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DEVELOPMENT OF A READ-IN ROUTINE TO AID QUANTITATIVE ANALYSIS OF CARDIAC ^{1}H-MRS DATA

by

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DEVELOPMENT OF A READ-IN ROUTINE TO AID QUANTITATIVE ANALYSIS OF CARDIAC ^{1}H-MRS DATA

Desenvolvimento de Uma Rotina de Leitura para Análise Quantitativa de Dados de ^{1}H-ERM Cardíaca

Thesis presented to Escola Superior de Biotecnologia of the Universidade Católica Portuguesa to fulfill the requirements of Master of Science degree in Biomedical Engineering

by

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March 2013

RESUMO

A Espectroscopia por Ressonância Magnética (ERM) é a única técnica não-invasiva e não radioactiva que permite investigar o metabolismo dos tecidos vivos. A ERM do protão ^{1}H, que proporciona a maior sensibilidade de todos os núcleos visíveis por RM, é um método capaz de detectar e quantificar biomoléculas cardíacas específicas. No entanto, os metabolitos estudados com ERM estão presentes em concentrações que são várias ordens de grandeza inferiores às dos protões da água, o que faz com que a ^{1}H-ERM não seja ainda utilizada na prática clínica devido a desafios metodológicos. Muitos estudos têm vindo a ser realizados a fim de melhorar os meios de quantificação e o BMRU tem estado na vanguarda do desenvolvimento de uma plataforma de análise de dados de ERM. Portanto, este projecto tem como objectivo desenvolver uma rotina capaz de ler dados de ERM da Siemens no formato específico TWIX e realizar uma análise piloto sobre dados ^{1}H-ERM provenientes de coração humano.

O trabalho realizado comtemplou diversas etapas. Inicialmente foi feito um estudo detalhado em relação aos ficheiros TWIX da Siemens, recorrendo a um algoritmo escrito em Matlab que tem o propósito de ler este tipo de ficheiro. De seguida, foi desenvolvida em IDL (*Interactive Data Language*) a rotina para leitura de ficheiros TWIX da Siemens que devolve os dados de ERM não processados no domínio do tempo. Foram analisados em jMRUI 14 conjuntos de dados de Espectroscopia por Resonância Magnética ^{1}H relativos a coração humano, no domínio do tempo, usando *Lorentzian line shape*, e comparou-se os resultados para o sinal de água com os obtidos anteriormente por Rial *et al.* (2011), para os mesmos dados. Os mesmos espectros foram re-analisados utilizando o mesmo modelo mas no domínio da frequência, utilizando o *software bmru_mrsw*, onde foi incorporada a rotina desenvolvida. Finalmente foram analisados os mesmos conjuntos de dados usando *Voigt line shape*, no domínio da frequência, novamente com o software *bmru_mrsw*.

Houve conformidade entre os resultados obtidos na análise dos dados de ERM com o *sofware* jMRUI e os publicados por Rial *et al.* (2011), com um coeficiente de correlação de 0,997. A diferença média entre as duas medições do sinal de água foi de 0,06, segundo o gráfico *Bland-Altman*. A análise dos dados, no domínio da frequência, com o *software bmru_mrsw* demonstrou concordância com os resultados obtidos pelo jMRUI, tendo-se obtido um coeficiente de correlação de 0,716 entre os resultados. A quantificação do sinal de água utilizando *Voigt line shape*, no *software bmru_mrsw*, demonstrou diminuição dos resíduos gerados.

Deste modo, foi possível com este projecto a criação de uma rotina capaz de ler data de Espectroscopia por Ressonância Magnética da Siemens no formato específico TWIX. Esta rotina conferiu ao *software bmru_mrsw* compatibilidade com este formato de ficheiros, tendo deste modo contribuído para o aperfeiçoamento das técnicas de quantificação de sinais de espectroscopia. A potencialidade da abordagem de análise no domínio do tempo e frequência foi também demonstrada. Os objectivos propostos neste projecto foram alcançados com sucesso, podendo este ter contribuído para o papel da Espectroscopia por Ressonância Magnética no campo da cardiologia clínica.

ABSTRACT

Magnetic Resonance Spectroscopy (MRS) is the only non-invasive and non-radiation technique for investigating the metabolism of living tissue. Proton (^{1}H)-MRS, which provides the highest sensitivity of all MR-visible nuclei, is a method capable of detecting and quantifying specific cardiac biomolecules. However, metabolites studied with MRS are present in concentrations that are several orders of magnitude lower than those of water protons, which make ^{1}H-MRS not being used yet in clinical practice, due to fundamental methodological challenges. Many studies are being carried out in order to increasingly improve the means of quantification and BMRU has been at the forefront of developing a novel analysis framework for MRS data. Therefore, this project aims to develop a read-in routine capable of reading Siemens MRS data in the specific TWIX format and to conduct a pilot analysis on a human cardiac ^{1}H Siemens MRS data.

The work carried out contemplated several phases. Initially a detailed study regarding the Siemens TWIX files was conducted, using an algorithm written in Matlab that aims to read this type of file. Then, it was developed in IDL (Interactive Data Language) the read-in routine for Siemens TWIX files that returns the unprocessed time domain MRS data. 14 human cardiac ^{1}H-MRS data sets were analyzed in the time domain using Lorentzian line shape in jMRUI, and the results for the water signal were compared with those previously obtained by Rial *et al*. (2011), for the same data. The same unsuppressed spectra were re-analyzed using the same model but in the frequency domain, using the software *bmru_mrsw*, where had been incorporated the developed read-in routine. Finally the same data sets were analyzed using Voigt line shape, in the time domain frequency domain, again with software *bmru_mrsw*.

There was agreement between the obtained results in the analysis of MRS data with jMRUI software and the ones published by Rial *et al*. (2011), with a correlation coefficient of 0.997. The mean difference between the two measurements for the water signal was 0.06, according to the Bland-Altman plot. The data analysis with *bmru_mrsw* software in the frequency domain, agreed with the results obtained by jMRUI, yielding a correlation coefficient of 0.716. Quantification of the water signal using Voigt line shape in *bmru_mrsw* software originated lower residues.

Thus, it was possible with this project to create a routine able to read the Siemens Magnetic Resonance Spectroscopy data in the specific format TWIX. This routine conferred to the *bmru_mrsw* software compatibility with this file format, and thereby contributed to the improvement of techniques for quantifying spectroscopy signals. The capability of the time domain frequency domain fitting approach was also demonstrated. The proposed objectives in this project were successfully achieved, and the project may has contributed to the role of Magnetic Resonance Spectroscopy in clinical cardiology.

ACKNOWLEDGMENTS

I would like to express my thankfulness to Dr Jurgen Schneider for the opportunity of developing a project in such a professional group. I am also very grateful to his precious teachings, orientations and confidence.

I am also extremely indebted to Dr Mahon Maguire, Ms. Vicky Thornton, Dr Kiterie Faller, Mr. Kyle Caldock, Mrs. Lee-Anne Stork, Dr Maélène Lohezic and Dr Irvin Teh for all the support and kindness during my stay in Oxford.

My sincere thanks to Professor João Paulo Ferreira for all his support and availability before and during my Erasmus experience.

I would like to deeply thank the unconditional support of my parents, brothers and grandparents, so that I could reach one more stage of success.

Finally I would like to warmly thank Neuza Silva, for having closely followed all the steps of this project and having been a precious help and motivation in every moment of this journey.

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NOMENCLATURE

AMARES	Advanced Method for Accurate, Robust and Efficient Spectral
BMRU	British Heart Foundation Experimental Magnetic Resonance Unit
CSI	Chemical Shift Imaging
DICOM	Digital Imaging and Communications in Medicine
FD	Frequency domain
FID	Free Induction Decay
FT	Fourier transformation
GUI	Graphical User Interface
HLSVD	Hankel Lanczos Singular Value Decomposition
HLTLS	Hankel Lanczos Total Least Squares
IDL	Interactive Data Language
jMRUI	Java-based Magnetic Resonance User Interface
LPSVD	Linear Prediction Singular Value Decomposition
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
NMR	Nuclear Magnetic Resonance
ppm	parts per million
PRESS	Point Resolved Spectroscopy
QUEST	Quantification Based on Quantum Estimation
RF	Radiofrequency
SE	Spin echoe
SNR	Signal-to-noise ratio
STEAM	Stimulated Echo Acquisition Mode
SVS	Single Voxel Spectroscopy
TD	Time domain
TDFD	Time domain Frequency domain fitting
TLS	Total Lineshape
VARPRO	Variable Projection

CHAPTER 1

INTRODUCTION

1.1 Background

1.1.1 Nuclear Magnetic Resonance

The Nuclear Magnetic Resonance (NMR) is a non-invasive technique that uses the physical properties of nuclei to create anatomical images of the body as well as to access metabolic information of living tissue and organs.

The following subsections describe physical principles of NMR and its variants, allowing a scientific contextualization of this technique, based on authors Athey *et al*. (1982), Fullerton (1982), Karstaedt *et al*. (1983), Gadian (1995), and Hillary and DeLuca (2007).

Magnetic Field

Some atoms have nuclei with a property called angular momentum or spin, which can be described in a simplified and classic form as the spinning motion of the nucleus about its own axis. Associated with the spin is a magnetic momentum, which contains both magnitude and direction expressing the strength and direction of the magnetic field surrounding the nucleus. When placed in a static magnetic field B_0, nuclei tend to orientate their spins in the same direction of this external magnetic field axis, acquiring a precession of the magnetic moments about the external magnetic field with an angular frequency, called Larmor Frequency. This frequency is proportional to the magnetic field. Alignment can occur in two different orientations according to the nucleus spin quantum number, parallel corresponding to low energy state and antiparallel referent to high energy state. Both orientations occur with a small angle of deviation in relation to the magnetic field position, resulting in an angular momentum.

Slightly more spins will align parallel to the B0 direction resulting in longitudinal magnetization (Mz) of the sample.

Human's body is mainly composed of water molecules, each of which is composed for one oxygen and two hydrogen atoms. The proton (^{1}H), the main isotope of hydrogen, is ideal to perform high-resolution MR imaging due to the abundance of hydrogen atoms in the human body. However, there are also other atoms with relevance for studies of living tissues, such as ^{13}C, ^{31}P, ^{23}Na, ^{15}N, ^{39}K and ^{19}F.

NMR signal

After the nuclei are placed in a static magnetic field, these achieve an equilibrium state, creating a net magnetization in which the nuclear spins are polarized in the direction of the applied field. At this state, the number of transitions between the lower and the upper energy levels are equal in both directions. To measure the net magnetization it needs to be disturbed out of its equilibrium. After that perturbation the nuclei relaxation to its initial state after that perturbation is analyzed. Perturbation consists in the application of an oscillating perpendicular radiofrequency (RF) field to the static magnetic field B_0 in a process called resonance phenomenon. This oscillating magnetic field rotates in resonance at nuclei specific Larmor frequency and perturbs the magnetization of the sample, flipping the longitudinal magnetization to an arbitrary angle (flip angle) with respect to the B0, resulting in transverse magnetization (Mxy). After the RF pulse is applied, a signal with Larmor frequency and a certain amplitude value is generated. Amplitude value decays to zero according to a characteristic time constant. Thereby, the signal is referred to as a Free Induction Decay (FID) presenting a damped sinusoidal shape. This is a time domain (TD) signal that can be converted to an understandable form in frequency domain (FD), i.e. a spectrum or image, using the mathematical operation Fourier transformation (FT).

Relaxation Times

As mentioned above, following the magnetization of the sample and its perturbation, the rotation of magnetization in the xy-plane (Mxy) occurs. It is this transverse magnetization that gives rise to the NMR signal in a receiver coil. However, the signal rapidly becomes weaker, due to relaxation processes that reduce the transverse magnetization over time, causing the return of the spins to equilibrium. This relaxation process is characterized by two time constants T_1 and T_2.

Time constant T_1 is the Spin-lattice Relaxation Time and is related with the recovery of magnetization along the direction of the static magnetic field, i.e. longitudinal direction. After the free precession magnetization regrows along longitudinal orientation with a time constant T_1. Thus, T_1 is the time necessary for the excited spins recover and be available for the next excitation. This spin-lattice relaxation process is characterized by energy dissipation of the nuclear spins to their surroundings while the nucleus returns to its lowest energy state and is, once again, oriented along the magnetic field. On the other hand, time constant T_2 is called the Spin-spin Relaxation Time and relates to the decay of magnetization component in the plane transversal to the static magnetic field. After the RF pulse transverse component of magnetization decays to zero. During relaxation processes interactions between nuclear spins are present acting as boosters to the magnetization net orientation changes. In case of Spin-lattice energy exchange between the nuclear spins and their molecular framework are also intervening.

Both time constants are intrinsic nuclei properties and their values differ between tissues, which can lead to different image properties and contrast.

1.1.2 Magnetic Resonance Spectroscopy

The Magnetic Resonance Spectroscopy (MRS) is the only non-invasive technique to characterize biochemical composition of tissue and metabolic function. Based on similar acquisition principles to those of NMR, this technique takes advantage over other existing ones by the absence of radiation doses from radioactive trackers (Singhal *et al.*, 2009; Neubauer, 2003, 2010). However, in *in vivo* analysis, the low concentration of metabolites leads to weak signal intensities compared to the water signal used for MRI. Hence, many research studies have being carried out to achieve better quality and well-resolved MR spectra (Gerothanassis *et al.*, 2002).

Magnetic Resonance Spectroscopy signal is measured as a function of time represented by a set of complex data points, which is then Fourier transformed to a function of frequency, i.e. the spectrum. Such spectrum is a depiction of the plot of chemical shifts of different metabolites relative to the frequency of a reference compound (Singhal *et al.*, 2009; Gadian, 1995). Chemical shifts are expressed in parts per million (ppm) being their values calculated as a frequency shift difference between the substance and the reference divided by the reference one. Reference is chosen depending on nuclei type under analysis (Drost *et al.*, 2002). The area under each peak is representative of the metabolite concentration to which it refers and several peak fitting algorithms are available to quantify the metabolites (Singhal *et al.*, 2009).

Cardiac ^{1}H-MRS

Improvements in the proton Magnetic Resonance Spectroscopy (^{1}H-MRS) have allowed the non-invasive study of myocardial compounds, such as creatine (phosphorylated and unphosphorylated), carnitine, taurine and lipids (Schneider *et al.*, 2004). Analysis of these metabolites can characterize the myocardial metabolism enabling the identification of dysfunctions and diseases (Rial *et al.*, 2011; Singhal *et al.*, 2009; Neubauer, 2007). However, proton spectroscopy remains methodological challenging as metabolites are present in concentrations that are several orders of magnitude lower than those on water protons. Therefore, in order to detect the weak signals from metabolites is necessary to perform effective water suppression (Schneider *et al.*, 2004; Rial *et al.*, 2011).

Localized spectroscopy

In vivo MRS typically requires localization in order to obtain metabolic information of tissue. This can be achieved by Single Voxel Spectroscopy (SVS) or Chemical Shift Imaging (CSI). In SVS, a spectrum is acquired from a single volume, while CSI provides simultaneous acquisition of spectra from multiple voxels within a single (2D CSI) or multiple slices (3D CSI) (Jansen *et al.*, 2006).

Single voxel spectroscopy

A variety of techniques based on the successive selection of three orthogonal slices by means of frequency-selective RF pulses in conjunction with magnetic field gradients have been developed for obtaining spectra from single volume elements of interest. Thus, only spins in the intersecting volume of all three slices contribute to the acquired NMR signal (Gadian, 1995). Spin echoes (SE) for Point Resolved Spectroscopy (PRESS) and stimulated echoes for Stimulated Echo Acquisition Mode (STEAM) are the two most common approaches to MRS volume selection (Barker *et al.*, 2001).

PRESS uses a slice-selective RF pulse sequence consisting of one 90° pulse followed by two 180° refocusing pulses. Under this approach the amplitude of the double spin echo originating for the voxel of interest is two times greater than the stimulated echo obtained by STEAM, conferring to PRESS better signal to noise ratios. Conversely, STEAM uses a sequence of three slice-selective 90° RF pulses. Although it is a less sensitivity method than PRESS, it can generally achieve shorter echo times, which is useful to detect some specific metabolites with short T_2 (Frahm *et al.*, 1989).

Chemical shift imaging

In contrast to SVS, Chemical shift imaging technique offers the possibility to measure MR spectra from multiple voxels within one or multiple slices, which is desirable to monitor the metabolic state of numerous regions (Brown *et al.*, 1982; Maudsley *et al.*, 1983). A series of free induction decays are collected following the application of phase-encoding gradients in as many directions as CSI dimensions are desired. Data can be displayed in the form of metabolic images showing the spatial distribution of signals from each of the metabolites (Gadian, 1995). This technique requires longer acquisition times, but has generally higher resolution than SVS (Chan *et al.*, 2001; Hsu *et al.*, 2001).

1.1.3. MRS data analysis

There is need to analyse the measured data with the greatest possible accuracy (Boogaart *et al.*, 1994). In this context, MRS data analysis can comprise two steps, namely pre-processing and quantification, both of which can be performed in time domain and frequency domain. Pre-processing procedures, such as eddy current compensation, offset corrections, noise filtering, data points zero filling, residual water suppression, phase corrections and baseline corrections, are carried out due to the non-ideal *in vivo* experimental conditions. These conditions, e.g. physiological motions, fast decaying signals from immobile components, overlapping signals, truncation of data before FID has decayed to noise level, presence of residual water peaks, lead to data imperfections which should be corrected to minimize errors associated with metabolites quantification (Mandal, 2012). Spectral analysis can be performed in either the time domain (TD) or in the frequency domain (FD).

Below in Figure 1.1 are presented, in a schematic form, the main steps of the MRS technique in order to contextualize the work carried out in this project.

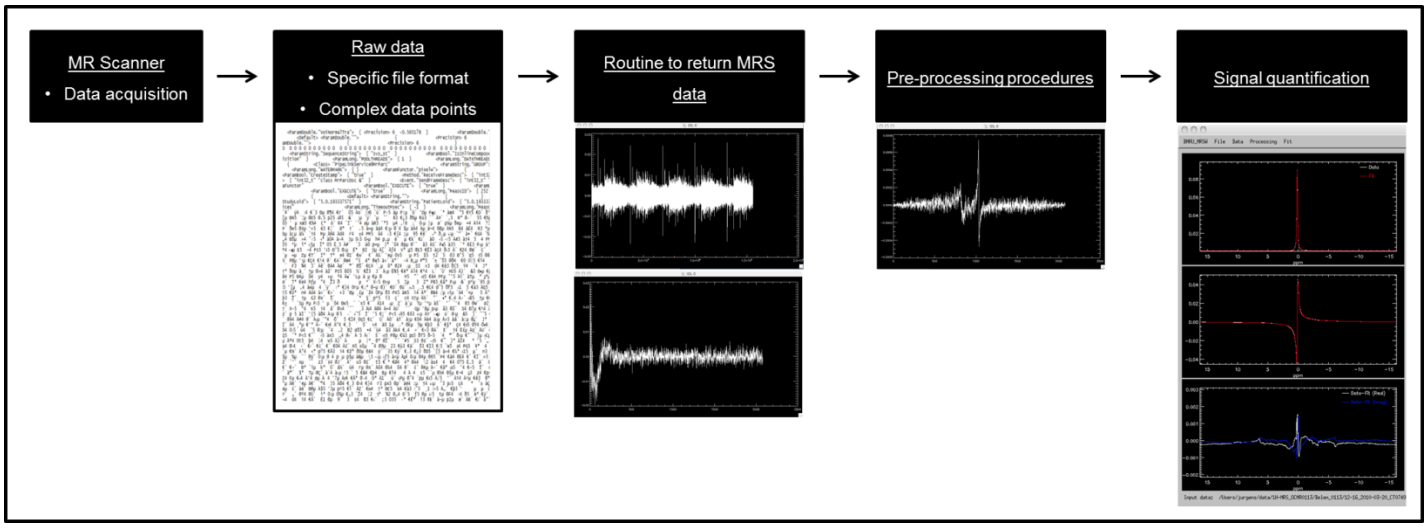

Figure 1.1. Outline of the main steps of MRS data analysis.

The developed routine has a central role in this process as it allows the connection between the raw data coming from the MR scanner and the MRS data analysis software.

Quantification of MRS data in time domain

To quantify metabolite concentrations in the time domain, two approaches can be adopted, iterative and non-iterative methods. Between the iterative ones, those stand out, which use non-linear least squares methods to minimize the difference between the data and the model function using local or global optimization (Mandal, 2012).

Iterative methods have the interesting feature of allowing for the implementation of prior knowledge, e.g. frequencies, damping factors, phases of metabolic resonances (Ala-Korpela *et al.*, 1995), by means of linear equations (Mierisova and Ala-Korpela, 2001). VARPRO (Variable Projection) was one of the early methods to incorporate prior knowledge, based on a Lorentzian line shape for the individual components of the resonances fitting corresponding decaying exponentials in time domain (Boogaart *et al.*, 1994; Vanhamme *et al.*, 1997). Later, AMARES (Advanced Method for Accurate, Robust and Efficient Spectra) was developed as a method able to more prior knowledge incorporation, pre-programmed to switch between Lorentzian and Gaussian model line-shapes (Vanhamme *et al.*, 1997, 2001).

On the other hand, non-iterative methods are less flexible for the implementation of prior knowledge. In these methods, model functions generally confined to only exponential decay and model

parameters are chosen in one single step. Hence, non-iterative approaches are suitable only in cases of almost ideal signal acquisition. However these methods are faster than the iterative ones. HLSVD (Hankel Lanczos Singular Value Decomposition) and LPSVD (Linear Prediction Singular Value Decomposition) are one of the most used non-iterative methods and show potentiality in removing dominating resonances from solvents (Boogaart *et al.*, 1994).

Quantification of MRS measured signal in time domain has the advantage to easily handle problems related with missing data points and truncated data sets (Slotboom *et al.*, 1998).

Quantification of MRS signal in frequency domain

Two different methods are available to accomplish the quantification of metabolites in frequency domain, through peak area integration or by non-linear least square fitting using model functions. As mentioned before, experimental conditions of *in vivo* experiments are not ideal, which makes it difficult to use the peak area integration methods, due to presence of complex spectra. Most of this complexity is related to the overlap of different resonances of interest, rolling baseline and low SNR (Mierisova and Ala-Korpela, 2001). Thus, non-linear least squares methods are preferred, using model-based optimization algorithms (Provencher, 2001; Mierisova and Ala-Korpela, 2001).

Ideally, the FID is expressed as a sum of exponentially decaying sinusoids. Applying the Fourier transformation to this time domain signal leads to the frequency domain MR spectrum. This spectrum consists of a sum of pure complex Lorentzian lines. Thus, in model lineshape fitting, model functions composed of Lorentzian lines are fitted to the experimental spectra. Both real and imaginary parts of these complex lines can be used for the parameter estimation (Mierisova and Ala-Korpela, 2001). However, when a large background component (the baseline) is present along with a low SNR accurate quantification of MR spectra is a challenging problem. Thus, it is common to use pre-processing methods, i.e. filtering to separate baseline and spectra of different metabolites and achieve an accurate MRS quantification. After filtering, the accuracy and the robustness of MRS quantification are improved (Guo *et. al*, 2009). In reality it is found that experimental spectra approximate to a mixture of Lorentzian and Gaussian, such as Voigt line shape (Slotboom et al., 1998) or other distorted shapes (Mandal, 2012). There are many fitting algorithms operating in the frequency domain that have been developed in recent years, which allow the incorporation of prior knowledge as well as implementation of information about relations between spectral components (Graaf and Bovee, 1990; Slotboom *et al*., 1998).

TLS (Total Lineshape) is a frequency domain advanced line shape fitting algorithm, Lorentzian based, capable of estimating frequencies, intensities, line widths and phases of individual peaks with the possibility of including prior knowledge on the estimated parameters (Mierisova and Ala-Korpela, 2001).

The possibility of perform frequency-selective fitting can be a very useful feature of frequency domain approaches representing an advantage of these methods over the time domain based ones (Vanhamme *et al.*, 1998).

The Time domain frequency domain fitting approach

Published work (Marshall *et al.*, 1997; Slotboom *et al.*, 1998) reports on frequency domain fitting algorithms. These algorithms use time domain models and prior knowledge, which combines both advantages of time and frequency domain approaches, often allowing more accurate parameters estimation. This approach, termed Time domain Frequency domain (TDFD) fitting, allows fitting spectra through the use of Voigt line shape, a combination between Lorentzian and Gaussian ones, with flexible contribution of each one, in order to obtain the most accurate estimation. The possibility to use both analytic and non-analytic lineshapes as well as experimental reference lineshapes is an appreciated feature of this approach conferring a useful versatility to the TDFD fitting.

Fitting itself is regarded as a minimization of the sum of least squares calculated in the FD setting up the TD model. Minimization can be performed using Levenberg-Marquardt algorithm, a method that reduce the sum of the squares of the errors between the function and the measured data points (Press *et al.*, 1992).

Analysis software – jMRUI

jMRUI is a Java-based Magnetic Resonance User Interface software to analyze the time domain MRS data (Naressi *et al.*, 2001). This software allows time domain MRS single voxel as well as in multiple voxel data quantification and can handle large data sets (Helms, 2008). Capacity of conversion of the file structure of commercial NMR spectrometer data files into a structure readable by jMRUI is a powerful feature of the software, which makes jMRUI attractive for the analysis MRS data of the most influent brands in the market (Naressi *et al.*, 2001). Moreover jMRUI is compatible with Digital Imaging and Communications in Medicine (DICOM) format for MRS data (Stefan *et al.*, 2009).

MRS data processing using jMRUI comprises in a first approach data pre-processing followed by the quantification. In terms of pre-processing methods, several options are offered by jMRUI. In order to the suppression of water molecules signal, HLSVD filter is available. Time domain QUALITY deconvolution method deals with magnetic field inhomogeneity problems. Peak extraction and dynamic phase correction are achieved using Gabor filters. Some mathematical operators are also available for the normalization of a signal or over a series of signals. Conversion of an echo signal to an FID signal can also be performed as a pre-processing step in jMRUI (Mandal, 2012).

jMRUI offers two different methods for quantification, i.e. black box quantification algorithms, such as HLSVD, HLTLS (Hankel Lanczos Total Least Squares) and LPSVD, and interactive quantification methods like VARPRO, AMARES and QUEST (Quantification Based on Quantum Estimation) (Mandal, 2012; Stefan *et al.*, 2009; Vanhamme *et al.*, 1997).

1.2 Thematic framework

Diagnostic methods developments have a strong role in medicine acting as a crucial tool in the increased rates of success in treating diseases. Assessment of cardiac metabolism is fundamental in the evaluation of cardiac dysfunctions. Thus, Magnetic Resonance Spectroscopy is the only non-invasive, non-destructive and non-radiation technique able to perform this evaluation. However, despite the results that have been published in the recent years on metabolic studies of normal and diseased hearts of animal models and humans, cardiac ^{1}H-MRS remains an under-used method in cardiology clinical practice. In fact, are many the fundamental methodological challenges hindering its use, such as the low concentrations of metabolites under study, dominant water signals, long scan times and cardiac and respiratory motion. Due to these reasons, becomes extremely important to develop efficient algorithms and software capable to perform data processing treatments and quantification with a suitable confidence degree. One focus of the work at the British Heart Foundation Experimental MR Unit (BMRU) is on advancing cardiac MRS in animal models of heart disease and in humans. Specifically, BMRU is developing a novel framework for analyzing MRS data. The aim of this project was to contribute to this framework, called *bmru_mrsw*.

The first objective of the project was to develop a routine that reads Siemens MRS data, including Siemens specific format TWIX. This routine was then incorporated into *bmru_mrsw*. Afterwards, it was intended to validate the developed routine conducting a TDFD pilot analysis on a human cardiac ^{1}H-MRS data and compare the results with the ones obtained from the commonly used analysis software jMRUI, for the same data sets.

CHAPTER 2

METHODOLOGY

2.1. Read-in routine development

In order to develop an efficient read-in routine to read Siemens MRS data stored in TWIX format, a detailed study of the structure and organization of TWIX files was made. For this purpose, it was necessary to resort to the MATLAB written routine read_meas_dat.m, which allowed for reading Siemens Twix data, as it was the only available code / documentation to read this type of data. This evaluation aimed the knowledge of TWIX files content as well as understanding the way in which the data is stored in these files. This knowledge was a crucial step to design the read-in algorithm, to facilitate the access of relevant parameters and allowing to a readout of MR spectroscopy data.

The read-in routine was to be developed using the scientific programming language IDL (Interactive Data Language) from Exelis. *bmru_mrsw* uses IDL for data handling, reconstruction and also to provide the Graphical User Interface (GUI) and a c-code for fitting. The read-in routine comprises several functions, which are called along the code in order to achieve greater clarity and high efficiency. An approach to obtain relevant acquisition parameters and data was implemented in IDL.

2.2. Validation

To validate MRS analysis with *bmru_mrsw*, a comparison with the approach developed by B. Rial was performed, which used MATLAB and jMRUI. Validation of the developed routine contemplated to distinct steps, Validation 1 and Validation 2. Validation 1 consisted in an initial spectral analysis using jMRUI, having the results for water amplitude been validated with those previously published by Rial *et al.* (2011).

14 ^{1}H-MRS data sets were acquired by B. Rial prior to this project on a 3T MR scanner (Tim Trio, Siemens Healthcare, Germany) using syngo MR B15 software and refer to multiple breath-hold SVS. A modified STEAM sequence was used to achieve a short TE (echo time) of 10 ms. Signal acquisition was performed resorting to 15 channels of data constituted for two phased-array body coils (Rial *et al.*, 2011).

Spectral analysis was composed for several steps, frequency and phase correction, combination of signal from multiple receiver coils, averaging and spectral quantification. MATLAB algorithm performs the coil combination based on Roemer *et al*. (1990) and Natt *et al*. (2005). Combination of individual signals is performed using the amplitude and phase of the time domain signal without water suppression to represent the weighting and phase correction factors required for weighted signal summation. Acquisitions are phase-corrected with the zero-order phase of the dominant peak in the spectra (the

residual water peak). AMARES algorithm, contained in jMRUI analysis software, was used to perform spectral quantification in time domain, using Lorentzian line shape to fit the metabolite contributions in the water-suppressed spectra. Water amplitude values were recorded for all data sets. To aid the quantification process, prior knowledge information, starting values and overall phases information were incorporated.

Validation 2 aimed the validation of *bmru_mrsw* analysis results against the ones obtained from jMRUI. The same 14 data sets (previously analysed with jMRUI) were analyzed using the software *bmru_mrsw*, where the read-in routine had been incorporated.

Fitting was carried out as a time domain approach, using Lorentzian lineshape. Coil combination was performed similarly as reported by Rial *et al.* (2011). In order to determine the correlation between jMRUI and *bmru_mrsw,* results for water amplitude were compared. During the quantification, prior knowledge information, starting values and overall phases information were incorporated.

2.3. *bmru_mrsw* TDFD fitting

After the read-in routine validation, by comparison with the results obtained with jMRUI, analysis of the 14 ^{1}H-MRS scans were worked out using the TDFD fitting approach available in *bmru_mrsw*. This approach uses the Voigt line shape to fit the spectra, which has different contributions of Gaussian and Lorentzian lines. The benefit of Voigt profile was showed in the determination of water amplitude and its residuum. The percentage of Gaussian contribution was initialised to 50% and fitted as an additional parameter.

Comparison between the obtained results for water amplitude residuum was made by statistical test t-test for paired samples. The results were considered statistically significant for a p-value of less than 0.01. All statistical analysis was evaluated using software SPSS 19 for Windows.

CHAPTER 3

RESULTS

3.1 Read-in routine development

A Siemens TWIX file consists of three parts. The first entry the TWIX file contains binary information concerning the location of the remaining binary data in the file. This is followed by an ASCII header that contains information and features related to the equipment from where the data was acquired, patient, scanning parameters, and others. Subsequently, in the position of the byte specified in the first entry of the file, is the binary data, arranged in the form of a binary header (128 bytes) followed by a data line (variable size), successively. Figure 3.1 presents schematically the content of a TWIX file.

TWIX file structure		Content	Size (bytes)
Binary line		Long integer containing the byte location of binary data in the file	4
ASCII header		Information about patient, scan parameters and other relevant information	Variable
Binary data (MDH and ADC lines alternately, until the end of file)	MDH line	Binary header containing information about scan data set	128
	ADC line	Scan data line of one of types described on Figure 3.2	Variable

Figure 3.1. Siemens TWIX file contents.

The data lines contained in the binary part of TWIX files can be of various types. For this study, only lines containing spectroscopy data were of interest. These lines were identified through the bit value of certain EvalInfoMask fields. NOISEADJSCAN, PATREFSCAN, PHASCOR, PHASESTABSCAN and REFPHASESTABSCAN fields equal to zero and MDH_ONLINE field equal to one are the conditions to represent a data line. In the following picture (Figure 3.2) are described all the possible types of a binary data (ADC) line.

Line	Type/Enumerator	Description
ADC line	NOISEADJSCAN	Noise adjust scan
	PATREFSCAN	PAT reference scan
	PHASCOR1D	Phase correction 1D data
	PHASCOR2D	Phase correction 2D data
	PATREFSCAN_PHASCOR	PAT reference scan, Phase correction data
	PHASESTABSCAN	Phase stabilization scan
	REFPHASESTABSCAN	Reference phase stabilization scan
	DATA	Spectroscopy data line

Figure 3.2. Types of different ADC (binary data) lines in a Siemens TWIX file.

Detailed knowledge about Siemens TWIX files organization, particularly the location of parameters of interest and spectroscopy data, was crucial to the development of the read-in routine for spectroscopy data. In a first stage, was designed a routine to perform the extraction of interest parameters from the ASCII header. These parameters are required for the subsequent calculation of the dimensions of spectroscopy data. The routine that performs the parameters extraction was designed as a function and then integrated into the main routine. The flowchart of the routine for the extraction of interest parameters is shown in Figure 3.3.

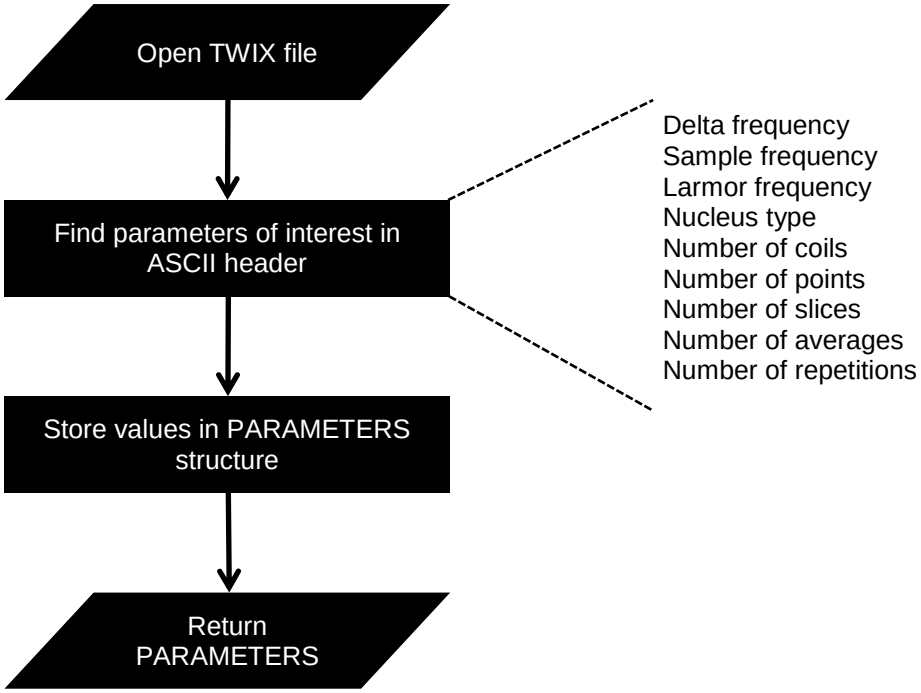

Figure 3.3. Flowchart of the routine for interest parameters extraction.

After the implementation of the routine to extract the parameters of interest, the main read-in routine for Siemens TWIX files was developed. The routine has the purpose of returning the unprocessed time domain MRS data. Figure 3.4 presents the flowchart of the read-in routine.

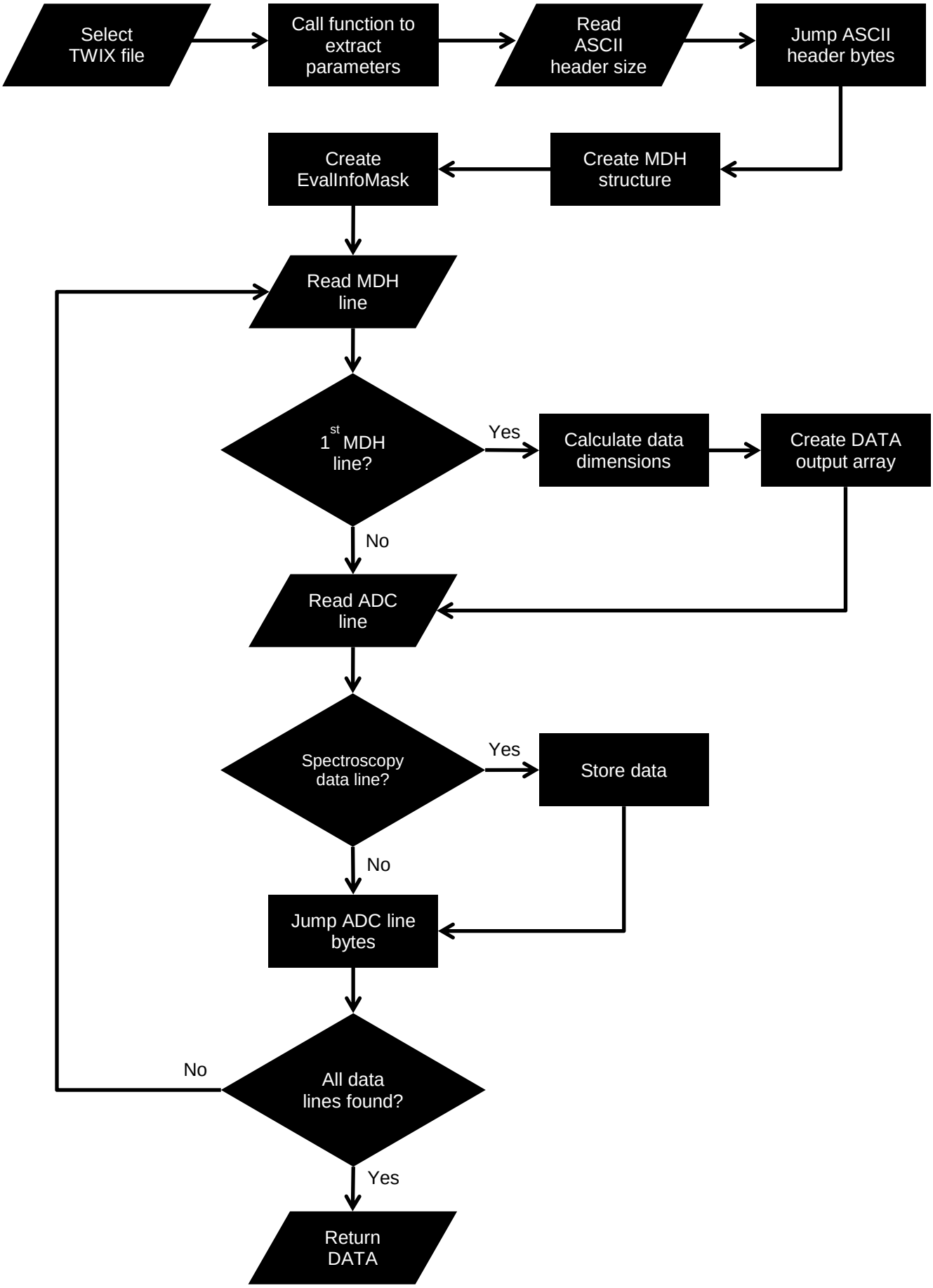
Select TWIX file
Call function to extract parameters
Read ASCII header size
Jump ASCII header bytes
Create MDH structure
Create EvalInfoMask
Read MDH line
1st MDH line?
Yes
Calculate data dimensions
Create DATA output array
No
Read ADC line
Spectroscopy data line?
Yes
Store data
No
Jump ADC line bytes
All data lines found?
No
Yes
Return DATA

Figure 3.4. Flowchart of the read-in routine to return unprocessed time domain Siemens MRS data.

3.2 jMRUI analysis – Validation 1

Before testing the developed read-in routine, it was necessary to analyse 14 ^{1}H-MRS data sets using the software jMRUI to obtain values for further comparison. Obtained results for water amplitude were validated against the ones previously published by Rial *et al.* (2011) and its correlation is shown below in Figure 3.5 and Figure 3.6. Figure 3.5 is a Bland-Altman plot for the obtained water amplitude values showing a bias of 0.06. In turn, Figure 3.6 shows a direct correlation curve between Rial´s results and obtained results, with a correlation factor of 0.997.

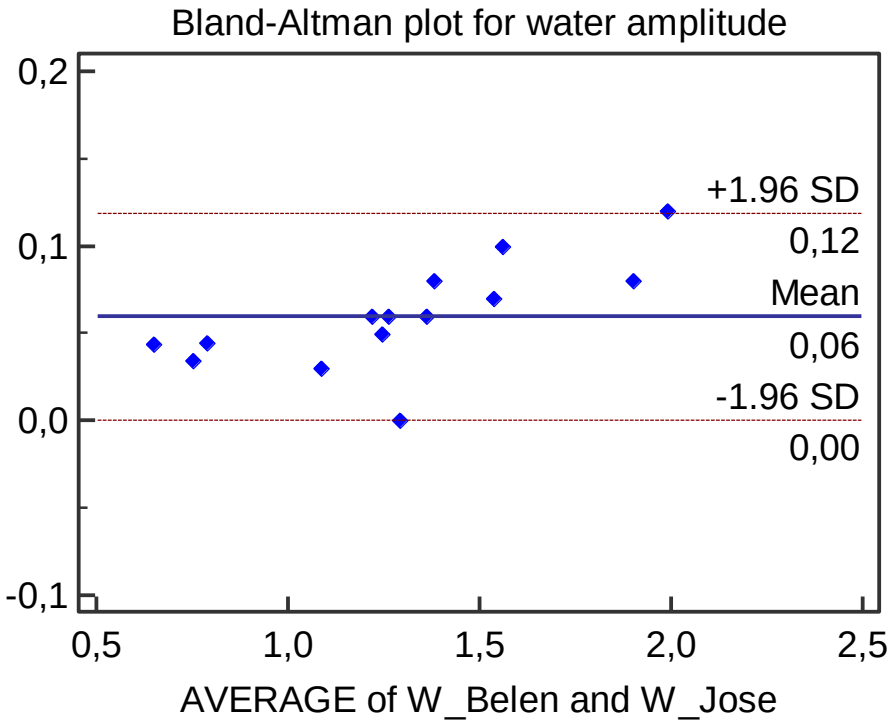

Figure 3.5. Bland-Altman plot of obtained results and Rial´s ones, using jMRUI, for water amplitude.

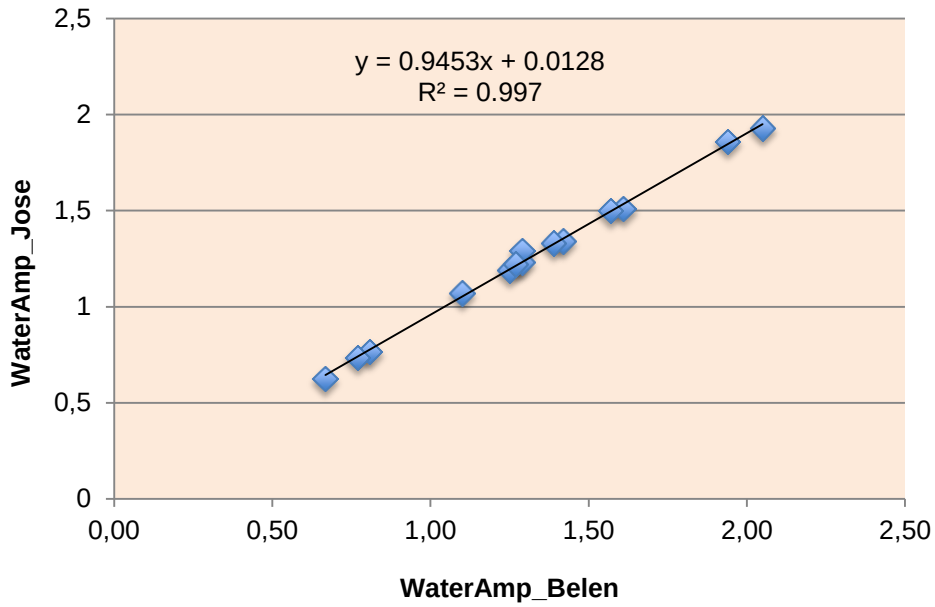

Figure 3.6. Correlation curve between the obtained results and Rial´s ones, for water amplitude, using jMRUI.

3.3 BMRU software pilot analysis – Validation 2

After a successful development of the read-in routine and its implementation in the analysis software *bmru_mrsw*, validation tests of the routine performance were conducted. Analysis of the same 14 ^{1}H-MRS data sets stored in TWIX format with *bmru_mrsw* software were performed, using Lorentzian line shape, and their water amplitude values were determined. These results were compared with those previously obtained through jMRUI. Below is displayed in Figure 3.7 the correlation curve between the results obtained by *bmru_mrsw* and jMRUI, with a correlation factor of 0.716.

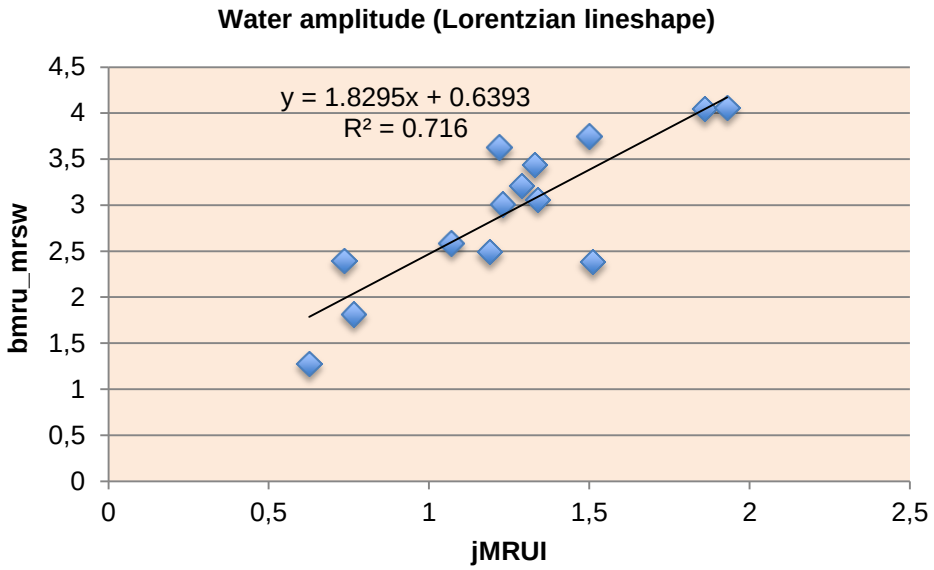

Figure 3.7. Correlation curve of water amplitude values obtained with *bmru_mrsw* and jMRUI.

Finally, in order to demonstrate the advantage of using the Voigt profile available in *bmru_mrsw* software, analysis of the same data sets were carried out using Voigt lineshape. Below is presented the layout of *bmru_mrsw* software performing a Voigt lineshape based MRS data analysis (Figure 3.8).

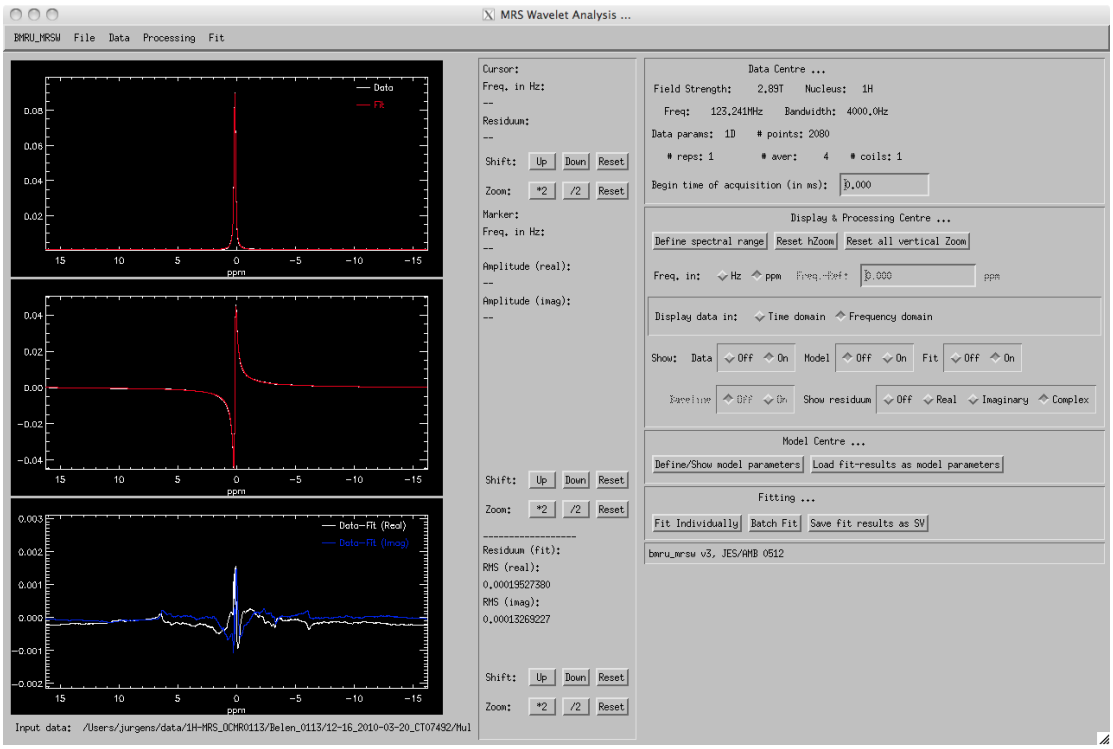

Figure 3.8. *bmru_mrsw* analysis software layout.

Residuum values (real and imaginary) results of the analysis using Lorentzian and Voigt line shapes are presented below in Figure 3.9 and Figure 3.10.

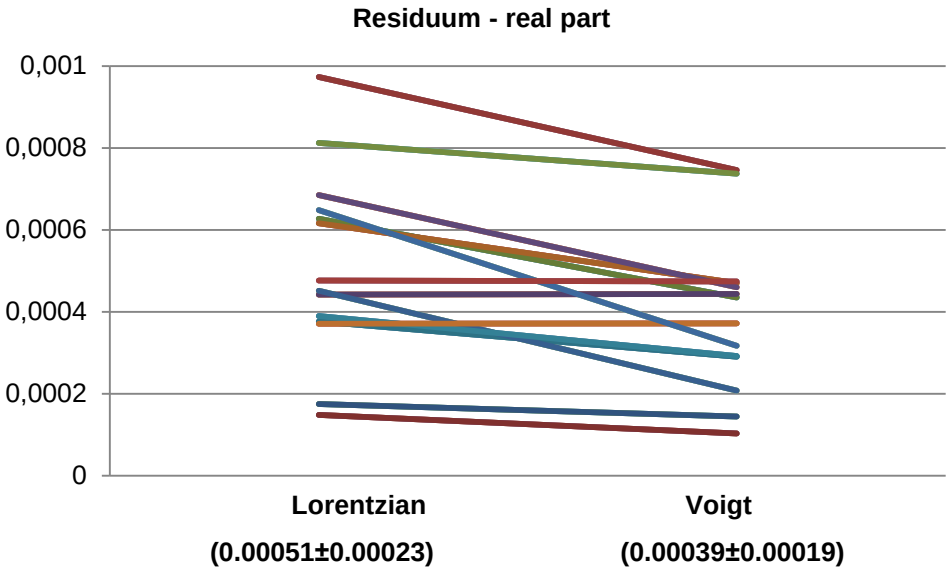

Figure 3.9. Residuum after Lorentzian (TD) and Voigt (TDFD) fittings, real part.

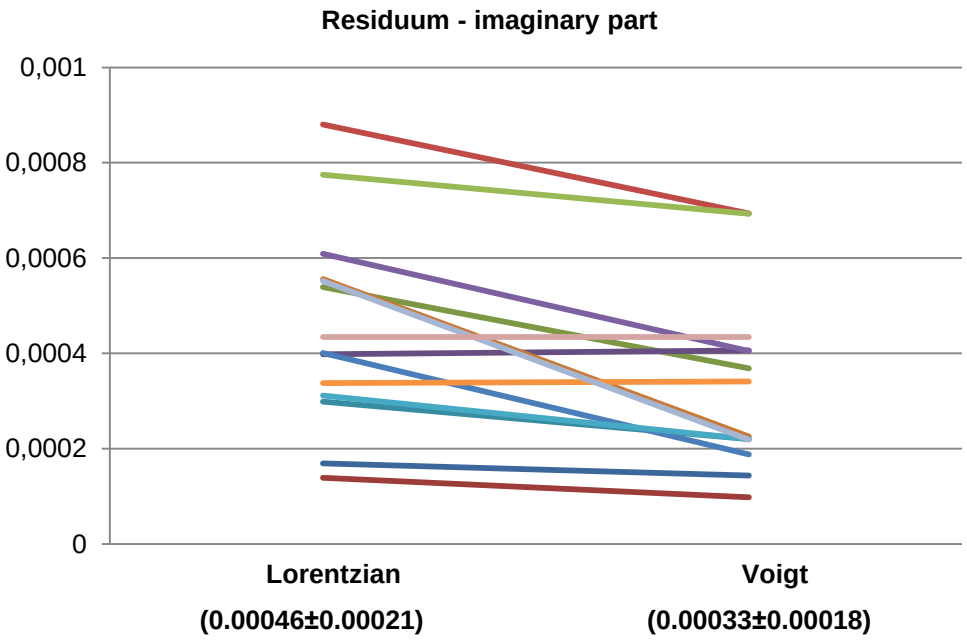

Figure 3.10. Residuum after Lorentzian (TD) and Voigt (TDFD) fittings, imginary part.

After statistical tests, significant differences in the real ($p<0.001$) and imaginary ($p<0.002$) residue values, generated by the two fitting approaches, were found (Tables A.1 – A.6). In both cases, Voigt line shape fitting approach originates lower values. Integral results of statistical t-test are presented in Appendix A.

CHAPTER 4

DISCUSSION

The potential of Magnetic Resonance Spectroscopy (MRS) as a means of investigating the metabolism of living tissues are well known and is extremely relevant. However, many challenges still exist regarding the quantification of the signals obtained by spectroscopy. The aim of this project was to develop a read-in routine that reads TWIX Siemens MRS data to be incorporated into the BMRU´s software *bmru_mrsw*. The validation and demonstration of increased accuracy over other existing software was also an objective pursued.

The programming language used to develop the read-in routine, namely IDL, was chosen because it is the language that is used for the software *bmru_mrsw*. Throughout the process of development the language proved to be adequate to the needs and allowed a clear and efficient writing of the algorithms.

Due to the fact that there was no official documentation of the Siemens TWIX data format, it was necessary to reverse-engineer the Matlab c-code read_meas_dat.m, whose purpose is to read this type of data, and post it into IDL. Thus, initially a detailed study of the content and structure of Siemens TWIX files was made. It was then possible to notice how the TWIX files are organized in a standardized way, as shown in Figure 3.1 of the results. This step was crucial for the development of the read-in routine. In programming terms, the first binary line, ASCII header and ADC lines spectroscopy in binary data were the key elements of TWIX files to build the routine. The ASCII header contains the parameters of interest that are required to calculate the dimensions of the spectroscopy data contained in the file, including number of points, number of coils, number of slices, number of repetitions and the number of averages. The data is stored in a 2D array, because it is the most appropriated form for subsequent processing. The reading of the first binary line allows to know the number of bytes that is necessary forward to a position in the file at which the binary data starts, where is allocated the data of interest - spectroscopy data. Finally the ADC spectroscopy lines are the lines containing the relevant date for this study.

In terms of routine´s aim, it was successfully achieved once it results from the routine the return of the unprocessed time-domain MRS data together ready to be read by *bmru_mrsw* software.

14 ^{1}H-MRS data sets were analyzed using the software jMRUI in order to subsequently carry out a comparison between this software and the *bmru_mrsw*. The obtained results using jMRUI were compared with those previously obtained by Rial *et al*. (2011). This comparison was made using the Bland-Altman plot for water amplitude values, once it is a method for comparing two different measuring techniques. The obtained mean value of 0.06, for a limit of agreement of 95%, i.e. 1.96 x SD, is satisfactory and translates good approximation between the both analysis software. This difference occured due to the fact that the used begin time in the analysis was different, i.e. a correct begin time of -

1.1 ms was used, while Rial used a begin time of 0 ms. However, as observed by the correlation curve shown in Figure 3.6, there was a good agreement between the values obtained for water amplitude, translating into an R^2 value of 0.997. Thus the values can be used as a reference for future results.

In order to validate the developed read-in routine, a pilot analysis was conducted on the same 14 human cardiac ^{1}H-MRS data sets using *bmru_mrsw* software. Initially, when was used an approach for fitting in the time domain, using Lorentzian line shape, a good linear correlation was followed between the results and those previously obtained using jMRUI, for water amplitude, with a correlation coefficient of 0.716. The deviation is most likely caused by differences in data pre-processing and scaling.

After a successful validation of the routine, analysis of the 14 ^{1}H-MRS data sets were conduted, this time using a TDFD fitting approach, since it is a differentiating capacity of *bmru_mrsw* software. In this approach, the fitting was carried out using Voigt line shape, with an initial contribution of 50% of Gaussian line. This method yielded a fitting residue, both real and imaginary, smaller than the ones generated using the TD approach, as proved by the statistical tests. This decrease can be seen in the graphs presented in Figure 3.9 and Figure 3.10. It was thus possible to demonstrate the advantage of the approach TDFD using the Voigt profile in the analysis of MRS data.

CHAPTER 5

CONCLUSIONS

This project successfully established and validated a routine to read Siemens MRS TWIX data, which was incorporated into the software *bmru_mrsw*. This allows now to use *bmru_mrsw* for analyzing clinical MRS data. It was also possible to demonstrate the potential of the TDFD fitting approach, the key approach present in *bmru_mrsw* software, by comparing it with another, commonly used fitting approach.

Thus, the extension of *bmru_mrsw* software compatibility to Siemens TWIX format represents an important step for the further improvement of techniques for quantification of MRS signals.

CHAPTER 6

FUTURE RESEARCH

This project, although being isolated with restricted objectives, is part of a global project that aims to create a novel analysis framework for MRS data. Thus, after creating a read-in routine for Siemens TWIX data and incorporated it into *bmru_mrsw* software, the next step will be to adapt this routine to read DICOM data. Compatibility with the DICOM format is essential in any treatment and analysis software for data acquired by imaging techniques, since this is an universal format used in medicine throughout the world.

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APPENDIX

Appendix A

The following tables present the results obtained after conducting the statistical test t-test, demonstrating the existence of significant differences between the residuum originated using Lorentzian and Voigt line shapes in *bmru_mrsw* software. All statistical analysis was evaluated using software SPSS 19 for windows.

Table A.1. Paired samples statistics for real residuum values of Lorentzian and Voigt fitting approaches.

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Lorentzian_real	,000513543	14	,0002292964	,0000612820
	Voigt_real	,000392357	14	,0001904925	,0000509113

Table A.2. Paired samples correlations for real residuum values of Lorentzian and Voigt fitting approaches.

		N	Correlation	Sig.
Pair 1	Lorentzian_real & Voigt_real	14	,886	,000

Table A.3. Paired sample t-test between real residuum results obtained with Lorentzian and Voigt line shapes.

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	99% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Lorentzian_real - Voigt_real	,0001211857	,0001069854	,0000285930	,0000350556	,0002073158	4,238	13	,001

Table A.4. Paired samples statistics for imaginary residuum values of Lorentzian and Voigt fitting approaches.

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Lorentzian_imaginary	,000457164	14	,0002112543	,0000564601
	Voigt_imaginary	,000332614	14	,0001847299	,0000493711

Table A.5. Paired samples correlations for real residuum values of Lorentzian and Voigt fitting approaches.

		N	Correlation	Sig.
Pair 1	Lorentzian_imaginary & Voigt_imaginary	14	,834	,000

Table A.6. Paired sample t-test between imaginary residuum results obtained with Lorentzian and Voigt line shapes.

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	99% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Lorentzian_imaginary - Voigt_imaginary	,0001245500	,0001167843	,0000312119	,0000305311	,0002185689	3,990	13	,002