Rician noise were generated (dataset #1) for each simulated condition (concentration and pH) resulting in 2500 Z-spectra for each noise level from which ground truth CEST contrast maps were calculated at 4.2 (Figure 3a) and 5.5 ppm. We then applied the denoising approaches to the corrupted data, resulting in a marked reduction of noisiness for the NLmCED approach in comparison to all the other methods (Figures 3b-3f). In addition, the NLmCED method provided less blurred images in respect to the Gaussian or to the BM3D approaches.

To quantitatively compare the denoising algorithms, two different metrics for assessing both the improvement in SNR and the similarity between the ground truth and the denoised Z-spectra were calculated: the PSNR and the SSIM indexes for the CEST contrast calculated at both 4.2 ppm and at 5.5 ppm and the SSIM index for the calculated pH maps. Figure 4 illustrates the evaluation of the four investigated denoising methods for the simulated data (dataset #1) contaminated with different levels of Rician noise. All the denoising approaches provided marked improvements in the parameter estimation at all the Rician noise levels in comparison to the noisy data, with a general trend: Gaussian filtering < Smoothing Cubic Spline < BM3D, NLmCED (Figure 4a-d). At the lowest levels of Rician noise (1% to 3%) almost similar results were observed for both BM3D and NLmCED methods that were both superior to the Gaussian and to the Smoothing Cubic Spline approaches. On the other hand, at the highest level of Rician noise (5% and 7%), the NLmCED filter provided the highest PSNR values. To further evaluate the denoising capabilities of the proposed method, we compared denoised parametric pH images that are obtained by rationing the two denoised contrast ST maps at 4.2 and 5.5 ppm, respectively, hence introducing a more stringent test. As shown in Figure 4e, the NLmCED denoising method provided the highest similarity to the ground-truth pH map at all the investigated noise levels in comparison to the other denoising methods. Figures 3g-3l shows corresponding calculated pH maps after denoising at 3% Rician noise with a clear improvement in the calculated pH values for the proposed NLmCED filter. Moreover, besides providing the highest PSNR and similarity in both the CEST contrast and pH maps, the NLmCED filter resulted in an increased number of pixels in which it was possible to calculate the pH values (92%), surpassing all the other methods and therefore providing more accurate and reliable pH quantification (Figure 4f).

An additional synthetic dataset was generated to assess the performance of the NLmCED method by using circular shapes (dataset #2). Overall a better performance of the proposed NLmCED denoising approach in comparison to all the other methods was observed (Supporting Information Figures S6-S7).

Additionally, the robustness of the proposed method was assessed by creating 20 realizations for 1%

and 3% of Rician noise for dataset #2. We observed that all the calculated metrics (CEST-based and similarity ones) were stable, with analogous values independently from the noise realization number (Supporting Information Figures S8-S9).

A more realistic phantom with unnormalized raw Z-spectra corrupted with the same noise levels and comprising areas with irregularly shaped edges and randomly distributed values of iopamidol concentrations, pH and ranges of ssMT amplitudes (dataset #3) was also exploited for assessing the performance of the NLmCED method in a in vivo-like model (Supporting Information Figure S4). CEST contrast maps (ΔST at 4.2 ppm, Figures 5a-5f) and calculated pH maps (Figures 5g-5l) clearly show a marked restoration of the ground truth images after denoising of the added 1% of Rician noise. For most of the calculated metrics (PSNR and SSIM) the NLmCED method was superior in recovering the original images (Figure 6) with a marked improvement in the fraction of pixels in which Iopamidol was detectable (Figure 6f).

4.2. Experimental results with in vitro data

The proposed method was then tested with in vitro data that were obtained by dissolving iopamidol at different pH values (keeping constant the concentration) or at several concentration values (titrated at the same pH), resulting in eight different conditions (phantom #1). The vials at different concentrations were used to assess the detection sensitivity of the denoising methods, whereas those at different pH values to evaluate the accuracy in pH estimation. First, we estimated the level of noise in this data in order to set the optimum number of iterations for the NLmCED filter. So according to the above presented observations and to the measured value of 1.1% of noise level, we set the number of iterations of our proposed method to 3 iterations.

The calculated CEST contrast maps at the two frequencies (4.2 and 5.5 ppm) and the corresponding ratiometric values for in vitro data and denoised ones are shown in Figure 7. CEST contrast maps calculated after denoising by the proposed method (NLmCED) are less noisy and more homogeneous without reduction in the measured ST contrast with respect to the maps obtained by all the other methods.

A quantitative comparison was performed by calculating the average values and the standard deviations (as an index of noise removal) of the CEST contrast (ST values at 4.2 and 5.5 ppm) and of the ratiometric values for all the vials or conditions. For the contrast ST at 4.2 ppm (Figure 8a and 8c), all the investigated denoised methods provided similar average CEST contrast values to the original data, but the NLmCED filter showed marked homogenous value for all the concentrations and pH conditions where the whiskers boxplots extend to the most extreme data values. Both BM3D and Gaussian filtering provided similar average values but higher standard deviations in comparison

to the other methods, whereas the smoothing cubic spline method produced the smallest standard deviation values but at the expenses of reduced CEST contrast quantification. Similar results were obtained for the CEST ST values at 5.5 ppm (Figure 8b and 8d). Corresponding ratiometric values calculated from the ST at 4.2 and 5.5 ppm values showed a similar trend for the denoising approaches (Figure 8e and 8f). Average ratiometric values were similar among all the denoised data, but only the NLmCED and the Smoothing Cubic Splines methods provided the smallest standard deviations.

We investigated the capability of the denoising methods to increase the iopamidol detectability in a phantom with iopamidol diluted at several concentrations (phantom #2). We observed similar CEST contrast values between the several denoising approaches, with a detection threshold for iopamidol of ca. 1-2 mM (Supporting Information Figure S10). Despite the NLmCED did not provide a clear advantage in terms of detectability of the agent, this approach delivered the more homogeneous contrast inside each vial, as observed by the smallest range of the boxplots. CEST contrast images for the two pools (4.2 ppm and 5.5 ppm) for the raw and denoised data are shown in the Supporting Information Figure S11.

4.3. Experimental results with in vivo data

Further validation of the denoising methods was performed with in vivo data, following iopamidol injection in an orthotopic prostate tumor murine model. Since the CEST contrast for in vivo experiments is measured as the difference (ΔST) between pre and post-injection ST CEST contrast images of iopamidol for both the two pools, the denoising methods were applied to both pre- and post-injection Z-spectra. According to the level of noise measured in vivo (2.5 %) we set the number of iterations to 6 for the NLmCED method. All the methods provided similar average CEST contrast calculated inside the tumor region but with reduced standard deviations when compared to the original data (Figure 9a-9c). Of note, the NLmCED filter provided consistent higher fraction of pixels for both the two pools when compared to the other denoising approaches (Figure 9d-e). Because of the enhanced detectability, the NLmCED filter also provided the highest fraction of pixels for mapping pH (Figure 9f), thus improving both the accuracy for tumor pH measurements and the assessment of tumor pH heterogeneity since the availability of a higher number of pixels, among which it is possible to calculate the pH. Figure 10 shows CEST contrast maps of a representative tumor bearing mouse for the two pools of iopamidol and the calculated tumor pH maps superimposed to the T_2-weighted morphological image by applying the investigated denoising methods. Visually, we can observe that the NLmCED provides more homogenous parametric maps and with more colored pixels in comparison with the other denoising methods.

When evaluated in a group of mice (n = 4), we obtained a marked and statistically significant increase

in the fraction of pixels in which Iopamidol was detectable and a statistically significant increase in the number of pixels in which it was possible to measure tumor pH (Figure 9g-i). Overall, the proposed denoising method increased the in vivo iopamidol detectability and improved tumor pH measurements.

Finally, we tested the runtime of each denoising algorithm method on a desktop computer equipped with a 3.10 GHz Intel® Core i5-2400 CPU with 8 GB of RAM using Matlab R2015b. The comparison of the computation time was performed for in vivo data, consisting of two sets of CEST images (before and after iopamidol injection, matrix: 96 x 96 x 39) and is presented in Supporting Information Figure S12. The Gaussian filter was shown to be the fastest method (2 seconds), followed by the BM3D filter (10 seconds), the proposed NLmCED filter (22 seconds) and by the Smoothing Cubic Spline method (117 seconds).

5. DISCUSSION

In this study, we presented a novel denoising method that combines Non-local mean and Anisotropic Diffusion Tensor filter for CEST-MRI pH imaging. We have shown that this NLmCED filter improves both contrast quantification and accuracy of pH measurements. To the best of our knowledge, this is the first time that a denoising approach that takes benefits from redundancy information in the image has been applied to contrast-enhanced CEST-MRI images following exogenous contrast media administration.

CEST imaging is naturally affected by low SNR because of the inherent CEST mechanism of the transfer of saturates spins that decreases the measured bulk water signal, specifically at frequencies close to the water pool due to compelling direct water saturation effects. Furthermore, common approaches to improve SNR, such as signal averaging, are hampered due to concurrent long saturation times (to increase the labelling, up to several seconds), number of images acquired at several offsets or Z-spectrum sampling (to provide specific selectivity toward the injected contrast agent) and required spatial resolution.

In our previous work, we proposed a combination between the Non-Local Means (NLM) filter and the anisotropic diffusion tensor and we succeed to improve the quality of the image and to preserve more details and edges in MRI images (54). The NLM filter is a powerful method based on the redundancy of information in the image while the anisotropic diffusion tensor is a suitable enhancing process that allows efficiently to reduce noise and preserve edges in an image. In this paper, we extended the application of our method to CEST-MRI images with optimized Coherence Enhancing

Diffusion parameters to improve both the contrast quantification and the pH accuracy in tumor pH imaging following iopamidol injection.

First, we evaluated the performance of the proposed NLmCED method by comparison with Smoothing Cubic Spline, Gaussian and BM3D filters in several simulated datasets contaminated with different levels of Rician noise. Overall, the results reported herein show that all the denoising methods succeed to remove the added noise, but our proposed method outperformed all the others. Despite the performance of the proposed approach decreases when increasing the noise levels, it provided the highest quantitative measurements in term of SSIM and PSNR when compared to all the other methods. The consistent results obtained with our method in the several simulated datasets that resemble realistic situations, including the semisolid component and heterogeneous regions with different properties (both in terms of iopamidol concentrations and pH values), support its exploitation in in vivo applications.

We further applied the NLmCED method to quantify the contrast originating from iopamidol in in vitro data with different concentrations and pH values. The results of all the denoising approaches were promising in terms of noise reduction. Visually, the NLmCED filter provided high homogenous areas in the vials prepared with different conditions, showing comparable contrast but markedly reduced standard deviations. In vivo, the NLmCED algorithm provided the highest fraction of pixels inside the tumor where it was possible to detect the injected contrast agent, hence improving the detection sensitivity for the CEST images.

Several post processing fitting and interpolation methods have been applied to improve the SNR or the CEST quantification in the CEST-MRI field. For example, Stancanello et al. (60) used a smoothing spline interpolation in order to attenuate the noise in the z-spectrum. Zhou et al. (66) used a symmetric 12th-order polynomial to fit endogenous z-spectra. Lorentzian line shapes have been used by Zaiss et al. to analyze z-spectrum as a combined fitting of several components (62). Moreover, Downsampling Expedited Adaptive Least-squares (IDEAL) fitting was proposed based on initial values from multi-pool Lorentzian fitting to improve the reliability of in vivo CEST-MRI quantification (67). However, all those fitting procedures are sensitive to both the initial values and to the chosen boundaries which may lead to inaccurate fitting results if not properly selected (68). In addition, fitting procedure are also computational demanding since they require long calculation times. Recently, a Principal Component Analysis (PCA) approach has been applied for the denoising of CEST-MRI images (52,69). The advantage of this PCA-based post processing procedure is to exploit the capability of PCA to reduce the dimensionality of the acquired data, retaining only the principal components that describe the higher data variability and discarding those more related to

the noise. However, such approach does not take into account the spatial information and the spatial correlations inside the acquired images. Recently, a new approach using multilinear singular value decomposition (MLSVD) that exploits spatiotemporal correlations has been proposed for denoising CEST-MRI images (70). The MLSVD method succeeded to recover CEST contrast from in vivo data, although a robust validation with corrupted ground-truth data was not performed. In contrast, our proposed approach combines both an efficient noise reduction algorithm and the redundancy of spatial information inside the image to improve the CEST contrast quantification. Finally, we think that the proposed approach could potentially also be widely exploited for the denoising of other CEST-MRI applications both for pH imaging as well as for GlucoCEST or APT imaging (71-73) after optimizing and evaluating the proposed denoising method for each CEST modality. Moreover, we want to emphasize that prior to any application, either at a human whole-body scanner or in conjunction with parallel imaging, further investigations concerning the influence of B_0 and B_1 inhomogeneities and non-Rician noise distribution are required to verify the applicability of the approach.

6. CONCLUSIONS

In summary, this study demonstrates for the first time that the proposed NLmCED denoising method effectively reduces the noise in the acquired CEST-MRI images and increases both the sensitivity detection following iopamidol injection and the accuracy of pH measurements for tumor acidosis imaging.

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DATA AVAILABILITY

The data and the matlab scripts that support the findings of this study are available at the following links:

https://github.com/Feriel87/Denoising-CEST-MRI

http://doi.org/10.5281/zenodo. 4320953

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FIGURE LEGENDS

Figure 1. Block diagram of the proposed denoising NLmCED algorithm.

Figure 2. Representative simulated Z-spectrum for the simulated dataset #1 (iopamidol at 30 mM and pH = 7.4, solid line) and noisy data after applying 3% Rician noise (dots) and corresponding denoised Z-spectrum by applying the four investigated denoised approaches: Smoothing Cubic Spline (a), Gaussian filter (b), BM3D filter (c) and NLmCED (d).

Figure 3. CEST contrast ST at 4.2 ppm and pH maps for the simulated dataset #1: (a, g) Original data, (b, h) Noisy data with 3% of Rician noise, (c, i) Smoothing Cubic Spline, (d, j) Gaussian Filter, (e, k) BM3D Filter and (f, l) NLmCED method.

Figure 4. Quantitative evaluation of the denoising methods for the simulated dataset #1 at several noise levels in terms of PNSR for the ST 4.2 ppm map (a), the ST 5.5 ppm map (b) and for the SSIM metric for the ST 4.2 ppm map (c), the ST 5.5 ppm map (d) and for the pH map (e). Bargraph showing the fraction of pixels in which is possible to calculate pH values (f) after denoising 3% of Rician noise.

Figure 5. CEST contrast ST at 4.2 ppm and pH maps for the simulated dataset #3: (a, g) Original data, (b, h) Noisy data with 1% of Rician noise, (c, i) Smoothing Cubic Spline, (d, j) Gaussian Filter, (e, k) BM3D Filter and (f, l) NLmCED method.

Figure 6. Quantitative evaluation of the denoising methods for the simulated dataset #3 at several noise levels in terms of PNSR for the ΔST 4.2 ppm map (a), the ΔST 5.5 ppm map (b) and for the SSIM metric for the ΔST 4.2 ppm map (c), the ΔST 5.5ppm map (d) and the pH map (e). Bargraphs showing the fraction of pixels in which is possible to calculate pH values (f) after denoising 1% of Rician noise.

Figure 7. Calculated ST images at 4.2 ppm (a), at 5.5 ppm (b) and ratiometric images (c) from in vitro data (phantom #1). From left to right: original data and denoised parametric maps obtained by applying the smoothing cubic spline, the Gaussian filter, the BM3D filter and the proposed NLmCED method.

Figure 8. Quantitative results for in vitro data (phantom #1) calculated from original data and after applying the denoising methods: (a) ST at 4.2 ppm at different pH values, (b) ST at 5.5 ppm at different pH values, (c) ST at 4.2 ppm at several concentrations, (d) ST at 5.5 ppm at several

concentrations, (e) ratiometric values for different pH values and (f) ratiometric values for several concentrations. Data are presented as box-plot showing mean and standard deviations.

Figure 9. Quantitative results for in vivo data. Bargraphs showing average values for a representative mouse: ΔST CEST contrast (measured as ST post iopamidol injection minus ST pre-injection) at 4.2 ppm (a) and 5.5 ppm (b), tumor pH values (c), fraction pixels at 4.2 ppm (d) and at 5.5 ppm (e) corresponding to the detection of iopamidol within the tumor region and fraction pixels in which it was possible to calculate the pH values (f). Average values for a group of mice (n = 4): fraction pixels at 4.2 ppm (g), at 5.5 ppm (h) and for pH values (i). Data are presented as mean and standard deviations.

Figure 10. CEST contrast ΔST maps at 4.2 (a) and 5.5 (b) ppm and tumor pH maps (c) superimposed to the anatomical image for the original data and the proposed denoising algorithms: Smoothing cubic spline, Gaussian filtering, BM3D and NLmCED (from left to right) for a representative tumor-bearing mouse and corresponding zoom-in for the tumor region.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Table S1 Simulation parameters.

Figure S1 Optimization of the NLmCED number of iterations as a function PSNR according to the added Rician noise levels to dataset #1.

Figure S2 Optimization of the **σ** parameter for the BM3D filter as a function PSNR according to the added Rician noise levels.

Figure S3. Illustration of the shapes and composition (Iopamidol concentration and pH) of the realistic phantom exploited for dataset #3.

Figure S4. Simulated dataset #3 showing the investigated ranges of iopamidol concentration, pH and ssMT amplitudes randomly distributed in the four ROIs (as shown in Figure S3) and representative normalized Z-spectra taken from the four regions.

Figure S5. Illustration of the in vitro phantoms composition. (a) phantom #1 with Iopamidol at 30 mM concentration titrated several pH values and at pH = 6.7 with several iopamidol concentrations. (b) phantom #2 with Iopamidol at several concentrations all titrated at pH = 6.7.

Figure S6. Quantitative evaluation of the denoising methods for the simulated dataset #2 at several noise levels in terms of PNSR for the ST 4.2 ppm map (a), the ST 5.5 ppm map (b) and for the SSIM metric for the ST 4.2 ppm map (c), for the ST 5.5 ppm map (d) and for the pH map (e). Bargraphs

showing the fraction of pixels in which is possible to calculate pH values (f) after denoising 3% of Rician noise.

Figure S7. CEST contrast ST maps at 4.2 (a) and 5.5 (b) ppm and calculated pH maps (c) for the original data and the proposed denoising algorithms: Smoothing cubic spline, Gaussian filtering, BM3D and NLmCED (from left to right) for the simulated dataset #2.

Figure S8. Quantitative evaluation of the denoising methods at several realizations (n = 20) of noise levels in terms of PNSR for the ST 4.2 ppm map at 1% (a), 3% (b), the ST 5.5 ppm map at 1% (c), 3% (d) and for the SSIM metric for the ST 4.2 ppm map at 1% (e), 3% (f), for the ST 5.5 ppm map at 1% (g), 3% (h) for the simulated dataset #2 with volume size (256 x 256) and 201 offsets.

Figure S9. Average of quantitative evaluation of the denoising methods at several realization (n = 20) of noise levels for the simulated dataset #2: Average PNSR for the ST 4.2 ppm map (a), Average PNSR for the ST 5.5 ppm map (b), Average SSIM for the ST 4.2 ppm map (c), Average SSIM for the ST 5.5 ppm map (d), Average SSIM for pH (e).

Figure S10. Calculated ST values 4.2 and 5.5 ppm for Iopamidol diluted at several concentrations from phantom #2 after applying the investigated denoising methods: (a) ST 4.2 ppm, (b) ST 5.5 ppm.

Figure S11. Calculated ST CEST contrast images at 4.2 (a) and 5.5 (b) ppm from phantom #2. From left to right: original data and denoised parametric maps obtained by applying smoothing cubic spline, Gaussian filter, BM3D filter and the proposed NLmCED method.

Figure S12. Average computation time of the different denoising methods for *in vivo* data (two Z-spectra data corresponding to before and after Iopamidol injection, matrix size: 96x96x39).