

Quantitation with QUEST of ER-Filtered HRMAS-NMR Signals

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Abstract—Quantitation of High Resolution Magic Angle Spinning (HRMAS) Nuclear Magnetic Resonance (NMR) signals enables establishing reference metabolite profiles of *ex vivo* tissues. Signals are often contaminated by a background signal originating mainly from macromolecules and lipids. We show that automatic quantitation of HRMAS signals, even in the presence of a background, can be achieved by the *semi-parametric* algorithm QUEST based on prior knowledge of a metabolite basis-set. The region of interest of spectra is a small part of the full spectral bandwidth. Reducing the computing time inherent to the large number of data-points is possible by using ER-Filter in a preprocessing step. Through Monte-Carlo studies, we analyze the performances of quantitation without and with ER-Filtering. Applications of QUEST to quantitation of ¹H *ex vivo* HRMAS-NMR data of rat brain are demonstrated.

Keywords— High Resolution Magic Angle Spinning (HRMAS) NMR, Semi-parametric Estimation, Background, Monte-Carlo simulations.

I. INTRODUCTION

High Resolution Magic Angle Spinning (HRMAS) Nuclear Magnetic Resonance (NMR) is playing an increasingly important role for diagnosis [1–6]. This technique enables setting up metabolite profiles of *ex vivo* pathological and healthy tissue, *i.e.* biopsies [7]. Automatic quantitation of HRMAS signals will provide reliable reference profiles to monitor diseases and pharmaceutical follow-up.

¹H HRMAS signals of *ex vivo* tissues contain about five hundred overlapping spectral components from many metabolites. Moreover, when acquired without a Carr-Purcell-Meiboom-Gill (CPMG) sequence, there is also an additional background signal from macromolecules and lipids. We show that automatic quantitation of HRMAS signals, even in the presence of a background, can be achieved by the *semi-parametric* algorithm QUEST based on a metabolite basis-set [8–10]. QUEST is part of the freely available MRS software package jMRUI [11, 12]; see <http://www.mrui.uab.es/mrui/>. The spectral region of interest is small compared to the

full spectral bandwidth and can be extracted with ER-Filter [13]. Through Monte-Carlo studies, we analyze the performances of quantitation without and with ER-Filtering.

This paper is set up as follows. In Sec.II, we first treat the signal processing methods. Then, in Sec.III Monte-Carlo studies are described and applications of QUEST to quantitation of ¹H HRMAS signals of rat brain are demonstrated.

II. METHOD

A. Experiments

Biopsies of 15 to 20 mg of tissue were split from rat-brain left hippocampus and rapidly placed in zirconium oxide 4 mm rotors with spherical insert, and 50 μ l of a 3 mM TSP solution in pure D₂O was added. The rotors were immediately transferred into the HRMAS probe and acquisition started after 5 min of rotation/temperature equilibrium. The HRMAS ¹H-NMR experiments were performed at 4°C on a Bruker DRX avance at ultra-high field of 9.4 Tesla. Samples were spun at 4000 Hz. Signals with 8192 data-points were acquired, see Fig. 1.

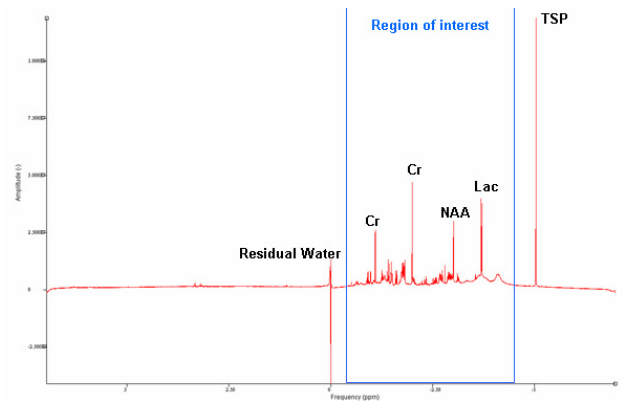


Fig. 1. Full HRMAS spectrum of a rat brain with 8192 data-points. The region of interest (0.5 to 4.5 ppm) within the blue lines was extracted with ER-Filter.

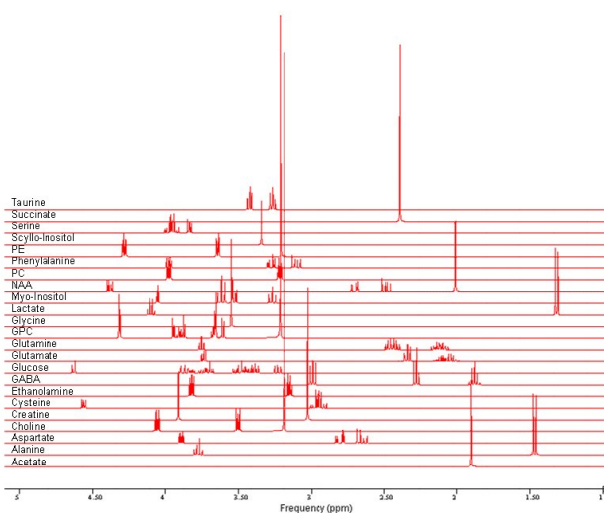


Fig. 2. Fourier transform of a metabolite basis-set at 9.4 Tesla, simulated by Quantum Mechanics with NMR-SCOPE for a one pulse sequence. This basis set was used in QUEST for quantitation of HRMAS signals. Lorentzian lineshapes were used.

B. Metabolite Quantitation

B.1 Metabolite Basis-set

The basis-set signals were simulated with NMR-SCOPE [14] using spin parameters given in [15]. Twenty-three 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), glycine (Gly), glycerophosphoryl-choline (GPC), lactate (Lac), myo-Inositol (mI), N-acetylaspartate (NAA), phosphoryl-choline (PC), phosphocreatine (PCr), phenylalanine (Phe), scyllo-inositol (sI), 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 HRMAS signals is shown in Fig. 2.

B.2 Preprocessing

In a preprocessing step, the spectral region of interest (0.5 to 4.5 ppm) was selected using ER-Filter, see Figs. 1 and 3, leading to filtered signals of about 2200 data-points. Note that ER-Filter avoids water-removal. *Filtered* basis models were then fitted to *ex vivo* filtered signals.

For comparison, raw signals (8192 data-points) were

used too. But, in this case, water removal was performed by HLSVD-Filter [16].

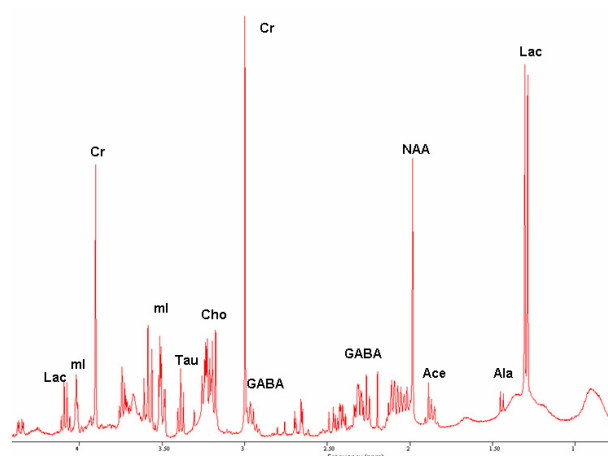


Fig. 3. ER-Filtered HRMAS spectrum of a rat brain. The spectral region of interest (0.5 to 4.5 ppm) (2200 data-points) exhibits many overlapping spectral components from many metabolites.

B.3 Quantitation with Subtract-QUEST

The algorithm Subtract-QUEST, for optimal fitting of basis-models to (contaminated) data is based on a semi-parametric approach. Subtract-QUEST sequentially uses 1) untangling of the background from the metabolite signal, 2) separate modelling, and 3) a parametric nonlinear least-squares fitting of the untangled metabolite signal knowing the background [9, 10]. ER-filtered and HLSVD-Filtered signals were quantitated with Subtract-QUEST [7]. Disentangling the metabolite from the background signals was achieved using the first 16/50 data-points respectively for modelling the background by 8 spectral components.

C. Monte-Carlo Studies

To assess the performances – including bias – of QUEST for quantitation of HRMAS signals, Monte-Carlo studies were performed. We simulated three ^1H HRMAS signals (8192 data-points) at 9.4 Tesla mimicking the spectrum in Fig. 6. The first signal (metabolite-only) comprised twenty-three metabolite-signals whose amplitudes corresponded to metabolite concentrations in a healthy rat-brain hippocampus. The second signal comprised the metabolite signals plus a 'true' macromolecule/lipid background signal estimated from quantitation results. The third signal mimicking a real-world signal contained the metabolite signals, plus the background and a water signal. To these simulated noiseless

signals 256 different realizations of white Gaussian noise were added. The noise level was chosen as in *ex vivo* conditions so that the SNR of the Cr singlet be 60:1. Thus, the noise standard deviation was 0.4. For quantitation of each set, we used 1) HLSVD-filtered signals with the initial number of data-points (full spectral bandwidth), 2) ER-Filtered signals (spectral region of interest (0.5, 4.5 ppm)).

The aim of the first Monte-Carlo study (set 1) was to insure that quantitation of metabolite-only signals with so many overlapping metabolites was possible and to determine how reliable quantitation of each metabolite was. That of the second study (set 2) was to determine if the nuisance background hampered the metabolite quantitation and led to biased estimated amplitudes. Finally, the third study (set 3) aimed at analyzing the quantitation performances of the proposed method on signals mimicking real-world ones.

To assess the performances of QUEST, we computed for each metabolite the mean values of the estimated amplitudes over all simulations and the corresponding standard deviations.

III. RESULTS

A. Monte-Carlo Results

Figs. 4 and 5 show the main Monte-Carlo results. The mean amplitudes of metabolites and two standard deviations estimated from the 256 ER-Filtered and HLSVD-Filtered signals are displayed.

Fig. 4 shows that:

- For metabolite-only signals, there is no bias on amplitude estimates. Quantitation of metabolites with low concentrations such as Eth or Suc is not significant.
- In the presence of macromolecules and lipids, metabolites strongly correlated with the background such as Asp, Gln, GABA, have slightly biased amplitudes. Note that the standard deviations increase compared to those obtained for metabolite-only signals. Nevertheless, most of metabolites (more than 15) are reliably quantitated, with standard deviations less than 10%.

Moreover, for signals mimicking real-world signals (containing important residual water), see Fig. 5, the metabolite amplitude-estimates were not significantly different compared with those of Fig. 4 obtained without and with ER-Filtering.

B. ^1H HRMAS Signals of Rat Brain

Our method was applied to automatically quantitate series of signals from biopsies of healthy and diseased rat brains (hippocampus, piriform cortex, cerebellum). Clear

specific differences in the metabolite profiles were observed.

An example of HRMAS signals of biopsies split from the hippocampus of a rat brain and quantitated with Subtract-QUEST is shown in Fig. 6. The metabolite spectrum (middle) is well estimated. Moreover, the background signal is well modelled; both lipid resonances (0.9 and 1.3ppm) and the four principal resonances of macromolecules (around 2.1ppm, 2.3ppm, 3.2ppm and 3.6ppm) are well identified.

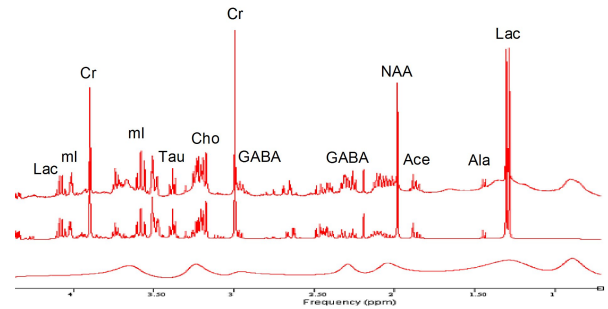


Fig. 6. ER-Filtered HRMAS spectra of a rat brain, quantitated with Subtract-QUEST. Measured (top), estimated (middle) spectra and estimated background (bottom). The estimated signal was used in the Monte-Carlo studies.

The algorithm QUEST is computationally fast. Furthermore, ER-Filtering enables reducing the computing time inherent to the large number of data-points. Quantitation of ER-Filtered signals requires only twenty seconds on a laptop PC with a Centrino 1.6 GHz processor and 1GHz RAM, running Windows XP. That of HLSVD-Filtered signals, requires about two minutes. It is then advantageous to use ER-Filter in a preprocessing step, mainly if large series of signals need to be automatically quantitated.

IV. CONCLUSIONS

About sixteen metabolites are reliably quantitated with QUEST, even in the presence of a background. Thus, signals acquired without suppression of macromolecule and lipids to avoid metabolite signal loss, can be handled. Using ER-Filter in a preprocessing step enables to reducing the computing time without hampering quantitation quality.

V. ACKNOWLEDGEMENTS

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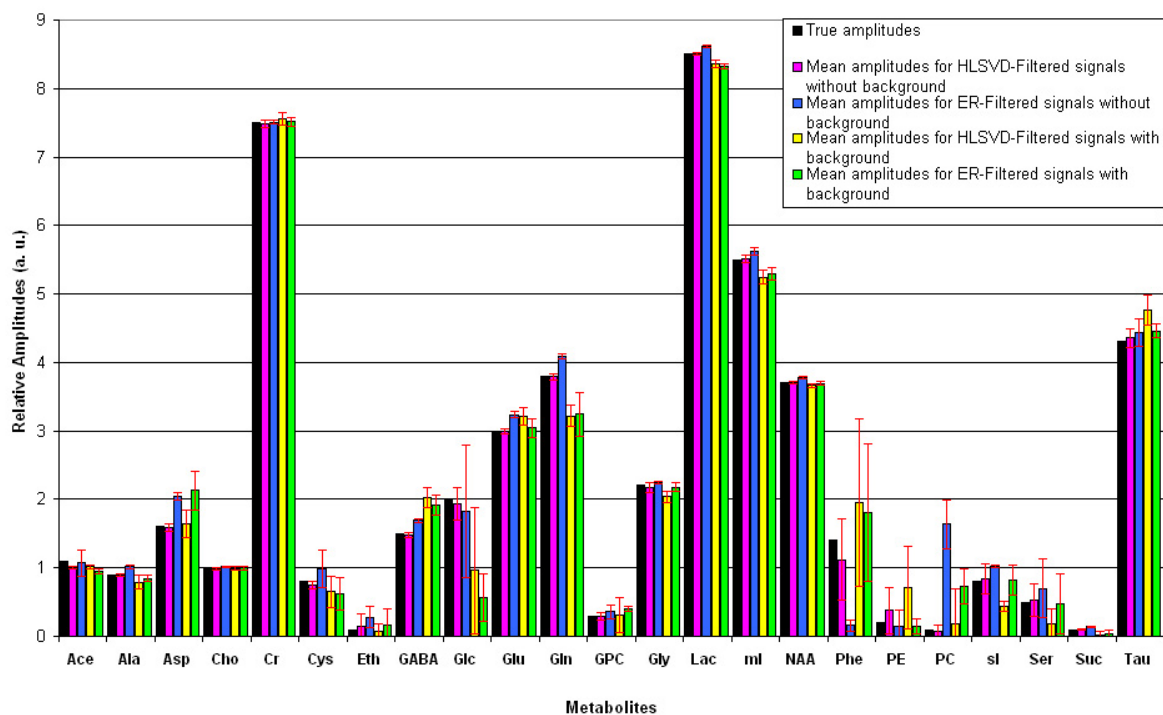


Fig. 4. Monte-Carlo studies for set 1 (metabolite-only) and set 2 (metabolites plus background). True amplitudes (black) and mean amplitudes of metabolites estimated from 256 HLSVD-Filtered (pink and yellow) and ER-Filtered (blue and green) signals quantitated with Subtract-QUEST. The error bars correspond to two standard deviations.

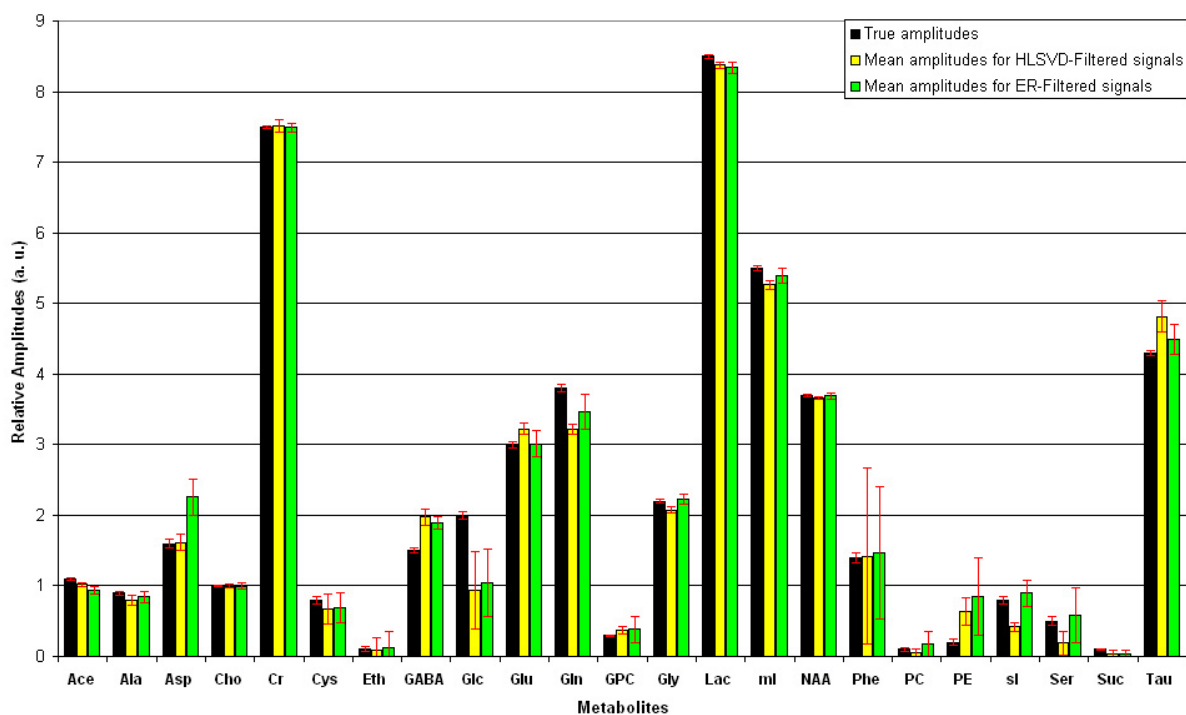


Fig. 5. Monte-Carlo studies for set 3 mimicking a real-world signal. True amplitudes and two Cramér-Rao lower bounds (black) and mean amplitudes of metabolites estimated from 256 HLSVD-Filtered (yellow) and ER-Filtered (green) signals quantitated with Subtract-QUEST. The error bars correspond to two standard deviations.

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