CHAPTER 1

INTRODUCTION

Chapter Highlights

- ***Magnetic Resonance***
- ***Magnetic Resonance Spectroscopy (MRS)***
    - ***Single Voxel Spectroscopy (SVS)***
    - ***Magnetic Resonance Spectroscopy Imaging (MRSI)***
    - ***Important Biochemicals (Metabolites and Macromolecules)***
- ***Overview Of the Research Problems***
- ***Thesis Objectives***
- ***Thesis Contribution***
- ***Thesis Organization***

1.1. Magnetic Resonance: An Introduction

The method of observing the stimulated absorption and emission of energy from nuclei put under a magnetic field has historically been referred to by several different names and abbreviations. This phenomenon was referred to as nuclear induction in the original 1940s physics literature and as nuclear paramagnetic resonance in the early 1950s [1-3]. The preferred terminology for this identical physical process has been nuclear magnetic resonance (NMR) since the late 1950s [4]. The phrase "NMR imaging" was used to refer to early imaging techniques that used the NMR signal [4]. By the middle of the 1980s, the phrase "nuclear" had been generally abandoned when referring to these imaging techniques, at least in part because of patients' worries over the risks of nuclear energy, radioactive radiation, and similar phenomena. The preferred name for this novel radiologic technology has been magnetic

resonance imaging (MR imaging), or simply MRI. The vocabulary has since been further broadened to include concepts like functional magnetic resonance imaging (fMRI), magnetic resonance spectroscopy, and magnetic resonance angiography (MRA) [5].

Magnetic resonance is a resonant effect which appears when a magnetic dipole (protons/nuclei) is under the exposure of a static magnetic field (B_o) with another magnetic field of oscillating nature (B_1). Protons/ nuclei possess angular momentum due to their intrinsic quantum property of 'spin'. Under static magnetic fields, the protons experience a torque perpendicular to both the field and the direction of momentum. This results in a circular motion called 'precession' and the frequency of this precession is directly proportional to the strength of B_o. The famous Larmor relationship comes from it as: Larmor frequency, $f_0 = \frac{\gamma B_0}{2\pi}$, where γ is gyromagnetic ratio and is specific to size, mass, and spin of the particle in consideration [6-9]. The generation of MR signal, a small current produced due to precession of the net magnetization (M) under resonance, is a direct manifestation of Faraday's Law of Induction. The phenomenon of MR signal generation can be summarized as:

- Each nucleus of a particle has a characteristic wave function, comprising low-energy and high-energy spin states.
- Protons/nuclei shows precession at their specific Larmor frequency (f_o), when placed under an external B_o.
- At static equilibrium, the wave function distribution of the system prefers a low energy state resulting in a net magnetization (M) along B_o.
- When this system is stimulated with a strong, short time, oscillating transverse magnetic field (B_1) matched at f_o, magnetic resonance occurs. The B_1 field is called RF-pulse.

- Now the net *M*, aligned with B_o previously, gets tipped and precess around B_o developing a transverse component (M_{xy}). The z-component of the net magnetization is the longitudinal component, M_z.
- When RF-pulse B_1 is turned off, *M* continue to precess around B_o till it again reaches the equilibrium state. The change in transverse components, as it oscillates back to ground state, causes magnetic flux changes which is captured by a receiver coil. According to Faraday-Lenz induction principle, this generates a small current signal in the receiver coil and is referred as 'NMR' or simply 'MR signal'.

According to Bloch, these signals would decay due to interaction between spin system nuclei and the surroundings, by releasing its excitation energy to reach equilibrium state. This was termed as relaxation. In 1946, Bloch proposed relaxation time constants to account for the net magnetization to reach the equilibrium after MR signal generation: *T1* (to account for the growth of M_z to reach its initial value), and *T2* (to characterize for M_{xy} decay). Both the relaxations were assumed to follow first-order kinetics of exponentials describing the transverse and longitudinal magnetization in equations 1.1, 1.2 with corresponding diagram below (Figure 1.1), respectively:

$$M_{xy}(t) = M_0 e^{\frac{-t}{T2}} \tag{1.1}$$

$$M_z(t) = M_{xy0}\left(1 - e^{\frac{-t}{T1}}\right) \tag{1.2}$$

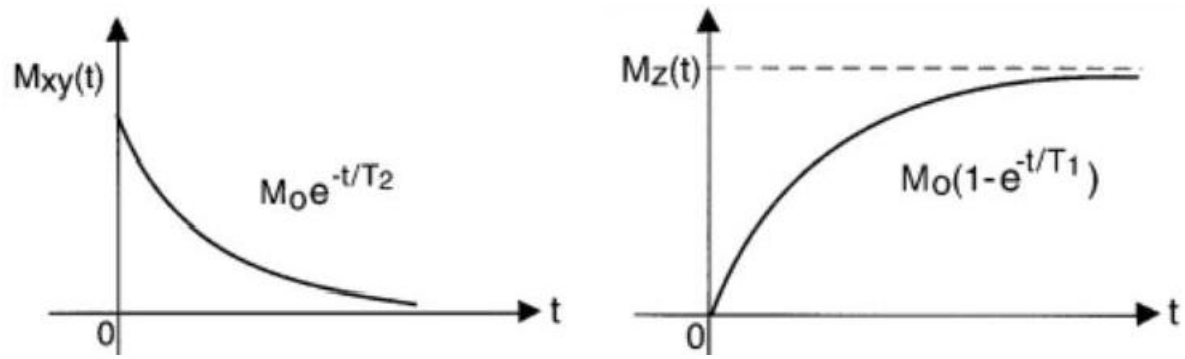

Figure 1.1: *Left:* Transverse magnetization plot; *Right:* Longitudinal magnetization plot

Here, M_0 is the initial magnetization along B_0, and M_{xy0} is the transverse magnetization at t=0. This implies that M_{xy} along xy-plane reduces to zero and M_z keeps growing till it reaches along z-axis (in parallel). Due to B_0-inhomogeneity in MR scanner, effective relaxation along transverse plane (xy) is different to T2, and referred commonly as, T2*. For homogenous B_0, T2=T2*, and otherwise, the relation is T2*<T2.

There are some atomic nuclei which possess non-zero net spin quantum number of which Hydrogen/Proton (^{1}H), Carbon (^{13}C), Sodium (^{23}Na), and Phosphorous (^{31}P) are prominent [10]. The human body has an abundance of ^{1}H making ^{1}H-MR the most prominent among others. The Larmor frequency for ^{1}H equals f_o=42.6 MHz/T [11].

1.1.1. Magnetic resonance spectroscopy (MRS)

MRS is a non-invasive, ionizing radiation-free analytical MR technique to obtain information about a range of biochemicals, usually referred to as "metabolites" and “macromolecules” signals, from MR scanner. Proton MRS (^{1}H) is the most common technique used for tissue characterization of an organ in clinical setup complementing with MRI, which provide anatomical information. The signals obtained are the sum of damped exponentials in time domain, called free induction decay (FID) [12]. Each individual exponential is associated with a particular ^{1}H nucleus of resonant frequency, uniquely defined by the local magnetic field depending upon the environment in which the molecule resides. The difference in these resonant frequencies is termed as ‘Chemical Shift’ [13-15] and is the main basis of differentiation among metabolites in MRS. It is defined as:

$$f_{ppm} = \frac{(f_{Hz} - f_{ref}) \times 10^6}{f_{ref}} \qquad (1.3a)$$

The chemical shift or the resonant frequency of individual metabolite is presented in the units of parts per millions (ppm) to discard the dependence on the spectrometer frequency. It is calculated by transforming as:

$$f_{ppm} = \frac{f_{Hz} \times 10^6}{f_s} + f_{ppmref} \qquad (1.3b)$$

Here, f_{Hz}: resonant frequency in Hz, f_s: spectrometer frequency in Hz, f_{ref}, f_{ppmref}: Hz, ppm value of reference carrier frequency respectively, in general, at water resonance of 4.7 ppm in ^{1}H-MRS.

When Fourier transform is performed on the FIDs, it unravels the individual biochemical components into their corresponding peaks at specific chemical shift location (Figure 1.2).

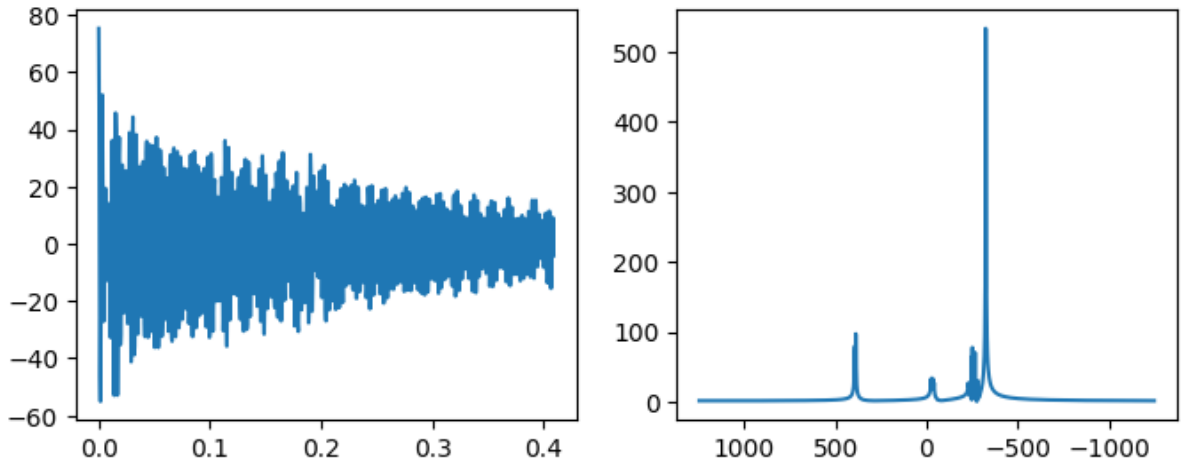

Figure 1.2: MR acquisition of the metabolite NAA. *Left*: real part of FID of metabolite 'NAA' (time-domain). *Right*: Real part of the Fourier transformed metabolite 'NAA' peaks.

MRS acquisitions comprising metabolite peaks are used to estimate concentration by calculating the area of the peaks present in the signal. These estimated values of concentrations (absolute or relative) are used to examine pathologies like metabolic changes in brain tumors, strokes, seizure disorders, Alzheimer's disease, depression, and other conditions that affect the brain. Other than brain, MRS has also found applications in other organ specific studies, such

muscles, liver, breast. In addition to the more popular magnetic resonance imaging (MRI), magnetic resonance spectroscopy is a complementary method that can be used to characterize tissue [16-18].

1.1.2. Single Voxel MRS and MR Spectroscopic Imaging

Based on the coverage area, MR spectra are acquired in different ways of which Single Voxel Spectroscopy (SVS) and Magnetic Resonance Spectroscopic Imaging (MRSI), also called Chemical Shift Imaging (CSI) are important. SVS is the acquisition technique which takes a single voxel/volume of interest from a small tissue region (spatial resolution ~ 1 cm^3) and produce a clean with good SNR spectrum from it. MRSI/CSI gives advantages over SVS by mapping over a multi-voxel slab for a larger coverage area and can provide a set of multiple signals from diverse tissue types in a single scan, which would be better in differentiating lesions like tumor [19-23] (See Table 1).

Table 1.1: A comparison between SVS and MRSI acquisition technique

Parameter	SVS	MRSI/CSI
Operation set up	Simple and fast	Difficult and slower than SVS
Field Shimming	Good shimming achievable because of small volume of interest.	Poor shimming covering entire region of interest
Spectral peak quality	High SNR achievable	Very low SNR

Contamination in spectra	Due to effects of partial volume and chemical shift displacement	Spectral bleeding due to aliasing from adjacent voxels
Acquisition time	3-5 minutes per voxel	5-15 minutes depending on resolution (2D/3D)
Application type	Homogenous lesions of medium size in large organs	Heterogenous lesion in large organ or small organ lesions

1.1.3. Acquisition parameters

There are several other parameters to consider before an MRS protocol is defined for acquisition of FIDs using either SVS or MRSI. The protocol parameters can be defined as:

a) Pulse Sequence: It is the design of the set of sequences in which the order and number of RF pulses, are to be used for voxel selection. The combination of RF pulses used determine the orientation of slice to be selected under the scanner. The most common pulse sequences for ^{1}H-MRS in clinical set up are: i) Point-RE Solved Spectroscopy (PRESS): it consists of a 90° RF-pulse followed by two 180° pulse along the x, y, and z coordinate gradients to obtain cuboidal voxel. This sequence can be used in SVS as well as CSI acquisitions by just adding a phase-encoding gradient to the sequence [24]. ii) STimulated Echo Acquisition Mode (STEAM): It uses three 90° pulses with x, y, z coordinate gradients applied concurrently to obtain cuboidal voxel where the slices overlap [25]. iii) Inversion Recovery: it applies a 180° RF pulse first, followed by a 90° pulse. The first pulse inverts the magnetization and the following pulse sets the longitudinal magnetization in the xy-plane to be detected later by receiver coil [26].

This sequence is used to null metabolite contributions to measure macromolecular and lipid components. the time taken to recover from 180° pulse to the start of 90° pulse is called recovery time (TI).

b) Echo time (TE) and Repetition time (TR): These parameters are important parts of a pulse sequence design. TE represents the time between first pulse and start of data acquisition (in milliseconds) or the time between RF pulse and corresponding echo, and TR is the time between consecutive RF pulses or echoes.
c) Number of Averages: it is needed to define the minimum number of acquisitions in a protocol to average for an expected SNR required for a specific study.
d) Number of data points: the spectra to be acquired is required to sampled for a fixed number of points for any analysis.
e) T1 and T2: these are magnetization times in longitudinal and transverse planes under the effect of a pulse sequence of RF-pulses. These are explained in above sub-section.

1.1.4. Metabolites and Macromolecules

In clinical settings, ^{1}H-MRS is the most preferable technique for diagnosis or pathological assessment of a patient especially in brain related analysis. From previous studies, it has been reported that after a good water suppression routine, and Fourier transforming the FIDs, around 16-18 metabolites peaks and 12-17 macromolecules peaks overlapping the metabolites in the same ppm range can be characterized from a 3T MR acquisition. Each biochemical has a unique signature of resonance which places it at a specific position on a chemical shift (ppm) scale and has important role in metabolism and disease related profiling of an organ [16-18, 27]. A brief information about metabolites and macromolecules has been presented below.

1.1.5. ^{1}H-MRS metabolites of interest

Some of the clinically relevant metabolites generally studied in diagnosis or pathological progression in brain disorder are given below with their main resonance and role.

- Alanine (Ala): It is an amino acid having the main resonance at around 1.4 ppm. It is linked with glycolysis cycle and related to tumor growth such as glioma in brain.
- Aspartate (Asp): It is an excitatory neurotransmitter with resonance peak at 3.89 ppm. It is important in urea cycle and participates in gluconeogenesis.
- Choline (Cho), Phosphorylcholine (PCho), Glycerophosholyl choline (GPC): The combination of the three compounds is denoted as total choline (tCho). It contributes to myelin and fluid-cell membrane structures in brain and has MRS resonance peak at 3.2 ppm. Increased tCho level in brain indicates of cancer growth, multiple sclerosis, trauma etc, whereas reduced concentration is the indicator of stroke or liver diseases.
- Creatine (Cr), Phosphocreatine (PCr): The main peak is at 3.03 ppm and the two compounds are practically difficult to separate because of very short *T2* time and overlap with nearby metabolite peaks. These compounds server as the energy delivery buffers from mitochondria to sites of energy consumption like nerve ending, myofibrils. These are associated with chronic pathologies of tumor having lower levels of concentration than normal.
- ɣ-Aminobutyric acid (GABA): It is an important inhibitory neurotransmitter with a peak resonance at 3.0 ppm. It is one of the metabolites which is difficult to estimate from a clinical MR spectrum due to overlap with other metabolite peaks. Depression related issues, seizures, bipolar disorders see reduced level of GABA in the patient.
- D-Glucose (Glc): It is a universal energy source (provides in the form of phosphates). It has resonance peaks at 3.88 ppm, 5.22 ppm and it is elevated in patients with

Alzheimer's. It has found application in ^{13}C-MRS as well for metabolic pathways related study.

- Glutamine (Gln): It has resonance peaks in 2.2-2.4 ppm range and at low magnetic field, Gln is close to Glu making the quantification difficult. It is important precursor to Glu storage.
- Glutamate (Glu): It is the most abundant excitatory neurotransmitter with peaks in 2.2-2.4 ppm range. The combined peak of Gln and Glu, referred as Glx, can be accurately quantified, where increased levels correspond to bipolar disorders and reduced levels relate to depression cases. It is an important precursor to GABA and glutathione synthesis.
- Glycine (Gly): Exhibiting a peak at 3.55 ppm, this neurotransmitter is in proximity with myo-inositol. It shows elevated levels in brain tumor cases.
- Lactate (Lac): It is produced in anaerobic glycolysis with peak at 1.31 ppm. It is found elevated in cases of tumor, hypoxia, and stroke.
- Myo-inositol (mI): It has a peak at 3.52 ppm and related to osmotic regulation and glucose storage. Mild cognitive damages, brain injury and Alzheimer's patients shows elevated mI.
- N-acetyl aspartate (NAA): This biomarker is spread through the brain and is related to neuronal functionalities. Its resonance peak is at 2.01 ppm and its concentration changes with neuronal dysfunction, tumor, stroke etc.
- N-Acetylaspartylglutamate (NAAG): Combined with NAA, it gives an overall estimate of NAA-containing compounds showing neuronal integrity. It has the resonance peak at 2.04 ppm, shouldering to NAA.
- Phosphorylethanolamine (PE): It has multiple peaks at 3.22 ppm and 3.98 ppm.

- Taurine (Tau): it has resonance peaks at 3.25 ppm and 3.42 ppm, and is responsible to modulate neurotransmitters. Its concentration reduces with age and the peak overlaps with mI and Cho peaks.

1.1.6. Macromolecules of interest in ^{1}H-MRS

^{1}H MR spectra of human brain manifest line-broadened signals of macromolecules (MM) and lipids (Lip) at short echo-time (TE) underlying the metabolite components of potential interests. MM signals in human brain find their origin from different amino acid protons, and have shorter T_1 and T_2 times compared to the metabolites. In frequency domain representation, these components overlap with the metabolite peaks complicating the parameterization of the metabolites as well as the macromolecular peaks. The standard nomenclature protocol for an MM peak is $M_{xx,}$ where subscript 'xx' implies the resonant frequency of a component in ppm. At short TEs, these peaks cover the whole frequency range underlying the metabolites and the variation in these MMs have been shown to be related to tumors, aging, stroke, multiple sclerosis [27]. The MM components of interest are:

- $M_{0.94}$: It has a peak at 0.94 ppm and the probable biochemicals contributing are Leucine, Isoleucine and valine.
- $M_{1.22}$: Threonine is the amino acid responsible for this peak at 1.22 ppm.
- $M_{1.43}$: At 1.43 ppm, Alanine is the MM component seen in the spectrum.
- $M_{1.70}$: This is the nomenclature given to a combination of amino acid peaks of Lysine, Arginine, Leucine at 1.63 ppm, 1.68 ppm, and 1.81 ppm.
- $M_{2.05}$: This is the combination of glutamine and glutamate peaks at 1.99 ppm and 2.04 ppm.
- $M_{2.27}$: This peak at 2.27 ppm is also the combined result of glutamine and glutamate.

- $M_{2.54}$: This peak resonance at 2.54 ppm is contributed by β-methylene protons of aspartyl groups.
- $M_{3.00}$: Lysine is the compound responsible for this peak at 3.00 ppm.
- $M_{3.21}$: Valine-H_{β}, αCH protons of protein amino acids are responsible for the peaks at 3.11 ppm, 3.22 ppm, 3.27 ppm, which presents as a single broad peak named as $M_{3.21}$.
- $M_{3.71,3.79}$ and $M_{3.97}$: These peaks at 3.71 ppm, 3.79 ppm, 3.97 ppm are because of the αCH protons of protein amino acids.

1.2. Thesis Objectives

At clinical strength acquisitions (in general, B_0=3T), MRS/MRSI spectra are highly degraded with low SNR, overlapped with macromolecular and residual water baseline. This makes the task of non-invasive quantitation post-acquisition quite difficult. Also, traditional approaches like standard curve-fitting lacks in scalability factor and capturing inter-spectral information especially when spectral data size is large and multiple voxels have been used from an ROI to obtain spectra, like MRSI acquisitions for tumor area localization. The main objective of this thesis is to design and develop novel post-acquisition scanner-independent methods in terms of viability for the analysis of degraded MR spectra systematically to improve non-invasive quantitation of biomarkers. In this thesis, different approaches using wavelets, machine/deep learning models have been presented to efficiently tackle these issues, thereby, improving the quantitation accuracy of biomolecules of concern.

1.3.Thesis Contribution

In this thesis, wavelets, machine- and, deep-learning based methodologies have been designed to address the issues of noise, MM contribution and isolation from the spectra and parameterization of each biomarker and creating models which are able to learn from wavelet-based feature descriptors to achieve an efficient model for MRS spectral peak estimation from a post-acquisition data.

The traditional approaches used till date have some shortcomings: (i) the analysis is performed on the spectra sequentially one at a time, which means these methods are unable to learn inter-spectral relationships and dependencies among the voxel's information acquired from different location of an organ, if present, or learn temporal patterns from different spectra to assign strong weightage based on importance of certain feature sets relevant across dataset and supressing the redundant features by weak weighting. (ii) for large dataset, evaluation and optimization of a traditional method is difficult and may require manual intervention and time-consuming.

Machine/Deep learning based proposed approaches, working along with wavelet feature descriptors in the current thesis, can counter these issues mentioned in the above section by handling multiple spectra at once; optimization over large or new dataset is less intensive and at the same time learning inter-spectral relationships from a large dataset to capture even compounds with small peaks from the spectra and discriminate from the noise/artifact background thereby increasing the overall efficiency of our proposed model. Also, in cases where patients' data of multi-voxel MRS is available, it can act as a classifier for identification of area/voxel based on specific compound concentration/peak amplitude helping in mitigating voxel contamination.

1.4. Thesis Organization

The thesis has been organized in following components which vividly discuss each of the objectives mentioned above.

Chapter 1 gives an introduction about the quantum mechanical phenomena of magnetic resonance, and magnetic resonance spectroscopy, the generation of MR signals, parameters to define a standard protocol for signal acquisitions, the composition of signals, and their respective characteristics relevant to pathological assessments and diagnostics.

Chapter 2 introduces MR spectroscopy signals in post-acquisition set up. This chapter provides a brief background and relevance of each approach used during different studies from MR signal acquisition to quantitation. Also, a review of the literature relevant and helpful to our studies have been presented in this section.

Chapter 3 discuss development of a novel approach to removal of echo and denoising the MR spectra using sparsity-based thresholding over wavelet coefficients. RADWT was used as the transform for multi-scale decomposition of spectra and norm-based sparsity metric was implemented to determine the thresholding criterion for efficient and robust noise and spurious echoes removal.

Chapter 4 focusses on a very important issue of macromolecular baseline overlap over the metabolite peaks throughout the spectral range which makes the quantitation of individual biomolecules difficult. A gradient boosted regression tree ensemble model for post-acquisition macromolecular baseline isolation using wavelet features of Brain MR spectra was designed to isolate MM spectra from acquired MR signal. The effective ideas of norm-based measures over wavelet coefficients, inferred from previous study was also incorporated while designing the machine learning model architecture for this study.

Chapter 5 builds on the advantages and knowledge gained from previous studies and combines them within a framework of deep learning architecture with the aim to achieve a complete

parameterization of MR spectra by estimating individual metabolite and MM components peak characteristics.

Chapter 6 gives a conclusion to the studies undertaken towards the goal of achieving an effective, noise robust, and efficient strategy for accurate quantitation of all possible biomolecules present in an MR spectrum, and presents some of the probable future paths which could be taken forward from the work presented in this thesis.