Simple Correction of Chemical Shift Changes in Magnetic Resonance Spectroscopy Quantitation

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***Abstract*—High-resolution magic angle spinning (HRMAS) ^{1}H spectroscopy is playing an increasingly important role for diagnosis. This technique enables setting up metabolite profiles of *ex vivo* pathological and healthy tissue. Automatic quantitation of HRMAS signals provides reliable reference profiles to monitor diseases and pharmaceutical follow-up. Nevertheless, for several metabolites chemical shifts may slightly differ according to the micro-environment in the tissue or cells, in particular its pH. This hampers accurate estimation of the metabolite concentrations mainly when using quantitation algorithms based on a metabolite basis-set. In this work, we propose a user-friendly way to circumvent this problem based on stretching of the metabolite basis-set signals and maximization of the correlation between the HRMAS and basis-set spectra prior to quantitation.**

***Index Terms*—Magnetic Resonance Spectroscopy, HRMAS, Quantitation, Stretching, jMRUI Software Package.**

I. 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 [1–8]. Automatic quantitation of HRMAS signals [9–12] will provide reliable reference profiles to monitor diseases and pharmaceutical follow-up.

^{1}H HRMAS signals of *ex vivo* tissues contain several overlapping spectral components from many metabolites, see Fig 1. For several metabolites chemical shifts may slightly differ according to the micro-environment in the tissue or cells, in particular its pH, see e.g. [13]. This hampers accurate estimation of the metabolite concentrations mainly when using quantitation algorithms based on a metabolite basis-set [14–16]. The values of mismatch (around $2 \cdot 10^{-2}$ ppm) between the *ex vivo* signal and those of the metabolite basis-set are not significant for *in vivo* (low-resolution) spectroscopy. But for HRMAS spectroscopy, this increases the error of quantitation to tens percents for certain metabolites (e.g. Creatine, Lactate). In this work, we propose a user-friendly way to circumvent this problem based on stretching of some metabolite basis-set signals prior to quantitation with the method QUEST [15].

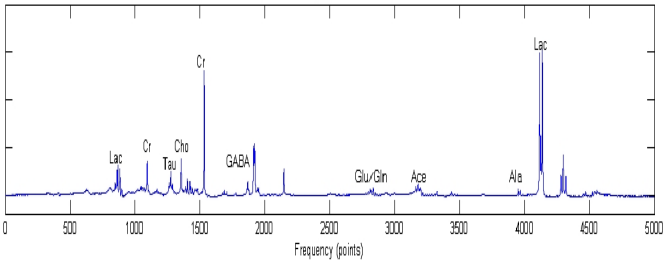

Fig. 1. Region of interest of an HRMAS spectrum from a tissue sample of a human brain with an oligodendroglioma, acquired at 11.7T.

II. Method

A. Quantitation with QUEST

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

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

where

- $\hat{x}^m$, m being a superscript, 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 metabolites signals $\hat{x}^m$ in the signal x rather than the amplitudes of individual spectral components.
- $\Delta\alpha_m, \Delta\omega_m, \Delta\phi_m$ represent small changes of the damping factors, angular frequencies, and phase shifts respectively. These changes – relative to the initial values in

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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 – included in the estimation – and t_s the sampling interval,
- ϕ_0 is an overall phase, included in the estimation,
- $i^2 = -1$.

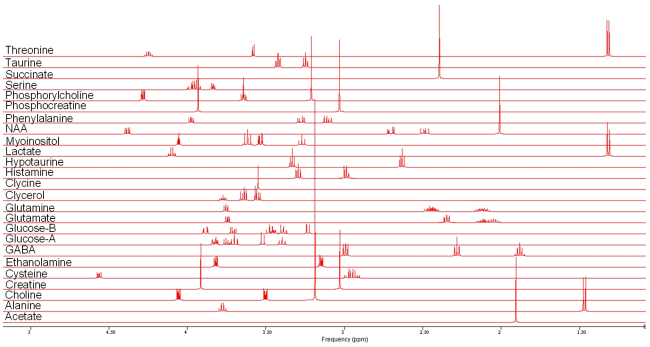

Fig. 2. Fourier transform of a metabolite basis-set at 11.7 Tesla, simulated by Quantum Mechanics with NMR-SCOPE for a one pulse sequence. This basis set was used in QUEST for quantitation of human brain HRMAS signals. Lorentzian lineshapes were used.

The basis-set signals were simulated directly in the time-domain with NMR-SCOPE which is based on the density matrix and product operator formalism [17]. Spin parameters were taken from [18]. We refined them. 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, Hypotaurine, Lactate (Lac), Myo-Inositol (mI), N-acetylaspartate (NAA), Phosphoryl-choline (PC), Phosphocreatine (PCr), Phenylalanine (Phe), Serine (Ser), Succinate (Suc), Taurine (Tau) – 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. The metabolite basis-set computed with NMR-SCOPE and used in QUEST for quantitation of human brain HRMAS signals is shown in Fig. 2. 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.

B. Stretching of the Basis-set Signals

We propose to modify the metabolite basis-set signals $\hat{x}^m$, sensitive to pH changes, by just slightly stretching (expansion/contraction) the corresponding spectrum. This is illustrated in Fig. 3 where the lactate spectrum of the basis-set differs from the *ex vivo* spectrum. Stretching can easily be done by introducing frequency scaling parameters κ_m for given metabolites and by directly using the inverse scale change of the $\hat{x}^m$ time-domain signals (frequency scale expansion results in contraction of the time scale and vice versa) [19]. This avoids splitting of the metabolite 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. Either κ_m can be estimated prior to quantitation by maximizing the correlation between the *ex vivo* signal and $\hat{x}^m$ or by introducing κ_m in the quantitation procedure.

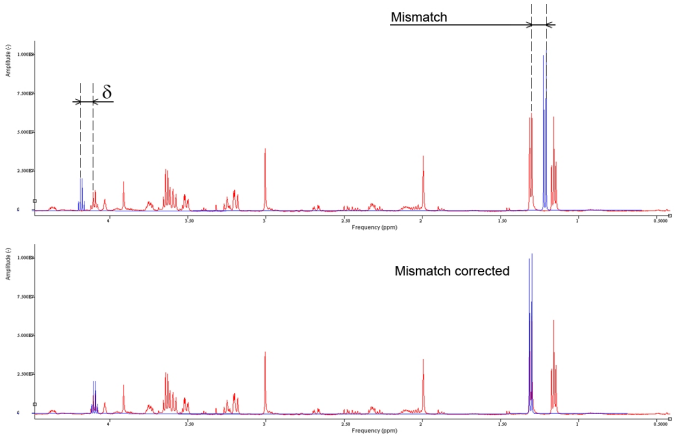

Fig. 3. Needs for stretching of a basis-set spectrum/signal. Red: oligodendroglioma spectrum, blue: Lactate basis-set spectrum presenting a mismatch with respect to the corresponding *ex vivo* Lactate spectrum.

C. Theoretical Limitations

Small changes of chemical shifts do not induce changes of J-coupling values, but the proposed method changes the distances between the peaks in each multiplet. This leads to some limitations of the stretching values with respect to the J-coupling values and distances between the peaks. A stretching coefficient can be defined as $\kappa = (\Delta + \delta)/\Delta$ where Δ corresponds to the distance between two multiplets (or to the difference between two chemical shifts) and δ corresponds to the frequency shift resulting from the stretching of the spectrum. Mathematically, the limitation can be expressed as $J(\kappa - 1) < \alpha/2$ where α represents the linewidth of a peak *i.e.* $J((\Delta + \delta)/\Delta - 1) < \alpha/2$ for the general case. This expression can easily be simplified to $\delta < \alpha\Delta/2J$. A schematic illustration of the stretching consequences on J coupling values is given in Fig. 4. It is then possible to establish the maximum limit of corrections possible, for instance, for Alanine, Aspartate, Citrate, Creatine and Lactate, see table I. Theses values were calculated for a resonance frequency of 500 MHz and a damping factor of 2 Hz corresponding to HRMAS spectra. As an example, for Alanine δ values less than or equal to 160 Hz can be corrected with the proposed method. For AB spectra, the distortions related to stretching of the signal became more important and lead to more significant

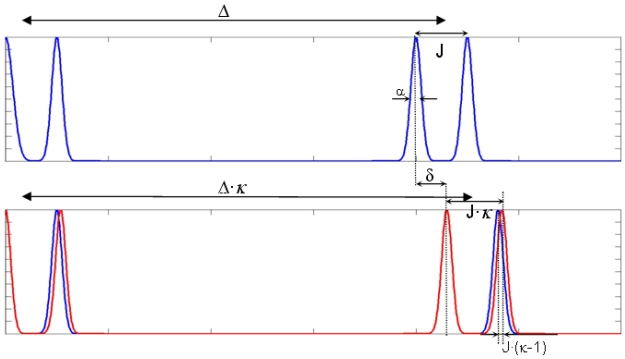

Fig. 4. Schematic illustration of the stretching consequences: Blue: spectrum with changeable peak positions, red: stretched spectrum.

limitations of the method's applications, *e.g.* the limit of correction of peak positions in the Citrate spectrum is about 4 Hz. Certain metabolites (*e.g.* Creatine) have no significant limitations as their proton groups are singlets. In this case, the artificial changes of the spectral linewidth is the only consequence of the method and it is insignificant.

TABLE I
METABOLITES WELL-PROCESSED WITH THE METHOD. THE LIMIT OF CORRECTION WAS CALCULATED FOR A RESONANCE FREQUENCY OF 500MHZ AND A DAMPING FACTOR OF 2HZ; - UNCOUPLED PROTONS.

Metabolite	AX-distance, ppm	δ_{Max} in Hz/ppm
Alanine	2.31	160 / 0.32
Aspartate	1.09	31.3 / 0.063
Citrate	0.13	4.1 / 0.008
Creatine	0.89	-
Lactate	2.78	167 / 0.334

III. RESULTS

Thirty spectra from tissue samples of human brains with oligodendroglioma acquired at 11.7T, were quantitated. Mismatches between the basis-set signals and some *ex vivo* signals were observed. For the latter, adaptation of the basis-set signals was necessary. Results are illustrated on such a spectrum, see Fig. 1 and Fig. 5. Lactate, Creatine, Alanine and others signals of the basis set were automatically stretched prior to the quantitation procedure as mentioned above by maximizing the correlation between the *ex vivo* and basis-set signals. It can easily be seen that, thanks to stretching, one can independently "shift" the different multiplets of the spectrum. This enabled us to adapt artificially the chemical shifts variations due to pH. Stretching coefficients automatically found for Alanine, Creatine and Lactate for the considered oligodendroglioma and basis-set spectra, are given in Table II. Quantitation was significantly improved.

IV. CONCLUSION

We proposed a very simple method to account for chemical shift changes related to pH in quantitation methods based on a metabolite basis-set and used for quantitation of HR-MAS or high resolution signals. The method avoids splitting of the metabolite basis-set signals of given metabolites into basis sub-components thus decreasing user involvement. It is well suited to improve quantitation of AB and AX spin systems like Citrate, Lactate, Creatine, Ethanolamine, etc.. For the more complicated spin systems like Glutamate and Glutamine, the method is less suited but still improves the quantitation results. A more accurate method based on Quantum Mechanics is under development and will be implemented in the version 4.x of the jMRUI software package [20].

TABLE II
STRETCHING COEFFICIENTS FOUND FOR ALANINE, CREATINE AND LACTATE FOR THE CONSIDERED OLIGODENDROGLIOMA AND BASIS-SET SPECTRA.

Metabolite	Ala	Cr	Lac
Stretching coefficient	1.004	0.994	1.009
Shift, Hz	5.9	0.55	0.32

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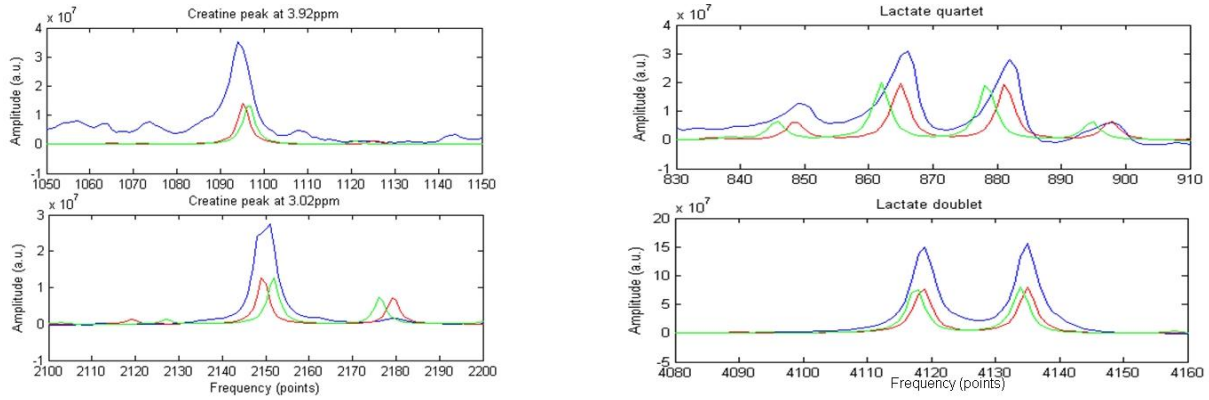

Fig. 5. Zoom in on the multiplets of Creatine and Lactate spectra. Blue: oligodendroglioma spectrum; green: initial basis-set spectrum and red: corrected (stretched) basis-set spectrum of Cr and Lac respectively.

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