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Optimization of metabolite basis sets prior to quantitation in magnetic resonance spectroscopy: an approach based on quantum mechanics

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Abstract
High-resolution magic angle spinning (HRMAS) nuclear magnetic resonance (NMR) is playing an increasingly important role for diagnosis. This technique enables setting up metabolite profiles of *ex vivo* pathological and healthy tissue. The need to monitor diseases and pharmaceutical follow-up requires an automatic quantitation of HRMAS ^{1}H signals. However, for several metabolites, the values of chemical shifts of proton groups may slightly differ according to the micro-environment in the tissue or cells, in particular to its pH. This hampers the accurate estimation of the metabolite concentrations mainly when using quantitation algorithms based on a metabolite basis set: the metabolite fingerprints are not correct anymore. In this work, we propose an accurate method coupling quantum mechanical simulations and quantitation algorithms to handle basis-set changes. The proposed algorithm automatically corrects mismatches between the signals of the simulated basis set and the signal under analysis by maximizing the normalized cross-correlation between the mentioned signals. Optimized chemical shift values of the metabolites are obtained. This method, QM-QUEST, provides more robust fitting while limiting user involvement and respects the correct fingerprints of metabolites. Its efficiency is demonstrated by accurately quantitating 33 signals from tissue samples of human brains with oligodendroglioma, obtained at 11.7 tesla. The corresponding chemical shift changes of several metabolites within the series are also analyzed.

Keywords: magnetic resonance spectroscopy, HRMAS-NMR, quantitation, quantum mechanics, jMRUI software package

1. Introduction

High-resolution magic angle spinning (HRMAS) nuclear magnetic resonance (NMR) is playing an increasingly important role for diagnosis. This technique enables setting up metabolite profiles of *ex vivo* pathological and healthy tissue, i.e. biopsies [1–10]. The need to monitor diseases and pharmaceutical follow-up requires an automatic and reliable quantitation of HRMAS ^{1}H signals [11–14].

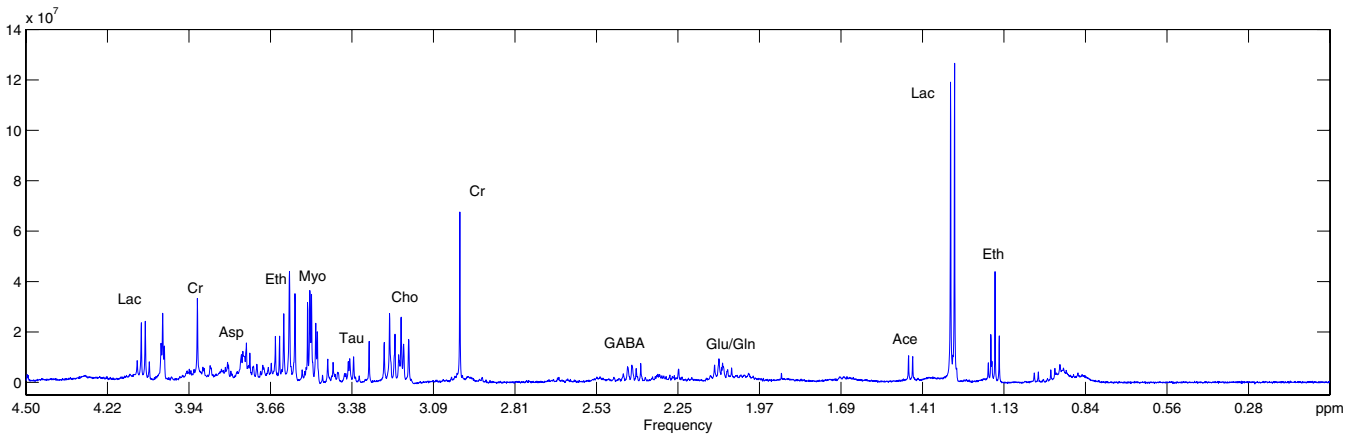

Figure 1. Region of interest of an HRMAS spectrum from a tissue sample of a human brain with an oligodendroglioma, acquired at 11.7 tesla.

Proton HRMAS signals of *ex vivo* tissues contain several overlapping spectral components (many hundreds) from many metabolites, see figure 1. For many metabolites, the values of chemical shifts of proton groups may slightly differ according to the micro-environment in the tissue or cells, see e.g. [15–17]. In fact, it is widely known that the exact values of chemical shifts are not only subject to the molecular geometry but also depend on the molecular environment. Thus, for example, the temperature dependence of certain peak positions enables temperature mapping [18–20], the pH dependence gives the possibility of analyzing acidity maps [15]. The influence of temperature on chemical shifts can also be predicted by quantum chemistry calculations [21]. These changes of chemical shift values hamper the accurate estimation of the metabolite concentrations mainly when using quantitation algorithms based on a metabolite basis set [22–24]: the metabolite fingerprints are not correct anymore.

The values of mismatches (around some Hz) between the signal to be fitted and those of the metabolite basis set are often not significant for *in vivo* (low-resolution) spectroscopy. But for HRMAS spectroscopy, this increases the error of quantitation to tens of percent for certain metabolites (e.g. creatine, lactate). Several methods were proposed to circumvent this problem, see for instance [25] and references herein. Subdividing the basis-set signals of given metabolites into basis sub-components according to chemical groups and adding appropriate constraints (prior knowledge) to the parameters of the groups were proposed in [13]. To limit user involvement, a simple method for chemical shift correction based on signal processing and stretching/shrinking of the metabolite basis-set signals was previously reported in [26, 27]. In *in vivo* MR spectroscopy, chemical shift recalibration based on linear interpolation was recently proposed [28]. In this work, we propose a more accurate method QM-QUEST, based on coupling quantum mechanical simulations and quantitation algorithms to handle basis-set changes, thus respecting the correct fingerprints of metabolites. The method is applied to the quantitation of 33 signals from tissue samples of human brains with oligodendroglioma, obtained at 11.7 tesla.

This paper is set up as follows. In section 2, we first describe the experiments and the quantitation method QUEST and then detail the quantum mechanics methods used to optimize the chemical shifts of metabolites. In section 3, our method is applied to automatically quantitate series of ^{1}H HRMAS signals from biopsies of human brain with oligodendroglioma. The obtained chemical shift changes of several metabolites within the series are analyzed. In section 4, results and the method are discussed.

2. Method

2.1. Experiments

1D ^{1}H HRMAS NMR spectra were obtained on a Bruker Avance 500 spectrometer operating at a proton frequency of 500.13 MHz using a 4 mm double resonance (^{1}H, ^{13}C) gradient HRMAS probe and a CPMG sequence as described previously [29]. The amount of tumor tissue used ranged from 16 to 20 mg. The whole HRMAS study was performed at 4 °C and was started immediately after the temperature inside the probe reached the equilibrium condition (5 min). Thirty-three spectra from tissue samples of human brains with oligodendroglioma were acquired (sampling interval: 0.143 ms, number of data points $N = 16\,384$).

2.2. Quantitation with QUEST

In magnetic resonance spectroscopy, quantitation based on a metabolite basis set has become very popular in the last 10 years. In this work, we used the method QUEST [23]. The complex-valued time-domain model signal is written as a linear combination of the M weighted metabolite models $\hat{x}^m$—either quantum mechanically simulated or *in vitro* measured—of the basis set, see figure 2. The model samples, $\hat{x}_n, n = 1, 2, \ldots, N$, where N is the number of data points, can be written as

$$\hat{x}_n = \exp(\imath\phi_0) \sum_{m=1}^{M} a_m \hat{x}_n^m \exp[(\Delta\alpha_m + \imath\Delta\omega_m)t_n + \imath\Delta\phi_m], \quad (1)$$

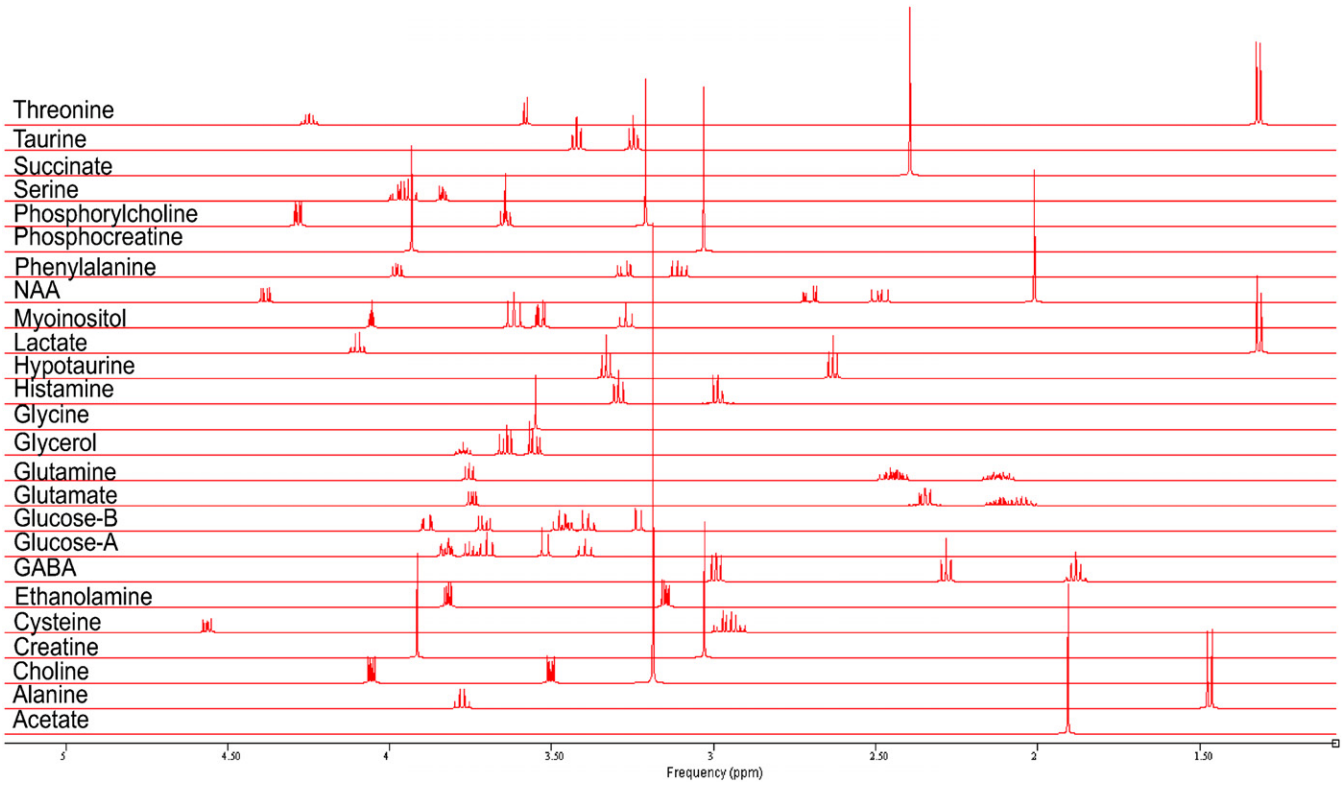

Figure 2. Fourier transform of a metabolite basis set at 11.7 tesla, simulated by quantum mechanics with NMR-SCOPE for a one-pulse sequence. This basis set was used in QUEST for the quantitation of human brain HRMAS signals. Lorentzian lineshapes were used.

where

- $\hat{x}^m$ represents the metabolite basis-set signals.
- a_m are M amplitudes to be estimated. Note that these amplitudes represent the relative proportions of the M metabolite signals $\hat{x}^m$ in the signal x rather than the amplitudes of individual spectral components.
- $\Delta\alpha_m$, $\Delta\omega_m$ and $\Delta\phi_m$ represent small changes of the damping factors, angular frequencies and phase shifts, respectively. These changes—relative to the initial values in the metabolite basis set—are included in the estimation procedure to automatically compensate for the effect of magnetic field inhomogeneities. Most often $\Delta\phi_m = 0$. Soft constraints on $\Delta\alpha_m$ and $\Delta\omega_m$ have been used in the minimization procedure.
- $t_n = nt_s + t_0, n = 1, 2, \ldots, N$, are the sampling times, in which t_0 is the dead-time of the receiver—fixed to 0 in this study—and t_s is the sampling interval.
- ϕ_0 is an overall phase, fixed to 0 in this study.
- $\imath^2 = -1$.

2.3. Optimization of the basis-set signals

Two quantum-mechanical simulators were used to simulate the basis sets. The first one is the NMR-SCOPE algorithm based on density matrix formalism [30], the second one, used in the optimization procedure, is based on the conventional approach [31].

2.3.1. Density matrix approach: NMR-SCOPE.

NMR-SCOPE is based on the density matrix and the product operator (super operators) formalism, and commutator algebra. It handles a product-operator description of strongly coupled spin $\frac{1}{2}$ systems and enables the simulation of signals of the metabolites in response to magnetic resonance pulse-sequences. It can handle various pulse sequences such as STEAM and PRESS and provides directly the time-domain signals. It is applicable to ^{1}H, ^{13}C, ^{19}F, ^{31}P, ..., nuclei and arbitrary field strength. The equation of motion of the density matrix operator $\rho(t)$ under the influence of a time-independent Hamiltonian $\mathcal{H}$ is given by

$$\rho(t) = \exp(-\imath\mathcal{H}t)\rho(0)\exp(\imath\mathcal{H}t) \tag{2}$$

where $\rho(0)$ is the density operator at the beginning of the experiment. Then, the evolution of the density matrix product operators was computed according to equation (2) for each pulse-sequence event by adapting $\mathcal{H}$ and t. During free-precession time intervals, $\mathcal{H}$ was taken equal to the strong coupling spin Hamiltonian

$$\mathcal{H} = \sum_{i}^{N_{\text{spins}}} \delta_i I_{iz} + \sum_{i<j}^{N_{\text{spins}}} \sum_{j}^{N_{\text{spins}}} 2\pi J_{ij} \mathbf{I}_i . \mathbf{I}_j , \tag{3}$$

where N_{spins} is the number of spins of the molecule/metabolite under consideration, I_{ix}, I_{iy}, I_{iz} represent the components of the spin $\mathbf{I}_i$, δ_i represent the chemical shifts and J_{ij} represent the coupling constants of the metabolite spins.

For radio-frequency (RF) pulses, $\mathcal{H}$ is equal to

$$\mathcal{H}_{\mathrm{RF}} = \theta \sum_{i}^{N_{\mathrm{spins}}} (I_{ix} \cos\phi + I_{iy} \sin\phi), \tag{4}$$

where θ is the magnetization flip angle and ϕ is the phase of the RF pulses. The Hamiltonian for selective RF pulses also includes the free-precession Hamiltonian.

In the detection period, the sampled signal is directly simulated in the time-domain leading to $\hat{x}^m$.

2.3.2. Conventional approach. In the optimization procedure, the conventional quantum mechanical approach [31] is used to speed up the calculations as at this step, one needs signals with accurate frequencies. The method is based on the time-independent Schrödinger equation

$$\mathcal{H}\Psi = E\Psi \tag{5}$$

where Ψ are the wavefunctions and E are the energy levels of $\mathcal{H}$. The matrix representation of $\mathcal{H}$ and eigenvectors in the spin system were obtained by working in the appropriate direct product space. Thus, for two spins $\frac{1}{2}$, $|\alpha\alpha\rangle$, $|\alpha\beta\rangle$, $|\beta\alpha\rangle$, $|\beta\beta\rangle$ were used as basis set, where α and β represent the $\frac{1}{2}$ and $-\frac{1}{2}$ states, respectively. The frequencies and corresponding amplitudes of the spectral components are obtained after diagonalization of the matrix representation of $\mathcal{H}$ corresponding to equation (3) on the mentioned basis set thus enabling the computation of $\hat{x}^m$.

2.3.3. Metabolite basis sets. The basis-set signals were simulated with NMR-SCOPE. Spin parameters were initially taken from [32] and we refined them to fit rodent brain HRMAS spectra [33]. Twenty-five metabolites—acetate (Ace), alanine (Ala), aspartate (Asp), creatine (Cr), choline (Cho), cysteine (Cys), ethanolamine (Eth), γ-amino-butyric acid (GABA), glucose (Glc), glutamate (Glu), glutamine (Gln), glycerol, glycine (Gly), histamine (Hist), hypotaurine (HTau), lactate (Lac), myo-inositol (mI), N-acetylaspartate (NAA), phosphoryl-choline (PC), phosphocreatine (PCr), phenylalanine (Phe), serine (Ser), succinate (Suc), taurine (Tau) and ethanol (Eth) as a trace of biopsy procedure—were included in the basis set. Signals modelling the lipids (Lip) at 0.9 and 1.3 ppm were not included in the basis set, considering that their model function is insufficiently known. They are estimated with the background signal.

As mentioned above, for several metabolites, chemical shifts can slightly differ according to the micro-environment in the tissue or cells, in particular with its pH. That means that prior knowledge based on the metabolite basis-set signals is not entirely correct anymore: the fingerprints of metabolites slightly differ.

2.3.4. Optimization of metabolite chemical shifts. The goal was to correct the possible mismatches between the signals of the simulated basis set and the experimental signal and to optimize the chemical shift values of the metabolites.

As mentioned above, the basis-set signals $\hat{x}^m$ depend directly on the spin parameters (chemical shifts δ_i^m and coupling constants J_{ij}^m) of the metabolite m:

$$\hat{x}^m = f\left(\delta_i^m, J_{ij}^m\right), \quad i, j = 1, 2, \ldots, N_{\mathrm{spins}}^m. \tag{6}$$

We propose to modify the metabolite basis-set signals $\hat{x}^m$, sensitive to pH and/or temperature changes, before quantitation by maximizing the normalized cross-correlation between each of these signals and the investigated HRMAS signal x as a function of chemical shift values:

$$\mathrm{corr}(x, \hat{x}^m) = \sum_{i=1}^{N} x_i^* \hat{x}_i^m \tag{7}$$

where * indicates complex conjugation. This means that the chemical shifts δ_i^m, initially provided to the quantum mechanical simulation procedure for metabolite subject to pH and/or temperature changes, will be optimized and new $\delta_{i,\mathrm{opt}}^m$ estimated. The normalized cross-correlation, chosen as the cost function, avoids signal normalization.

To the best of our knowledge, such work has never been reported. If fitting of spin parameters is a well-known problem in high-resolution NMR, the problem tackled here is much more complicated as the signals under analysis show rich biomedical profile from a *mixture* of metabolites with unknown concentrations and overlapping spectral components. Minimization of the residue between the fitted and theoretical signals of a metabolite, usually used in high-resolution NMR, could not be used anymore.

2.3.5. Algorithm scheme. The optimization scheme of MQ-QUEST can be summarized as follows:

(1) simulation with NMR-SCOPE of the basis set $\{\hat{x}^m, m = 1, 2, \ldots, M\}$ using the initial spin parameters $\left\{\delta_i^m \text{ and } J_{ij}^m, i, j = 1, 2, \ldots, N_{\mathrm{spins}}^m\right\}$;
(2) selection of metabolites that are subject to pH changes leading to M_{pH} metabolite signals to be optimized;
(3) for each of the M_{pH} metabolites: maximization of $\mathrm{corr}(x, \hat{x}^m) \Longrightarrow \left\{\delta_{i,\mathrm{opt}}^m, i = 1, 2, \ldots, N_{\mathrm{spins}}^m\right\}$ and $\hat{x}_{\mathrm{opt}}^m$;
(4) simulation with NMR-SCOPE of the optimized basis set $\left\{\hat{x}_{\mathrm{opt}}^m, m = 1, 2, \ldots, M_{\mathrm{pH}}\right\}$ using the spin parameters $\left\{\delta_{i,\mathrm{opt}}^m \text{ and } J_{ij}^m\right\}, i, j = 1, 2, \ldots, N_{\mathrm{spins}}^m\}$;
(5) QUEST quantitation using the optimized basis set $\left\{\hat{x}_{\mathrm{opt}}^m, m = 1, 2, \ldots, M\right\}$.

Note that the conventional quantum mechanical simulation algorithm is inside the maximization procedure (based on the steepest descent algorithm) of the cost function. The optimization was performed in Matlab (©MathWorks) using the conventional quantum mechanical algorithm in C [34] and quantitation was performed with the jMRUI software package [20].

As a quality criterion of the method, we chose the estimated standard deviations based on the estimated Cramér-Rao lower bounds on the amplitudes provided by QUEST [23] for metabolites subject to pH and/or temperature changes.

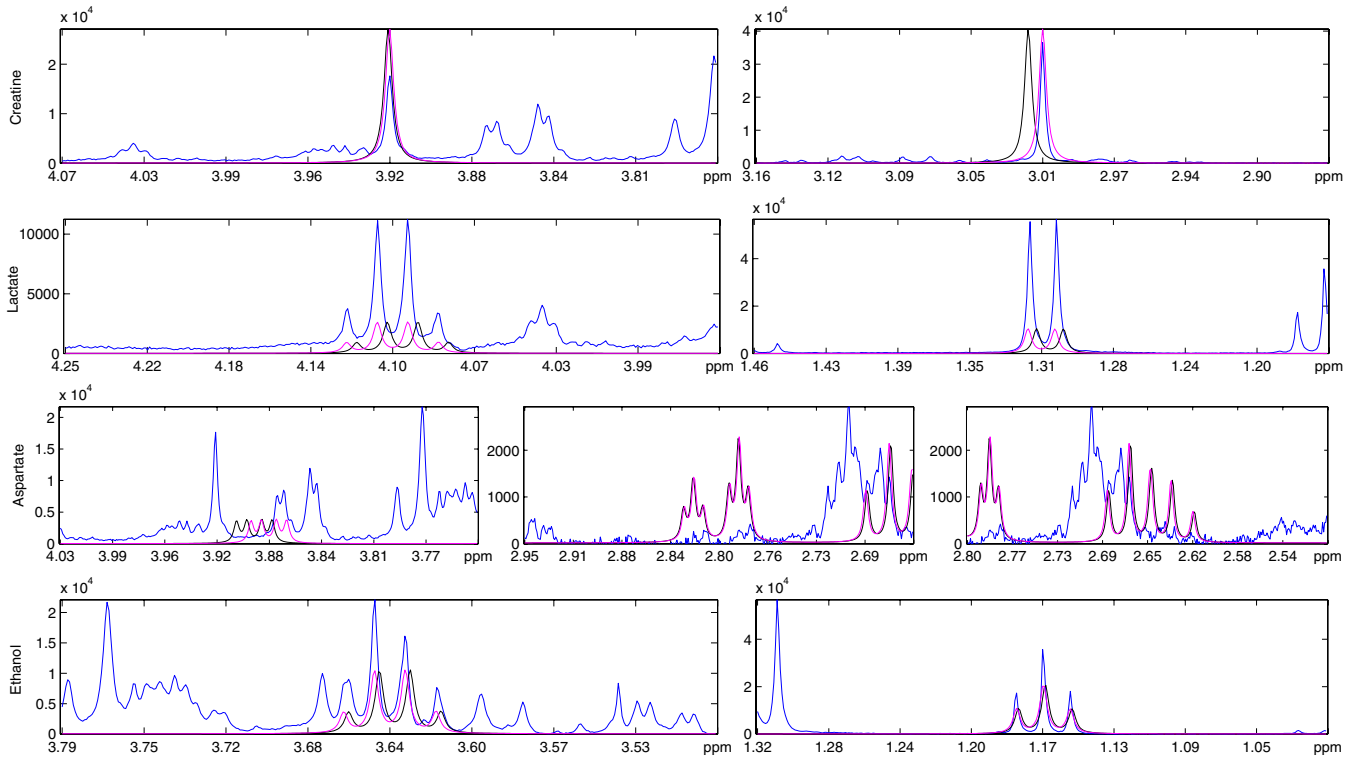

Figure 3. Zoom in on the Cr, Lac, Asp and Eth multiplet regions in an HRMAS spectrum from a tissue sample of a human brain with an oligodendroglioma, acquired at 11.7 tesla and the basis-set ones (blue: raw spectrum, black: original basis-set spectra, magenta: optimized basis-set spectra).

3. Results

The proposed method was first tested on simulated signals mimicking HRMAS-NMR ones (not reported in this paper) and then on HRMAS-NMR signals. Thirty-three spectra from tissue samples of human brains with oligodendroglioma, acquired at 11.7 tesla were quantitated. The region of interest of the spectra was first extracted with ER-Filter [35] as mentioned in [33]. Mismatches between the initial basis-set signals and some *ex vivo* signals were observed. For the latter, adaptation of the basis-set signals was necessary. Results are illustrated for an oligodendroglioma spectrum of the series in figure 3. Lac, Cr, Asp, Eth, the latter as a trace of the biopsy procedure, and other signals of the basis set were automatically optimized prior to the quantitation procedure as mentioned above by maximizing the correlation between the *ex vivo* and basis-set signals. The method enables independent movements of the different multiplets of the spectrum, keeping all strong-coupling effects, contrary to methods which subdivide the basis-set signals of given metabolites into sub-components. This enabled us to adapt the chemical shift variations. It can easily be seen in figure 3 that the mismatches between the basis-set and HRMAS spectra have been reduced for Cr, Lac, Eth. Asp multiplets at 2.80 and 2.65 ppm have amplitudes of the same order as the noise level which makes the recognition of peak positions more difficult.

The optimized chemical shifts for Lac and Cr for the 33 spectra are shown in figure 4. The left plots represent the chemical shifts of two spin groups (or of two chosen groups for Asp in figure 5). The red dots correspond to the starting values provided to the optimization procedure. In the absence of pH-dependence, a straight line (corresponding to a global spectrum shift) would be expected. The 'cloud points' represent independent changes of the chemical shifts of the different multiplets due to the environment changes. Note that the Cr chemical shift at about 3 ppm is nearly the same for all biopsies, in agreement with [36]. On the right, the series of optimized chemical shift differences are plotted as a function of the experiment number. Again, it can be easily seen that independent chemical shift corrections are needed to adapt the distance between the metabolite multiplets. From the estimated chemical shifts of Cr in the series and the titration curves given in [36], one could cautiously estimate that the pH in these biopsies was about 7–8. Comparing the results obtained for Cr and Lac to those obtained for Asp, one can see the poor optimization quality for the latter, see figure 5. The Asp spectra after optimization are not (or slightly) changed. This is due to the small amplitude of the Asp signal in the investigated spectra. For more complicated spin systems such as Glu and Gln, the residue of the fit decreases but the commonly low concentrations of these metabolites do not allow us to conclude about the efficiency of the proposed method.

To establish possible correlation between the obtained variations in chemical shifts across different metabolites among the 33 investigated biopsies, the differences in chemical shifts $\delta_1 - \delta_2$ of the two proton groups for Lac were sorted in ascending order, see figure 6, left. On the right the differences in chemical shifts $\delta_1 - \delta_2$ of the two proton

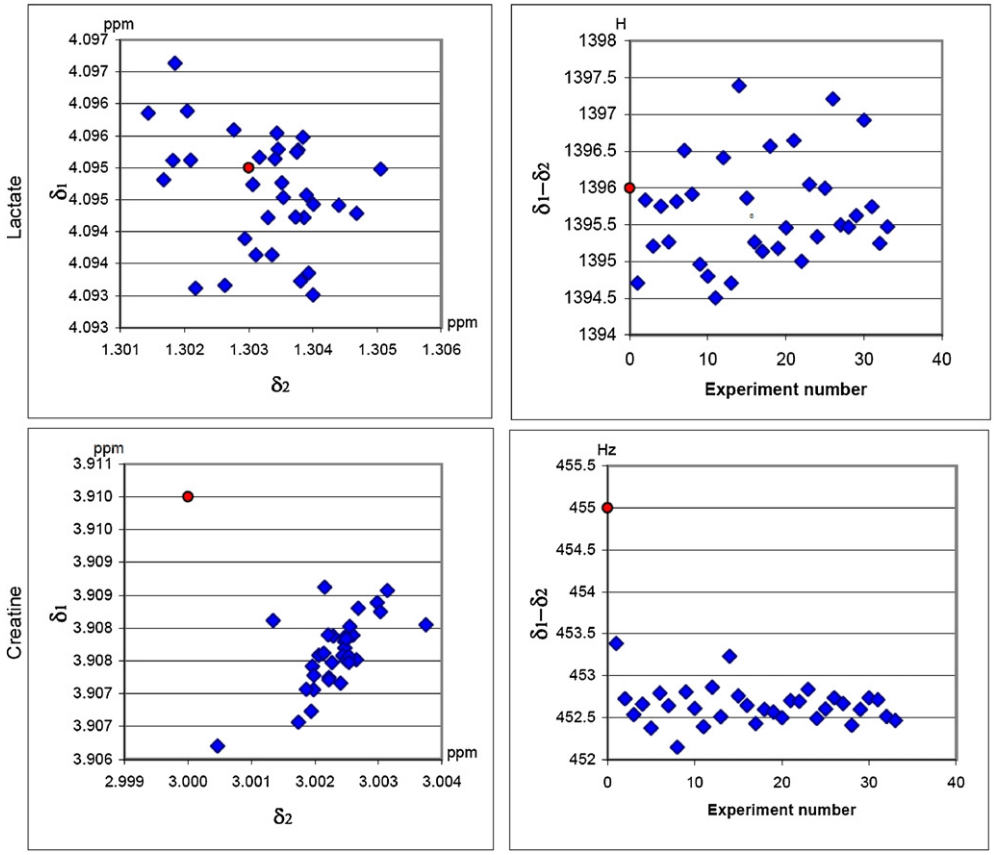

Figure 4. Optimized chemical shift values of Lac and Cr for 33 spectra from tissue samples of human brains with oligodendroglioma, acquired at 11.7 tesla. Left: chemical shifts δ_1 and δ_2 of the two proton groups; right: differences $\delta_1 - \delta_2$. The red dots correspond to the starting values provided to the optimization procedure.

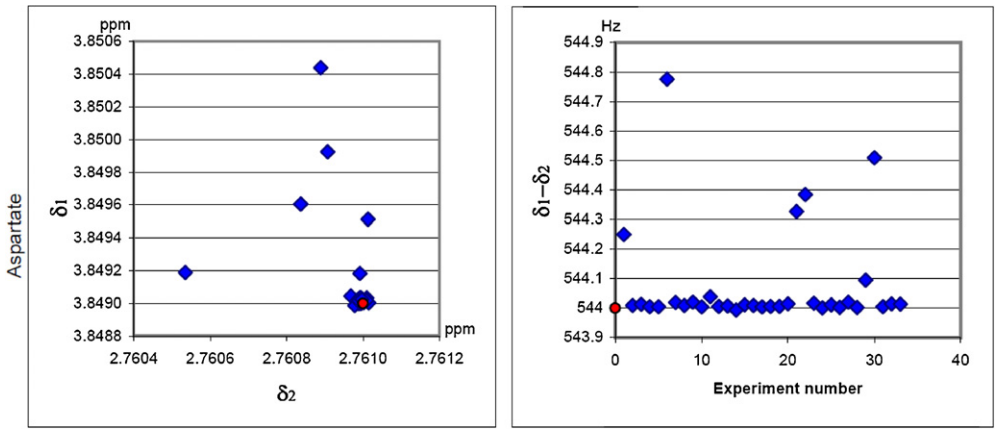

Figure 5. Optimized chemical shift values of Asp for 33 spectra from tissue samples of human brains with oligodendroglioma, acquired at 11.7 tesla. Left: chemical shifts δ_1 and δ_2 of the two proton groups at about 2.8 and 3.9 ppm; right: differences $\delta_1 - \delta_2$. The red dots correspond to the starting values provided to the optimization procedure.

groups for Cr for the corresponding sorted experiments are shown. A certain dependence exists. The Pearson product-moment correlation r between the two series is 0.29 corresponding to a small correlation. But note that the trends of pH curves for different chemical shifts and various metabolites are different [16, 36]. Pearson's r between Eth and Lac, and between Eth and Cr series were also computed and found equal to 0.52 and 0.47, respectively, showing a rather large correlation. That means that differences in chemical shift changes are in part due to pH as expected.

Results show an improvement of quantitation quality when using the optimized basis set. As a quality criterion of the method, we chose the estimated standard deviations on the amplitudes provided by QUEST for metabolites subject to pH and/or temperature changes. Comparing these values obtained using the initial and optimized basis sets, one finds decreases of as much as 30–40%. Moreover, the parameters

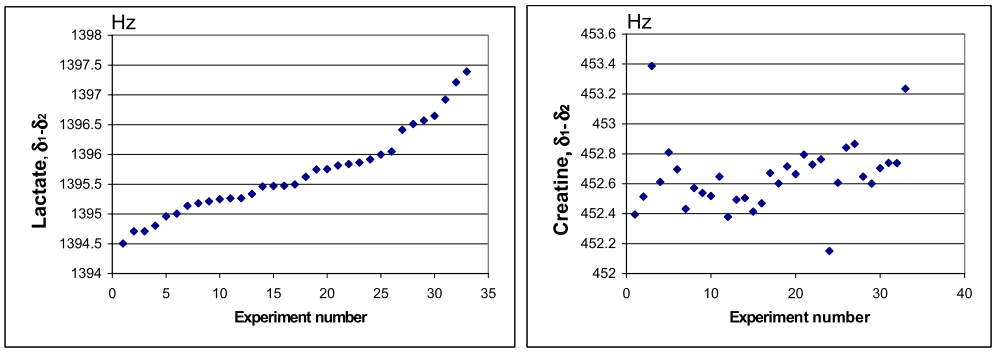

Figure 6. Left: optimized chemical shift differences $\delta_1 - \delta_2$ of the two proton groups at about 1.3 and 4.1 ppm of Lac for 33 spectra from tissue samples of human brains with oligodendroglioma, acquired at 11.7 tesla and sorted in ascending order. Right: differences $\delta_1 - \delta_2$ of the two proton groups at about 3 and 3.91 ppm for Cr corresponding to sorted experiments.

$\Delta\omega_m$ of the model function (equation (1)) become redundant after the optimization of the chemical shifts, thus reducing the number of free parameters in the quantitation procedure.

The computing time for the optimization procedure depends mainly on the spin number of the considered metabolite as the quantum mechanical simulation is iteratively computed in the cost function. It was about 8 s for Cr, 63 s for Lac, 44 s for Asp and 67 s for Eth, on a laptop PC with an Intel Core Duo with a 2.4 GHz processor and 2 GHz of RAM, running Windows XP.

4. Discussion

A novel method based on quantum mechanical simulations was developed that provides an *automatic* approach for the accurate quantitation of the metabolite concentrations in high-resolution signals from biopsies. As previously demonstrated, the method when combined with a quantitation algorithm (for instance, QUEST, leading to the method QM-QUEST) provides more robust fitting. In particular, it is well suited to improve the quantitation of metabolites with well-resolved spectra (Lac, Cr, Asp, mI, Eth as a trace of the biopsy procedure, etc) in HRMAS spectra. Moreover, it limits user involvement as only metabolites for which chemical shifts have to be optimized must be selected, whereafter optimized chemical shift values for these metabolites are provided automatically. Compared to methods based on subdividing the basis-set signals of given metabolites into basis sub-components according to chemical groups and adding appropriate constraints (prior knowledge) to the parameters of the groups [13], the proposed method limits user interaction and the number of parameters used in the quantitation routine. The simple method for chemical shift correction based on signal processing and stretching/shrinking of the metabolite basis-set signals, we previously reported in [26, 27], does not provide optimized chemical shifts.

The reliability achieved in the final fits was assessed using the estimated Cramér–Rao bounds on amplitudes and shows decreases of as much as 30–40% for metabolites for which the basis-set signals have been optimized. For a given metabolite, results depend on the signal-to-noise ratio (SNR) related to the spin numbers and concentration and on the overlap between the spectral components of other metabolites. For example, the scyllo-inositol singlet appears near other metabolite multiplets around 3.3 ppm, which makes its chemical shift hardly optimizable. For several metabolites, the estimated changes in the chemical shifts were unique; this result differed from one biopsy to the other. Only the Cr chemical shift at about 3 ppm was nearly the same for all biopsies. No strong correlation has been observed between changes in the $\delta_1 - \delta_2$ of pairs of metabolites, indicating that these changes are due not only to pH.

Besides important improvements, the method has some limitations. For metabolites with very similar spectra such as Cr and PCr, it was not possible to adjust their chemical shifts separately and only total Cr was considered. Note that in most of the spectra of the series, PCr was not identifiable. For more complicated and overlapping spin systems such as Glu and Gln, α and β Glc, the method works well if these metabolites have sufficient concentrations. When the SNR is low, the quantum mechanical optimization procedure should be included into the quantitation algorithm. This would enable quantitation of signals with an optimized basis set but the computing time would substantially increase. As mismatches of peak positions between the basis-set signals and the signal under analysis are of the order of some Hz, the optimization algorithm is also sensitive to the presence of local minima of the correlation function.

The method would also be well suited for the accurate quantitation of prostate tissues, mainly because of citrate chemical shift changes according to pH [16].

5. Conclusion

We proposed a new quantum mechanics-based method accounting for pH- and/or temperature-related chemical shift changes prior to quantitation. Its advantages are as follows: (1) it is the only method which respects the correct fingerprints of metabolites; (2) it limits user involvement, (3) the parameters $\Delta\omega_m$ of the model (equation (1)) in QUEST become redundant after the optimization of the chemical shifts, thus reducing the number of free parameters in the quantitation procedure. The proposed method QM-QUEST is well suited to improve the quantitation of metabolites with well-resolved

spectra (lactate, creatine, aspartate, myo-inositol, ethanol as a trace of the biopsy procedure, etc). For more complicated spin systems such as glutamate and glutamine, the method works well if these metabolites have sufficient concentrations. The proposed method QM-QUEST will be implemented in the coming version 4.2 of the jMRUI software package.

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