Quantitation and Classification of HR-MAS Brain Biopsy Tissue Spectra

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INTRODUCTION: *Ex-vivo* high resolution magic angle spinning (HR-MAS) NMR spectroscopy might help for brain tumor diagnosis. Quantitation of HR-MAS spectra is particularly challenging due to the inherent complexity of such spectra (lot of overlapping peaks). However, in order to use as much information as possible, it is necessary to disentangle the metabolite contributions, which is not feasible with a method such as "peak integration". In this study, we propose a full procedure (preprocessing, quantitation, classification) for classifying HR-MAS spectra.

METHODS: Brain tumor biopsies were obtained from 46 patients and stored at -80°C until use. The biopsies were gathered in 5 brain tumor classes: 17 glioblastoma (GBM), 4 Glioma grade III (GIII), 6 Glioma grade II (GII), 8 metastasis (MET) and 11meningioma (MEN). HR-MAS data were acquired at 11.7 T (500 MHz for ^{1}H) using a BRUKER Analytik GmbH spectrometer. The 1D "presat" spectra were normalized (divided by the norm of the frequency domain signal between 0.25 and 4.2 ppm), aligned and corrected for the baseline (by subtracting the product of the signal and an apodization function). Quantitation was performed with AQSES [1] which uses a basis set of metabolite profiles like QUEST [2]. The 16 metabolite profiles (Acetate, Alanine, Aspartate, Choline, Creatine, Glutamate, Glutamine, Glycine, Glycerophosphocholine, Lactate, Myo-Inositol, N-Acetylaspartate, Phosphocreatine, Phosphorylcholine, Succinate and Taurine) of the simulated basis set were quantum mechanically simulated using the method described in [3]. The estimated amplitudes were then used in a least-squares support vector machine (LS-SVM) classifier [4]. The leave-one-out (LOO) method was used for classifier validation. The classifier results obtained with AQSES as processing method were compared with those obtained with 'peak integration' (using the same preprocessings) where the integrals were calculated in the frequency intervals [1.46 1.5], [1.85 1.95], [2.01 2.05], [2.09 2.13], [2.22 2.32], [3.01 3.05], [3.18 3.2], [3.2 3.23], [3.234 3.244], [3.4 3.44], [3.52 3.56], [3.74 3.78], [3.91 3.95], [4.08 4.16] ppm. The lipids at 0.9 ppm and 1.3 ppm were not included in the basis set or in the frequency intervals since their contributions were strongly attenuated due to the 'baseline' correction.

RESULTS: To illustrate the results of the quantitation step, the filtered original and filtered estimated spectra (GIII) are plotted in Figure 1 (Bottom). The residue is displayed in the top plot. The fatty acids in the frequency region around 0.9 ppm are not completely removed with the baseline correction.
Systematic residuals were obtained in the choline compound frequency region, which suggests that there is a slight mismatch in the resonance pattern of the basis and ex vivo spectra.
The classification results are reported in Table 1 for the different pairs of brain tumors. Using AQSES as processing method (top row), most of the binary classifiers provide good results (larger than 85 % of correctly classified data). Discrimating between GBM and MET, and between GIII and GII turns out to be more complicated. The results obtained with 'peak integration' are worst than with AQSES except for classifying GIII vs GII, where the number of samples (or data) may be too small for drawing conclusions.

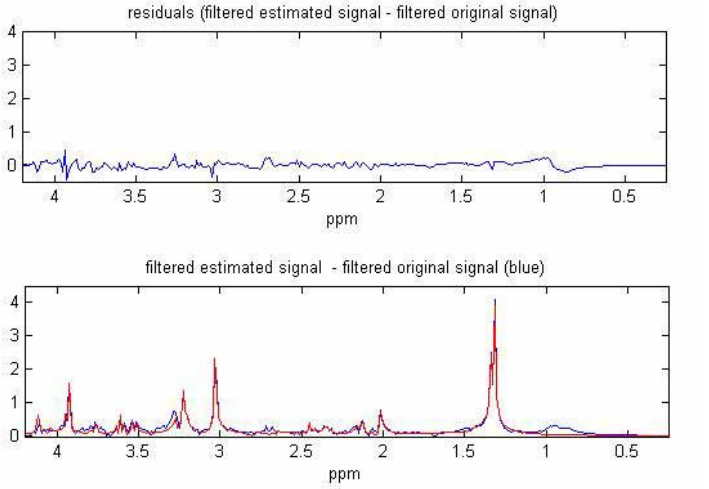

Figure 1: AQSES quantitation results. Top: real part of the residue (filtered estimated signal-filtered original signal). Bottom: Filtered estimated signal (in red) and filtered original signal in absolute values.

CONCLUSIONS: A full procedure for quantifying and classifying HR-MAS data has been proposed. More advanced processing methods like AQSES are recommendable in comparison with the common 'peak integration' method.

ACKNOWLEDGMENTS: eTUMOUR (FP6-2002-LIFESCIHEALTH 503094), Healthagents (FP6-2005-IST 027214)

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GIII vs GBM	GIII vs MEN	GIII vs MET	GIII vs GII	GBM vs MEN	GBM vs MET	GBM vs GII	MEN vs MET	MEN vs GII	MET vs GII
~86 % (18 out of 21)	100 % (15 out of 15)	~92 % (11 out of 12)	70 % (7 out of 10)	~96 % (27 out of 28)	84 % (21 out of 25)	~96 % (22 out of 23)	~95 % (18 out of 19)	~88 % (15 out of 17)	~86 % (12 out of 14)
~81 % (17 out of 21)	~73 % (11 out of 15)	~83 % (10 out of 12)	80 % (8 out of 10)	75 % (21 out of 28)	80 % (20 out of 25)	~78 % (18 out of 23)	~79 % (15 out of 19)	~76 % (13 out of 17)	~71 % (10 out of 14)

Table 1: Percentage of correctly classified data for each possible brain tumor pair. Top row: results with AQSES. Bottom row: results with 'peak integration'.