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Quantification and classification of high-resolution magic angle spinning data for brain tumor diagnosis

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***Abstract*— The goal of this work is to propose a complete protocol (preprocessing, processing and classification) for classifying brain tumors with proton high-resolution magic-angle spinning (^{1}H HR-MAS) data. The different steps of the procedure are detailed and discussed. Feature extraction techniques such as peak integration, including also the automated quantitation method AQSES, were combined with linear (LDA) and non-linear (least-squares support vector machine or LS-SVM) classifiers. Classification accuracy was assessed using a stratified random sampling scheme. The results suggest that LS-SVM performs better than LDA while AQSES performs better than the standard peak integration feature extraction method.**

I. INTRODUCTION

Ex-vivo high resolution magic angle spinning (HR-MAS) magnetic resonance (MR) spectroscopy might help for brain tumor diagnosis. Cheng *et al.* [1] demonstrated that proton magnetic resonance spectroscopy (^{1}H MRS) with high-resolution magic-angle spinning (HR-MAS), a technology introduced in 1997, can preserve tissue histopathology features while producing well-resolved spectra of cellular metabolites. Tugnoli *et al.* [2] and Martinez-Bisbal *et al.* [3] showed that HR-MAS is very helpful for the assignment of the resonances *in-vivo* of human meningiomas and glioblastomas and for the validation of the quantitation procedure of *in-vivo* MR spectra. They concluded that HR-MAS MRS is a complementary method to strongly support *in-vivo* MR spectroscopy and increase its clinical potentiality.

In this paper, we propose a complete procedure to diagnose brain tumors using HR-MAS data. To our knowledge nothing similar has been achieved in the literature. This procedure contains three main parts: preprocessing, processing and classification of the data. The preprocessing methods are used to clean the spectra from the unwanted underlying components such as the residual water resonances or the macromolecular baseline contributions. They also ensure that the spectra are standardized so that we can compare them. The purpose of the processing techniques is to extract relevant features from the filtered spectra, which will then be used as inputs in the binary classifier.

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II. MATERIALS AND METHODS

Brain tumor biopsies were obtained from 59 patients and stored at -80C until use. The biopsies were gathered in 4 brain tumor classes: 22 glioblastomas (GBM), 21 grade II and grade III gliomas (GLI), 7 metastasis (MET) and 9 meningiomas (MEN). 1D PRESAT (pulse-and-acquire) data were acquired at 11.7 T (500 MHz for ^{1}H) at 0-4 ^{o}C and 4,000 Hz spinnning rate using BRUKER Analytik GmbH spectrometers.

By looking at the linewidth and SNR differences between *in-vivo* and *ex-vivo* spectra (see Fig. 1), we may reasonably think that different strategies are required to (pre)process the data. Quantitation of HR-MAS spectra is particularly challenging due to the inherent complexity of such spectra (a large amount of peaks that may overlap). However, HR-MAS spectra present much higher signal-to-noise ratios than *in-vivo* MR spectra and the macromolecular contribution is not spread over the whole spectrum in the region of interest, but bumps due to fatty acids appear instead in several well-known frequency regions (mainly around 0.9, 1.3 and 2.05 ppm). Furthermore, eddy current effects are negligible in HR-MAS spectra simplifying the preprocessing.

The water components were removed by HLSVD-PRO [4]. The filtered 1D "presat" signals were normalized (divided by the L2 norm of the frequency domain signal between 0.25 and 4.2 ppm), aligned with respect to the Alanine doublet at 1.47 ppm, and corrected for the baseline (by subtracting the product of the signal and an apodization function) to obtain the preprocessed signals.

We investigated different processing or feature selection methods: whole spectrum in the region of interest, peak integration and an automated quantitation method designed for short-echo time MR spectra (AQSES) (see [5] and references therein).

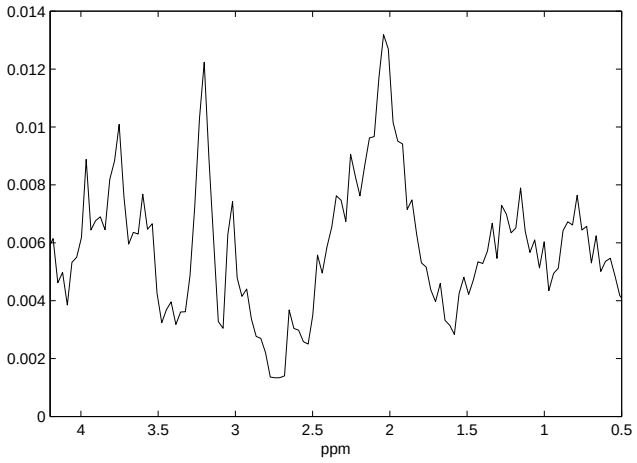
The whole spectrum in the region of interest

Every point of the magnitude spectra in the region of interest (defined as the frequency interval [0.25 4.2] ppm) is considered as an individual feature.

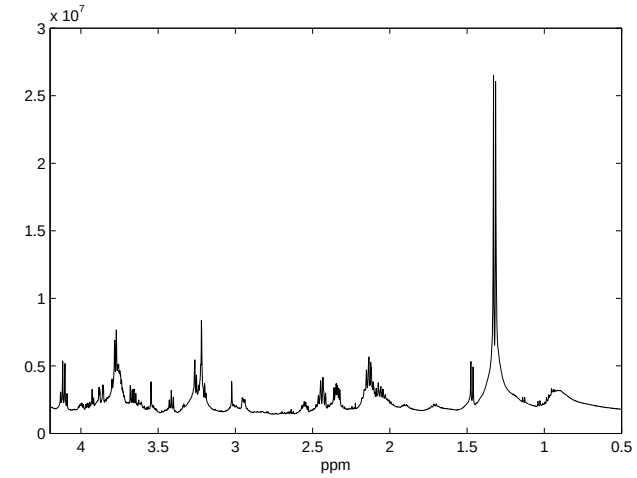
Peak integration

18 peak integrated values are extracted from each magnitude

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(a) *In-vivo*



(b) *Ex-vivo*

Fig. 1. Magnitude (arbitrary units) *in-vivo* and *ex-vivo* spectra in the region of interest from the same meningioma patient.

spectrum in the following intervals in ppm: [1.45 1.47], [1.88 1.94], [1.99 2.03], [2.1 2.14], [2.22 2.32], [2.39 2.49], [3.01 3.05], [3.19 3.21], [3.21 3.23], [3.23 3.25], [3.255 3.275], [3.4 3.44], [3.52 3.56], [3.58 3.64], [3.75 3.79], [3.91 3.95], [4.03 4.07], [4.095 4.155]. This method, largely used in *in-vivo* MRS, is less sensitive to the noise and misalignment issues than the previous one.

AQSES

AQSES is a time domain quantitation method originally designed for short-echo time *in-vivo* MR spectra. The quantitation problem is mathematically formulated as a separable nonlinear least-squares fitting problem, which is numerically solved using a modified variable-projection procedure. The parameters of interest, *i.e.* the metabolite amplitudes, are the weighting coefficients of a linear combination of 18 simulated metabolite profiles constituting the basis set (see Fig. 2): Acetate (Ace), Alanine (Ala), Aspartate (Asp), Choline (Cho), Creatine (Cr), Glucose-α (Glc-α), Glucose-β (Glc-β), Glutamate (Glu), Glutamine (Gln), Glycine (Gly), Glycerophosphocholine (GPCh), Lactate (Lac), myo-Inositol (mI), N-Acetylaspartate (NAA), Phosphocreatine (PCr), Phosphocholine (PCh), Succinate (Succ) and Taurine (Tau). These were quantum mechanically simulated using the method described in [6]. Two methylene resonances were filtered out from PCh and GPCh to reduce the number of overlapping peaks around 3.65 ppm. The phases of the metabolite profiles are imposed to be equal, damping corrections and frequency shifts are free. The main advantage of this method with respect to the previous ones is to allow to disentangle overlapping metabolites. In most of the selected regions, peak integration will integrate the contribution of several metabolites together, loosing thereby information about the individual metabolites.

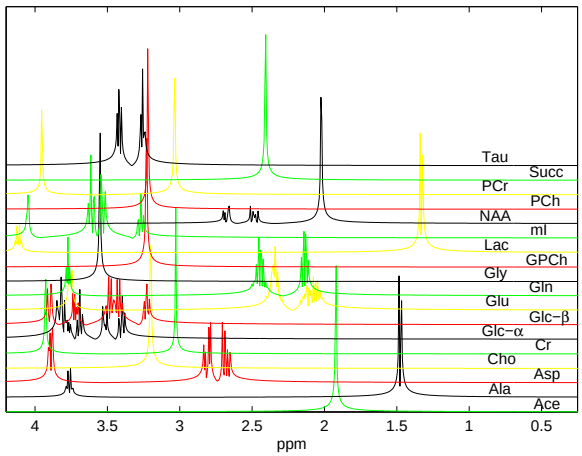


Fig. 2. Basis set of metabolite profiles used in AQSES (magnitude spectra).

Two supervised learning methods are used for classification: the classical linear discriminant analysis (**LDA**) and the least-squares support vector machine (**LS-SVM**) [7], a kernel-based method able to handle high dimensional input vectors. For each pair of classes, the feature extraction and classification methods are compared using stratified random sampling. The data obtained after processing the signals are 100 times randomly split in a stratified way in a set for training and validation (*i.e.* 2/3 of the data), and one for testing (*i.e.* 1/3 of the data). Classification accuracy is given for each pair of classes and each procedure.

III. Results

A representative fit obtained with AQSES is given as illustration in Fig. 3. The preprocessed spectrum (in black) is the result of the preprocessings applied to the signal plotted in Fig. 1(b). We observe a good fit in the frequency region between 1 and 3 ppm (Cr peak included) while it presents more residuals from 3 to 4 ppm. The larger residuals around 3.6 ppm are probably due to the low amplitude estimate of mI, which can be explained by the fact that the contribution

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of mI at 4.05 ppm is almost non-existent in the spectrum. A similar observation can be made for Asp around 3.88 ppm. The Asp contribution at 2.8 ppm has probably been removed with the baseline correction due to its overlap with fatty acids. Adding the methylene resonances in the profiles of PCh and GPCh might improve the fit around 3.65 ppm, but this will decrease the quality of the fit around 3.2 ppm (where the largest peaks of PCh and GPCh are located) since the resonances around 3.65 ppm are more largely spread in the frequency domain, and so more reduced by the baseline correction, than the resonances around 3.2 ppm.

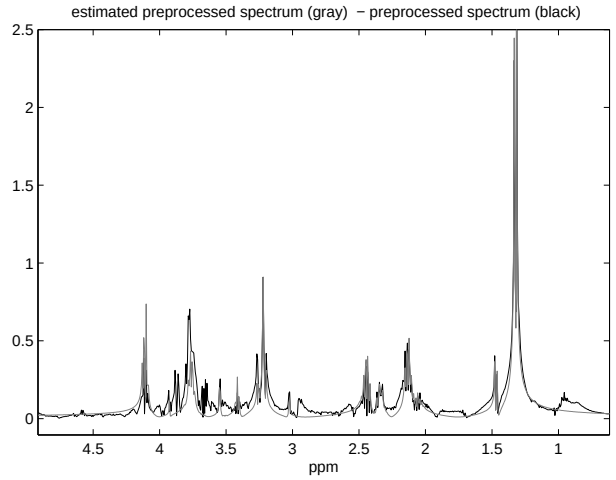


Fig. 3. Region of interest of the preprocessed spectrum vs estimated processed spectrum. Spectra are shown in magnitude and the y axis in arbitrary units.

The classification results are reported in Table I. Concerning the processing or feature extraction methods, AQSES and the whole spectrum provide generally better results than peak integration and are comparable. LS-SVM seems however to improve the performance of the classifier when compared with the standard linear method LDA.

IV. Discussion

Although the question of the diagnosis of brain tumors using long-echo or short-echo time *in-vivo* MRS data has been largely studied (see, *e.g.*, [8], [9], [10], [11]), no complete procedure (preprocessing, processing and classification steps) has been proposed for HR-MAS data. The methods proposed in this paper, already used in *in-vivo* MRS, have been adapted for HR-MAS data.

Preprocessing HR-MAS is made easier by the almost complete absence of eddy current effects. The large-damping components (fatty acids) are also more localizable (not spread over the whole region of interest as in *in-vivo* MRS). The baseline correction used in this paper to remove these components has two main effects: increasing the signal-to-baseline ratio (SBR) and decreasing the signal-to-noise ratio (SNR). In contrast with *in-vivo* MRS, HR-MAS spectra present high SNRs, allowing a larger increase of the SBR and thus a better suppression of the fatty acids.

Although the baseline correction allows to disentangle the metabolites of interest from the lipids (or fatty acids), it also prevents from using the information relative to the lipids. In *in-vivo* MRS, the lipids at 0.9 and 1.3 ppm are known to be potential means for separating certain tumor groups (see, *e.g.*, [12]). The baseline modeling of AQSES has not been used since it is not appropriate for HR-MAS data. However, we may reasonably think that other baseline or lipid modeling might improve the classification accuracy in several classification problems.

Selecting the features (processing step) is more tricky for HR-MAS data than *in-vivo* MRS data since the number of available features (number of points) and the number of visible and overlapping resonances are much larger, making methods like peak integration more sensitive to their initial parameters (peak intervals, etc). The interest of using peak integration instead of the whole spectrum is limited by the fact that the disturbing noise and artifacts are rather small in HR-MAS spectra, and averaging out these components will not be as important as in *in-vivo* MRS. Magnitude spectra have been chosen to avoid phasing problems, which might result in poor performances of peak integration. The results show that the whole spectrum or AQSES should be preferred to peak integration. This contrasts with *in-vivo* MRS where peak integration is often advised compared to other quantitation methods like LCModel (see, *e.g.*, [13]).

Better classification accuracies are obtained with the non-linear kernel-based classification method LS-SVM than with LDA. Similar observations were noted in [8] for short-echo time *in-vivo* MRS. A good separation in general, and especially between GBM and MET, suggests that HR-MAS might be an interesting method as complement of *in-vivo* MRS and MR imaging. Better results are generally obtained for GBM vs MET than GBM vs MEN, which contrast with the results in *in-vivo* MRS (see, *e.g.*, [8]). This might be due to the strong reduction of lipids at 0.9 and 1.3 ppm by baseline correction, these lipids being not taken into account when using peak integration or AQSES.

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TABLE I

CLASSIFICATION ACCURACY IN PERCENTAGE FOR EACH PAIR OF CLASSES AND EACH PROCEDURE.

Processing	Classification	GBM vs GLI	GBM vs MET	GBM vs MEN	GLI vs MET	GLI vs MEN	MET vs MEN
Whole spectrum	LS-SVM	89.81	93.10	90.71	92.86	94.53	87.38
Peak integration	LDA	85.63	86.79	86.58	84.04	88.53	79.38
Peak integration	LS-SVM	88.37	91.66	91.48	90.86	88.67	85.75
AQSES	LDA	89.67	86.48	87.42	84.75	86.33	88.50
AQSES	LS-SVM	91.95	91.86	90.19	92.93	92.40	89.88

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