Quantification of *in vivo* ^{1}H Magnetic Resonance Spectroscopy (MRS) signals with baseline and lineshape estimation

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Abstract. *In vivo* quantification of Magnetic Resonance Spectroscopy (MRS) signals is a method to estimate metabolite concentrations of living tissue. Obtaining reliable concentrations is still a challenge due to the experimental conditions affecting spectral quality. Additionally, lipids and macromolecules overlap with the metabolites of interest, affecting their reliable estimation. In this study, we propose to combine the self-deconvolution lineshape estimation method, which accounts for spectral shape distortions, with two different approaches for taking into account the macromolecular baseline contribution: (*a*) based on macromolecules and lipids measured *in vivo* using an inversion recovery technique, and (*b*) based on simulation of macromolecular resonances using prior knowledge from a database of inversion recovery signals. The ultimate goal is to measure macromolecular and lipid data only once as described in (*a*) to create macromolecular and lipid profiles. These profiles then can be used as described in (*b*) for data measured under the same conditions. The method is evaluated on *in vivo* ^{1}H MRS signals at 9.4 T from mouse hippocampus. Results show that better metabolite fits are obtained when lineshape and baseline estimation are simultaneously performed and that baseline estimation based on prior knowledge from macromolecular measured signals can be reliably used to replace time-consuming individual macromolecular and lipid acquisitions.

Keywords: Magnetic Resonance Spectroscopy (MRS), Metabolite Quantification, Lineshape, Macromolecules, Baseline

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1. Introduction

Magnetic Resonance Spectroscopy (MRS) is a non-invasive technique used for the diagnosis of cancer, epilepsy and metabolic diseases. ^{1}H MRS signals exhibit the biochemical information of tissues containing metabolites that give rise to specific single or multiple resonances. However, different metabolites might resonate at nearby frequencies causing overlap between peaks of different metabolites. Their concentration can be estimated using quantification methods. MRS measurements are performed in the time domain characterized by a decaying signal also called Free Induction Decay (FID). To better observe individual metabolites, MRS signals are Fourier transformed to the frequency domain and are called spectra.

The goal of MRS quantification is to reliably determine the concentration of individual metabolites to be used as a complementary tool for diagnosis. Numerous methods have been developed to estimate metabolite concentrations, based on either the time or frequency domain signals. The state-of-the-art techniques are based on prior information about the specific metabolites known to be present in the tissues, where a basis set of individual metabolites is created to fit to the experimental signals [20–22,27].

Reliable quantification of ^{1}H MRS signals is still a challenge due to unwanted distortions affecting their quality and adequate fitting. Signals measured at short echo time (TE) (*i.e.* $<$50 ms) provide more metabolite information compared to those measured at long TE, however they are more sensitive to measurement and tissue conditions. Major challenges are related to the lineshape distortions and baseline contamination.

On the one hand, the lineshape is defined in the frequency domain as the shape of the spectral components ideally represented by a Lorentzian, Gaussian or Voigt function. In *in vivo* studies, the natural lineshape of MR spectra is highly affected by field inhomogeneities, tissue heterogeneities and small movements of the patient, causing lineshape distortions; therefore, several signal processing methods have been proposed to correct for field inhomogeneities, eddy currents and other unwanted distortions [1, 8, 19, 21, 26, 27].

On the other hand, besides the metabolites there are also other kind of components of potential interest captured in the MR spectra, pertaining to lipids and macromolecules (MMs). In normal brain tissue a small contribution from MMs in the frequency regions between 0.5 and 2 ppm can be observed, while in different brain tumours and metabolic diseases lipid concentration increases and this presence also provide useful diagnostic information [9]. Nevertheless, in measurements close to the skull and the scalp, MR spectra from healthy tissue contain unavoidable high lipid contamination.

In the frequency domain, the lipid and macromolecular contributions are observed as an underlying profile called baseline (see bottom curve in Fig.1), which overlaps with the metabolite peaks and complicates quantification. Because there exist no ideal model solutions for macromolecules and lipids, different studies focused on the detection, suppression, evaluation and modeling of the baseline. Most approaches

employ advanced acquisition techniques (*e.g, in vivo* measurements using an inversion recovery pulse [13]) and post-processing methods to model MMs using mathematical functions (*e.g.,* Lorentzians, splines, wavelets or polynomials) [2, 7, 22–25].

The measurement time of MM depends on the acquisition parameters (TI, TR, number of averages etc.) and took approximately 50 minutes in this study. Although the MM signal acquired by inversion recovery is known to provide a good approximation of the macromolecular contamination it is not reproducible when the conditions of the region of interest are affected by acquisition problems and various diseases. Moreover, it also contains some unsuppressed metabolites causing underestimation of metabolites if this inversion recovery signal is subtracted from the MR signal to be quantified [15]. Therefore, it is important to be able to avoid a separate inversion recovery measurement for each *in vivo* MRS signal, and instead rely on automatic signal processing with prior knowledge on MMs. Along this idea, in [2] the position of specific macromolecular resonances was fixed and empirically determined values for the damping terms were used. However, in other studies [3, 12], the whole physiological macromolecular content detected with a metabolite suppression method was added to the basis set for the quantification as an additional model component.

In this study we perform automatic quantification of *in vivo* MRS signals using a novel extension of the quantitation method AQSES [20] that is aimed at combining the self-deconvolution lineshape estimation described in [26] with two approaches for dealing with the macromolecular baseline contamination: (a) To each measurement of an *in vivo* MRS signal we added a separate measurement of the macromolecules and lipids signal by inversion recovery. (b) Avoiding the time-consuming inversion recovery in (a), we instead simulated the resonances of the macromolecules and lipids signal with the aid of prior knowledge. This prior knowledge was extracted from a database of MM + Lip signals measured under similar conditions and in the same location of the mouse brains. By computing the spectral parameters of the acquired macromolecular signals we can simulate individual peaks or sums of peaks and include them in the basis set of metabolites.

Improved quantitation using the combination of lineshape and baseline estimation is shown and the advantages of including prior knowledge in the estimation of the macromolecular components is stressed.

2. Materials and Methods

2.1. Materials

2.1.1. Animals. Twelve wild-type mice were used for this study. Mice were anesthetized by using 1.25% isoflurane and their heads were immobilized during experiments. Breathing was measured during the experiment for monitoring the physiological status of the animals. Respiration and body temperature were measured with the MR compatible small animal monitoring and gating device model 1025 from

S.A. Instruments (Stony Brook, NY, USA). Hereby, respiration is measured using a small pneumatic pillow placed under the abdomen of the animal. Measured pressure changes are digitized and transmitted to the control/gating module using optical fibres. Body temperature was measured using a rectal thermometer, the room temperature was maintained around 31°C. All animal experiments were performed by certified researchers conforming to regional, national and European regulations concerning animal welfare and animal experimentation, authorized and supervised by the university animal welfare commission (Ethische Commissie Dierenwelzijn, KULeuven, approval number 2008/060). We formally declare that we comply to the European FP7-Decision 1982/2006/EC, Article 6§1, *i.e.* all research activities is carried out in compliance with fundamental ethical principles and all experiments are approved and overlooked by the respective Animal Welfare Commissions.

2.1.2. In vivo ^1H MRS signals. Single Voxel Spectroscopy (SVS) MRS signals were acquired on a 9.4 T Bruker Biospec small animal MR scanner (Bruker BioSpin MRI, Ettlingen, Germany) with a magnet bore of 20 cm using a 7 cm linear body resonator as transmitter combined with a circularly polarized ^{1}H mouse brain surface coil for signal reception. *In vivo* SVS signals from mouse brains were obtained using the PRESS pulse sequence [4] with implemented pre-delay Outer Volume Suppression as well as the water suppression method, VAPOR [30]. MRS parameters were: repetition time of TR=4 s, TE=12 ms, bandwidth of SW=4KHz and 256 averages. Spectra were corrected for B_0 instability due to eddy currents as well as B_0 drift using the Bruker built-in routines. Remaining shimming problems, line broadening and non-Lorentzian character of the peaks may still be present after correcting for eddy currents. Shimming was performed using FASTMAP [11]. SVS and FASTMAP Volumes of Interest (VOI) was positioned close to the magnet iso-center and was placed on the mice hippocampus. The voxel size was 3x1.75x1.75 mm^3. The widths of unsuppressed water lines were between 20 and 25 Hz. A typical spectrum of a ^{1}H MRS signal is shown in Fig.1.

For studying one of the ways to estimate the macromolecular contributions, we measured metabolite-nulled *in vivo* signals using inversion recovery with a 1ms Hermitian inversion pulse.

Macromolecules are characterized by shorter T1 and T2 relaxation times than those of metabolites. Therefore, signals from macromolecules can be separated by introducing a T1 selective encoding of the signal which can be achieved by non-selective inversion pulse and an appropriate time delay prior to the measurement sequence (inversion time, TI). This time can be adjusted to suppress signals from metabolites; however, a full suppression of metabolites is not possible due to their different T1s. In particular, the short T1 of Cre [18] caused an incomplete suppression at any TI for the Cre/PCr peak at 3.9 ppm which was further filtered out in preprocessing (see 2.2.1). The best suppression was achieved with the selected TI of 800 ms, the repetition time was TR=3 s with 1024 averages. The spectrum of macromolecules (MM) was measured individually for each mouse.

2.1.3. Metabolite basis set. The following metabolites were measured *in vitro* to be used as basis set: Alanine (Ala), Aspartate (Asp), Creatine (Cre), Gamma-Aminoburytic acid (GABA), Glucose (Glc), Glutamine (Gln), Glutamate (Glu), Glycerolphosphorylcholine (GPC), Glutathione (GSH), Lactate (Lac), Myo-Inositol (m-Ins), N-Acetyl Aspartate (NAA), Phosphorylcholine (PCh), Phosphocreatine (PCr), Phosphoryl Ethanolamine (PE) and Taurine (Tau). 50 mM solutions of these metabolites were dissolved in Phosphate Buffer Solution (PBS) and 5 mM DSS was added as a chemical shift reference. GPC and PCh were dissolved in 100mM NaCl containing 5mM DSS. pH for every phantom was adjusted to 7.20±0.10. The VOI was positioned almost in the middle of the phantom containing the metabolites. The basis set of reference metabolites is shown in Fig.2.

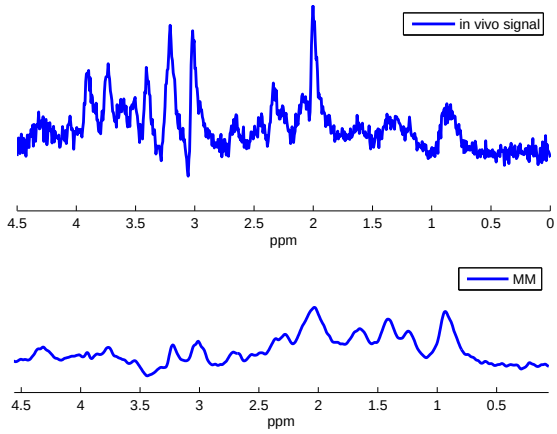


Figure 1. Real part of the spectra of an *in vivo* MRS signal and MM background signal. Top: *in vivo* mouse signal measured at 9.4 T. Acquisition parameters: PRESS sequence, TR=4 s, TE=12 ms, SW=4 KHz and 256 averages in a VOI of size 3x1.75x1.75 mm^3. Bottom: *in vivo* metabolite-nulled signal acquired with inversion recovery and post-processed as described in Section 2.2.1. Acquisition parameters: TI=800 ms, TR=3 s and 1024 averages. The measurement time for the MM and MRS signal was approximately 50 and 15 minutes respectively.

2.2. Methods

2.2.1. Preprocessing MRS signals. Due to an analog-digital filter incorporated in the Bruker system, a time circular shift is necessary. The circular shift consists of removing data points from the beginning and adding them to the end of the signal, thus the data is shifted N points. The time circular shift is performed in the time domain (FID) and is normally used for Bruker data which have been digitally filtered and decimated before saving the FID. The first data points of the FID are initially zero and then increase, alternating in sign, until the actual FID starts. Thus, a shift must be performed before the Fourier Transform [5]. A shift of 68 points (provided in the acquisition parameters of the manufacturer) was applied to all *in vitro* and *in vivo*

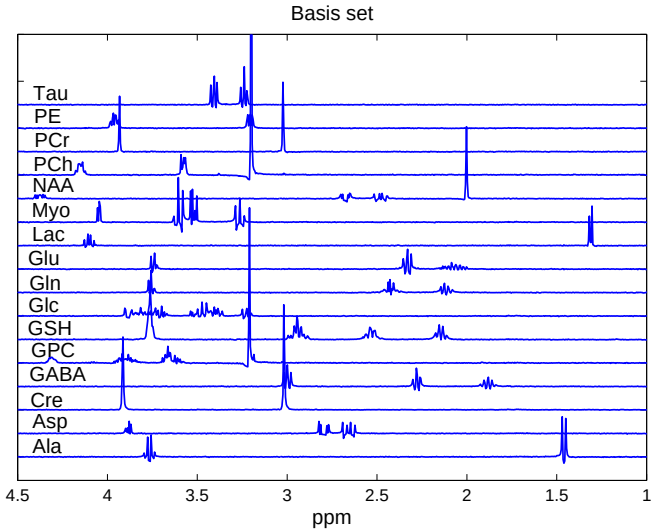


Figure 2. Real part of the spectra of the *in vitro* basis set of metabolites acquired at 9.4 T used for quantification of MRS signals. Metabolites used were: Alanine (Ala), Aspartate (Asp), Creatine (Cre), Gamma-Aminoburytic acid (GABA), Glucose (Glc), Glutamine (Gln), Glutamate (Glu), Glycerolphosphorylcholine (GPC), Glutathione (GSH), Lactate (Lac), Myo-Inositol (m-Ins), N-Acetyl Aspartate (NAA), Phosphorylcholine (PCh), Phosphocreatine (PCr), Phosphoryl Ethanolamine (PE) and Taurine (Tau). Acquisition parameters: PRESS sequence, TR=8 s, TE=20 ms, SW=4 KHz and 64 averages.

signals using the jMRUI software package [29]. Further processing consisted of filtering out the residual water located at 4.7 ppm and for the *in vitro* signals the components corresponding to the DSS and buffer solution. To filter without affecting the metabolite resonances, we applied HLSVD-PRO [16]. MM signals were similarly preprocessed with an additional Lorentzian linebroadening of 15 Hz. Moreover, the remaining resonance at 3.9 ppm corresponding to (Cre + PCr), which is due to fast relaxation, was filtered using HLSVD-PRO.

2.2.2. Quantification. The quantification method used for analyzing the signals was AQSES [20], a time-domain method that estimates metabolite concentrations by fitting a linear combination of metabolite profiles to the experimental data and includes the modeling of the baseline using splines. The basis set of metabolite profiles can be measured *in vitro* or simulated using quantum mechanics [6, 28, 29]. In this study, we used *in vitro* signals and an additional signal (or set of signals) corresponding to MM was added to the basis set for quantification.

Since the baseline is not flat in the water region and the measured MM does not completely take into account all baseline problems, we allowed AQSES to make use of a smooth non-parametric baseline incorporated in the method via penalized splines.

The model used in AQSES to describe the MRS signal under investigation in the

time domain is:

$$y(t) = \sum_{k=1}^{K} a_k e^{(j\phi_k)} e^{(-d_k t + 2\pi j f_k t)} v_k(t) + B(t) + \epsilon(t) \quad (1)$$

where $y(t)$ is the experimental signal, K is the number of metabolites, $v_k(t)$ the metabolite signal k in the basis set, a_k the amplitude, ϕ_k the phase shift, d_k the damping correction, f_k the frequency shift due to B_0 inhomogeneity, pH, temperature or chemical composition of the tissue, $B(t)$ denotes the non-parametric baseline modeled with splines and $\epsilon(t)$ denotes white noise with a certain standard deviation σ.

Baseline estimation. Characterization of the macromolecular contamination was addressed in two ways:

(a) *Measured via inversion recovery.* For each of the 12 mice, an MM signal was acquired, leading to a database of MM signals. An example of a measured macromolecular signal is shown in Fig.4 obtained with TI=800 ms and TR=3 s and pre-processed as described in section 2.2.1. From these signals the major resonances were observed at a frequency location around: 0.89 (MM1), 1.20 (MM2), 1.36 (MM3), 1.63 (MM4), 2.02 (MM5), 2.29 (MM6), 2.65 (MM7), and 3.03 (MM8), 3.21 (MM9), 3.75 (MM10), and 4.31 ppm (MM11). These resonances are in accordance to [3, 18, 24] and extended with the MM7 which appeared to be also present in all individual MM signals. The top curve in Fig.4 shows a prominent peak at 3.92 ppm corresponding to the incompletely suppressed (Cre + PCr) methylene resonance, which was filtered out using the time domain method HLSVD-PRO. All spectra were further apodized by multiplication with an exponential function of Lorentzian type (e^{-xt}) of 15 Hz. Because the MM signal does not account for all baseline issues, the spline baseline from AQSES was used in combination with the MM signal to account for extra baseline contamination. In this case, the smoothing parameter λ from AQSES was set high indicating a very smooth spline function, while in cases when the MM signal is not available, λ is set low to allow a good modelling of lipid and macromolecular resonances.

(b) *Computed using prior knowledge from a database of the inversion recovery signals.* A second approach to estimate the baseline consisted of computing individual lipid and macromolecular peaks using the whole database of MM signals from item (a) as prior knowledge. To this end we used AMARES [31] in jMRUI [29] to compute individual spectral components. From the twelve available MM signals from different mice, the mean of amplitudes, frequency locations and linewidths were used to create the individual macromolecule and lipid resonances that were further included in the basis set of AQSES, see Fig.3. It is important to notice that the model (1) used in AQSES allows small parameter variations to better accommodate individual conditions of each signal under analysis. The simulated MM components are also subject to such corrections. Moreover, the lineshape

estimation combined with AQSES (2) also fits a flexible common lineshape to all these components.

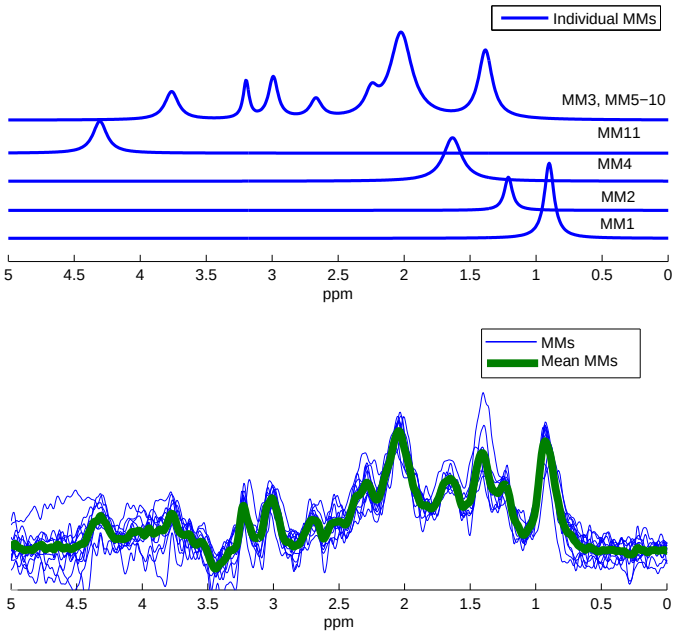


Figure 3. Real part of the MM spectrum obtained with AMARES using prior knowledge from the measured inversion recovery signals. Top: individual MMs or combination of MMs simulated based on the mean of the AMARES estimates of all MM signals. Small variations are expected for different signals which are then corrected by AQSES. Bottom: All MM spectra, which show the MM locations and variability of data.

Lineshape estimation. Due to unavoidable spectral distortions that can not always be corrected with advanced shimming techniques, lineshape corrections of ^{1}H MRS signals measured at high magnetic field are necessary. In this study, we include in the algorithm the case when the lineshape is distorted and has a profile differing from the ideal Lorentzian shapes. Within one measurement the same lineshape is assumed for all components; thus, for taking into account lineshape distortions in the quantification method, we consider a common decay for all spectral components in the model of AQSES, with the exception of the macromolecular signal measured *in vivo*. Note that the contribution of local inhomogeneity on the already fast decay of B(t) is only marginal. Moreover the measured MM has the same properties as the signal when measured in the same location and therefore it is not necessary to correct its lineshape. The exponential decays $e^{(-d_k t)}$ in (1) are then replaced by the common factor $g(t)$, of arbitrary shape, resulting in:

In (a) the model is:

$$y(t) = g(t) \sum_{k=1}^{K} a_k e^{(j\phi_k)} e^{(2\pi j f_k t)} v_k(t) + \tilde{a} e^{(j\tilde{\phi})} e^{(2\pi j \tilde{f} t)} MM(t) + B(t) + \epsilon(t) \qquad (2)$$

and $g(t)$ is estimated as:

$$g(t) = \frac{y(t) - \tilde{a}e^{(j\tilde{\phi})}e^{(2\pi j\tilde{f}t)}MM(t) - B(t)}{\sum_{k=1}^{K} a_k e^{(j\phi_k)}e^{(2\pi j f_k t)}v_k(t)} \tag{3}$$

In (b) the model is:

$$y(t) = g(t)\left(\sum_{k=1}^{K} a_k e^{(j\phi_k)}e^{(2\pi j f_k t)}v_k(t) + \sum_{i=1}^{m} \tilde{a}_i e^{(j\tilde{\phi}_i)}e^{(2\pi j\tilde{f}_i t)}MM_i(t)\right) + B(t) + \epsilon(t) \tag{4}$$

and $g(t)$ is estimated as:

$$g(t) = \frac{y(t) - B(t)}{\sum_{k=1}^{K} a_k e^{(j\phi_k)}e^{(2\pi j f_k t)}v_k(t) + \sum_{i=1}^{m} \tilde{a}_i e^{(j\tilde{\phi}_i)}e^{(2\pi j\tilde{f}_i t)}MM_i(t)} \tag{5}$$

where in the numerator $y(t)$ is the experimental signal and $B(t)$ is the non-parametric baseline from the previous iteration, MM is either (a) measured or (b) modeled using m profiles; and in the denominator K is the number of metabolites, $v_k(t)$ the metabolite signal k in the basis set, and the amplitudes a_k, $\tilde{a}_i$, frequency shifts f_k,$\tilde{f}_i$ and phase shift ϕ_k,$\tilde{\phi}_i$ are estimated from AQSES computation in the previous iteration. Thus, $g(t)$ can be estimated at the same time as the spectral parameters (a_k and f_k) using an iterative method similar to the methods described in [17, 26].

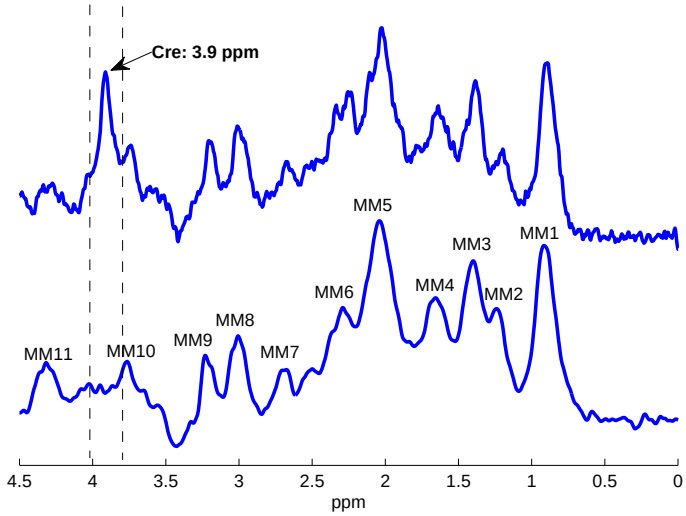


Figure 4. Real part of the mean of *in vivo* metabolite-nulled MM spectra acquired at 9.4 T, TE=12 ms, TI=800 ms, TR=3 s and 1024 averages. Spectra were post-processed with a 15 Hz Lorentzian linebroadening. Top: Due to a shorter T1, the marked (Cre + PCr) resonance at 3.9 ppm was not completely minimized, therefore this peak was filtered out using HLSVD-PRO. Macromolecular resonances are labeled by MM from 1 until 11 at the following central frequencies: 0.89, 1.20, 1.36, 1.63, 2.02, 2.29, 2.65, 3.03, 3.21, 3.75, 4.31 ppm. Bottom: Mean of filtered MM signals.

3. Results

3.1. Lineshape.

Satisfactory results were obtained when using AQSES with lineshape estimation, in particular for those signals that have a noticeable lineshape distortion. Fig.5 shows the fits with and without lineshape estimation for one of the mouse signals. These lineshape distortions are unavoidable and it is recommendable to correct them in order to improve quantification and thus metabolite estimates. Due to the fact that the model in AQSES assumes that spectral components have a Lorentzian shape, the quantification with the conventional AQSES presents some problems which can be observed at the lineshape of the spectral components (especially visible at around 3 ppm). By allowing a free lineshape model in AQSES, $g(t)$ is adjusted to fit such non-ideal shapes.

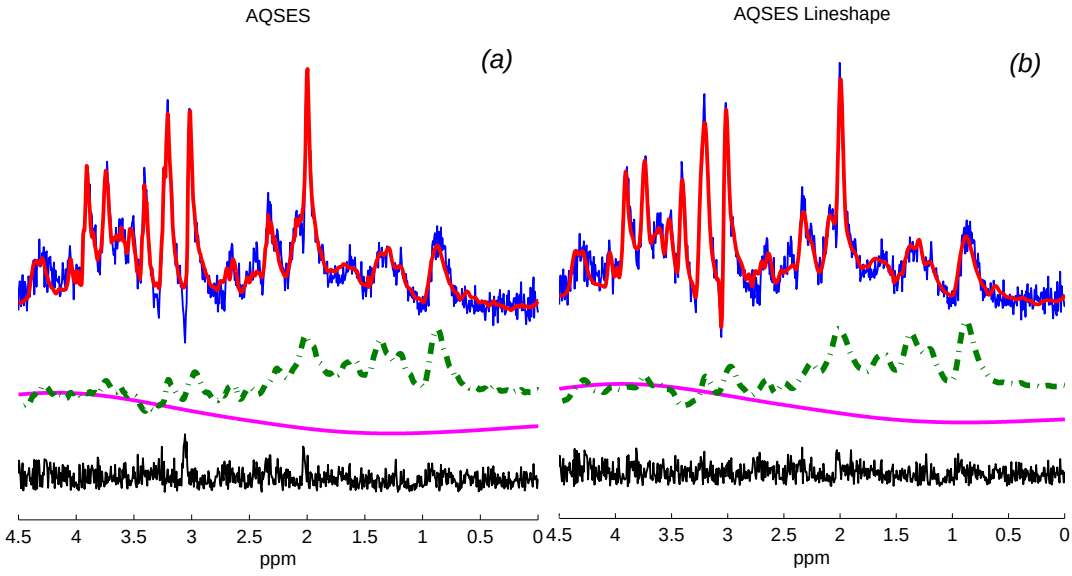


Figure 5. AQSES fit for one of the short TE ^{1}H MRS *in vivo* signals from mouse brains with lineshape distortion. Real part of the *in vivo* spectrum (noisy signal) together with the fit using AQSES (bold line on top of signal), MM measured signal (dotted curve below the signal), spline baseline (smooth curve below the MM) and the residual (curve beneath). This quantification was made including the measured macromolecular signal in the basis set and the splines function to fit any additional baseline.

3.2. Baseline.

Table 1 shows the results of macromolecule fitting using AMARES. Macromolecule resonances are simulated independently, however, to avoid mis-quantification as suggested in [24], MMs overlapping with metabolites were combined in a single spectrum to fix the ratio between those resonances. We have tested the case when

Table 1. Results from AMARES for the mean of amplitudes, frequencies and linewidths obtained from the fit of all macromolecular signals

Components	Position (ppm) ± SD	Linewidth (Hz) ± SD	Amplitude/MM1 ± SD
MM1	0.901 ± 0.007	109.377 ± 26.529	1.000
MM2	1.210 ± 0.000	89.681 ± 26.959	0.363 ± 0.123
MM3	1.384 ± 0.010	156.800 ± 36.492	1.301 ± 0.799
MM4	1.633 ± 0.022	211.219 ± 37.678	1.119 ± 0.722
MM5	2.023 ± 0.008	248.888 ± 27.485	2.601 ± 0.892
MM6	2.247 ± 0.012	145.477 ± 107.953	0.394 ± 0.333
MM7	2.668 ± 0.013	132.942 ± 70.944	0.309 ± 0.301
MM8	2.992 ± 0.020	107.262 ± 34.717	0.543 ± 0.369
MM9	3.198 ± 0.017	66.016 ± 41.417	0.299 ± 0.286
MM10	3.762 ± 0.032	154.517 ± 84.011	0.530 ± 0.475
MM11	4.306 ± 0.028	161.256 ± 102.208	0.625 ± 0.737

the macromolecules are let free, thus frequencies, amplitudes and dampings can vary independently. This resulted in MMs fitting metabolites peaks (*e.g.* NAA) leading to mis-quantification. To avoid this problem, we combined MM3 with MM5-MM11 to obtain a single profile containing these seven resonances located in the region between 2 ppm - 4.1 ppm where all important metabolites are resonating.

Fig.6 shows the results of fitting the mean of spectra using both approaches: *a*) the mean of measured MMs signals (1 MM profile) and *b*) the mean of individual MMs obtained with AMARES (5 MM profiles). The corresponding fitting of the MMs is shown to demonstrate that both estimations reflect similar MM contributions. Moreover, in Fig.7 we present the amplitude estimates for the corresponding fittings, which confirm that using both approaches we can obtain similar estimations for the metabolites of interest with either of the two approaches. The goodness of the fit is reflected in the small residuals and in plausible amplitude estimates with acceptable Cramér-Rao Lower Bounds, *i.e.*, below 25%. The present implementation of AQSES Lineshape contains the over-optimistic Cramér-Rao bounds computed under the assumption that the final $g(t)$ is the true lineshape of the model. These results indicate that the simulated MMs contained all relevant lipid and macromolecular spectral contributions and quantification results approximated well those obtained when measuring an inversion recovery signal.

4. Discussion

In this study we utilized a data set of 12 signals measured in the hippocampus of mouse brains, which is a rather problematic region for achieving good homogeneity of the magnetic field using shimming techniques, in animals as well as in human brain [14]. This implies that lineshape distortions are likely to occur in such signals, justifying the development of lineshape estimation methods, such as those proposed

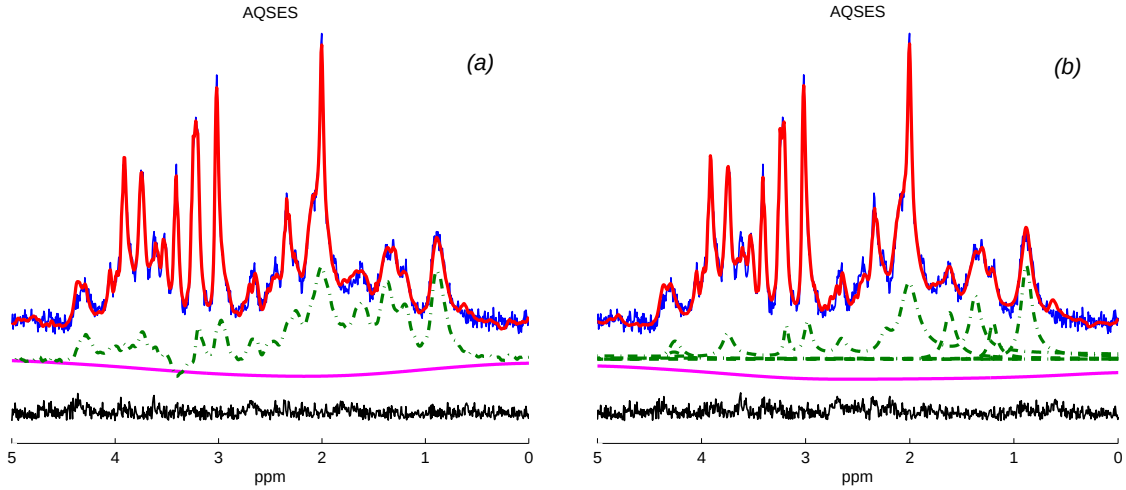


Figure 6. AQSES fit for the mean spectra of short TE ^{1}H MRS *in vivo* from mouse brains. Real part of the *in vivo* spectrum (noisy signal) together with the fit using AQSES (bold line on top of signal), MM (dotted curve below the signal), spline baseline (smooth curve below the MM signal) and the residual (curve beneath). This quantification was made including the MM signal (measured (*a*) and simulated (*b*)) in the basis set and the splines function to fit any additional baseline.

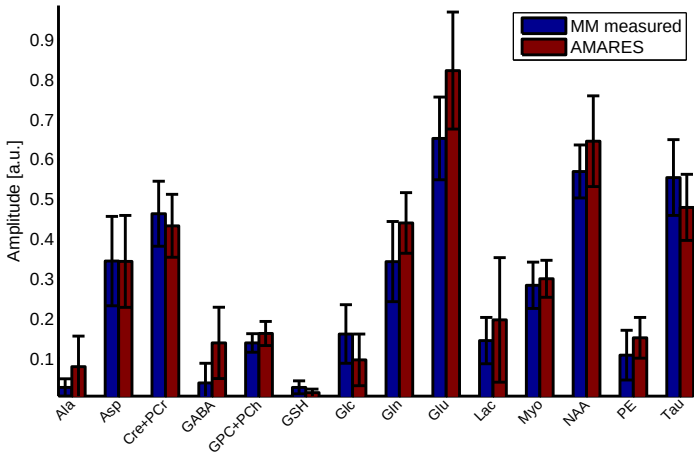


Figure 7. Amplitude estimates of metabolites and corresponding Cramér-Rao lower bound quantified using AQSES with: *a*) the measured and *b*) simulated set of MMs (obtained from AMARES) to fit the macromolecular baseline.

in [1, 8, 19, 21, 26, 27]. In particular, the self-deconvolution method proposed in [26] has been originally developed under the assumption that the signal under investigation is free of baseline contaminations. Here we have further extended this method by allowing it to be combined either with measured macromolecular background signals, or with a set of simulated macromolecular components. There is an important difference in how the two approaches have been combined with lineshape estimation. In the first case, the measured MM signal is measured in the same location and it is influenced by the same tissue heterogeneities as the analyzed MRS signal. In this case, this MM signal has been included in the model (2) without multiplying it with the common decay $g(t)$. In the second case, the modeled individual MM components have an ideal shape, and should be corrected with the common decay $g(t)$.

Although the MM signal acquired by inversion recovery is known to provide a good approximation of the macromolecular contamination, it is also true that it requires a long acquisition time and is not reproducible when the conditions of the region of interest are affected by acquisition problems and various diseases. Moreover, it also contains some unsuppressed metabolites; thus this part of metabolite contributions are attributed to the baseline, those metabolites will be underestimated by the model fitting algorithm. A promising new acquisition technique based on diffusion weighted spectroscopy has recently been proposed [15], and was shown to be less contaminated by unsuppressed metabolites.

In order to build up prior knowledge about the MM contributions in mouse hippocampus we extracted eleven peaks from the *in vivo* ^{1}H MRS spectra measured with inversion recovery at 9.4 T corresponding to prominent resonances of macromolecules and lipids. The quantification method AMARES has been used to compute the MM parameters (amplitudes, frequencies and linewidths) from all available inversion recovery signals. The mean of those estimates shown in Table 1 was then used to create individual MMs to be included in the basis set. This prior knowledge contains essential information about the MM of healthy mice which provides spectral parameters close to the MM of *in vivo* signals. Such information can be further used for obtaining the MM of mouse brains under similar measurement and health conditions. Quantification of the MM spectra in AMARES using both Gaussian and Lorentzian shapes has been evaluated. Results show only small differences although the shapes of the peaks were not identical in all signals. Thus, for some signals the resonance at 0.89 ppm was better fitted with a Gaussian shape, while most of the peaks between 2 ppm and 4.3 ppm were better fitted with a Lorentzian shape. Moreover, because we also include a method to estimate the lineshape, the shape of all individual metabolites and MM profiles will also be corrected according to the shape of the *in vivo* signal (*i*.e. $g(t)$ will also correct MM profiles). Using this approach, the shape of the individual MMs will not play an important role, but good prior knowledge about approximate values for frequency locations, amplitudes and linewidths will be beneficial for AQSES.

Due to low SNR of the individual MM signals, all MM components could not always be clearly localized and therefore some variability has been observed. In *in vivo* MRS

signals, this variability in the data is also expected, therefore the quantification method AQSES allows small variations in damping and frequency which finally corrects this difficulty for all metabolites and in this case also for the individual MMs which are added to the basis set of metabolites.

Several papers have been focused on comparing inversion recovery macromolecular baselines with mathematical models for background accommodation. At lower magnetic fields (human MR scanner at 3 T) it has been shown that using Subtract-QUEST [22] gave similar quantification results in terms of metabolite concentrations, but had lower precision than using measured macromolecular signals [10]. In [7], the spline fitting of LCModel [21] resulted in a smooth approximation of the *in vivo* macromolecules, and could not reproduce completely all features of the *in vivo* spectrum of macromolecules at 14.1 T. However, when the knot spacing is reduced beyond the default value, the LCModel spline model is more flexible to follow rather closely the measured MM at 9.4 T, as illustrated in [18].

In this study, metabolite amplitude estimations shown in Fig.7 indicate that small differences are obtained when using a measured or a simulated MM signal, but these differences are not statistically significant. Note that a data base of metabolites is hardly complete as only metabolites of highest concentration are included in the data base.

At high magnetic field the macromolecular contamination plays an important role; however, even at 1.5 T the resonances of lipids and macromolecules affect the reliable estimation of the relevant metabolites. Therefore, this macromolecular estimation is essential in the quantification of metabolites in *in vivo* ^{1}H MRS spectra of human brain. The location and characterization of all individual MMs and lipids in mice and human brain has been studied previously at various magnetic field strengths, *e.g.*, at 8.4 T [3] and 1.5 T [24]. Resonances similar to those observed in the mouse brains have been observed in the *in vivo* ^{1}H MRS spectra of human brain [10, 12, 24]. However, we decisively had to include 11 compared to a lower number (8-10) macromolecular components [18,24], due to a pronounced peak at 2.66 ppm. When analyzing the values provided in Table 1, we notice that the variability of the central frequency locations and of the estimated linewidths is relatively low. A similar table is provided by [24] for the case of MRS signals measured at 1.5 T; note however that the difference in magnetic fields translates to a different scale for the linewidth values, since these are reported in Hz.

5. Conclusion

In this study we evaluated the quantification of *in vivo* MRS mouse brain signals simultaneously perturbed by a macromolecular baseline and by an arbitrary, unknown lineshape distortion due to inhomogeneity of the static magnetic field B_0. We used an extension of the quantification method AQSES, which optimally exploits prior knowledge and can handle both perturbations. Moreover, the lineshape handling has been automated. The latter capability is crucial when processing a series of signals, not

all of which have a distorted lineshape.

One important aspect to consider is the fact that metabolites might be underestimated, depending on *a*) the measurement conditions for the case when MM is obtained *in vivo* or *b*) setting the AMARES parameters for the case when the MM components are computed via modelling. We have shown that lipid contamination becomes more important at high magnetic field and that adequate prior knowledge on lipids is beneficial for improving quantification.

Finally, we address the fact that good starting values and prior knowledge are advantageous for the quantification methods, providing a strong motivation for using simulated macromolecular and lipid components that should be appropriate for specific measurement and experimental conditions.

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