Water Diffusion in Soft Matter by Means of NMR: from Conventional to Anomalous Behaviour

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by

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Dedicated to
Mum and Dad
**Abstract**

Molecular diffusion is the principal phenomenon regulating the system dynamics in soft matter. Self-diffusive properties of simple fluids in bulk solutions are fully characterised by the diffusion constant, which reflects their mobility under the assumption of a linear dependence between the mean squared displacement and the diffusion time. In the framework of the classical Brownian theory, this is based on the hypothesis that the probability of molecular displacements follows a Gaussian distribution in an isotropic regime. Conversely, dynamics within complex media may be hindered by several factors that can affect the diffusive motion.

Estimation of the effective diffusion properties of fluids in random heterogeneous media is a frequent task in materials and biological physics, since knowledge about the environment the molecules are diffusing in may be indirectly gathered. Proton Nuclear Magnetic Resonance (NMR) can be made sensitive to dynamical displacements of water molecules between 10 nm and 0.1 mm, thus offering a unique tool to address a wide range of problems, encountered in material sciences, in a non-invasive way.

In the last years, diffusion NMR has been applied to characterise anomalous dynamics on several systems. Nevertheless, the literature regarding this very specific branch of science is often unsatisfactory and incomplete. On one hand, anomalous diffusion formalism was developed mostly from the theoretic point of view; on the other hand, the stretching exponential model was borrowed for medical applications as a phenomenological tool to reach a better agreement with the measured decay curves. Such shortcomings are indeed possible since NMR is a widely interdisciplinary technique.

Aim of this thesis was to characterise water anomalous diffusion dynamics, and other related issues linked to diffusion, in a formal, consistent and comprehensive fashion. For this purpose, water dynamics in selected systems were characterised by means of spectroscopic and imaging diffusion measurements. Measurements were performed on a high field tomographer (9.4T), using strong magnetic field gradients and high-resolution probe, as well as on a clinical scanner (3T) devoted to research. Data has been analysed with both conventional post-processing software and home-made scripts written *ad hoc* in Matlab.

Three systems with different topological features were considered: styrene aqueous suspensions at high sphere packing, bone marrow in the trabecular network and in diaphysis and eventually human brain investigated *in vivo*.

Styrene suspensions at different beads concentrations were used to clarify the link between local geometry/susceptibility of the media and the stretching parameters, which are introduced to account for the non-linearity of the relationship between the mean squared displacement and the diffusion time.

Bone marrow was investigated by means of both conventional NMR technique, to assess the role of internal gradients due to susceptibility differences at interfaces, and anomalous diffusion protocol, which allows to calculate the stretching parameter. The stretching parameter turned out to be in turn dependent on the internal gradient, providing an innovative contrast tool to characterise trabecular bone.
Finally, the stretching parameter was used as a new source of contrast to highlight micro-structural property of cerebral tissue. In parallel, a new method to account for media anisotropy in the calculation of the stretching exponent was proposed, particularly suitable to brain investigations. The combination of the stretching exponent proposed in literature and the innovative approach to media anisotropy provided a promising tool to achieve a comprehensive characterisation of cerebral white matter.

The results reported in this thesis ambitiously aim at filling the gap between anomalous dynamics paradigms proposed by theoreticians and experimental approaches followed by medical scientists. At the same time, the stretching parameters as defined and characterised in this work open new interesting perspectives for the early diagnosis of muscle-skeletal pathologies and the characterisation of brain microstructure in both normal and abnormal development.
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## Contents

### Introduction

1 Basic NMR principles

1.1 Quantum-mechanical approach

1.1.1 Density operator and expectation values

1.1.2 Populations and Coherences

1.1.3 The Liouville-von Neumann equation and time evolution of a physical variable

1.1.4 Spin Dynamics

1.1.5 Relaxation Theory

1.2 Semi-classical formalism

1.2.1 The rotating frame

1.2.2 Relaxation: a phenomenological approach

1.2.3 Signal detection

1.3 Principles of Imaging

1.3.1 Conjugate Space

1.3.2 Selective Excitation

1.3.3 Image Reconstruction

2 Molecular Diffusion

2.1 Classic theory of Diffusion

2.1.1 Fick’s laws

2.1.2 Self-diffusivity

2.1.3 Brownian motion and Random walk

2.1.4 The Diffusion Tensor

2.2 Diffusion and NMR

2.2.1 The Spin Echo

2.2.2 Diffusion in the presence of a gradient

2.2.3 Pulsed Gradient Spin Echo

2.2.4 The q-space

2.2.5 Diffusion Tensor Imaging

2.3 Anomalous Transport

2.3.1 Continuous Time Random Walk

2.3.2 Subdiffusion

2.3.3 Superdiffusion

2.3.4 The competition between long rest and long jumps
## CONTENTS

3 From conventional to anomalous behaviour 51
   3.1 Dynamical ranges .................................. 52
      3.1.1 Multi-exponential decays ......................... 52
      3.1.2 Single tensor model ............................. 55
   3.2 Evidences of anomalous behaviour .................... 57
      3.2.1 Water diffusion decay in human brain .......... 57
      3.2.2 Anomalous diffusion in materials and biological matter 58
   3.3 The stretching exponential model ..................... 60
      3.3.1 Applications in brain ........................... 61
      3.3.2 Theoretical models ................................ 62
      3.3.3 Hall and Barrick’s approach to anisotropy .... 64
      3.3.4 Original contribution: Anomalous diffusion imaging .... 65

4 Experimental results I 69
   4.1 Internal gradients in spongy bone: role of diffusion ...... 69
      4.1.1 Problem statement ................................ 70
      4.1.2 Methods ........................................... 71
      4.1.3 Results ............................................ 76
   4.2 High b-values main frame in cerebral white matter .......... 85
      4.2.1 Problem statement ................................ 86
      4.2.2 Methods ........................................... 87
      4.2.3 Results ............................................ 90

5 Experimental results II 101
   5.1 Anomalous diffusion in Styrene beads .................. 102
      5.1.1 Problem statement ................................ 102
      5.1.2 Methods ........................................... 103
      5.1.3 Results ............................................ 105
   5.2 Anomalous diffusion in Bone Marrow .................... 109
      5.2.1 Problem statement ................................ 109
      5.2.2 Methods ........................................... 110
      5.2.3 Results ............................................ 111
   5.3 Anomalous diffusion imaging in Human brain ............. 114
      5.3.1 Problem statement ................................ 114
      5.3.2 Methods ........................................... 115
      5.3.3 Results ............................................ 116

Conclusions and Perspectives 127

Bibliography 129
Molecular diffusion is the principal phenomenon regulating the system dynamics at the mesoscopic lengthscales. In the past years, there have been numerous reports on the study of water in heterogeneous systems using a variety of techniques [1–5]. What makes the investigation of water dynamics so attractive to the scientific community is the possibility of obtaining indirect information on the environment in which diffusion takes place [6].

Diffusion Nuclear Magnetic Resonance (NMR) is nowadays the only technique able to measure diffusion in vivo in a non invasive way, having direct access to diffusivity timescales in the range of ten milliseconds up to seconds. The mean squared displacement due to translational diffusion of water ranges from some $\mu m$ up to several tens of $\mu m$, depending on the strength of the interaction between water molecules and the surrounding environment. For this reason, diffusion NMR is particularly suitable to investigate the differential behaviour of water in a variety of different environments, hence furnishing important pieces of information about the system under investigation.

Ideal systems, characterised by isotropy and homogeneity, in which the molecular displacements can be considered random with no memory effects, long rests or long jumps, are completely described by a single diffusion coefficient, as introduced by A. Fick in 1855 and by A. Einstein in 1905. However, real systems are often characterised by complex architectures in which water dynamics cannot be described simply by means of the classic formalism. In these cases, theoretical frameworks suitable to take into account the interaction with the environment have been proposed.

The aim of this work was to investigate water diffusion in complex environments and to demonstrate its capabilities for gaining insight into structure and dynamics of soft matter systems. Three systems with different topological features were chosen.

A simple two-phases system was realised by dispersing styrene beads of different sizes in deionized water, achieving high concentration (70%) with the aid of a surfactant. In this system, water interaction with the beads is controlled by a limited number of measurable parameters and important insight can be gathered to validate the effect the environment geometry and properties on water molecules’ displacement.

The following step has been to investigate the interaction between water and fat molecules in a peculiar biological system, the bone marrow. Bone marrow can be regarded as a privileged biological system to investigate water diffusion, since it is a relatively simple two-phases system composed by water and several other particles of spherical/elliptical shape and average size ranging from $6 \ \mu m$ to approximately
100 µm. On the other hand, the characterisation of water diffusion can offer new insights on the bone marrow properties themselves, in the perspective of the search for alternative markers for bone pathologies diagnosis.

Finally, water diffusive properties were investigated *in vivo* in human brain. The central nervous system is doubtless regarded as a privileged system among all the other apparatuses of human and mammalian body, due to the special functions of which it is in charge. The importance of its role within the body is reflected by an highly complex microstructure, characterised by areas of homogeneous, bulk-like diffusion and areas of restricted, directional and tortuous diffusion. Besides, brain contains a high percentage of water (about 80%), hence water diffusion investigations can be of particular interest.

In this work, the approach to the proposed problems has been twofold: on one hand, a theoretical framework was proposed to account for anomalous dynamics in highly anisotropic environments by means of a rotationally-invariant description. On the other hand, experimental investigations by means of *ad hoc* diffusion sequences spanning several diffusion ranges were collected.

The overall conclusion was that conventional and unconventional diffusive dynamics could be used to obtain important pieces of information on a number of systems characterised by intrinsic complexity. On the basis of the findings reported in this thesis, new protocols can be developed to introduce new potential markers of specific pathologies/status, in order to obtain a more comprehensive characterisation of the investigated peculiar soft matter systems.
Chapter 1

Basic NMR principles

Nuclear Magnetic Resonance (NMR) spectroscopy and imaging is a powerful quantitative technique especially suitable to soft matter investigations.

The 1952 Physics Nobel Prize winners Bloch and Purcell independently carried out the first NMR experiments during the 1940s. In the following decades, NMR spawned several major breakthroughs. During the 1960s, NMR sensitivity was strongly improved with the use of short, intense, radio frequency pulses, which led to the development of Fourier transform NMR. In the 1970s, two-dimensional NMR emerged as a revolutionary technique. Ernst, 1991 Chemistry Nobel Prize winner, played a key role in these developments. In the 1970s, a major medical breakthrough came with magnetic resonance imaging (MRI), a technique relying on the water content of the sample to provide detailed images without invasion.

Nowadays, due to its capability of imaging soft tissue in vivo non-invasively with detailed anatomical resolution, it is successfully applied to monitor a number of pathological conditions and it is one of the most used medical imaging techniques. In parallel, it is applied to a large variety of soft, amorphous, inorganic, or organic supra-molecularly organised materials: in addition to providing a powerful structural characterisation tool, NMR spectroscopy has become invaluable in providing access to dynamic information, leading to essential mechanistic interpretations.

NMR technique is able to detect energy transitions between different levels. These transitions are induced by specific stimulations, i.e. electromagnetic fields that oscillate at the frequency that match the difference between the energy levels. Those levels are indeed produced by a static magnetic field, which is able to reveal the hyperfine structure of spin ensemble.

NMR deals with the nuclear spin that characterises paramagnetic atomic nuclei. As a matter of fact, nuclear spins are inherently quantum-mechanical in their behaviour. Besides, in NMR one usually is interested in exceedingly large numbers of nuclei ($\sim 10^{22}$), observing their mean response, which is determined by an average over the states of each individual spin. The correct description, which will be reviewed in the first part of the chapter, involves thus the properties of a quantistic ensemble of spins.

Notwithstanding, in the majority of the cases and specifically for the purpose of this thesis, the spins belonging to liquid-like systems can be regarded as weakly interacting, so that at the macroscopic level the collection of particles appears continuous. In such cases, the observables of interest may be treated with a semi-
classical formalism, without losing in information. This formalism is very easy to handle, especially when the specific design of an NMR experiment is concerned. The semi-classical formalism is reviewed in the second part of this chapter.

What made NMR so widespread is the fact that the signal depends on several measurable parameters that provide different chemical-physical information. Besides, the great success in the field of medical imaging is due to the possibility of encoding the spatial information within the signal, thus allowing images reconstruction. In the last part of this chapter, the basic principles that allow for the image reconstruction will be introduced.

1.1 Quantum-mechanical approach

Here, the properties of a quantistic ensemble of spins will be reviewed. A single spin, interacting with a static magnetic field, can be described by a ket $|\psi(t)\rangle$ that determines the possible spin states of the system. To obtain the time evolution of the system, one needs to apply the Hamiltonian operator to the state at $t = 0$ $|\psi(0)\rangle$. When dealing with a Zeeman interaction such as that of a single spin with the external magnetic field, it can be easily shown that the time evolution describes a precession of the spin about the static field.

To describe instead a system constituted by a large number of spin, it is mandatory to define the density operator, which introduces the concept of probability associated to each possible state within the range of allowed configurations.

1.1.1 Density operator and expectation values

In order to define the density operator $\rho$ of the quantum mechanical system and to derive its equation of motion, we start with the time-dependent Schrödinger equation for the evolution of a state function $|\psi(t)\rangle$,

$$\frac{d}{dt} |\psi(t)\rangle = -i\mathcal{H}(t) |\psi(t)\rangle \quad (1.1)$$

$\mathcal{H}(t)$ is the Hamiltonian operator of the system, which may itself be time-dependent. We may expand the state function $|\psi(t)\rangle$ in terms of a complete orthonormal base $|i\rangle$, $i = 1, 2, ..., n$

$$|\psi(t)\rangle = \sum_{i=1}^{n} c_i(t) |i\rangle \quad (1.2)$$

where the time-dependence of $|\psi(t)\rangle$ is expressed by the time-dependent coefficients $c_i(t)$ and $n$ is the dimension of the Hilbert space.

With regard to the definition of the density operator, two cases can be distinguished.
1.1. QUANTUM-MECHANICAL APPROACH

• In an idealized pure state, all the spin systems of the ensemble are in the same state and can be described by the same normalized state function $|\psi(t)\rangle$ with $\langle \psi(t)|\psi(t)\rangle = 1$. The corresponding density operator $\rho$ is defined by the product of the ket $|\psi(t)\rangle$ and the bra $\langle \psi(t)|$,

$$\rho(t) = |\psi(t)\rangle \langle \psi(t)| = \sum_i \sum_j c_i(t)c_j^*(t) |i\rangle \langle j|.$$  \hspace{1cm} (1.3)

• The situation is different for an ensemble in a mixed state, e.g. for an ensemble in thermal equilibrium. Here we can only indicate probabilities $p_k$ that a spin system of the ensemble is in one of several possible states $|\psi_k(t)\rangle$. The density operator is then understood as an average over the ensemble,

$$\rho(t) = \sum_k p_k |\psi_k(t)\rangle \langle \psi_k(t)| = \sum_k p_k \sum_i \sum_j c_k^i(t)c_k^{*j}(t) |i\rangle \langle j|,$$  \hspace{1cm} (1.4)

where $\sum_k p_k = 1$ and the bar denotes the ensemble average.

Because the state functions are assumed to be normalised, i.e. $\langle \psi | \psi \rangle = \sum_{r=1}^n |c_r(t)|^2 = 1$, the trace of the density operator is equal to unity

$$tr\{\rho\} = \sum_{r=1}^n \langle r | \rho | r \rangle = \sum_{r=1}^n \rho_{rr} = 1.$$  \hspace{1cm} (1.5)

The density operator in thermal equilibrium at temperature $T$ is given by

$$\rho_0 = \frac{1}{Z} \exp\{-\frac{H\hbar}{kT}\},$$  \hspace{1cm} (1.6)

where

$$Z = tr\{\exp\{-\frac{H\hbar}{kT}\}\}.$$  \hspace{1cm} (1.7)

is the partition function of the system. By evaluating $\rho_0$ in the eigenbasis $\{|r\rangle\}$ of the Hamiltonian, one easily verifies that the probability distribution of the eigenstates $|r\rangle$ correctly describes a Boltzmann distribution

$$\langle r | \rho_0 | r \rangle = \frac{1}{Z} \exp\{-\frac{E_r\hbar}{kT}\}.$$  \hspace{1cm} (1.8)

In NMR it is usually sufficient to calculate expectation values of a restricted set of
operators that act exclusively on nuclear spin variables. The remaining degrees of freedom are referred to as the 'lattice'. For the calculation of such expectation values, knowledge of the complete density operator \( \rho(t) \) is not required. It is sufficient to define a reduced spin density operator \( \sigma(t) \), which is obtained from \( \rho(t) \) by trace formation over all degrees of freedom of the lattice. If we write the basis function \( |i\rangle \) of the entire system as product of functions \( |f\rangle \), depending on the lattice variables only, and functions \( |s\rangle \), depending exclusively on the spin coordinates \( |i\rangle = |f\rangle |s\rangle \), the reduced density operator can be defined as

\[
\sigma(t) = \sum_f \langle f | \rho(t) | f \rangle = tr_f \{ \rho(t) \}.
\] (1.9)

The calculation of the expectation values \( \langle A \rangle \) of an arbitrary observable operator \( A \) is now straightforward. For normalized state functions:

\[
\langle A \rangle = \sum_k p_k(t) \left\langle \psi^k(t) | A | \psi^k(t) \right\rangle
\] (1.10)

can be expressed by \( \sigma(t) \)

\[
\langle A \rangle = \sum_k p_k(t) \sum_r \sum_s c_r^k(t) c_s^k \langle r | A | s \rangle
\]

\[
= \sum_r \sum_s \sigma_{rs} A_{rs},
\] (1.11)

leading to the expression

\[
\langle A \rangle = tr \{ A \rho(t) \}.
\] (1.12)

Thus the expectation value is found by evaluating the trace of the product of the observable operator and the density operator.

### 1.1.2 Populations and Coherences

Consider an ensemble of non-interacting spins-\( \frac{1}{2} \) in the presence of an high external magnetic field \( \mathbf{B}_0 = B_0 \mathbf{\hat{z}} \). The matrix representation of the corresponding density operator (the density matrix) is given by the following:

\[
\sigma = \begin{pmatrix}
\sigma_{\alpha\alpha} & \sigma_{\alpha\beta} \\
\sigma_{\beta\alpha} & \sigma_{\beta\beta}
\end{pmatrix} = \begin{pmatrix}
c_{\alpha} c_{\alpha}^* & c_{\alpha} c_{\beta}^* \\
c_{\beta} c_{\alpha}^* & c_{\beta} c_{\beta}^*
\end{pmatrix}
\] (1.13)

The diagonal elements of the spin density operator \( \sigma_{\alpha\alpha} \) and \( \sigma_{\beta\beta} \) are called the populations of states \( |\alpha\rangle \) and \( |\beta\rangle \), and are equal to the probability that the spin system is found in these states respectively. The off-diagonal elements \( \sigma_{\alpha\beta} \) and \( \sigma_{\beta\alpha} \) are called the coherences between states \( |\alpha\rangle \) and \( |\beta\rangle \) and can be associated with a transition between these states.

What is the physical interpretation of the components of the density operator, in terms of the microscopic states of the individual spins?
We start with the populations. Since the sum of the populations is always equal to one (eqn 1.5), only the difference in populations between the two states has physical significance. The difference in spin state populations indicates net longitudinal spin polarization, i.e. magnetisation of the sample in the direction of the field. If the population of the two states are equal, then there is no net polarization in the direction of the field.

The presence of coherences $\sigma_{\alpha\beta}$ and $\sigma_{\beta\alpha}$ indicates transverse spin magnetisation, i.e. a net spin polarization perpendicular to the external field. Coherences require the existence of spins which have transverse polarization vectors, i.e. spins which are in superposition states. However, this is not sufficient. For coherences to exist, the transverse polarizations must also be partially aligned. Polarization vectors which are uniformly distributed in the $xy$-plane provide no coherences. Since $\sigma_{\alpha\beta}$ and $\sigma_{\beta\alpha}$ are complex numbers, they have a phase as well as an amplitude. The phase of the coherences indicates the direction of the transverse spin polarization in the plain perpendicular to the external field. Thus for a statistical mixture of kets, the off-diagonal elements of the density matrix may vanish due to the random distribution of their phases in the mixture.

1.1.3 The Liouville-von Neumann equation and time evolution of a physical variable

The equation of motion for the density operator is given by the Liouville-von Neumann equation. The starting point for establishing this equation is the Schrödinger equation (1.1) for $|\psi\rangle$. The same equation for the conjugated bra is:

$$\frac{d}{dt} \langle \psi | = i\mathcal{H} \langle \psi (t) | .$$

We obtain therefore, for the time derivative of the projection operator on $|\psi\rangle$:

$$\frac{d}{dt} |\psi\rangle \langle \psi | = |\dot{\psi}\rangle \langle \psi | + |\psi\rangle \langle \dot{\psi} |$$

$$= -i\mathcal{H} |\psi\rangle \langle \psi | + i |\psi\rangle \langle \psi | \mathcal{H}$$

$$= -i[\mathcal{H}, |\psi\rangle \langle \psi |].$$

As this relation is true whatever the projection operator, it is also true for a linear combination of projection operators. We then obtain for the density operator its equation of motion

$$\frac{d}{dt} |\rho\rangle = -i[H, \rho],$$

called Liouville-von Neumann equation. Its formal solution may be written

$$\rho(t) = U(t)\rho(0)U(t)^{-1}; U(t) = T \exp\{-i \int_0^t \mathcal{H}(t')dt'\},$$

where the Dyson time-ordering operator $T$ [7] defines a prescription for evaluating the exponential functions in case where the Hamiltonian at different times do not commute \footnote{[$\mathcal{H}(t'), \mathcal{H}(t'') | \neq 0$]}. By selecting a suitable rotating frame, the Hamiltonian can often be
made time-independent within finite segments of time. In liquid-like systems, this is possible either using the so called hard pulses, i.e. RF pulses that move all the signals at different Larmor frequencies away from equilibrium, or under specific approximations, using soft pulses In these cases, the evolution can be expressed by a sequence of unitary transformations of the type

$$\rho(t + \sum_{k=1}^{n} \tau_n) = \exp(-i\mathcal{H}_n \tau_n) \ldots \exp(-i\mathcal{H}_1 \tau_1) \rho(t) \exp(i\mathcal{H}_1 \tau_1) \ldots \exp(i\mathcal{H}_n \tau_n), \quad (1.18)$$

with the propagator $\exp(-i\mathcal{H}_k \tau_k)$. In arrow notation [8] it runs

$$\rho(t) \xrightarrow{H_1 \tau_1, H_2 \tau_2, \ldots, H_n \tau_n} \rho(t + \sum_{k=1}^{n} \tau_n). \quad (1.19)$$

Now, let us consider a physical variable and its operator $O$. The time evolution of the average value of this operator is, according to eqn (1.16), given by:

$$\frac{d}{dt} \langle O \rangle = tr\{O \frac{d\rho}{dt}\} = tr\{O(-i[\mathcal{H}, \rho])\}. \quad (1.20)$$

By using the property $tr\{A[B,C]\} = tr\{[A,B]C\}$ this becomes:

$$\frac{d}{dt} \langle O \rangle = tr\{-i[O, \mathcal{H}]\rho\} = \langle -i[O, \mathcal{H}] \rho \rangle. \quad (1.21)$$

If we look now at the value of $O$ at time $t$, we have in the general case:

$$\langle O \rangle(t) = tr\{OU(t)\rho(0)U^\dagger(t)\} = tr\{U^\dagger(t)OU(t)\rho(0)\}. \quad (1.22)$$

In the particular case when $\mathcal{H}$ is time-independent, this become according to eqn (1.17)

$$\langle O \rangle(t) = tr\{\exp(i\mathcal{H}t)O \exp(-i\mathcal{H}t)\rho(0)\} \quad (1.23)$$

1.1.4 Spin Dynamics

In the following, we clarify the main terms that compose the spin Hamiltonian in the general case of an NMR experiment. Among the various atomic nuclei, about a hundred isotopes possess an intrinsic angular momentum (spin) $\hbar \vec{I}$ and a magnetic moment $\vec{\mu} = \gamma \hbar \vec{I}$ collinear with it. With few exceptions, the order of magnitude of these moments is between $10^{-3}$ and $10^{-4}$ Bohr magnetons. The coefficient $\gamma$, which characterise of each nuclear species, is called the gyromagnetic ratio. We will consider nuclear magnetic resonance experiments performed on diamagnetic samples, that is on substances without either spin or orbital electron paramagnetism.
1.1. QUANTUM-MECHANICAL APPROACH

Effect of the Zeeman interaction

The Zeeman Hamiltonian of a magnetic moment $\vec{\mu}$ in a static magnetic field $\vec{B}_0$ is:
\[ \mathcal{H} = -\gamma \vec{I} \cdot \vec{B}_0. \] (1.24)

The evolution of, say, the component $\mu_x$ of the magnetic moment is described by the equation:
\[
\frac{d}{dt} \langle \mu_x \rangle = \gamma \hbar \frac{d}{dt} \langle I_x \rangle = -i\gamma \hbar \langle [I_x, \mathcal{H}] \rangle = i\gamma^2 \hbar \{B_{0x} \langle I_z, I_x \rangle + B_{0y} \langle I_x, I_y \rangle + B_{0z} \langle [I_x, I_z] \rangle \},
\]
that is, according to the commutation relations characteristic of angular momentum operators:
\[
\frac{d}{dt} \langle \mu_x \rangle = -\gamma \{B_{0y} \langle \mu_z \rangle - B_{0z} \langle \mu_y \rangle \}. \tag{1.25}
\]

The term inside the curly brackets on the right hand side of the last line is equal to the component along $\hat{x}$ of the vectorial product $\vec{B}_0 \wedge \langle \vec{\mu} \rangle$. By generalizing the former result to the other component of the magnetic moment, we obtain:
\[
\frac{d}{dt} \langle \vec{\mu} \rangle = -\gamma \vec{B}_0 \wedge \langle \vec{\mu} \rangle. \tag{1.25}
\]

Each component of this moment has a large quantum uncertainty, but if we consider simultaneously a very large number of identical spins we know, from the central limit theorem, that their bulk magnetic moment $\vec{M} = \sum_i \langle \vec{\mu}_i \rangle$ has simultaneously well-defined components and can be treated as a classical vector. The evolution of this bulk momentum is given by
\[
\frac{d}{dt} \langle \vec{M} \rangle = -\gamma \vec{B}_0 \wedge \langle \vec{M} \rangle, \tag{1.26}
\]
which describes a precession of $\vec{M}$ around $\vec{B}_0$ of frequency equal to $-\gamma B_0$. The rotation (or precession) frequency:
\[
\nu_0 = -\frac{\gamma B_0}{2\pi} \tag{1.27}
\]
is called the Larmor frequency.

The chemical shift

The electrons in the molecules cause the local magnetic fields to vary on a submolecular distance scale. The magnetic fields experienced by nuclei at two sites in the same molecule are different if the electronic environments are different. Thus
the Zeeman interaction is modified by this chemical shielding. The modified Zeeman Hamiltonian of a nuclear spin is then:

$$\mathcal{H}_Z = -\sum_k \gamma_k \vec{I}_k (E - \vec{\sigma}_k) \vec{B}_0,$$

(1.28)

$\vec{\sigma}$ is a tensor, called the chemical shift tensor. In isotropic and homogeneous liquid-like systems, molecular rotations produce a variation in the course of time of the chemical shift tensor, which is so fast that the spins 'respond' only to the time average of the chemical shift Hamiltonian $\mathcal{H}_C = \gamma \vec{\sigma} \cdot \vec{B}_0$. Now $\sigma$ is a number called the chemical shift. It is generally expressed in p.p.m.

**Effect of a radio-frequency field**

In addition to a steady field $B_0$ applied along the direction $\hat{z}$, let us apply a second field $B_1$ much smaller than $B_0$, perpendicular to it and rotating around it at the frequency $\omega$. The Hamiltonian of a spin is

$$\mathcal{H} = -\gamma B_0 I_z - \gamma B_1 (I_x \cos \omega t + I_y \sin \omega t)$$

$$= \omega_0 I_z + \omega_1 (I_x \cos \omega t + I_y \sin \omega t),$$

(1.29)

where we use the notations: $\omega_0 = -\gamma B_0$ and $\omega_1 = -\gamma B_1$. In order to replace the time-dependent Hamiltonian, eqn (1.29), by a time-independent Hamiltonian, we use the method of the interaction representation. We define the unitary operator $U(t) = \exp(i\omega t I_z)$ and the transformed density operator $\tilde{\sigma} = U(t) \sigma U(t)$. Let us look at the equation of evolution of the transformed density operator $\tilde{\sigma}$; we have:

$$\frac{d\tilde{\sigma}}{dt} = \frac{d}{dt} U(t) \sigma U(t)^\dagger$$

$$= \dot{U} \sigma U(t)^\dagger + U \dot{\sigma} U(t)^\dagger + U \sigma \dot{U} U(t)^\dagger,$$

that is, according to eqn (1.15):

$$\frac{d\tilde{\sigma}}{dt} = i\omega I_z U(t) \sigma U(t)^\dagger - iU(t)[\mathcal{H} \sigma, \dot{U}] - iU(t) \sigma U(t)^\dagger \omega I_z.$$ 

It follows from the unitarity of $U(t)$ that:

$$\frac{d\tilde{\sigma}}{dt} = -i[\{\tilde{\mathcal{H}}, \omega I_z\}, \tilde{\sigma}].$$

(1.30)

In the new representation the evolution of the density operator $\tilde{\sigma}$ is the same as if the system were subjected to an effective Hamiltonian $\mathcal{H}_{eff} = \tilde{\mathcal{H}} - \omega I_z$. If one notes that in eqn (1.29) the time-dependent term on the right-hand side can be written:

$$I_x \cos \omega t + I_y \sin \omega t = \exp(-i\omega_0 t I_z) I_x \exp(i\omega_0 t I_z) = U(t) I_x U(t),$$

the effective Hamiltonian is:

$$\mathcal{H}_{eff} = (\omega_0 - \omega) I_z + \omega_1 U U(t) I_x U(t)^\dagger$$

$$= (\omega_0 - \omega) I_z + \omega U I_x U(t).$$

(1.31)
1.1. QUANTUM-MECHANICAL APPROACH

The passage to the interaction representation is equivalent to rotating the reference axes around \( \hat{z} \) at the frequency \( \omega \), as we will clarify later on. The evolution of the nuclear magnetic moment in the rotating frame is a precession around an effective field \( \vec{H}_{\text{eff}} \) with a frequency \( \omega_{\text{eff}} \) of components \( \omega_0 - \omega \) along \( \hat{z} \) and \( \omega_1 \) along \( \hat{x} \). In particular, when \( \omega_0 = \omega \), the effective field in the rotating frame reduces to \( H_1 \), i.e., it is purely transverse.

**Nonlinear interactions in the spin operators**

By now we have introduced the most important linear interactions in the spin operators. For the sake of completeness, in what follows we will briefly review some common bilinear and quadratic interactions in the spin operator.

The contribution of the direct dipolar coupling to the nuclear Hamiltonian has the form

\[
\mathcal{H}_D = \sum_{k<l} D_{kl} \{ \vec{I}_k \cdot \vec{r}_l - 3\frac{(\vec{I}_k \cdot \vec{r}_l)(\vec{I}_l \cdot \vec{r}_k)}{r_{kl}} \} 
\]  

(1.32)

with \( D_{kl} = \mu_0 \gamma_k \gamma_l / (4\pi r_{kl}^3) \) in SI units. The internuclear vector \( \vec{r}_{kl} \) can be expressed in polar coordinates \( \theta_{kl} \), \( \phi_{kl} \) to obtain a representation of the dipolar Hamiltonian in terms of irreducible tensor operators (??)

\[
\mathcal{H}_D = \sum_{k<l} \sum_{q=-2}^{2} F^{q}_{kl} A^{q}_{kl}.
\]  

(1.33)

The functions \( F^{q}_{kl} \) describe the orientation and \( A^{q}_{kl} \) contain the spin operators:

\[
\begin{align*}
A_{kl}^0 &= D_{kl} \{ I_{kz} I_{lz} - \frac{1}{3} (I_{k}^+ I_{l}^- + I_{k}^- I_{l}^+) \}, & F_{kl}^0 &= 1 - 3 \cos^2 \theta_{kl}, \\
A_{kl}^1 &= -\frac{3}{4} D_{kl} (I_{k}^- I_{l}^+ + I_{k}^+ I_{l}^-), & F_{kl}^1 &= \sin \theta_{kl} \cos \theta_{kl} \exp \{-i\phi_{kl}\}, \\
A_{kl}^{-1} &= -\frac{3}{4} D_{kl} (I_{k}^+ I_{l}^- + I_{k}^- I_{l}^+), & F_{kl}^{-1} &= \sin \theta_{kl} \cos \theta_{kl} \exp \{+i\phi_{kl}\}, \\
A_{kl}^{2} &= -\frac{3}{4} D_{kl} I_{k}^- I_{l}^+, & F_{kl}^{2} &= \sin^2 \theta_{kl} \exp \{-2i\phi_{kl}\}, \\
A_{kl}^{-2} &= -\frac{3}{4} D_{kl} I_{k}^+ I_{l}^-, & F_{kl}^{-2} &= \sin^2 \theta_{kl} \exp \{2i\phi_{kl}\},
\end{align*}
\]

(1.34)

where \( \theta_{kl} \) is the angle between the magnetic field \( \vec{B}_0 \) and the internuclear vector \( \vec{r}_{kl} \), and \( \phi_{kl} \) is the azimuthal angle respect to the \( x \)-axis. Comparing these terms with the conventional 'dipolar alphabet' [9, 10], we can make the identifications:

\[
\begin{align*}
A + B &= \frac{1}{D_{kl}} A_{kl}^0 F_{kl}^0, \\
C &= \frac{1}{D_{kl}} A_{kl}^1 F_{kl}^1, \\
D &= \frac{1}{D_{kl}} A_{kl}^{-1} F_{kl}^{-1}, \\
E &= \frac{1}{D_{kl}} A_{kl}^{2} F_{kl}^{2}, \\
F &= \frac{1}{D_{kl}} A_{kl}^{-2} F_{kl}^{-2}.
\end{align*}
\]  

(1.35)

In the high-field approximation, it is normally possible to neglect non-secular contributions and retain only the term with \( q = 0 \) (term \( A + B \) of dipolar alphabet). In heteronuclear spin systems further simplification can be obtained due to weak coupling by dropping all terms that involves transverse spin operators.

\[
\tilde{\mathcal{H}}_D = \sum_{k<l} D_{kl} (1 - 3 \cos^2 \theta_{kl}) I_{kz} S_{kz}.
\]  

(1.36)
Nuclear spins are coupled together even because of the influence of the bonding electrons on the magnetic fields running between the nuclear spins. The electronic magnetisation resulting from the perturbation produced by a given nuclear spin is proportional to its magnetic moment, that is to its spin. It gives rise to an extra field at the sites of the nearby nuclear spins. The resulting interaction is bilinear with respect to the spin operators of two nuclei. It is called indirect dipolar interaction or homonuclear J-coupling\(^2\) and for two spins is of the form:

\[
\mathcal{H}_J = 2\pi \sum_{k<l} \vec{I}_k \cdot \hat{J}_{kl} \vec{I}_l
\]  

with the indirect spin-spin coupling tensor \(\hat{J}_{kl}\). As for the case of the chemical shift, the only effective part of this interaction in liquids is its average over all relative orientations of the spin space. In isotropic liquids it is of the form \(\mathcal{H}_J = J \vec{I}_1 \cdot \vec{I}_2\). \(J\) is called the indirect interaction constant. It is independent of the applied magnetic field and is expressed in Hertz.

The most important quadratic interaction in the spin operator is the so called Quadrupolar interaction. Quadratic terms arise from the electric quadrupole interaction and may be interpreted as spin \(I_k \geq 1\) interactions with electric field gradients. The contribution to the Hamiltonian has the form

\[
\mathcal{H}_Q = \sum_k \vec{I}_k \hat{Q}_k \vec{I}_k
\]  

with the quadrupole coupling tensor \(\hat{Q}_k\), which may be expressed in terms of the electric field gradient tensor \(\hat{V}_k\) at the site of the nucleus \(k\)

\[
\hat{Q}_k = \frac{e Q_k}{2I_k(2I_k-1)\hbar}\hat{V}_k.
\]  

\(Q_k\) is the nuclear quadrupole moment of nucleus \(k\).

In terms of the quadrupolar frequency \(\omega_{Q_k}\),

\[
\omega_{Q_k} = \frac{3e^2Q_k}{4I_k(2I_k-1)\hbar}Q_k = V_{k,zz}
\]  

and the asymmetry parameter \(\eta_k = (V_{k,xx} - V_{k,yy})/V_{k,zz}\), the quadrupolar Hamiltonian of nucleus \(k\) can be written, in its principal-axis coordinate system, in the convenient form

\[
\mathcal{H}_Q = \sum_k \omega_{Q_k}\{(I_{kz}^2 - \frac{1}{3}I_k^2) + \frac{\eta}{3}(I_{kx}^2 - I_{ky}^2)\}.
\]  

The principal axis values of the electric field gradient tensor \(\hat{V}_k\) are arranged in the order \(|V_{k,xx}| \leq |V_{k,yy}| \leq |V_{k,zz}|\). Then, the asymmetry parameter is within the order \(0 \leq \eta \leq 1\).

Note that in general, different nuclei in a molecule will have different principal-axis systems.

\(^2\)It should be mentioned that there exists another kind of indirect dipolar interaction, namely the omonuclear J-coupling, which instead is a linear interaction in the spin operator

\(^3\)\(I_k^2 = I_{kx}^2 + I_{ky}^2 + I_{kz}^2\)
1.1.5 Relaxation Theory

The equations shown in previous sections predict that in the absence of a radio-frequency field, the populations do not change, and the coherences oscillate indefinitely at the Larmor frequency corrected by the chemical shift. When a radio frequency is applied, deviations from this behaviour are observed:

- The populations are not time-independent, but gradually drift towards their thermal equilibrium values.
- The coherences do not last for ever, but gradually decay to zero.

These effects are due to relaxation. The radio-frequency pulse causes the state of the spin system to depart from thermal equilibrium. Over a sufficiently long time, the fluctuating molecular surroundings cause the thermal equilibrium state to be gradually re-established. In the phenomenological approach of Bloch [11], two relaxation time constants are introduced. The time constants $T_1$ (the longitudinal relaxation time constant, or spin-lattice relaxation constant) takes into account the drift of the populations towards their thermal equilibrium values. The time constant $T_2$ (the transverse relaxation time constant, or spin-spin relaxation constant) takes into account the decay of the coherences.

The relaxation of populations is clearly due to the thermal exchanges between the spin system and the surrounding molecules, thus it involves energy transfers. On the other hand the coherences decay is of different origin. Coherence requires a consistent polarization direction of the spin ensemble. Each spin precesses around the $z$-axis according to the strength of the local magnetic field. On the average, all spins experience the same field in a liquid, because of motional averaging, which creates identical conditions for all the spins, on the average. However at any particular instant in time, the fields are slightly different on different spins, which causes a gradual loss of synchronization. The decay of coherence does not involve any exchange of energy with the surroundings. Coherence decay does, however, increase the entropy of the spin ensemble.

To determine the expectation values $\langle Q \rangle$, it is not necessary to know the complete density operator $\rho(t)$. It is sufficient to define a reduced spin density operator $\sigma(t)$, which is obtained from $\rho(t)$ by trace formation over all degrees of freedom of the lattice. The dynamics of the reduced density operator proceed according to the equation

$$\frac{d}{dt}\sigma(t) = -i[H^s, \sigma(t)] - \hat{\Gamma}\{\sigma(t) - \sigma_0\}. \quad (1.42)$$

which is often called the ‘quantum mechanical master equation’. Here $H^s$ is the spin Hamiltonian acting only on the spin variables, and is obtained by averaging the full Hamiltonian over the lattice coordinates,

$$H^s = \sum_{f} \langle f | H | f \rangle = tr_f\{H\}. \quad (1.43)$$

The relaxation superoperator $\hat{\Gamma}$ accounts for the dissipative interactions between the spin system and the lattice and drives the density operator towards its equilibrium value $\sigma_0$ [8].
The most direct brute force approach to solve the master equation proceeds via explicit matrix representations of the operators. Let us assume an arbitrary set of basis functions \( \{|r\rangle\} \) and evaluate the matrix elements \( \sigma_{rs} = \langle r | \sigma | s \rangle \). The relaxation superoperator \( \hat{\Gamma} \) may transform any matrix element \( \sigma_{tu} \) into \( \sigma_{rs} \), thus requiring a representation with to pairs of indices

\[
\frac{d}{dt} \sigma_{rs} = -i \sum_k (H_{rk} \sigma_{ks} - \sigma_{rk} H_{ks}) - \sum_{tu} \Gamma_{rstu} \{ \sigma_{tu} - (\sigma_0)_{tu} \}. \tag{1.44}
\]

If we define the supermatrix elements \( H_{rstu} = H_{rt} \delta_{us} - \delta_{rt} H_{us} \) of the commutator superoperator \( \hat{\mathcal{H}} \) the eqn (1.44) becomes

\[
\frac{d}{dt} \sigma_{rs} = -i \sum_{tu} (H_{rstu} \sigma_{tu} - \sum_{tu} \Gamma_{rstu} \{ \sigma_{tu} - (\sigma_0)_{tu} \}). \tag{1.45}
\]

If the \( n^2 \) elements \( \sigma_{rs} \) are arranged in the form of a column vector \( \vec{\sigma} \), eqn (1.45) can be expressed in matrix form

\[
\frac{d}{dt} \vec{\sigma} = -i \hat{\mathcal{H}} \vec{\sigma} - \hat{\Gamma} (\vec{\sigma} - \vec{\sigma}_0). \tag{1.46}
\]

If we assume a time-independent Hamiltonian we obtain the formal solution

\[
\vec{\sigma}(t) = \vec{\sigma}^{ss} + \exp \{ (-i \hat{\mathcal{H}} - \hat{\Gamma}) t \} \{ \vec{\sigma}(0) - \vec{\sigma}^{ss} \}. \tag{1.47}
\]

Several strategies have been proposed to solve this equation. Nevertheless, for the purpose of the present thesis, we do not need to solve the master equation but, as it will appear later, the semi-classical formalism will be enough to account for relaxation in all the investigated cases.

### 1.2 Semi-classical formalism

As stated at the beginning of this chapter, the correct description of nuclear spin dynamics involves a quantum-mechanical formalism. Nevertheless, if we are interested in the expectation value of a macroscopic observable such as the resulting magnetisation of a spin ensemble, the semi-classical formalism allow for its calculation in a simple way.

In Eisenberg description, the time dependence of the expectation value of the angular momentum \( \vec{I} \) is indeed:

\[
\frac{d}{dt} \vec{I} = -i \left[ \hat{\mathcal{H}}, \vec{I} \right] = -i \left[ -\gamma B \cdot \vec{I}, \vec{I} \right] \tag{1.48}
\]

For the \( z \) component, one obtains

\[
\frac{d}{dt} I_z = i \gamma \{ B_x [I_x, I_z] + B_y [I_y, I_z] \} = \gamma \{ I_x B_y - I_y B_z \} = \gamma \left[ \vec{I} \wedge \vec{B} \right]_z \tag{1.49}
\]

which is equivalent to the classic cardinal equation describing the rotational motion of a rigid body. This simple equivalence, which is indeed justified by the fact that at the macroscopic level a collection of non-interacting spins appear continuous, allow us to use a semi-classical formalism without loosing in information.

\(^4n\) being the dimension of the spin space.
1.2. SEMI-CLASSICAL FORMALISM

1.2.1 The rotating frame

The rotating frame picture is very useful to get a quick understanding of magnetic resonance, especially as far as the pulse sequences are concerned. The Zeeman interaction, which was introduced in section 1.1.4 starting from the behaviour of the single spin (bottom-up), can be also derived with semi-classical considerations about the resulting macroscopic magnetisation vector (top-bottom), which may always describe an ensemble of non-interacting spins. This vector is subject to a torque exerted by the external field that tries to line up the magnetisation parallel to the field itself. By equating the torque to the rate of change of angular momentum we obtain

\[ \frac{d}{dt} \vec{M} = -\gamma \vec{M} \wedge \vec{B} \]  

which as stated in eq 1.26, when \( \vec{B} \) is a static magnetic field of amplitude \( B_0 \), corresponds indeed to a precession of the magnetisation about the field at the Larmor frequency. The resonance phenomenon results on application of a transverse magnetic field oscillating at \( \omega_0 \), as stated in section 1.1.4. To obtain the same expression for the spin evolution, we need retain only the circularly polarised component of the oscillating transverse field that is rotating in the same sense as the spin precession, namely

\[ \vec{B}_1(t) = B_1 \cos \omega_0 t \hat{i} - B_1 \sin \omega_0 t \hat{j} \]  

It is easy to show that the solution, under the initial condition \( \vec{M}(t) = M_0 \hat{k} \), is

\[ M_x = M_0 \sin \omega_1 t \sin \omega_0 t \]
\[ M_y = M_0 \sin \omega_1 t \cos \omega_0 t \]
\[ M_z = M_0 \cos \omega_1 t \]  

Figure 1.1. Evolution of the nuclear spin magnetisation in the presence of a longitudinal static field \( B_0 \) and a transverse rotating field \( B_1 \).
CHAPTER 1. BASIC NMR PRINCIPLES

Equation 1.52 implies that on application of a rotating magnetic field of frequency $\omega_0$, the magnetisation simultaneously precesses about the longitudinal polarizing field $B_0$ at $\omega_0$ and about the radio-frequency field $B_1$ at $\omega_1$. The phenomenon is illustrated in fig.1.1. Of course this means that in the reference frame rotating with $B_1$ about $B_0$, the motion is simply a precession about $B_1$, as shown in fig.1.2.

1.2.2 Relaxation: a phenomenological approach

The effect of a resonant radio-frequency pulse, as seen in previous sections, is to perturb the spin system from its thermal equilibrium state. That equilibrium is restored by the spin-lattice relaxation. In section 1.1.5 the master equation and its formal solution were displayed.

Relaxation can be described at four increasingly fundamental levels of physical significance. Among these, we will focus here on the simplest approach that leads to the phenomenological Bloch equations.

The longitudinal and transverse relaxation times $T_1$ and $T_2$ have been introduced [11] on purely phenomenological grounds, leading to the Bloch equations for the magnetisation vector $\vec{M}(t)$ which are conveniently written in vector notation

$$\frac{d}{dt} \vec{M}(t) = \gamma \vec{M}(t) \times \vec{B}(t) - \hat{R}\{\vec{M}(t) - \vec{M}_0\}. \quad (1.53)$$

The relaxation matrix $\hat{R}$ has the form

$$\hat{R} = \begin{pmatrix}
1/T_2 & 0 & 0 \\
0 & 1/T_2 & 0 \\
0 & 0 & 1/T_1
\end{pmatrix}. \quad (1.54)$$

$T_1$ is known as the spin-lattice or longitudinal relaxation time, while $T_2$ is known as the spin-spin or transversal relaxation time. The former is associated to the energy
exchanges between the lattice and the spin system whilst the latter describes the process whereby nuclear spins come to thermal equilibrium among themselves.

1.2.3 Signal detection

In principle, the signal detection should be described quantum-mechanically with the proper detection operator. However, for most of what follows, however, the semi-classical magnetisation approach will be enough. The detection process is governed by Faraday’s law and depends on the motion of the magnetisation vector. A coil is placed around the sample with its symmetry axis transverse to the static field $B_0$. In the laboratory frame, any transversal magnetisation precessing at the Larmor frequency will induce an oscillatory e.m.f. at frequency $\omega_0$. The mathematics of the detection process is conveniently handled using complex numbers where the real part is used to represent the $x$-direction in the rotating frame whilst the imaginary part the $y$-direction.

Consider a simple experiment which consist in applying a radio-frequency resonant pulse to the equilibrium spin magnetisation $M_0 \hat{k}$, such that the magnetisation is rotated of $90^\circ$ around the $\hat{y}$ axis. The laboratory frame magnetisation at time $t$ following the pulse is, in cartesian notation

$$\vec{M}(t) = \left[ M_0 \cos \omega_0 t \hat{i} + M_0 \sin \omega_0 t \hat{j} \right] \exp (-t/T_2)$$

(1.55)

Usually, radio-frequency receivers work by mixing the signal with the output from a reference radio-frequency oscillator, a process known as heterodyning. This method is inherently phase sensitive: it means that by separately mixing the acquired signal with two heterodyne references each $90^\circ$ out of phase, we obtain separate in-phase and quadrature phase output signals, which are each respectively proportional to the magnetisation in $x$ and $y$ planes. In complex number notation, the resulting magnetisation in the plane orthogonal to the static field becomes

$$M_\perp(t) = M_0 \exp (\omega_0 t) \exp (-t/T_2)$$

(1.56)

The heterodyne signal at phase offset $\Delta \omega$ is therefore

$$S(t) = S_0 \exp (i\phi)M_0 \exp (\Delta \omega t) \exp (-t/T_2)$$

(1.57)

where $\phi$ is the absolute receiver phase and $S_0$ is the signal amplitude immediately following the pulse, which is proportional to $M_0$. This signal is commonly addressed as Free Induction Decay (FID). The FID is then Fourier transformed into the frequency domain to obtain the so called absorption and dispersion spectra, which correspond respectively to the real and the imaginary part of the transform.

The FID is a simple example of the spin manipulation that is usually performed in NMR. The main goal is to obtain a signal that is weighted in the parameter of interest and remove the dependence from the others. This goal is obtained by applying proper radio-frequency and, as it will be shown later on, gradient pulse schemes to the sample, called sequences.

A number of different sequences have been proposed since the introduction of NMR spectroscopy. In the following chapter, the main sequences used to obtain diffusion-weighted signal will be reviewed.
1.3 Principles of Imaging

One of the most powerful resources that made NMR so popular is the possibility to encode the spatial information in the recorded signal, thus allowing to obtain images of the investigated sample, i.e. maps in which each volumetric element or "voxel" is proportional to the desired NMR parameter, rather than to its mean value over the entire volume. This goal is reached by means of a linearly varying magnetic field across the sample, which causes the Larmor frequencies of the spin to show the same spatial dependence. In the following, the basic principles of the imaging technique will be reviewed.

1.3.1 Conjugate Space

At first instance, a magnetic field gradient applied along the \( z \) axis, i.e.

\[
\frac{d}{dz} B \neq 0 \quad (1.58)
\]

When a field gradient parallel to \( \vec{B}_0 \) is applied, the local Larmor frequency becomes:

\[
\omega(\vec{r}) = \gamma B_0 + \gamma \vec{G} \cdot \vec{r} \quad (1.59)
\]

where \( \vec{G} \) is defined as the gradient of the field component parallel to \( \vec{B}_0 \). Consider now the nuclear spins at position \( \vec{r} \) in the sample, occupying an element of volume \( dV \). Assuming that \( T_2 \) relaxation is much slower than the transversal dephasing due to the spread in frequencies, then the signal arising from a volume element is:

\[
dS(\vec{G}, t) = \rho(\vec{r}) dV \exp \left[ i(\gamma B_0 + \gamma \vec{G} \cdot \vec{r}) t \right] \quad (1.60)
\]

if we choose the reference frequency to be \( \gamma B_0 \), the signal obtained oscillated at \( \gamma \vec{G} \cdot \vec{r} \) and so the integral of eq. 1.60 becomes:

\[
S(\vec{G}, t) = \iiint \rho(\vec{r}) \exp \left[ i\gamma \vec{G} \cdot \vec{r} t \right] d\vec{r} \quad (1.61)
\]

If we introduce the concept of a reciprocal space vector \( \vec{k} \):

\[
\vec{k} = (2\pi)^{-1} \gamma \vec{G} t \quad (1.62)
\]

the sum of oscillating terms in eq. 1.61 has the form of a Fourier transformation:

\[
S(\vec{k}) = \iiint \rho(\vec{r}) \exp \left[ i2\pi \vec{k} \cdot \vec{r} \right] d\vec{r} \quad (1.63)
\]

Eq. 1.63 states that the signal \( S(\vec{k}) \) and the spin density \( \rho(\vec{r}) \) are mutually conjugate.

To derive such equation, we made the assumption that the signal was simply proportional to the spin density (as shown in eq. 1.60). Nevertheless, as seen previously, there are many physical parameters which can affect the NMR signal and which are of interest. This issue can be easily considered introducing a contrast factor \( C(\vec{r}) \) in eq. 1.60, which means that what is imaged will be the term \( C(\vec{r}) \rho(\vec{r}) \) rather than \( \rho(\vec{r}) \). Clearly, by normalizing images obtained with and without the contrast effect, a map of \( C(\vec{r}) \) can be obtained.
1.3.2 Selective Excitation

Selective excitation involves applying an r.f. pulse which affects only a specific region of the NMR frequency spectrum. In the presence of a magnetic field gradient, the selective r.f. pulse may be used to excite only those spins within some specified layers of the sample. This phenomenon is based on the principle that the frequency bandwidth of an r.f. pulse is inversely proportional to the pulse duration, given that the turn angle is determined by the product $\gamma B_1 T$. To understand what happens to the magnetisation, we write the Bloch equations for a field gradient applied along $z$ (neglecting the relaxation):

\[
\begin{align*}
\frac{dM_x}{dt} &= \gamma M_y G_z z \\
\frac{dM_y}{dt} &= \gamma (M_z B_1(t) - M_x G_z z) \\
\frac{dM_z}{dt} &= -\gamma M_y B_1(t)
\end{align*}
\]  

(1.64)

Now we consider the situation from the frame of reference that rotates about the $z$-axis at an angular frequency of $\gamma G_z z$, $x'y'z'$. Under the linearity assumption, i.e. $z$-component of magnetisation changing only slightly so that $dM_z/dt = 0$ and $M_z' = M_0$:

\[
\begin{align*}
\frac{dM_x'}{dt} &= -\gamma M_0 B_1(t) \sin[\gamma G_z z(t + T)] \\
\frac{dM_y'}{dt} &= \gamma M_0 B_1(t) \cos[\gamma G_z z(t + T)] \\
\frac{dM_z'}{dt} &= 0
\end{align*}
\]  

(1.65)

if we treat $M_{x'}$ and $M_{y'}$ as real and imaginary part of a complex number, $M'_+$, eq. 1.65 becomes:

\[
\frac{dM'_+}{dt} = i\gamma M_0 B_1(t) \exp[i\gamma G_z z(t + T)]
\]  

(1.66)

Integrating and returning to the rotating frame ($M_+(T) = M'_+(T) \exp(-i\gamma G_z z 2T)$) gives:

\[
M_+ = i\gamma M_0 \exp(-i\gamma G_z z T) \int_{-T}^{T} B_1(t) \exp[i\gamma G_z z t] dt
\]  

(1.67)

The integral in eq. 1.67 is simply the Fourier transform of the r.f. pulse. Thus, the equation states that the FID signal is proportional to the amplitude of the r.f. spectrum at $z$. If we want to excite a rectangular slice, then we will need a rectangular spectrum. Besides, eq. 1.67 contains a net phase shift, $\gamma G_z z T$, which is a nuisance in a plane normal to $z$ and which will be removed by applying an opposite sign $z$ gradient of magnitude $-G_z$.

1.3.3 Image Reconstruction

To perform the in-plane reconstruction, we need to sample the signal in presence of a gradient, obtaining points along a single line in $\vec{k}$-space. The gradient direction associated to this sampling is referred as the read direction. Usually, the $x$-coordinate
is ascribed to this direction. The intercept of this line along the orthogonal axis can
be changed by imposing a $G_y$ gradient for a fixed period before the sampling begins.
This gradient is called the phase gradient since it imparts a phase modulation to
the signal, dependent on the position of the volume elements along the $y$-axis. The
signal will be then:

$$S(k_x, k_y) = \int_{-\alpha/2}^{\alpha/2} \left\{ \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \rho(x,y,z) \exp\left[i2\pi(k_xx + k_yy)\right] dx dy \right\}$$  \hspace{1cm} (1.68)

To obtain the density $\rho(x, y, z)$ or, more interestingly, the contrast factor defined in
sec. 1.3.1, one needs to calculate the inverse Fourier transform of eq. 1.68:

$$\rho(x, y) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} S(k_x, k_y) \exp[-i2\pi(k_xx + k_yy)] dk_x dk_y$$  \hspace{1cm} (1.69)

where the outer integral, which simply represents the process of averaging across
the slice, was neglected. Since the density is a real quantity, the signal is subject to
symmetry $S(-k_x, -k_y) = S^*(k_x, k_y)$ so in principle, only two out of four quadrants
of the $(k_x, k_y)$ plane must be sampled.
Chapter 2

Molecular Diffusion

Diffusion is a spontaneous phenomenon in any fluid whose absolute temperature is greater than zero Kelvin. The molecules constituting the fluid possess kinetic energy and are therefore constantly moving: the greater the energy, the faster the movement. Their motion can be considered random due to the fact that their trajectories are continuously deviated by collisions with other solvent particles.

Diffusion is often thought of as the process by which concentration gradients are flattened out, but the principle is equally applicable to the movement of molecules within a fluid composed of a single type of molecule. In the latter case, which is the most interesting in the context of this thesis, the process is known as self-diffusion. In this chapter, the basic principles of diffusion will be briefly reviewed.

NMR is one of the most powerful tools to investigate diffusion non-invasively. The basis of the so-called Diffusion-Weighted NMR (DW-NMR) and Diffusion Tensor Imaging (DTI) will be addressed in the second part of the chapter.

According to these techniques, diffusion is modelled as an ideal process happening in an homogeneous environment. When water molecules interact with heterogeneous environments, some of the hypotheses that allow a simple description fail and more specific models must be introduced to have a comprehensive description. In this regard, in the last part of the chapter, the issue of the anomalous transport will be addressed in dept.

2.1 Classic theory of Diffusion

Even though the Scottish botanist R. Brown had been observing the random movement of particles suspended in a fluid (which was named Brownian after him) since 1827, a formal theory of diffusion was introduced by A. Fick in 1855 and by A. Einstein in 1905.

2.1.1 Fick’s laws

If an isotropic fluid is considered, a flux of molecules shifting from one side to another is described by the 1st Fick’s law:

\[ J = -D \nabla C \]  \hspace{1cm} (2.1)
i.e. the flux density $\vec{J}$, the number of diffusing particles per unitary time and surface, is proportional to the concentration gradient $C$ of the diffusing species. In homogeneous environments, the factor of proportionality, $D$, is the diffusion coefficient and depends on the characteristics of the diffusing particle and on those of the solvent. The presence of a minus ensures that the particles move from the highest populated region to the lowest populated one. Consider now the concentration of some molecule at location $x$ and time $t$, $C(x,t)$. In one dimension, the flux along the $x$ direction will be:

$$J_x = -D\frac{\partial C(x,t)}{\partial t} \quad (2.2)$$

As a result of this flux, however, the local concentration gradient will decrease, and so a time-dependent aspect needs to be introduced to describe the picture more fully. The equation:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \quad (2.3)$$

describe the conservation of the total mass inside the liquid and is called 2nd

Figure 2.1. Volume element inside a liquid sample

Fick’s law. For each volume element $2dx \; 2dy \; 2dz$ described in fig. 2.1, the number of particles crossing the A face of the parallelepiped in the time unity is:

$$4dydz \left( J_x - \frac{\partial J_x}{\partial x} dx \right) \quad (2.4)$$

where $J_x$ is the flux density through the unitary surface in the plane containing $P$, the parallelepiped centre.

In the same way, the number of particles exiting the volume through $A'$ is:

$$4dydz \left( J_x + \frac{\partial J_x}{\partial x} dx \right) \quad (2.5)$$

Considering the equations 2.4 and 2.5, the net flux into the parallelepiped is

$$-8dx dy dz \frac{\partial J_x}{\partial x} \quad (2.6)$$
2.1. CLASSIC THEORY OF DIFFUSION

To this net particles flux the following concentration variation is associated:

\[ 8dx dy dz \frac{\partial C}{\partial t} \quad (2.7) \]

Since the conservation of the mass implies eq.2.6 to be equal to eq.2.7:

\[ \frac{\partial C}{\partial t} + \frac{\partial J_x}{\partial x} + \frac{\partial J_y}{\partial y} + \frac{\partial J_z}{\partial z} = 0 \quad (2.8) \]

which is just the conservation of the mass. If the diffusion coefficient is constant, we can derive the second Fick’s law by inserting the expression if \( J \) of eq.2.2:

\[ \frac{\partial C}{\partial t} = D \left( \frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial y^2} + \frac{\partial^2 C}{\partial z^2} \right) \quad (2.9) \]

It is easy to show that a solution of the 2.9 is given by:

\[ C(t) = \frac{M}{2\pi Dt^{1/2}} \exp(-x^2/4Dt) \quad (2.10) \]

which is a Gaussian centred in \( x = 0 \) whose width is proportional to \( \sqrt{4Dt} \), i.e. it increases with increasing \( t \).

It is important to underline the hypotheses of isotropy and homogeneity of the fluid, which lead to the Fick’s laws. If the former is violated, i.e. we are dealing with heterogeneous environments, then the behaviour will be direction-dependent and a single diffusion coefficient will not be able to characterise the diffusion. If instead the latter hypothesis is violated, then the diffusion coefficient will vary as a function of spatial and temporal coordinates. The attention of the NMR community in the last 20 years has been focussed on the first phenomena, since anisotropy proved to be a powerful contrast tool to discriminate between different tissues.

2.1.2 Self-diffusivity

To derive the Fick’s laws of the previous paragraph, one needs to define a concentration gradient across the liquid sample, i.e. at least two species must be present, the diffusing particles and the solvent. Nevertheless, due to their kinetic energy, also the particles inside a monodisperse solution are subject to a translational motion. To describe this phenomena, which is called self-diffusion, it is useful to introduce the self-correlation function, \( P_s(r|r',t) \), which gives the probability that a molecule initially at \( r \) will have moved to \( r' \) after a time \( t \).

For self-diffusion, there is no net concentration gradient. Nonetheless, the Fick’s law description is possible using, in place of concentration, the total probability defined as:

\[ \Psi(r',t) = \int \Psi(r',0)P_s(r|r',t)dr \quad (2.11) \]

where \( \Psi(r',0) \) is just the particle density. Thus, the first Fick’s law may be expressed as

\[ J = -D \nabla'P_s \quad (2.12) \]
Because the total conditional probability is conserved, the continuity theorem applies and

$$\nabla' \cdot J = -\frac{\partial P_s}{\partial t}$$

(2.13)

Combining eq.2.12 and 2.13 we obtain the analogous of the Fick’s second law:

$$\frac{\partial P_s}{\partial t} = D \nabla'^2 \cdot J$$

(2.14)

The solution of eq.2.14 is straightforward for the special boundary condition which applies for unrestricted self-diffusion, i.e. \( P_s \to 0 \) as \( r' \to \infty \):

$$P_s(r|r', t) = (4\piDt)^{-3/2} \exp\left\{-(r' - r)^2/4Dt\right\}$$

(2.15)

which is again a normalized Gaussian function of the net displacement \( r' - r \).

2.1.3 Brownian motion and Random walk

We can recover the self-correlation function of the previous paragraph by using the so called random walk model, which decomposes the motion of a single particle as a sequence of random jumps driven by the collisions with the others. This derivation will be useful in the next sections, since allows one to stress the hypotheses that are made to obtain a Gaussian-shaped self-correlation function. A typical Brownian

Figure 2.2. Schematic representation of a Brownian random walk in 2D. The walker jumps at each time step \( t = 0, \Delta t, 2\Delta t, ..., n\Delta t \) to a randomly selected direction, thereby covering the distance \( \Delta x \), the lattice constant. Reproduced from [12]

walk is schematically displayed on a two-dimensional lattice in Fig.2.2. In discrete time steps of span \( \Delta t \), the test particle is assumed to jump to one of its nearest neighbour sites, here displayed on a square lattice with lattice constant \( \Delta x \), the direction being random. Such a process can be modelled by the master equation:

$$P_j(t + \Delta t) = \frac{1}{2}P_{j-1}(t) + \frac{1}{2}P_{j+1}(t)$$

(2.16)
2.1. CLASSIC THEORY OF DIFFUSION

in the one-dimensional analogue, the index denoting the position on the underlying one-dimensional lattice. Eq.2.58 define the probability density function (or pdf) to be at position $j$ at time $t + \Delta t$ in dependence of the population of the two adjacent sites $j \pm 1$ at time $t$. In the continuum limit $\Delta t \to 0$ and $\Delta x \to 0$, Taylor expansions in $\Delta t$ and $\Delta x$,

$$P_j(t + \Delta t) = P_j(t) + \Delta t \frac{\partial P_j}{\partial t} + O([\Delta t]^2) \quad (2.17)$$

and

$$P_{j \pm 1}(t) = P(x, t) \pm \Delta x \frac{\partial P}{\partial x} + \frac{(\Delta x)^2}{2} \frac{\partial^2 P}{\partial x^2} + O([\Delta x]^2) \quad (2.18)$$

lead to the diffusion equation:

$$\frac{\partial P}{\partial t} = D \frac{\partial^2 P}{\partial x^2} \quad (2.19)$$

on taking along the lowest orders in $\Delta t$ and $\Delta x$. The continuum limit thereby has to be drawn such that the quotient

$$D = \lim_{\Delta x \to 0, \Delta t \to 0} \frac{\Delta x^2}{2\Delta t}$$

is finite. The diffusion equation 2.19 is a direct consequence of the central limit theorem. Under the condition that the first two moments of the pdf, describing the appropriately normalised distance covered in a jump event and the variance, $X = \sum_i X_i$ and $X^2$, as well as the mean time span $\Delta t$ between any two individual jump events, exist, the central limit theorem assures that the random walk process is characterised by a mean velocity $V = \overline{X}/\Delta t$ and a diffusion coefficient $D = \frac{1}{(2\Delta t)^{-1}[\overline{X^2} - \overline{X}^2]}$. Furthermore, for long times, i.e., a large enough number of steps, the pdf of being at a certain position $x$ at time $t$, is governed by the diffusion equation 2.19, and it is given by the Gaussian shape, as seen in the previous section.

2.1.4 The Diffusion Tensor

In sec.2.1.1, the Fick’s laws were derived under the hypothesis of isotropy and homogeneity. This means that the diffusion dynamics can be described by the same scalar quantity, disregarding the direction across which the motion is observed. This is unlikely to happen in most of the system that are usually investigated by means of NMR and, in particular, in the biological tissue examined in this thesis.

When dealing with anisotropic means, the flux is not necessarily parallel to the normal of the surface of constant concentration and so $J$ can be not parallel to $\nabla C$. The Fick’s law 2.1 should thus be modified in the following way:

$$\begin{align*}
-J_x &= D_{xx} \frac{\partial C}{\partial x} + D_{xy} \frac{\partial C}{\partial y} + D_{xz} \frac{\partial C}{\partial z} \\
-J_y &= D_{yx} \frac{\partial C}{\partial x} + D_{yy} \frac{\partial C}{\partial y} + D_{yz} \frac{\partial C}{\partial z} \\
-J_z &= D_{zx} \frac{\partial C}{\partial x} + D_{zy} \frac{\partial C}{\partial y} + D_{zz} \frac{\partial C}{\partial z}
\end{align*} \quad (2.21)$$

where the coefficients $D_{ij}$ that appears in the formula express the contribution to the flux along $i$ due to the concentration gradient along $j$. These coefficients are
the 9 components of the diffusion tensor $\mathbf{D}$:

$$
\mathbf{D} = \begin{pmatrix}
D_{xx} & D_{xy} & D_{xz} \\
D_{yx} & D_{yy} & D_{yz} \\
D_{zx} & D_{zy} & D_{zz}
\end{pmatrix}
$$

Due to its physical interpretation, diffusion tensor is required to be positive-definite and symmetric, so that the effective independent elements reduces to 6. Besides, it is always possible to diagonalise the diffusion matrix of 2.22, i.e. to find a reference frame in which the diffusion is completely characterised by three diffusivities, which corresponds to the three matrix eigenvalues. The eigenvectors associated to the diagonal form of the diffusion matrix are called the principal axes of diffusion.

In this main reference frame, the diffusion tensor assumes the diagonal form:

$$
\mathbf{D} = \begin{pmatrix}
\lambda_1 & 0 & 0 \\
0 & \lambda_2 & 0 \\
0 & 0 & \lambda_3
\end{pmatrix}
$$

Diffusion is conveniently visualised by means of an ellipsoid in which the lengths of the axes are related to the three eigenvalues, and their orientation is given by the associated eigenvectors. The ellipsoid shape can be either prolate, showing the existence of one prevalent diffusion direction, oblate, suggesting instead the presence of different enhanced diffusivities which lie in a plane, or isotropic, indicating the lack of a specific privileged path for water diffusion.

The concept that in anisotropic environments the diffusive dynamics may be expressed by a tensor is at the basis of the development of diffusion tensor imaging. As shown in the following section, DTI is based on the evaluation of scalar invariants of the diffusion tensor, which are tissue-specific and can furnish important pieces of information about the local microstructure.

### 2.2 Diffusion and NMR

NMR is the most versatile non-invasive technique to investigate diffusion dynamics, providing information about a wide range of specimens going from liquid solutions to biological material.

The idea that lies at the basis of the diffusion weighted NMR is the possibility of impress a molecular label via the characteristic Larmor frequencies of the nuclei. This label is the phase of the transverse magnetisation: if a spatial label is given to nuclei at one instant of time, the motion can be in principle deduced by checking this label at a later time. Moreover, by choosing a specific time spacing, faster or lower dynamics contribution can be in turn exalted or depressed.

In the followings, the Spin-echo (SE) sequence will be introduced as a method to measure phase differences. Then, the spatial label will be impressed using a couple of magnetic field gradients, leading to the Pulse-Gradient Spin-Echo (PGSE), which is the most commonly used sequence to measure diffusion.

In the last part of the section, diffusion anisotropy will be taken into account in reviewing the basis of Diffusion Tensor Imaging, which allows to discriminate tissues on the basis of their diffusivity properties.
2.2. **DIFFUSION AND NMR**

2.2.1 **The Spin Echo**

The Spin echo is one of the most widely diffused sequences and it represents the starting point for obtaining diffusion-weighted images. Even when no magnetic field gradient is deliberately applied, the inhomogeneity of the polarizing magnet will result in a field spread across the sample. Following a 90° r.f. pulse, i.e. a magnetic field pulse in which the parameters had been set to rotate the magnetisation of 90° towards the \( x \) axis, the transverse magnetisation will be progressively dephased by such a spread. This loss of coherence is indeed reversible: application of a second 180° r.f. pulse after a time delay \( \tau \) will cause refocusing at \( 2\tau \), as showed in fig. 2.3. The intensity of the echo relative to the initial signal is given by:

\[
I(t) = \exp\left(-\frac{2\tau}{T_2}\right)
\]

where \( T_2 \) is the time constant for spin-spin relaxation.

The formation of the echo is predicated on the assumption that nuclei experience the same local Larmor frequency during the successive dephasing and rephasing parts of the cycle. Since there is a correspondence between the nuclear position and the local magnetic field, this assumption is equivalent to requiring that the nuclei do not move in translation along the gradient direction. But since the molecular diffusion cannot be removed, it is possible to take advantage of this phenomenon to quantify the molecular displacement spectrum, as will be shown in the next paragraph.

2.2.2 **Diffusion in the presence of a gradient**

The most convenient way to depict molecular self-diffusion is to model it as a succession of discrete hops with motion resolved in one dimension, i.e. the direction of the field gradient. Let the main time between steps be \( \tau_s \) and the root mean square displacement in one dimension be \( \xi \). A molecule has an equal probability
of jumping to the left or right so that the distance travelled after \( n \) jumps at time \( t = n\tau \) is given by:

\[
Z(n\tau_s) = \sum_{i=1}^{n} \xi a_i
\]  
(2.25)

where \( a_i \) is a random number equal to \( \pm 1 \). The mean square displacement is then:

\[
\overline{Z^2(n\tau_s)} = \sum_{i=1}^{n} \sum_{j=1}^{n} \xi^2 a_{ij}
\]  
(2.26)

Since \( a_i \) is chosen randomly, \( a_{ij} = 0 \) unless \( i = j \), so all the cross-terms cancel:

\[
\overline{Z^2(n\tau_s)} = \sum_{i=1}^{n} \xi^2 a_i^2 = \xi^2 \sum_{i=1}^{n} a_i^2 = n\xi^2
\]  
(2.27)

Defining the self-diffusion coefficient as

\[
D = \frac{\xi^2}{2\tau_s}
\]  
(2.28)

we obtain:

\[
\overline{Z^2(t)} = 2Dt
\]  
(2.29)

which is the same result obtained in sec.2.1.1.

Within this formalism, it is possible to calculate the influence of the Brownian motion on the coherence of transverse magnetisation in the case where a magnetic field gradient is present \[13\]. We consider the influence of diffusion along \( z \) on the transverse magnetisation of spins originating at \( z = 0 \). The local Larmor frequency is:

\[
\omega(n\tau_s) = \gamma B_0 = \gamma G \sum_{i=1}^{n} \xi a_i
\]  
(2.30)

so that the cumulative phase angle after time \( \tau = n\tau_s \) is:

\[
\phi(t) = \gamma B_0 n\tau_s = \sum_{m=1}^{n} \gamma G \tau_s \sum_{i=1}^{m} \xi a_i
\]  
(2.31)

The first term is just the Larmor precession, while the second one is more interesting since it contains the dephasing. It is possible to write it as:

\[
\Delta \phi(t) = \gamma G \tau_s \xi \sum_{i=1}^{n} (n - i)a_i
\]  
(2.32)

as illustrated in fig.2.4.

What one needs to calculate is the term \( \exp(i\Delta \phi) \), the coefficient by which the ensemble-averaged transverse magnetisation will be phase modulated as a result of the diffusional motion in the presence of a gradient:

\[
\exp(i\Delta \phi) = \int_{-\infty}^{\infty} P(\Delta \phi) \exp(i\Delta \phi) d(\Delta \phi)
\]  
(2.33)

At this point, one needs to assume its distribution to be Gaussian (this issue will be largely addressed in the following), so:

\[
\exp(i\Delta \phi) = \exp(-\Delta \phi^2/2)
\]  
(2.34)
In order to calculate $\Delta \phi^2$, one needs to square eq.2.32 and take the ensemble average.

Once again, cross terms disappear, so:

$$\Delta \phi^2 = \gamma^2 G^2 \tau_s^2 \xi^2 \sum_{i=1}^{n} (n + 1 - i)^2$$

$$= \gamma^2 G^2 \tau_s^2 \xi^2 \sum_{j=1}^{j} j^2$$

$$= \frac{1}{3} \gamma^2 G^2 \tau_s^2 \xi^2 n^3$$

(2.35)

where the last sum is evaluated assuming that $n$ is large. Substituting eq.2.28 one finds:

$$\exp(i \Delta \phi) = \exp(-\frac{1}{3} \gamma^2 G^2 Dt^3)$$

(2.36)

Consider now the spin echo sequence. As shown in the previous section, the $180^\circ$ reverses all the phase shifts which existed before its application. The phase step diagram is shown in fig.2.5.

The second evolution period contains a section which completely cancels the net phase shift which occurred before the $180^\circ$ pulse; the residual phase shift is given by the remaining region and is twice the value found in eq.2.36.

This result is extremely important in the presence of *internal gradients*, i.e. local perturbations of the magnetic field due mostly to differences in magnetic susceptibility at the interface between different regions within the sample. In fact, these perturbations are able to influence spin relaxation by varying the local Larmor frequency by a factor

$$\sqrt{\Delta \omega_0^2} \sim \gamma \Delta \chi B_0$$

(2.37)
In presence of internal gradient, due to their dephasing effects the signal after a Spin Echo sequence is thus:

\[ S(t) = S_0 \exp \left( -\frac{t}{T_{2\text{true}}} - \frac{1}{12} \gamma^2 G^2 D t^3 \right) \quad (2.38) \]

where \( T_{2\text{true}} \) is the spin-spin relaxation time in which the effects of diffusion and field inhomogeneities are averaged out and \( t = 2\tau \), as defined in fig.2.3. To obtain \( T_{2\text{true}} \), a specific sequence called Carr-Purcell-Meiboom-Gill (CPMG) should be used. This sequence is able to average out the effects of diffusion by means of an echo train.

### 2.2.3 Pulsed Gradient Spin Echo

The dephasing of the transversal magnetisation in presence of magnetic field gradients is used to measure diffusion by inserting on purpose a couple of rectangular gradients in the dephasing and rephasing part of the echo sequence. The obtained scheme is called Pulse Gradient Spin Echo (PGSE) sequence [15] and is shown in fig.2.6.

Following the previous arguments, from fig.2.5 it is apparent that the phase shifts associated with the unshaded areas cancel and the net phase shift is obtained by summing two uncorrelated triangular regions, each with mean square phase shift \( \frac{1}{3} \gamma^2 g^2 \tau_s^2 \xi_n^2 n^3 \), along with one uncorrelated rectangular region with mean square phase shift \( \frac{1}{3} \gamma^2 g^2 \tau_s^2 \xi_n^2 (p - n) \). The net mean square shift is therefore:

\[ \Delta \phi^2 = \gamma^2 g^2 \tau_s^2 \xi_n^2 (p - n + \frac{2}{3} n) \]

\[ = 2\gamma^2 g^2 \delta^2 D (\Delta - \delta/3) \quad (2.39) \]
The attenuation of the signal in the presence of a couple of magnetic field gradients is then described by:

\[ S(g) = S(0) \exp[-\gamma^2 g^2 \delta^2 D(\Delta - \delta/3)] \] (2.40)

which is known as Stejskal-Tanner equation [15]. Usually, this expression is written as a function of the \( b \)-factor:

\[ b = (\gamma g \delta)^2(\Delta - \delta/3) \] (2.41)

\[ S(g) = S(0) \exp(-bD) \] (2.42)

Eq.2.42 provides a precise description of the influence of self-diffusion in the PGSE experiment and is the basis of a considerable literature pertaining to this technique.

### 2.2.4 The q-space

In the previous paragraph, it has been shown how to impose a label, codifying the spin position, on the phase of the transverse magnetisation. The Stejskal-Tanner equation was derived modelling the spin trajectories as a succession of discrete hops resolved in one dimension.

Here, we derive the signal attenuation adopting a more analytical approach, showing how the PGSE method can be used to give information about the self-correlation function \( P_s \) defined in section 2.1.2. In the so called narrow-pulse approximation, i.e. \( \delta \ll \Delta \), the effect of the gradient pulse is to impart a phase shift \( \gamma \delta g \cdot r \) to a spin located in a position \( r \) at the instant of the pulse. In the PGSE sequence, this phase shift is subsequently inverted by the 180\( ^\circ \) pulse. If the spin has moved to \( r' \) at the time of the second pulse, the net phase shift will be \( \gamma \delta g \cdot (r' - r) \).

The total signal is given by the superimposition of transverse magnetisations, an
ensemble average in which each phase term \( \exp[i\gamma \delta \mathbf{g} \cdot (\mathbf{r}' - \mathbf{r})] \) is weighted by the probability for a spin to begin at \( \mathbf{r} \) and to move to \( \mathbf{r}' \), i.e. \( \rho(\mathbf{r})P_s(\mathbf{r}|\mathbf{r}', \Delta) \):

\[
S_{\Delta}(\mathbf{g}) = \int \rho(\mathbf{r}) \int P_s(\mathbf{r}|\mathbf{r}', \Delta) \exp[i\gamma \delta \mathbf{g} \cdot (\mathbf{r}' - \mathbf{r})] d\mathbf{r} d\mathbf{r}'
\]  

(2.43)

Defining a reciprocal space \( q \), where:

\[
q = (2\pi)^{-1}\gamma \delta \mathbf{g}
\]  

(2.44)

eq(2.43) can be written as:

\[
S_{\Delta}(q) = \int P_s(\mathbf{R}, \Delta) \exp(i2\pi \mathbf{q} \cdot \mathbf{R}) d\mathbf{R}
\]  

(2.45)

where \( \mathbf{R} \) is the relative displacement between initial and final position. Eq.2.45 states that \( S_{\Delta}(q) \) and \( P_s(\mathbf{R}, \Delta) \) are linked by a simple Fourier relationship. This means that, by means of NMR, one can measure the signal obtained after a diffusion-sensitized sequence and get information about the self-diffusivity of the molecules constituting the sample.

The parameter \( \Delta \) is explicit on purpose since it sets the range of the mean path travelled by the spins, according to the relation 2.29. Giving some quantitative measures, if we are observing water free diffusion \( (D = 2.27 \cdot 10^{-9} \text{m}^2/\text{s}) \), for a typical value of \( \Delta = 100 \text{ms} \) we obtain a mean path of 20\( \mu \text{m} \).

### 2.2.5 Diffusion Tensor Imaging

When dealing with homogeneous and isotropic media, the diffusive motion can be described by a single scalar value \( D \) and the PGSE sequence allow for its evaluation by means of eq.2.42. When the media exhibit organisational anisotropy, the measurements with gradients applied along different directions can give very different results. In such cases, the diffusive motion should be described by the diffusion tensor \( \Rightarrow D \) of sec.2.1.4.

Diagonal and non-diagonal elements of \( \Rightarrow D \) can be related to the measured echo intensity in a PGSE experiment [16]. In fact, eq.2.42 can be generalized for the diffusion tensor \( \Rightarrow D \) in the following way:

\[
S(TE) = S(0) \exp(- \sum_{i=1}^{3} \sum_{j=1}^{3} b_{ij} D_{ij}^{eff})
\]  

(2.46)

the \( b\)-matrix performing the role of the scalar b-factor defined in the previous paragraph. Since \( \Rightarrow D \) has only 6 independent elements, at least 6 different measurements are necessary to reconstruct it, i.e. 6 PGSE realized with 6 non-collinear gradient directions. Generally, the magnitude of the diffusion weighting which is impressed along the different gradient directions is the same for each direction and is optimized to match with the expected diffusivity value, as previously demonstrated [17]. Moreover, a \( b_0 \) image is also acquired for normalization, i.e. an image with no diffusion weighting.

Usually, anisotropic samples are investigated by means of imaging techniques, i.e. the diffusion behaviour is spatially resolved as shown in sec.1.3. In those cases,
to classify the diffusion, scalar invariants extracted from the tensor are displayed as parametric maps. The two most popular invariants are the mean diffusivity (\(MD\)) and the fractional anisotropy (\(FA\)) [18], which are defined according to the following:

\[
MD = \frac{Tr \vec{D}}{3} = \frac{\sum_{i=1}^{3} \lambda_i}{3} \tag{2.47}
\]

\[
FA = \sqrt{\frac{\sum_{i=1}^{3} (\lambda_i - MD)^2}{\sum_{i=1}^{3} \lambda_i^2}} \tag{2.48}
\]

where \(\lambda_i\) are the eigenvalues of the diffusion tensor.

In fig.2.7, an example of \(MD\) and \(FA\) parametric maps calculated on human brain is shown.

**Figure 2.7.** \(MD\) (left) and \(FA\) (right) maps obtained on a healthy volunteer. The mean diffusivity map reveal the presence of regions in which the diffusion is nearly unrestricted, which appear lighter, and regions in which the diffusion is highly constrained. The fractional anisotropy instead highlight the white matter fibres, i.e. areas in which, due to the presence of fibre bundles, the diffusion is highly oriented along the fibre axis, i.e. is anisotropic. These areas are lighter in the map. Reproduced from [19].

Usually, in brain imaging the obtained signal is due to the protons belonging to water molecules. \(MD\) is the mean value of the eigenvalues, i.e. the diffusivity along the three principal axes. Since the diffusivity of unrestricted water at room temperature is equal to \(2.27 \times 10^{-9} \text{m}^2/\text{s}\), the lower the value, the higher the barriers which are encountered during the diffusive motion.

\(FA\) quantify instead the amount of anisotropy, i.e. the difference between each of the three eigenvalues and their mean value. It ranges from 0, which means isotropic diffusion, to 1, which means complete anisotropy.

It is worth to stress that, even though the image resolution depends on the reconstruction procedure, i.e. the k-space imaging, and its lower bound is about 1 \(mm\) so far, in each voxel DTI parameters are usually selected to focus on molecular displacements, which are of the order of few \(\mu m\) in liquid systems. Experimental
parameters can be tuned by the choice of the diffusion time $\Delta$ and the gradient strength $g$, which determine the b-value as shown in the previous section. It is useful to anticipate here that by changing those parameters, different dynamics can indeed be highlighted, leading to different characterisations of the same system and thus a deeper understanding of its dynamical properties.

2.3 Anomalous Transport

Diffusion NMR has proven so far a versatile technique able to characterise the diffusive dynamics of a wide range of specimens in a totally non-invasive way. When applied to reconstruct parametric maps as in DTI, it is able to furnish information on a sub-voxel level, due to the possibility of tuning the diffusion time to catch either faster or slower dynamics. This is one of the main reasons of its widespread popularity.

It has been largely shown that the hypotheses on which DTI model relies are those of the classical diffusion theory, which dates back to the beginning of the last century. The main assumption on deriving the form of the signal attenuation is a Gaussian-shaped self-correlation function or propagator, i.e. the self-correlation function calculated for initial condition $\delta(x)$. Even though this assumption is reasonable in most of the cases, due to the central limit theorem, nature often violates the Gaussian universality. This phenomenon is mirrored in experimental results that do not follow the Gaussian predictions.

In the last years, several models have been introduced to account for the typical anomalous features which are observed in many systems. The typical signature of anomalous transport regime is a non-linear relationship between the root mean squared displacement and the diffusion time, i.e.:

$$<x^2(t)> \sim t^{2\theta}$$

(2.49)

where $\theta$ is the anomalous exponent. In this section, one of the frameworks used for modelling anomalous transport phenomena, i.e. the Continuous Time Random Walk (CTRW), will be revised.

2.3.1 Continuous Time Random Walk

For the generalisations to anomalous transport, we choose the continuous time random walk (CTRW) scheme as the starting point. In parallel to the complementary, dual approach in the standard diffusion problem, we will then develop a generalised diffusion equation of fractional order on the basis of the CTRW [12].

The CTRW model is based on the idea that the length of a given jump, as well as the waiting time elapsing between two successive jumps are drawn from a pdf $\psi(x,t)$ which will be referred to as the jump pdf. From $\psi(x,t)$, the jump length pdf

$$\lambda(x) = \int_0^\infty dt \psi(x,t)$$

(2.50)

and the waiting time pdf

$$w(t) = \int_{-\infty}^{\infty} dx \psi(x,t)$$

(2.51)
2.3. ANOMALOUS TRANSPORT

can be deduced. If the jump length and waiting time are independent random variables, one finds the decoupled form $\psi(x, t) = w(t)\lambda(x)$.

Different types of CTRW processes can be categorised by the characteristic waiting time:

$$T = \int_0^\infty dt w(t)t$$

and the jump length variance:

$$\Sigma^2 = \int_{-\infty}^\infty dx \lambda(x)x^2$$

being finite or diverging, respectively. The following equation:

$$\eta(x, t) = \int_{-\infty}^\infty dx' \int_0^\infty dt' \eta(x', t')\psi(x - x', t - t') + \delta(x)\delta(t)$$

relates the pdf $\eta(x, t)$ of just having arrived at position $x$ at time $t$, with the event of having just arrived at $x'$ at time $t'$, $\eta(x', t')$. The second summand in eq.2.54 denotes the initial condition of the random walk, here chosen to be $\delta(x)$. Consequently, the pdf $W(x, t)$ of being in $x$ at time $t$ is given by:

$$W(x, t) = \int_0^t dt'\eta(x, t')\Psi(t - t')$$

i.e., of arrival on that site at time $t'$, and not having moved since. The latter is being defined by the cumulative probability:

$$\Psi(t) = 1 - \int_0^t dt'w(t')$$

assigned to the probability of no jump event during the time interval $(0, t)$. In Fourier-Laplace space, the pdf $W(x, t)$ can be written as:

$$W(q, u) = \frac{[1 - w(u)]W_0(q)}{u[1 - \psi(q, u)]}$$

where $W_0(q)$ denotes the Fourier transform of the initial condition $W_0(x)$.

If both characteristic waiting time and jump length variance, $T$ and $\Sigma^2$, are finite, the long-time limit corresponds to Brownian motion. Let us consider, for instance, a Poissonian waiting time pdf $w(t) = \tau^{-1}\exp(-t/\tau)$ with $T = \tau$, together with a Gaussian jump length pdf $\lambda(x) = (4\pi\sigma^2)^{-1/2}\exp(-x^2/4\sigma^2)$, leading to $\Sigma^2 = 2\sigma^2$. Then, the corresponding Laplace and Fourier transforms are of the forms:

$$w(u) \sim 1 - u\tau + O(\tau^2)$$

$$\lambda(k) \sim 1 - \sigma^2q^2 + O(q^4)$$

any pair of pdfs leading to finite $T$ and $\Sigma^2$ leads to the same result, to lowest orders, and thus in the long-time limit [20]. Substituting eq.2.58 and eq.2.59 into eq.2.57 leads to:

$$W(q, u) = \frac{1}{u + Dq^2}$$
where \( D = \sigma^2 / \tau \). Back-transformed to \((x,t)\)-coordinates, this is but the well-known Gaussian propagator.

However, this framework is extremely useful to model all those diffusion processes that instead cannot be described by regular (i.e. non-diverging) distributions of jump lengths and/or waiting time. Several processes are in fact associated to long rests and/or long jumps. In the following, we will consider different cases of the CTRW model defined through the decoupled jump pdf \( \psi(x, t) = w(t) \lambda(x) \), taking as examples real systems where anomalous diffusion dynamics have been largely recognised and characterised.

### 2.3.2 Subdiffusion

Let us take as an example the diffusion of a protein in the cytoplasm. It is known that the cytoplasm is an heterogeneous viscous solution as differently sized proteins, lipids, and sugars constitute up to 40% of the cytoplasmic volume. The protein diffusional mobility in the cytoplasm is affected by the crowded environment and as a consequence, it is reasonable to model the distribution of the waiting times between two consecutive jumps as diverging. In this way, one can take into account the possible trapping phenomena occurring in the cytoplasm [21].

When the characteristic waiting time \( T \) diverges, but the jump length variance \( \Sigma^2 \) is finite, we are in the condition of subdiffusion. To this end, a long-tailed waiting time pdf with the asymptotic behaviour:

\[
w(t) \sim A_\alpha \left( \frac{\tau}{t} \right)^{1+\alpha}
\]

for \( 0 < \alpha < 1 \) is introduced, which has the corresponding Laplace space asymptotics:

\[
w(u) \sim 1 - (u\tau)^\alpha
\]

The pdf in Fourier Laplace space becomes:

\[
W(q, u) = \frac{W_0(q)/u}{1 + K_\alpha u^{-\alpha} q^2}
\]

It is also possible to reconstruct the analogous of the diffusion equation in the case of subdiffusion:

\[
\frac{\partial W}{\partial t} = 0 D_t^{1-\alpha} \frac{\partial^2}{\partial x^2} W(x, t)
\]

where the Riemann-Liouville operator \( D_t^{1-\alpha} \) is defined through the relation:

\[
0 D_t^{1-\alpha} W(x, t) = \frac{1}{\Gamma(\alpha)} \frac{\partial}{\partial t} \int_0^t dt' \frac{W(x, t')}{(t - t')^{1-\alpha}}
\]

The generalised diffusion constant \( K_\alpha \) is defined by:

\[
K_\alpha = \sigma^2 / \tau^\alpha
\]

A closed-form solution of eq.2.65 can be found in terms of Fox functions and is reported in [12]. Employing some standard theorems of the Fox function, one can
2.3. ANOMALOUS TRANSPORT

derive the asymptotic stretched Gaussian behaviour:

\[ W(x,t) \sim \frac{1}{\sqrt{4\pi K_\alpha t^\alpha}} \left[ \frac{1}{2 - \alpha} \left( \frac{2}{\alpha} \right)^{(1-\alpha)/(2-\alpha)} \left( \frac{|x|}{\sqrt{K_\alpha t^\alpha}} \right)^{-(1-\alpha)/(2-\alpha)} \right. \\
\exp \left( \frac{2 - \alpha}{2} \left( \frac{\alpha}{2} \right)^{(\alpha/(2-\alpha))} \left[ \frac{|x|}{\sqrt{K_\alpha t^\alpha}} \right]^{1/(1-\alpha/2)} \right) \]

(2.67)

valid for \(|x| \gg \sqrt{K_\alpha t^\alpha}\). The propagator for the subdiffusive case \(\alpha = 1/2\) is shown in fig.2.8.

In the q-space, the space in which NMR signal is observed, the individual modes decay in accordance to:

\[ W(q,t) = E_\alpha(-K_\alpha q^2 t^\alpha) \]

(2.68)

This typical Mittag-Leffler behaviour of the mode relaxation replaces the exponential mode relaxation occurring for normal diffusion: the Mittag-Leffler function interpolates between the initial stretched exponential form:

\[ E_\alpha \left[ -(t/\tau)^\alpha \right] \sim \left[ \frac{(t/\tau)^\alpha}{\Gamma(1 + \alpha)} \right] \]

(2.69)

and the long-time inverse power-law behaviour:

\[ W(q,t) \sim \frac{1}{K_\alpha q^{2\alpha} \Gamma(1 - \alpha)} \]

(2.70)

Figure 2.8. Propagator \(W(x,t)\) for subdiffusion with anomalous exponent \(\alpha = 1/2\), drawn for the consecutive times \(t = 0.1, 1, 10\). Reproduced from [12].

2.3.3 Superdiffusion

Let us consider instead the price fluctuations in financial markets. Due to the peculiar dynamics associated to financial practice, short as well as very long sudden
jumps in the price values are expected. These fluctuations are better described by a diverging jump length variance.

The opposite case of finite characteristic waiting time $T$ and diverging jump length variance $\Sigma^2$ can be modelled by a Poissonian waiting time and a Levy distribution for the jump length, i.e.:

$$\lambda(q) = \exp(-\sigma|q|^\mu) \sim 1 - \sigma|q|^\mu$$

(2.71)

The motion propagator is given by:

$$W(q,u) = \frac{1}{u + K^\mu|q|^\mu}$$

(2.72)

The diffusion equation is easily reconstructed in its fractional form:

$$\frac{\partial W}{\partial t} = K^{\mu}_{-\infty}D^{\mu}_x W(x,t)$$

(2.73)

The operator $-\infty D^{\mu}_x$ is called the Weyl operator and is defined in [12]. The generalised diffusion constant $K^\mu$ is defined by:

$$K^\mu = \sigma^\mu/\tau$$

(2.74)

The Fourier transform of the propagator reads:

$$W(q,t) = \exp(-K^\mu t|q|^\mu)$$

(2.75)

Note that by definition, the mean squared displacement diverges in a Levy flight. This problem is often circumvented by the consideration of $(x,t)$ scaling relations, or measuring the width of the pdf $W = (x,t)$ rather than its variance.

### 2.3.4 The competition between long rest and long jumps

If one is dealing with a system in which both long rests and long jumps coexist, a method to investigate the dynamics has been proposed [22]. In this model, the walker is considered in an imaginary, growing box, leading to:

$$<x^2(t)>_L \sim \int_{L_1 t^{1/\mu}}^{L_2 t^{1/\mu}} dxx^2W(x,t) \sim t^{2/\mu}$$

(2.76)

The imaginary box spans the spatial interval $\Delta t = (L_1 - L_2)t^{1/\mu}$ which grows in the course of time. It gives a measure, that a finite portion of the probability is gathered within the given interval $\Delta t$. By use of such relations, one can consider a random walk characterised through broad pdfs for both waiting time and jump length, thus leading to infinite $T$ and $\Sigma^2$. The diffusion equation in this case is expressed by:

$$\frac{\partial W}{\partial t} = -\infty D^{1-\alpha}_x K^\mu_{\alpha} \Delta t^\mu W(x,t)$$

(2.77)

with the generalized diffusion coefficient equal to:

$$K^\alpha_{\mu} = \sigma^\mu/\tau^\alpha$$

(2.78)
2.3. ANOMALOUS TRANSPORT

Figure 2.9. Phase diagram $\alpha - \mu$ for eq.2.79. Three different domains can be distinguished, on the basis of the value of $T$ and $\Sigma^2$: subdiffusive for $0 < \alpha < 1$ and $2\alpha < \mu$; superdiffusive for $0 < \mu < 2$ and $2\alpha > \mu$ and Brownian diffusion for $\alpha > 1$ and $\mu > 2$ and on the line $2\alpha = \mu$.

In this case, the appropriately defined quantity $<x^2(t)>_L$, which we call the pseudo or imaginary mean squared displacement, reveals the temporal form:

$$<x^2(t)>_L \sim t^{2\alpha/\mu}$$

where the result of eq.2.49 is found for $\theta = \alpha/\mu$. Fig.2.9 synthesises the different domain which can be distinguished on the basis of the value of $T$ and $\Sigma^2$. 
Chapter 3

From conventional to anomalous behaviour

In the previous chapter, it has been demonstrated that diffusion dynamics can be characterised in liquids by means of NMR. It has also been shown that by tuning the experimental parameters, different dynamical ranges can be highlighted.

Ideal systems, characterised by isotropy and homogeneity, in which the molecular displacements can be considered random with no memory effects, long rests or long jumps, are completely described by a single diffusion coefficient which do not depend on the choice of the experimental parameters.

Conversely, real systems are often characterised by complex architectures in which water dynamics cannot be described simply by means of the formalism introduced in the first part of the previous chapter.

Here, we will focus on the latter kind of systems. The leitmotiv of this chapter will be the link between the investigated diffusion range and the information that can be extracted.

In the first part of this chapter, some aspects of the so called high b-values dynamics will be introduced and discussed.

It has been shown that the diffusion weighted NMR signal is linked to the motion propagator by means of a simple Fourier transform. If the motion can be expressed by a Gaussian propagator, then the NMR signal is an exponential decay as a function of the diffusion weighting and the diffusion coefficient - or tensor, if we are in three dimensional space. When higher diffusion weightings are investigated, specific situations in which the exponential decay proves unsuitable to fit the experimental results are found, suggesting that the hypotheses lying at the basis of its derivation are, for some reasons, violated.

In this regard, the CTRW framework will be proposed to describe the dynamics in terms of sub- and super-diffusion, and NMR signal decay will be shown to have a stretched exponential shape within this framework.

In the last section of the chapter, a model will be proposed to extend the stretched exponential to thee dimensional anisotropic environments, in the same way in which DTI is an extension to three dimensions of diffusion weighted imaging. This approach represents one of the original contributions brought by this thesis and is going to be published as a full paper [23].
CHAPTER 3. FROM CONVENTIONAL TO ANOMALOUS BEHAVIOUR

3.1 Dynamical ranges

As mentioned in the chapter introduction, by tuning specific experimental parameters in a diffusion NMR experiment, different dynamical ranges can be highlighted. The dependence of the results on the experimental parameters, specifically the b-value, can furnish important pieces of information about the system under investigation.

This section will be focused on systems where the conventional framework used to derive the NRM signal decay is supposed to hold, at least at first approximation, as opposed to next section, which instead will take into account advanced model of diffusion, leading to different shapes for the signal decay.

3.1.1 Multi-exponential decays

When two or more compartments, characterised by different diffusion constants (and usually different relaxation times), coexists in the same sample/voxel (for either spectroscopic or imaging techniques), the diffusion decay reflects this heterogeneous composition. In such cases, the recorded signal is composed by two or more exponential decay that are superimposed.

Those components can be distinguished on the basis of their diffusivity values. Examples of diffusivity values for different species of protons investigated in this thesis are reported in Table 5.1.

<table>
<thead>
<tr>
<th>Diffusivity range</th>
<th>Free water</th>
<th>Weakly bound water</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$2 \cdot 10^{-9}$ m$^2$/s</td>
<td>$10^{-11} - 10^{-10}$ m$^2$/s</td>
<td>$5 \cdot 10^{-12}$ m$^2$/s</td>
</tr>
</tbody>
</table>

Table 3.1. Diffusivity range for protons belonging to a free unrestricted water compartment, a bounded compartment and for protons belonging to fat macromolecules

In systems such as bone marrow, one of the soft matter examples investigated in this thesis, different peaks are distinguishable in the spectrum, as shown in the upper panel of Fig.3.1. Bone marrow is in fact composed by water and fatty acid triglycerides at different relative percentages. The relative concentration of each bone marrow component is dependent on both, its anatomical skeletal location and the age of considered individuals [24]; however, in a typical spectrum, one can resolve the fat peak at 1.3 ppm and olefinic fat peak at 5.3 ppm, which usually overlaps the broad water peak centred at 4.7 ppm.

Thus, fat and water components are often superimposed in the decay. Even though fatty acids are contained in the adipocytes, i.e. white fat cells contain a large lipid droplet surrounded by a layer of cytoplasm, as far as the experimental designs considered in this thesis are concerned, fat molecules can be regarded as spherical objects characterised by slow diffusion as opposed to the fast diffusion of water molecules. These two components can thus be distinguished on the basis of their different diffusive properties, as shown in Table 5.1.
3.1. DYNAMICAL RANGES

Figure 3.1. Bone marrow spectrum at 9.4T (upper panel). The broad peak in the left side of the spectrum is characterised by two different components: water centred at 4.7 ppm and olefinic fat centred at 5.3ppm. Lower panel: peak attenuation versus gradient strength or TE values shows a clear bi-exponential trend. Conversely the attenuation of fat peak at 1.3ppm (in the right side of the spectrum) is best fitted by a mono-exponential. Reproduced from [25].

Also the same molecular species can be associated to different dynamical properties that are reflected by two (or more) different diffusivity values. In particular, water interacting with biological membranes, macromolecules or other structures usually shows more than one diffusion coefficient [26]. The portion of water which lies more closely to the object manifest a reduced mobility and a diffusion coefficient from one to two orders of magnitude lower compared to that of free water. This component is usually called weakly bound water to discriminate with the hydration layer (which is invisible to NMR).

Another system characterised by different water compartments is the human brain. The central nervous systems (CNS) is doubtless regarded as a privileged system among all the other apparatuses of human and mammalian body, due to the special functions of which it is in charge. The importance of its role within the body is reflected by a highly complex microstructure, which will be widely
described in the following chapters. For the moment, it is important to stress that the brain contains a high percentage of water (about 80%), and that investigating the diffusion of water protons within the different areas can offer a powerful tool to understand the local structural properties.

By means of NMR, water diffusion signal is collected voxel-wise and parametric maps of the brain are obtained, as those shown in fig.2.7.

DTI images of the brain are commonly acquired using diffusion weightings or b-values up to \( b = 10^9 \text{ s m}^{-2} \). The b-value is optimised for the typical water diffusivity values found in brain parenchyma. Since the average diffusivity is of the order of \( 10^{-9} \text{m}^2\text{s}^{-1} \), under the assumption of a mono-exponential decay of the DW signal as a function of the b-value, the optimal b-value is \( 10^9 \text{ s m}^{-2} \) [17, 27].

It has been shown in sec.2.2.5 that a single diffusion weighting is enough to perform DTI reconstruction. However, if the exponential decay is governed by a single term, as predicted by DTI theory, the data points acquired at higher b-values should dispose along a line in the \( \log(S) \) vs. \( b \) plot. As will be shown in detail in the next section, for most of the brain region this is not the case. Initially, a bi-exponential model was introduced to account for this behaviour, as reported in fig. 3.2 [28].

![Figure 3.2](image-url)

**Figure 3.2.** Logarithmic plot of MR signal intensities relative to noise vs. b-factor along different diffusion-encoding directions for an individual pixel located within the right internal capsule. Along all directions the diffusion-related signal decay clearly deviates from a basic mono-exponential decay. The solid curves represent the optimal bi-exponential fits. Reproduced from [28].

Although there have been lots of controversies regarding the bi-exponential model, in most cases data have been fitted with a bi-exponential function corresponding to two water diffusion pools or phases in slow exchange, with a fast and a
3.1. DYNAMICAL RANGES

slow diffusion coefficient \([29, 30]\):

\[
S = S_0 f_{\text{slow}} \exp(-bD_{\text{slow}}) + S_0 f_{\text{fast}} \exp(-bD_{\text{fast}})
\] 

(3.1)

Where \(D_{\text{slow}} \sim 10^{-10} \text{ mm}^2/\text{s}\) and \(D_{\text{fast}} \sim 10^{-9} \text{ mm}^2/\text{s}\). It has been often considered that the extracellular compartment might correspond to the fast diffusing pool, as water would be expected to diffuse more rapidly there than in the intracellular, more viscous compartment corresponding to the slow diffusing pool. However, the volume fractions of the two water phases obtained using the bi-exponential model do not agree with those known for the intra- and extracellular water fractions (\(F_{\text{intra}} > 0.80\) and \(F_{\text{extra}} < 0.20\)) \([31]\), even by taking into account differences in \(T_2\) relaxation contributions between those compartments, so that the nature of those phases has yet remained unclear. Furthermore, some careful studies have shown that such a bi-exponential diffusion behaviour could also be seen solely within the intracellular compartment, pointing out that both slow and fast diffusing pools probably coexist within the intracellular compartment. Such studies were conducted with ions or molecules much larger than water, such as N-acetyl-aspartate \([32]\), fluoro-deoxy-glucose, or in particular biological samples, such as giant oocytes \([33]\).

LeBihan proposed a fascinating interpretation of the bi-exponential diffusion decay found in brain \([34]\), according to which the \(\sim 70\%\) fast diffusion pool correspond to the tissue bulk water in fast exchange with the water hydration shell around proteins and macromolecules, while the \(\sim 30\%\) slow diffusion water pool would originate from packets of highly structured water molecules which are trapped within a membrane-bound water network and the three- dimensional cell micro-trabecular network (see fig. 3.3). Although appealing, this interpretation seems to be an oversimplification and still one should be careful in assigning physical relevance to the parameters obtained by the bi-exponential model.

What is certainly true is that the bi-exponential model "philosophy" can be generalised assuming that for different diffusion weightings, different diffusion pools are characterised. Specifically the higher the b-value, the slower the diffusive behaviour. This means that, without any specific hypothesis about the number of components and the origin of such different pools, information about different dynamical ranges can be easily obtained by tuning the experimental parameters. This is true either in brain or in all other tissues/materials where water is interacting with larger molecules.

3.1.2 Single tensor model

In some cases, even though spin displacements are characterised by a single diffusion coefficient, DTI framework offers a partial characterisation of water dynamics, due to the presence of multiple privileged diffusivity directions. Again, one of the most important examples of this behaviour is the cerebral white matter.

In white matter, bundles of nerve fibres act like obstacles for the diffusing water molecules and the presence of axons creates privileged diffusive patterns. Due to the typical resolution of DTI scans, different fibre orientations may coexist in a single voxel, thus resulting in an intra-voxel orientational heterogeneity, which will depend on the anatomical region \([35, 36]\). In pure crossing fibres regime, the dynamic is conveniently described by two different tensors, which generate an oblate diffusion
ellipsoid (i.e. two equal bigger axes and one smaller), rather than the conventional prolate ellipsoid (i.e. one bigger axis and two smaller). In more complicated architectures, it is hard to find a comprehensive mathematical synthesis and the results will in general depend on the chosen diffusion time.

The poor resolution of crossing fibres at a single voxel level represents one of the major limitations of DTI tractography algorithms, which are based on the single-tensor model. Acquisition strategies such as HARDI [38, 39] allow overcoming some limitations of the single tensor model. Nevertheless, such strategies are extremely demanding in terms of hardware and post-processing.

Given that the angular dependency of signal is more pronounced at higher b-values [40, 41], exploring higher diffusion weightings within conventional DTI framework may thus help in this sense.
3.2 Evidences of anomalous behaviour

The notions and concepts of anomalous dynamical properties, such as long-range spatial or temporal correlations manifested in power laws, stretched exponentials or non-Gaussian probability density functions (PDFs), have been predicted and observed in numerous systems from various disciplines including physics, chemistry, engineering, geology, biology, economy, meteorology, astrophysics and others.

Here, the analysis on the failure of the regular diffusion framework on the human brain will be deepened, opening possible scenarios for other models.

The section will then span a wide range of specimens, going from materials to living matter, in which evidences coming from diffusion NMR, but also from different techniques, demonstrate the presence of an anomalous diffusion behaviour.

3.2.1 Water diffusion decay in human brain

In the previous section, it has been anticipated that if the exponential decay is governed by a single term, as predicted by DTI theory, the data points acquired at higher b-values should dispose along a line in the log(S) vs. b plot. Fig.3.5 shows instead the discrepancy between the expected decay and the measured one, as the b-value is increased above the optimal value of $10^9$ s m$^{-2}$.

In the last few years several experiments highlighted the impossibility of fitting the diffusion data with a single mono-exponential curve. This outstanding evidence has been reported in a number of brain studies, both in animal model [42] and in human [43,44]. Several approaches have been suggested to give a deeper insight into the non-trivial diffusive phenomenon in the CNS, thus finding a better agreement with the experimental curves [45–49].

The shortcomings related to the bi-exponential model have already been exam-
CHAPTER 3. FROM CONVENTIONAL TO ANOMALOUS BEHAVIOUR

Figure 3.5. Log of the acquired NMR signal (a.u.) versus the diffusion weighting or b-value, expressed in s m$^{-2}$. The experimental points are plotted as empty circles, while the mono-exponential decay predicted by regular diffusion is shown in black. Reproduced from [19].

ined. Since its introduction, few other models have been proposed. For example, the bi-exponential model was implemented adding an exchange term between compartments, obtaining interesting results [47]. Nevertheless, it has been difficult to synthesise the information into an easy to handle formalism, suitable for the clinical practice.

One of the most popular model is the Diffusional Kurtosis Imaging (DKI) [48, 49]. In this model, the deviation from Gaussian behaviour is quantified using a convenient dimensionless metric called the excess kurtosis, which is determined from the first three terms of an expansion of the logarithm of the NMR signal intensity in powers of b. At the moment, DKI proposes parametric maps to be used in combination with DTI conventional outputs. However, since it is based on a series expansion, DKI is suitable for b-values up to $2 \cdot 10^9$ s m$^{-2}$. Despite its increasing popularity, DKI is thus unable to explain the peculiar contrast obtained at very high b-values.

Only recently, it has been proposed that this intrinsic non mono-exponential behaviour can be described by a specific non-Gaussian PDF [50–53]. This hypothesis is fascinating since it relates the local diffusion dynamics to the macroscopic signal acquired by means of NMR. This issue will be deepened in the following paragraphs.

3.2.2 Anomalous diffusion in materials and biological matter

In the last twenty years, diffusion in disordered media has been deeply investigated from the theoretical point of view and by means of simulations [54, 55]. In the context of diffusion the term disordered is used to designate a medium where it is not
possible to define a geometry. In the cases of interest for this thesis, it may contain randomly distributed or geometrically generated fractal obstacles, which perturb the trajectory of the diffusing object tracer. This subject has been extensively studied by computer simulation, exact enumeration and renormalization methods.

From the experimental point of view, anomalous diffusion was demonstrated to occur in many complex systems. These range from turbulent fluids, to chaotic dynamical systems, to disordered media. In these systems, anomalous sub- and super-diffusion mechanisms, closely related to normal diffusion but with some qualitatively different properties, drive the physics of the transport processes.

Peculiar systems where sub-diffusion properties have been predicted theoretically [56, 57] and verified experimentally [58] are the porous media near the percolation threshold. Ternary microemulsions undergo a transition from a connected bicontinuous water-oil system to a disconnected water-in-oil, as volume fraction is varied. The anomalous transport phenomena are manifested in a non-linear relationship between the mean squared displacement and the diffusion time [58]. In a similar system, constituted by water molecules flowing through disordered porous glass, the crossover from sub-diffusive to super-diffusive power laws of the mean squared displacement as expected in the scaling window of disordered media upon the onset of advection was reported [59].

The translocation of biomolecules through membrane pores (channels) is one of the most vital processes within or across biological cells, serving both delivery and signaling purposes. If strong memory effects prevail during the translocation, the distribution of translocation times is broader than predicted by simple Markovian models based on Brownian motion and the diffusion becomes anomalous [60].

Brownian motion is also affected by molecular crowding that induces sub-diffusion of proteins and larger structures, thereby compromising diffusive transport and the associated sampling processes. Contrary to the naive expectation that sub-diffusion obstructs cellular processes, computer simulations have shown that sub-diffusion rather increases the probability of finding a nearby target [61]. Consequently, important events like protein complex formation and signal propagation are enhanced as compared to normal diffusion. Hence, cells indeed benefit from their crowded internal state and the associated anomalous diffusion. Crowded fluids are a natural phenomenon in living cells with considerable impact on intracellular chemical reactions. Indeed, the cytoplasm and nucleoplasm of living cells are crowded with a plethora of macromolecules, often rendering the diffusion in these intracellular fluids anomalous. While the interior of cells is very complex and heterogeneous, the phenomenon of crowding-induced sub-diffusion has also been observed in more controlled in vitro approaches [62, 63]. In all of these cases, a nonlinear growth of the mean square displacement has been observed.

All two diffusion regimes, i.e. sub- and super-diffusion, may be observed for the surfactant self-diffusion in systems of wormlike micelles, also called living polymers, depending on small variations in the sample composition [64, 65]. The living polymers are polymers with stable, polymerization-active sites formed by a chain polymerization in which irreversible chain transfer and chain termination are absent. The physical interpretation of the sub-diffusion behaviour is that the dominating diffusion mechanism corresponds to a lateral diffusion along the contour of the wormlike micelles. Super-diffusion is obtained near the overlap concentration.

\[3.2. \text{EVIDENCES OF ANOMALOUS BEHAVIOUR}\]
where the average micellar size is smaller so that the centre of mass diffusion of the micelles contributes to the transport of surfactant molecules.

Interestingly, some years ago the super-diffusion was proposed as a possible mechanism underlying adsorption of liquid molecules on surfaces such as in pores of porous glass [66]. This surface diffusion mechanism can indeed be described as a special form of a Levy walk [67]. In the strong-adsorption/short-displacement limit, the Cauchy propagator typical for Levy walks was verified and it was shown that the displacements effectively taking place along surfaces follow a super-diffusive time dependence of the mean square.

3.3 The stretching exponential model

Recently, the increasing number of evidences of anomalous behaviour in biological tissues suggested this peculiar transport dynamics to be the cause of non mono-exponential behaviour observed on human brain. As anticipated in sec.3.2.1, this is a very fascinating hypothesis since it links the local diffusion dynamics, due to the geometric configuration, to the macroscopic signal acquired by means of NMR.

Nevertheless, when this hypothesis was drawn, i.e. when the nonlinear relationship between root mean squared displacement and diffusion time was addressed as the cause of non mono-exponential behaviour, rather than applying the anomalous transport formalism to derivate the shape of the signal recorded by a PGSE sequence, in most cases only some general considerations were reported [50, 52]. As a consequence, the stretched exponential model for the signal following a PGSE sequence was at first introduced as a phenomenological model to fit the experimental data, when the single exponential decay proves unsuitable. In the first part of this section, these models will be reviewed briefly.

Few other Authors attempted instead to introduce more rigorous frameworks to describe anomalous diffusion [51, 53]: their works will be reported in the second part of the section.

Even though several issues remain to be clarified regarding the stretched exponential method, the contrast between different tissues provided by the stretching exponent proved so far absolutely promising for a wide range of applications.

Nevertheless, to characterise biological materials, one needs to take into consideration the anisotropy of the diffusion, i.e. the fact that the resulting diffusion decay may show a dependence from the spatial direction.

A first important experimental approach, introduced by Hall and Barrick [52], tried to quantify the anisotropy using values of the stretching exponent obtained along different directions. Values lower than one were recorded on human brain, but their results are compromised by the dependency on the gradient directions. In the following, this method will be briefly described.

One of the purposes of this work has been instead to develop an alternative method to account for the dependence of the stretching exponent from the spatial direction. The starting point was Hall and Barrick’s work, which was criticised as inherently dependent on the chosen measurement directions. The original approach proposed in this thesis will be discussed here from the theoretical point of view, while the results will be discussed in the last chapter.
3.3.1 Applications in brain

To describe the behaviour of the same signal attenuation analytically as a function of b-value, Bennett and coworkers [50] propose the use of the following stretched-exponential model:

\[ S(b) = S(0) \exp[-(b \cdot DDC)^\alpha] \] (3.2)

Here \( \alpha \) is the stretching parameter which characterises the deviation of the signal attenuation from mono-exponential behaviour, and is limited to values between zero and one. A value of \( \alpha \) that is near one indicates high homogeneity in apparent diffusion, namely a highly mono-exponential attenuation curve. Lower values of \( \alpha \) result from non-exponential behaviour caused by the addition of multiple separable proton pools within the voxel. This model was introduced following pure qualitative considerations, mainly based on the fact that the stretched exponential has been applied extensively in other fields of physics to similar problems.

![Figure 3.6. a) Histogram of stretched-exponential heterogeneity measure \( \alpha \) in rat cerebral cortex from a nonlinear least-squares fit. Data was obtained from DWI of six male Sprague-Dawley rats. b) Color map of the heterogeneity index \( \alpha \) in a stretched-exponential fit to signal attenuation from ROI voxels in cerebral cortical ribbon. Reproduced from [50].](image)

This model was applied to image rat brain. The diffusion gradients were applied in the frequency-encoding direction and the signal was spatially resolved, so for each voxel the stretching parameter was calculated and displayed as a map. The histogram of the stretching exponent values and the map are reported in fig.3.6.
CHAPTER 3. FROM CONVENTIONAL TO ANOMALOUS BEHAVIOUR

The measurements were performed across one direction only because the Authors believe water diffusion heterogeneity index in the human brain to be insensitive to the orientation of applied magnetic field gradients [68].

Similar qualitative ruminations led to the work of Hall and Barrick [52]. The relationship between mean-squared displacement and time was used as a definition of diffusion constant $D$ via

$$D(t) \sim \frac{\langle r^2(t) \rangle}{t} \quad (3.3)$$

these Authors proposed that, if the signal is observed as a function of the b-value, a similar relationship holds due to the linear dependence of $b$ vs $t$:

$$\text{ADC}(b) \sim \frac{\langle r^2(b) \rangle}{b} \quad (3.4)$$

When diffusion is anomalous one obtains:

$$\frac{\langle r^2(b) \rangle}{b} \sim b^\gamma \quad (3.5)$$

and thus

$$S(b) \sim S(0) \exp \left[ -bA \frac{\langle r^2(b) \rangle}{b} \right] \sim S(0) \exp(-Ab^\gamma) \quad (3.6)$$

It is clear that there is no formal justification behind this derivation. Besides, it is mathematically wrong. If one supposes that the propagator is Gaussian, then the signal decay would show an anomalous dependence in $t$ rather than in $b$, as a consequence of eq.2.49 and it would be impossible to observe a stretched exponential decay for experiments in which $b$ is varied through the gradient amplitude (and $t$ is thus a constant). On the other way, if the propagator is not Gaussian, the linear dependence of $b$ vs $t$, which follows by the definition of $b$ (eq.2.41), does not hold anymore in the case of anomalous diffusion, since the signal is no longer expressed by a simple exponential. In that case, a correct approach might be the CTRW, as will be shown later.

3.3.2 Theoretical models

From the previous chapter, it is evident that the derivation of the signal expression after a PGSE sequence has to be derived by Fourier transforming a proper motion propagator. If the conditions of anomalous transport hold, the motion propagator should reflect specific characteristics; for example, the root mean square displacement has to show the proper dependence on the diffusion time, with the exponent larger or smaller than unity in case of super- and sub-diffusion, respectively. One of the most useful frameworks to derive the motion propagator for anomalous dynamics is the CTRW introduced in sec.2.3.1, but also other approaches can be used.

Ozarslan and co-workers [51, 69] introduced two scaling exponents to characterise excised human neural tissue samples and red blood cell ghosts: the exponent which appears in the relationship between the root mean square displacements and diffusion time

$$\langle r^2(t) \rangle \propto t^{2/d_w} \quad (3.7)$$
3.3. **The Stretching Exponential Model**

and the return-to-origin-probability for diffusing particles, which obeys the following power-law relationship

\[
P(r = 0, t) \propto t^{-d_s/2}
\]

(3.8)

The relationship between these three exponents is simply

\[
d_f = \frac{d_sd_w}{2}
\]

(3.9)

note that \(d_s\) and \(d_f\) play the role of \(2\alpha\) and \(\mu\) of eq.2.79. These scaling relationships were incorporated into a particular form for the diffusion propagator:

\[
P(r, t) \propto \frac{r^{d_s-d}}{t^{d_s/2}} \Phi \left( \frac{r}{t^{1/d_w}} \right)
\]

(3.10)

The two scaling exponents were measured by means of diffusion NMR in the q-space domain. Diffusion in neural tissue turned out to be in the sub-diffusive regime whereas in the red blood cell ghosts it was very close to normal, as expected.

![Figure 3.7. \(\mu\) vs \(\alpha\) calculated according to the results showed in [69]. Note that the diffusion is normal for red blood cell sample while it is anomalous for both the tumor tissue and the normal gray matter.](image)

Magin et al. [53] were the first to link the CTRW formalism to the stretched exponential model in an explicit way. One of the merit of this work has been to derive the complete expression for the signal decay following a PGSE sequence, starting from the Bloch equations. They investigated two cases:

- \(\alpha = 1\) and \(1/2 < \beta < 1\), where the diffusion term is assumed to follow fractional order dynamics in space
- \(0 < \alpha < 1\) and \(\beta = 1\), where the spin dynamics are assumed to follow fractional order behaviour in time

i.e. the two cases addressed by Metzler and Klapfer, where \(\alpha\) and \(\beta\) are the fractional order derivative in time and in space respectively [12]. In the first case, they found
the following expression for the signal decay:

\[ S = S(0) \exp \left[ -D\mu^{2(2\beta-1)}(\gamma G_z \delta)^{2\beta} \left( \Delta - \frac{2\beta - 1}{2\beta + 1} \delta \right) \right] \]  

(3.11)

The second case was investigated only for a fixed gradient and the signal expression was:

\[ S = S(0)E_\alpha \left[ -i\gamma G_z z\tau (t/\tau)^\alpha \right] \exp \left[ -B(t/\tau)^{3\alpha} \right] \]  

(3.12)

where \( \mu \) and \( \tau \) are fractional order space and time constants needed to preserve units.

Magin and co-workers were the first to offer a theoretical justification for the stretched exponential used in other works [52, 68]. Nevertheless, one issue is completely overlooked in their works. The stretched exponential model corresponds to the first investigated case, i.e. the fractional order behaviour in time, but this means that the Authors are hypothesising a finite characteristic waiting time \( T \) and diverging jump length variance \( \Sigma^2 \) (see sec.2.3.3). Thus, since they are supposing the dynamics in time to be regular, Magin et al. actually proposed super-diffusion as the microscopic mechanism to lead to the stretched exponential signal decay. Conversely, all other works regarding anomalous diffusion, and also common sense, hypothesized the diffusion to be slower in biological systems compared to free water (i.e. sub-diffusion).

This very important issue will be addressed in the last chapter, where experimental results on samples and biological tissues will be furnished to clarify the role of the fractional dynamics in space and in time to determine sub- or super-diffusive phenomena.

### 3.3.3 Hall and Barrick’s approach to anisotropy

Hall and Barrick [52] developed a method to quantify not only the magnitude of the stretching exponent, but also its anisotropy. Their approach was based on the acquisition of diffusion weighted images at different b-values and across several gradient directions. From the decay curves, they measured the anomalous exponent \( \gamma \) fitting the decay with a stretched exponential model. In this way, they reconstructed parametric maps of the mean value of the anomalous exponent:

\[ \langle \gamma \rangle = \frac{1}{N} \sum_{i=1}^{N} \gamma_i \]  

(3.13)

and its spread along different directions, which they called *anomalous anisotropy*

\[ AA = \sqrt{\frac{N \sum_{i=1}^{N} (\gamma_i - \langle \gamma \rangle)^2}{\sum_{i=1}^{N} \gamma_i^2}} \]  

(3.14)

where \( N \) is the number of the directions, \( \gamma_i \) is the anomalous exponent measured in the i-th direction and \( \langle \gamma \rangle \) is the mean exponent.

According to Hall and Barrick, anomalous anisotropy should be regarded as an equivalent of the FA. However, while FA quantifies the mean squared gap between three eigenvalues of the diffusion tensor and their mean value, the anomalous
anisotropy estimates the difference between the stretching exponents as measured in $n$ different directions and their correspondent mean values. The main difference between the outputs of the DTI analysis and the anomalous exponent indices is that, while the former are defined as scalar invariants of a tensor (i.e. the diffusion tensor) and are by definition independent of the reference frame in which they are measured, the latter instead depend on the directions along which the average is quantified.

### 3.3.4 Original contribution: Anomalous diffusion imaging

In this section, a different approach to derive the $\gamma$ anisotropy, which is similar to that used for FA estimation in DTI analysis, will be proposed. This method aims at accounting for the tensorial nature of the anomalous diffusion, with a description that does not depend on the laboratory reference frame but which is intrinsic of the system. The method is an original contribution brought by this thesis and is reported in a paper in press [23].

The tensorial nature of ordinary diffusion has been fully demonstrated. The tensorial structure comes from the properties under rotations of quadratic forms. The *Spectral Theorem* affirms in fact that:

- Each symmetric matrix is diagonalisable by means of a rotation
- The resulting eigenvalues are real
- The resulting eigenvectors form an orthonormal basis

The tensorial nature of anomalous diffusion has not been demonstrated yet but in the following approach, it represents an hypothesis that is driven by reasonableness criteria.

For this purpose, an analogy with the ordinary diffusion dynamics, where the diffusion tensor is diagonal only along three main directions, was draws. According to the spectral theorem, in a three-dimensional space it is always possible to decompose the motion into its projections along three main axes. In this case, the stretched exponential model is valid along each of the three principal diffusive directions. When considering a generic direction, one hypothesises the total signal to be due to a combination of the behaviours along each of the three main axes, according to the following formula:

$$S(b) \propto \prod_{i=1}^{3} \exp (-A_i b^{\gamma_i})$$  \hfill (3.15)

where $A_i$ is a generalization of the diffusion constant and $b$ is the b-value. The signal $S(b)$ is simply a productory of three terms since it derived by Fourier-transforming the motion propagator. Indeed, if the spin displacements along the three main directions are independent from each other, the propagator can be decomposed as a productory of three terms. In the same way, its Fourier-transform will be a productory of three terms.

The b-value in eq.3.15 is calculated along the chosen measurement direction in the reference frame of the principal axes, i.e. it contains the director cosines of the
measurement direction with respect to the principal reference frame. See Fig.3.8 for a visual example of the method.

It is not possible to know a priori the directions associated to the principal diffusion axes, which are thereby linked to the local geometrical structure and therefore supposed to be voxel-dependent. In principle, the complete solution for this problem requires the estimation of 12 parameters: 3 for the $A_i$, 3 for the $\gamma_i$ and 6 to define the principal reference frame.

To implement the fitting routine, this model was simplified by separating the analysis into three steps:

1. first, the principal reference frame was calculated by using the DTI analysis. From the map of the diffusion eigenvector, the director cosines of the principal reference frame expressed in the laboratory frame were found on a voxel by voxel basis.

2. For each voxel, the rotation matrix which links the laboratory frame with the principal frame was calculated. The rotation matrix was then used to express, for each applied gradient direction, the corresponding b-value in the principal reference frame.

3. Once the main reference frame was known, it was possible evaluate the remaining six parameters (3 for the $A_i$, 3 for the $\gamma_i$) of eq.3.15 by measuring the signal in at least six non collinear directions. This implies as an approximation
that the principal reference frame is the same for both DTI and anomalous
diffusion framework.

This method has been applied to 10 healthy volunteers’ brain and compared the
results to both conventional DTI and Hall and Barrick’s analysis. The experimental
results will be shown in the next chapter.

It is worth saying that this approach in dealing with anisotropy when dynam-
ics are described by non-Gaussian pdfs is completely general and can be applied
to different forms of the signal decay. This means that in this thesis, a general
prescription was introduced, which can prove useful in characterising anisotropic
structures where the motion spans from sub- to super-diffusion.
Chapter 4

Experimental results I

In the previous chapter, several issues have been raised, all of those were linked to the diffusion of water as an advanced tool to investigate the tissue/material microstructure.

This chapter and the following one will try to deepen some of the unanswered questions and to verify the hypotheses that have been introduced. All the topics covered in the next two chapters are related to very general problems of the soft matter physics. However, particular emphasis will be given to the practical applications of the proposed methods.

For the sake of clarity, the experimental results are separated into two parts: the first one regards findings obtained under the hypothesis of Gaussian diffusion (this chapter), while the second one deals with contexts in which anomalous diffusion regime is supposed to hold (next chapter).

Diffusion properties turned out to be the key to understand the peculiar behaviour of porous systems in which internal gradients are present, like the spongy bone. These internal gradients are generated by the difference in magnetic susceptibility between bone marrow and solid bone plates. By investigating the properties of fat and water molecules in the bone marrow, it is possible to obtain important information on the bone properties that can be useful for the diagnosis of bone pathologies like osteoporosis. This innovative strategy will be discussed in the first part of this chapter.

The importance of dynamics range has been highlighted in the last chapter. In the last part of this chapter, those considerations will be applied to investigate the orientation of the main diffusion axes as a function of increasing diffusion weightings. Interesting outcomes suggest that at high b-values, oblate ellipsoids contribution is enhanced with respect to prolate ellipsoids in areas characterised by a non-negligible fibre dispersion within the same voxel. In other words, the peculiar contrast observed in brain at high b-values is modulated by a stronger influence of fibre crossing dynamics.

4.1 Internal gradients in spongy bone: role of diffusion

As introduced in the previous chapter, spongy bone is a porous system characterised by a solid trabecular network immersed in bone marrow, which is characterised by
a different relative percentage of water and fats. By investigating the response of these two components to a simple Spin Echo sequence, due to the strong influence of internal gradients at the bone/bone marrow interface, it is possible to infer information on the micro-architecture of the solid matrix.

To understand which kind of information is encoded in the internal gradient trend measured in different specimens, the key point is the characterisation of the differential effects of fat and water diffusion. Specifically, water dynamics turn out to be responsible for the sensitivity of the spin echo decay to the internal gradients. This is the main result of this section that will be presented in the followings.

This innovative approach to the bone marrow investigation can be applied to assess the status of spongy bone. It will be shown, by means of in vitro experiments, that water $G_i$ magnitude from calf samples is directly proportional to their TB density. Similar behaviour is also observed in the clinical measures of the calcanei of individuals characterised by different known TB densities. Conversely, fat $G_i$ did not provide any information on spongy-bone density. Water $G_i$ may thus be considered as a reliable marker to assess the status of spongy bone.

The results of this section had been published in abstract form [70] and as a full paper [25]

4.1.1 Problem statement

Bone tissue is a complex biomaterial composed of a solid mineral matrix filled by bone marrow (the liquid interstitial phase). The solid matrix is constituted mainly of mineral components, while bone marrow is composed mainly of hematopoietic marrow and fatty acid triglycerides at different relative percentages. The relative concentration of each bone marrow component is dependent on both, its anatomical skeletal location and the age of the individual [24].

Mammalian and human bone may be classified as cortical or trabecular. The former is mainly present in the shaft of long bones and is much denser (with a porosity ranging from 5% to 10%) than trabecular bone (TB). Conversely, TB, or spongy bone, is much more porous (with a porosity ranging from 50% to 90%) and is metabolically more active. From a physical point of view, spongy bone is a porous system that can be described as a solid with holes and cavities (i.e. presenting as connected void spaces randomly distributed within a solid matrix).

In approaching this issue, the main aim was to characterise the differential behaviour of water and fat. The starting point was a joint measure of the diffusion curve and of the spin echo decay. In presence of internal gradients, the latter is governed by in eq.2.38, from which the value of $G_i$ can be estimated. Taking into consideration the scenario depicted in sec.3.1.1, water and fat contributions are overlapped in the measured signal decay. The contributions of the two species can indeed be discriminated, allowing for the estimation of two different diffusivity values and two values of $G_i$.

$G_i$ is a key parameter in the context of bone pathologies investigations since it is strictly associated with TB density and structural rearrangements, but also depends on the bone marrow quality (i.e. specific composition), as will be shown in this section.

Osteoporosis is indeed a highly diffuse disease that typically affects elderly indi-
individuals. It is a systemic skeletal disease characterised by low bone density, micro-architectural deterioration of the bone tissue and by an increase in bone porosity [71], leading to bone fragility and increased susceptibility to fracture (WHO Scientific Group Report 2000). From an epidemiological viewpoint, in elderly individuals the occurrence of bone fractures mainly affects those skeletal locations that are particularly rich in TB, such as the vertebrae and femoral head. The evaluation of bone mineral density (BMD) based on dual-energy x-ray absorptiometry (DXA) is currently considered the gold standard for clinical diagnosis of osteoporosis [72]. However, only a poor correlation between BMD assessments and the relative risk of bone fracture has been reported [73, 74], suggesting that other factors besides low BMD likely contribute to determine bone fragility. This lack of information on the risk of bone fracture, which is critical for clinical purposes, has prompted intense research to identify new parameters with the ability to assess spongy-bone status and to provide reliable measures of bone’s resistance.

One of the aims of the present thesis was thus to clarify the role of water and fat diffusion in the measurement of the internal gradients in a specific porous system as the spongy bone. These insights highlighted the potential ability of $G_i$ to describe spongy bone status as related to its TB density and quality. For this purpose, using a micro-imaging probe at high magnetic field (9.4 T), $G_i$ behaviour inside each single pore of calf spongy bone samples was investigated in vitro as a function of both TB density and relative fat and water-bone marrow concentrations. To test the methodological feasibility of this approach, mean $G_i$ was first quantified in calf samples characterised by different TB densities. The potential suitability of $G_i$ as a measure able to assess the TB status in vivo was then investigated. For this purpose, using a 3T clinical scanner, the calcanei of subjects with different TB densities were examined.

### 4.1.2 Methods

As previously reported, in systems like spongy bone it is possible to assess the intensity of mean $G_i$ from the attenuation of the spin echo signal as a function of different TEs. Furthermore, a measure of the diffusion coefficient $D$ (i.e. a measure of the apparent diffusion coefficient ($ADC$)) and/or a measure of $T_2^{\text{true}}$ are needed to reduce the number of unknown parameters and improve the fit quality. In all in vitro and in vivo experiments reported in this section, the $ADC$ was measured and inserted in eq.2.38 to extract $G_i$ and $T_2^{\text{true}}$ using a fitting procedure.

#### In vitro experiments

Experiments were performed using a Bruker Avance-400 high-resolution spectrometer operating at 9.4 T. XWINNMR and ParaVision 3.0 software were employed for data acquisition and analysis. Five ex vivo spongy-bone samples, excised from calf femur head, were cut into pieces of approximately 20 mm high and 7 mm deep in order to fit into the micro-imaging probe bore. The long axis of each sample was located parallel to the main direction of the static magnetic field (the z-axis). The temperature of each sample was fixed to 291 K. $T_2$-weighted (SE) images were obtained for initial evaluation of TB density. All samples were characterised by a non-uniform trabecular density.
As shown in fig.4.1, TB density varies continuously along the height of each sample, moving from the upper to the lower zone. The upper zone, which is close to the cortical bone, is characterised by higher trabecular density and smaller spongy bone pores (fig.4.1a). Conversely, the lower zone is characterised by a larger intertrabecular space (fig.4.1c). The middle zone (located between upper and lower zones) shows intermediate characteristics of TB density (fig.4.1b).

These three anatomical locations will be referred as follows: (a) HTD (high trabecular density; upper zone), (b) ITD (intermediate trabecular density; middle zone) and (c) LTD (lower trabecular density; lower zone). In each sample, for each considered location, three 0.2 mm thickness slices were identified to measure quantitative MR parameters. Data extracted from each of the three slices were then averaged to obtain mean values of each MR parameter in the three locations (HTD, ITD and LTD). Five analysed samples were selected such that they were characterised by similar TB densities and similar fat-to-water ratio quantities in bone marrow. TB density and bone marrow water content (together with the relative content of different types of fat in bone marrow) are known to be dependent on age, race and skeletal site. To correct for these potential biases, all samples were obtained from 10- to 12-month-old calves of German race, bred in Germany and butchered in Rome (Italy). In all fresh samples (analysed immediately after slaughter) the same area of the femur head was selected for MR investigation.

Spectroscopic experiments

In the first step of these experiments, a validation experiment was performed under ideal circumstances where molecular diffusion and $T_2$ information were obtained for each of the two individual spectral components (water and fat molecules).

A spectroscopic CPMG sequence (repetition time $TR = 1$ s, number of averaged scans $NS = 8$, $N = 64$ data points) and a spectroscopic PGSE ($TE/TR = 18/3000$ ms, diffusion gradient pulses delay $\Delta = 400$ ms, diffusion gradient pulses duration $\delta = 4.6$ ms and diffusion gradient strength $g$ applied along the x-axis were used to recognise and discriminate $T_2$ and $ADC$ of water and fat molecules in the samples.

Spatially resolved experiments

A MSME (multi-slice multi-echo) imaging sequence ($TR = 2000$ ms, field of view $FOV = 7$ mm, matrix 256x256, slice thickness 0.2 mm, $NS = 8$) at various TEs ($4.8, 6, 8, 10, 12, 14, 16, 18, 20, 24, 30, 40, 50, 60, 70, 80, 100, 120$ ms) was used to obtain the SE decay in the three regions of each sample characterised by a different TB density. A PGSTE imaging sequence was also employed ($TE/TR = 21.9/3000$ ms, $FOV = 7$ mm, matrix 256x256, $\Delta = 40$ ms, $\delta = 4$ ms, using eight $b$ values ranging from 400 to 40 000 s mm$^2$, slice thickness = 0.2 mm, $NS = 8$) in order to measure the $ADC$ along the x-axis for each of the three selected regions of the considered samples. The x-axis was arbitrarily chosen to assess molecular diffusion perpendicular to trabecular surfaces (see fig.4.1).

After the micro-imaging investigation of the bone marrow’s water and fat behaviour within pores of spongy bone, an equivalent acquisition protocol at lower TEs ($4.8, 10, 20, 40, 60, 80, 100$ ms) and $b$ values (ranging from 200 to 10 000 s mm$^2$) was set up. This additional protocol was added to obtain a protocol potentially
Figure 4.1. An example of the calf spongy bone sample used for experiments in vitro. GE localizer and SE images (TE = 4.3 ms) selected perpendicularly to the static magnetic field at three different depths in the sample show a non-uniform TB density. In each experimental sample, trabeculae are closely spaced in the upper zone (a). Conversely, they show an intermediate density in the middle zone (b) and a larger inter-trabecular space in the lower zone (c). Reproduced from [25].

suitable for clinical application (i.e. lower scan time, lower b values). However, due to a reduced number of experimental points, this acquisition provides SE decays and ADCs that do not clearly discriminate between water and fat.

Data processing and statistical analysis

$T_2$ and $ADC$ of water and fat components were obtained using a Levenberg-Marquardt (L-M) fit taking into account the peak area decays respectively centred at 4.7 ppm (large from 4 to 5.4 ppm) for water and at 1.3 ppm (large from 0.1 to 2.5 ppm) for fat. For the reasons explained in the previous chapter, the attenuation of this broad peak (from 6 to 4 ppm) was fitted versus the diffusion gradient strength or the TE value using a bi-exponential function. This took into account the olefinic and water component separately.

Two different in vitro analyses were performed using the data extracted from the imaging experiments. In both cases only the central zone of each image was considered (i.e. excluding the image border), in order to exclude or limit the presence of air in bone marrow cavities. Indeed, this represents one of the major sources of artefacts when performing imaging on excised samples. The influence of surface relaxation was not taken into account in this analysis, due to negligible values of
surface relaxivity reported for bone [75].

In the first analysis, the signal deriving from water and fat protons in T₂-weighted images was investigated as a function of each voxel location with respect to the bone-bone marrow interface. In each slice of the three considered locations, the signal nearby the boundaries between bone marrow and solid bone was identified using a software (written in Matlab) (fig.4.2) written in-house.

![Figure 4.2. Example of selection of a three pixel wide boundary region in three considered locations, respectively, characterised by lower (LTD), intermediate (ITD), and higher trabecular density (HTD). The red mask, which represents the boundary region, is superimposed over SE images obtained at TE = 4.3 ms. Reproduced from [25].](image)

Spin echo signal properties and $G_i$ behaviour were then assessed in the boundary and inner regions of the trabecular pores. The inner regions of the pores were identified by subtracting the boundary regions from the whole pore area. An L-M fit was performed using the signal as a superimposition of two components: one belonging to fats and another belonging to water molecules. Each component contributes to the total signal as one exponential term of eq.2.38. The signal intensity at TE = 0, $T_{2}^{\text{true}}$, $G_i$ and the ADC of water and of fat components were obtained. Using this procedure, mean and SD values of $G_i$ from water and fat were respectively derived. Water ADC and fat ADC were measured from diffusion-weighted images, while $T_{2}^{\text{true}}$ values were considered a free parameter in the fit.

Conventional T₂ relaxation times of water and fat components were also evaluated from the same images, executing a bi-exponential L-M fit of water and fat components. This analysis was repeated at different widths (measured in voxels) from the boundary region of each pore. The mean values of water and fat $G_i$ were calculated by averaging the results obtained in all pores contained in three image slices for each specific location (HTD, ITD and LTD).

In order to have a more objective quantification of TB density, the ratio between the perimeter and the area of each pore in the slices was calculated. As a result, the ratio (Np/Na) between the number of voxels defining each pore perimeter (Np) and the number of voxels constituting the corresponding area (Na) was obtained from all pores included in each of the slices. Then, the mean values of Np/Na and $G_i$ in different bone locations (characterised by different TB densities as defined by Np/Na) were obtained from each sample. Moreover, mean values of both magnetisation associated with water and fat extracted from the bi-exponential fit as a function of the boundary region width were evaluated. The averages were calcu-
lated in all five samples. Pearson’s correlation coefficient (r) and a paired Student’s t-test were used to assess the linear correlation between $G_i$ and TB densities and differences between water and fat magnetisation as a function of boundary distances measured in voxels.

In a second experiment, $G_i$ was derived using the signal arising from each entire slice. In this case, the aim was to identify an acquisition protocol at high magnetic field which might also be suitable for clinical application (using relatively short acquisition times, conventional sequences and a lower image resolution compared to that obtained by micro-imaging apparatus). Distinguishing water from fat components was not a priority in this case. Therefore, a mono-exponential L-M fit was employed to obtain the mean $ADC$ along the x-axis. A mono-exponential L-M fit was performed including a constant which took into account the deviation from mono-exponential behaviour. Mean and SD values of $G_i$ were obtained in the three bone locations (HTD, ITD and LTD) performing an average of $G_i$ values across the five samples together with their corresponding values of $T_2^\text{true}$. Moreover, conventional $T_2$ relaxation time was estimated from each slice using a mono-exponential L-M fit plus a constant. Again, mean and SD values of $T_2$ were obtained in the three zones (HTD, ITD and LTD) by performing an average of $T_2$ values across the five samples.

### In vivo experiments

Six subjects were recruited for this study, three healthy females (24, 42, 52 years old, respectively), one osteopenic and one osteoporotic female (both subjects 62 years old), and one male (33 years old). Healthy, osteopenic or osteoporotic status of the 52- and 62-year-old females was established on the basis of their last (within 6 months from our MR investigation) DXA medical report. The mean T-score in L1 to L4 vertebrae of the 52- and of the two 62-year-old females were -1.30, -1.81 and -3.51, respectively. The study was approved by the Local Ethics Committee. Each subject gave her/his informed written consent to participate in this study.

A progressive reduction of TB density across aging has been well described in the previous literature [76, 77]. In humans, bone mass reaches a maximum peak at the approximate age of 25 years, and then progressively decreases over the following decades. As a consequence, it is reasonable to assume that both the 42- and the 52-year-old females had a lower TB density when compared to the 24-year-old female. Gender-related differences are also reported [78]. Men are known to have a higher bone mass peak than age-matched women, thus suggesting the male subject to have the highest TB density. Moreover, the osteoporotic female (T-score = -3.51) included in this study had a lower TB density than the age matched osteopenic (T-score = -1.81) female. As expected, both 62-year-old females (with osteopenia and osteoporosis) had a lower TB density than the 52-year-old healthy woman (T-score = -1.30).

Each subject underwent an MRI examination of the right foot (fig.4.3), using a 3T MR system (Siemens Allegra, Erlagen, Germany). A FLASH (Fast Low-Angle SHot) sequence (TR = 1500 ms, field of view FOV = 192 mm, matrix 128x128, slice thickness 5 mm, flip angle 30°) was acquired using various TEs (5, 7, 10, 20 ms) to estimate $T_2$. Sagittal SE images (TR = 1500 ms, FOV = 192 mm,
matrix 256x256, slice thickness 5 mm) were also collected using a SEMC (Spin Echo Multi Contrast) sequence at various TEs (20, 30, 40, 50, 80, 100 ms). The ADC was evaluated from sagittal diffusion-weighted images using a SE segmented-EPI (Echo Planar Image) sequence at two different b values (0 and 8 \times 10^9 \text{ s m}^{-2}) with diffusion gradient applied along the anterior-posterior direction and TE/TR = 89/2500 ms. \( G_i \) was obtained by the same mono-exponential L-M fitting procedure (using eq.2.38 without discriminating between water and fat components) described in the previous section by selecting the entire calcaneal area of each subject as shown in fig.4.3. Single voxel spectroscopy (PRESS sequence) with TE = 22 ms, TR = 5 s, NS = 32, was used to obtain bone marrow proton spectra to extract fat and water content percentage. A single voxel (size of 15x15x15 mm$^3$) was positioned in the centre of the calcaneus of each subject.

**Figure 4.3.** MR sagittal view of the foot examined in a 24-year-old female. The region outlined in black shows the calcaneal area on the SE image used to assess the mean value of \( G_i \) after fitting based on the L-M procedure. Reproduced from [25].

### 4.1.3 Results

**In vitro experiments**

Spectroscopic $T_2$ and $ADC$ mean values and their SD of water and fat components in excised bone samples are reported in table 4.1. Data show that both $T_2$ and $ADC$ of the water component (centred at 4.7 ppm) are significantly lower than those of fat components (centred at 5.3 ppm and 1.3 ppm). This allows to discriminate clearly between the two components (water and fat). Specifically, $T_2$ values of water and fat are consistent with those previously reported [79]. Similarly, $ADC$'s of fat components, which are approximately two order of magnitude lower than those of water molecules, fit well with those recently reported by other authors [80].
4.1. INTERNAL GRADIENTS IN SPONGY BONE: ROLE OF DIFFUSION

\[
\begin{align*}
(T_2 \pm SD) & \quad (ADC \pm SD) \\
\text{Water at 4.7 ppm} & \quad 19 \pm 2 \quad 4.2 \pm 1.8 \cdot 10^{-10} \\
\text{Fat at 5.3 ppm} & \quad 72 \pm 36 \quad 5.0 \pm 0.6 \cdot 10^{-12} \\
\text{Fat at 1.3 ppm} & \quad 65 \pm 16 \quad 5.3 \pm 0.3 \cdot 10^{-12}
\end{align*}
\]

Table 4.1. Spectroscopic evaluation of excised calf samples.

In Table 4.2a, mean values (and their SD) of \(T_2\), \(ADC\), \(G_i\) and \(T_2^{true}\) are reported for the water component obtained in LTD, ITD and HTD locations of one sample (performing an average of values extracted from each slice belonging to a single location). In table 4.2b, the corresponding results for the fat component are reported.

<table>
<thead>
<tr>
<th>Water %</th>
<th>((T_2 \pm SD)) (ms)</th>
<th>((ADC \pm SD)) ((10^{-10} m^2 s^{-1}))</th>
<th>(G_i \pm SD) ((mTm^{-1}))</th>
<th>(T_2^{true} \pm SD) (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTD 55</td>
<td>17.0 \pm 3.0</td>
<td>19.0 \pm 0.4</td>
<td>222 \pm 131</td>
<td>17.9 \pm 1.0</td>
</tr>
<tr>
<td>ITD 47</td>
<td>15.0 \pm 2.3</td>
<td>7.0 \pm 0.4</td>
<td>282 \pm 134</td>
<td>15.2 \pm 0.5</td>
</tr>
<tr>
<td>HTD 97</td>
<td>14.8 \pm 0.4</td>
<td>1.6 \pm 0.1</td>
<td>803 \pm 181</td>
<td>16.0 \pm 0.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fat %</th>
<th>((T_2 \pm SD)) (ms)</th>
<th>((ADC \pm SD)) ((10^{-10} m^2 s^{-1}))</th>
<th>(G_i \pm SD) ((mTm^{-1}))</th>
<th>(T_2^{true} \pm SD) (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTD 45</td>
<td>42.3 \pm 6.2</td>
<td>5.3 \pm 0.3</td>
<td>760 \pm 195</td>
<td>43.0 \pm 4.8</td>
</tr>
<tr>
<td>ITD 53</td>
<td>39.7 \pm 4.0</td>
<td>5.9 \pm 0.3</td>
<td>727 \pm 437</td>
<td>44.0 \pm 5.3</td>
</tr>
<tr>
<td>HTD 3</td>
<td>31.6 \pm 6.0</td>
<td>5.4 \pm 0.2</td>
<td>904 \pm 119</td>
<td>37.5 \pm 9.8</td>
</tr>
</tbody>
</table>

Table 4.2. Spectroscopic evaluation of excised calf samples.

The water \(ADC\) showed a decreasing trend proportional to the increase of trabecular density; thus, water mobility is more restricted in regions where the trabeculae are smaller. Conversely, it is evident from Table 4.2 that the fat \(ADC\) is not sensitive to increase in trabecular density. Water \(G_i\) increases proportionally with the increase in TB density, while fat \(G_i\) is characterised by an opposite trend and does not allow discrimination between LTD and ITD.

Fig. 4.4 displays different types of behaviour of water and fat \(G_i\) as a function of the distance (in voxels) between the TB marrow interface and the internal part of the trabecular pore. \(G_i\) values were derived using a fitting procedure, while \(ADC\) values were derived from diffusion-weighted images.

According to these findings, water \(G_i\) is characterised by a decreasing trend when moving from zones adjacent to the trabecula to zones located in the centre of the inter-trabecular space. This behaviour was observed in each of the three locations characterised by different TB densities. When comparing \(G_i\) magnitude between the three locations, it was otherwise evident that \(G_i\) in the HTD location is higher than that extracted from the other two locations (ITD and LTD). Conversely, fat compared to water \(G_i\) revealed an opposite behaviour. In the latter case, \(G_i\)
was characterised by an increasing trend when moving from voxels adjacent to trabeculae to those located in the centre of the inter-trabecular space. In the insets of fig.4.4, mean magnetisation intensities are reported for fat and water components as derived from the fitting procedure. Water concentration is higher in the HTD slices and decreases proportionally with the reduction of trabecular density (i.e. in ITD and in LTD).

Figure 4.4. behaviour of water (left) and fat $G_i$ (right) as a function of increasing voxel number ranging from regions with one pixel (located close to the bone surface) to regions constituted by five pixels (from the bone surface to the pore centre). Results are reported for HTD (empty squares), ITD (empty circles) and LTD (filled circles). In regions with HTD, the last two points are missing because the trabecular interspaces were not large enough to allow a boundary region (which is wider than three pixels) to be drawn. Reproduced from [25].

Figure 4.5. Water $G_i$ (left) and fat $G_i$ (right) behaviour for LTD in the boundary region (filled circles) and in the inner region (empty circles) as a function of increasing boundary region width (which corresponds to a decrease of the inner region width). The size of each pixel is 27 $\mu$m. Reproduced from [25].

$ADC$ values and their magnetic susceptibility are the key parameters accounting for the different types of behaviour of water and fat $G_i$ (fig. 4.4, 4.5). As reported in the previous chapter, the water $ADC$ is approximately two orders of magnitude
higher than the fat ADC. As a consequence, water molecules compared to fat molecules are characterised by a faster motion. According to eq.2.20, water spins travel a distance of the order of tenths of microns (calculated using the diffusion times \( \Delta \) employed in this study). Conversely, the displacement of protons in fat lies in the sub-micron range. Moreover, water and fat are characterised by different magnetic susceptibilities: \( \chi_{\text{water}} = -9.05 \cdot 10^{-6} \text{ (SI units)} \) and \( \chi_{\text{fat}} = -8.44 \cdot 10^{-6} \) [81], while bone tissue is believed to be characterised by \( \chi_{\text{bone}} = -11 \cdot 10^{-6} \) [82].

The \( G_i \) belonging to protons in water is sensitive to differences in magnetic susceptibility in the range of the pixel dimension. As expected, \( G_i \) shows a decreasing trend when moving from the bone-bone marrow interface to the pore centre. Indeed, the difference in water-bone susceptibility represents the main source of water \( G_i \).

Conversely, protons in fat are sensitive to differences in magnetic susceptibility ranging at least from two orders of magnitude less than the pixel size. For this reason, fat \( G_i \) measured in this study cannot be attributed to the bone-bone marrow interface. Fat \( G_i \) magnitude is better explained by the difference in magnetic susceptibility between fat and water. The increasing trend of fat \( G_i \) values (when moving from the bone-bone marrow interface to the pore centre) can thus be ascribed to a different rearrangement of fat molecules. In the central zone of each pore, there is a higher concentration of fat molecules characterised by a lower molecular motion than that of water molecules. As a consequence, fat \( G_i \) in the centre of the pore assumes higher values than those observed nearby the bone surface. Indeed, in this latter location, there is a reduced concentration of fat molecules. This means that a correspondingly higher concentration of water molecules (characterised by faster motion) modulates fat \( G_i \) by reducing its values. This scenario fits well with the hypothesised distribution of bone marrow filling trabecular pores, for which it is possible to say that "water wets" the surface of bone pores.

On the other hand, fat molecules, due to their hydrophobic nature, are mainly rearranged to lie in the central zone of the pore. As shown in fig. 4.5, water and fat \( G_i \) behaviour in the boundary and inner regions of LTD pores support this hypothesis. In the figure, water and fat \( G_i \) behaviour (expressed as a function of increasing pixel width) of the boundary region are compared to those measured from the inner region. \( G_i \) values of both water and fat bone marrow components move toward an asymptotic value which is very close to that obtained in the central zone of the trabecular pore. This observation confirms that water \( G_i \) variation is mainly due to bone-bone marrow interface susceptibility properties. Therefore, water \( G_i \) might be considered as a surrogate marker of the TB structure.

The asymptotic \( G_i \) values reached by the fat component were higher when compared to those reached by the water component. Again, the difference in the diffusion coefficient between fat and water might explain these results. As water molecules, compared to fat molecules, are associated with a faster diffusive motion during the experimental diffusion time, water spins are more dependent on motional averaging. As a consequence, the susceptibility dephasing effect which affects the SE signal of water is partially averaged. This peculiar behaviour of water/fat systems has already been investigated and verified elsewhere [83].

To support the hypothesis that water concentration is higher in the boundary zone of pores, while fat is more concentrated in the central zone of pores, mean mag-
Mean magnetisation values arising from the bi-exponential fit associated with water (left) and fat (right) versus boundary region width, expressed as a distance in voxels from the bone/marrow boundary. The average is performed across all samples. LTD location was considered in all experimental samples. Reproduced from [25].

Figure 4.6. Mean magnetisation values and their corresponding SD values were obtained from all five samples belonging to different calves. Mean magnetisation values associated with each voxel’s zone of the boundary region are statistically different (p = 0.05). Data reported in fig.4.6 demonstrate that in each pore of spongy bone, the quantities of water molecules at the bone-bone marrow interface are higher than that of fat molecules. As a consequence, in spongy bone (filled with bone marrow), the measurements of water MR parameters (such as $G_i$, $T_2^*$ or ADC) best provide information on TB density.

In fig.4.7 mean $G_i$ values and their SD, obtained from the five samples as a function of their TB density, are reported. The graph in the inset of fig.4.7 shows a positive linear correlation between water $G_i$ and TB densities (Pearson’s correlation coefficient $r = 0.71$). The large SD associated with water $G_i$ mean values is mainly due to differences in water content between the five samples. The strong variation of water ADC is likely due to the small differences in water-fat content percentages across samples. The larger the ADC SDs, the larger are the SDs of $G_i$. Conversely, the fat ADC changes less as a function of water-fat percentage variation.

<table>
<thead>
<tr>
<th></th>
<th>$T_2 \pm SD$ (ms)</th>
<th>$(ADC \pm SD)$ ($10^{-10} m^2 s^{-1}$)</th>
<th>$G_i \pm SD$ (mT m$^{-1}$)</th>
<th>$T_2^{true} \pm SD$ (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTD</td>
<td>22.6 ± 9.7</td>
<td>4.0 ± 0.7</td>
<td>263 ± 68</td>
<td>30.7 ± 3.3</td>
</tr>
<tr>
<td>ITD</td>
<td>22.0 ± 5.7</td>
<td>3.8 ± 1.2</td>
<td>411 ± 102</td>
<td>28.1 ± 2.9</td>
</tr>
<tr>
<td>HTD</td>
<td>8.7 ± 2.8</td>
<td>5.3 ± 1.6</td>
<td>675 ± 183</td>
<td>8.3 ± 1.2</td>
</tr>
</tbody>
</table>

Table 4.3. Results obtained from excised calf samples without discriminating between water and fat.

Finally, $T_2$, $ADC$, $G_i$ and $T_2^{true}$ mean values and their SDs obtained from the
4.1. INTERNAL GRADIENTS IN SPONGY BONE: ROLE OF DIFFUSION

Figure 4.7. Water $G_i$ behaviour (empty circles) and fat $G_i$ behaviour (filled circles) as a function of trabecular density obtained as the ratio between the number of voxels belonging to the boundary perimeter ($N_p$) and the number of voxels of the whole area ($N_a$). The mean $G_i$ values were calculated in a boundary region of three voxels wide. In the figure inset a zoom is displayed for TB densities ranging from 0.25 to 0.34. Reproduced from [25].

entire slice area from each of the selected locations (averaging on the five samples) are reported in tab.4.3. $G_i$ and $T_2^{true}$ were extracted from the fit by eq.2.38, while $T_2$ and $ADC$ represent values obtained by direct measures. Results reported in tab.4.3 are from an additional analysis in which we did not discriminate between fat and water components. This means that both components contribute, with different modalities, to the total fitted signal. However, the acquisition parameters chosen for this experiment were intentionally optimized to focus on the water signal (for example, b values from 0 to $10^9$ s m$^{-2}$ were chosen to detect the typical water diffusion decay but not the fat one). This means that there is only a minimal weight of the fat component in the determined results reported in tab.4.3.

$T_2$ and $ADC$ values did not discriminate between LTD, ITD and HTD. Conversely, $T_2^{true}$ was characterised by a decreasing trend when moving from LTD to HTD, while $G_i$ showed different values in the three locations with different TB densities. In bone marrow, $T_2$ is mainly dependent on the relative proportion of fat and water protons (which are characterised by different transverse relaxation times), as well as from the TB density. The $ADC$ depends on the interstitial spaces between bone and fat where water diffuses. $G_i$ assumes a higher value for higher trabecular densities, as expected by water $G_i$ values reported in tab.4.3, which are due to differences in magnetic susceptibility at the bone-water interface. Results reported in tab.4.3 represent a preliminary test at high field to investigate the feasibility of
including $G_i$ measurements within clinical protocols (i.e. collection of $T_2$-weighted images and $ADC$ maps). $G_i$ would add useful information by discriminating between different TB densities.

**In vivo experiments**

Mean $T_2^*$, $T_2$, $ADC$, $G_i$ and $T_2^{true}$ with their corresponding SD values are summarized in tab.4.4, together with the bone marrow fat content percentage extracted from the calcanei of six subjects. These findings indicate that $T_2^*$, $T_2$ and $ADC$ do not discriminate between the six different subjects presenting with different TB densities, while $T_2^*$ discriminates between a healthy female of 52 years, and subjects with osteopenia and osteoporosis. Conversely, $G_i$ discriminates between calcanei with different TB densities as shown in fig.4.8. Moreover, $T_2^{true}$ values seem to be more associated with fat percentage than with TB densities.

According to the previous literature, the only male included in the current study showed a higher fat fraction when compared to females [84]. The two youngest females (24 and 42 years old respectively) and the subject with osteopenia, showed a similar fat bone marrow percentage content with no evident differences related to aging. $T_2^*$ was lower in the youngest female, characterised by a higher TB density when compared with the 42-year-old female. However, since $T_2^*$ depends on both TB density and fat bone marrow concentrations, the male subject showed a higher $T_2^*$. Moreover, younger subjects were characterised by $T_2^*$ values equal to those from a 52-year-old healthy subject characterised by a higher fat percentage value when compared to the 24-year-old female. Conversely, $G_i$ values were remarkably different among subjects with different TB densities, independently from their bone marrow fat content.

The relationship between $ADC$ in vivo and age/gender is less clear: a decreasing $ADC$ for reduced BMD was reported in the vertebrae [85,86], while other works reported a lack of correlation between diffusion indexes and BMD [87]. The presence or absence of this relationship seems, therefore, to be related to different anatomical locations. Since the fat content in the calcaneus is higher than that observed in the spine [24], the two sites are likely to be affected by BMD modifications in a different way. In the specific case of calcaneus spongy bone, restricted diffusion experienced by water confined between fat and bone could be hypothesized, as suggested by the $ADC$ values reported in tab.4.4, which are of the order of $10^{-11}$ m$^2$ s$^{-1}$. Moreover, a lower restricted diffusion due to increasing age or decreasing TB density could also be expected. However, it is not possible to conclude any general trend for all parameters listed in tab.4.4 due to the small sample size.

Several studies reported an increase of $T_2^*$ values for decreasing trabecular densities. However, the high inter-subject variability observed across studies is likely to be due to differences in water/fat ratios which affect $T_2^*$ decay. Remarkably, higher values of $T_2^*$ have been reported for fat as compared to the water component, either in vitro [88,89] or in vivo [90]. Furthermore, several kinds of fat molecules are present in bone tissue [91], with relative concentration again varying across subjects. In tab.4.4, $T_2^*$ values of healthy subjects are very close to each other and overlap when considering their SDs. Moreover, the large $T_2^*$ value reported for the male subject is more likely to be linked to a higher fat concentration rather than to a
### Table 4.4. Spectroscopic evaluation of human calcanei.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age</th>
<th>Status</th>
<th>Fat %</th>
<th>$T_1^*$ ± SD ($ms$)</th>
<th>$T_2$ ± SD ($ms$)</th>
<th>(ADC ± SD) ($10^{-10} m^2 s^{-1}$)</th>
<th>$G_i$ ± SD ($m T m^{-1}$)</th>
<th>$T_2^{true}$ ± SD ($ms$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M 33 H</td>
<td>93</td>
<td>12.5 ± 3.1</td>
<td>34.6 ± 6.0</td>
<td>4.5 ± 0.9</td>
<td>894 ± 113</td>
<td>55.3 ± 7.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F 24 H</td>
<td>87</td>
<td>9.8 ± 1.7</td>
<td>41.0 ± 3.7</td>
<td>4.3 ± 0.5</td>
<td>477 ± 40</td>
<td>41.3 ± 1.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F 42 H</td>
<td>87</td>
<td>12.7 ± 2.3</td>
<td>35.3 ± 2.8</td>
<td>3.7 ± 0.7</td>
<td>415 ± 56</td>
<td>39.5 ± 1.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F 52 H</td>
<td>88</td>
<td>9.5 ± 1.8</td>
<td>41.2 ± 3.2</td>
<td>5.0 ± 0.5</td>
<td>399 ± 44</td>
<td>40.6 ± 1.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F 62 OPE</td>
<td>87</td>
<td>15.1 ± 2.3</td>
<td>42.0 ± 2.7</td>
<td>7.3 ± 0.5</td>
<td>311 ± 29</td>
<td>41.3 ± 0.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F 62 OPO</td>
<td>90</td>
<td>21.1 ± 2.5</td>
<td>38.0 ± 2.5</td>
<td>6.6 ± 0.6</td>
<td>240 ± 47</td>
<td>47.0 ± 2.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
reduced TB density, which is not supported by any evidence from young healthy men. According to our experimental results, \( T_2^* \) turned out to be affected by the water/fat ratio, a parameter that shows a huge spread across different subjects. Some authors have suggested introducing corrections to account for variations in marrow composition \([92]\). However, to the best of the Author’s knowledge, no-clinical studies involving these corrections have been published so far.

TB density is believed to be higher in male than in female subjects and to be lower in young than in elderly females. Moreover, TB density is lower in osteopenic subjects than in healthy subjects with the lowest values in females with osteoporosis. In these experiments, \( G_i \) has shown the ability to clearly discriminate between six subjects with different ages and different TB densities. Furthermore, \( G_i \) does not seem to suffer from the aforementioned limitation, which does not currently allow the clinical use of MR parameters on a single subject basis for diagnostic purposes. Clearly, the discussion about the potential clinical use of \( T_2^* \) and \( G_i \) parameters requires caution due to the small sample size recruited for the current study. In fig.4.8, \( G_i \) values presented are obtained from the central slice of the calcanei in six subjects, who presumably had different TB densities as expected in individuals with different sex, age and T-score values.

![Figure 4.8.](image)

Figure 4.8. Clinical measures of \( G_i \) obtained from human calcanei as a function of individual supposed TB densities. Reproduced from \([25]\).

These results illustrate a smooth decrease of \( G_i \) values in the five females from the youngest to the oldest (linear correlation coefficient \( r = 0.81 \)). Considering that age is a well-known factor affecting TB density, we can conclude that preliminary data obtained in vivo demonstrate a progressive reduction of \( G_i \) across aging. With this perspective, one can speculate that \( G_i \) decreases proportionally with the physiological reduction of TB density. Future studies, including both large populations of healthy subjects and subjects with osteopenia and osteoporosis, are needed to
clarify the potential diagnostic role of $G_i$. Moreover, it should be noted that these results, obtained in vivo by conventional $T_2$ and ADC measurement performed on a clinical scanner, fit well with those obtained in vitro (see fig.4.7).

In summary, in this section the properties of $G_i$ for bone marrow’s fat and water molecules were investigated in the framework of conventional (i.e. Gaussian) diffusion. Thanks to the ability of discriminating the properties of the two diffusion ranges, it has been demonstrated that water $G_i$ is directly proportional to TB densities, while fat $G_i$ does not provide specific information on bone’s structural features. Moreover, it was shown that the concentration of water molecules at the bone-bone marrow interface is higher than that of fat molecules.

On the basis of these in vitro findings, a new strategy to assess bone’s status in vivo has been proposed. Data obtained in the calcaneus of human subjects at different ages and with different DXA T-scores demonstrated a progressive reduction of $G_i$ across aging, thus suggesting that $G_i$ decreases proportionally to the physiological reduction of TB density. Future studies, including both large populations of healthy subjects and patients with osteopenia and osteoporosis, are needed to clarify the potential diagnostic role of $G_i$ in clinical protocols.

4.2 High b-values main frame in cerebral white matter

The second part of this chapter is devoted to another issue regarding the sampled diffusivity range. As outlined in the previous section, the poor resolution of crossing fibres at a single voxel level represents one of the major limitations of DTI on the brain. On the other hand, in the last decade some authors showed that additional information concerning brain microstructures could be obtained by selecting b-values higher than $10^9$ s m$^{-2}$, thus emphasising slower diffusion pools. Such investigations were mainly focused on the different contrasts of white and gray matter in mean diffusivity and fractional anisotropy maps.

In this section, specific aim was to characterise the principal frame of reference as a function of increasing diffusion weighting. This could be very important since data regarding eigenvectors rotational shift can be combined with data coming from new advanced methods, performed at high angular resolution, to increase the ability in resolving crossing fibres phenomena at a single-voxel level and hence obtain additional information for the optimization of fibre track methods. For this purpose, DTI eigenvectors rotational shift (ES), induced by high b-values, were quantified with respect to the eigenvectors calculated for conventional b=$10^9$ s m$^{-2}$. The ES trend due to a b-value increase was compared to ES caused by the signal-to-noise ratio decrease, which is known to affect high b-values acquisitions. In parallel, the bias due to low anisotropy was accounted for by means of repeated measures on one subject, while the bias introduced by a low number of gradient directions was quantified by comparing the 6 to 20 direction schemes. Moreover, different diffusion regimes were characterised by investigating the eccentricity of the diffusion ellipsoid at increasing b-values.

Experimental data collected at 3T showed that in regions characterised by heterogeneous fibre directionality the b-value increase is an intrinsic cause of the measured eigenvector shift since noise alone cannot account for the observed trend.
Eccentricity distributions suggest that high b-value DTI highlight the contribution of oblate geometry, which is considered an evidence of fibre crossing.

### 4.2.1 Problem statement

In the previous section, it has been shown that without any specific hypothesis about the number of components and the origin of such different pools, information about different dynamical ranges can be easily obtained by tuning the diffusion weighting.

By sampling at b-values greater than \( b = 10^9 \text{ s m}^{-2} \) some authors observed a reversal pattern of gray-white matter contrast compared to that usually obtained at \( b = 10^9 \text{ s m}^{-2} \) [93, 94]. Indeed, when the b-value increases a stronger decrease of ADC is detected in white matter compared to that observed in gray matter. Conversely, fractional anisotropy is affected to a lesser extent by the b-values range. At higher b-values, DTI has been reported to have an increased sensitivity to certain pathological abnormalities [95, 96] as well as changes due to brain maturation [97, 98]. Furthermore, some Authors reported a correlation between the extent of the estimated WM tracts in DW and the b-value; specifically, at a b-value of \( 10^9 \text{ s m}^{-2} \), WM tracts are overestimated compared to those detectable in the correspondent \( T_2 \) reference images [99].

In the DTI framework, the information contained in diffusion tensor eigenvectors can be used to reconstruct WM tracts by means of tractography algorithms [100, 101]. Recently, high-angular resolution diffusion imaging (HARDI) combined with appropriate acquisition schemes improved the resolution of fibre crossing within a voxel by using multiple orientation and higher b-values (over \( 3 \cdot 10^9 \text{ s m}^{-2} \)) [38, 39]. Indeed, the angular dependency of signal is more pronounced at higher b-values [40, 41].

The poor resolution of crossing fibres at a single voxel level represents one of the major limitations of DTI tractography algorithms based on a single-tensor model. Acquisition strategies such as HARDI overcome some of the limitations in the single tensor model. However, high angular resolution imaging techniques remain, so far, clinically untenable due to their long scanning times. For this reason, high b-values DTI is still a much investigated research topic. In this regard, the investigation of main diffusion axes behaviour at increasing b-values could give important information to better evaluate the opportunity of extending the b-value range commonly used in clinical applications. Some of the current diffusion models that attempt to explore higher b-values in a clinically feasible scanning time consider the same principal axes for both slow and fast diffusion subgroups [102]. This is a reasonable assumption whenever the imaging voxel contains homogeneous and coherent WM fibres. However, it needs further validation in the case where different fibre populations with different orientations are present within the same voxel (i.e. fibre crossing).

When probing higher diffusion weightings, an important aspect to take into account is the role of noise. In the following, any contrast difference that is not due to b-value increasing will be classified as noise. First, increasing b-values can substantially reduce the signal-to-noise ratio (SNR) where the signal from some voxels can be approximately equal to the background noise [103]. In this case,
noise alone can partially explain some of the effects reported at high b-values, such as the reversal pattern of gray-white matter contrast. For this reason, the issue of signal versus noise contribution to the estimation of eigenvectors orientation at high b-values was addressed. Specifically, Rician noise was added to the lowest b-value scan to generate images at $b = 10^9 \text{s m}^{-2}$, but with the SNR of higher b-value scans.

Furthermore, Laun and co-workers [104] pointed out that a limited number of gradient directions could introduce a bias the estimation of eigenvectors. To keep the experimental time acceptable, the analysis was performed with 6 gradient directions, which is the minimum to perform DTI reconstruction, but this gradient scheme was compared with a 20 directions scheme in one selected subject.

Finally, for isotropic geometries, each tern of eigenvectors is equivalent. Ideally, in such cases, one can obtain different orientations for repeated measures in the same subject. This is expected to introduce a bias for tissues characterised by low anisotropy. This bias was accounted for by comparing three dataset obtained at the same b-value and for the same subject in three separate scans. To the Author’s knowledge, this potential source of noise has never been taken into account in the main axes investigations that have been proposed so far.

The eigenvectors rotational shift at 6 b-values ranging from 1.5 to 5, with respect to the eigenvectors calculated at $b = 10^9 \text{s m}^{-2}$, was investigated for each of the three eigenvectors in selected GM and WM brain regions. To assess the contribution of a lower SNR in the resulting trend, Rician noise was added to the $b = 10^9 \text{s m}^{-2}$ ($b_1$) images. This allowed to simulate 6 datasets replicating the SNR observed in images obtained at higher diffusion weightings. Further, the effect of the bias introduced by the limited number of gradient directions was evaluated comparing a dataset acquired with a higher number of directions (20) with one obtained considering the six gradients dual scheme. Moreover, in one subject, the same protocol was applied in three separate sessions, thus quantifying the contribution of the low anisotropy bias. Finally, the corresponding change in the diffusion ellipsoid shape was evaluated by investigating the eccentricity distribution across three different planes containing the three eigenvectors.

To the best of the Author’s knowledge, a systematic study on the changes occurring in the diffusion principal reference frame, as obtained by means of DTI reconstruction at increasing b-values, has not been previously reported in literature.

### 4.2.2 Methods

To quantify the angular shift between the diffusion main axes recorded as a function of increasing b-values and the diffusion main axes at $b_1$, one needs to define a quantitative parameter that is able to overcome the inherent sign ambiguity of each eigenvector [105]. For this reason, two different orientations are usually compared by evaluating the absolute value of the scalar product between the vectors that define them [36, 106]. In this study, the eigenvector shift (ES) in each voxel was calculated using the following formula:

$$ES_i = 1 - |V_{b_1} \cdot V_i|$$  (4.1)
where \(i = b_{1.5}, b_{2}, b_{2.4}, b_{3}, b_{4}, b_{5}\). According to formula 4.1, when \(ES=0\) there is no change between the eigenvectors orientation, while when \(ES=1\) the two eigenvectors are perpendicular to each other.

In order to understand which dynamics are emphasised at high \(b\)-values as compared to \(b_{1}\), the eccentricity was calculated as a function of \(b\)-value for each of the three planes containing the eigenvectors, following the eccentricity expression:

\[
e_{ij} = \sqrt{1 - \frac{\lambda_i^2}{\lambda_j^2}}
\]

where \(\lambda_i < \lambda_j\) and \(i, j = 1, 2, 3\).

Ten healthy volunteers (F/M = 4/6, mean age 24 ± 3 years) participated in this study after giving informed consent, according to the national laws and to the local ethics committee guidelines. All subject were scanned on 3.0T scanner Siemens Magnetom Allegra (Siemens Medical Solutions, Erlangen, Germany), equipped with a circularly polarized transmit-receive coil. The maximum gradient strength was 40 mT m\(^{-1}\), with a maximum slew rate of 400 mT m\(^{-1}\) ms\(^{-1}\). T1-weighted sagittal images were acquired for anatomical reference and brain segmentation, using a MPRAGE (magnetisation-Prepared Rapid Acquisition with Gradient Echo sequence) [TR/TE/TI = 2000/4.38/910 ms, flip angle = 8\(^{\circ}\), matrix = 448×512, in-plane resolution = 0.5×0.5 mm\(^2\), slice thickness = 1 mm, 176 adjacent slices, field of view (FOV) of 224×256 mm\(^2\)]. Diffusion weighted spin-echo echo planar imaging (DW SE EPI) sequence was used to acquire the DW data to cover the whole brain using the following parameters: TR/TE= 6400/107 ms, \(\Delta/\delta = 107/35\) ms, bandwidth=1860 Hz/px, slice thickness=3mm, in plane resolution=1.8×1.8 mm\(^2\). The diffusion gradients were applied along six non-collinear directions (dual gradient scheme) at seven different \(b\)-values: 1, 1.5, 2, 2.4, 3, 4 and 5 · 10\(^9\) s m\(^{-2}\). Images with \(b = 0\) were also acquired to perform DTI reconstruction. In order to rule out the contribution of the low anisotropy bias in the estimation of the eigenvectors shift, the protocol was repeated three times in one of the ten subjects. Furthermore, in the same subject a dataset with 20 non-collinear gradient directions was acquired to quantify the bias due to the 6 gradient directions scheme.

All DW images were corrected for eddy currents distortions and subject motion artefacts (affine registration), using FSL version 4 (http://www.fmrib.ox.ac.uk/fsl) software. As a first step, FA, MD, \(\lambda_1\), \(\lambda_2\), \(\lambda_3\), \(V_1\), \(V_2\) and \(V_3\) maps were derived after tensor calculation, using FSL DTIFIT routine, for each subject and for each of the seven considered \(b\)-values. Anatomical scans were then co-registered to FA maps and segmented into WM, GM and cerebrospinal fluid (CSF) using SPM version 5 (http://www.fil.ion.ucl.ac.uk/spm). A binary mask was obtained by combining WM, GM and CSF segments and retaining only those voxels with intensity greater than 0.8 on the resulting image. A custom script (Matlab, The Mathworks, Natick, MA, USA) was implemented to evaluate voxel-wise ES and \(e_{ij}\) as reported in formula 4.1 and 4.2 respectively.

For each subject, eighteen ES maps were computed: one for each of the three eigenvectors \((V_1, V_2, V_3)\) reporting the ES between \(b\) values of 1 and 1.5 · 10\(^9\), between \(b\) values of 1 and 2 · 10\(^9\), between \(b\) values of 1 and 2.4 · 10\(^9\), between \(b\) values of 1 and 3 · 10\(^9\), between \(b\) values of 1 and 4 · 10\(^9\), and between \(b\) values of 1 and 5
Moreover, nine eccentricity maps were calculated for each subject, showing the eccentricity for three selected b-values \((1, 3 \times 10^9)\) and for each plane as defined by a couple of eigenvectors: \(V_1 - V_2, V_1 - V_3\) and \(V_2 - V_3\). To perform a statistical analysis, regions of interest (ROIs) were selected on the b0 images as previously described [107]. Rectangular ROIs were positioned manually subject by subject. Rectangular ROIs were placed bilaterally in the following WM regions: the occipital and temporal lobe, the anterior pericallosal areas, the parietal and frontal lobe, the posterior pericallosal areas, the genu and the splenium of the corpus callosum. An ROI was also placed in GM in the head of the caudate nucleus. Conversely to the original paper [107], in which ROIs were differently sized depending on the structure investigated, the volume of each ROI was set to 233 mm\(^3\). The ROIs were grouped on the basis of their FA values. Four WM groups and one GM group were selected: low FA (occipital and temporal lobe), intermediate FA 1 (parietal and frontal lobe), intermediate FA 2 (pericallosal areas), high FA (corpus callosum) and GM (head of the caudate nucleus). These regions will be referred as lowFA, intFAa, intFAb, highFA and GM respectively throughout the section.

Mean values and standard deviations of ES were calculated for each group by means of a home-made script written in Matlab. Besides, for each brain region, histograms were obtained by adding up the contribution of eccentricity calculated in each voxel for every subject. Histogram analysis was preferred to a simple average for the purpose of investigating the distribution of eccentricity within each ROI, which can provide some information on fibre populations. Eccentricity histograms were compared by evaluating their distribution peaks. The peak value was obtained for each curve by fitting the histograms with an empirical asymmetric peak function in OriginPro v.8 (OriginLab Corporation), namely an asymmetric double sigmoid.

Noise simulation

In order to investigate the contribution of a reduced SNR as in high-b data, the DW images obtained at \(b1\) were used to create six simulated datasets that replicated the SNR at \(b1.5, b2, b2.4, b3, b4\) and \(b5\) images, respectively. The measured signal intensity, \(M\), in MR images is described by a Rician distribution, according to the following expression:

\[
P(M|A, \sigma) = \frac{M}{\sigma^2 I_0 \left( \frac{A \cdot M}{\sigma^2} \right)} \exp\left( -\frac{M^2 + A^2}{2\sigma^2} \right)
\] (4.3)

where \(A\) and \(\sigma\) represent the image pixel intensity in the absence of noise and the standard deviation of the Gaussian noise in the real and imaginary channel, respectively. In image areas where only noise is present, the Rician distribution reduced to the Rayleigh distribution [108].

The following procedure was used in each subject’s dataset:

- Standard deviation of background noise was obtained as the background average intensity divided by \((\pi/2)^{1/2}\), as stated by the Rayleigh distribution
- SNR was calculated for each ROI and for each considered b-value as the ratio between mean ROI intensity and background noise standard deviation
• Given the original SNR of the b1 scan and six target SNRs, corresponding to the SNRs of the other b-values, six σ parameters were obtained for each ROI: σ1,5, which was used to simulate noise on the b1 images returning synthetic images with the SNR of the b1.5 scans, σ2, used to simulate noise on b1 images to obtain in turn synthetic images with SNR values observed at b2 and so on, up to σ5.

• Finally, Rician noise was added to each voxel inside the ROIs belonging to the b1 images, using as A the input voxel intensity and as σ, σ1,5, σ2, σ2.4, σ3, σ4 and σ5, respectively. In this way, for each b1 image six synthetic images were generated with the SNR of b1.5, b2, b2.4, b3, b4 and b5 scans respectively.

This procedure contains two approximations: first, σ was calculated assuming that in background areas the total standard deviation is the sum of the original standard deviation plus the standard deviation of the added noise, but this is not the case for the Rayleigh distribution [108]. However, simulations were performed case by case to obtain the desired SNR as follows: given the standard deviation of the b1 image and the target standard deviation, a custom script generates trial values of the noise standard deviation which are accepted or rejected with the confidence level of 5%. Secondly, voxel intensity at \( b = 10^9 \) \( \text{s m}^{-2} \) was used as noise-free intensity A in the generation of synthetic datasets with increased SNR. This is justified by the higher SNR of the b1 scans as compared to the others.

In the end, ES was calculated on the synthetic images. The results were compared to those obtained with measured images.

Other noise sources: repeated scans and 20 directions dataset

Three repeated scans were performed on one selected subject were co-registered with respect to the first scan and processed in the way described previously. Nine ES maps were then calculated: one for each of the three eigenvectors (\( V_1, V_2 \) and \( V_3 \)) reporting the ES between b1 of the first and second run, between\( b1 \) of the first and third run and between b1 of the second and third run.

Mean values of ES and associated standard deviation were calculated for the same brain regions defined previously on the basis of FA. This analysis was repeated for two higher b-values, b3 and b5. The 20 directions dataset was processed as described previously and the results were compared with the 6 gradients dual scheme. Specifically, the ES of the two acquisition strategies were compared by means of paired t-tests, performed with SPSS 16.0 (SPSS Inc.).

4.2.3 Results

Fig. 4.9 illustrates the logarithm of the signal decay as a function of the considered b-values. This plot is the experimental proof of the non-monoexponential behaviour of the signal decay described throughout all previous chapter. The non-monoexponential nature of the signal is evident: the higher b-values data points do not lie on the straight line, i.e. the mono exponential decay in logarithmic scale (dashed line), but at the same time they are well above the noise floor (dotted line).

Fig. 4.10 shows an example of the FA, \( V_1, V_2 \) and \( V_3 \) maps obtained from a single subject for three different b-values: \( b1, b3 \) and \( b5 \). The eigenvectors maps
4.2. HIGH B-VALUES MAIN FRAME IN CEREBRAL WHITE MATTER

Figure 4.9. Plot of the logarithm of the signal decay versus the b-value in a selected WM ROI (genu of the CC), averaged over all pixels. The dashed line represents the predicted mono-exponential decay at \( b = 10^9 \) s m\(^{-2} \) while the dotted line represents the noise floor, calculated as the average of the pixel intensities in a ROI outside the brain, in the same slice. Reproduced from [109].

were masked with correspondent GM (left) and with WM maps (right), to discriminate between different dynamic regimes associated to the underlying architecture of the two tissues when increasing the diffusion weighting. From a visual inspection, GM voxels appear oriented in random directions with respect to all three eigenvectors. Orientation changes with increasing b-value, but no specific trend can be recognized. Conversely, WM tracts are characterised by highly uniform areas that remain unaffected by b-value increasing and areas in which the eigenvectors orientation is moderately dependent on b-values. \( V_1 \) is characterised by large areas of coherent orientation while \( V_2 \) is associated to the highest degree of randomness when compared to the other eigenvectors.

Fig. 4.11 shows ES (SD) values calculated (using formula 4.1) between \( b_1 \) and six higher b-values for the three eigenvectors \( V_1 \) (filled triangles), \( V_2 \) (filled circles) and \( V_3 \) (filled squares). Each panel shows the trend for a different WM group: lowFA (panel a), intFAa (panel b), intFAb (panel c) and highFA (panel d).

In the same plot, ES calculated between \( b_1 \) and the synthetic dataset with the SNR observed in the six higher b-values images, are shown (empty markers).
Figure 4.10. From left to right: FA maps, V₁, V₂, V₃ color coded maps in the GM and V₁, V₂, V₃ color coded maps in the WM, for one representative slice belonging to subject 1. The eigenvectors are displayed following the conventional colour code: red corresponds to left-right, green corresponds to front-back and blue represents head-foot direction. The maps are reported for increasing b-value: $b = 10^9$ s m$^{-2}$ (upper panel), $b = 3 \cdot 10^9$ s m$^{-2}$ (middle panel) and $b = 5 \cdot 10^9$ s m$^{-2}$ (lower panel. Reproduced from [109].)
4.2. HIGH $B$-VALUES MAIN FRAME IN CEREBRAL WHITE MATTER

Figure 4.11. Eigenvector rotational shift of the $b_1$, $b_2$, $b_2.4$, $b_3$, $b_4$ and $b_5$ images as compared to the $b_1$ reference scans. The ES are calculated following formula 4.1 and plotted as a function of the b-value. The 4 subplots show the results for the 4 WM regions, chosen on FA basis: lowFA with FA(SD)=0.29(0.08) (panel a), intFAa with FA(SD)=0.43(0.06) (panel b), intFAb with FA(SD)=0.51(0.15) (panel c) and highFA with FA(SD)=0.77(0.13) (panel d). In each plot, the different markers correspond to the three eigenvectors: $V_1$ (triangles), $V_2$ (circles), $V_3$ (squares). Filled markers correspond to eigenvector rotational shift calculated for the measured dataset while empty markers are referred to eigenvector rotational shift for the synthetic data artificially corrupted by Rician noise. Reproduced from [109].
In white matter regions, observed ES values are strongly dependent on FA range. For low and intermediate FA, ES increase with increasing b-values for all the three eigenvectors. For high FA instead, V2 and V3 ES values are similar to each other and shows a feeble dependence on the b-value, while V1 is associated to lower ES slightly increasing with b-value. SNR decrease affects ES values, particularly for low FA, where eigenvalues are closer in magnitude. This confirms simulations reported in literature [110]. According to these Authors, the higher the anisotropy, the lower the likelihood of misclassifying one of the eigenvalues.

However, even if SNR decrease plays a role in the resulting curve, the ES trend as a function of b-value is still more pronounced than that due to the increased Rician noise, particularly so for intFAa and intFAb. Thus, these data demonstrate that, in regions of moderate anisotropy, the change of eigenvectors’ orientation with increasing b-values cannot be merely accounted for by noise.

In gray matter, ES has a slightly increasing trend for increasing b-value, as shown in Fig. 4.12. When the effect of a lower SNR is taken into account in the simulated noisy images, a similar increasing trend was found, suggesting that in GM the ES change can be mostly accounted for by the noise contribution.

To evaluate the bias introduced by the dual gradient scheme used in this study, the ES trend for increasing b-value obtained with 6 gradient directions was compared with that obtained with 20 gradient directions by means of paired t-tests. Results obtained in each WM and GM region are shown in Tab.4.5. There were no statistical differences between the pattern measured with 6 versus 20 gradient directions (p>0.05), except for V3 in both lowFA and GM, V2 and V3 in highFA. This indicates that the effect of the number of gradient directions is small compared...
4.2. HIGH B-VALUES MAIN FRAME IN CEREBRAL WHITE MATTER

to the effect of increased diffusion weighting.

<table>
<thead>
<tr>
<th></th>
<th>FA</th>
<th>SD</th>
<th>$V_1$</th>
<th>$V_2$</th>
<th>$V_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>lowFA</td>
<td>0.29</td>
<td>0.08</td>
<td>0.936</td>
<td>0.177</td>
<td>0.003</td>
</tr>
<tr>
<td>intFAa</td>
<td>0.43</td>
<td>0.06</td>
<td>0.145</td>
<td>0.139</td>
<td>0.132</td>
</tr>
<tr>
<td>intFAb</td>
<td>0.51</td>
<td>0.15</td>
<td>0.563</td>
<td>0.708</td>
<td>0.106</td>
</tr>
<tr>
<td>highFA</td>
<td>0.77</td>
<td>0.13</td>
<td>0.745</td>
<td>0.010</td>
<td>0.005</td>
</tr>
<tr>
<td>GM</td>
<td>0.27</td>
<td>0.07</td>
<td>0.139</td>
<td>0.075</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Table 4.5. Paired t-tests performed in all brain regions comparing ES trend at different b-values, obtained with 6 and 20 gradient directions. There are no statistical differences between the trend measured with a reduced number of gradient directions ($p>0.05$), except for $V_3$ in both lowFA and GM, $V_2$ and $V_3$ in highFA.

Fig.4.13 displays ES$\pm$SD for the three eigenvectors ($V_1$, $V_2$, $V_3$) as a function of increasing FA. ES were calculated in one subject for b values $10^6$, $3\cdot10^6$, and $5\cdot10^6$, across three repeated scans. At high FA, $V_1$ shows high coherence between different scans, while $V_2$ and $V_3$ are associated with high ES across repeated scans. This is likely reflected by the arbitrariness of the definition of the second and third eigenvectors in a prolate geometry. For lower and intermediate FA, $V_1$ again is characterised by an ES different from zero.

The differences between the eigenvalues calculated on different images of the same geometry was interpreted as a consequence of the arbitrary choice of the eigenvectors term in anisotropic geometry. In fact, as the geometry approaches isotropy, the measured ES increases. This bias is independent of the b-value, as confirmed by paired t-tests (all $p > 0.05$). One can thus conclude that additional sources of noise gives rise to a constant bias that does not affect the relationship between ES changes and increasing b-values, rather it just affects the ES absolute values.

In order to characterise the shape of the diffusion ellipsoid at increasing b-values, the eccentricity of the three planes ($e_{12}$, $e_{13}$ and $e_{23}$) was assessed voxel-by-voxel and, for each ROI, eccentricity values for all voxels and all subjects were displayed as a histogram.

<table>
<thead>
<tr>
<th></th>
<th>FA</th>
<th>SD</th>
<th>$e_{12}$</th>
<th>$e_{13}$</th>
<th>$e_{23}$</th>
<th>$e_{12}$</th>
<th>$e_{13}$</th>
<th>$e_{23}$</th>
<th>$e_{12}$</th>
<th>$e_{13}$</th>
<th>$e_{23}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>lowFA</td>
<td>0.29</td>
<td>0.08</td>
<td>0.59</td>
<td>0.87</td>
<td>0.63</td>
<td>0.61</td>
<td>0.70</td>
<td>0.61</td>
<td>0.59</td>
<td>0.80</td>
<td>0.65</td>
</tr>
<tr>
<td>intFAa</td>
<td>0.43</td>
<td>0.06</td>
<td>0.93</td>
<td>0.97</td>
<td>0.81</td>
<td>0.80</td>
<td>0.95</td>
<td>0.74</td>
<td>0.67</td>
<td>0.93</td>
<td>0.76</td>
</tr>
<tr>
<td>intFAb</td>
<td>0.51</td>
<td>0.15</td>
<td>0.83</td>
<td>0.98</td>
<td>0.83</td>
<td>0.72</td>
<td>0.97</td>
<td>0.77</td>
<td>0.86</td>
<td>0.99</td>
<td>0.92</td>
</tr>
<tr>
<td>highFA</td>
<td>0.77</td>
<td>0.13</td>
<td>0.99</td>
<td>1.00</td>
<td>1.00</td>
<td>0.98</td>
<td>1.00</td>
<td>0.95</td>
<td>0.99</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Table 4.6. Peak position for eccentricity histograms obtained for three different b-values ($b1000$, $b3000$ and $b5000$), calculated in each of the selected brain regions.

Fig.4.13 reports the eccentricity distribution, $e_{i,j}$, for the four WM regions and for all three planes. In each plot, the histograms are shown for three different b-values ($b1$, $b3$ and $b5$). In Tab.4.6, the peak position for each histogram, as obtained
Figure 4.13. Eigenvector rotational shift for the three eigenvectors $V_1$ (triangles), $V_2$ (circles), $V_3$ (squares) as a function of increasing FA. ES is calculated between three images obtained in three repeated scans on the same subject for three selected $b$-values: $b_1$, $b_3$ and $b_5$. Paired t-tests (not reported) confirmed that there is no statistical difference between the ES trend calculated at the three different $b$-values. Reproduced from [109].

by fitting the data with an empirical asymmetric peak function, is reported. In lowFA and highFA regions, eccentricity peak shows no trend with the increasing of diffusion weighting. In contrast, in WM ROIs with moderate FA (intFAa and int-FAb), the $b$-value effect is to decrease eccentricity. The decrease in the eccentricity for increasing $b$-value is higher for $e_{12}$ as compared to the other planes for moderate FA, indicating that in these regions differences between the main and the medium eigenvector tend to decrease at high $b$-values. This trend suggests the phenomenon of fibre crossing, which is modelled in DTI as two major eigenvalues which are very close to each other, is emphasised at high $b$-value.

These results quantified in terms of angular shift the difference between the orientations of the main diffusion axes measured at two different $b$-values. Related observations were previously reported on phantoms [104], but they were ascribed to noise.

In vivo, the angular shift can be due to various different factors. For example,
4.2. HIGH B-VALUES MAIN FRAME IN CEREBRAL WHITE MATTER

Figure 4.14. Measured eccentricity histograms obtained collecting all the voxels for all the subjects in the same ROI for 4 WM regions, chosen on FA basis. From left to right: lowFA, intFAa, intFAb and highFA. The three different colours correspond to the three different b-values: \( b = 10^9 \) s m\(^{-2}\) (blue), \( b = 3 \cdot 10^9 \) s m\(^{-2}\) (red) and \( b = 5 \cdot 10^9 \) s m\(^{-2}\) (yellow). All results are showed separately for the three planes: \( e_{12}\) (upper line), \( e_{13}\) (middle line) and \( e_{23}\) (lower line). Reproduced from [109].

When higher b-values are selected keeping all the other experimental parameters fixed, a lower SNR is recorded. Noise indeed affects the DW images, causing artefacts such as the shift of eigenvectors towards attractive orientations of the gradient scheme [104]. In figs. 4.11 and 4.12 (empty markers), the effect of the SNR decrease determining an artifactual increase of ES was evaluated by means of simulations.

Another source of noise is the limited number of gradient directions used to perform DTI reconstruction. Employing more gradient directions reduces the angular shift due to noise [104]. Following their results, 20 directions were suggested as the minimum number for negligible angular shift. These experimental results indicate that, even though at a low number of gradient directions the angular shift due to noise increased, there are no statistical differences between the ES trend at increasing b-values measured with 6 and 20 gradient directions, except for few cases.

The third type of bias that was taken into account was the ES due to isotropic contribution. For pure isotropic geometry, each eigenvectors term is equivalent and different measures of the same object are expected to provide random results, thus ES different from zero. As a matter of fact, this uncertainty can cause a bias in the measurement even for tissues characterised by low anisotropy. The contribution of this effect was investigated by means of repeated measures of the same subject and reported in Fig.5. As expected, the lower the anisotropy, the higher the bias due
to this "random effect". Nevertheless, it has been shown that this bias affects the absolute ES value but not its trend as a function of b-value.

On the basis of the experimental results described in this section, a combination of these effects (SNR decrease, random effect and limited number of gradient directions) can explain the trend observed for lowFA (Fig. 4.11a) and highFA (Fig. 4.11d). Nevertheless, the patterns reported in fig. 4.11b and 4.11c for intFAa and intFAb, cannot be explained as the sum of these effects. These results suggest instead that for regions characterised by intermediate anisotropy, tuning the diffusion weighting can highlight different diffusion regimes and thus lead to a change in the main diffusion axes.

Due to the typical resolution of DTI scans, different fibre orientations may co-exist in a single voxel, thus resulting in an intravoxel orientational heterogeneity depending on the anatomical region [35, 36]. This heterogeneity is reflected in the abundance of oblate ellipsoid highlighted by DTI investigations [111]. The fact that in regions of moderate anisotropy, the diffusion axes at small diffusion weighting do not coincide to those obtained by increasing the b-value may be linked to the presence of intra-voxel fibre heterogeneity. This heterogeneity may be emphasised at high b-values due to an increased contrast between the low-diffusion component from one fibre and the high diffusion component from another fibre with a different orientation [36, 112, 113].

This hypothesis is confirmed by the eccentricity histograms of fig. 4.14. HighFA and lowFA regions are poorly affected by the b-value increasing. Conversely, for moderate FA (intFAa and intFAb) eccentricity distribution peaks shift to lower values. Specifically, this shift is more evident in $e_{12}$, which corresponds to $V_1 - V_2$ plane, thus indicating that differences between the main and the medium eigenvector tend to be reduced. This trend confirms the phenomenon of fibre crossing, which is modelled in DTI as two major eigenvalues that are very close to each other, is highlighted at higher b-values.

To summarize, this study demonstrates for the first time that in regions of high directional coherence between white matter fibres, such as in the corpus callosum, an increase in diffusion weighting does not return different information compared to conventional b-values. Conversely, in areas characterised by a non-negligible fibre dispersion within the same voxel, there exists an intrinsic effect due to increasing b-values in the orientation of the principal diffusion axes, even when the noise contribution is taken into account. Experimental results indicates that probing higher b-values can offer a way of highlighting dynamic regimes associated to complex fibre distributions. High b-value DTI is certainly not able to resolve fibre crossing, but can offer a deeper insight into the white matter architecture without resorting to more accurate but time consuming techniques such as HARDI. In parallel, attention should be paid when dealing with diffusion models involving fast and slow diffusion spin pools. In fact, in areas of intersection and dispersion of fibres within a single voxel, the orientation of main diffusion axes depends on the chosen b-value. Nevertheless, for areas where the uniaxial model holds, this study shows that for increasing diffusion weightings, the geometry probed by diffusing spins remains the same, thus validating the approach based on a fixed reference frame. These results can thus contribute substantially to the open debate regarding the opportunity of extending the b-value range to perform a DTI protocol, in the perspective of a more
comprehensive investigation of water dynamics in brain, which can prove useful to monitor both normal development and neural tissue alterations.
Chapter 5

Experimental results II

This last chapter is devoted to the investigation of anomalous dynamics that arise when water is diffusing in specific local micro-architectures, in which conventional Brownian motion theory is inadequate to synthesise the properties of the path travelled by the spins.

As previously reported, the stretching exponential model was introduced in NMR to account for the recorded deviation from the mono-exponential decay of water in brain, predicted by means of regular diffusion theory. In parallel, anomalous dynamics have been largely investigated in a variety of systems.

Specific aim of this chapter is to characterise the phenomenon of anomalous diffusion in depth on different systems, going from controlled ad-hoc phantoms to more complex environments like those characterising biological matter.

The first step was thus the investigation of styrene beads solutions constituted by beads of different dimensions. By understanding water diffusion dynamics for different geometrical configurations, it has been possible to clarify some of the controversial aspects of the results that has been reported in literature during the last years.

Results obtained on samples were crucial to validate the proposed framework. Further step was the characterisation of water diffusion in bone marrow, which can be considered a natural biphasic system (constituted by water and fat - see sec. 4.1).

The last step was the validation of the proposed anomalous diffusion imaging framework (as introduced in sec. 3.3.4) on human brain. Interesting findings will be linked to the local characteristics of cerebral white matter and innovative perspectives will be opened, in order to show that the anomalous behaviour of water in biological environments can offer a new tool to characterise tissue properties.

Part of the ruminations and experimental results contained in this chapter were submitted as a review paper [114]. The experimental results concerning bone marrow diffusion were submitted as a full paper [115]. Besides, the experimental results obtained in human brain were publishes as a full paper [23].
5.1 Anomalous diffusion in Styrene beads

As reported in sec.3.2, the stretched exponential model has been introduced on pure phenomenological basis, with some generic considerations regarding the non linear relationship between the root mean squared displacement and the diffusion time. A formal approach [53] was instead able to derive the shape of the NMR signal in heterogeneous environments, using fractional order calculus framework. Nevertheless, even though the formal derivation of the stretched exponential has been derived, the link between the supposed fractional dynamics and the effective biological environment characteristics has not been clarified.

Aim of this section is to focus on a phantom built ad hoc to characterise water diffusion in a controlled environment. For this purpose, styrene beads solutions constituted by beads of different dimensions and characterised by different polidispersity conditions were realised and investigated.

5.1.1 Problem statement

As anticipated in sec.3.3.2, even though the approach proposed by Magin and co-workers [53] was able to explain the relationship between the stretching exponent and the NMR signal following a diffusion-sensitised sequence, the link between the local microstructure and the characteristics of the mathematical formalism used to derive the model was somehow missing.

Specifically, a stretched exponential decay was obtained under the hypothesis of a fractional order behaviour in time, which corresponds to a finite characteristic waiting time $T$ and diverging jump length variance $\Sigma^2$ (see sec.2.3.3). Thus, since Magin et al. supposed the dynamics in time to be regular, they actually proposed super-diffusion as the microscopic mechanism leading to the stretched exponential signal decay. Conversely, all other works regarding anomalous diffusion, and also common sense, hypothesised the diffusion to be slower in biological systems compared to free water (i.e. sub-diffusion).

Diffusion NMR offers indeed a powerful tool to give new insights into this issue. Conventionally, the diffusion weighting is expressed as a function of the b-value (see sec. 2.2.3). The b-value contains indeed both the diffusion time $\Delta$ and the squared gradient strength $g^2$. Usually, in human-scale spectrometer, the b-value is varied by changing the gradient strength rather than the diffusion time, for pure practical reasons (such as to reduce as much as possible TE and get a higher SNR).

When stretched exponential model is applied to fit the data, the stretching exponent in the formula usually is referred to the entire b-value. In such cases however, it is NOT equivalent to measure the decay using a sequence in which the gradient strength is varied or using a sequence in which the diffusion time is varied.

In the case of a fractional order behaviour in time, as stated in sec.2.3.2, the NMR signal (i.e. the Fourier transform of the motion propagator) has a typical Mittag-Leffler behaviour, expressed as a stretched exponential form in time ($\propto \exp(-\lambda t^\alpha)$) for sufficiently short diffusion time values. If a diffusion NMR sequence is applied with varying diffusion time, the NMR signal as a function of the b-value ($\propto t$) will be a stretched exponential containing $\alpha$, i.e. $S(b) = S(0) \exp(-B_b b^\alpha)$.

Conversely, in the case of a fractional order behaviour in space, as stated in sec.
2.3.3 and as explicitly derived in [53], the NMR signal is expressed by a stretched exponent in the squared gradient strength \( \propto \exp(-A_{\gamma} g^2) \). If a diffusion NMR sequence is applied with varying gradient strength, the NMR signal as a function of the b-value \( \propto g^2 \) will be a stretched exponential containing \( \gamma \), i.e. \( S(b) = S(0) \exp(-B_{\gamma} b^\gamma) \).

Aim of this section was to investigate the differential behaviour in space and time of a model system constituted by highly packed styrene spherical beads suspended in water. In high-field spectrometers, it is possible to control both the diffusion time and the diffusion gradient strength independently to obtain spectroscopic measurements. As a consequence, the stretching exponent was measured for both \( g \)-varying and \( \Delta \)-varying sequences.

Beads suspensions have been taken into consideration quite often to investigate diffusion. In particular, Callaghan’s works largely dealt with styrene phantom [116], introducing a pore hopping theory developed on the basis of the assumption that diffusion within pores is very much faster than diffusion between pores. As a consequence, for sufficiently long diffusion times all molecules, irrespective of starting position, may be found with equal probability anywhere in the pore, a condition termed pore equilibration. However, in this section the experimental parameters will be tuned to focus on the diffusion within a pore, avoiding the conditions of pore equilibration.

Experimental results will show that, even for a simple and symmetrical sample as styrene suspensions, anomalous dynamics are measured both in space and in time. Those dynamics are influenced indeed by different geometrical factors. As a consequence, when applied to complex systems as biological matter, the stretching exponents \( \gamma \) and \( \alpha \) can offer different levels of information.

In vivo applications of diffusion sequences in which the diffusion time is varied are challenging, due to several experimental factors. The results of this section demonstrate indeed that, despite the required efforts, new insight can be gained by investigating both the anomalous exponents \( \gamma \) and \( \alpha \).

### 5.1.2 Methods

Eight different beads sizes were used to prepare eight different samples, ranging from 6 \( \mu \text{m} \) to 140 \( \mu \text{m} \). Specifically, SPHEROMERS CA (polymethylmethacrylate) CA6, CA10, CA15, CA20, CA30 and DYNOSEEDS TS (polystyrene) TS40, TS80, TS140 were purchased by Microbeads AS (Norway).

Samples were prepared dispersing dry styrene beads in deionized water (characterised by a conductivity of the order of \( 10^{-6} \text{mho/cm} \)). In order to stabilise the solution, a surfactant was added to the mixture (Tween-20, polyoxyethylene-sorbitan-mono-laurat) in a concentration of \( 10^{-6} \text{M} \). The solutions were then inserted in glass tubes of 1 cm in diameter and 18 cm in length. The filled volume was about 2 cm³.

For each sample, beads concentration was measured directly by means of a pycnometer containing 25 ml \( V_P \) of the solution. The pycnometer mass was measured at \( T = 25^\circ \text{C} \) and then inserted into the oven at \( T = 50^\circ \text{C} \), in order to let all the water evaporate. The mass was measured again and the difference was attributed to the evaporated water mass \( V_{H_2O} \). The beads concentration \( \nu \) was eventually
obtained from the following relation:

$$\nu = \frac{V_P - V_{H_2O}}{V_P}$$  \hspace{1cm} (5.1)

The mean value of $\nu$ or sphere packing was 70% for all the investigated samples.

\textbf{Figure 5.1.} Image of styrene beads configuration obtained with Mathematica (Wolfram). The beads were disposed in the so-called cannon ball configuration.

NMR measurements were performed on a Bruker Avance-400 high-resolution spectrometer operating at 9.4 T, according to the protocol that follows. First of all, a Spin Echo sequence was applied to evaluate internal gradients, following the same procedure described in sec.4.1, using the following parameters: TR = 2500 ms, NS=32 values of TE ranging from 1 to 1400 s. A Pulsed-field gradient STimulated Echo (PGSTE) sequence (TR = 5000 ms, diffusion gradient pulses delay $\Delta = 80$ ms, diffusion gradient pulses duration $\delta = 4.4$ ms, NS=48 experimental points obtained varying linearly the diffusion gradient strength from 0 to 102 mT/m) was applied across three directions corresponding to the three spectrometer’s axes $x$, $y$ and $z$. A modified version of the same PGSTE sequence, in which the b-value was varied by increasing the diffusion time $\Delta$ rather than the gradient intensity, was then applied (TR = 5000 ms, diffusion gradient pulses ranging from 20 to 1010 ms, diffusion gradient pulses duration $\delta = 4.4$ ms, NS=48 experimental points, gradient strength $g= 12$ mT/m. The temperature was monitored to lie in the range $18.0 \pm 0.5 ^\circ C$.

A Levenberg-Marquardt algorithm was used to fit the signal decay. To obtain the internal gradient, the SE decay was fitted to eq.2.38. The diffusion coefficient was obtained by means of a fitting procedure of data obtained from the first PGSTE sequence, according to the conventional mono-exponential decay.

The first diffusion-weighted dataset ($g$-varying) was fitted to the following function:

$$S(g) = S(0) \exp(-A_g g^{2\gamma})$$  \hspace{1cm} (5.2)
where $A_g$ is a coefficient depending on the experimental design and intrinsic diffusivity while $g$ is the gradient strength.

The second diffusion-weighted dataset ($\Delta$-varying) was instead fitted according to the following function:

$$S(\Delta) = S(0) \exp(-A_\Delta \Delta^\alpha)$$

where $A_\Delta$ is a coefficient depending on the experimental design and intrinsic diffusivity. The influence of surface relaxation was not taken into account in this analysis, due to negligible values of surface relaxivity reported for styrene [117, 118]. In this way, for each sample and for each gradient direction estimates of $\gamma$ and $\Delta$ were collected. Data were processed separately for each of the three gradient directions and the results were then averaged.

5.1.3 Results

In fig.5.2, the stretching exponent $\gamma$ is plotted as a function of beads dimension. For small beads dimensions, $\gamma$ decreases for increasing beads dimension. Conversely, for larger dimensions ($> 40\mu m$), an opposite trend is reported.

**Figure 5.2.** Stretching exponent $\gamma$ values and associated standard deviations as obtained by means of Levenberg-Marquart fit of the signal decay, according to formula 5.2.

In fig.5.3, there is reported the stretching exponent $\alpha$, plotted as a function of beads dimension. Decreasing values of $\alpha$ are measured for increasing beads radius. A significant correlation ($p < 0.05$) is observed between $\alpha$ and beads dimension ($r = -0.926$). No correlation is instead observed between $\alpha$ and internal gradient values.

Fig 5.4 shows the internal gradient values, reported as a function of the stretching exponent $\gamma$, in logarithmic scale. For increasing values of the internal gradient, lower values of $\gamma$ are measured, indicating an increasing effect of phenomena generating Levy walks. The logarithmic correlation between $\gamma$ and internal gradient is indeed significant ($r = 0.907, p < 0.05$). Conversely, correlation between internal gradient and $\alpha$ is not significant.
Finally, fig. 5.5 shows the phase diagram $\gamma - \alpha$. The dashed line represents the Gaussian diffusion limit, which separates the sub-diffusion regime ($\gamma < \alpha$) from the super-diffusion regime ($\gamma > \alpha$), as illustrated in fig.2.9. The pattern reported in the phase diagram indicates that depending on the beads dimension, water in some samples has a sub-diffusive behaviour while in others it has a super-diffusive behaviour.

The first important result achieved in this section is thus that water dynamics in structured environments can be described by two different stretching exponents, which are referred to different properties and hence assume different values.

To interpret the reported trends, one needs to consider the properties of styrene beads that influence $\gamma$ and $\alpha$ values.

First of all, beads dimension sets the dimension of the void spaces in which water diffuses. A reasonable model of sphere packing can be the Hexagonal Close Packing (HCP), reported in fig.5.6. In this model, two differently sized void spaces are present: the larger ones, whose maximum extension is equal to the pore diameter, and the smaller ones, characterised by a maximum extension of $\sqrt{3} - \frac{1}{2}d$, where $d$ is the bead diameter (see fig.5.6). However, independently of the exact disposition of the beads, the larger the sphere radius, the larger the void spaces in which water diffuses. The stretching exponent $\alpha$ is highly negatively correlated with beads dimension, indicating the sensitivity of this exponent to the geometric configuration.

Another important property to be considered is the internal gradient. Styrene has a magnetic susceptibility higher than water ($\chi_{\text{water}} = -9.05 \cdot 10^{-6}$ vs $\chi_{\text{styrene}} = -7.46 \cdot 10^{-6}$ in SI units) [119], hence magnetic field gradients are generated at the interface between water and beads.

Internal gradients (just like every magnetic field gradient) can change the phase of moving spins on a timescale defined by the spin dynamics. The beads/water interface can thus improve the effect due to the diffusion weighting gradients, supplying an additional phase, which results in an apparent boost of the moving molecules.
Figure 5.4. Internal gradient values and associated standard deviations, obtained according to formula 2.38, as a function of stretching exponent $\gamma$ values as obtained by means of Levenberg-Marquart fit of the signal decay, according to formula 5.3. The $y$ axis is shown in logarithmic scale.

From a quantum mechanics point of view, during a diffusion weighted sequence, the inhomogeneous loss of coherence, induced by the internal gradients, will reduce the number of spin refocusing, as if the diffusion coefficient of some of them was larger. Since the only contrast mechanism in a diffusion sequence is the spin phase, from the point of view of the diffusive motion this is equivalent to water molecules experiencing long jumps. The formalism associated to such mix of long and short trajectories has been largely described in literature by means of Levy flights [12].

For this reason, the stronger the internal gradients, the larger the 'apparent' jumps, the lower the value of $\gamma$.

In specimens where either fractional dynamics in space and in time are present, a single exponent is not enough to establish if sub- or super-diffusion regimes are actually taking place. Values of $\alpha$ lower than one correspond to water trajectories undergoing trapping processes, characterised by a diverging waiting time distribution between two consecutive steps. From the point of view of the environment in which water diffusion takes place, this corresponds to a system with high trap densities on different length scales. In porous systems, these traps can be represented by interconnected cavities of different volumes, where the residential time tends to infinity.

Conversely, $\gamma$ is linked to the 'jump' length, i.e. the extent of the displacements happening during the time unit. Values of $\gamma$ lower than one describe Levy-type processes, during which the square displacement diverges. Such processes can take place in systems characterised by cavities with interconnection of variable length, in BMSD under particular limits as reported in sec.3.2.2 or, as seen before, as a consequence of internal gradient dephasing action.

However, both of the exponents contribute to determine whether the dynamics are slower or faster than those of pure water, according to eq.2.79 where $\mu = 2\gamma$. The balance between $\alpha$ and $\gamma$ can be reported in a phase diagram, such as in fig.5.5,
Figure 5.5. Phase diagram $\gamma - \alpha$. The dashed line represents the Gaussian diffusion limit, which separates the sub-diffusion regime ($\gamma < \alpha$) from the super-diffusion regime ($\gamma > \alpha$). The white data point represents the behavior of distilled water at 25°C.

Figure 5.6. Unit cell and correspondent geometry for HCP model. Left: in plane schematisation of the void spaces between the beads and the different regimes can be discriminated.

Interestingly, some of the analysed samples lie in the portion of the graph indicating super-diffusion. It is noticeable that the samples experiencing super-diffusion are those whose beads (and consequently holes) dimensions are comparable to the mean free path travelled by the water molecule ($\sim 15\mu m$ according to the selected diffusion time and typical diffusivity values).

Experimental results reported in this section explain the role of the two stretching exponents and clarify the link between $\gamma$ and super-diffusive phenomena. Specifically, results obtained on cerebral tissue by means of the anomalous diffusion imaging method reported a value of $\gamma$ lower than one, but this alone does not imply super-diffusion conditions. Measurements of $\alpha$ in the same b-value range would have been required. Unfortunately, these are challenging to implement on a clinical scanner.

However, it is noticeable that $\gamma$ in these simple spherical systems is strongly correlated to the internal gradient. On the basis of these experimental results, one
can speculate that in more complex systems as certain biological environments, the value of gamma can be related to the dephasing effects generated by internal gradients at the boundaries between water and other biological material and thus to the architecture itself.

5.2 Anomalous diffusion in Bone Marrow

In this section, anomalous diffusion measurements based on the evaluation of $M_γ$ parameter to quantify anomalous diffusion processes in bone marrow are proposed. In sec.4.1, the properties of bone marrow in trabecular bone were described; here the attention is focused on both bone marrow in femoral epiphysis, where it fills pores generated by the trabecular bone network, and bone marrow in femoral diaphysis, where it is not forced in pores.

Ex-vivo specimens extracted from calves were investigated at 9.4T. AD experiments and conventional apparent diffusion coefficient (ADC) measurements were performed in samples characterised by different relative percentage of water and fat.

Experimental results show that water in bone marrow undergo an anomalous diffusion regime characterised by values of $\gamma$ lower than one. $M_γ$ of water component shows a decreasing trend as bone marrow water decreases in spongy-bone specimens. Conversely $M_γ$ values do not depend on the water percentage in diaphysis bone marrow. Conventional ADC does not discriminate nor water in diaphysis and epiphysis, or spongy-bone characterised by different trabecular bone network, while $M_γ$ does.

These results confirm that anomalous diffusion dynamics characterise different biological systems and suggest potential applications of water $M_γ$ to investigate and to diagnose bone marrow and spongy-bone pathologies such as osteoporosis and cancer.

The results reported in this section had been presented in several abstracts [120–123].

5.2.1 Problem statement

As reported in sec.4.1, bone marrow can be considered a natural biphasic system constituted by water and fat. From a micro-structural point of view, both bone marrow in spongy bone and bone marrow free in the larger bone cavities are soft tissues characterised by several particles of spherical/elliptical shape and average size ranging from 6 $\mu$m (red blood cells) to approximately 100 $\mu$m (fat globules). Moreover, spongy bone is characterised by a trabecular bone network which has been reported as having fractal characteristics related to its bio-mechanical properties (??). In turn, the spongy bone can be discriminated in epiphysis, which represents the upper part of the spongy bone, and metaphysis, which instead lies between the epiphysis and the diaphysis. The trabeculation is well known to vary in different anatomic regions, being densest in the epiphysis, but less so in the metaphysis, with little or no trabeculation in the diaphysis [124].

For all the bone marrow features listed so far, and taking into account of the potentiality provided by an anomalous diffusion investigations, in this section $M_γ$ was measured on bone marrow calf specimens, both belonging to the trabecular
network and to the diaphysis, where it is not constrained into pores. The results were then compared with conventional ADC measurements.

The goal was to investigate anomalous diffusion dynamics in this peculiar biphasic system, where water interacts both with the fat molecules and with the solid matrix.

In sec. 4.1 it has already been underlined that, due to the severity of the disease which affect the muscle-skeletal apparatus, new parameters to describe spongy bone status are highly required. Further development of this technique can be used to test Mγ as new potential MR parameter to investigate bone marrow and spongy bone. As a consequence, Mγ and ADC results, obtained in different bone marrow and spongy bone specimens, were compared. Finally, ADC and Mγ were investigated as a function of different percentage of water in bone marrow and as function of two different kinds of trabecular bone network.

5.2.2 Methods

A total of thirty-four samples were excised from different femoral locations (as shown in fig. 5.7) of calves characterised by different ages, ranging from 6 to 24 months. The goal was to obtain samples characterised by various architectures and water concentration. Fresh samples (analysed immediately after slaughter) were investigated at a temperature fixed to 293 K.

![Figure 5.7. Femoral locations selected for bone marrow investigations: epiphysis (1), metaphysis (2) and diaphysis (3) (left). NMR tube positioning with respect to the external magnetic field Hz (right).](image)

Twenty ex-vivo spongy bone samples excised from two different parts of femoral head, were cut into pieces of approximately 15mm high and 7mm deep. The long axis of each sample was located parallel to the main direction of the static magnetic field (z axis) as depicted in fig. 5.7. Ten of the twenty specimens were excised from femoral epiphysis while the other ten samples were extracted from femoral metaphysis (see fig. 5.7). As a consequence former spongy bone specimens are characterised by a more oriented and compact trabecular bone structure than that of latter samples. Moreover, thirteen bone marrow specimens were extracted from femoral diaphysis. Specifically the central zone of bone marrow in femoral cavity
were put in capillaries of 1 cm in diameter operating a carotage in femoral diaphysis slices of 2 cm thickness (see fig. 5.7).

All measurements were performed on a Bruker 9.4T Avance system described in sec. 4.1.2 1H-spectra were collected from each sample to derive water and fats content, using a single 90° pulse with TR=5 sec and NS=8.

A spectroscopic PGSTE (TE/TR=18/3000ms, diffusion gradient pulses delay ∆=80ms, diffusion gradient pulses duration δ=4.4ms and diffusion gradient strength g was applied to each samples using 64 gradient amplitude steps from 6mT/m to 1000mT/m) along x, y and z axis. Then a b-values range from 4 to 110000 s/mm² was used. This sequence employs bipolar diffusion gradients to minimise eddy currents effects.

Water and single fat percentage were extracted from spectra collected from all samples, following ref. [125]. Fat resonances at 0.9, 1.3, 2.0, 2.25, 2.77, 4.3 and 5.3 ppm were evaluated.

Molecular diffusion behaviour of each of the two individual spectral components (water+fat at 4.7ppm and fat molecules at 1.3ppm) was investigated. Peak area decays of the former peak (water+fat) was fitted as a function of g to a bi-exponential decay:

\[ S = S_{\text{water}}(0) \exp(-b \cdot ADC_{\text{water}}) + S_{\text{fat}}(0) \exp(-b \cdot ADC_{\text{fat}}) \]  

and using the stretched-exponential function:

\[ S = S_{\text{water}}(0) \exp(-b^\gamma \cdot AAC_{\text{water}}) + S_{\text{fat}}(0) \exp(-b \cdot ADC_{\text{fat}}) \]

where AAC is the apparent anomalous diffusion coefficient.

γ and ADC of water component and ADC of fat component were thus obtained as a function of water percentage.

All statistical analysis were performed using SPSS 15.0 (Chicago, IL). To identify significant differences in different groups of samples investigated, one way analysis of variance was applied. Student’s t-test was employed to evaluate significant differences between samples. A p-value less than 0.05 was considered statistically significant. Moreover, ADC and Mγ values were correlated with water content percentage using the Pearson’s correlation coefficient (r).

5.2.3 Results

In fig. 5.8, a typical bone marrow spectrum with peaks assignment is reported on the left. On the right, the fat composition averaged across the sample, separately for trabecular and free bone marrow, is shown. No significant differences in composition between bone marrow located in the femoral head (trabecular) and in the diaphysis (free) are measured.

Fig. 5.9 shows the ADC values as a function of water percentage for free bone marrow, trabecular bone marrow in metaphysis and trabecular bone marrow in epiphysis . A non-significant correlation (p > 0.05) is observed for both trabecular bone marrow vs water percentage and free bone marrow vs water percentage. The ADC range for trabecular and bone marrow is the same, reflecting the homogeneity of composition between the two systems. Besides, ADC values do not allow the
Figure 5.8. Bone marrow spectrum with peaks assignment (left). Fat composition averaged across the samples, for free and trabecular bone marrow (right). Reproduced from [120]

Figure 5.9. ADC values in mm$^2$/s (water component) vs water content for trabecular in epiphysis (black), trabecular in metaphysis (gray) and free (white) bone marrow discrimination between trabecular and free bone marrow or between different kinds of location within the trabecular network (epiphysis or metaphysis).

In fig.5.10, the values of $\gamma$ obtained from the fit are shown for for free bone marrow, trabecular bone marrow in metaphysis and trabecular bone marrow in epiphysis. Free and trabecular bone marrow show a different trend for increasing water content. Trabecular bone marrow $\gamma$ values are positively correlated with water content ($r = 0.56$, $p < 0.05$), while in free bone marrow, $\gamma$ ad water content are uncorrelated. Besides, $\gamma$ in free bone marrow is closer to one as compared to $\gamma$ in trabecular bone marrow, i.e. larger deviations from the Gaussian behaviour are observed in bone marrow when it is constrained inside the pores of the bone network. $\gamma$ trends for increasing water content as measured in metaphysis and in epiphysis separately are positively (logarithmic) correlated to water content, as shown in fig.5.10 ($p < 0.05$ for both curves). Besides, analysis of variance confirmed
that $\gamma$ values discriminate between bone marrow belonging to metaphysis respect to bone marrow belonging to epiphysis.

![Figure 5.10. $\gamma$ values vs water content for both trabecular in epiphysis (black), trabecular in metaphysis (gray) and free (white) bone marrow](image)

If the bone marrow composition does not change significantly between different locations, i.e. in the trabecular network or in the diaphysis, then the major difference between the two systems (trabecular vs free bone marrow) considered in this section is the presence or the absence of the bone matrix. As largely shown in sec4.1, internal magnetic field gradient due to differences in magnetic susceptibility are generated at the interface between bone and bone marrow. According to findings reported in the previous section (see fig.5.4), $\gamma$ values are supposed to be sensitive to the internal gradients which affect the spin phase.

This important result is confirmed by the differential behaviour of bone marrow water diffusion when it is enclosed into bone cavities or when it is free, which is reported in fig.5.10. Besides, the increasing trend of $\gamma$ values as a function of increasing water content is coherent with the findings reported in sec.4.1, according to which water wets the pore’s surface while fat is rearranged principally in the central zone of the pore. For very small water concentrations, the water molecules are mostly found at the bone interface. At the bone interface, internal gradients dephase water molecules and a strong effect on the diffusion decay is recorded. As the water concentration increases, only a fraction of water molecules is in contact with the pore surface and the $\gamma$ exponent increases towards unity.

Interestingly, $\gamma$ values can discriminate between different locations within the trabecular network. Trabeculae are known to be denser in the epiphysis as compared to metaphysis, thus a stronger internal gradient is generated. As a consequence, lower values of $\gamma$ can be recorded.

The sensitivity of $\gamma$ parameter to the presence of trabecular bone network and to water content is a promising result in the perspective of the need of new specific tools to characterise trabecular bone network.
5.3 Anomalous diffusion imaging in Human brain

The anomalous diffusion imaging method has been introduced in sec. 3.3.4 as a way to quantify both the tissue anomalous diffusion and its anisotropy, independently of the reference frame of the experiment.

Here, experimental findings to validate the method will be presented, together with a discussion about its potential application to cerebral white matter characterisation. Aim of this section is to show the ability of anomalous diffusion indices to characterise white matter structures, whose complexity is only partially accounted by DTI indices.

Experimental results, obtained on 10 healthy subjects at 3T, show that the new parameters are highly correlated to intrinsic local geometry when compared to indices derived in [52]. Moreover, they offer a different contrast in white matter regions when compared to DTI. Specifically, the new indices show a higher capability to discriminate among areas of the corpus callosum associated to different distribution in axonal densities, thus offering a new potential tool to detect more specific patterns of brain abnormalities than DTI in the presence of neurological and psychiatric disorders. These results had been presented in abstract form [19, 126] and as a paper [23].

5.3.1 Problem statement

The theoretical aspects underlying the anomalous diffusion method were introduced in sec. 3.3.4. The stretching exponent sensitivity to the microstructural degree of heterogeneity makes this parameter especially suitable to investigate white matter regions. In white matter, bundles of nerve fibres act like obstacles for the diffusing water molecules and the presence of axons creates privileged diffusive patterns. For this reason, anomalous exponent values strongly dependent on the direction of measurement are expected.

Using the strategy proposed in sec. 3.3.4, it has been possible to quantify the mean values of the anomalous exponents and their anisotropy, which will be define as $M_\gamma$ and $\gamma A$ respectively throughout this section. These indices are thus similar to those defined in [52], but the dependence on the laboratory frame has been removed.

Motivation to establish a correct model to extract $M_\gamma$ and $\gamma A$ as scalar invariant indices, is related to the potential ability of $\gamma$ parameter to reflect the degree of structural disorder and complexity in cerebral tissues. In this perspective, $M_\gamma$ could provide a new source of contrast in diffusion maps, different from that of MD.

In performing this analysis, one of the aims has been testing the $\gamma$ parameter as a potential marker for specific tissue compositions, in the perspective of future clinical applications. Changes in white matter structural organisation are known to occur during normal and abnormal development, as well as in the presence of neurological and psychiatric disorders. Under certain pathological conditions, FA and MD still remain poorly informative about the specific pathophysiological substrate underlying different pathological conditions. With this regard, a promising application of $\gamma$ parameter investigations could be $M_\gamma$ measurements in WM areas, such as the corpus callosum, which are characterised by different axonal densities and diameters. Experimental findings suggest a specific sensitivity of $\gamma$ parame-
ter in detecting changes in axonal diameter distributions, which may be selectively affected by pathological conditions.

Specific aims of the current section are thus to define a new procedure to obtain in vivo $M\gamma$ and $\gamma A$, to compare these measures with DTI indices and with anomalous diffusion parameters as defined by Hall and Barrick [52] and eventually to discuss the results in terms of possible applications for white matter characterisation in future clinical applications.

### 5.3.2 Methods

Ten healthy volunteers (F/M=4/6, mean age and standard deviation 24 ± 3 years) participated in this study after giving informed consent, according to the national laws and to the local ethics committee guidelines. All imaging was obtained using a head-only 3.0T scanner (Siemens Magnetom Allegra, Siemens Medical Solutions, Erlangen, Germany), equipped with a circularly polarized transmit-receive coil. The maximum gradient strength is 40 mT m$^{-1}$, with a maximum slew rate of 400 mT m$^{-1}$ ms$^{-1}$. T1-weighted sagittal images were acquired for anatomical reference and brain segmentation, using a MPRAGE (magnetisation-Prepared Rapid Acquisition with Gradient Echo sequence) [TR/TE/TI=2000/4.38/910 ms, flip angle=8°, matrix=448x512, in-plane resolution=0.5x0.5 mm$^2$, slice thickness=1 mm, 176 adjacent slices, field of view (FOV) of 224x256 mm$^2$]. Diffusion weighted (DW) SE EPI were acquired to cover the whole brain using the following parameters: TR/TE= 6400/107 ms, $\Delta/\delta=107/35$ms, bandwidth=1860 Hz/px, slice thickness=3mm, in plane resolution=1.8x1.8 mm$^2$. The encoding gradients were applied along 6 non collinear directions at 16 different b-values: $0, 0.1 \cdot 10^9, 0.2 \cdot 10^9, 0.3 \cdot 10^9, 0.4 \cdot 10^9, 0.5 \cdot 10^9, 0.7 \cdot 10^9, 0.8 \cdot 10^9, 10^9, 1.2 \cdot 10^9, 1.5 \cdot 10^9, 2 \cdot 10^9, 2.4 \cdot 10^9, 3 \cdot 10^9, 4 \cdot 10^9, 5 \cdot 10^9$ s/m$^2$.

### Data Analysis

All DW images were corrected for eddy currents distortions using FSL version 4 (http://www.fmrib.ox.ac.uk/fsl) software. As a first step, FA and MD were derived after tensor calculation by means of FSL DTIFIT routine using the $b=1000$ s/mm$^2$ subset of the acquired data. Besides, the three eigenvectors that define voxel-wise the principal reference frame were obtained. Anatomical scans were then co-registered to FA maps and segmented into white matter, gray matter and Cerebrospinal Fluid using SPM version 5 (http://www.fil.ion.ucl.ac.uk/spm). A binary mask was obtained by combining WM, GM and CSF segments and retaining only voxels with intensity greater than 0.8 on the resulting image.

A custom script (Matlab, The Mathworks, Natick, MA, USA) was implemented to perform the image reconstruction. The $\gamma_i$ values were obtained by means of a non-linear least-squares (utilizing Levenberg-Marquardt minimization) multi-dimensional estimation procedure of formula 3.15, in the subspace of $b$ and $A_i$.

Parametric maps based on $M\gamma$ and on $\gamma A$ were then obtained. To compare the results with the anomalous exponent analysis proposed by Hall and Barrick, maps
of the mean value of the anomalous exponent (AE), i.e.

$$AE = \langle \gamma \rangle = \frac{1}{N} \sum_{i=1}^{N} \gamma_i$$  \hfill (5.6)

and of the anomalous anisotropy (AA), i.e.

$$AA = \sqrt{\frac{N}{N-1} \sum_{i=1}^{N} (\gamma_i - \langle \gamma \rangle)^2 \sum_{i=1}^{N} \gamma_i^2}$$  \hfill (5.7)

where $N$ is the number of the directions, $\gamma_i$ is the anomalous exponent measured in the $i$-th direction and $\langle \gamma \rangle$ is the mean exponent, were obtained. AE and AA were derived by means of a custom script, written following the prescriptions found in the published paper [52].

MD, FA, $M_\gamma$, $\gamma_A$, AE and AA were measured in 13 regions of interest (ROIs) selected on the $b_0$ images as previously described [107]. Data have not been normalized into a stereotaxic space but kept in their own native one, so the ROIs were placed manually for every subject. Rectangular ROIs of variable volume (range 68.4-420.3 mm$^3$), depending on the anatomical region studied, were placed bilaterally in the following areas: the occipital(a) and temporal lobe(b), the anterior pericallosal areas(c), the genu(d) and the splenium(e) of the corpus callosum, the posterior pericallosal areas(f), the frontal(g) and parietal lobe(h), the thalamus(i), the putamen(l), the head of the caudate nucleus(m), the posterior limb and the genu of the internal capsule(n). An additional control ROI was defined within each lateral ventricle (o). Figure 5.11 illustrates the location of all parenchymal ROIs. The ROIs were eventually transferred onto all considered quantitative maps for each subject, and average measures were calculated for every ROI. Two kind of statistical analysis were performed to evaluate the proposed method. First of all, Pearson correlation coefficient ($r$) was obtained between FA, $\gamma_A$ and AA and between MD, $M_\gamma$ and AE. Besides, mean and SD were obtained in each ROI and ANOVA was used to test the efficacy of the new parameters in discriminating between considered regions, compared to DTI. As a first step, Lilliefors test was performed to confirm the normality of the distribution across subjects. Then a two-way ANOVA was performed, in which the two factors were the regions (12 levels) and the methods used (two levels, DTI or our approach). The ANOVA was performed separately for the methods FA-$\gamma_A$ and for MD-$M_\gamma$. Following significant interaction in the ANOVA for both FA-$\gamma_A$ and MD-$M_\gamma$, post-hoc t-tests were performed for each couple of regions.

### 5.3.3 Results

Fig.5.12 shows an example of MD, FA, $M_\gamma$, $\gamma_A$, AE and AA maps obtained from one studied subject. A different contrast was evident between MD/FA and anomalous diffusion maps ($M_\gamma$, $\gamma_A$, AE and AA). Interestingly, within the framework of the anomalous diffusion model, the two analyses (i.e. $M_\gamma$, $\gamma_A$ vs AE, AA) lead to different contrasts. From a visual inspection of the images, in both MD and FA and our anomalous diffusion maps $M_\gamma$ and $\gamma_A$ there are present anatomical landmarks which are not visible in AE and AA maps.
5.3. ANOMALOUS DIFFUSION IMAGING IN HUMAN BRAIN

Figure 5.11. Location of the selected regions of interest (ROIs) in a healthy volunteer: 
b1-b2 temporal lobe, a1-a2 occipital lobe, l1-l2 putamen, l1-l2 thalamus, n1-n2 posterior 
limb of the internal capsule, d-e genu and splenium of the corpus callosum, c1-c2 anterior 
pericallosal areas, f1-f2 posterior pericallosal areas, m1-m2 head of the caudate nucleus, 
g1-g2 frontal lobe, h1-h2 parietal lobe. All the ROIs are superimposed on B0 images. 
Reproduced from [23].

As a first step of the data analysis, specific aim was to investigate the correlations 
between DTI indices and anomalous diffusion parameters, derived with both 
methods. In Fig.5.13A and 5.13B there are reported the correlation plots across 
ROIs between FA and $\gamma_A$ and between FA and AA. The plot ??A clearly shows 
that there is a high positive correlation between FA and $\gamma_A$ ($r = 0.91$, $p < 0.0001$ 
without CSF, $r = 0.92$, $p < 0.0001$ with CSF). Conversely, the linear correlation 
between FA and AA is less remarkable ($r = 0.47$, $p = 0.063$ without CSF, $r = 0.54$, 
$p = 0.028$ with CSF), and the error bars associated to data points overlap in the 
AA axis, thus suggesting that poor additional information can be gathered by esti-
mation of AA.

The correlation plots across ROIs between MD and $M_\gamma$ and between MD and 
AE are shown in Fig.5.13C and ??D respectively. The resulting trend between MD 
and $M_\gamma$ is positive ($r = 0.45$, $p = 0.069$ without CSF, $r = 0.92$, $p < 0.0001$ with 
CSF) but moderate, especially when the CSF contribution is not considered. It is 
noticeable that some regions are clearly discriminated by $M_\gamma$, while their MD values 
overlap when the error bars are considered. For example, in the corpus callosum the 
splenium has a $M_\gamma$ value significantly lower than the genu, as shown in Fig.5.13C. 
Conversely, MD and AE are poorly correlated to each other ($r = 0.34$, $p = 0.14$ 
without CSF, $r = 0.39$, $p = 0.096$ with CSF).
Figure 5.12. From top to bottom: mean diffusivity (MD), fractional anisotropy (FA), mean $\gamma$ ($M\gamma$), $\gamma$ anisotropy ($\gamma A$), mean anomalous exponent (AE) and anomalous anisotropy (AA) maps of the slices 3-12-16-23-27-36 from a healthy subject. Reproduced from [23]
Figure 5.13. Correlation plots in which every point represents the value calculated for each of the 12 ROIs. Left: correlation between FA and $\gamma_A$ (A) and between FA and AA (B). Right: correlation between MD and $M\gamma$ (C) and between MD and AE (D). Mean values were derived from the ROIs illustrated in Fig. 5.11: occipital lobe(a), temporal lobe(b), anterior pericallosal areas(c), genu(d) and splenium(e) of the corpus callosum, posterior pericallosal areas(f), frontal lobe(g), parietal lobe(h), thalamus(i), putamen(l), head of the caudate nucleus(m) and posterior limb of the internal capsule(n). In the inserts, the data point corresponding to the ROI drawn in the CSF(o) is added to the plot. Reproduced from [23].
Since the correlation plots were calculated between the mean values of MD, FA, M\gamma, \gamma A, AE and AA, averaged across all subjects, to investigate if those correlations are significant (i.e. found also in each single subject) or if they are merely an effect of the average, the correlation coefficient for each of the ten subjects was calculated. In Fig. 5.14 there is reported the correlation coefficient for MD and M\gamma, MD and AE, FA and \gamma A, FA and AA. The correlation coefficient which relates the DTI indices with M\gamma and \gamma A is always positive and larger than 0.6, showing that the positive correlations reported in Fig. 5.13A and 5.13C reflects the trend found at single subject level. Conversely, the correlation coefficient calculated between MD and AE and between FA and AA fluctuates between positive and negative values. This means that the moderate correlation which is reported in Fig. 5.13B and 5.13D is not found in every single subject but it is just a consequence of the values averaging.

**Figure 5.14.** Correlation coefficient for mean diffusivity (MD) and mean \gamma (M\gamma) (blue), fractional anisotropy (FA) and \gamma anisotropy (\gamma A) (red), mean diffusivity (MD) and mean anomalous exponent (AE) (yellow), fractional anisotropy (FA) and anomalous anisotropy (AA) (green), plotted for each of the 10 subjects. Reproduced from [23].

In the second part of the study the capability of our new indices M\gamma and \gamma A to discriminate between different cerebral tissue was evaluated and compared to DTI indices. In Fig. 5.15 there are reported the mean values and SD averaged across all subjects into the considered ROIs, of FA, \gamma A, AA, MD, M\gamma and AE. In the histogram 5.15A there are reported FA values (left), \gamma A and AA (right), all in adimensional units. In the histogram 5.15B there are reported MD values (in m^2/s, left), M\gamma and AE (in adimensional units, right).

In order to investigate the ability in discriminating among different cerebral regions of FA compared to \gamma A and of M\gamma compared to MD, two-way ANOVA tests were performed. The F-value associated to the levels FA-\gamma A was 54 (9 degrees of freedom, P < 0.001) while the F-value associated to the levels MD-M\gamma was 9 (9
degrees of freedom, \( P < 0.001 \). Following significant interaction in the ANOVA, paired t-tests are reported in Tab.5.1A for FA, Tab.5.1B for \( \gamma A \), Tab.5.2A for MD and Tab.5.2B for \( M\gamma \). By comparing Tab.5.1A with Tab.5.1B, it is evident that most of the regions which are highly discriminated \( (P < 0.001, \text{in gray}) \) by \( \gamma A \) are highly discriminated by FA as well. Moreover, FA is able to highly discriminate some regions which are not discriminated or moderately discriminated by \( \gamma A \), i.e. FA values have more discriminating power compared to \( \gamma A \). An exception to this trend is represented by the highest anisotropic structures, i.e. the genu and the splenium of the corpus callosum, which instead are better discriminated by \( \gamma A \) than by FA.

Conversely, several regions which are not statistically discriminated \( (P > 0.05) \) by conventional MD, turned out to be discriminated on the basis of \( M\gamma \). As an example, the splenium is always associated to a \( P_{M\gamma} < 0.05 \) when correlated to each one of the other ROIs (see Tab.5.2A), while \( P_{MD} > 0.05 \), except for the putamen, the occipital and temporal lobe (see Tab.5.2B). Besides, couples of regions associated to highly significant p-values \( (P < 0.001, \text{in gray}) \) on \( M\gamma \) basis are different to those highly discriminated by MD. For example, the genu and the splenium are discriminated by MD with a p-value of 0.05 but are better discriminated by \( M\gamma \) with \( P_{M\gamma} < 0.001 \).

A first result is that the cerebral tissue is anisotropic also respect to \( \gamma \). Bennett and co-workers [50] introduced the stretched exponential model as a fitting function to obtain maps of the \( \gamma \) exponent across one selected direction only, in analogy with DWI. The sensitivity to the chosen direction was further tested by the same group [68], showing that the stretching exponent is insensitive to the orientation of the applied magnetic field gradient. These Authors measured the stretching exponent across three orthogonal directions reporting a small anisotropy which was roughly constant in both GM and WM tissues. Conversely, other Authors [52] reported a difference between GM, WM and CSF with respect to their anisotropy measured across 12 different directions. In this regard, this work confirmed that there exists an anisotropy in the stretched exponent, on whose basis it is possible to discriminate between different cerebral structures.

The high correlation found between \( \gamma A \) and FA confirms that in the framework of the anomalous diffusion, the definition of three main diffusivity directions may be a good strategy to obtain a quantification of anisotropy which is independent from the reference frame. Their high correlation indicates that both quantities, i.e. FA and \( \gamma A \) refer to intrinsic geometrical properties of brain tissues, which are independent from the reference frame in which the gradient directions are expressed. Moreover, this indicates that the hypothesis of the tensorial structure holding also in the case of anomalous diffusion is reasonable. Post-hoc t-tests highlighted that the highest anisotropic structures, i.e. the genu and the splenium of the corpus callosum, are better discriminated by \( \gamma A \) than by FA.

Conversely, the anisotropy index obtained by considering each direction as characterised by a single stretched exponential decay, i.e. AA, revealed a poor correlation with FA, which fluctuates between positive and negative values at single-subject level. The low correlation found between AA and FA, i.e. between two measures that are supposed to be dependent on the geometry, confirm that Hall and BarrickÕs method suffers from the dependence on the reference frame in which
Table 5.1. Post-hoc t-tests for FA (A) and $\gamma$A (B). For each couple of regions, there are reported the obtained p-values. The cells corresponding to regions discriminated with high significance ($P < 0.001$) are coloured in gray. The statistical test was performed between the following selected ROIs: occipital lobe(a), temporal lobe(b), anterior pericallosal areas(c), genu(d) and splenium(e) of the corpus callosum, posterior pericallosal areas(f), frontal lobe(g), parietal lobe(h), thalamus(i), putamen(l), head of the caudate nucleus(m) and posterior limb of the internal capsule(n).
Table 5.2. Post-hoc t-tests for MD (A) and Mγ (B). For each couple of regions, there are reported the obtained p-values. The cells corresponding to regions discriminated with high significance ($P < 0.001$) are coloured in gray. The statistical test was performed between the following selected ROIs: occipital lobe(a), temporal lobe(b), anterior pericallosal areas(c), genu(d) and splenium(e) of the corpus callosum, posterior pericallosal areas(f), frontal lobe(g), parietal lobe(h), thalamus(i), putamen(l), head of the caudate nucleus(m) and posterior limb of the internal capsule(n).
the measurement is performed. Nevertheless, if the number of chosen directions is enough to sample the space uniformly, then these effects are likely to be smoothed. To reduce the experimental time, data were acquired using gradients applied along 6 directions only, which is the minimal number required to perform DTI calculation. Conversely, in the work published by Hall and Barrick, 12 different directions were chosen. Even though these Authors did not show any correlation plot, the contrast-to-noise ratio of the obtained maps seems to be higher as compared to these results, thus confirming the relevance of the number of directions.

On the other hand, one of the main limitations of the proposed method is its approximation in considering the principal directions of diffusion as the same obtained using the DTI model. This may have enhanced the resulting correlations that we found between FA and $\gamma_A$.

However, this approximation is reasonable. In fact, since the magnitude of $\gamma$ is always slightly lower than 1 (ranging from 0.7 to 1, where for $\gamma = 1$ the Stejskal-Tanner mono-exponential decay holds), only a small difference in the spatial orientation of the two reference frames is expected. Moreover, this approximation is expected to hold if the main diffusive axes are independent of the b-value. DTI reconstruction is in fact performed at a relatively low b-value of 1000 s/mm$^2$ while the anomalous diffusion method uses higher b-values (up to $b = 5000$ s/mm$^2$). This issue is crucial since exploring higher b-values means probing slower dynamics, which can be linked to different spatial arrangements, as demonstrated in sec. 4.2.

On the basis of the results of sec. 4.2, in regions of high fibre coherence like the corpus callosum, the eigenvectors of the diffusion tensor remain unchanged when the b-value range is extended from $10^9$ s m$^{-2}$ to $5 \cdot 10^9$ s m$^{-2}$.

In order to overcome this approximation, a future work is needed in which more gradient directions are selected. In this case, in the multi-dimensional fit, also the director cosines associated with the main diffusion axes can be estimated, thus avoiding to assume any a priori information about the principal reference frame orientation. This improvement may also offer an experimental validation of the hypothesis underlying the tensorial description of anomalous diffusion, which is a theoretical issue still to be resolved.

Even though the results concerning $M_\gamma$ need further studies and a future validation, experimental findings suggest the anomalous exponent to be related to specific white matter features. The lack of a high correlation between MD and $M_\gamma$ is encouraging because it indicates that the two measures provide different structural information. That is to say, there exist regions in which the diffusion is restricted but not anomalous.

The two properties indeed correspond to different physical phenomena. The restricted diffusion is due to barriers which constrain the water molecules motion inside a portion of the space that is smaller than that travelled if the environment is barrier-free, as reflected by a reduction of the MD [127]. Conversely, the anomalous diffusion is associated to the complexity of the path travelled by the spins, which depends on the shape and size distribution of the barriers. For example, it has been demonstrated that neurons have a fractal-like appearance [51, 128–130]. As a consequence, two situations characterised by the same average mean free path but different barriers distributions might be better discriminated by $M_\gamma$, as confirmed by comparing the post-hoc t-tests reported in Tab.5.2.
In this regard, this study reports an interesting difference in $M\gamma$ values associated to two distinct areas of the corpus callosum, i.e. the genu and the splenium, which are instead overlapped in the MD axis (fig.5.13C) and for which also in literature similar diffusivity values are reported [131, 132].

Since the stretching exponent is postulated to be sensitive to the presence of traps and obstacles on many different length scales, one can speculate that a broader distribution of axonal diameters would result in a lower $M\gamma$. A number of publications underlined the presence of uneven distributions of fibre types along the corpus callosum [133]. A recent work introduced a powerful method to evaluate the axon diameter distribution by means of diffusion MRI [134]. As this method was applied in vivo to the corpus callosum of the rat brain [135], it showed different axonal density distributions, which are moreover characterised by different widths. In particular, the genu was associated to a narrower distribution compared to the splenium, i.e. in the splenium different axonal diameters coexist. On the basis of the experimental results, it is possible to speculate that this diameters heterogeneity can explain the difference in the $M\gamma$ observed between the splenium and the genu of the corpus callosum, as reported in fig.5.13C. If confirmed, these findings can offer a new non-invasive tool to investigate the axon diameter distribution in normal and abnormal development [136, 137]. In fact, the axonal diameter is believed to play an important role in determining axonal properties. As an example, in myelinated axons, nerve conduction speed is directly proportional to axon diameter. Besides, the axonal diameter seems to be relevant for understanding the pathophysiology of specific neurological and psychiatric conditions. For example, in amyotrophic lateral sclerosis, larger axons are preferentially damaged [137]; conversely, autism is dominated by involvement of smaller diameter fibres [137]. In this contest, the $\gamma$ parameter can thus offer a non-invasive tool to investigate white matter tracts according to their composition/distribution. Different patterns of rearrangements in axonal diameters within white matter tracts, can indeed be detected by changes in mean $\gamma$. 
Figure 5.15. Mean values and associated standard deviations of fractional anisotropy (FA), $\gamma$ anisotropy ($\gamma_A$), anomalous anisotropy (AA) (A) and mean diffusivity (MD), mean $\gamma$ ($\gamma_M$), mean anomalous exponent (AE) (B) derived from the ROIs illustrated in Fig. ??: occipital lobe(a), temporal lobe(b), anterior pericallosal areas(c), genu(d) and splenium(e) of the corpus callosum, posterior pericallosal areas(f), frontal lobe(g), parietal lobe(h), thalamus(i), putamen(l), head of the caudate nucleus(m), posterior limb of the internal capsule(n) and ventricle(o). Reproduced from [23].
Conclusions and Perspectives

In this thesis, conventional and anomalous diffusion dynamics occurring to water molecules in different soft matter samples were investigated by means of diffusion NMR techniques, using both high field and clinical spectrometers. At first stage, some aspects of conventional diffusion were elucidated. Specifically, the role of diffusion of water in bone marrow belonging to spongy bone was clarified. As a consequence, internal gradients were linked to the trabecular density of the spongy bone and proposed as a new parameter to assess the status of spongy bone. In the framework of conventional diffusion, the potentiality of DTI performed at high b-value was explored by means of experiments and simulations. High b-values turned out to highlight regions of complex fibres distribution, providing important pieces of information to resolve the fibre composition within a voxel. Finally, anomalous diffusion of water was investigated theoretically and experimentally. The role of the two stretching exponent, associated to anomalous dynamics in time and space respectively, was discussed and clarified in styrene phantoms at high beads concentration. Specifically, the former exponent is strongly influenced by internal magnetic while the latter is linked to specific length-scales of the system. The sensitivity of $\gamma$, the stretching exponent linked to fractional dynamics in space, to the internal gradient was developed to test its potential discrimination power in bone marrow contained in epiphysis, metaphysis and diaphysis. Finally, a theoretical formalism was introduced to account for $\gamma$ anisotropy by means of scalar invariant indices and their discrimination ability between different white matter regions was tested on healthy human brains.

On the basis of all the results reported so far, this thesis opens new interesting perspectives for the early diagnosis of muscle-skeletal pathologies and the characterisation of brain microstructure in both normal and abnormal development. The first step will be to investigate the correlation between local susceptibility distribution in human brain and the $\gamma$ parameter. New data were recently acquired using a increased number of gradient directions (in order to avoid any approximation regarding the diffusion main axes), accompanied by $T_2$ weighted images ($T_2$ is sensitive to the local differences in magnetic susceptibility). This new dataset can definitively confirm the origin of the contrast in $\gamma$ reported in literature in the last years. Further step will be to develop a diffusion sequence able to evaluate $invivo$ the stretching exponent linked to fractional dynamics in time, i.e. $\alpha$. This aim is challenging for practical reasons due to scanner hardware but may offer a completely different contrast and, thus, may be able to catch other aspects of the anomalous diffusion dynamics, in order to depict the complete panorama of water interacting with biological systems.
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