

## Two-dimensional fitting for *in vivo* NMR spectra quantification

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**Introduction:** NMR 2D spectroscopy is a powerful and versatile approach to correlate signals arising from different positions within a molecule. Experiments based on this type of spectroscopy are commonly used in *in-vitro* NMR applications, providing information such as connectivity [1] and molecular distance [2]. The indirect dimensions are encoded by incrementing one or more evolution periods and acquiring the signal repetitively by multiple excitations. In *in vivo* applications this technique can improve unambiguous identification of resonances in spectra with signal overlap that may arise from line broadening. However, the use of multi-dimensional spectroscopy is less widespread in *in vivo* applications and post-processing techniques are still not as sophisticated as they are for 1D-spectroscopy. The present work is based on the development and implementation of a software package that makes it possible to process and quantify 2D spectroscopic data obtained in *in vivo* experiments. The strategy on which the data analysis is based, involves the combined use of the chemical shift and the signal modulation arising from homonuclear *j*-coupling. This is a particularly powerful way to distinguish different resonances, because these two parameters make it possible to determine the underlying spin system from which the signal arises [3]. Currently, LcModel [4] is the most widely used software for metabolite quantification in *in vivo* magnetic resonance spectroscopy. Based on the data fitting strategy of LcModel and a recent 2D fitting technique [4], our software aims to fit a real 2D spectrum by using a linear combination of 2D model spectra of individual metabolites to provide absolute metabolite concentrations and their respective  $T_2$  values in a reasonable processing time.

**Methods:** This fitting program uses a basis data set that is either acquired using standard solutions in phantom samples or obtained from simulations. In a first approach we have generated a basis data set by simulations since this strategy is the least time consuming one. In addition, the simulated data set is not affected by signal attenuation due to relaxation process and by eddy current distortions. The signal evolution of six metabolites [NAA, Cho, Cre, Glu, Lac, mIns] was calculated using Spinevolution [5]. For each metabolite 73 PRESS (Point RESolved Spectroscopy) experiments were simulated with bandwidth=2500 Hz, number of points=2048 and echo times ranging from 36 to 180 ms in 2 ms increments. To match the performance of our whole body scanner, the TE values in the PRESS simulation were changed by keeping the time delay  $TE_1$  between the  $\pi/2$  and the first  $\pi$  pulse fixed at 10 ms and by varying only the time delay  $TE_2$ . Using this basis set the experimental spectrum of a mixture of metabolites is fitted taking into account further physical parameters. These are: apodization ( $\epsilon_{m,2}$ ),  $T_2$  decay ( $T_2^m$ ), concentrations ( $C_m$ ), shifts in both dimensions ( $\gamma_{m,2}$ ,  $\gamma_{m,1}$ ), phase distortions due to eddy currents ( $\Phi(t_2)$ ) where the distortion model was truncated at  $n=2$ . The software uses the gradient descendental method and minimizes the difference function ( $\Delta(\omega_1, \omega_2)$ ) between the model data and the real data.

$$\phi(t_2) = \left[ \sum_n \sum_{m=1}^{N_M} \Omega_m(n) e^{-\frac{t_2}{T_2^m}} \right] \quad \hat{\psi}(t_1, t_2) = \exp[-i(\phi_0)] \left[ \sum_{m=1}^{N_M} C_m \widehat{M_m}(t_2, t_1; \gamma_{m,2}, \gamma_{m,1}, \epsilon_{m,2}, e^{-\frac{t_2}{T_2^m}}) \exp[-i(\phi(t_2))] \right] \quad \Delta(\omega_1, \omega_2) = \sum_{\omega_1} \sum_{\omega_2} (\hat{\psi}(\omega_1, \omega_2) - \psi_R(\omega_1, \omega_2))^2 + \text{regularizers}$$

In order not to compromise the  $T_2$  values and the concentrations obtained, no terms were used to regularize them. Nevertheless, secondary parameters such as apodizations, phases and shifts, were included into the regularizers. Before the model starts the 2D fitting algorithm, only a few 1D spectra are utilized to recover the seed values for the subsequent 2D fitting routine. This avoids the problem of initializing the starting values for the most prominent resonances.

Finally, the software fits the water signal and computes its concentration value. Such computation is achieved through a separate simulation for the water, instead of calculating mathematically its area. The estimate is used to scale the metabolite concentrations and to obtain absolute concentration values. The software package is implemented using MATLAB.

**Materials:** 2D spectra were acquired on a 3T Philips Achieva scanner using a SENSE coil. A J-PRESS (TE=36-180 ms, 2 ms steps) sequence was exploited (TR=3 s; BW=2500 Hz; NSA=16; Number of Points=2048) to scan a General Electric brain metabolite phantom (model 2152220). The data were phased with jMRUI package (3.2 version). Suppressed and unsuppressed water spectra were only phased to zero order.

**Results:** The robustness of the fitting strategy was tested by analyzing one data set several times using different initialization parameters. The fitting routine converged to the same final concentrations independently to the choice of the initialization values. A representative run of the fitting program requires currently 90min. The final value of the concentrations was obtained by scaling to the water concentration value according to the formula:

$$[metabo] = \left( \frac{C_{metabo}}{C_{water}} \right) \times [water]$$

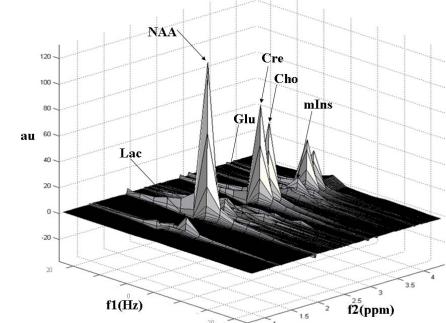
where the values in bracket are the millimolar concentrations. Our results show a good accordance with the  $T_2$  values reported in the literature and in the GE phantom information sheet. In addition, the concentration values are very good for the NAA, Cho, Cre and mIns. The Glu and Lac concentrations are slightly biased, probably because of localization effects not included into the simulations [6]. The goal of the approach is providing an absolute concentration of the metabolites and in addition correcting them by  $T_2$  relaxation.

**Conclusions:** We propose a new 2D fitting software that allows to simultaneously determinate both the absolute concentrations and the  $T_2$  values of all major cerebral metabolites as successfully demonstrated in a brain phantom. The extension to further metabolites is straightforward however with the penalty of increasing the processing time. Further improvements are planned by use of C++ in some subroutines to speed up the computation but without changing the main structure of the software package. Localization effects can also be included into the simulations to better match the real spectra shape. Furthermore, to make the fitting strategy useful for *in vivo* data, a correction for macromolecules and baseline distortions will be implemented following the same approach as used for LcModel. An optimisation of the numbers of eddy current phase distortion terms will also be performed.

**Bibliography:** 1 Mol. Phys., (1980); 41:95, 2. Magn. Reson. Med., (2001); 46:855-63, 3. NMR Biomed. (2006); 19: 255-263, 4. Magn. Reson. Med., (1993); 30:672-679, 5. J. Magn. Reson. (2006); 178: 248-282, 6. J. Magn. Reson. (2008); 195:67-75, 7. Clinical Neurol. and Neuros. (2005); 107:379-384, 8. J. Korean Soc. Magn. Reson. Med. (2008); 12(1):20-26.

	Determined conc.(mM)	Conc. Phantom (mM)*	Determined. $T_2$ (ms)	Literature $T_2$ (ms)	Ref. for $T_2$
<b>NAA</b>	12.7±0.4	12.5	452±80	468±74	[8]
<b>Cho(3.18ppm)</b>	3.2±0.4	3.0	177±66	182±44	[8]
<b>Cre(3.02ppm)</b>	10.5±0.3	10.0	296±51	275±57	[8]
<b>Glu(~2.3ppm)</b>	9.8±1.5	12.5	139±21	-	
<b>Lac(1.3ppm)</b>	2.9±0.4	5.0	468±187	-	[7]**
<b>mIns(-3.5ppm)</b>	7.5±1.0	7.5	65±17	75	*

**Table1:** Calculated and theoretical values for concentration and  $T_2$  for the 6 metabolites.\*The value shown is the one reported in the GE phantom information sheet.\*\*The values are *in vivo*.



**Figure1:** Typical example of a 2D fitted spectrum, where the arbitrary units (au) on the z axis are the values before the water scaling.