Diffusion tensor MRI of human ischaemic stroke: quantitative measurements, acquisition and registration issues

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I declare that I composed this thesis and the work contained in it is my own. Where work has been published that has been done by other people associated with the research group, acknowledgement is made. This thesis has not been submitted for any other degree or professional qualification.
A mis padres

"La verdadera ciencia enseña, por encima de todo, a dudar y a ser ignorante."
Miguel de Unamuno y Jugo (1864–1936)
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Abstract

The value of diffusion-weighted magnetic resonance imaging (DW-MRI) for the early diagnosis of human ischaemic stroke has triggered the rapid development of this imaging technique. The introduction of the diffusion tensor (DT) model provides a range of tools that permit the quantitative assessment of this and many other neurological disorders. This thesis addresses some of the methodological issues encountered when using DT-MRI to image acute stroke patients.

To demonstrate the use of this technique in investigating the pathophysiology of ischaemic stroke, the first part of the thesis presents results from a study of DT-MRI derived scalar water diffusion parameters in a large group of stroke patients scanned serially. These data demonstrate for the first time differences in the evolution of grey and white matter water diffusion parameters within the ischaemic lesion. The thesis then focuses on a number of problems met during the acquisition and analysis of this patient data.

New DT-MRI acquisition protocols specifically designed to image acutely ill stroke patients are presented. These incorporate optimised image acquisition parameters that address problems related to scan time and patient motion. The effect of patient movement on the acquisition protocol is investigated by means of computer simulations.

Patient motion also increases the importance of image registration to enable voxel-by-voxel calculation of the water diffusion tensor. The next section of this thesis is therefore dedicated to the development and testing of tools that permit the quantitative assessment of DT-MRI image registration. These tools are then applied to data acquired in healthy volunteers to evaluate the performance of three methods used for the correction of image distortions. The advantages and pitfalls found in each method are discussed.

The development of advanced white matter tractography techniques that require high values of diffusion weighting to resolve complex fibre structures may help the understanding of the changes occurring during ischaemic stroke. Using the image registration assessment tools developed, this thesis concludes with a series of numerical simulations that establish the limits of maximum diffusion weighting and minimum signal-to-noise ratio that permits the registration of diffusion-weighted images within error tolerance for two image-based registration methods.
Resumen

RM-TD de la isquemia cerebral humana: análisis cuantitativos y consideraciones en la obtención y el registro de las imágenes.

La importancia de la resonancia magnética (RM) de difusión en el diagnóstico precoz de la isquemia cerebral humana ha desembocado en un rápido desarrollo de esta modalidad de diagnóstico por imagen. La introducción del modelo de tensor de difusión (TD) proporciona además, una amplia gama de herramientas que permiten valorar cuantitativamente éste y otros desórdenes neurológicos. Esta tesis trata algunos de los aspectos metodológicos que surgieron al aplicar RM-TD a pacientes con isquemia aguda.

Para demostrar la aplicabilidad de esta técnica en la investigación de la patofisiología de la isquemia cerebral, la primera parte de esta tesis presenta los resultados de un estudio sobre la evolución de los parámetros escalares derivados de RM-TD. Éstos se midieron en imágenes procedentes de un extenso grupo de pacientes con isquemia que fueron examinados en serie. Los resultados demuestran, por primera vez, diferencias en la evolución de las materias blanca y gris dentro de la lesión isquémica.

A continuación, esta tesis presenta nuevos protocolos para la obtención de imágenes RM-DT, diseñados específicamente para el uso en pacientes con isquemia aguda. Estos protocolos optimizan los parámetros de adquisición teniendo en consideración problemas relacionados con la duración del examen y el movimiento del paciente. Los efectos de movimiento durante el examen en los nuevos protocolos se investigan por medio de simulaciones numéricas.

El movimiento del paciente refuerza la importancia del registro de las imágenes que facilita el cálculo del tensor de difusión vóxel a vóxel. Por consiguiente, parte de esta tesis se dedica al desarrollo y evaluación de herramientas que permiten el examen cuantitativo del registro de imágenes RM-TD. Usando imágenes adquiridas en voluntarios sanos, estas herramientas son utilizadas para evaluar el funcionamiento de tres métodos usados para corregir distorsión de las imágenes. Finalmente se discuten las ventajas y desventajas de cada método.

El desarrollo de algunas técnicas de tractografía avanzadas requiere la adquisición de imágenes con valores altos de potenciación en difusión que permiten resolver estructuras de fibras cerebrales complejas. Ésto puede ayudar a entender los cambios que ocurren durante la isquemia. Esta tesis concluye con una serie de simulaciones numéricas que
estudian los límites de potenciación en difusión máxima y relación señal-ruido mínima que permiten el registro de las imágenes potenciadas en difusión dentro de la tolerancia de error. Se establecen estos límites para dos métodos de registro basados en la imagen, utilizando para ello las herramientas de evaluación del registro de imágenes desarrolladas anteriormente.
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Introduction

Background
Diffusion tensor magnetic resonance imaging (DT-MRI) is a non-invasive technique that provides a macroscopic measure of the random microscopic diffusion of water molecules within brain tissue. Conventional brain imaging methods rely on the detection of gross structural changes for the diagnosis of disease. However, water molecule diffusion is extremely sensitive to alterations in brain physiology, and can be used to investigate the structure of the brain in both health and disease. One of the main clinical applications of DT-MRI is in the study of human ischaemic stroke, where changes in water diffusion parameters have been observed as little as 30 minutes after stroke onset. Yet, although DT-MRI shows considerable promise as a tool to investigate brain structure, more work is required to devise acquisition protocols that allow this modality to be used routinely in acutely ill patients and image processing strategies that maximise the information content of the data. To this end, this thesis deals with the issues met during both acquisition and analysis of DT-MRI data acquired in a study of acute stroke patients.

Aim and scope
The aim of this thesis is to develop image acquisition and processing methods to optimise clinical DT-MRI studies, specifically with regard to ischaemic stroke. It is organised into the following seven chapters.

Chapter 1 provides the basic theoretical description of diffusion, and explains how MRI can measure tissue water molecule diffusion. This is followed by a discussion of the background physics and development of DT-MRI. Finally, the visualisation of DT-MRI data and the calculation of scalar parameters derived from the apparent diffusion tensor of water are explained, and the main clinical application areas reviewed.

Chapter 2 describes a new study investigating the pathophysiology of ischaemic stroke. The temporal evolution of scalar water diffusion parameters was measured in grey and white matter within the ischaemic lesion of a group of stroke patients scanned serially. It was found that the two tissue types evolved differently after
stroke. During this study, issues related to the length of the examination and partial volume averaging of different tissue types were identified. Artifacts in the temporal evolution of the measured water diffusion parameters of normal contralateral tissue were also observed. Work was undertaken to investigate and address these problems.

Chapter 3 presents new DT-MRI acquisition protocols optimised to image acute stroke patients, in particular reducing scan times and dealing with patient motion. The advantages of the new protocols were assessed with numerical simulations.

Chapter 4 introduces quantitative tests to assess the performance of image registration methods that compensate for patient movement and eddy current induced geometric distortions in DT-MRI data.

Chapter 5 reviews general methods for minimizing eddy current induced distortions in echo-planar diffusion-weighted images, and compares the performance of three different methods using the registration assessment tools developed in Chapter 4. A quantitative analysis showed that the artifacts found in the temporal evolution of normal contralateral brain water diffusion parameters may be explained by the use of 3D image-based registration methods.

Chapter 6 investigates, using numerical simulations and registration assessment tools, the limits of maximum diffusion weighting and minimum signal-to-noise ratio that permit the registration of DT-MRI data for two different image registration techniques. This analysis is a precursor to future studies that may use high $b$-value diffusion MRI and tractography methods to resolve complex white matter fibre structures and characterize how these are affected during ischaemic stroke.

Chapter 7 summarises and discusses the results of this thesis and makes recommendations for future work.
Chapter 1 Background to DT-MRI

1.1 Introduction

This chapter commences with a brief introduction to the basic principles of molecular diffusion and MRI necessary to understand the development of diffusion MRI. §1.5 describes how diffusion was first measured using nuclear magnetic resonance (NMR) and the first analytical approaches. The first pulse sequence specifically designed to measure diffusion and the development of DT-MRI are also explained in this section. §1.6 describes the quantitative measurements derived from DT-MRI such as mean diffusivity (\(D\)) and fractional anisotropy (FA), which will be used in later chapters of this thesis. §1.7 illustrates different methods for displaying diffusion data, including a brief description of tractography techniques, and §1.8 presents briefly some of the image artifacts commonly found in DT-MRI images. §1.9 describes new approaches to diffusion MRI that deal with some of the limitations found in the diffusion tensor model. Finally, section §1.10 describes various clinical applications of DT-MRI.

1.2 Description of diffusion

1.2.1 Isotropic diffusion

Self-diffusion describes the random motion of molecules that have thermal energy, in the absence of any gradients that would cause a mass flux. This phenomenon, known as Brownian motion, was first described by the botanist Robert Brown in 1827, who observed the rapid motions of small particles that he found inside the pollen of plants when they were immersed in water (1).

Living tissues contain 60-95% of water by volume in liquid phase and diffusion (comprising both the motion of one type of particle among others, and the motion through cell membranes) plays an important role in the transport of biochemical components. In solids, diffusion is very slow since the thermal energy is extremely small when compared to the energy barriers separating atomic sites.

The first theoretical description of Brownian motion was made in 1905 by Albert Einstein in his PhD thesis "On a new determination of molecular dimensions". He
proposed that random motion of particles was the same process as diffusion, and derived the formula for the average distance moved in a given time during Brownian motion, known as the *Einstein relation*. In an isotropic medium, such as unrestricted water, diffusion of molecules moving a mean microscopic length \( r \) in a time \( \tau \) can be described with the following Gaussian probability distribution

\[
\rho(r, \tau) = \frac{1}{\sqrt{D(4\pi \tau)^3}} \exp\left(-\frac{r^2}{4D\tau}\right), \tag{1.1}
\]

where \( D \) is the self-diffusion coefficient given by the Einstein relation

\[
D = \frac{1}{6\tau} \langle r^2 \rangle, \tag{1.2}
\]

and \( \langle r^2 \rangle \) is the mean-squared displacement of the particle.

Macroscopically, diffusion can be characterised by the transport diffusivity, described by Fick’s Laws (2). Fick’s first law states that the amount of a substance crossing a given area is proportional to the spatial gradient of concentration and to the diffusion constant (which is related to molecular size, viscosity and temperature) giving the equation

\[
F_s = -D \frac{\partial C(x,t)}{\partial x}, \tag{1.3}
\]

where \( F \) is the rate of transfer per unit area of section (flux density), \( C \) the concentration of the diffusing substance and \( x \) the spatial coordinate measured normal to the section. If both \( F \) and \( C \) are expressed in terms of the same unit of mass, from Eq. 1.3 \( D \) has dimensions of \((\text{length})^2 \ (\text{time})^{-1}\), e.g. mm\(^2\)/s.

Generalising diffusion to the three dimensions, Eq. 1.3 becomes

\[
\vec{F} = -D \vec{\nabla} C(\vec{r},t), \tag{1.4}
\]

or Fick’s First Law in isotropic media.

To derive the second of Fick’s laws, we use the continuity equation which expresses a conservation law by equating a net flux over a surface with a loss or gain of material within the surface

\[
\vec{\nabla} \cdot \vec{F} = -\frac{\delta C(\vec{r},t)}{\delta t}. \tag{1.5}
\]

Taking the divergence of Eq. 1.4 and substituting Eq. 1.5, we obtain Fick’s Second Law of diffusion (2)

\[
\frac{\delta C(\vec{r},t)}{\delta t} = D \nabla^2 C(\vec{r},t), \tag{1.6}
\]
assuming $D$ is equal in all directions. This law states that the rate of change of concentration in a volume element within the diffusion field is proportional to the rate of change of concentration gradient at that point in the field.

The two equations given by Fick’s laws describe the diffusion process in fluids where the movement of particles is not restricted. However, when studying biological systems the underlying cellular structure presents barriers that limit the free movement of water in any direction. The diffusion in this case becomes anisotropic and the formalism needs to be generalised.

### 1.2.2 Anisotropic diffusivity

Anisotropic media have different diffusion properties in different directions. To account for the different diffusion coefficients that would be measured in each direction Eq. 1.4 must be replaced by

$$F_x = D_{11} \frac{\partial C}{\partial x} + D_{12} \frac{\partial C}{\partial y} + D_{13} \frac{\partial C}{\partial z}$$

$$F_y = D_{21} \frac{\partial C}{\partial x} + D_{22} \frac{\partial C}{\partial y} + D_{23} \frac{\partial C}{\partial z}.$$  \hspace{1cm} \text{Eq. 1.7}

$$F_z = D_{31} \frac{\partial C}{\partial x} + D_{32} \frac{\partial C}{\partial y} + D_{33} \frac{\partial C}{\partial z}.$$  \hspace{1cm} \text{Eq. 1.8}

$D$ therefore can no longer be expressed as a scalar parameter, but as a second-order tensor,

$$\bar{F} = -D \bar{V} C,$$  \hspace{1cm} \text{Eq. 1.9}

where $D$, the effective diffusion tensor, is defined as

$$D = \begin{pmatrix} D_{11} & D_{12} & D_{13} \\ D_{21} & D_{22} & D_{23} \\ D_{31} & D_{32} & D_{33} \end{pmatrix}.$$  \hspace{1cm} \text{Eq. 1.10}

All elements of $D$ are real since it is hermitian and symmetric, given that for uncharged substances, such as water, the principle of reciprocity applies (3,4), and

$$D_{12} = D_{31}; D_{13} = D_{31}; D_{23} = D_{32}.$$  \hspace{1cm} \text{Eq. 1.10}

In this new formalism $D$ fully describes the molecular diffusion along each direction and the correlation between directions.
1.3 **Introduction to magnetic resonance imaging**

1.3.1 Bloch equations

The property of the nucleus that causes it to interact with magnetic fields is the intrinsic spin $I$ which gives rise to an associated magnetic dipole moment $\vec{\mu} = \gamma I$, where $\gamma$ is the gyromagnetic ratio for that nucleus and $\hbar$ the Plank constant divided by $2\pi$. When placed in a static magnetic field $B_0$, the nuclei adopt one of the $2I+1$ possible orientations corresponding to the allowed energy levels determined by the $z$-component of the spin $I_z = m\hbar$, where $m = \pm 1/2$ for protons. By convention in NMR experiments the static magnetic field is along the $z$-axis. Due to quantum mechanical selection rules, transitions between the allowed energy states will occur only when electromagnetic radiation is applied with the right frequency to obtain a change in $m$ of $\pm 1$. This frequency is called the resonant or Larmor frequency and is given by

$$\omega_L = -\gamma B_0.$$  \hspace{1cm} (Eq. 1.11)

The static magnetic field causes a small excess in nuclei in the lower energy level state given by the Boltzmann distribution, leading to a net magnetisation $\vec{M}$ which is used in the NMR experiment. At equilibrium $\vec{M}$ does not have a component perpendicular to $B_0$. However, if perturbed from its equilibrium state it will precess at angular frequency $\omega_L$ about $z$-axis according to

$$\frac{d\vec{M}}{dt} = -\omega_L \times \vec{M} = \gamma \vec{M} \times \vec{B}_0.$$  \hspace{1cm} (Eq. 1.12)

Perturbation of the system is achieved by applying a radiofrequency (RF) electromagnetic pulse $B_1$ for a time $t$. This is an oscillating magnetic field of frequency $\omega_L$ applied perpendicular to $B_0$ which causes excitation of the nuclei. The precessing magnetisation generates its own rotating magnetic field that induces a current of frequency $\omega_L$ in the receiver coil, permitting detection of the signal.

In the rotating frame of reference, which rotates about the axis parallel to $B_0$ at a frequency $\omega_L$, Eq. 1.12 becomes

$$\frac{d\vec{M}}{dt} = \gamma \vec{M} \times \left( \vec{B}_0 + \frac{\omega_L}{\gamma} \right).$$  \hspace{1cm} (Eq. 1.13)
Therefore the effect of \( B_0 \) cancels out, being replaced by the effective magnetic field applied to the sample, in this case \( \vec{B}_1 \). In this frame of reference, \( \vec{M} \) does not precess and after excitation passes from initial state \( \vec{M}_0 \), parallel to \( \vec{B}_0 \), to a state tilted at an angle \( \alpha \) (flip angle) from the \( z \)-axis (Figure 1.1). The flip angle is determined by the magnitude of \( B_1 \) and the duration of the RF pulse \( t \).

![Figure 1.1 Trajectory of the magnetisation after an RF pulse providing a flip angle \( \alpha \) in the rotating frame of reference](image)

After switching off \( \vec{B}_1 \), the magnetisation \( \vec{M} \) relaxes to the initial state parallel to \( \vec{B}_0 \) by means of two mechanisms: spin-lattice and spin-spin relaxation. Spin-lattice relaxation returns the magnetic moments back to their equilibrium state by transference of the excitation energy to the magnetic and nuclear environment with which the nuclei interact. \( \vec{M} \) gradually realigns with \( \vec{B}_0 \), in an exponential decay with time constant \( T_1 \). Spin-spin relaxation is caused by interactions between the individual nuclear magnetic moments. The interactions between neighbouring nuclear magnetic moments causes the precession rates of individual nuclei to vary slightly, with the result of a loss of phase coherence and reduction of the components of \( \vec{M} \) perpendicular to \( \vec{B}_0 \) (\( M_x, M_y \)) with time constant \( T_2 \).

The excitation magnetic field \( \vec{B}_1 \) and the two relaxation mechanisms can be incorporated into Eq. 1.13 resulting in the Bloch equations for nuclear induction that describe the evolution of the magnetisation in the rotating frame of reference (5)

\[
\begin{align*}
\frac{dM_x}{dt} &= \gamma (M_x B_z - M_z B_x) \frac{M_y}{T_2} \\
\frac{dM_y}{dt} &= \gamma (M_y B_z - M_z B_x) \frac{M_y}{T_2} \\
\frac{dM_z}{dt} &= \gamma (M_z B_z - M_z B_z) \frac{(M_z - M_0)}{T_1}
\end{align*}
\]

**Eq. 1.14**
These equations describe the magnetisation of the system considering relaxation effects. Unfortunately, it is not always possible to solve them analytically. However, examples of solutions to these equations will be shown in following sections of this chapter.

1.4 Measurement of relaxation times

The water molecules in tissues are not entirely free but are affected by other tissue constituents, which can constrain their motion and change $T_1$ and $T_2$ relaxation times. The amplitude of the NMR signal of water protons in tissues is therefore determined by the biophysical environment of water. The different relaxation properties of different tissues is the basis for the creation of contrast in MR images, where the brightness is given by NMR signal amplitude, modulated by proton density or characteristic $T_1$, or $T_2$ of the tissues. Table 1.1 shows typical values of $T_1$ and $T_2$ relaxation times in the human brain at $B_0 = 1.5$ Tesla.

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<th>$T_1$ (ms)</th>
<th>$T_2$ (ms)</th>
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<td>White matter</td>
<td>720</td>
<td>90</td>
</tr>
<tr>
<td>Grey matter</td>
<td>1130</td>
<td>100</td>
</tr>
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$T_1$ and $T_2$ relaxation times can be measured by employing sequences of RF pulses. The pulse sequence commonly used to measure $T_1$ is called Inversion Recovery. This consists of an RF pulse providing a flip angle of $180^\circ$ (magnetisation inversion) followed by a $90^\circ$ pulse at a specified time later (TI) (Figure 1.2). After the first pulse the magnetisation vector lies antiparallel with the $z$ axis and relaxes due to spin-lattice interaction processes during TI back to its equilibrium position, parallel to the $z$-axis. The $90^\circ$ pulse converts the remaining magnetisation along $z$ into transverse ($M_x$, $M_y$) magnetisation, generating a signal collected by the RF coil known as free induction decay (FID).
Figure 1.2 Basic Inversion Recovery pulse sequence. The time TI is the time between the end of the 180° pulse and the middle of the 90° pulse.

Since the RF pulse duration $t$ is much smaller than the relaxation time constants, $T_1$ and $T_2$, we can assume that no relaxation takes place during the application of $\vec{B}_1$, allowing the Bloch equations to be simplified. Between the RF pulses, Eq. 1.14 can be written as

$$
\begin{align*}
\frac{dM_x}{dt} &= -\frac{M_x}{T_2} \\
\frac{dM_y}{dt} &= -\frac{M_y}{T_2} \\
\frac{dM_z}{dt} &= -\frac{(M_z - M_0)}{T_1}
\end{align*}
$$

Eq. 1.15

The solution after the 90° pulse gives the magnitude of the signal measured. This is proportional to the component of the magnetisation parallel to the static magnetic field ($M_z$) at time $TI$.

$$M(TI) = M_0\left(1 - 2e^{-\frac{TI}{T_1}}\right).$$

Eq. 1.16

By repeating the experiment for different values of $TI$, the relaxation time can be calculated by fitting a curve to a plot of $M$ vs $TI$.

It can be seen from Eq. 1.16 that for a particular value of $TI = TI_{null}$ the magnetisation will be $M_z(TI_{null}) = 0$ when the 90° pulse is applied. The $TI_{null}$ is dependent on the $T_1$ relaxation time and therefore this technique can be used to suppress unwanted signals such as fat or CSF.

The spin-spin relaxation time $T_2$ is usually measured using the Spin-Echo pulse sequence developed by Hahn (7). This consists of a 90° pulse followed by an 180° pulse applied after a time $\tau$ (Figure 1.3).
The first pulse flips the magnetisation into the transverse plane where it decays at a rate determined by the combination of spin-spin interactions and inhomogeneities in the main magnetic field ($T_2^*$ relaxation time). The 180°, or refocusing, pulse reverses the dephasing effects of magnetic field inhomogeneity leaving only the signal loss caused by $T_2$ relaxation. As all the individual magnetisation vectors associated to each isochromat\(^1\) refocus, they build up a signal or spin echo in the transverse plane, which is collected by the RF coil. The peak of the spin echo occurs at a time $t = 2\tau$, usually known as echo time (TE), after which the magnetisation vectors dephase again. The maximum amplitude of the spin echo will be given by

$$M(\text{TE}) = M_0 e^{-\text{TE}/T_2}. \quad \text{Eq. 1.17}$$

The $T_2$ of an unknown sample can therefore be calculated by repeating the experiment for different values of TE and fitting the curve of $M$ vs TE. A time of at least 5 $T_1$ should be left between consecutive experiments to allow complete relaxation of the magnetisation and avoid $T_1$ relaxation effects in the signal.

As mentioned above, the 180° pulse is added to the Spin-Echo pulse sequence to refocus the signal decay caused by magnetic field inhomogeneities. This refocusing is complete if each individual spin remains in the same position within the magnetic field between the 90° and 180° pulses. However, spins experience random displacements caused by molecular diffusion that prevent complete refocusing and decrease the echo amplitude. This effect led to preliminary observations of magnetic resonance sensitivity to diffusion \(\text{(7,8)}\), as will be discussed in §1.5.1.1.

### 1.4.1 Magnetic field gradients

Spatial discrimination in MR images is achieved by applying magnetic field gradients in different directions, to encode the spatial information in the signal. Fourier transform methods are then used to post-process the acquired data. The

---

\(^1\) An ensemble of spins with volume much smaller than $B_0$ microscopic inhomogeneities which can be assigned a classical physical magnetisation vector.
application of a gradient $G$ in any direction causes the magnetic field strength to vary with position in that direction. Since the NMR resonant frequency is proportional to the strength of the magnetic field, the application of a gradient along $x$ ($G_x$) will make the resonance frequency vary in space according to

$$\omega(x) = \gamma (B_0 + xG_x).$$ \hspace{1cm} \text{Eq. 1.18}$$

Hence, the frequency of the NMR signal emitted will encode the position information in that direction. Similarly applying a gradient in another direction, for example along the $y$-axis, the position information along $y$ can be encoded in the phase of the signal. The gradient changes the frequency of precession of each position of the sample as before. When it is switched off after a time $\tau$, all spins precess again at the same frequency. However, each one will now have a relative phase dependent on the position given by

$$\phi(y) = \gamma y \int_0^\tau G_y(t) dt.$$ \hspace{1cm} \text{Eq. 1.19}$$

Position in the third orthogonal direction can be encoded by limiting the volume of spins to be excited to a chosen slice within the sample or patient. This is done by selective excitation, consisting of the application of a shaped RF pulse simultaneously with a gradient perpendicular to the slice. The resonant frequency of each spin will depend on $z$ due to the presence of a gradient $G_z$, as in Eq. 1.18. The pulse is amplitude modulated so that it contains a narrow spread of frequencies close to $\omega_L$, and therefore only the spins whose frequency lies within this range will be excited. The width of the slice is given by

$$\Delta z = \frac{\Delta \omega}{\gamma G_z},$$ \hspace{1cm} \text{Eq. 1.20}$$

where $\Delta \omega$ is the bandwidth of frequencies in the selective RF pulse. The slice can be made thinner by decreasing $\Delta \omega$ (increasing pulse duration), or by increasing the gradient amplitude.

### 1.4.2 Spin Warp imaging

The basic technique used to obtain spatial localization of NMR signals in three dimensions combines the three techniques for spatial discrimination explained in §1.4.1 in what is termed the Spin Warp imaging pulse sequence (9) (Figure 1.4)
The frequency encoding, phase encoding and slice-selection gradients are not necessarily fixed to x, y and z directions. However, here they will be denoted $G_x$, $G_y$ and $G_z$ for simplicity.

- **Slice selection** is achieved by applying a shaped RF pulse in combination with the magnetic field gradient $G_z$ followed by a negative gradient pulse to bring the spins back into phase across the slice.
- **Frequency encoding** is achieved by applying a readout gradient $G_x$. To avoid missing the start of the FID when measuring the signal immediately after excitation, either a $180^\circ$ RF pulse, to generate a spin echo, or a reversal gradient, as shown above, are applied before the readout gradient. The negative gradient dephases the spins and the positive brings them back into phase generating a gradient echo (GRE). The spin echo or gradient echo are sampled $n$ times during readout, with $n$ being the number of voxels of the image in the $x$ direction.
- **Phase encoding** is achieved by performing $m$ separate excitations with a different size phase encoding gradient $G_y$ applied each time. The number of image voxels in the $y$ direction is then $m$. As opposed to frequency encoding, where signal is sampled $n$ times in the presence of the gradient, the signal is sampled only once for each of the $m$ phase encoding excitations.

The MRI signal $S(t)$ for a given slice is collected using a quadrature detector with two orthogonal channels. The raw digitised signals are stored as a complex data array with the real and imaginary parts corresponding to each detector channel. Each of the $m$ rows of the array corresponds to the $n$ signal samples collected during the readout of a phase encoding step. These raw data are processed into images by applying the Fourier transform in two dimensions.
1.4.3 Echo planar imaging

Conventional MRI sequences using Spin Warp methods collect a single line of data in each ‘shot’ or application of RF excitation, needing \( m \) excitations with changing phase encoding gradients to collect a whole image. Echo planar imaging (EPI) techniques can encode all the information to reconstruct an image in one single shot (10). Figure 1.5 illustrates a GRE-EPI sequence. This is an extension of the conventional GRE imaging sequence illustrated in Figure 1.4, where an oscillatory gradient is applied in the frequency encoding direction so that a train of echoes is generated. In Figure 1.5, phase encoding is achieved by a ‘blip’ of short duration applied at the end of each frequency-encoding gradient pulse. It can also be achieved by applying a constant phase-encoding gradient over the entire duration of the EP readout. The RF pulse sequence preceding the EPI spatial encoding will determine the image contrast. For example, a 90° pulse followed by a 180° pulse with subsequent EPI data collection results in an image with mainly \( T_2 \) contrast and minimized \( T_{2*} \) contrast.

![Figure 1.5 Gradient echo EPI pulse sequence.](image)

EPI acquisition is faster than conventional MRI, thus minimising image artifacts caused by subject movement during the scan. However, the long readout period and the rapid gradient switching of EPI make it more sensitive to other image artifacts than conventional MRI (11).

1.4.4 Fourier transform and k-space

The idea of Fourier transform methods for image reconstruction is based on the fact that the simple one-dimensional projection of the sample (obtained by frequency encoding) is the Fourier transform of the signal as it evolves with time in the presence of a magnetic field gradient. The further encoding in the orthogonal
direction obtained by the phase encoding gradient introduces a second dimension of time or ‘pseudo-time’ as the signal evolves in each phase encoding step.

The FID after a $90^\circ$ pulse in the presence of a constant gradient $G_x$, neglecting the effects of T2 relaxation, can be expressed as (12)

$$S(t_s) = \int \int \rho(x, y) \exp[i \gamma G_x x t_s] dx dy,$$  \hspace{1cm} \text{Eq. 1.21}

where $\rho(x, y)$ is the spin density function.

Introducing the second time dimension $t_y$ during which another perpendicular gradient $G_y$ is applied, the corresponding signal will be

$$S(t_s, t_y) = \int \int \rho(x, y) \exp[i \gamma (G_x x t_s + G_y y t_y)] dx dy,$$  \hspace{1cm} \text{Eq. 1.22}

and therefore the values of $S(t_s, t_y)$ are the two-dimensional Fourier coefficients of the spin density, where $(\omega_x = \gamma G_x x, \ t_s)$ and $(\omega_y = \gamma G_y y, \ t_y)$ are the conjugate variables. The inverse Fourier transform of $S(t_s, t_y)$ returns the spin density, producing the image. The effects of relaxation can be easily incorporated in the analysis. T2-weighting, for example, is added by including the term $\exp\{-t/T_2\}$ (§1.4)

$$S(t_s, t_y) = \int \int \rho(x, y) \exp[i \gamma (G_x x t_s + G_y y t_y) - (t_s + t_y)/T_2] dx dy.$$  \hspace{1cm} \text{Eq. 1.23}

k-space is the Fourier transform domain of ordinary space, being related in the same way as $\omega$ and t. $\vec{k}$ is given by the change of phase of the spins with position when a set of gradients $\vec{G} = (G_x, G_y)$ is applied between $t = 0$ and $t = \tau$

$$\vec{k} = \gamma \int_0^\tau \vec{G} dt.$$  \hspace{1cm} \text{Eq. 1.24}

This formula represents the point in k-space reached by the spin system at the time $\tau$, assuming that all the spins in the system are in phase at $t = 0$. The path in k-space can then be traced by applying this formula to the magnetic field gradients in a MRI pulse sequence. Since the spin density function of a single slice of a sample can be represented as a k-space distribution by the Fourier transform, the image is produced by the combination of gradients in the acquisition sequence yielding a path that samples k-space.

As examples, the path for conventional Spin Warp and EPI sequences are shown in Figure 1.6.
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1.5  Diffusion MRI

1.5.1  Diffusion measurements using NMR

1.5.1.1  Preliminary observations

Hahn first observed the self-diffusion effect of molecules within tissues during his MR free precession experiments (7). He observed that for some liquids the maximum of secondary echo signals was attenuated in a time much shorter than $T_1$. This could be explained by considering the diffusion of the molecules through the inhomogeneous static magnetic field, since this effect was not observed in substances with higher viscosity and smaller self-diffusion coefficients, such as glycerine. Hahn confirmed qualitatively the effects of diffusion by applying previously published values of the self-diffusivity constant of distilled water to explain the envelope of decay of the echo that he measured in his experiments.

Carr and Purcell extended Hahn’s analysis of diffusion effects in the description of their technique to measure the $T_2$ relaxation time (Carl-Purcell sequence) (8). They simplified the formulation of the problem by considering diffusion as a random walk of discrete steps and taking the limit of infinitesimal steps, obtaining the same distribution of the spin phases after a $90^\circ$ pulse as Hahn. They extended this formulation to the case in which a sequence of $n$ $180^\circ$ pulses are applied after the initial $90^\circ$ pulse and found that the effect of interpolating a large number of $180^\circ$ pulses in a time $t$ was to reduce the spins’ phase dispersion $\phi_D$ by a factor determined by $\frac{1}{n^2}$

$$S(t) = S_0 \exp(-t/T_2) \exp(-\frac{\gamma^2 G^2 D t^3}{12n^2}). \tag{1.25}$$

As an application of this formulation, Carl and Purcell provided the first measurement of self-diffusivity of water from the slope of a plot of...
\[ \ln(S/S_0) + t/T_2 \text{ vs } t^3, \] obtaining a diffusion constant of \( 2.5 \pm 0.3 \times 10^{-6} \text{ mm}^2/\text{s} \) at 25°C which was in good agreement with published values.

1.5.1.2 Bloch equations with diffusion terms

Torrey (13) introduced a new term into the Bloch equations (Eq. 1.14) to incorporate the effect of the motion of the particles caused by diffusion on the evolution of the magnetisation of a system of spins, giving

\[
\begin{align*}
\frac{\partial M_x}{\partial t} &= \gamma (M_y B_z - M_z B_y) - \frac{M_x}{T_2} + \hat{\nabla} \cdot \hat{\nabla} (M_x - M_{x0}) \\
\frac{\partial M_y}{\partial t} &= \gamma (M_z B_x - M_x B_z) - \frac{M_y}{T_2} + \hat{\nabla} \cdot \hat{\nabla} (M_y - M_{y0}) \\
\frac{\partial M_z}{\partial t} &= \gamma (M_y B_x - M_x B_y) - \frac{M_z - M_{z0}}{T_1} + \hat{\nabla} \cdot \hat{\nabla} (M_z - M_{z0})
\end{align*}
\]

where \( D \) depends on the direction to describe anisotropic diffusion, and \( M_{x0} = (n\mu^2/kT)B_z \) (and similarly for \( M_{y0} \) and \( M_{z0} \)) are the components of the equilibrium magnetisation derived from the drift velocity of the \( n \) spins in the system. The drift terms are generally quite small and their effects almost negligible (13).

Wayne et al. (14) and Neuman (15) derived analytical solutions of the Bloch-Torrey equations for diffusion in restricted geometries, and Stejskal and Tanner (16) solved them for the case of free, anisotropic diffusion in the principal frame of reference under the application of a constant magnetic field gradient \( \vec{G} \).

The method for measuring self-diffusion based on Hahn spin-echo experiments as developed by Carr and Purcell required the presence of a magnetic field gradient at all times. As the amplitude of this was increased in order to observe smaller diffusivities the NMR linewidth broadened, decreasing the duration of the echo and creating experimental limitations for its detection (16). Some observations on restricted diffusion had also suggested that the time during which diffusion occurs previous to collection of the echo should be clearly defined so it is known whether the diffusion measurement is reflecting the geometrical arrangement of barriers, or just the viscosity of the medium (17). Stejskal and Tanner (16) proposed a technique...
to measure diffusion where the gradients applied during the RF pulses are minimised by using short pulses of gradients where the time of diffusion is well defined.

The resulting magnetisation evolution in the transverse plane in the presence of a magnetic field gradient $\tilde{G}$ could be formulated as (18)

$$\frac{dM_x}{dt} = \gamma M_x \hat{r} \cdot \tilde{G} - \frac{M_x}{T_2} + D \nabla^2 M_x,$$

$$\frac{dM_y}{dt} = \gamma M_y \hat{r} \cdot \tilde{G} - \frac{M_y}{T_2} + D \nabla^2 M_y,$$

with $\tilde{G} = \tilde{G}(t)$ and assuming uniform throughout the sample. Using

$$M_x + iM_y = \psi \exp[-(i\omega_0 + 1/T_2)t],$$

which represents the behaviour of the components of $\tilde{M}$ in the x-y plane perpendicular to the applied magnetic field $\tilde{B}_0$, Eq. 1.27 can be rewritten as

$$\frac{\partial \psi}{\partial t} = -i\gamma(\hat{r} \cdot \tilde{G})\psi + D \nabla^2 \psi.$$

This equation, without the diffusion term in the case of a spin echo sequence, with a $90^\circ$ pulse at $t = 0$ followed by a $180^\circ$ pulse at $t = \tau$, can be solved in two parts. Between the two pulses ($0 < t < \tau$), the function $\psi$ is given by

$$\psi = A \exp(-i\tilde{F} \cdot \hat{F}),$$

where

$$\tilde{F}(t) = \int_0^t \tilde{G}(t')dt',$$

is the phase, and the boundary condition that $\psi = A$ after the first pulse is met. The $180^\circ$ pulse then will set back the phase of $\psi$ twice the amount it advanced after the $90^\circ$ pulse. Hence at $t > \tau$, after the second pulse, we have

$$\psi = A \exp(-i\tilde{F} \cdot (\tilde{F} - 2\tilde{f}) + i\phi),$$

where

$$\tilde{f} = \tilde{F}(\tau).$$

The phase angle $\phi$ depends on the phase of the $180^\circ$ pulse relative to the phase of the $90^\circ$ pulse, and can be set to zero without lost of generality (16). The echo would be expected at a time $t = \tau'$ such that $\tilde{F}(\tau') = 2\tilde{f}$, when the exponent cancels and $\psi = A$.
for all values of $\tilde{r}$. However, depending on $\tilde{G}$ this condition might never occur and the maximum reached by $\psi$ would be less than $A$. Considering the case in which the function reaches the maximum $\psi = A$, we can express the behaviour of $\psi$ from the $90^\circ$ pulse to the echo as

$$\psi = A \exp\left(-i\tilde{F} \cdot (\tilde{F} - (\tilde{x} - 1) \tilde{f})\right)$$

with

$$\tilde{x} = +1 \text{ for } 0 < t < \tau,$$

$$\tilde{x} = -1 \text{ for } t > \tau.$$  \hspace{1cm} \text{Eq. 1.34}

Now, taking into account the diffusion term, we can assume a solution to Eq. 1.29 identical to Eq. 1.34 in which $A$ is a function of time, $A(t)$. Substituting into Eq. 1.29 we obtain an equation for the amplitude of the signal

$$\frac{dA}{dt} = -\gamma^2 D \left[\tilde{F} + (\tilde{x} - 1) \tilde{f}\right]^2 A.$$  \hspace{1cm} \text{Eq. 1.35}

The integration of this equation between $t = 0$ and $t = \tau$ gives

$$\ln \left(\frac{A(\tau)}{A(0)}\right) = -\gamma^2 D \left[\int_0^\tau \tilde{F}^2 dt - 4 \tilde{f} \cdot \tilde{F} dt + 4 \tilde{f}^2 (\tau - \tau)\right].$$  \hspace{1cm} \text{Eq. 1.36}

Since $\psi = A(0)$ immediately after the $90^\circ$ pulse and $\psi = A(\tau)$ at the echo maximum, the ratio of the two represents the effect of diffusion on the amplitude of the echo. The quantity in the brackets is only dependent on the diffusion time $\tau$ and the magnetic gradient $\tilde{G}$. Thus, it is possible to measure $D$ for a substance if the rest of the parameters are known. However, when the diffusion is measured in an anisotropic system in the presence of barriers, the degree of mobility of a substance appears to depend on the length of the observation time. Even if a fluid is immersed in a restrictive environment, at very short times diffusing molecules might not reach the barriers and the diffusion observed is only characteristic of the viscosity of the medium. Conversely, at longer measurement times, the diffusion observed reflects the geometric arrangement of the barriers. To take this effect into account, Tanner (19) defined an effective diffusion coefficient $D_{\text{eff}}$, which is averaged over the echo time. Thus, Eq. 1.36 can be written

$$\ln \left(\frac{A(\tau)}{A(0)}\right) = -bD_{\text{eff}}.$$  \hspace{1cm} \text{Eq. 1.37}

This is known as the Stejskal-Tanner equation where
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\[ b = \gamma^2 \left[ \int_0^\tau F^2 dt - 4 \bar{f} \cdot \int_\tau^{\tau_1} \bar{F} dt + 4 f^2 (\tau' - \tau) \right], \]  \hspace{1cm} \text{Eq. 1.38}

is the diffusion attenuation factor that depends only on the set of gradient pulses (20).

1.5.1.3 Pulsed gradients

We can illustrate the use of Eq. 1.37 in the case of two gradient pulses of constant amplitude (Figure 1.7) and calculate its effect on the amplitude of the signal. The nuclei are subjected to a steady gradient \( \vec{G}_0 \), caused in part by the inhomogeneities in the static magnetic field \( \vec{B}_0 \), and a sequence of two pulsed gradients of amplitude \( G \) and short duration \( \delta \) placed around the 180\(^\circ\) pulse, the first one at a time \( t_1 \) and the second at a time \( t_1 + \Delta \). With this gradient configuration, the echo will occur at a time \( t = 2\tau \).

![Figure 1.7 Pulsed gradient spin echo NMR sequence for measurement of the diffusion coefficient.](image)

During the first pulsed gradient, the magnetic field varies slowly along one direction introducing a phase that ‘labels’ the water protons according to their spatial location. The 180\(^\circ\) RF pulse inverts the spins and therefore the second gradient pulse, of equal amplitude and duration to the first, will introduce a phase equal but in the opposite direction that should reverse the effects of the first gradient. However, these phase changes do not cancel out completely if diffusion causes the water molecules to change their location. From Eq. 1.37 and Eq. 1.38, the reduction in the echo amplitude due to diffusion will be

\[ \ln \left( \frac{A(2\tau)}{A(0)} \right) = -\gamma^2 D_{\text{eff}} \begin{bmatrix} 2\tau^3 G_0^2 + \delta^2 \left( \Delta - \frac{1}{3} \delta \right) G^2 \\ -\delta \left( t_1^2 + t_2^2 \right) + \delta \left( t_1 + t_2 \right) + \frac{2}{3} \delta^2 - 2\tau^2 \end{bmatrix} \cdot \vec{G} \cdot \vec{G}_0, \]  \hspace{1cm} \text{Eq. 1.39}
where \( t_2 = 2\tau - (t_1 + \Delta + \delta) \) is the time between the second gradient pulse and the echo peak. In the case \( G = 0 \), i.e. without the gradient pulses, the result is the same as that obtained for the spin-echo experiment (21). If \( G_0 \) is sufficiently small, the term in \( G \) is predominant and the echo attenuation will be given by

\[
\ln \left( \frac{A(2\tau)}{A(0)} \right) = -\gamma^2 D_{\text{eff}} G^2 \delta^2 \left( \Delta - \frac{1}{3} \delta \right),
\]

which is known as the Stejskal-Tanner equation.

Early measurements of water diffusion in biological tissues using NMR were made using this technique (22,23). However, in the derivation of the Stejskal-Tanner equation it was assumed that an anisotropic sample could be physically aligned so its principal axes coincide with the laboratory coordinate system - an assumption very unlikely to be met in reality. A more general scheme was needed to make possible the measurement of the entire diffusion tensor in the laboratory frame of reference.

1.5.2 Diffusion tensor imaging (DT-MRI)

1.5.2.1 Development of diffusion imaging

The basic principles of diffusion MRI were developed in the mid-1980’s combining the principles of MRI with the principles of diffusion sensitisation introduced by Stejskal and Tanner to create diffusion-weighted (DW) images. Merboldt et al. performed NMR imaging of molecular diffusion for the first time by introducing a pair of pulsed gradients into the first and third intervals in the stimulated-echo acquisition-mode (STEAM) sequence (24). They obtained DW images in aqueous solutions and reconstructed the first images where the voxel intensity represented the effective self-diffusion coefficient. Taylor and Bushell also introduced a pair of pulsed gradients into a conventional spin-echo imaging sequence to image biological samples, and acquired DW images of an egg (25). The self-diffusion coefficient values measured with these imaging methods were in good agreement with the values obtained previously with other techniques.

Using similar principles, Le Bihan et al. measured the diffusion coefficient of biological tissues in vivo for the first time (20). They introduced the \( b \)-value, describing the product of the gradient and timing constants (Eq. 1.38), and the concept of the apparent diffusion coefficient (ADC) to take into account other
intravoxel incoherent motions as well as diffusion. Their human studies showed that images with ADC contrast highlighted pathologies such as metastatic carcinoma, peritumoural oedema and some brain tumours, thereby showing the diagnostic potential of this new MRI technique.

As expected from the results obtained in previous diffusion measurements, some of the first diffusion MRI studies revealed that in anisotropic biological tissues the diffusion measurements were dependent on the direction of the gradients applied (26,27). An example of this effect is shown in Figure 1.8, where the diffusion data were acquired in three orthogonal directions. It is clear how signal intensity and contrast between tissues is different for the three images, especially in white matter where the amount of diffusion is greater along the fibres than across them (see arrows). These images also show an area of hyperintensity next to the right sylvian fissure corresponding to an infarct. This and more clinical applications of diffusion imaging will be described later in §0.

![Figure 1.8 DW images acquired with the diffusion gradients in directions (a) xz, (b) yz and (c) xy.](image)

The effect of directionality of diffusion seen in these images is caused by the underlying cellular structure of tissue that influences the overall mobility of diffusion molecules by providing barriers and creating individual compartments (28). Figure 1.9 sketches two types of samples, one grey matter, where diffusion is isotropic with little preference for diffusion in any particular direction, and the other white matter or skeletal muscle fibres, where the geometry of the barriers favours larger displacements parallel to fibre bundles. In a variety of fibre types and species it has been observed that diffusion parallel to the fibres was 2-4 greater than that measured perpendicular to them using typical diffusion times of 20-40 ms (28).
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Figure 1.9 Illustration of diffusion in two types of samples, (a) with similar displacements in all directions (isotropic diffusion) (b) with preferential diffusion in one direction (anisotropic diffusion)

These observations lead to the development of a new MRI technique capable of characterising the directionality of water diffusion in tissues, and providing information on the structure and geometrical organisation of tissues.

1.5.2.2 Principles of DT-MRI

DT-MRI estimates the effective diffusion tensor in each voxel of the image, including the off-diagonal components that had not been considered before but which are necessary to describe completely the mean displacements of the water molecules in all tissue types (29). As shown in Eq. 1.7 for anisotropic media, Fick’s First Law becomes a system of equations where the diffusion is represented by a tensor (Eq. 1.9), which can be expressed as

$$\begin{bmatrix}
F_x \\
F_y \\
F_z
\end{bmatrix} = -\begin{bmatrix}
D_{11}^{\text{eff}} & D_{12}^{\text{eff}} & D_{13}^{\text{eff}} \\
D_{21}^{\text{eff}} & D_{22}^{\text{eff}} & D_{23}^{\text{eff}} \\
D_{31}^{\text{eff}} & D_{32}^{\text{eff}} & D_{33}^{\text{eff}}
\end{bmatrix} \begin{bmatrix}
\frac{\partial C}{\partial x} \\
\frac{\partial C}{\partial y} \\
\frac{\partial C}{\partial z}
\end{bmatrix}^T.$$

Eq. 1.41

One consequence of this representation is that the concentration gradient $\nabla C$ is not necessarily parallel to the flux $\vec{F}$ and therefore the diffusion depends on direction. Stejskal made a modification to the Bloch-Torrey (Eq. 1.26) equations to describe free diffusion in anisotropic media including new terms to take into account the directionality of the diffusion (30)

$$\frac{\partial \vec{M}}{\partial t} = \gamma \vec{M} \times \vec{B} - \frac{(M_x \vec{i} + M_y \vec{j})}{T_2} + \frac{(M_0 - M_z)\vec{k}}{T_1} - \vec{v} \cdot \vec{M} + \vec{v} \cdot \vec{D} \cdot \nabla \vec{M}.$$

Eq. 1.42

where $\vec{B} = \vec{B}_0 + (\vec{r} \cdot \vec{G})$ in the laboratory frame of reference, the term in $\vec{v}$ represents the velocity of the spins due to flow of the medium within which they are embedded and, for simplicity, the effective diffusion tensor is denoted as $\vec{D}$. Both $\vec{v}$ and $\vec{D}$ are
functions of time and the magnetic field gradient vector is \( \mathbf{G}(t) = [G_x(t), G_y(t), G_z(t)]^T \).

Integration of Eq. 1.42 between \( t = 0 \) (after the 90° pulse) and \( t = \tau' \) (the time of the echo) gives the effect of diffusion in the amplitude of the echo (30)

\[
\ln \left( \frac{A(\tau')}{A(0)} \right) = -\gamma^2 \int_0^{\tau'} \tilde{F} \cdot \mathbf{D} \cdot \tilde{F} \, dt - 4\zeta \int_0^{\tau'} \tilde{F} \cdot \mathbf{D} \cdot \tilde{f} \, dt + 4\zeta \int_0^{\tau'} \tilde{f} \cdot \mathbf{D} \cdot \tilde{f} \, dt,
\]

Eq. 1.43

with \( \tilde{F} \) and \( \tilde{f} \) defined in Eq. 1.31 and Eq. 1.33 and \( \zeta = 0 \) for \( 0 < t < \tau \) and \( \zeta = 1 \) for \( t > \tau \). This can be rewritten in matrix form as (29,31)

\[
\ln \left( \frac{A(\tau')}{A(0)} \right) = -\gamma^2 \int_0^{\tau'} \left[ \tilde{F}(t) - 2\tilde{f} \right] \mathbf{D} \left[ \tilde{F}(t) - 2\tilde{f} \right]^T \, dt.
\]

Eq. 1.44

By analogy with the definition of the scalar effective diffusion coefficient (§1.5.1.2) the effective diffusion tensor can be defined as the mean value of the term on the right in Eq. 1.44 over the interval \( t = [0, \tau'] \) (32).

We can establish a linear relationship between the logarithm of the echo attenuation and \( \mathbf{D} \) equivalent to Eq. 1.37

\[
\ln \left( \frac{A(\tau')}{A(0)} \right) = -\sum_{i=1}^{3} \sum_{j=1}^{3} b_{ij} D_{ij}^{eff} = -\mathbf{b} \cdot \mathbf{D},
\]

Eq. 1.45

where the \( \mathbf{b} \)-value now takes the form of a matrix:

\[
\mathbf{b} = \gamma^2 \int_0^{\tau'} \left[ \tilde{F}(t) - 2\tilde{f} \right] \left[ \tilde{F}(t) - 2\tilde{f} \right]^T \, dt.
\]

Eq. 1.46

The \( \mathbf{b} \)-matrix can be calculated either numerically or analytically from the scheme of gradient pulses used in the image sequence, and summarises the effect of all the gradients applied in all three directions \( x, y \) and \( z \). The \( \mathbf{b} \)-matrix should include all interactions between imaging and diffusion gradients applied in orthogonal directions and even between imaging gradients applied in orthogonal directions that can result in ‘cross-terms’ when measuring diffusion in anisotropic media (33,34).

1.5.2.3 Estimation of the diffusion tensor

The elements of \( \mathbf{D} \) are estimated from Eq. 1.45 and Eq. 1.46. This requires the collection of images with diffusion gradients applied in several different directions, along with an image acquired with no diffusion weighting (baseline image), and the calculation of the elements of the \( \mathbf{b} \)-matrix for that particular acquisition scheme.
As already mentioned, $\mathbf{D}$ is a symmetric positive definite tensor and therefore only the six independent elements need to be calculated. In the case that the diffusion images are collected with the diffusion gradients applied in non-collinear directions, Eq. 1.45 represents a system of seven linear equations with seven unknowns (the six independent elements of $\mathbf{D}$ and $A(0)$). The simplest method to measure the diffusion tensor uses seven MR images, six DW images and a baseline image. This is the only case where exact analytical expressions for the coefficients of the diffusion tensor can be calculated (35). As the orientation of barriers within the tissues is not known a priori, the diffusion gradients directions should be uniformly distributed so that the measurements are not biased along a particular direction.

An example of a six gradient direction scheme is the oblique double gradient (ODG) encoding scheme, where the gradients are oriented in the directions of the vertices of a 14-sided regular polyhedron. Gradients can be represented as gradient amplitude ($G_0$) times the direction vector,

$$
\mathbf{G}^0 = \{0,0,0\}^T, \quad \mathbf{G}^1 = G_0/\sqrt{2}\{1,0,1\}^T, \quad \mathbf{G}^2 = G_0/\sqrt{2}\{-1,0,1\}^T, \\
\mathbf{G}^3 = G_0/\sqrt{2}\{0,1,1\}^T, \quad \mathbf{G}^4 = G_0/\sqrt{2}\{0,1,-1\}^T, \\
\mathbf{G}^5 = G_0/\sqrt{2}\{1,1,0\}^T, \quad \mathbf{G}^6 = G_0/\sqrt{2}\{-1,1,0\}^T
$$

Eq. 1.47

If cross-terms are eliminated by refocusing the imaging gradients after they are applied, the $b$-matrix for DW image in the $i^{th}$ orientation simplifies as (35)

$$
b^i = \alpha^2 \mathbf{G}^i \mathbf{G}_i^{\text{T}} = \alpha^2 G_0^2 \bar{r}_i \bar{r}_i^{\text{T}},
$$

Eq. 1.48

where $\alpha$ is a constant function of the gyromagnetic ratio, gradient pulse shape and timing parameters of the pulse sequence (calculated from the integration of equation Eq. 1.46), and $\bar{r}_i$ is the direction vectors (32). We can simplify Eq. 1.45 for the $i^{th}$ image ($IM_i$)

$$
IM_i = \ln \left( \frac{A(\mathbf{G}^i)}{A(\mathbf{G}^0 = 0)} \right) = -\alpha^2 G_0^2 \sum_{s=1}^{3} \sum_{r=1}^{3} r_s^i r_r^i D_{sr}^{\text{eff}}.
$$

Eq. 1.49

$IM_i$ is therefore proportional to diffusion measured in the direction $\bar{r}_i$. By substituting the gradient directions from Eq. 1.47 in Eq. 1.49 and solving the system of linear equations, we obtain the analytical expressions for each of the six independent elements of $\mathbf{D}$ as function of $IM_i$ in Eq. 1.50.
This gradient-encoding scheme is useful for clinical applications where rapid scanning and processing are required since the number of acquired DW images is minimised. However, it is more susceptible to noise and systematic artifacts than other schemes where a larger number of diffusion gradient directions are acquired, and its does not provide estimates of the uncertainty of the measured parameters.

\[
D_{11}^{\text{eff}} = \frac{-IM_1 - IM_2 + IM_3 + IM_4 - IM_5 - IM_6}{2\alpha^2 G_0^2},
\]
\[
D_{22}^{\text{eff}} = \frac{IM_1 + IM_2 - IM_3 - IM_4 - IM_5 - IM_6}{2\alpha^3 G_0^2},
\]
\[
D_{33}^{\text{eff}} = \frac{-IM_1 - IM_2 - IM_3 - IM_4 + IM_5 + IM_6}{2\alpha^2 G_0^2},
\]
\[
D_{12}^{\text{eff}} = \frac{-IM_5 + IM_6}{2\alpha^2 G_0^2}; \quad D_{13}^{\text{eff}} = \frac{-IM_1 + IM_2}{2\alpha^2 G_0^2},
\]
\[
D_{23}^{\text{eff}} = \frac{-IM_1 + IM_4}{2\alpha^2 G_0^2}.
\]

Eq. 1.50

To estimate \( \mathbf{D} \) from a larger number of DW images statistical methods are used. All methods minimise the value of \( \chi^2 \) given by the sum of squares of the deviations between observed \( x_i \) and predicted intensities \( \xi_j \) in the equation

\[
\chi^2 = \sum_i w_i (x_i - \xi_i)^2,
\]

Eq. 1.51

where \( w_i \) is a weighting factor that introduces the signal variance into the equation so that signal variability produced by image noise is taken into account. The multivariate least squares linear regression model is the most commonly applied (32). The optimal \( \mathbf{D} \) is estimated by minimizing the sum of the squares of the differences between the logarithms of the intensities measured for each of the DW images (obtained in several non-collinear directions), and their theoretically predicted values given by Eq. 1.45. The weighting factor used is the squared signal measured in each trial divided by the signal variance

\[
w_i = \frac{(A(\hat{G}))^2}{\sigma^2}.
\]

Eq. 1.52

In practice, \( \sigma^2 \) corresponds to the root mean square of the background noise in the corresponding DW image.
In a DT-MRI experiment, images are collected in \( n \) non-collinear gradient directions, performing \( m \) measurements of \( A(G_i) \) per direction at different gradient strengths, as well as a measurement with no diffusion weighting \( A(0) \). For each voxel in the image, \( D \) can be calculated as follows (29).

The \( n \times m \) measurements of \( \ln\left[A_j(G_i)/A(0)\right] \) \((i = 1\ldots n, j = 1\ldots m)\) are stored in a \( nm \times 1 \) column vector \( x \), and the six parameters to be estimated are stored in another vector \( D_{est} \).

\[
\mathbf{D}_{est} = \left\{ D_{11}^{eff}, D_{22}^{eff}, D_{33}^{eff}, D_{12}^{eff}, D_{13}^{eff}, D_{23}^{eff} \right\}^T. \tag{Eq. 1.53}
\]

Each of the elements of the \( b \)-matrix are calculated either analytically or numerically from Eq. 1.46 for each gradient direction and strength, obtaining an \( nm \times 6 \) matrix \( \mathbf{B} \), with each row \((i \times j)\) is given by

\[
\mathbf{B}_{(i\times j)} = b_j \begin{bmatrix} 2g_{1i}g_{2j} & 2g_{1i}g_{3j} & 2g_{1i}g_{2i} & 2g_{1i}g_{3i} \end{bmatrix}, \tag{Eq. 1.54}
\]

where \( b_j \) determines the gradient strength and \( \{g_{1i}, g_{2j}, g_{3j}\} \) the gradient direction in each measurement.

Defining a vector of predicted outcomes \( \mathbf{\xi} \) as the product of \( \mathbf{B} \), and \( D_{est} \)

\[
\mathbf{\xi} = \mathbf{BD}_{est}, \tag{Eq. 1.55}
\]

the parameter to minimise is

\[
\chi^2(\mathbf{\xi}) = (\mathbf{x} - \mathbf{\xi})^T \mathbf{\Sigma}^{-1} (\mathbf{x} - \mathbf{\xi}), \tag{Eq. 1.56}
\]

where \( \mathbf{\Sigma} = \{ \sigma_{ij} \} \) is the signal covariance matrix whose diagonal elements provides the weighting factors \( 1/\sigma^2 \).

The minimisation of \( \chi^2 \) with respect each of unknowns in \( \mathbf{\xi} \) gives six linear equations that can be written in matrix form from as

\[
\left( \mathbf{B}^T \mathbf{\Sigma}^{-1} \mathbf{B} \right) \mathbf{D}_{est} = \left( \mathbf{B}^T \mathbf{\Sigma}^{-1} \mathbf{x} \right) \tag{Eq. 1.57}
\]

and therefore the optimal parameters \( \mathbf{D}_{est} \) can be calculated from

\[
\mathbf{D}_{est} = \left( \mathbf{B}^T \mathbf{\Sigma}^{-1} \mathbf{B} \right)^{-1} \left( \mathbf{B}^T \mathbf{\Sigma}^{-1} \mathbf{x} \right) = \mathbf{M}^{-1} \left( \mathbf{B}^T \mathbf{\Sigma}^{-1} \mathbf{x} \right) \tag{Eq. 1.58}
\]

\( \mathbf{M}^{-1} \) is a \( 6 \times 6 \) matrix and the diagonal elements of which provide the error associated to each of the six estimated parameters.

Other methods to estimate \( \mathbf{D} \) have been proposed, with some being more robust and less sensitive to the presence of outliers than linear least-squares. Mangin et al., for instance, used an approach that iteratively re-weights the least-squares fitting, using in each iteration new weights that are function of the residuals of the previous
iteration (36). A similar tactic was proposed by Chang et al. with RESTORE, another iterative robust estimation of $D$ based on non-linear least-squares fitting (37). They identify and reject the outliers from data corrupted by, for instance, cardiac pulsation or subject motion that could result in erroneous calculation of $D$.

Once $D$ is obtained for each voxel, it is possible to calculate parameters that can characterise microstructural features and the physiological state of the tissue (38,39).

### 1.6 Quantitative parameters obtained by DT-MRI

#### 1.6.1 Eigenvectors and eigenvalues of the water diffusion tensor

For every estimated $D$, we can construct a local coordinate system, $E = \{\vec{e}_1, \vec{e}_2, \vec{e}_3\}$, in which the fluxes and concentration gradients in different directions are decoupled (Eq. 1.41), with $D$ in its diagonal form denoted by $A$. Due to the symmetric and positive definite properties of $D$, the three eigenvectors defining this principal coordinate system are orthonormal, $\vec{e}_i \cdot \vec{e}_j = \delta_{ij}$.

Associated with each eigenvector of $D$ there is an eigenvalue. The three eigenvalues are known as principal diffusivities (31) such that

$$D\vec{e}_i = \lambda_i \vec{e}_i \text{ for } i = \{1, 2, 3\},$$

which can be rewritten in matrix form as

$$DE = EA,$$

or

$$\begin{pmatrix}
D_{11}^{\text{eff}} & D_{12}^{\text{eff}} & D_{13}^{\text{eff}} \\
D_{21}^{\text{eff}} & D_{22}^{\text{eff}} & D_{23}^{\text{eff}} \\
D_{31}^{\text{eff}} & D_{32}^{\text{eff}} & D_{33}^{\text{eff}}
\end{pmatrix}
\begin{pmatrix}
e_{1x} & e_{2x} & e_{3x} \\
e_{1y} & e_{2y} & e_{3y} \\
e_{1z} & e_{2z} & e_{3z}
\end{pmatrix}
= \begin{pmatrix}
e_{1x} & e_{2x} & e_{3x} \\
e_{1y} & e_{2y} & e_{3y} \\
e_{1z} & e_{2z} & e_{3z}
\end{pmatrix}
\begin{pmatrix}
\lambda_1 & 0 & 0 \\
0 & \lambda_2 & 0 \\
0 & 0 & \lambda_3
\end{pmatrix},$$

Eq. 1.60

where $\{x, y, z\}$ is the laboratory frame of reference. In ordered structures such as brain white matter or skeletal muscle, the principal coordinate system is determined by microstructural barriers, such as fibre membranes, that restrict the diffusion in any direction, constraining eigenvectors to the inherent directions of that tissue. In particular, the eigenvector associated with the largest eigenvalue is commonly related to the tissue fibre-tract axis (31) although this might not be true in areas of crossing fibres where they run in different directions.
1.6.2 Measures of water diffusivity and diffusion anisotropy

Analysis of $D$ provides quantitative measurements of the isotropic and anisotropic parts of diffusion intrinsic to the medium. These are found to be very useful diagnostic markers for clinical applications (§0) and will be widely used in this thesis. These quantities are independent of the laboratory frame of reference and are rotationally invariant.

As shown by Basser and Pierpaoli (39), $D$ can be decomposed into two summands, one attributed to isotropic diffusion and another one attributed to anisotropic diffusion,

$$
D = \langle D \rangle I + \left( D - \langle D \rangle I \right) = \langle D \rangle I + A,
$$

where $I$ is the identity tensor and $\langle D \rangle$ is the mean diffusivity defined as

$$
\langle D \rangle = \frac{Tr(D)}{3} = \frac{D_{11} + D_{22} + D_{33}}{3} = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}.
$$

Using the decomposition from Eq. 1.61, the magnitude of the isotropic part of $D$ will be proportional to the mean diffusivity

$$
\sqrt{\langle D \rangle I \cdot \langle D \rangle I} = \langle D \rangle \sqrt{I \cdot I} = \sqrt{3}\langle D \rangle,
$$

where $\mathbf{D} \cdot \mathbf{D} = \sum_{i=1}^{3} \sum_{j=1}^{3} D_{ij}^2 = \lambda_1^2 + \lambda_2^2 + \lambda_3^2$ is the definition of the dot product for tensors.

The quantity $\langle D \rangle$ characterises the overall mean squared displacement of molecules or bulk diffusivity. It is rotationally invariant since it is only a function of the trace of the tensor, which is also a rotationally invariant quantity. In anisotropic media, however, complete characterisation of $D$ requires all six components (40).

The magnitude of the anisotropic diffusion is given by

$$
\sqrt{A \cdot A} = \sqrt{\sum_{i=1}^{3} \sum_{j=1}^{3} \left( D_{ij} - \langle D \rangle I_{ij} \right)^2}.
$$

In the principal frame of reference, defined by the eigenvectors, the off-diagonal elements of $D$ vanish and the diagonal elements are replaced by the eigenvalues. Eq. 1.64 can therefore be rewritten as
This quantity gives the sum of the squares of the deviations between the principal diffusivities and \( \langle D \rangle \). Taking the magnitude of \( \mathbf{A} \) and dividing by the isotropic part of \( \mathbf{D} \), we obtain the relative anisotropy RA, a diffusion anisotropy index (DAI),

\[
RA = \frac{\sqrt{\mathbf{A} \cdot \mathbf{A}}}{\sqrt{\langle \mathbf{D} \rangle \cdot \langle \mathbf{D} \rangle}} = \frac{1}{\sqrt{3}} \sqrt{\frac{(\lambda_1 - \langle D \rangle)^2 + (\lambda_2 - \langle D \rangle)^2 + (\lambda_3 - \langle D \rangle)^2}{\langle D \rangle}}.
\]

Another DAI is the fractional anisotropy FA, which is obtained by dividing the anisotropic part by the total diffusion

\[
FA = \frac{\sqrt{\mathbf{A} \cdot \mathbf{A}}}{\sqrt{\mathbf{D} \cdot \mathbf{D}}} = \frac{3}{2} \sqrt{\frac{(\lambda_1 - \langle D \rangle)^2 + (\lambda_2 - \langle D \rangle)^2 + (\lambda_3 - \langle D \rangle)^2}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}.
\]

Both parameters are quantitative and dimensionless with ranges from \( RA = FA = 0 \) for an isotropic medium to \( RA = \sqrt{2} \) and \( FA = 1 \) for a cylindrically symmetric anisotropic medium (\( \lambda_1, \lambda_2 = \lambda_3 = 0 \)).

Although both FA and RA are rotationally invariant, they are still susceptible to noise contamination. At low and intermediate \( b \)-values, this results in an overestimation of diffusion anisotropy, especially for isotropic structures, when the signal-to-noise ratio (SNR) of the diffusion images is low. Using the orientational information contained in the eigenvectors of \( \mathbf{D} \) and performing a local average over a specified number of neighbouring voxels another DAI can be defined, the lattice anisotropy index. The basic element of the lattice index is given by (41)

\[
LI_N = \frac{3}{8} \left( \sqrt{\frac{\mathbf{A}_{ref} \cdot \mathbf{A}_N}{\mathbf{D}_{ref} \cdot \mathbf{D}_N}} + \sqrt{\frac{\mathbf{A}_{ref} \cdot \mathbf{A}_N}{\mathbf{D}_{ref} \cdot \mathbf{D}_N}} \right),
\]

where \( ref \) indicates a reference voxel and \( N \) an arbitrary neighbouring voxel. The dot product is maximal when the eigenvectors of the component tensors are collinear.

The total lattice index is calculated as the average of \( LI_N \) for all the voxels contiguous to the reference one, weighted by a factor defined by the distance between them. This
anisotropy measure is less biased towards high values in the presence of noise since it compensates for the apparent increase in anisotropy with the loss of coherence between the neighbour voxels as noise increases. The spatial averaging causes, however, a partial volume effect in the calculation of the anisotropy index. Time domain averaging from repeated measurements could avoid this effect (42).

1.7 Visualisation of DT-MRI data

1.7.1 Diffusion weighted images and parametric maps

The images collected using diffusion sensitised imaging sequences, before further processing is performed, are known as DW images. Their contrast is not due entirely to diffusion effects; in particular it will also depend on $T_2$. An example of a DW image of the human brain is shown in Figure 1.10 (a). This image was constructed from the average of the DW images acquired in six non-collinear directions to avoid the directionality effects that were demonstrated in Figure 1.8. As expected, in areas where the water motion is less restricted, such as the cerebrospinal fluid (CSF) in the ventricles and sulci, signal decay is greater due to increased diffusivity, and as a result these regions appear darker in the DW image. These images are greatly useful for some clinical applications as will be illustrated in §0.

Post-processing of the DT-MRI data from the DW images and baseline $T_2$-image, as explained above, allows $D$ to be calculated for each voxel in a subject’s brain. Quantities such as eigenvectors, eigenvalues, $\langle D \rangle$ or DAI allow the local structure of tissues to be characterized. This data can be displayed on a voxel-by-voxel basis to form parametric maps, the most common of which show $\langle D \rangle$ and FA (Figure 1.10).

![Figure 1.10](a) DW image; (b) $\langle D \rangle$ and (c) FA maps of a human brain.)
Maps of \( \langle D \rangle \) show high signal intensity in the ventricles and sulci and lower intensity in other brain tissues, according to the mobility of water protons in each environment. On the other hand, diffusion anisotropy maps show bright areas of white matter where diffusion is preferentially orientated parallel to the fibre bundles.

1.7.2 Diffusion ellipsoids

In each voxel, \( D \) can be represented by an ellipsoid whose semi-major axes are related to the eigenvalues and eigenvectors of the tensor. Equivalent to Eq. 1.1, the diffusion ellipsoid can be interpreted using the Gaussian conditional probability density function (PDF) (30),

\[
\rho(\vec{r} | \vec{r}_0, \tau) = \frac{1}{\sqrt{|D(\vec{r})|} (4\pi)^{3/2}} \exp \left[ -\frac{(\vec{r} - \vec{r}_0)^T D^{-1}(\tau)(\vec{r} - \vec{r}_0)}{4\tau} \right],
\]

Eq. 1.69

which gives the probability that a molecule at \( \vec{r}_0 \) at \( t = 0 \) reaches position \( \vec{r} \) at \( t = \tau \). It is a function of time since, as explained in §1.5.1.2, the effective value \( D \) can vary with the diffusion time. The diffusion ellipsoid can be calculated by setting the quadratic form of the exponent in Eq. 1.69 to \( \frac{1}{2} \) (31)

\[
\frac{(\vec{r} - \vec{r}_0)^T D^{-1}(\tau)(\vec{r} - \vec{r}_0)}{2\tau} = 1
\]

Eq. 1.70

The explicit equations of the ellipsoid are obtained by transforming the coordinates into the principal coordinate system within each voxel, by using the matrix of eigenvectors,

\[
\vec{r}' = E^T (\vec{r} - \vec{r}_0),
\]

Eq. 1.71

and, with Eq. 1.60, we obtain

\[
\frac{\vec{r}'^T \Lambda^{-1} \vec{r}'}{2\tau} = 1.
\]

Eq. 1.72

This can be expanded to give the equation of the effective diffusion ellipsoid in a particular voxel

\[
\left( \frac{x'}{\sqrt{2\lambda_1 \tau}} \right)^2 + \left( \frac{y'}{\sqrt{2\lambda_2 \tau}} \right)^2 + \left( \frac{z'}{\sqrt{2\lambda_3 \tau}} \right)^2 = 1.
\]

Eq. 1.73

The shape of this ellipsoid defines a surface of constant mean displacement of the water molecules at time \( t = \tau \), with the ellipsoid axes being the mean effective diffusion distances in the principal directions at time \( \tau, \sqrt{\langle x' \rangle} = \sqrt{2\lambda_1 \tau} \), etc.
Maps of diffusion ellipsoids can be created to represent diffusion in each voxel (41). Figure 1.11 shows an example of the diffusion ellipsoids of a region of interest in the corpus callosum, with the ellipsoid orientation illustrating the direction of water diffusion along the fibres in this area.

![Figure 1.11 Diffusion ellipsoid representation of D in each voxel.](image)

The eccentricity of each ellipsoid represents the anisotropy, with spherical surfaces indicating isotropic diffusion. Anisotropic tissues with one of the principal diffusivities significantly larger than the other two are represented by prolate ellipsoids with the largest axis oriented in the principal direction of diffusion. Oblate ellipsoids may appear in areas of crossing tracts, where fibres running in different directions are averaged in the same voxel. In these regions, the first and second eigenvalues are considerably larger than the third, and the ellipsoids only characterise the plane of diffusion. The derived principal eigenvector in this case might give an erroneous indication of tract direction since the eigenvectors of both tracts average out. Averaging of crossing fibres also causes error in the calculation of DAI, which appear lower in these voxels. This effect is noticeable in Figure 1.10 (c) in areas such as the anterior or posterior forceps of the corpus callosum. Therefore, in cases where fibres intersect, a second order tensor is not the correct model to represent diffusion processes (43). Some new techniques, such as high angular resolution diffusion MRI combined with multitensor analysis, have been suggested as a way to resolve multiple fibres in such voxels (43,44).

### 1.7.3 Eigenvector and colour maps

Other methods for displaying the diffusion information in two and three dimensions have been proposed. A two dimensional representation of \( \mathbf{D} \) makes the in-plane direction of diffusion easier to visualise. This is achieved by the use of headless
arrows that correspond to the in-plane components of the principal eigenvector. Figure 1.12 shows the direction of the fibres running along the corpus callosum.

Figure 1.12 Representation of the principal diffusion direction in each voxel using 2D arrows.

In this example, green arrows represent prolate diffusion ellipsoids and red arrows represent oblate ellipsoids that might occur in areas of crossing fibres. A threshold of FA > 0.25 was applied to this map to display only vectors corresponding to anisotropic tissues. However, as discussed in the previous section, areas of crossing fibres may incorrectly show lower anisotropy and care must be taken when applying thresholds. In this representation of diffusion, the out-of-plane information could also be added by colour coding of the arrows (45).

To visualise the diffusion data in anisotropic tissues, colour maps can also be employed (46). The orientation of the fibres is characterised by assigning a colour to each of the components of the principal eigenvector. In the example of Figure 1.13 the fibre bundles running in the \( x \) direction are represented in red, the \( y \) direction in green and the \( z \) direction in blue. Combinations of these colours correspond to oblique directions.

Figure 1.13 Axial, coronal and saggital representation of fibre direction using colour coding (\( x \)-direction is red, \( y \)-direction is green and \( z \)-direction is blue).

The degree of anisotropy is also coded in the image by weighting the brightness of the colour in each voxel by the measured DAI (46).
1.7.4 Tractography

DT-MRI provides a unique tool for investigating fibre structures *in vivo* by using the orientation information contained within $\mathbf{D}$ for each voxel of the image. There are two main applications of tractography; the direct assessment of connectivity between different areas of the brain and the segmentation of white matter regions-of-interest (ROI) for the specific study of this tissue.

Several tractography methods have been proposed so far (47). Vector field based approaches connect each voxel to the adjacent one to which the principal eigenvector points. A streamline is propagated in this way from an initial starting (seed) point until a stopping criterion is met, usually a threshold in DAI, that prevents the tract propagating into isotropic areas such as grey matter or CSF (48-51). Streamline methods have various disadvantages, however. Firstly they are susceptible to noise, which can lead to errors in the principal direction producing erroneous tracts. Moreover, since they are deterministic, there is no information about the confidence associated with the calculated tract. However, recent application of bootstrapping techniques (resampling with replacement from the original sample) to streamline tractography has added confidence information to the calculated fibre tracts (52,53).

Another problem found in streamline tractography is that most algorithms generate a single tract per seed point, restricting the possibility of large fibres branching into small ones, with the consequent lost of information. This has been partly addressed by using an approach where tracts are 'seeded' in every point of the brain, then selecting those that pass through a specified ROI (47).

Other tractography methods such as probabilistic and front propagation techniques attempt to overcome the problems with streamline tractography. Front propagation works through the evolution of a 'front' over time that propagates from a seed point, with the speed of propagation in each direction determined by the nature of $\mathbf{D}$. Examples of algorithms using this approach are fast marching tractography (54) and front evolution tractography (55). The random walk method calculates the possible tracts from a seed point by taking successive steps in random directions uniformly distributed on the sphere. The tract propagates along a regular curve that models the statistical nature of the diffusion process. To favour propagation along the principal direction, the random vector is weighted by $\mathbf{D}^\alpha$, where $\alpha > 1$ is an exponent that...
enhances the anisotropic character of the diffusion tensor and tightens the
distribution along the main eigenvector (56).

Behrens et al. (57,58) suggested a probabilistic approach using Bayesian inference
(a statistical inference in which probabilities are interpreted as a degree of belief) to
characterise the uncertainty in the orientation of the underlying fibre structure in a
diffusion model. They represent the uncertainty in every voxel of the brain image as
a PDF of the fibre orientation using a simple partial volume model with one fibre
direction. Given a seed point, a sample from the PDF in that voxel is randomly
selected to obtain a possible fibre orientation. The tract then moves along that
direction a small distance before reaching a new point in the image where the process
starts again. A probabilistic streamline is then generated until a stopping criterion is
met. The initial seed point sampling is repeated many times and a likelihood of
connection to the seed location is calculated by dividing the number of probabilistic
streamlines crossing that voxel by the total number. Behrens' technique was later
extended to the case of two different fibre orientations within each voxel by using
high angular diffusion imaging (59).

Examples of tracts reconstructed from diffusion MRI data are shown below. A
virtual 3D visualisation of brain white matter fibres calculated by a streamline
tractography technique is shown in Figure 1.14 (a). Each track is displayed as a
stream tube representing a fibre bundle; colour coding corresponds to fibres
connecting different areas of the brain, but no information about the confidence of
each tract is given. Figure 1.14 (b) shows tracts from a seed point in the posterior
limb of the internal capsule calculated using Behrens' probabilistic algorithm and
superimposed on an FA map. The colour scale from white to red represents high to
low probability of the tract passing through the voxel.
1.8 Artifacts in DT-MRI

1.8.1 Patient movement

Ill patients are more likely to move during an MRI scan than healthy volunteers. Chapter 3 will investigate the effects of bulk motion of the subject during DT-MRI acquisition and how it can affect the estimation of $D$ due to the acquisition of data with a suboptimal set of diffusion sampling directions. Other effects of subject motion during the scan are the appearance of ghosting due to phase shifts and misalignment between baseline and DW images. This latter effect prevents the accurate voxel-by-voxel calculation of $D$, since the same voxel in each image may correspond to a different location in the brain. Using immobilisation devices and keeping the length of the imaging protocol as short as possible can help to avoid large patient movements. However, slight movements still may cause phase shifts and a small misregistration of the DW images prior to calculation of $D$, causing loss of contrast on diffusion anisotropy maps. Phase shifts can be addressed in part by incorporating navigator echoes into the DW sequence (60,61): an additional, non-phase encoded line of data is acquired as well as the imaging data in each phase-encoding step. Both echoes are diffusion-weighted, but changes in the phase of the navigator echo from one phase encoding-step to the following are caused by bulk motion, assuming translational motion. The phase information from the raw navigator data is therefore used to correct the phase of the imaging data in later steps.

Figure 1.14 (a) Visualisation of the brain white matter structures in 3D calculated by using a streamline method (image courtesy of Martin Connell). (b) Projection in a coronal plane of probabilistic tracts connecting brain cortex to brain stem using seed points in internal capsules with colour encoded confidence (image courtesy of Jonathan Clayden).
(60). This method has been extended to correct for rotational motion (61). Another technique to obtain DW images free from motion artifacts uses periodically rotated overlapping parallel lines with enhanced reconstruction (PROPELLER) (62) which acquires the data in a series of rotating strips in the k-space, each one passing through the centre. Since there is a central circular region that is sampled by all strips, comparing the central data acquired by each of them allows phase inconsistencies to be detected and removed before reconstruction of the image.

Some imaging strategies are intrinsically insensitive to bulk motion and do not need correction of the acquired data. To avoid the use of phase encoding, and therefore the effects from phase shifts caused by motion, line scan diffusion imaging (LSDI) (63) uses two slice-excitation pulses to image a column of spins in the intersection of the slices. The 2D image is then obtained by acquiring the appropriate set of columns in the plane. To avoid saturation of the spins and loss of signal in the imaged plane the two associated slices used to select a column must lie outside the imaging plane. One disadvantage of this technique is that it is relatively slow. The time of acquisition can be halved by acquiring two columns from two separate slices in each shot (64). However, another drawback of this method is the loss of sensitivity compared to a conventional 2D Fourier Transform experiment that is reduced by a factor of $\sqrt{N}$, where $N$ is the number of scan lines (65).

The standard methods used for diffusion MRI are single shot acquisition techniques, such as DW-EPI. These are free from motion induced ghost artifacts since all data are acquired with a single application of the diffusion gradients pulses and the effects are uniform over the dataset. However, EP images are especially vulnerable to susceptibility and eddy current induced distortions, and still need to be re-aligned between acquisitions.

1.8.2 Eddy current induced distortions

The large pulsed gradients used to introduce diffusion sensitisation in the imaging sequence create eddy currents (EC) in the electrically conductive structures of the MRI scanner. These produce additional decaying magnetic fields that remain during the EPI readout period, causing magnification, translation, and shearing of the image.
in the phase encoding direction, depending on the direction of the residual EC. An example of EC induced distortions in a water phantom is shown in Figure 1.15.

![Figure 1.15](image.png)

Figure 1.15 (a) $T_2$-weighted undistorted image of a water phantom, (b) uncorrected DW image of the same slice distorted by residual eddy currents during acquisition.

Several solutions have been suggested to avoid or correct for EC distortions, addressing the problem at the acquisition, processing or post-processing level. These correction techniques will be described in more detail in Chapter 5.

Another effect of EC is to cause inaccuracies in the $b$-matrix. However, this tends to be negligible and the $b$-matrix generally only needs to be corrected for bulk patient motion (66).

1.8.3 Magnetic susceptibility effects

Large changes in the magnetic susceptibility at boundaries between materials cause local magnetic gradients as illustrated in Figure 1.16 (a). The same effect occurs in the subject’s head when it is placed in the static magnetic field of the MRI scanner, at boundaries between soft tissue and air, e.g. in regions of the brain near the sinuses, or soft tissue and bone. The susceptibility gradients interact with the imaging gradients that encode the position of the signal, causing distortion of the DW images as shown in Figure 1.16 (b).
The effect is usually small and in vivo studies using the pulsed gradient spin echo sequence at 1.5 T suggest that susceptibility effects do not make a substantial contribution to the diffusion measured in the boundaries between bone and brain parenchyma or to the diffusion anisotropy measured in brain white matter (67). For higher magnetic fields (3T) susceptibility effects are more pronounced, but it has been shown that they can be reduced by using sensitivity encoding techniques (SENSE) (68). These use an array of multiple, simultaneously operated receiver coils for signal acquisition. Each element of the array has different spatial sensitivity producing an encoding effect analogous to gradient encoding (69).

1.8.4 Signal-to-noise ratio

As the diffusion weighting is increased, the signal level of the DW images approaches the noise level. At this level, the sorting of the eigenvalues of D for calculation of DAI introduces a bias in their value. This sorting bias, makes isotropic media appear anisotropic and anisotropic media appear more anisotropic (70,71). To increase SNR of the DW images and avoid the sorting-bias effect, averaging of multiple acquisitions in every gradient direction is usually performed. The effect of low SNR is shown in Figure 1.17. Effects of noise on FA will be further investigated in §2.4 and §3.4.2.
1.8.5 Nyquist ghost

Nyquist artifacts arise due to an asymmetry between odd and even echoes when positive and negative read gradients are used to sample alternate lines of the k-space. This causes a ghost in the image, which is displaced by half the field of view as shown in Figure 1.18. Usually a Nyquist ghost is easily identified and does not affect image interpretation. However, cases have been reported where artifacts in the image caused by Nyquist ghosting appear as regions of apparently low diffusion resembling a lesion (72). These artifacts can be avoided by using spatial presaturation or fluid suppression in the acquisition protocol (72) or performing phase correction of the EPI data (73).

1.8.6 Systematic errors

Systematic errors in the acquisition and processing of DT-MRI data must be avoided, especially for quantitative studies. Errors might arise from hardware issues such as
the presence of background gradients, gradient miscalibration or non-linearity that would lead to inaccuracies in the calculation of \( D \). Errors in the calculation of the \( B \)-matrix or in the pulse programming would also spread systematically to the final values of the water diffusion parameters. Accuracy of the DT-MRI data acquired can be assessed by phantom studies using water or other fluids of known self-diffusion coefficients and comparing measurements with the published values (74). Diffusion anisotropy is more difficult to assess, as there is as yet no standard anisotropic diffusion phantom.

1.9 New approaches in diffusion MRI

The introduction of stronger magnetic field gradients that permitted the use of diffusion MRI at high \( b \)-values showed that the signal decay of water in the brain as a function of \( b \) was multi-exponential, suggesting the existence of more than one diffusion component (75,76). However, since the Stejskal-Tanner equation (Eq. 1.40) assumes a single ADC, a new strategy was necessary to analyse multicomponent diffusion data. Another major limitation of the diffusion tensor model emerges in voxels containing crossing fibres with different principal directions since the model assumes a single Gaussian diffusion compartment in each voxel and, therefore, only a single principal orientation. Alternative diffusion MRI methods have been suggested to tackle these problems.

The \( q \)-space approach to diffusion imaging (77,78) provides displacement probability profiles that offer non-invasive structural information about the sample without using a model for diffusion. The echo attenuation in a pulsed gradient diffusion experiment (see Figure 1.7) is related to the displacement probabilities as

\[
E_\Delta(q) = \int \overline{P}(R, \Delta) \exp(i2\pi q \cdot R) dR, \quad \text{Eq. 1.74}
\]

where \( R \) is the net displacement vector, \( \overline{P}(R, \Delta) \) the displacement probability and \( q \) the reciprocal spatial vector defined as

\[
q = \frac{1}{(2\pi)^3} \gamma \delta G , \quad \text{Eq. 1.75}
\]

Eq. 1.74 implies an inverse Fourier transform relationship between \( q \) and \( R \). Thus, Fourier transformation of the echo attenuation yields the displacement probability, and the full width half maximum (FWHM) of \( \overline{P}(R, \Delta) \) is directly related to the root mean square value of the dynamic displacement. Disadvantages of this approach are
the need for large pulsed field gradients and a substantial number of data samples to fit the model, which increases the scanning time significantly. Also, although this method provides a better description of the diffusion within tissues, it does not provide, for example, the orientation of the fibres within the voxel.

Other approaches to describe diffusion in areas of crossing fibres use high angular resolution diffusion imaging (HARDI), which involves sampling of the signal in a large number of orientations uniformly distributed over a sphere (79). Different models and fitting procedures have been proposed to relate the signal in a voxel to the underlying diffusion profile using this technique. These include the fitting of multiple diffusion tensors to the data to extract the volume fractions and orientations of the fibres (79), or the decomposition of the diffusion with spherical harmonics to describe non-Gaussian profiles that reveal the complexity of the structure of tissues in some areas of the brain (44,80). Persistent angular structure (PAS-) MRI, introduced by Jansons and Alexander (81), uses HARDI to calculate a particle displacement PDF, named PAS, that represents the relative mobility of particles in each direction. This has a non-Gaussian profile with many peaks that represent the orientation of the different fibre bundles within a voxel and is calculated using the principle of maximum entropy; tests of this technique in synthetic data and human brain have shown that the principal directions of the PAS reflect the actual orientation of the underlying structures. All these methods reduce the number of measurement compared to q-space imaging. However, they do require a model for the diffusion. Model-free approaches to analyse the HARDI signal, such as Q-ball imaging (82), spherical deconvolution (83) and diffusion spectrum MRI (84), have also been suggested.

Q-ball imaging (82) calculates the diffusion orientation density function (ODF) using an extension of the Funk-Radon transform of the diffusion signal (which maps the three-dimensional Cartesian space into the sphere), which implies that the ODF in a given direction is approximated by the sum of the diffusion signal over the equator around that direction, providing a model-free approach to calculate the diffusion probability from the diffusion signal sampled over a sphere. Spherical deconvolution was used by Tournier et al. (83) to infer the ODF by assuming that the signal attenuation caused by diffusion weighting measured over the surface of a
sphere is given by the convolution of the ODF with the signal attenuation profile for a typical fibre bundle (response function). The DW signal attenuation is obtained using HARDI sampling and then represented in a new basis function set, given by the spherical harmonics, in order to calculate the ODF by spherical deconvolution.

The most recently proposed model-free approach proposed is diffusion spectrum MRI (84), which calculates the ODF from its relationship with the orientation maxima of the diffusion spectrum. In their paper, Wedeen et al. (84) proved under
which conditions the diffusion contrast is positive and using that result they
reconstructed the diffusion spectrum from the Fourier transform of the modulus of
the measured signal. Examples of ODFs calculated from these three methods are
shown in Figure 1.19. As opposed to the diffusion tensor model, these approaches
can represent intersections of two or three fibres, with the colour coding in the
figures depending on the diffusion orientation.

1.10 Clinical applications of DT-MRI

1.10.1 Cerebral ischaemia

The first and most important application of diffusion imaging emerged in 1990 when
Moseley et al. found that the apparent diffusion coefficient of water in brain
markedly decreased early after ischaemia in cat models (85). This effect was
corroborated in the human brain and many diffusion studies in ischaemic stroke have
been performed since then, demonstrating its clinical value. It was found that $\langle D \rangle$
was decreased by 30-50% as early as 30 minutes after onset of ischaemia (86-88),
which resulted in abnormalities on DW images before they could be detected with
CT or conventional MRI. Studies of diffusion parameters at different time points
after an ischaemic event also demonstrated the ability of DT-MRI to distinguish
between acute and chronic infarcts, thus improving management of patients.

An extensive study of DT-MRI parameters in patients with ischaemic stroke will
be presented in Chapter 2.

1.10.2 Multiple sclerosis

DT-MRI is increasingly being used to quantify in vivo the extent and severity of
multiple sclerosis (MS) lesions. MS pathology may alter the permeability or
geometry of structural barriers to water motion in the brain, optic nerve and spinal
cord (89). Diffusion imaging allows quantification of the amount of tissue damage
cased by MS and can identify even small changes in grey and white matter not
detectable by conventional T2-weighted MRI. For example, studies have shown that
$\langle D \rangle$ of normal appearing white matter on T2-weighted images is higher in MS
patients than in controls, while diffusion anisotropy is lower, possibly indicating
myelin and axonal loss (89). Tractography techniques could also identify areas of axonal disruption that may occur during the pathological process of MS (90).

1.10.3 Wallerian degeneration

The degeneration of white matter fibres at a distance from a primary lesion in the brain has also been investigated using DT-MRI. Secondary white matter degeneration shows a large reduction of diffusion anisotropy and a moderate increase or no change in $D$ (91,92), whereas in longstanding ischaemic lesions $D$ is markedly increased. This allows differentiation between the primary lesion and associated Wallerian degeneration which is not possible with conventional MRI. Changes in FA can be detected as early as 6 to 10 days after stroke onset in brain white matter near the main lesion (93). The generation of eigenvector maps or fibre tracks also provides a means of comparing the trajectories of affected white matter fibres showing significant changes in the measured orientation and pathways (91,92).

1.10.4 Brain tumours

Brain tumours modify the water content and architecture of brain tissue causing changes in the measured $D$ and diffusion anisotropy. This might provide useful information in the diagnosis of patients, in particular with specification and grading of tumours that is still limited when using conventional MRI techniques. Some studies have investigated the correlation of diffusion values with tumour type and grade. For example, it was observed that low-grade astrocytomas have significantly higher ADC than other tumours and ADC values correlated with cellularity for some tumour types (94).

Tractography and eigenvector colour maps have important clinical use for preoperative planning of brain tumours. These methods can show the relationship between tumours and surrounding white matter tracts. Intact tracts displaced by the tumour can be identified in their new location or orientation and potentially be preserved during resection (95). Tracts disrupted by the tumour, or presenting tumour infiltration, may also be distinguished by decreased FA and abnormalities in colour maps (95).
1.10.5 Developing brain and normal ageing

During normal brain maturation there is a decrease in $\langle D \rangle$ and increase in FA that correlate with a decrease of total water content and increase in organisation during white matter myelination. Abnormalities in the normal evolution of these parameters during brain development may be a marker of brain injury in newborns. For example, diffusion studies have shown that in babies with brain injury, $\langle D \rangle$ fails to decrease and may even increase, with no increase in diffusion anisotropy of white matter (96).

DT-MRI has also been used for the study of the normal ageing brain. An increase in $\langle D \rangle$ and a decrease of FA with age is generally found in white matter of healthy subjects (97,98). Several studies using diffusion imaging have also shown correlation between the extent of the changes in these parameters and the cognitive ability in older people (99,100).

1.10.6 Other applications

Diffusion imaging has proved to be a potentially useful tool for the diagnosis of other pathologies. For example, in patients with Alzheimer’s disease it has been found that white matter tracts associated with cognitive function, such as the splenium of the corpus callosum, have reduced diffusion anisotropy, presumably showing axonal degeneration related with progression of the disease (101). DT-MRI is also used for the study of other types of cognitive aspects such as reading disabilities. A relationship was found between reading performance and diffusion anisotropy values of white matter within the left temporo-parietal regions of the brain in both adults (102) and children (103).

In general, diffusion parameters such as $\langle D \rangle$ and FA are able to demonstrate loss of structural organisation in brain tissue caused by many different pathologies. Other quantities derived from diffusion data can also help diagnose diseases causing demyelinating disorders. For example, axial ($\lambda_1$) and radial ($(\lambda_2+\lambda_3)/2$) diffusivities have been directly linked to axon and myelin integrity (104). Different forms of DT-MRI data display, such as colour or vector maps and tractography techniques, are useful in assessing changes in orientation, localisation or disruption of fibre tracks that may occur in the diseased brain.
Chapter 2 Quantitative Diffusion MRI of Ischaemic Stroke

2.1 Introduction

This chapter illustrates one of the main applications of DT-MRI, the study of ischaemic stroke. §2.2 briefly introduces the pathophysiology of ischaemic stroke and gives an overview of previous diffusion MR studies of this disease. The results from a study on the temporal evolution of water diffusion parameters derived from DT-MRI data are presented and discussed in §2.3. Other aspects to consider in the measurement of these parameters, such as the SNR of the component DW-EP images and CSF contamination are also investigated and discussed in §2.4 and §2.5.

2.2 Diffusion MRI of ischaemic stroke

2.2.1 Introduction

Ischaemic stroke is caused by a reduction in cerebral blood flow (CBF) to a region of the brain, usually as a consequence of thrombolic or embolic occlusion of a cerebral artery. Ischaemia occurs when CBF is too low to supply enough oxygen and energy substrates to support cellular function. Normal CBF in the brain is approximately 50 ml per 100 grams of brain tissue per minute (ml/100g/min), although it varies due to neuronal activity. Experimental models of ischaemic stroke have identified a threshold of flow of about 35 ml/100g/min below which some cell functions are altered resulting in electrical failure (105). The critical threshold of CBF for irreversible cell damage is about 10 ml/100g/min. At this threshold, neurones stop functioning and tissue becomes infarcted if perfusion is not restored in a short period of time (105). In some cases, the CBF to the tissue adjacent to the infarcted area falls between these two thresholds, where electrical failure has occurred but cellular integrity is still maintained. Stroke therapy, applied early after stroke onset, might be capable of saving this area from also becoming infarcted, thus reducing final lesion size and improving patient outcome (106).

Precise imaging techniques are required to make early differential diagnosis of ischaemic stroke and define whether there is tissue at risk that could benefit from
otherwise potentially harmful therapies. The imaging technique conventionally used to help the diagnosis of stroke is CT. However, changes in the normal appearance of CT scans are mainly associated with the change in electron density in tissue or to morphological changes, such as intracellular oedema, causing cell swelling. Therefore its sensitivity is relatively poor during the first hours after onset, particularly for small ischaemic lesions (107). MRI has proved to be more sensitive than CT in the diagnosis of stroke, in particular advanced MRI techniques, such as perfusion-weighted MRI (PW-MRI) or DW-MRI can detect abnormalities at a very early stage. PW-MRI provides maps of relative CBF that can identify hypoperfused areas in the brain. DW-MRI detects areas of decreased water diffusivity seen early after the onset of stroke, which is attributed to cellular energy failure. Water diffusion parameters are thought to be useful markers of tissue cellular integrity while perfusion parameters can potentially identify tissue at risk of permanent damage. Knowledge of the temporal evolution of these parameters provides signatures that could help to identify reversible or irreversible cellular damage in the brain after stroke (108), as well as differentiation of acute from non-acute infarcts (109,110). This knowledge may have important implications in the clinical management of stroke patients.

2.2.2 Diffusion MRI studies of ischaemic stroke

After the introduction of DW-MRI, one of the main applications for this new technique emerged when a rapid decrease of water diffusion in brain tissue affected by ischaemic stroke was observed. This effect was first seen by Moseley et al. using DW-MRI in cat brain models (85). They observed regions of hyperintensity in the DW images corresponding to the areas of ischaemia as early as 45 min after permanent middle cerebral artery occlusion. At this time, changes still could not be seen by other conventional imaging modalities such as CT or T2-weighted MRI. These results were confirmed in rats (111-113) and it was demonstrated that the diffusion abnormalities were reversed when the occlusion was removed early on (112).

After these preliminary studies in animal models of stroke many other authors showed similar results in the human brain. Many of them investigated the temporal evolution of the ADC of water in ischaemic stroke lesions (87,108-110,114-120). All
these studies found that the ADC decreases immediately after onset. The exact mechanism responsible for this reduction is still not well defined. However, it is generally believed that the most likely cause is the redistribution of water from the extra-cellular to the intra-cellular space, where diffusion is more restricted, after failure of cell membrane function (cytotoxic oedema) (121,122). A relationship has also been observed between initial ADC and CBF values after stroke onset, with ADC decreasing rapidly at a CBF threshold of 21 ml/100g/min (123).

The initial reduction of ADC is followed by slow recovery or “pseudo-normalisation” attributed to the rupture of the blood-brain barrier and development of vasogenic oedema (124,125). Finally, the ADC becomes elevated, as the tissue proceeds towards necrosis, remaining higher than normal thereafter. There are, however, inconsistencies in the results of these studies. For example, some authors have reported a time of pseudo-normalisation between 5 to 10 days (110,115,116,118,120,126), while others have found that the ADC reaches normal values between 24 to 48 hours (127) or at about one month (87,109,117).

Other authors also measured the evolution of DAI after ischaemic stroke (88,118,128), and again different results were obtained. A reduction in diffusion anisotropy was found in some cases consistent with loss of tissue structural integrity, although some studies reported an increase in diffusion anisotropy in the early stage after onset (118,128).

Differences in patient cohorts and imaging methodology between studies, such as measurement of orientation dependent parameters and use of different diffusion gradient sampling schemes, may partly explain the conflicting results (129). However, another possible cause could be heterogeneity of diffusion values within the lesion in the acute stage, potentially arising from the different water diffusion properties of grey and white matter (130-132). Some investigators have considered this issue, and measured diffusion parameters separately in ischaemic grey and white matter (118-120,133,134). However, the results from these studies reporting changes in grey and white matter water diffusion parameters again showed contradictory results. Several authors found that $D_{R}$ ($(D)_{\text{lesion}}/(D)_{\text{contralateral}}$) were significantly more reduced in white than grey matter at < 3 days (118,134). Conversely, another study reported that $D_{R}$ was significantly more reduced in grey
matter (133), while two other investigations did not show significant differences between grey and white matter at this stage (119,120). In two out of the three previous studies that have investigated the temporal evolution of grey and white matter diffusion parameters, it was observed that $\langle D \rangle_R$ of both grey and white matter remained low during the first ~4 days, increasing thereafter, and reaching normal contralateral values at day ~10 (118,120). The third study only found this behaviour in grey matter, which started to rise at ~7 days, while no significant change was observed in white matter water $D_R$ during the first 14 days after the initial reduction following stroke (119). However, all these studies observed a slightly earlier renormalisation of grey matter water diffusivity.

Some of the studies also measured DAI in these tissues and again the results disagree. One group found that grey matter FA remained unchanged at < 24 hours while white matter FA decreased significantly in 50 patients (133), although only 10 of them had lesions affecting both grey and white matter. Conversely, the study by Yang et al. reported changes in both grey and white matter diffusion anisotropy (118). In their longitudinal study of 26 patients, most subjects showed an increase of diffusion anisotropy for both tissue types at < 12 hours, after which the diffusion anisotropy declined. At approximately 90 days, the diffusion anisotropy was further reduced in all lesions. However, for most patients in this study the diffusion anisotropy was assessed by means of an orientation-dependant parameter derived from ADC values, rather than a rotationally invariant index derived from the eigenvalues of $D$, such as FA.

Unfortunately, not many of these studies scanned individuals serially at each time point to control for between subject variability (133,134), and most did not measure $D$ (118-120). Studies of the temporal evolution of $\langle D \rangle$ used snapshots of different individuals at different times and are likely to be confounded by the considerable variability in the individuals response to ischaemia and recovery pattern. To assess $\langle D \rangle$ change over time requires the same individuals to be imaged serially at the same time points. Furthermore, some studies only imaged a small number of patients (119,120,134), while not all were specifically designed to analyse differences between the two tissue types (118,133). The inconsistencies in these results suggest that more work is needed in this area to characterise the changes in water diffusion
parameters of grey and white matter after stroke and determine whether there are any significant differences between the temporal evolution of grey and white matter $\langle D \rangle$ and FA.

2.3 Temporal evolution of water diffusion parameters after stroke

2.3.1 Introduction

As mentioned in §2.2.2, some previous studies have investigated water diffusion parameters after stroke separately in grey and white matter. However, there has been no longitudinal study specifically designed to analyse differences in $\langle D \rangle$ and FA between the two tissue types including a large number of patients. The purpose of the present study was to measure changes in water diffusion parameters of grey and white matter after stroke and determine whether there are any significant differences between the temporal evolution of grey and white matter $\langle D \rangle$ and FA by following the same group of patients from onset to 3 months.

For this purpose DT-MRI data from patients imaged since January 2001 from the department database were used. The patients were recruited retrospectively from the database (but had been prospectively recruited in the original study (135)) with the following inclusion criteria: (a) the patient had been imaged initially as soon as possible on admission, within a maximum of 24 hours after symptom onset, (b) patient did not receive any stroke thrombolysis or experimental drug treatment. Most of these patients underwent repeat DW- and PW-MRI at around 4-7, 10-14 days, 1 and 3 months after stroke. Data from patients that were scanned at least twice, whose visible ischaemic lesion on DW-EP images affected both grey and white matter and with no evidence of haemorrhage in initial scan were selected for the present study. A total of thirty-two patients, the majority with medium to large cortical infarcts, were included in the study. The demographics of the patient group and the number of patients scanned at each time point are summarised in Table 2.1.
Table 2.1 Number of scans, demographics, and stroke syndromes for the patients included in this study.

<table>
<thead>
<tr>
<th>Number of Patients</th>
<th>Number of scans</th>
<th>M/F</th>
<th>Mean age (years)</th>
<th>Stroke syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>5</td>
<td>12/6</td>
<td>67.8 ± 13.9</td>
<td>3 13 1 1</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>4/2</td>
<td>80.5 ± 7.1</td>
<td>4 2 - -</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>2/3</td>
<td>77.6 ± 7.2</td>
<td>2 2 - 1</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>2/1</td>
<td>85.3 ± 13.4</td>
<td>2 - - 1</td>
</tr>
<tr>
<td>32</td>
<td>135</td>
<td>20/12</td>
<td>73.9 ± 13.3</td>
<td>11 17 1 3</td>
</tr>
</tbody>
</table>

TACS = Total anterior circulation syndrome, PACS = Partial anterior circulation syndrome, LACS = Lacunar syndrome, POCS = Posterior circulation syndrome.

All MRI data were obtained using the GE Signa LX 1.5 T clinical scanner. The MRI examination consisted of a standard fast spin-echo (FSE) T₂-weighted sequence, a GRE T₁-weighted sequence, and a DT-MRI protocol. In the DT-MRI experiment, DW-EP images were acquired using a single-shot spin-echo EP imaging sequence in which two symmetric trapezoidal gradient pulses of duration δ = 32.2 ms, separation Δ = 39.1 ms and rise time η = 1.2 ms were inserted around the 180° refocusing pulse in the required gradient channel. Sets of axial DW-EP images (b = 0 and 1000 s/mm²) were collected with diffusion gradients applied sequentially along six non-collinear directions (136). Five acquisitions (NEX) consisting of a baseline T₂-weighted EP image and six DW-EP images, a total of 35 images, were collected per slice position. The acquisition parameters for the DW-EP imaging sequence were 15 axial slices of 5 mm thickness and 1.0 mm slice gap, a field-of-view of 240 × 240 mm, an acquisition matrix of 128 × 128 (zero filled to 256 × 256), a TR of 10 s and a TE of 98.8 ms. To ensure that the slice locations used in the follow-up scans corresponded as closely as possible to those in the first, the subject's head position and tilt in the first scan were recorded and the patient repositioned as close as possible to this location in the follow-up scans.

Bulk patient motion and EC induced artefacts were removed from all component EP images using a 3D image-based alignment technique, namely affine FLIRT (137). All DW-EP image volumes were aligned to the first T₂-weighted EP image volume acquired in the first examination. The set of five EP images collected for each gradient direction was averaged to give seven high SNR images for each slice. Typical SNR values for normal brain parenchyma in the T₂-weighted and DW EP images were 40 and 20 respectively.
From this MRI data, $D$ was calculated in each voxel from the signal intensities in the component EP images as explained in §1.5.2.3. Maps of $\langle D \rangle$ and FA were generated on a voxel-by-voxel basis from the sorted eigenvalues of $D$ as explained in §1.6.2 and converted into Analyze format.

### 2.3.2 Region-of-Interest Analysis

For each patient, the area affected by the stroke at less than 24 hours was outlined in each slice where the lesion was visible by tracing around the region of signal hyperintensity on the first DW-EP scan (Figure 2.1 (a)). The lesion outlines were copied onto the first scan $T_2$-weighted EP images and multiple circular ROI of 6 mm diameter were placed in grey and white matter within the lesion using anatomical and imaging knowledge (Figure 2.1 (b)). This ROI size was chosen to enable selection of regions of grey and white matter, while minimising the effects of mixed tissues partial-voluming and CSF contamination. ROI were also placed in comparable contralateral grey and white matter to be used as control regions for each patient. As many ROI as possible were placed in each slice and overlaid onto the co-registered DW-EP, $\langle D \rangle$ and FA parametric maps for the same time point (Figure 2.1 (c, d)). A total of 1518 ROI were drawn for the 32 patients, with 14 ROI placed in lesion grey matter and 10 in lesion white matter on average. When tissue structures sampled by ROI chosen at the first time point were displaced in subsequent scans by lesion swelling or atrophy, the ROI positions were corrected to sample the same anatomical piece of tissue at all time points. Areas within periventricular white matter disease and haemorrhagic transformation of the infarct at later time points were avoided. A neuroradiologist verified the positions of all ROI and ischaemic lesion outlines.
Figure 2.1 Examples of grey and white matter ROI drawn on a representative slice from a 79-year-old male patient with right total anterior circulation stroke. (a) Lesion outline drawn on the first scan DW-EP image. (b) Circular ROI drawn in areas of grey and white matter in the lesion and in contralateral tissue using the co-registered T2-weighted EP images. All ROI copied onto (c) D and (d) FA maps for all time points to characterise the temporal evolutions of these parameters. Crossed ROI indicate grey matter while open ROI indicate white matter.

Mean values of \( \langle D \rangle_{\text{lesion grey}} \), \( \langle D \rangle_{\text{lesion white}} \), \( \text{FA}_{\text{lesion grey}} \), \( \text{FA}_{\text{lesion white}} \), \( \langle D \rangle_{\text{contr.grey}} \), \( \langle D \rangle_{\text{contr.white}} \), \( \text{FA}_{\text{contr.grey}} \), and \( \text{FA}_{\text{contr.white}} \) were calculated from all ROI for every patient at each time point. However, while it is important to characterise the evolution of \( \langle D \rangle \) and FA in order to understand how stroke affects brain tissue structure, these absolute parameters also reflect the intrinsic difference between grey and white matter (\( \langle D \rangle_{\text{lesion grey}} > \langle D \rangle_{\text{lesion white}} \) and \( \text{FA}_{\text{lesion grey}} < \text{FA}_{\text{lesion white}} \) for normal brain), and inter-patient variability. To control for these differences and allow comparisons among individuals at each time point, the ratio of lesion to contralateral \( \langle D \rangle \) and FA (\( \langle D \rangle_R \) and \( \text{FA}_R \)) were also calculated (119,133,134). Finally, a global mean for each time point for grey and white matter was determined from the individual patient mean.
data. Thus, all lesions were weighted equally regardless of the number of ROI in each.

Differences between $\langle D \rangle_R$ and $\text{FA}_R$ values of grey and white matter at each time point for each individual patient were then assessed using a two-tailed Student’s paired $t$-test (null hypothesis: $\langle D \rangle_R\{\text{grey}\} - \langle D \rangle_R\{\text{white}\} = 0$; $\text{FA}_R\{\text{grey}\} - \text{FA}_R\{\text{white}\} = 0$), with $p < 0.05$ considered as statistically significant.

2.3.3 Results

Figure 2.2 (a) and (b) shows the variation in the absolute values of lesion and contralateral $\langle D \rangle$ and FA after stroke for grey and white matter. After the initial reduction from normal values, both $\langle D \rangle\{\text{lesion grey}\}$ and $\langle D \rangle\{\text{lesion white}\}$ increase. The general trend in the evolution of $\langle D \rangle\{\text{lesion grey}\}$ and $\langle D \rangle\{\text{lesion white}\}$ is similar, although between time points two (4-7 days) and three (10-14 days) the rate of increase of $\langle D \rangle\{\text{lesion grey}\}$ is faster than $\langle D \rangle\{\text{lesion white}\}$. $\langle D \rangle\{\text{contr. grey}\}$ and $\langle D \rangle\{\text{contr. white}\}$ also increased between time points one and two but no more changes were observed at later time points. $\text{FA}\{\text{lesion white}\}$ decreases rapidly during the first week, then more gradually over the next three weeks. $\text{FA}\{\text{lesion grey}\}$ decreases less rapidly than $\text{FA}\{\text{lesion white}\}$ during the first week, but then has a similar evolution over the next three weeks. Between one and three months there is little change in either $\text{FA}\{\text{lesion grey}\}$ or $\text{FA}\{\text{lesion white}\}$. However, although $\text{FA}\{\text{lesion white}\}$ decreases more than $\text{FA}\{\text{lesion grey}\}$ it is still higher at 3 months. $\text{FA}\{\text{contr. grey}\}$ and $\text{FA}\{\text{contr. white}\}$ also decrease between time points one and two, but remaining constant at later time points.
Figure 2.2 Temporal evolution of the absolute values of lesion and contralateral grey and white matter (a) $\langle D \rangle$ and (b) FA.

Figure 2.3 shows the time course of $\langle D \rangle_R$\{grey\}, $\langle D \rangle_R$\{white\}, $FA_R$\{grey\}, and $FA_R$\{white\} during the first three time points where different patterns of change for grey and white matter are observed. Values of these parameters are shown in Table 2.2 for all five time points.
Figure 2.3. Evolution of (a) $\langle D \rangle_R^{\text{grey}}$, (b) $\langle D \rangle_R^{\text{white}}$, (c) $FA_R^{\text{grey}}$ and (d) $FA_R^{\text{white}}$ during the first 14 days after stroke. Open circles plot individual patient data and close circles the mean value for each time point. Error bars indicate the standard deviation on the mean.

Table 2.2. Mean ratios of ischaemic lesion to contralateral normal brain for mean diffusivity ($\langle D \rangle_R$) and fractional anisotropy ($FA_R$) values (Mean $\pm$ SD) for grey and white matter at each time point. Bold type indicates a significant difference ($p < 0.05$) between grey and white matter values at each time point.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>$\langle D \rangle_R$</th>
<th>$FA_R$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grey</td>
<td>White</td>
</tr>
<tr>
<td>Window</td>
<td>Mean ± SD</td>
<td></td>
</tr>
</tbody>
</table>
| < 1         | 0.3 ± 0.3   | 0.74 ± 0.13 | 0.69 ± 0.14 | 0.02     | 1.00 ± 0.19 | 0.86 ± 0.13 | < 0.001 
| 4 - 7       | 5.5 ± 1.1   | 0.79 ± 0.13 | 0.79 ± 0.18 | 0.99     | 0.90 ± 0.17 | 0.54 ± 0.14 | < 0.001 
| 10 - 14     | 11.7 ± 1.7  | 1.05 ± 0.19 | 0.97 ± 0.19 | 0.03     | 0.80 ± 0.22 | 0.52 ± 0.15 | < 0.001 
| 30          | 30.0 ± 3.4  | 1.32 ± 0.23 | 1.28 ± 0.29 | 0.47     | 0.73 ± 0.26 | 0.46 ± 0.17 | < 0.001 
| 90          | 94.5 ± 7.1  | 1.60 ± 0.25 | 1.60 ± 0.42 | 0.97     | 0.69 ± 0.20 | 0.46 ± 0.17 | < 0.001 

Both $\langle D \rangle_R^{\text{grey}}$ and $\langle D \rangle_R^{\text{white}}$ are significantly reduced during the first day after stroke onset ($p < 0.001$). $\langle D \rangle_R^{\text{grey}}$ is significantly higher than $\langle D \rangle_R^{\text{white}}$ at this time point ($p = 0.02$), with an average reduction of 26% for grey matter and 31% for white matter. During first and second time points there is no significant increase in $\langle D \rangle_R^{\text{grey}}$ ($p = 0.10$), while $\langle D \rangle_R^{\text{white}}$ starts to rise slowly. $\langle D \rangle_R^{\text{grey}}$ and
are identical at time point two. However, between time points two and three \( \langle D \rangle_R \{ \text{grey} \} \) increases at a faster rate and pseudo-normalises earlier than \( \langle D \rangle_R \{ \text{white} \} \). \( \langle D \rangle_R \{ \text{grey} \} \) is again significantly higher than \( \langle D \rangle_R \{ \text{white} \} \) at 10-14 days after stroke \((p = 0.03)\). This demonstrates the faster recovery of \( \langle D \rangle \{ \text{lesion grey} \} \) shown by the evolution of the absolute values between time points two and three. Thereafter, both \( \langle D \rangle_R \{ \text{grey} \} \) and \( \langle D \rangle_R \{ \text{white} \} \) increased slowly over time becoming elevated and of the same value at 3 months.

Table 2.2 and Figure 2.3 also show that \( FA_R \{ \text{white} \} \) is significantly reduced in the first day after stroke \((p < 0.001)\), while \( FA_R \{ \text{grey} \} \) remains unchanged. At 4-7 days, \( FA_R \{ \text{grey} \} \) is reduced by 10\%, while \( FA_R \{ \text{white} \} \) is reduced by 46\%. At later time points both \( FA_R \{ \text{grey} \} \) and \( FA_R \{ \text{white} \} \) decrease gradually, remaining depressed at 3 months. \( FA_R \{ \text{grey} \} \) is significantly greater than \( FA_R \{ \text{white} \} \) at all time points \((p < 0.001)\), although the absolute value of white matter FA was higher than grey matter at all time points (Figure 2.2).

### 2.3.4 Discussion

The above results show that there are differences in the temporal evolution of \( \langle D \rangle \) and FA of grey and white matter in ischaemic lesions affecting both tissue types. Although the overall evolution of \( \langle D \rangle \{ \text{lesion grey} \} \) and \( \langle D \rangle \{ \text{lesion white} \} \) is similar, subtle differences were observed during the first 15 days after stroke, specifically the rate of increase of \( \langle D \rangle \{ \text{lesion grey} \} \) was faster during time points two and three. Furthermore, as in previous studies it was found that \( \langle D \rangle_R \{ \text{white} \} \) was significantly more reduced than \( \langle D \rangle_R \{ \text{grey} \} \) within 24 hours of stroke \((118)\), and the rate of recovery of \( \langle D \rangle_R \{ \text{grey} \} \) was faster than \( \langle D \rangle_R \{ \text{white} \} \) between days 6 to 12 \((119, 120)\). Differences in the evolution of grey and white matter FA were more marked than those seen for \( \langle D \rangle \), with the loss of \( FA \{ \text{lesion white} \} \) being significantly greater than \( FA \{ \text{lesion grey} \} \) during the first five days after stroke, as had been shown previously \((133)\). Values of relative fractional anisotropy showed that \( FA_R \{ \text{white} \} \) was significantly more reduced than \( FA_R \{ \text{grey} \} \) at all time points.

Elevated \( \langle D \rangle \) and reduced FA are thought to indicate destruction of membrane integrity and progression towards necrosis \((138)\). Interestingly, Figure 2.2 shows that grey and white matter \( \langle D \rangle \) are still increasing at three months, while FA does not
change significantly after one month. Increasing water diffusion at later time points has been observed in other studies, and has been attributed to gliosis and CSF increase secondary to encephalomalacia (114), or flow dephasing effects in regions that have undergone cystic degeneration (115). However, no comparable measurements of diffusion anisotropy were made in these studies. Another important observation is that despite the greater loss of white matter FA, it is still significantly higher than grey matter at three months, indicating that some white matter structure remains. It was also noticed that the standard deviation of FA\{lesion white\} was larger than FA\{lesion grey\}. This was investigated by looking at individual patient data and two sub-groups of patients were identified according to their FA\{lesion white\} evolution (Figure 2.4).

![Figure 2.4](image)

Figure 2.4. FA\{lesion white\} of individual patients for the two patient sub-groups. (a) FA\{lesion white\} decreases rapidly during the first five days; (b) FA\{lesion white\} decreases less and more gradually.

In patients from sub-group (a), FA\{lesion white\} decreased more than the patients in sub-group (b). The amount of FA reduction during stroke could potentially be a signature of tissue damage, in particular related to the amount of white matter structures remaining at later times after the stroke. This could have implications in predicting patient outcome. For instance, tractography methods could be used to measure how changes in tract volume and integrity following stroke relate to clinical dysfunction and outcome.

Changes in the contralateral absolute (D) and FA values were also observed. This was not expected, although Ahlhelm et al. (126) have reported changes in the normal contralateral regions during the first 2-3 days after stroke onset. They attributed these to true changes in normal brain caused by the stroke. To corroborate this observation they measured the temporal evolution of CSF as internal control, and found no statistically significant changes. To verify whether changes in the contralateral side
in the present study were real or were caused by an artifact during imaging or processing, we also measured the temporal evolution of $\langle D \rangle$ and FA in CSF in four patients chosen randomly from the group (Figure 2.5).

Figure 2.5 shows that rather than having a flat evolution, the diffusion parameters also change in CSF between time points one and three. To analyse if these changes are significant, a two-tailed Student’s paired t-test was performed between the values at these time points (Table 2.3).

![Figure 2.5 Temporal evolution of (a) $\langle D \rangle$ and (b) FA in CSF (Means ± SD).](image)

Table 2.3 Mean values of $\langle D \rangle$\{CSF\} and FA\{CSF\} for the first three time points and $p$-values for the comparison between time points one and two, and two and three. $\langle D \rangle$ values are in $10^{-6}\text{mm}^2/\text{s}$.

<table>
<thead>
<tr>
<th>Time point</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\langle D \rangle$</td>
<td>3065.7 ± 239.3</td>
<td>2924.4 ± 195.7</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>FA</td>
<td>0.127 ± 0.060</td>
<td>0.116 ± 0.052</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>$\langle D \rangle$</td>
<td>2924.4 ± 195.7</td>
<td>2969.0 ± 182.7</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>FA</td>
<td>0.116 ± 0.052</td>
<td>0.123 ± 0.054</td>
<td>0.07</td>
<td></td>
</tr>
</tbody>
</table>

Since $\langle D \rangle$\{CSF\} changes significantly between the first two time points we cannot attribute all the changes in contralateral areas of grey and white matter to true changes caused by stroke. It is not clear what causes this artefact. Later in this thesis we investigate the hypothesis that it is potentially caused by the image co-registration.
process of serial DT-MRI data. Large corrections due to bulk patient motion or head tilt which need to be realigned across more than one slice would have to interpolate voxels across slices, causing smoothing and averaging of voxels corresponding to different tissues or CSF. This may cause the effect observed in the diffusion parameters for all the co-registered scans (time points two to five). This could be tested by using a different time point as a reference scan for the registration and repeating the measurements. A registration artifact would then produce decreased values of $\langle D \rangle$ and FA in normal contralateral brain at the time point taken as reference for registration. This possibility will be further investigated in §5.5.3. Since this artifact would affect both lesion and contralateral values, the relative values used in this study to assess the statistical significance of changes and differences between grey and white matter parameters would not be affected.

2.4 Effects of SNR on water diffusion parameters

2.4.1 Introduction

Noise contamination in the DT-MRI data artificially increases the value of the diffusion anisotropy measured. This is caused by a systematic bias introduced when sorting the eigenvalues before the calculation of DAI (139,140). In the presence of noise, the values of the first and third eigenvalues become systematically larger and smaller than their true values, and therefore the measured diffusion anisotropy increases. There is also the possibility that negative eigenvalues will be obtained, which will produce erroneous measurements of diffusion anisotropy. This effect is larger at lower SNR and also depends in the diffusion properties of the medium. When noise introduces differences in the eigenvalues of isotropic media, which otherwise would be approximately equal, the isotropic structures appear anisotropic. However, in anisotropic media where the first eigenvalue is much larger than the other two, the relative effect of noise is less pronounced, and the artifactual increase in diffusion anisotropy is smaller.

The effect of noise on diffusion anisotropy measurements is clearly undesirable. An approach to remove the systematic bias is to use DAI insensitive to the order of the eigenvalues, such as RA and FA. However, although rotationally invariant DAI are less sensitive to noise than DAI which are asymmetric functions of the diffusion
eigenvalues, FA is still slightly biased, with the result that all media appear more anisotropic at low SNR (139). To increase the SNR and diminish this effect, image averaging is usually performed with multiple NEX.

To assess whether the level of noise contamination in DT-MRI data used in the above study may have modified the diffusion anisotropy measurements in grey and white matter we measured FA at different SNR levels.

### 2.4.2 Methods

DT-MRI data from four stroke patients with large cortical infarcts were chosen. For each patient, the lesion was defined as the hyperintense region on DW-EP images. T2-weighted images were then used to draw ROI in the following brain areas: lesion grey matter \{lesion grey\}, contralateral grey matter \{contr.grey\}, lesion white matter \{lesion white\}, contralateral white matter \{contr.white\}, CSF \{CSF\} and whole brain \{brain\}. CSF ROI were placed in the ventricles in areas free of pulsatile motion artifacts as indicated by a neuroradiologist. Whole brain ROI were drawn by outlining the brain image in the slice avoiding areas of susceptibility artifacts. ROI in these areas were chosen to represent the wide range of FA values encountered in the human brain, from anisotropic (white matter) to nearly isotropic (CSF).

In the routine DT-MRI scanning protocol used for the experiments described in this chapter, the sets of baseline and six DW-EP images were repeated five times (5 NEX). Each DW-EP image of the set had an SNR of approximately 15. After registration to compensate for patient motion between acquisitions, images were averaged to obtain high SNR images and processed to obtain \(\langle D\rangle\) and FA maps. To reproduce different levels of noise, we averaged in turn two, three, four and five sets to obtain DW-EP images with SNR \(\sqrt{2}, \sqrt{3}, \sqrt{4}\), and \(\sqrt{5}\) times the SNR of the non-averaged image respectively. After processing, \(\langle D\rangle\) and FA maps were constructed for each dataset. Values of FA were measured and compared in ROI in each of the maps to assess how diffusion anisotropy values are affected by the different levels of noise and whether the SNR achieved with the 5 NEX used in the study is satisfactory for accurate FA measurements.
2.4.3 Results

Figure 2.6 shows how noise in the FA maps is reduced, and the white matter tracks become more visible, as the number of averages from which the maps are calculated increases. This effect is most pronounced going from 1 to 3 NEX, while the image quality increases only marginally with 4 and 5 NEX.

![Figure 2.6](image)

Table 2.4 and Figure 2.7 show the values of FA measured in the five ROI.

<table>
<thead>
<tr>
<th>NEX</th>
<th>Contralateral grey</th>
<th>Lesion grey</th>
<th>Contralateral white</th>
<th>Lesion white</th>
<th>CSF</th>
<th>Whole brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.24±0.02</td>
<td>0.22±0.02</td>
<td>0.51±0.02</td>
<td>0.46±0.04</td>
<td>0.18±0.07</td>
<td>0.28±0.01</td>
</tr>
<tr>
<td>2</td>
<td>0.20±0.02</td>
<td>0.18±0.03</td>
<td>0.50±0.02</td>
<td>0.43±0.05</td>
<td>0.13±0.06</td>
<td>0.24±0.02</td>
</tr>
<tr>
<td>3</td>
<td>0.18±0.02</td>
<td>0.17±0.03</td>
<td>0.49±0.02</td>
<td>0.42±0.04</td>
<td>0.12±0.05</td>
<td>0.23±0.02</td>
</tr>
<tr>
<td>4</td>
<td>0.17±0.02</td>
<td>0.16±0.03</td>
<td>0.49±0.02</td>
<td>0.42±0.04</td>
<td>0.12±0.05</td>
<td>0.22±0.02</td>
</tr>
<tr>
<td>5</td>
<td>0.17±0.03</td>
<td>0.15±0.02</td>
<td>0.49±0.02</td>
<td>0.41±0.04</td>
<td>0.11±0.05</td>
<td>0.22±0.02</td>
</tr>
</tbody>
</table>

![Figure 2.7](image)

As predicted, the FA values are larger for smaller NEX. This results in nearly isotropic regions, such as CSF, being anisotropic with one NEX. For example, the
FA of CSF is increased by 64% when compared with the diffusion anisotropy value measured using five averages. This effect is less pronounced in regions with high anisotropy such as white matter, where FA just increased by 4%. FA measured in all regions converged to a constant value at 3 NEX, and does not change significantly with 4 or 5 NEX.

2.4.4 Conclusions
This experiment confirms that the stroke DT-MRI data were acquired with sufficient NEX to measure accurately water diffusion parameters. The results show that FA values do not change significantly with 3 or more NEX. This indicates that the acquisition time could potentially be reduced to increase patient compliance, or the available scanning time invested in increasing image quality in another way, such as improving the measurement accuracy of $D$ by using a more advanced gradient sampling scheme. New stroke protocols that consider this and other issues will be developed in Chapter 3.

2.5 Effects of CSF contamination

2.5.1 Introduction
The ROI used in §2.3 were drawn to minimise partial volume averaging effects. This issue has to be considered when measuring diffusion parameters since it has been shown that even a small amount of CSF within a voxel will significantly increase the diffusion value (141). In the present section, it is demonstrated that CSF averaging can also significantly change the measured temporal evolution of both $\langle D \rangle$ and FA after stroke. Since CSF contamination is more likely to occur in cortical grey matter than in white matter this problem was only investigated for grey matter using DT-MRI data from one patient analysed in §2.3. A patient with a stroke lesion conveniently placed near the Sylvian fissure was selected.

2.5.2 Methods
To investigate whether CSF has been included in the selected ROI, histograms of $\langle D \rangle$ values were analysed. Mean values of $\langle D \rangle$ measured in grey matter were $\langle D \rangle\{\text{contr. grey} \rangle = 960 \pm 101 \times 10^{-6}$ mm/s$^2$ and $\langle D \rangle\{\text{lesion grey} \rangle = 678 \pm 116 \times 10^{-6}$ mm/s$^2$ (for
all patients at the first time point), while \( \langle D \rangle_{\text{CSF}} = 2990 \pm 18 \times 10^{-6} \text{ mm/s}^2 \). Therefore, voxels of grey matter including CSF are shown in the histogram as outliers with very high \( D \), while other voxels values should be grouped around the mean value. A qualitative assessment can be performed to identify the outliers and determine whether there is CSF contamination. \( \langle D \rangle \) histograms were checked only at the first time point since at later time points, as \( \langle D \rangle \) starts increasing in the lesion, it is not possible to determine whether the high voxels values are caused by CSF contamination or by the increase of \( \langle D \rangle \) during the evolution of infarcted tissue.

One circular ROI was drawn in grey matter within a stroke lesion next to the Sylvian fissure and in the corresponding contralateral region. These two regions were initially placed in areas with minimal CSF signal as confirmed by the histograms. Then both ROI were moved exactly two voxels closer to the Sylvian fissure to include voxels more likely to be contaminated with CSF (Figure 2.8). These ROI were copied onto the follow up scans and sets of \( \langle D \rangle \) and FA values were obtained at the five time points for each of the ROI. To assess how the level of CSF contamination in either ROI will affect the temporal evolution of the relative values \( \langle D \rangle_R \{\text{grey}\} \) and \( \text{FA}_R \{\text{grey}\} \), the ratios of lesion to contralateral ROI were calculated as follows:

(a) lesion and contralateral ROI with minimal CSF contamination (baseline),
(b) lesion ROI with increased CSF signal and contralateral ROI with minimal CSF contamination,
(c) lesion ROI with minimal CSF signal and contralateral ROI with increased CSF contamination,
(d) lesion and contralateral ROI both with increased CSF contamination.

Statistical analysis for comparison of the \( \langle D \rangle_R \) and \( \text{FA}_R \) values from these ROI was not performed since the sample used for this study (only one of each ROI) was not large enough to test statistical significance. Only a qualitative analysis will be presented here to indicate what artifacts could be introduced in the evaluation of temporal evolution of water diffusion parameters after stroke. A fuller analysis would require, for example, performing the study with both the standard DT-MRI protocol and a CSF-suppressed DT-MRI protocol using fluid attenuated inversion recovery acquisition (FLAIR) in a larger number of subjects.
2.5.3 Results and discussion

Figure 2.9 shows the histograms from the ROI analysis with minimal (a) and increased (b) CSF contamination. Moving ROI slightly into the Sylvian fissure introduces contamination from CSF in some voxels causing the distribution of \( \langle D \rangle \) to spread towards higher values.

![Histograms from original lesion and contralateral ROI.](image)

Figure 2.10 shows clear differences in the temporal evolution for the relative diffusion parameters when the ROI included some CSF. The values of the data points from these graphs are in Table 2.5.
Table 2.5. Values of $D_R$ and $FA_R$ at the five time points for the four possible cases using ROI with and without CSF contamination. In cases (b), (c) and (d) the percentage change with respect to case (a) is shown.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>(a) Minimal CSF</th>
<th>(b) increased CSF in lesion ROI only</th>
<th>(c) increased CSF in contralateral ROI only</th>
<th>(d) increased CSF in both ROI</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 24 h</td>
<td>$D_R$</td>
<td>$FA_R$</td>
<td>$D_R$</td>
<td>$FA_R$</td>
</tr>
<tr>
<td>5</td>
<td>0.63</td>
<td>1.03</td>
<td>32%</td>
<td>-9%</td>
</tr>
<tr>
<td>12</td>
<td>0.92</td>
<td>0.65</td>
<td>8%</td>
<td>18%</td>
</tr>
<tr>
<td>28</td>
<td>1.37</td>
<td>0.45</td>
<td>7%</td>
<td>-16%</td>
</tr>
<tr>
<td>90</td>
<td>1.70</td>
<td>0.53</td>
<td>12%</td>
<td>-9%</td>
</tr>
</tbody>
</table>

Figure 2.10 Temporal evolution of $D_R$ and $FA_R$ calculated as explained in the text.

The evolution of $D_R$ and $FA_R$ is clearly affected by changing the degree of CSF signal in either of the ROI (Figure 2.10 (b) and (c)). The magnitude of the change...
depends on the amount of CSF contamination in each of the ROI at each time point. This may change slightly in each scan due to lesion swelling or small inaccuracies in image registration. As expected, increased CSF signal in lesion ROI increases the value of $\langle D \rangle_R$ at all time points (Figure 2.10 (b)), while increased CSF in the contralateral ROI decreases them (Figure 2.10 (c)). From Table 2.5 we see that in case (b) the largest change occurs at $< 24$ hours, with the value of $\langle D \rangle_R$ increasing by 32% at that time point and ~10% at later time points. This difference might be an effect of swelling of the infarcted tissue after the first day, which could force movement of the fluid out of the lesion, thus reducing the amount of CSF contamination in the ROI at later time points. An effect of this is a change in the slope of the curve between the first and second time points, which now appears to increase at a slower rate. When the CSF contamination is affecting the contralateral ROI more, all the values decreased by ~14 - 21%. The effects from CSF contamination could be potentially cancelled when both ROI are affected (case (d)); however, the different pattern of change found between cases (b) and (c) prevents that, as shown in Figure 2.10 (d) and in Table 2.5. In this particular case, the values of $\langle D \rangle_R$ at all time points decrease, except in the first time point where it increases by 11%, producing overall a temporal evolution of $\langle D \rangle_R$ different than that shown in Figure 2.10 (a) where minimal CSF was included.

$F_{AR}$ values are also affected in different way by CSF contamination in cases (b) and (c) and the overall effect when both ROI include larger amount of CSF was greater than for $\langle D \rangle_R$ (Figure 2.10 (h) and Table 2.5). $F_{AR}$ in the first time point was reduced by 13% and increased by 32% in the second time point as compared to the $F_A$ values from Figure 2.10 (a). This creates a completely different temporal evolution of $F_{AR}$ during the first 15 days; it appears to be reduced at $< 24$ hours while no initial reduction is seen in Figure 2.10 (a). It then decreases gradually until 5 days and then rapidly between 5 and 12 days.

This simple analysis indicates how values and temporal evolution of both $\langle D \rangle_R$ and $F_{AR}$ are affected by CSF contamination.
2.5.4 Considerations on CSF contamination in the study of temporal evolution of diffusion parameters.

An analysis of CSF contamination was also performed in the ROI used in the main stroke study presented in §2.3. Histograms of all lesion and contralateral grey matter ROI were plotted for each patient to assess the degree of potential CSF contamination. Even though care was taken when drawing the ROI to avoid obvious regions of CSF, in 14 out of the 32 patients analysed the \( \langle D \rangle \) histograms indicated some CSF contamination in either lesion or contralateral ROI. The position of all these ROI was corrected to reduce the number of voxels contaminated with CSF. However, as indicated by the final histograms small CSF contamination could not be eliminated completely due to the difficulty of placing the ROI in pure grey and white matter, avoiding mixed tissue and taking into account changes over time such as swelling. This was unlikely to cause significant effects in the temporal evolution of the diffusion parameters derived as an average from the entire group of patients.

The fact that the slices acquired were 5 mm thick increased the difficulty of avoiding CSF since averaging with underlying tissues in each voxel cannot be completely avoided. Thinner slices would be more appropriate to avoid the problem. One of the new DT-MRI acquisition protocols developed in Chapter 3 will consider this issue.

One way of avoiding CSF averaging is to use FLAIR DT-MRI, which has been shown to give reliable \( \langle D \rangle \) and FA measurements (142). Drawbacks of this technique are some loss of SNR and longer scanning times needed for the 180° inversion pulse, which make it less suitable for use in clinical studies. Another possible solution to partial volume averaging is the use of segmentation algorithms to identify grey and white matter regions with minimal CSF signal (143). For example, as described in §1.7.4, tractography techniques could be used to achieve segmentation of brain white matter avoiding contamination with other tissues.

2.6 Conclusions

A precise evaluation of the temporal evolution of water diffusion parameters in ischaemic stroke is mainly subject to the quality of the DT-MRI data and correct positioning of the ROI. We have shown that our DW-EP images were collected with
sufficiently high SNR to avoid the effects of systematic noise bias in the FA measurements. However, the acquisition protocol used to scan stroke patients could be optimised to produce better estimates of $D$ with shorter scan time or to minimise the amount of CSF contamination in ROI analyses.

By following individual patients over time, we have shown that the temporal evolution of $\langle D \rangle$ and FA is different for grey and white matter. Furthermore, the results indicate that studies quantifying changes in diffusion parameters to assess tissue damage or investigate progress of ischaemic stroke should take into account the extent of grey and white matter within the lesion.

The results of this stroke study have been published (144), and the paper is presented in Appendix D.
Chapter 3 Optimised DT-MRI acquisition protocols for stroke

3.1 Introduction

The stroke DT-MRI protocol used in Chapter 2 consisted of 6 directions repeated 5 times to increase SNR. As demonstrated in §2.4, the use of 3 NEX was only just satisfactory to reduce the noise bias in the measurement of FA. By using 3 NEX rather than 5, the scanning time could be reduced for very ill stroke patients. As an alternative, the scanning time could be invested in optimised acquisition protocols. For example, a larger number of diffusion measurements in different directions (N) could be made permitting calculation of $D$ using more robust statistical methods. Also thinner slices could be used to allow the application of tractography algorithms.

The aim of this chapter is to design two clinical acquisition protocols optimised for scanning stroke patients, one of them with near isotropic voxels for tractography applications.

Many different acquisition schemes and optimisation techniques for DT-MRI have been suggested in the literature, and these are reviewed in §3.2. Using examples of data from patients, §3.3 demonstrates the problems arising from scanning acutely ill patients, in particular restless patients that move during the DT-MRI acquisition. In §3.4, we analyse the problem of FA overestimation at low SNR and propose two new acquisition protocols optimised for the scanning of stroke patients based on the results of numerical simulations. Finally, §3.5 presents further numerical simulations that investigate the effects of patient motion during the scan on the accuracy of FA measurements for the original and optimised stroke DT-MRI acquisition schemes.

3.2 Background

3.2.1 The gradient sampling scheme

The minimum number of diffusion measurements in non-collinear directions required to estimate the six independent elements of $D$ is six. However, since the acquisition of DW signals is affected by noise, the use of more than six non-collinear gradient orientations improves the estimation of $D$. Statistical approaches, such as
multivariate linear least squares, can then be used to provide the estimated error for each tensor element (§1.5.2.3).

In addition to the number of gradient directions, their spatial distribution has also been shown to have a strong influence on the estimated $D$, in particular in the rotational variance of the parameters calculated from anisotropic diffusion tensors. Without a previous knowledge of the orientation of $D$ in the tissue of study, a diffusion gradient scheme with gradient directions uniformly distributed in the space should be used (145). Many gradient-encoding schemes have been developed to date, and they can be classified as follows:

3.2.1.1 Heuristic encoding schemes

These are the most commonly used schemes for DT-MRI, and employ a base set of directions defined by the logical $x$, $y$ and $z$ gradients of the scanner, which correspond to the faces of a cube. The unit cube with an edge length of 2, originating at (0 0 0) defines 13 non-collinear directions at the face centres, edge bisectors and body diagonal directions. The heuristic encoding schemes are therefore made of the following building blocks of gradient directions

$$
B_0 = \{(1 0 0) (0 1 0) (0 0 1)\},
B_1 = \frac{1}{\sqrt{2}} \{(0 1 1) (1 0 1) (1 1 0)\},
B_2 = \frac{1}{\sqrt{2}} \{(0 -1 1) (1 0 -1) (1 -1 0)\},
B_3 = \frac{1}{\sqrt{3}} \{(1 1 1) (-1 1 1) (1 -1 1) (1 1 -1)\}. 
$$

- Orthogonal gradient encoding (N = 6) combines the directions of the three orthogonal axes ($B_0$) with the directions of the off-diagonal edges ($B_1$). This scheme is also called ‘pyramidal encoding’ in chemical shift tensor spectroscopy (146).
- Oblique double gradient encoding (ODG) (N = 6) is the scheme described by Basser and Pierpaoli (35) and the one more commonly used in DT-MRI. It is formed by the entire set of edge bisectors ($B_1 + B_2$), and the analytical equations for calculation of the elements of $D$ using this scheme are given in Eq. 1.50.
- Orthogonal/tetrahedral hybrid encoding (N = 7) is a combination of the orthogonal and tetrahedral encoding schemes ($B_0 + B_3$) (148).
Decahedral gradient encoding (N = 10) is the composition of the ODG encoding with the corner directions of the cube (\(B_1 + B_2 + B_3\)), as proposed by Skare and Nordell (149).

Complete heuristic gradient encoding (N = 13): combines all face, edge and corner directions of a cube (\(B_0 + B_1 + B_2 + B_3\)) (150).

### 3.2.1.2 Numerically optimised encoding schemes

For an arbitrary number of acquisition directions used in a DT-MRI experiment, the orientation of the gradients in the scheme can be optimised with respect to a specific criterion. Optimisation criteria are usually intended either to achieve the most uniform distribution of the gradients in three-dimensional space, or to minimise the estimated error in the tensor elements. Some possible criteria are:

- **Minimum force** suggested by Jones *et al.* (145), which considers gradient directions as N pairs of opposing unit charges constrained on the surface of a sphere and maximises their separation by applying the criteria of minimum electrostatic repulsion force between neighbours

\[
F = \sum_{i>j} \sum_{j=1}^{2N} \frac{1}{r_{ij}^2}, \quad \text{Eq. 3.2}
\]

where \(r_{ij}\) is the distance between neighbouring charges. The acquisition schemes generated with this criterion are known as JonesN gradient schemes. A similar criteria was used by Papadakis *et al.* (151) using an ultra-repulsive model

\[
F = \sum_{i>j} \sum_{j=1}^{2N} \frac{1}{r_{ij}^n} \quad \text{with } n \to \infty. \quad \text{Eq. 3.3}
\]

- **Minimum energy**, which is related to the minimum force criterion, minimises the energy from Coulombic energy of the \(2 \times N\) unit charges

\[
E = \sum_{i>j} \sum_{j=1}^{2N} \frac{1}{r_{ij}}. \quad \text{Eq. 3.4}
\]

- **Minimum total variance** (\(TV\)), which minimises the sum of the variances of all the elements \(D_{ij}\) calculated from \(M^{-1}\) in Eq. 1.58, \(TV\) has the form

\[
TV = \sum_{i,j} \sigma_{D_{ij}}^2, \quad \text{Eq. 3.5}
\]

and has been used by Papadakis *et al.* to generate gradient schemes with 6, 12 and 24 orientations (152,153).
Minimum condition number of the encoding matrix was developed by Skare et al. (154). As described in §1.5.2.2, the signal intensity observed in the \( i^{th} \) orientation is given by Eq. 1.45, which can be rewritten as

\[
\ln \left( \frac{A_i}{A_0} \right) = -b a_i \mathbf{D}, \tag{3.6}
\]

or

\[
a_i \mathbf{D} = -\frac{1}{b} \ln \left( \frac{A_i}{A_0} \right) = ADC_i, \tag{3.7}
\]

where \( ADC_i \) is the apparent diffusion coefficient measured in the \( i^{th} \) direction and

\[
a_i = \left( g_{ix}^2, g_{iy}^2, g_{iz}^2, 2g_{ix}g_{iy}, 2g_{ix}g_{iz}, 2g_{iy}g_{iz} \right). \tag{3.8}
\]

For a DT-MRI acquisition scheme using \( N \) gradient directions

\[
\mathbf{A} = (\alpha_1, \alpha_2, \ldots, \alpha_N)^T, \tag{3.9}
\]

which is known as the design matrix, and can be calculated from the elements of \( \mathbf{D} \) by solving the system of equations

\[
\mathbf{D}^{-1} = \mathbf{A}^{-1} \mathbf{ADC}^1, \tag{3.10}
\]

where \( \mathbf{ADC} \) is the vector of the \( ADC_i \) measurements. The condition number associated with this linear equation is defined as

\[
\text{cond}(\mathbf{A}) = \left\| \mathbf{A} \right\| \left\| \mathbf{A}^{-1} \right\|, \tag{3.11}
\]

and provides a limit on how inaccurate the estimated elements of \( \mathbf{D} \) will be after numerical solution of the equation. The condition number also amplifies the error present in the diffusion measurements. Thus, if \( \text{cond}(\mathbf{A}) > 1 \), the error propagated into the elements of \( \mathbf{D} \) has become larger than the measurement error by an amount which depends only on the directions of the DW gradients. Thus, \( \text{cond}(\mathbf{A}) \) can be used to quantify the noise performance of the acquisition scheme, with a low \( \text{cond}(\mathbf{A}) \) indicating a good performance. The optimisation of the gradient scheme using the condition number criterion consists of a downhill simplex minimisation procedure (DSM) that reduces the value of \( \text{cond}(\mathbf{A}) \) for a given number of gradients \( N \) (154).
3.2.1.3 Geometric polyhedra schemes

These use directions defined by vertices, faces or edges of polyhedra with three-dimensional symmetry.

- The icosahedral family is composed of triangular faces, with five faces intersecting at each vertex. The vertices are the set $V_{\text{icosa}}$ of 12 points in $\mathbb{R}^3$ obtained from the vertices of three mutually perpendicular golden rectangles (155),

$$V_{\text{icosa}} = \{(\pm 1, \pm \tau , 0), (0, \pm 1, \pm \tau ) , (\pm \tau ,0, \pm 1)\}, \quad \text{Eq. 3.12}$$

where $\tau = \frac{1 + \sqrt{5}}{2}$ is the golden ratio. The 12 vertices give six non-collinear gradient encoding directions. Face triangulation can provide sets with $N = 5n^2 + 1$ for $n = 1, 2, 3\ldots$ ($N = 6, 21, 46\ldots$).

- The dodecahedron is the dual of the icosahedron, with pentagonal faces and three faces intersecting at each vertex. The vertices are given by the centre of the 20 faces of the icosahedron,

$$V_{\text{dodeca}} = \{(0, \pm 1/\tau, \pm \tau), (\pm \tau, 0, \pm 1/\tau), (\pm 1/\tau, \pm \tau, 0)\}. \quad \text{Eq. 3.13}$$

This set gives an $N = 10$ scheme (156).

- Other polyhedra generated from truncation of the icosahedron may also be used to design gradient encoding schemes. For example, truncation of the vertices produces the buckyball, which is constructed from 20 regular hexagons and 12 regular pentagons. It has 60 vertices giving an encoding scheme with $N = 30$ directions and produces the $C_{60}$ structure of pure carbon molecules known as fullerenes (155).

- Both icosahedra and dodecahedra have 30 edges which yield an $N = 15$ encoding scheme. Concatenation of directions given by each polyhedron or combination of directions from both structures produces encoding schemes with $N = 16$ (6+10), 21 (6+15), 25 (10+15), 36 (30+6), 40 (30+10), etc. (156). All the different configurations belong to the icosahedral group (Icosa) and have the same cond($A$) of 1.58.
3.2.2 Comparison of encoding schemes performance

Several authors have presented comparative studies of the performance of the different acquisition schemes described above, with the aim of establishing the optimal scheme in terms of number of diffusion measurements and configuration of the gradient directions (150-154,156,157). This issue was first addressed by Papadakis et al. in 1999 (152) who studied various DT-MRI sampling schemes in terms of the variance of DAI as a function of tensor orientation. They compared two well-established six-gradient direction schemes - orthogonal gradient encoding and ODG encoding (4 NEX each) - to a scheme with 24 non-collinear gradient directions (1 NEX). Their results showed that, using the same scanning time, the 24-direction scheme outperformed the 6-direction schemes improving the rotational invariance of the DAI, decreasing noise, improving SNR and decreasing noise-induced bias of the AI in low-anisotropy areas. These effects were reflected in the general quality of the anisotropy maps as shown in their paper.

Papadakis et al. (153) also compared the performance of different DT-MRI acquisition schemes used for the determination of the six independent components of $\mathbf{D}$. They assessed the precision of the acquisition by measuring the propagation of noise from the initial DW signals by means of a scalar index $\kappa$, which depends only on the gradient directions employed by the scheme. This index was proportional to the total variance of the elements of $\mathbf{D}$ for an isotropic medium, assuming the same $b$-value for each gradient direction. Therefore the smaller the index, the better the noise behaviour of the scheme. They tested three commonly used gradient schemes, ODG, orthogonal and a seven-direction gradient scheme (formed by the orthogonal plus the (1,1,1) direction), and found the ODG scheme had the best noise performance. In this study a new optimisation method using minimisation of the index $\kappa$ was introduced to develop a new acquisition scheme with $N = 12$ gradient directions. Simulations, phantom and human experiments showed that the optimised scheme gave the smallest errors and showed the smallest dependence of noise on the degree of diffusion anisotropy.

Skare et al. (154) also compared the performance of several encoding schemes (tetrahedral, ODG, decahedral, icosahedral and schemes developed by Jones (145,158) and Papadakis (153) with $N = 10,\ldots,30$). $\text{cond}(\mathbf{A})$, was used to rate the
performance of the previous schemes, and they found that the tetrahedral scheme had
the highest $\text{cond}(A)$ and produced the noisiest FA maps. The schemes obtained in
this study by minimising $\text{cond}(A)$ were not as robust as other schemes, such as Jones
or Icosa, in terms of orientation independence. The reason for this was that $\text{cond}(A)$
on its own does not fully characterise the errors in FA since they are also dependent
on the relative orientation between the tensor principal direction and the gradient
direction. Therefore $\text{cond}(A)$ may not be sensitive to the advantage of increasing the
number of directions (154,159). Skare et al. (154) concluded that to reduce the
rotational variance of FA, a large number of evenly distributed DW gradients is also
needed as well as a small $\text{cond}(A)$.

All these authors agreed that increasing the number of gradient directions in the
acquisition scheme reduces the rotational variance of FA. However, none of them
established a minimum number of directions above which increasing $N$ further does
not provide any significant benefit. Papadakis et al. (151), in a later study, optimised
the minimum number of gradient directions necessary for estimation of $D$ using the
ultrarepulsive force criteria for choosing the directions. They studied the variation of
SNR (defined as mean value/standard deviation) of three DAI (FA, RA, VR) with
increasing $N$. They found that SNR decreases asymptotically with $N$ reaching a
minimum $N_0$ such that for $N > N_0$ SNR shows negligible change. However, $N_0$
increased with the diffusion anisotropy of the tissue to be measured. They found that
$N_0$ was in the range $N = 18-21$ for a fibre model with $D$ eigenvalues corresponding to
the extreme values of the experimentally measured principal diffusivities in healthy
brain.

On the other hand, Hasan et al. (150) compared the performance of different
scheme-optimisation criteria (minimum TV, minimum force, minimum potential
energy and minimum $\text{cond}(A)$), and the regular polyhedra scheme including
variations of the icosahedron in terms of the variance of $D$. They found that the
commonly used 6-direction schemes (orthogonal and ODG) were suboptimal and
that any of the optimisation criteria could produce a 6 direction scheme functionally
equivalent to the icosahedron scheme. The results from their simulations suggested
that the icosahedron (or any equivalent scheme calculated through optimisation) is
optimum for 6 directions. However, in contradiction with previous studies, they
found no significant advantage in using more than 6 encoding directions once the encoding scheme is optimised.

Batchelor et al. (156) supported Hasan's result by proving that the icosahedral direction scheme has the same properties with respect to propagation of noise as ideal uniform infinite sampling, both producing the same $\text{cond}(A)$. However, the assumptions used to make this statement were later proved to be wrong (160). Experiments by Batchelor et al. (156) also showed that for icosahedral schemes with 30 directions the SD of FA is both low and nearly independent of the tensor orientation. They recommended the use of an icosahedral gradient scheme, using the largest number of directions achievable in the available scanning time.

All these studies have focused on the performance of the acquisition schemes and their influence on the measurement of diffusion anisotropy. To date only the study by Jones (157) has also looked at the effects of gradient encoding schemes on $\langle D \rangle$ and tensor orientation as well as on FA measurements. He used Monte Carlo simulations to study both the influence of the number of gradient directions on an optimised encoding scheme, and the advantage of using 'efficient' gradient schemes (which make efficient use of the gradient power) over conventional 'unit-sphere' schemes in the calculation of those parameters. To test the effect of varying the number of gradient directions he used the JonesN (145) with $N \in \{6,10,12,15,20,30,60\}$. To test the benefit of using these efficient schemes, he compared JonesN $(N/N\text{EX}) = (30/1)$ with an efficient double-gradient scheme, and an efficient icosahedral scheme, both $(N/N\text{EX}) = (6/5)$. To assess the effects on $\text{Tr}(D)$ and FA, the conventional mean and SD for all the simulations was calculated for each orientation of $D$ tested. The distribution of the principal eigenvector at each orientation was characterised by the 95% cone of uncertainty (CU), computed as in Jones et al. (161). The 95% CU was taken as the 95th percentile. He found that the benefit for the CU of increasing $N$ was more marked for higher FA and lower SNR. As in Papadakis et al. (151), he observed an asymptotic behaviour in the reduction of CU SD with increasing $N$. The value of $N$ after which an increase does not make a significant difference to SD was

\[ N_{\text{MC}} = 10000. \]

\[ N_{\text{MC}} \text{ values of the angle are sorted from smallest to the largest and the one in the 9500 position is the 95th percentile for } N_{\text{MC}} = 10000. \]
found to be 30. The reduction in the SD of FA for the largest FA tested (0.9) did not improve for N > 20. For Tr(D), the reduction in the SD did not improve for N > 30. These benefits were again more marked in more anisotropic tensors. When the 95% CU was compared using the 'unit-sphere' Jones30 scheme with the 'efficient' dual gradient and icosahedral schemes (both with N = 6), he found that Jones30 produces the flattest response. The efficient dual-gradient scheme reduced the mean CU while the efficient icosahedral scheme increased the CU. He concluded that, depending on the application, there is a trade off between minimising the measurements bias by using more directions or increasing the SNR by using efficient schemes. However, when the scanning time is limited and only the minimum number of 6 directions can be acquired an efficient scheme should be used.

### 3.3 Problems scanning acutely ill stroke patients: analysis of patient movement

As suggested in the literature, an optimised protocol for DT-MRI should acquire diffusion measurements in 20-30 uniformly distributed directions to obtain relatively rotational invariant DT-MRI data, even for a high degree of diffusion anisotropy (157). However, in a clinical situation where the scanning subjects might be very ill, the scanning protocol is limited by the tolerance time of the patient. This is considered to be 15-20 minutes (162). Moreover, due to patient movement during the acquisition, the actual relative direction of each measurement could be modified, affecting the uniform distribution of the acquisition gradients on the space. As an example of patient movement during a scan, Figure 3.1 shows the rotations around three orthogonal axes during the acquisition of 30 DW volumes in a restless stroke patient. The angles were calculated from the registration matrix obtained after registering each of the DW volumes to the initial baseline T₂-weighted volume using rigid body FLIRT (137). The diagram on the right identifies each of the angles plotted in the graph. Since the acquisition numbers on the abscissa are in order of acquisition, we observed that this patient kept nearly still during the first 12 DW volumes; beginning to move his/her head after that, mainly with rotations around the z-axis.
Figure 3.1 Rotation angles of the head of a stroke patient during the acquisition of 30 DW volumes

As a consequence of movement, the patient’s head position could change between acquisitions of the gradient orientations and thus the relative gradient orientations might be different to those in the intended acquisition scheme. This might produce a suboptimal acquisition, as compared with the theoretical one. We can compare the prescribed orientations with the actual ones after considering patient movement by plotting the gradient orientations on the sphere (Figure 3.2). We used the rotation angles for the patient in Figure 3.1 to illustrate how different acquisition schemes (ODG, Icosa6, Icosa10 and Icosa30) would be affected in a real case of patient random motion. The number of N × NEX gradient in each scheme was chosen to be 30 in total to match the motion data available. In each case, the direction of the \( i \)-th gradient \((g_{ix}, g_{iy}, g_{iz})^T\) was rotated by the \( i \)-th set of \((\theta, \phi, \psi)\) angles obtained from the patient using the composite rotation matrix in the pitch-roll-yaw convention (163)

\[
\begin{pmatrix}
\cos \psi_i & \sin \psi_i & 0 \\
-\sin \psi_i & \cos \psi_i & 0 \\
0 & 0 & 1 \\
\end{pmatrix}
\begin{pmatrix}
\cos \phi_i & 0 & -\sin \phi_i \\
0 & 1 & 0 \\
\sin \phi_i & 0 & \cos \phi_i \\
\end{pmatrix}
\begin{pmatrix}
1 & 0 & 0 \\
0 & \sin \theta_i & \cos \theta_i \\
0 & -\sin \theta_i & \cos \theta_i \\
\end{pmatrix}
\begin{pmatrix}
g_{ix} \\
g_{iy} \\
g_{iz} \\
\end{pmatrix}
= \begin{pmatrix}
g'_{ix} \\
g'_{iy} \\
g'_{iz} \\
\end{pmatrix} \quad \text{Eq. 3.14}
\]

In Figure 3.2, dark blue lines with filled dots represent the original gradient orientations, while the light colour lines with open dots represent the acquired orientations for each NEX. We noticed significant differences between prescribed and acquired sampling directions, especially in those acquired at later time as can be seen in those acquisitions with more than one NEX.

The change in gradient orientations can be assessed analytically by using the mean dot product (\(\bullet\)) between the orientation vectors of the acquired gradients and the
vectors of the corresponding original gradient schemes. Table 3.1 shows the results for each NEX of the four acquisition schemes. As expected, in this particular case, the change in the gradient orientations during the first 12 acquisitions was negligible, producing a dot product of 1.0 in NEX 1 and 2 of ODG and Icosa6, and NEX 1 of Icosa10. NEX of any scheme including later acquisitions reduced the mean dot product as a consequence of the misalignment between the prescribed and actual acquisition vectors.

Figure 3.2 Representation of the original gradient directions (dark blue) of different gradient schemes and of the new gradient directions acquired in each NEX modified by movement of the patient presented in Figure 3.1 during the acquisition. The total number of DW acquisitions is 30 with different number of NEX in each acquisition scheme. (a) 5 NEX acquired with oblique double gradient scheme, (b) 5 NEX acquired with a 6 orientation Icosahedral scheme, (c) 3 NEX acquired with a 10 orientation Icosahedral scheme, and (d) 1 NEX acquired with a 30 orientation Icosahedral scheme (buckyball)

The effect of the change in orientation of the sampling gradients on the calculation of D due to the motion of this patient during the scan can be evaluated using cond(A) (Eq. 3.11) and TV (Eq. 3.5). As explained in §3.2.1.2, cond(A) represents the amplification of the measurement error into D and TV is the sum of the errors in the estimated tensor elements. Therefore, increases in either of these parameters imply
larger inaccuracy in the calculation of $D$ with the modified acquisition scheme. Table 3.1 shows the results for the cases presented in Figure 3.2.

Table 3.1 Numerical analysis of the acquisition schemes shown in Figure 3.2. The second column provides the values of cond(A) and $TV$ for the prescribed acquisition scheme. The following columns show the values of the mean dot product, and the % change in cond(A) and $TV$ for each NEX of the schemes after modification of gradient orientations by patient movement.

<table>
<thead>
<tr>
<th>Original values for scheme</th>
<th>NEX 1</th>
<th>NEX 2</th>
<th>NEX 3</th>
<th>NEX 4</th>
<th>NEX 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>• ODG</td>
<td>1.000</td>
<td>1.000</td>
<td>0.988</td>
<td>0.988</td>
<td>0.984</td>
</tr>
<tr>
<td>cond(A)</td>
<td>2.00</td>
<td>2.5 %</td>
<td>1.3 %</td>
<td>10.1 %</td>
<td>1.6 %</td>
</tr>
<tr>
<td>$TV$</td>
<td>6.00</td>
<td>0.07 %</td>
<td>0.01 %</td>
<td>0.51 %</td>
<td>-1.05 %</td>
</tr>
<tr>
<td>• Icosa6</td>
<td>1.000</td>
<td>1.000</td>
<td>0.988</td>
<td>0.988</td>
<td>0.981</td>
</tr>
<tr>
<td>cond(A)</td>
<td>1.58</td>
<td>0.3 %</td>
<td>0.3 %</td>
<td>-0.4 %</td>
<td>2.0 %</td>
</tr>
<tr>
<td>$TV$</td>
<td>4.88</td>
<td>0.01 %</td>
<td>-0.12 %</td>
<td>0.19 %</td>
<td>0.83 %</td>
</tr>
<tr>
<td>• Icosa10</td>
<td>1.000</td>
<td>0.991</td>
<td>0.984</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cond(A)</td>
<td>1.58</td>
<td>0.5 %</td>
<td>6.0 %</td>
<td>8.1 %</td>
<td></td>
</tr>
<tr>
<td>$TV$</td>
<td>2.93</td>
<td>0.18 %</td>
<td>2.34 %</td>
<td>2.30 %</td>
<td></td>
</tr>
<tr>
<td>• Icosa30</td>
<td>0.991</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cond(A)</td>
<td>1.58</td>
<td>3.9 %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$TV$</td>
<td>0.98</td>
<td>1.17 %</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

cond(A) generally increases as compared with the values obtained in the original schemes, with the increase up to 8-10 % in some NEX. The $TV$ also increases in most cases, although this increase is smaller in NEX acquired within the first 12 DW volume acquisitions. Since patient movement is random, its effect on the acquisition scheme will also be unsystematic. However, since the intended acquisition schemes are numerically or spatially optimised, it is expected that a random modification of gradient orientations produces a less optimal acquisition, increasing cond(A) and $TV$, although reduction of these parameter could also happen as we found in the example.

The cond(A) results indicate that patient movement affects more the acquisition schemes with smaller N (ODG, Icosa6). Conversely, the increase in $TV$ in ODG and Icosa6 is generally small, which suggests that these acquisition schemes are less sensitive to the introduction of movement effects. These results were calculated using motion data from a single subject as an example and therefore they may not be completely representative. A complete analysis of the effect of patient movement on cond(A) and $TV$ using particular gradient schemes will corroborate these results (§3.5).
To estimate the range of patient movement, images from 20 stroke patients who were scanned acutely were randomly selected from those included in the stroke study in Chapter 2. Using rigid body FLIRT, all DW images in each patient were registered to the first T₂-weighted acquisition, and the rotation angles in each case were obtained from the registration matrix. The histograms in Figure 3.3 show the ranges of the absolute (θ,φ,ψ) angles in degrees obtained for the 20 patients, as defined in Figure 3.1. Thanks to the effective immobilisation of the patient's head during the scan, the rotations were in general very small with the median rotations being θ_{median} = 0.3°, φ_{median} = 0.2° and, ψ_{median} = 0.6°. However, we cannot ignore the presence of cases where rotations were much larger, in particular rotations around the z-axis. The worst case in this patient group was defined as that given by the 95th percentile of each angle, with those for the whole group of patients being θ_{95} = 3°, φ_{95} = 2° and, ψ_{95} = 7°.

A representation of patient movement data from 20 stroke patients with respect to the order of DW volume acquisition reveals that patients are more liable to move in later rather than earlier acquisitions (Figure 3.4). In Figure 3.4, the median of the absolute values of the angles obtained for all patients at each acquisition was used in order to avoid both the cancellation of positive with negative angles and the skewing of the data due to outliers. A short optimised acquisition with a small number of
gradients will therefore avoid the largest movement artifacts that might occur during the scan. However, this will imply a trade off with the higher SNR and directionality independence of the parameters derived from $D$ achieved when many gradient orientations are used.

![Figure 3.4 Median rotation values at each acquisition from a group of 20 stroke patients scanned acutely.](image)

**3.4 Optimised acquisition protocols for stroke subjects**

**3.4.1 Introduction**

As detailed in §2.3.1, the original stroke DT-MRI protocol consisted of 6 directions with ODG encoding repeated 5 times to increase SNR. The number of slices acquired was 15, with 5 mm thickness and 1 mm slice gap. This was designed to obtain matched slice positions between DT-MRI and PW-MRI stroke protocols so that diffusion and perfusion parameters could be measured simultaneously. The field-of-view was 240×240 mm$^2$ and the matrix size 128×128 (zero-filled to 256×256). This generated 0.94×0.94×5 mm$^3$ voxels suitable for the calculation of scalar water diffusion parameters, but not suitable for tractography methods that could potentially assess white matter tract volume and integrity following an ischaemic stroke. Previous studies looking at the performance of acquisition schemes (§3.2.2) also suggested that ODG sampling is not the optimal for calculation of $D$ due to its inferior noise propagation properties and rotational invariance.

Here we design two new stroke protocols using optimised gradient schemes, one of them with near-isotropic voxels that would allow the use of tractography methods.
The first optimised stroke protocol (Stroke_A) is a modified version of the original protocol with the same acquisition parameters and same slice thickness of 5 mm and 1 mm gap. The voxel size of $0.9 \times 0.9 \times 5$ mm$^3$ ensures good SNR and resolution properties which permits the display of conventional (D) and FA maps. This protocol will still match the slice positions of the PW-MRI protocol. A new optimised gradient direction scheme will be introduced with the aim of reducing scanning time while improving SNR, and rotational variance of the parameters calculated from D.

The second optimised stroke protocol (Stroke_B) is designed to acquire images with voxels as close to isotropic as possible on our clinical scanner, with the objective of enabling the use of tractography techniques in stroke patients. The minimum slice thickness and output matrix size achievable on our GE Signa LX 1.5 T scanner using single-shot spin-echo EPI are currently 2.8 mm and $128 \times 128$. These limitations make it impossible to acquire images with completely isotropic voxels. With these settings the best 'near-isotropic' voxel size for a conventional field-of-view of $240 \times 240$ mm is $1.9 \times 1.9 \times 2.8$ mm$^3$. Although the voxel dimensions are not ideal and a small bias towards the z-direction of the calculated tracts might be expected, tractography methods have proved to be successful using similar image resolution (58). Another advantage of using thinner slices is the reduction of through-slice CSF contamination of voxels, which increases the accuracy of $\langle D \rangle$ and FA measurements in brain tissues (§2.5) The use of the Stroke_B protocol in a combined DW- and PW-MRI study would involve the modification of the slice thickness in the PW-MRI acquisition to 5.6 mm, if matching slice positions and volume coverage were required.

3.4.2 Selection of the number of diffusion measurements

3.4.2.1 Background

The total number of DW images $N$ determines the SNR properties of the diffusion parametric maps. These can be acquired in different orientations or using repetitions of a small number of orientations. The effect of SNR on the measured FA obtained in the original stroke protocol was investigated in §2.4. It was found that the overestimation of FA in low anisotropy tissues was considerably reduced with 3 NEX, while not being significantly further reduced with either 4 or 5 NEX. The SNR
experiment shown in Figure 2.7 was duplicated in a healthy volunteer (Figure 3.5) using 1.9×1.9×2.8 mm³ image resolution suggested for the new Stroke_B protocol and ODG acquisition averaged different numbers of times; the abscissa represents the values of SNR produced by averaging the corresponding number of NEX. FA was measured in ROI in white matter, grey matter, CSF and the whole brain, avoiding areas with partial volume averaging, susceptibility artifacts or pulsatile motion. The graph shows that there is no further reduction in FA after 5 NEX for the less anisotropic tissues, which corresponds to a SNR of approximately 20.

![Figure 3.5 Variation of FA values with the SNR of the DW images acquired with 1.9×1.9×2.8 mm³ resolution. The corresponding number of NEX is indicated above each data point in the graph.](image)

The benefit of averaging in the calculation of FA and the principal diffusion direction can be checked using eigenvector maps (§1.7.3). Figure 3.6 shows maps of the corpus callosum calculated from the DT-MRI data acquired in the volunteer described above. Data acquired with 1 NEX produces many 'black' voxels in the underlying FA map that correspond to estimated D with negative eigenvalues. Vector arrows are represented only in voxels with FA > 0.3 which correspond mainly to white matter (Figure 3.5). However, using 1 NEX, many arrows appear in voxels in the ventricles where CSF should have a very low FA; this corroborates the overestimated FA measurements shown in Figure 3.5. The number of black voxels decreases with increasing number of NEX, as well as the number of voxels with high
FA in CSF areas. The white matter fibre orientation is better defined in the 5 NEX map than in the 1 NEX and 3 NEX maps, where arrows are more randomly orientated. No further improvement is observed when increasing the NEX to 10.

![1 NEX](image1)
![3 NEX](image2)
![5 NEX](image3)
![10 NEX](image4)

Figure 3.6 FA map of the corpus callosum with overlying principal diffusion direction vectors calculated in each voxel with FA threshold of 0.3. The corresponding number of NEX is indicated above each figure. Voxels where estimated D is not positive definite are filled in black.

The results presented above suggest that scanning time should be invested in acquiring several NEX to increase SNR of the DW images. Nevertheless, different authors have recommended that for a given scanning time a larger number of sampling directions should be preferred to repeating and averaging a low N (156,157). These studies investigated the rotational variance of FA and established that its SD decreases as N increases, which would reinforce the significance of patient-control differences in diffusion parameters (157). However, none of them determined whether the overestimation of FA was reduced when a larger N was employed in the acquisition. Only Papadakis et al. (151), using numerical simulations, investigated the changes in mean value with N for three different DAI, as well as SD and SNR of parametric maps. They found that both SD and SNR improved with increasing N, decreasing the former and increasing the latter. However, their results did not show noticeable improvement in the overestimated
mean value of any of the DAI with larger N. Papadakis et al. (151) only tested these parameters for a single FA value of 0.91. Unfortunately, the overestimation of FA at low SNR affects mainly low anisotropy values and it is less noticeable at such a high FA value, thus a minimal improvement would be expected from increasing N in that case. Moreover, the estimated mean values of the three DAI used for their experiments were always lower than the actual value. Hence their results exhibit underestimation at low SNR as opposed to the overestimation generally found by other studies (41,71,164,165).

3.4.2.2 Mean FA vs number of unique diffusion sampling orientations

We performed specific numerical simulations to assess the effect of increasing N on the calculated FA for Icosahedral acquisition schemes with $N \in \{6,10,15,21,30,45,60\}$ for five different levels of FA $\in \{0.1, 0.3, 0.5, 0.7, 0.9\}$. $\mathbf{D}$ was simulated using a constant trace value similar to that found in brain tissues $\text{Tr}(\mathbf{D}_0) = 2100 \text{ mm}^2/\text{s}$ (166) with the eigenvalues given by (157)

$$\lambda_i = \frac{\text{Tr}(\mathbf{D}_0)}{3} \left( 1 + \frac{2\text{FA}}{\sqrt{3 - 2\text{FA}^2}} \right),$$

$$\lambda_2 = \lambda_3 = \frac{\text{Tr}(\mathbf{D}_0)}{3} \left( 1 - \frac{\text{FA}}{\sqrt{3 - 2\text{FA}^2}} \right).$$

Eq. 3.15

$\mathbf{D}$ was rotated to produce 100 different orientations of the principal diffusion direction uniformly distributed over one hemisphere. Complex Gaussian noise was added in quadrature to the $T_2$-weighted ($S_0$) and DW ($S$) signals, the latter calculated with $b = 1000 \text{ s/mm}^2$. The noise factor was constant and equivalent to a SNR of 20 in $S_0$ and 11 in $S$. This was repeated 1000 times for each orientation of $\mathbf{D}$. Mean FA was calculated from $\mathbf{D}$ estimated from each of the noisy numerical signal samples for each value of N. The results are shown in Figure 3.7.
Our simulations showed overestimation of FA due to noise as expected, particularly at low FA values. When the number of diffusion sampling orientations increases, FA decreases towards the true value. FA of 0.1 and 0.3 decreases markedly with increasing N. However, they never reach the true FA value even for N up to 60. By contrast, mid to high FA converge to their true values at N of approximately 20. Previous studies have shown that using a large number of diffusion sampling orientations produces more rotationally invariant FA measurements (156,157). Our results demonstrated that this also produces more accurate mean FA values by reducing the overestimation caused by noise in the diffusion signals.

3.4.2.3 Selection of N

Based on the previous results and the background literature we select the number of unique gradient sampling directions for the new stroke protocols as follows:

*Stroke A*

The number of gradient orientations in this protocol is selected as a compromise between the accuracy of FA measurements and scanning time. The scan time for a single DW volume on our scanner is approximately 20 s, with TR = 17 s and 48 slices. Therefore N = 21 could be acquired in 7 min, a time well within the tolerance of 15-20 minutes for neurological patients (162), and shorter than the original stroke protocol (approx. 10 min). This would allow some spare time to perform other MRI
modalities in the same scan session if necessary. The SD of the FA does not decrease further with $N > 20$ (151,157) and, according to our simulations, the mean FA is close to the true value for $FA > 0.5$ and overestimated by less than 8 % for $FA = 0.3$. The overestimation of $FA = 0.1$ is larger, however in the normal brain this value will usually be found only in CSF.

3.4.3 Selection of the acquisition scheme

It is not uncommon that stroke patients participate in multi-modality MRI studies, which considerably increases the total scanning time, and hence the possibility that the scan will need to be abandoned before the whole set of diffusion directions has been collected. Since it is not generally possible to predict for how long a patient will tolerate an MRI scan, our new stroke acquisition protocols will be based on the idea suggested by Dubois et al. who used optimised subsets of gradient directions to acquire DT-MRI from restless patients (168).

Concatenation of orientations defined by elements of the icosahedral group produce sets of gradient directions with icosahedral symmetry, which have been shown to be optimised for the calculation of $D$ (156). We can use these combinations...
to generate a scheme with a larger number of orientations \( N \), built from smaller optimised subsets of gradient directions. In the situation where the scan was interrupted or patient motion increased before all the \( N \) orientations were acquired, \( D \) could be calculated using the diffusion samples from the first optimised subset/s. Some of the basic building blocks of orientations with icosahedral symmetry and combinations of them are listed in Table 3.2.

**Table 3.2 Possible gradient orientations with icosahedral symmetry**

<table>
<thead>
<tr>
<th>Building blocks of gradient orientation with icosahedral symmetry</th>
<th>Number of orientations</th>
<th>Described by</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 Vertices of the icosahedron</td>
<td>6</td>
<td>Vertices of the icosahedron</td>
</tr>
<tr>
<td>10 Vertices of the dodecahedron</td>
<td>10</td>
<td>Vertices of the dodecahedron</td>
</tr>
<tr>
<td>15 Edges of the icosahedron</td>
<td>15</td>
<td>Edges of the icosahedron</td>
</tr>
<tr>
<td>30B Vertices of the truncated icosahedron (buckyball)</td>
<td>30B</td>
<td>Vertices of the truncated icosahedron (buckyball)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gradient orientation built from combinations</th>
<th>Number of orientations</th>
<th>Built from</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 6 + 10</td>
<td>16</td>
<td>6 + 10</td>
</tr>
<tr>
<td>21 6 + 15</td>
<td>21</td>
<td>6 + 15</td>
</tr>
<tr>
<td>25 10 + 15</td>
<td>25</td>
<td>10 + 15</td>
</tr>
<tr>
<td>31 6 + 10 + 15</td>
<td>31</td>
<td>6 + 10 + 15</td>
</tr>
<tr>
<td>36 6 + 30</td>
<td>36</td>
<td>6 + 30</td>
</tr>
<tr>
<td>40 10 + 30</td>
<td>40</td>
<td>10 + 30</td>
</tr>
</tbody>
</table>

We use encoding schemes within this group of geometric polyhedra to define the acquisition of the stroke protocols as follows:

**Stroke A**: \( N = 21 \) orientations, built from Icosa6 and 15, named Icosa21.

**Stroke B**: \( N = 30 \) orientations can be obtained from the vertices of the truncated icosahedron (also known as buckyball). However, this set is optimised as a whole and we cannot guarantee that a subset of measurements will be optimised in case of interruption of the scan. Using the optimised subsets of 6, 10 and 15 orientations, we can define a 31-orientation scheme for this protocol, named Icosa31.

The sets of orientations chosen for the new protocols are represented in Figure 3.8. The ODG scheme used in the original protocol is also represented for reference.
To assess quantitatively the improvement in the calculation of $\mathbf{D}$ introduced by the use of Icosa21 and Icosa31, as compared with the ODG scheme, $\text{cond}(\mathbf{A})$ and $TV$ considering the measurement of noise unity (169), were calculated. The results are shown in Table 3.3.

Table 3.3 Theoretical values of condition number and sum of diffusion tensor variances for three gradient acquisition schemes

<table>
<thead>
<tr>
<th>Gradient scheme</th>
<th>$\text{cond}(\mathbf{A})$</th>
<th>$\sum_{i,j} \sigma_{D_{ij}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oblique double gradient</td>
<td>2.00</td>
<td>6.00</td>
</tr>
<tr>
<td>Icosa21</td>
<td>1.58</td>
<td>1.39</td>
</tr>
<tr>
<td>Icosa31</td>
<td>1.58</td>
<td>0.94</td>
</tr>
</tbody>
</table>

As described in §3.2.1.2, $\text{cond}(\mathbf{A})$ predicts the amplification of the error present in the diffusion measurements into $\mathbf{D}$ due to the choice of the gradient orientations. We found an improvement in $\text{cond}(\mathbf{A})$ with the acquisition schemes selected for the new protocols, which decreased from 2.00 to 1.58 in both cases. The improved estimation of $\mathbf{D}$ manifests itself in a reduction of the sum of the variances of the tensor elements. This last parameter also reflects the advantageous noise properties given by the increase of $N$ to which $\text{cond}(\mathbf{A})$ is insensitive (159).

3.4.4 Selection of $b$-values and number of baseline measurements

Kingsley and Monahan (170) recommend a $b$-value of 1000 s/mm$^2$ to obtain the optimum diffusion-to-noise ratio for discrimination of ischaemic and normal brain tissues in the acute imaging of stroke patients. They found that the optimum $b$-factor increases slightly when imaging stroke in subacute stages. However, their recommendation is to keep $b = 1000$ s/mm$^2$ if, for simplicity, just one $b$-value is to be used in all situations. This value is within the range of $b$-values [700,1000] s/mm$^2$ recommended by Alexander and Barker (171) for the recovery of one fibre direction.
with the typical diffusivity of brain tissue. This $b$-value is also within the recommended range to obtain the best estimates of FA, $[950,1100]$ s/mm$^2$, and just slightly lower that the range recommended for the estimation of Tr($\mathbf{D}$), $[1100,1500]$ s/mm$^2$. The $b$-value of 1000 s/mm$^2$ has shown in the current literature to be a good compromise for the calculation of various diffusion parameters and is, therefore, the value to be used in the two new stroke protocols.

Several authors have estimated the ideal number of baseline measurements per number of gradient directions to be acquired for the accurate calculation of $\mathbf{D}$ (145,164,171). The most recent simulations by Alexander and Barker (171) suggested a ratio of approximately 5 between high to low $b$ measurements as a good compromise to estimate $\mathbf{D}$. Therefore, the numbers of baseline $T_2$-weighted measurements chosen in the new stroke protocols are:

- Stroke_A: $M = 4$
- Stroke_B: $M = 6$

These will increase the scanning time of the protocols by approximately 1.3 min and 2 min respectively.

### 3.5 Effects of subject movement during scanning on the stroke DT-MRI acquisition protocols

#### 3.5.1 Introduction

Movement of the subject during the scanning process may produce suboptimal acquisition of the diffusion data. This would manifest itself as an increase in cond($\mathbf{A}$) and $TV$ of the estimated $\mathbf{D}$, as was shown in §3.3. In addition, the modification of the actual $\mathbf{B}$-matrix acquired, as compared to the prescribed $\mathbf{B}$-matrix associated to the acquisition scheme, introduces errors in the calculation of $\mathbf{D}$, which suggest that the $\mathbf{B}$-matrix should be corrected before the calculation.

The aims of the present section are:
- To check the effect of subject motion on parameters derived from $\mathbf{D}$ when the $\mathbf{B}$-matrix is not corrected.
- To check the effect of subject movement on parameters derived from $\mathbf{D}$ when the $\mathbf{B}$-matrix is corrected.
To check whether these effects are less significant using the new suggested DT-MRI stroke acquisition protocols as compared with the original acquisition.

### 3.5.2 Methods

Numerical simulations were performed using the acquisition schemes from the original stroke DT-MRI protocols (ODG) and the two new protocols (Icosa21 and Icosa31) for five different levels of FA \( \in \{0.1, 0.3, 0.5, 0.7, 0.9\} \). The flow chart in Figure 3.9 represents the operation steps in the simulation process and Table 3.4 the parameters used.

- For each sampling scheme and FA value tested, the original \( B_0 \)-matrix \( (B_0) \) and a diffusion tensor \( D_0 \) were calculated. \( D \) was simulated as in §3.4.2.

#### Table 3.4 Parameters used in the numerical simulations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Acquisition schemes</th>
<th>b-value (s/mm(^2))</th>
<th>FA values</th>
<th>( \alpha (^\circ) )</th>
<th># Random rotations of scheme</th>
<th># Rotations of ( D_0 )</th>
<th>Noise repetitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>ODG, Icosa21, Icosa31</td>
<td>1000</td>
<td>0.1, 0.3</td>
<td>0.5, 0.7, 0.9</td>
<td>1, 3, 5,..., 19</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Patient movement in each acquisition is simulated with equal probability in any direction. This is done by rotating each of the original orientations of the sampling gradients by a fixed angle \( \alpha \), with the direction of the rotation applied to each gradient being random and independent of the others. Figure 3.10 (a) shows 50 random rotations of the ODG acquisition scheme sampling directions with \( \alpha = 10^\circ \). Since the variation angle \( \alpha \) is fixed, all the random modifications of one original
gradient direction lay on the surface of a cone with opening angle $2\alpha$ as shown in Figure 3.10. A total of 100 random rotations of the scheme sampling directions were performed for each $\alpha$ tested with $\alpha = 1^\circ, 3^\circ, 5^\circ, \ldots, 19^\circ$.

- For each random rotation of gradient orientations, the new $\mathbf{B}$-matrix was calculated; this represents the actual acquisition matrix for a gradient scheme when random patient movement takes place during the scan. The mean $\text{cond}(\mathbf{A})$ and $\text{TV}$ for each of the modified acquisition schemes was calculated to compare the optimisation of the actual with the original acquisition scheme.

![Figure 3.10](a) Distribution of the random rotations of the sampling scheme directions in ODG for $\alpha=10^\circ$. (b) Orientations of $\mathbf{D}$ used in the simulations.

- With each new $\mathbf{B}$-matrix, new diffusion signals $\mathbf{S}$ were calculated from Eq. 1.45 using 100 different orientations of the principal diffusion direction of $\mathbf{D}$ uniformly distributed over the hemisphere as shown in Figure 3.10 (b). These were obtained by rotating $\mathbf{D}_0$.

- Complex Gaussian noise is added in quadrature to $\mathbf{S}_0$ and the new set of signals $\mathbf{S}$. The noise factor was set to obtain a SNR of 20 in the unweighted signal $\mathbf{S}_0$. This is repeated 1000 times for each orientation of $\mathbf{D}$.

- The diffusion tensor estimated from each of the noisy numerical signal samples $\mathbf{D}_{\text{est}}$ is calculated by multivariate linear regression using the original $\mathbf{B}_0$. This permits the assessment of the error present in the measurement of $\mathbf{D}$ if the $\mathbf{B}$-matrix is not recalculated when subject motion occurs during the scan.
• The mean $\text{FA}_{\text{est}}$ is calculated from the 1000 noisy $\mathbf{D}_{\text{est}}$, for each rotation of $\mathbf{D}_0$ in each random modification of the scheme. The mean and SD of $\text{FA}_{\text{est}}$ is then calculated for all rotations of $\mathbf{D}_0$.

• For each $\alpha$ and FA value selected, the mean and standard deviation of $\text{FA}_{\text{est}}$ is then calculated for all the rotations of $\mathbf{D}_0$ and the 100 random rotations of the sampling gradients to assess the rotational variance of this parameter.

3.5.3 Results and discussion

Figure 3.11 shows plots of mean $\text{FA}_{\text{est}}$ (first column) and its SD (second column) obtained from the numerical simulation of subject movement in ODG, Icosa21 and Icosa31 acquisition schemes using different levels of FA. This figure shows, therefore, the effects on the estimation of $\mathbf{D}$ without re-calculation of the $\mathbf{B}$-matrix when subject movement occurs during scanning. In these Mean($\text{FA}_{\text{est}}$) plots, the dotted lines represent the true FA and the markers the mean FA estimated during the simulations for the 100 random modifications of the scheme, 100 rotations of $\mathbf{D}_0$ and 1000 times iterations adding noise to the signals. At the lowest diffusion anisotropy values, $\text{FA}_{\text{est}}$ is overestimated as an effect of noise. This effect is reduced as the number of unique sampling directions in the scheme increases, corroborating the results from §3.4.2.2. At large values of $\alpha$, the mean $\text{FA}_{\text{est}}$ is underestimated and this effect is more pronounced when more sampling directions are used. This could be a consequence of the smaller angles between sampling directions when the number of directions is larger, meaning that when $\alpha$ is sufficiently large a random rotation of each of the sample gradient directions could make two or more gradient directions in the scheme coincide, thus providing the same measurement of $S$. When $\mathbf{D}$ is then estimated using the original $\mathbf{B}$-matrix the algorithm would 'believe' that diffusion is the same in different directions, which would result in $\mathbf{D}$ being more isotropic than it should be. No other justification was found for this effect. However, it suggests that in the case of very large subject movement during DT-MRI scanning (rotations above $10^\circ$) when using Icosa21 and Icosa31 the $\mathbf{B}$-matrix should be corrected to avoid underestimation of FA, especially in anisotropic brain structures.
Figure 3.11 Mean and SD values of $\text{FA}_{\text{est}}$ obtained in three different acquisition schemes with different degrees of simulated subject motion.

The SD of $\text{FA}_{\text{est}}$, and therefore the rotational variance of the estimated $\mathbf{D}$, increases with increasing $\alpha$ for the three acquisition schemes, particularly for larger diffusion anisotropy values, although the increase is less rapid for Icosa21 and Icosa31 compared to ODG. In particular with $\alpha = 20$ and for the same $\text{FA} = 0.9$, the $\text{SD}(\text{FA}_{\text{est}})$ is 40% lower for Icosa31 than for ODG. The rotational variance of acquisition schemes was previously found to improve with increasing number of unique sampling directions in the DT-MRI acquisition scheme (153,154,157). However, the effect of motion during the scan on optimised acquisition schemes has
not been investigated before. The present work demonstrates the advantage of using more sampling directions when subject movement occurs during the scan.

![Figure 3.12 Variation of FA\textsubscript{est} as a function of the tensor orientation for FA = 0.9 for (a) ODG, (b) Icosa21 and (c) Icosa31. (d) for a subject random rotations during the acquisition with $\alpha = 7^\circ$. Plot of the SD(FA\textsubscript{est}) in each case for different FA values](image)

The surface plots in Figure 3.12 represent the variation of FA\textsubscript{est} as function of the diffusion tensor orientation with FA = 0.9 for random rotations of the sampling gradients with $\alpha = 7^\circ$. This angle was chosen for this example since it was found to be the largest rotation in the patient group analysed in §3.3 given by \(\psi_{95}\). The polar and azimuthal angles ($\theta, \phi$) on the axes indicate the direction of the principal direction of $D$. The estimated value of FA is clearly less dependent on the orientation of $D$ for the two Icosa acquisition schemes as compared to ODG at this FA value. The graph in (d) represents mean SD(FA\textsubscript{est}) calculated from 100 random gradient direction rotations for this and the other FA values as a function of the number of sampling directions in the acquisition scheme. For FA = 0.9 the variation of FA decreases by approximately 50 % in the 21 and 31 direction schemes as compared to the 6 direction scheme showing the advantage of the two new DT-MRI acquisition protocols stroke_A and stroke_B over the original protocol to scan ill, restless patients.
The plots in Figure 3.13 show the mean and SD of the \( \text{cond}(A) \) and \( TV \) from the 100 random rotations of the gradient orientations of ODG, Icosa21 and Icosa31 as a function of the rotation angle \( \alpha \). These graphs reflect, therefore, the effects on the estimation of \( D \) when re-calculation of the \( B \)-matrix is performed. Both parameters depart from their original values, increasing as \( \alpha \) increases, particularly in ODG. The change in the mean total variance of the elements of \( D \) with \( \alpha = 20^\circ \) is 14 % and 10 % for Icosa21 and Icosa31 respectively with small standard deviations, while the increase with ODG is of 350 % with very large standard deviation. This demonstrates that the optimisation and noise propagation characteristics of the two Icosa acquisition schemes remain nearly unchanged even for larger \( \alpha \). The optimisation of ODG was initially poorer than that of the Icosa21 and 31 schemes as a consequence of the lower number of unique sampling directions. Furthermore, the data obtained from the simulations reveal that for large \( \alpha \) the optimisation of ODG deteriorates dramatically. The error bars show that random rotations of the ODG sampling gradients could produce an improvement in the acquisition scheme by producing lower \( \text{cond}(A) \) and \( TV \), as we observed in Table 3.1. However, the mean values show that they generally produce suboptimal acquisition schemes. These numerical simulations support the examples in §3.3 by showing an increase in \( \text{cond}(A) \) and \( TV \) caused by movement due to the acquisition of signals with a suboptimal set of sampling directions.

As mentioned above for the case of large patient motion, the \( B \)-matrix should be re-calculated when using Icosa21 and Icosa31 to avoid underestimation of high FA values. Figure 3.13 confirms that this will not affect significantly the error propagated into \( D \) estimated with the new \( B \). Neither \( \text{cond}(A) \) or \( TV \) increased significantly for Icosa21 or Icosa31 when using the recalculated \( B \)-matrix even for large \( \alpha \).
3.5.4 Conclusions

We have demonstrated with numerical simulations that subject motion during the scan process affects the robustness of the calculation of $D$. This is demonstrated by an increase in the rotational variance of the estimated FA values when the $B$-matrix is not re-calculated. This effect is caused by the use of a $B$-matrix different to the one corresponding to the actual acquisition sampling directions ($B_0$) and, as expected, the larger the angle of subject motion, the larger the increase in FA variance. The effects are less pronounced for the two new acquisition protocols proposed to scan acutely ill stroke patients. However, for very large patient rotations ($\alpha > 10^\circ$) it could produce underestimated values of FA in very anisotropic tissues. This degree of motion is unlikely to occur in properly immobilised patients since the largest rotation
found in a subgroup of 20 stroke patients was 7°. In addition, the simulations showed that when the \( B \)-matrix is re-calculated for Icosa21 and Icosa31 acquisition protocols, the error propagated into \( D \) remains unchanged compared to the originally optimised acquisition schemes. However, \( B \)-matrix recalculation of the ODG old stroke protocol would deteriorate significantly the optimisation of the acquisition scheme.

### 3.6 Conclusions

The effects of patient motion during the scan in an optimised acquisition scheme have been illustrated in this chapter using real data from stroke patients scanned acutely. Taking into account the limitations that scanning these patients involve, two new DT-MRI stroke acquisition protocols have been proposed, a summary of whose acquisition parameters are shown below.

**Table 3.5 New protocols for scanning acute stroke patients**

<table>
<thead>
<tr>
<th></th>
<th>( b ) (s/mm(^2))</th>
<th>N</th>
<th>Configuration</th>
<th>M</th>
<th>TR (ms)</th>
<th>Slice thickness (mm)</th>
<th>Matrix size</th>
<th>Voxel size (mm(^3))</th>
<th>Acquisition time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stroke_A</strong></td>
<td>1000</td>
<td>21</td>
<td>Icosa6+15</td>
<td>4</td>
<td>17000</td>
<td>5 +1 gap</td>
<td>256x256</td>
<td>0.9x0.9x5</td>
<td>8.3</td>
</tr>
<tr>
<td><strong>Stroke_B</strong></td>
<td>1000</td>
<td>31</td>
<td>Icosa6+10+15</td>
<td>6</td>
<td>17000</td>
<td>2.8</td>
<td>128x128</td>
<td>1.9x1.9x2.8</td>
<td>12.3</td>
</tr>
</tbody>
</table>

The first of these protocols, *Stroke_A*, is a legacy stroke protocol with improved acquisition scheme and acquisition time, permitting matching of DT-MRI and PW-MRI slice positions.

*Stroke_B* is designed to allow the use of tractography methods in the acquired diffusion data. It, therefore, has a voxel size as close to isotropic as possible on our GE Signa LX 1.5 T scanner.

Both protocols use a larger number of sampling orientations than the old stroke protocol. New numerical simulations revealed that this not only decreases the rotational variance of the calculated diffusion parameters, but also reduces the overestimation of FA at low SNR.

The acquisition schemes used in both protocols are composed from fully optimised smaller acquisition blocks which allow the estimation of \( D \) in case of interruption of the scan before the whole acquisition is completed. As soon as the first optimised block is acquired, \( D \) can be reconstructed thus avoiding wasting of data. It is worth observing, however, that the estimated \( D \), and parameters derived from it, in this case
would be less reliable than those obtained when the full set of sampling directions is acquired.

The performance of the acquisition schemes in both new DT-MRI stroke protocols, with respect to patient motion during the scan, was tested and compared to the acquisition scheme used by the original stroke protocol. Numerical simulations generally showed a more robust calculation of $D$ with the new schemes. It was observed that at a high degree of subject motion (rotations of over $10^6$), the FA of very anisotropic tissues could be underestimated by these acquisition schemes, in which case re-calculation of the $B$-matrix before calculation of $D$ should be performed. The simulations also show that the optimisation of the schemes is not significantly affected by the random rotations of the sampling directions that may occur during patient motion.
Chapter 4 Methods for quantitative assessment of image registration techniques

4.1 Introduction

One of the problems met during the acquisition and analysis of the stroke DT-MRI data was patient movement during the scan. Chapter 3 dealt with the optimisation of the acquisition protocol in order to reduce the errors in the calculation of $D$ due to motion. However, even with an optimized acquisition protocol, movement produces misalignment of the images that prevent the accurate voxel-by-voxel calculation of $D$. Eddy current induced distortions in the component EP images also introduce misalignment artifacts. Both subject motion and EC induced distortions can be corrected for at the post-processing stage by image registration.

When using registration techniques to correct for motion and EC induced artifacts in EP images, it is difficult to measure to what extent misalignments have been corrected for. The improvements in image registration given by the correction method are usually verified qualitatively. However, qualitative assessment does not usually give enough information to compare different methods and thus select the one with the best performance. The aim of this chapter is, therefore, to establish quantitative methods for the assessment of image registration techniques used to remove both bulk patient motion and EC induced artifacts.

4.2 Assessment of image registration methods

4.2.1 Qualitative assessment of image registration

Qualitative methods for assessing the registration of the component images of a DT-MRI study are the most straightforward. The aim is to find ways to identify misregistration artifacts either in the original images or in maps of scalar diffusion parameters derived from $D$. Some methods for qualitative assessment of the image registration are described below.
4.2.1.1 Image subtraction

Image subtraction is the simplest method since it highlights all the differences between two images and has been used to show improvement in the image alignment after applying a registration algorithm (172-174). As described in §1.8.2 EC induced distortions predominantly appear in the phase encoding direction of DW images while the baseline T₂-weighted images remain unchanged. Misalignment caused by EC is, therefore, easily identified as a halo at the top and the bottom of the brain when intensity normalised DW and T₂-weighted EP images are subtracted as shown in Figure 4.1.

![Figure 4.1 Subtraction of a DW from its corresponding T₂-weighted EP image](image)

Figure 4.1 Subtraction of a DW from its corresponding T₂-weighted EP image shows misregistration artifacts caused by EC in the phase encoding direction. Both images were normalised by their mean before subtraction.

4.2.1.2 Off-diagonal elements of the tensor

One of the effects of motion and EC distortions introduced in the DW images acquired with different gradient directions is that the spatial location of each voxel is different from one image to another and also different from the T₂-weighted image. These cause errors in the voxel-by-voxel calculation of D since it combines signals from all T₂- and DW images acquired in the dataset. This effect is more severe at the edges of the brain where D is calculated using signal from voxels that may be randomly located inside or outside brain tissue in DW images acquired with diffusion gradients applied in different directions. The off-diagonal elements of D represent correlations of water diffusion in two directions and clearly show these misregistration effects. These artifacts manifest themselves in the form of apparent correlations of water diffusion at the edges of the brain and tissue boundaries (173,175), as shown in Figure 4.2.
4.2.1.3 Diffusion anisotropy maps

Artifacts caused by incorrectly registered T2 and DW signals used for calculation of \( D \) are propagated into the calculation of the scalar parameters derived from it such as \( \langle D \rangle \) (172,176) and FA (173,175). These artifacts are manifested as a rim at the edge of the brain. In diffusion anisotropy maps, they appear as a bright rim consistent with high water diffusion anisotropy.

However, this rim does not correspond to any real anatomical structure (Figure 4.3 (a)). Other areas of high diffusion anisotropy corresponding to white matter fibres appear diffuse and ill defined and relatively high values of FA are found inside the
ventricles where low anisotropy from CSF is expected. After registration, the edge artifacts disappear and the fibres in the image look sharper and better defined (Figure 4.3 (b)).

4.2.1.4 $\chi^2$ and $R^2$

There are several approaches that can be used to calculate $D$ from $T_2^*$- and DW images, with the most widely used being linear least-squares fitting of the logarithm of the signals (§1.5.2.3). These methods are based on the minimisation of $\chi^2$, a function of the sum of the squares of the deviations between the measured and the predicted intensities weighted by a factor that depends on the fitting approach used. Maps of $\chi^2$ produced by the fitting procedure can be created. These will show higher intensity in voxels where the signals measured do not properly fit the tensor model, and therefore $\chi^2$ is not minimised. If the component DT-MRI images are misaligned, signals in different DW images might come from very different tissue type or even from background, preventing optimum fitting and producing an increased $\chi^2$. Figure 4.4 shows examples of $\chi^2$ maps from corrected and uncorrected images.

![Figure 4.4 $\chi^2$ maps obtained from the tensor fitting of uncorrected images, first row, and the same images after distortion and motion correction (from Rohde et al. (66))](image)

The correlation coefficient $R^2$ is a quantity that indicates the quality of a least-squares fitting. Its maximum value is 1, when the optimisation produces a perfect fit of the signals to the model, and decreases as quality of the fitting deteriorates. Like
Chapter 4 Methods for quantitative assessment of image registration techniques

\( \chi^2 \) maps, \( R^2 \) maps will show darker voxels where signals do not fit properly to the tensor model as consequence of DW image distortions.

4.2.2 Quantitative assessment of image registration

4.2.2.1 Consistency tests

The robustness of any general registration algorithm determines whether given the same source image \( I \), and reference image \( R \), the result is independent of the starting point or state of \( I \). Robustness is a necessary, but not sufficient, condition to ensure the good performance of the algorithm and other tests are necessary to give a measure of the accuracy of the registration. Robustness can be assessed quantitatively by testing the consistency of the algorithm, although it is important to note that consistency tests do not give an absolute estimate of robustness. However, they are useful for comparing different registration algorithms, and therefore provide tools to evaluate quantitatively the performance of image-based registration methods.

Consistency testing of a registration algorithm can be performed by applying several different affine transformations to the initial image, in order to obtain several different starting points of the registration (137). That is, given the initial image \( I \) and \( k \) transformations \( T_i \) for \( i = 1, \ldots, k \) we obtain \( k \) different initial source images

\[ T_i \cdot I = T_i \cdot I, \]

Eq. 4.1

to be registered to the reference image \( R \). If the transformations given by the registration for each of the starting images are \( \{ R_i : R_i \cdot T_i = R \} \), it can be combined with its respective initial affine transformation \( T_i \) as

\[ R_i \cdot T_i \cdot I = C_i \cdot I, \]

Eq. 4.2

and each composite transformation \( C_i \) is compared quantitatively with the transformation \( R_0 : R_0 \cdot I = R \) using the root mean square deviation between them. This is calculated in 2D as

\[ E_{\text{RMS}} = \sqrt{\frac{\int_{S_1} |\Delta x_1|^2 \, dx_1}{\int_{S_1} \, dx_1}}, \]

Eq. 4.3

in all voxel positions \( x_1 \) of \( I \) inside the surface \( S_1 \) that can be approximated by a circle in the case of a brain image (177). The error vector \( \Delta x_1 \) is the deviation in mm.
obtained when applying separately each of the two different transformations to be compared, in this case

$$\Delta x_i = (C_i - R_0)x_1.$$  \hspace{1cm} \text{Eq. 4.4}

A robust algorithm should not depend on the initial state of the registered image, and should yield transformations $C_i$ which are nearly identical to $R_0$ and therefore produce a very small $E_{\text{RMS}}$.

An easier way of testing the robustness of the correction algorithm is to find differences between the transformation given by registration and the transformation obtained by registration when the image to be registered and the reference image are swapped (178). Given a distorted image $I$ and reference image $R$, firstly $I$ is registered to $R$ yielding the forward correction transformation $T_{IR}$. If the registration algorithm is then applied to $R$ using $I$ as a reference image, the reverse transformation obtained $T_{RI}$ should ideally be equal to $T_{IR}^d$. In practise, the reverse transformation is not the inverse of the original (forward) transformation and the differences between them show inconsistencies of the correction algorithm. Quantification can then be achieved using the consistency error (178)

$$\epsilon_{IR} = \max_{x_i} \| (T_{RI} T_{IR}) x_1 - x_1 \|_2,$$  \hspace{1cm} \text{Eq. 4.5}

where $\| \|_2$ is the second order norm. Eq. 4.5 returns the maximum difference of voxel position in mm between $I$, and $T_{IR}T_{RI}$. When using a robust registration algorithm, the product $T_{RI}T_{IR}$ will be close to the identity and therefore $\epsilon_{IR}$ will be small. In practice, this error is calculated for all DW images in the data set (all NEX and diffusion directions). The mean and SD of the error is calculated in mm and quoted for each slice position.

When performing this test, we need to rule out the possibility that a small error is merely caused by insignificant transformations produced by an algorithm that is not in fact correctly registering the images. Therefore, we should verify that the mean displacement of every voxel position is not negligible when applying either the forward or the reverse transformation. The mean displacement is calculated using the equation (178)

$$r_{IR} = \max_{x_i} \left\{ \| T_{IR} x_1 - x_1 \|_2, \| T_{RI} x_1 - x_1 \|_2 \right\},$$  \hspace{1cm} \text{Eq. 4.6}
and we take the mean of $r_{IR}$ across all the NEX and diffusion directions for each slice position. To eliminate both bulk subject motion and EC induced distortions we would expect displacements of at least a few mm.

We introduce here another parameter that shows not only consistency errors in voxel position as detected by the consistency test described above, but also the effect of the registration on the intensity values of each voxel in the image. The mean intensity error is equivalent to the consistency error in the sense that the combined forward and reverse transformation are applied to the image. In this case the mean voxel-by-voxel difference of the signal intensity ($s_i$) between the double transformed and the original images is quoted as a percentage of the mean signal intensity of the original image ($\overline{s}$).

$$\overline{\sigma}_{IR} = \frac{(T_{IR}T_{RI})s_i - s_i}{\overline{s}} \times 100.$$ \hspace{1cm} Eq. 4.7

It is expected that a small intensity error will be obtained due to interpolation, as shown in Figure 4.5. The original image (a) was transformed using linear interpolation and a magnification factor $M$ of 0.96, a translation factor $T$ of -3 voxel and a shear factor $S$ of 0.01 voxels/column (typical values of EC induced distortions found in DW images (175)). The inverse transformation ($M = 1.04$, $T = 3$, $S = -0.01$) was applied to the resulting image and the double-transformed image (b) compared with the original.

![Figure 4.5](image_url)

**Figure 4.5** Synthetic image showing the effects of interpolation. (a) Original image, (b) image after applying a double transformation composed of a transformation and its inverse.

The two images are not identical since although the overall transformation applied is the identity matrix, the interpolation applied twice produces a smoothing effect on
image (b). Although visually apparent, the overall effect is very small and $\bar{\sigma}_{\text{IR}}$ in this case is -0.2 %. The mean intensity error in this case reveals how important the "smoothing" of the image that occurs due to the interpolation steps during image transformation is. The application of both the forward and reverse transformations would not be necessary if the only goal is to assess the effects of interpolation – a single transformation would then suffice. However, $\bar{\sigma}_{\text{IR}}$ defined as in Eq. 4.7 helps to identify the source of error that contributes to the consistency error since registration errors might become evident in the intensity of the signals. Examples of this will be shown in §5.4

### 4.2.2.2 Principal component analysis

**Introduction**

Principal component analysis (PCA) is a common technique for finding patterns in data of high dimensionality. Using PCA, the variables from a data set are transformed into a new reference frame where the variables are uncorrelated. This new system of co-ordinates is given by the eigenvectors of the covariance matrix sorted by the order of magnitude of its corresponding eigenvalues. The new uncorrelated variables called principal components (PC), are therefore ordered such that the first component holds the maximum variation present in the original data, the second PC holds the second maximum variation, and so on (179). A simple example of PCA in a data set with two dimensions is plotted in Figure 4.6. In the original data (a), the observations in both variables are highly correlated and the variation is large along both directions, although is slightly larger along $x_1$ than along $y_1$. Transforming into PC (b), the new variables become uncorrelated. Now most of the variance of the observations is along the direction of the first co-ordinate $x_2$ with little along $y_2$.

PCA can be applied to a dataset formed by all $T_2$- and DW images acquired in a DT-MRI experiment to obtain new uncorrelated images. These PC images will show patterns in the original images ordered according to their contribution to the total variance of the whole set of images. As we will see, the percentage of the total variance held by each of the PC can be used as an indicator of the accuracy of a registration algorithm. As in consistency testing, PCA does not provide an absolute
measurement of the accuracy. However, it does allow comparison of different methods to correct for motion and EC induced distortions in DT-MRI data.

**Figure 4.6 Principal component analysis**

finds a new reference frame where the first direction points to the maximum variance of the original variables (a). The transformed variables (b) are uncorrelated and ordered in terms of the variance in each one.

### Calculation of principal components

Given \( p \) vector random variables \( x_i, \ i=1\ldots p \), we can form a \( p \times 1 \) vector \( X = (x_1,\ldots,x_p)^T \). In PCA, \( p \) linear functions \( y_i \) of the elements of \( X \) are calculated

\[
y_i = a_i^T X = \sum_{j=1}^{p} \alpha_{ij} x_j , \tag{4.8}
\]

where each \( a_i \) is a vector of \( p \) coefficients, and each of the linear functions \( y_i \) is a PC such that \( y_1 \) is the linear function having the maximum variance and \( y_i \) is the linear function having the \( i^{th} \) maximum variance being uncorrelated (orthogonal) to all the previous \( y_1,\ldots,y_{i-1} \).

The PC will be given by an orthogonal transformation \( A \) such that when applied to the vector \( X \) the covariance matrix of the transformed vector is diagonal
\[ Y = A^T \cdot X \] such that \( \Lambda = \left( Y - \nu \right) \left( Y - \nu \right)^T \) = \( \begin{pmatrix} \lambda_1 & \cdots & \lambda_p \end{pmatrix} \), \[ \text{Eq. 4.9} \]

where \( Y = (y_1, ..., y_p)^T \) is the vector of PC, \( \nu \) is the expected value of \( Y \) and \( \Lambda \) the covariance matrix in its diagonal form. The \( (\lambda_1, ..., \lambda_p) \) eigenvalues represent the variances of each of the transformed elements in \( Y \) and all the off-diagonal elements of the new covariance matrix are zero. The new transformed variables are therefore uncorrelated with each other. The transformation \( A \) is given by the eigenvectors of \( \Lambda \) and calculated as follows.

The first step for the computation of the PC is the calculation of the covariance matrix from the set of variables as

\[ \Sigma = \left( (X - \mu)(X - \mu)^T \right), \] \[ \text{Eq. 4.10} \]

where \( \mu \) is the expected value of \( X \) given by the values of each of the component variables, \( \mu = (\mu, ..., \mu_x)^T \). The square matrix \( \Sigma \) of dimension \( p \) can be expressed in a new base of vectors such that the matrix is proportional to the identity, \( \Lambda = \lambda I \). Its eigenvalues \( (\lambda_1, ..., \lambda_p) \) are therefore given by the roots of the characteristic polynomial

\[ |\Sigma - \lambda I| = 0. \] \[ \text{Eq. 4.11} \]

The calculated eigenvalues of \( \Lambda \) must be sorted in magnitude such that \( \lambda_1 \) is the biggest and \( \lambda_p \) the smallest. The respective eigenvectors \( a_i \) can be calculated by using the relationship

\[ \Sigma a_i = \lambda_i a_i. \] \[ \text{Eq. 4.12} \]

The transformation between the original random variables and their PC is then formed by these eigenvectors

\[ A = (a_1, ..., a_p)^T, \] \[ \text{Eq. 4.13} \]

where \( a_1 \) is the vector corresponding to the highest variance in the data and \( a_p \) corresponds to the lowest. Therefore, the transformed variables expressed in the new set of base vectors will be the PC ordered so that \( y_1 \) holds the maximum variance of the set of variables and \( y_p \) the minimum variance.
PCA of DT-MRI images

PCA is commonly applied to find patterns in images, for example in the computation of eigenfaces for face recognition (180). An example of eigenfaces is shown in Figure 4.7. Each of these images represents one of the eigenvectors that forms the transformation that diagonalises the covariance matrix of a dataset formed by pictures of men's faces. This transformation identifies statistical patterns in the data and is useful for recognising faces.

![Figure 4.7 Example of eigenfaces (from Zhang et al. (180))](image)

The PC of a set of images will therefore show the uncorrelated patterns that are repeated in the whole set ordered from the most repeated pattern (first PC) to the least repeated pattern (last PC). The percentage that each component contributes to the total variance occurring in the set of images can be calculated from the eigenvalues of the covariance matrix as

\[
\text{% Variance}_j = \frac{\lambda_i}{\sum_{j=1}^{l} \lambda_j} \times 100.
\]

Eq. 4.14

In the case that all images in the analysed set are identical, the percentage of the variance of the first PC would be approximately 100 % (maximum correlation between images) and the contribution of the higher order components to the variance would be minimal, displaying only noise effects. Any difference between the images will be expressed as a new pattern in the PC, increasing the percentage contribution to the variance of the higher order components and decreasing that of the first PC. A simple example is illustrated in Figure 4.8. A set of six identical images of a grey
square on a black background (with added Gaussian noise of 1%) were analysed using PCA. As shown in (a) the first PC holds nearly all of the variance in the data set (99.9%), since this pattern is repeated in all the images and no other pattern exists in them. When some images in the set are modified so that they are different from the rest, (b) and (c), the total variance is shared between several components.

Analogously, PCA can be applied to a DT-MRI dataset to assess the quality of the registration between the component images. A DT-MRI data set is formed by a minimum of one $T_2$-weighted and six DW images. Commonly, however, the set includes more than seven images, since DW images are typically acquired in more than six directions, several $T_2$-weighted images are collected, and multiple averages are performed to increase SNR. A characteristic of this dataset is that the range of variation in intensity values in the $T_2$-weighted images is larger than in the DW images (e.g. about four times larger for $b = 1000 \text{ s/mm}^2$). This means that the variance in $T_2$-weighted signal intensity will be larger (about eight times for $b = 1000 \text{ s/mm}^2$) and will dominate the variance in rest of the images. Using the covariance matrix, PCA would give greater weight to the images with higher maximum intensity ($T_2$-weighted), and hence greater signal variability, and less to the images with lower intensities (DW). This effect can be avoided by using the correlation instead of the covariance matrix in the analysis. This is done by standardising, or dividing by the standard deviation, each of the variables in $X$ before the calculation

$$X^* = (x_1^*, \ldots, x_p^*)^T \text{ with } x_i^* = \frac{x_i}{\sigma_i}. \quad \text{Eq. 4.15}$$

PCA is then performed using standardised variables $X^*$. The properties of the PC calculated with the covariance matrix also hold for the correlation matrix, with the advantage that the PC do not depend now on the absolute value of correlations but only on their ratios (179).
Figure 4.8 PCA of a set of synthetic images. First column shows the original images, second column the PC images and the third column the percentage of the total variance held by each PC. If all the original images are identical the first PC holds all the variance of the data, while higher order PC only show noise effects (a). When image 5 is magnified in the x-direction, the second PC highlights the difference between this and the other five identical images (b). If more than one image is modified such as by translation (4th image), magnification (5th image) or rotation (6th image) more higher order PC increase their percentage variance, showing the new patterns in the set of images, while the % variance of the first PC decreases (c).
PCA is applied to the DT-MRI dataset as follows. Let $p$ be the total number of images in a DT-MRI dataset with image size $N \times N$. The vector of variables in this case can be computed by expressing each of the images as a vector $x_i$ of dimension $N^2$ where the rows of voxel intensity values in the image $(i_1,...,i_N)$ are placed one after another to form a one-dimensional image. The resulting vector of variables will look like

$$X = (x_1, ..., x_p)^T = \begin{pmatrix} i_{11} & \cdots & i_{1N} & i_{1N+1} & \cdots & i_{12N} & i_{12N+1} & \cdots & i_{1N^2} \\ \vdots & & \vdots & \vdots & & \vdots & \vdots & & \vdots \\ i_{p1} & \cdots & i_{pN} & i_{pN+1} & \cdots & i_{p2N} & i_{p2N+1} & \cdots & i_{pN^2} \end{pmatrix}.$$  

Eq. 4.16

The vector $X$ is then standardised as in Eq. 4.15, and the PC are calculated as described above from Eq. 4.8 to Eq. 4.13. The percentage of the total variance in each of the components is then calculated from Eq. 4.14 and used to compare the performance of different registration methods. In particular, the percentage of the total variance in the first PC ($\% \text{ var}(\text{PC}_1)$) can be used to compare quantitatively the accuracy of the registration.

When PCA is applied to a DT-MRI dataset, the PC obtained will show patterns within the component images. The most repeated pattern is expected to be related to the $T_2$-weighting contrast present in all baseline and DW-EP images, since they are all $T_2$-weighted to some extent. Differences between each of the images in the set will then be given by the diffusion weighting and diffusion anisotropy effects caused by changes in the direction of measurement for each DW image. Directionality effects, as well as misregistration effects and noise, are most likely related to higher order components since the effects are different for images acquired in different directions. If misregistration is eliminated, the contribution of the higher order components to the total variance will be reduced, increasing the percentage of the variance corresponding to the first components. This can be used as a tool to assess quantitatively the registration of the images in a DT-MRI dataset.

Synthetic DT-MRI data is used in Figure 4.9 as an example of PCA. The noise-free $T_2$- and DW images were generated using the Montreal Simulated Brain Database (181) as in Bastin (175) with no added noise.

(a) PCA was first applied to the original perfectly registered images (for convenience only six images, the $T_2$- and five DW, are shown in the figure). In the second row,
the PC show repeated patterns in the data, mostly related to the contrast in the images. The third row shows the percentage of the total variance in each of the first six PC. In the example shown in Figure 4.8, 99.9% of the variance was contained within the first PC. Here, though, since synthetic brain images present more patterns the variance is shared between the first three components with the percentage of the total variance held by the first PC being 83.8%.

(b) The synthetic DW images in the dataset were distorted to simulate EC effects using combinations of a magnification factor of 1.05, a translation factor of 5 voxels and a shear factor of 0.02 voxels/column. The distortion in each of the synthetic DW images was a different combination of these factors so none of them were registered either with each other or with the synthetic T_2-weighted image. The PC in this case show effects of misregistration between the different images in the set, which is especially noticeable at the edges of the brain and CSF tissue interfaces. Since these effects are caused by patterns that change between images, they are not only shown in the first three PC but are also propagated into the higher order components. This results in a redistribution of the total variance, thus decreasing the percentage held by the first PC to 60.7%. The share of the total variance between the PC can therefore be used to quantify the degree of registration between the images in the dataset.
Figure 4.9 PCA of a set of synthetic DT-MRI images. First row shows the original images, second row the PC images and the third row the percentage of the total variance in each PC. (a) Without distortions, the first three PC hold most of the variance of the data, and show only effects of different contrast between the images in the set. Higher order PC have very little contribution to the total variance. (b) Magnification, translation and shear are applied to the synthetic DW images. PC now show misregistration artifacts as new patterns in the set of images. These artifacts increase the percentage of the total variance held in the high order PC, while decreasing the variance of the first PC.

4.2.3 Region-of-interest analysis

Conventional ROI analysis can be used as a quantitative quality control test for registration techniques. The registration method should be able to compensate for misregistration effects without introducing significant changes in the images intensity values. However, as seen in Figure 4.5, interpolation in image-based
methods may cause small changes in the image such as smoothing. This effect is expected to be larger when the interpolation is performed along the directions where voxel dimension is larger, usually the $z$ direction that can be up to 5 or 6 mm and include slice gaps. This artifact is therefore expected to be more severe when using 3D registration algorithms that correct for bulk motion as well as EC induced distortions, since they may interpolate across thick slices in order to correct translations along the $z$-axis or rotations around the $x$- or $y$-axes.

ROI analysis can be also used to assess possible changes in signal intensity that the modification of the acquisition sequence may cause in methods that compensate for EC during acquisition.

Measurement of $D$ and FA in ROI will show whether any of the effects mentioned above are significant for a particular correction method. If they are, one should be careful if subsequent quantitative analysis of the corrected images is going to be performed.

### 4.3 Conclusions

Images acquired for the calculation of $D$ are prone to misregistration artifacts that will affect the accuracy of the calculation and therefore propagate errors into the scalar diffusion parameters derived from it. Various approaches to correct for these artifacts have been suggested, although no qualitative method has been established for systematic comparison of the different techniques. This chapter described some qualitative and quantitative methods to compare the performance of image registration techniques used to remove misregistration artifacts from the images. These tools will be applied to three such registration techniques in Chapter 5.
Chapter 5 Quantitative assessment of eddy current induced distortion correction methods

5.1 Introduction

In this chapter the quantitative and qualitative tools described in Chapter 4 to assess the performance of registration methods are used to assess three techniques that can compensate for EC induced distortions, namely ICC phantom calibration, FLIRT and DSE acquisition. Volunteer and phantom DT-MRI data were acquired with various slice thickness and $b$-values to check how these parameters affect the performance of these three correction methods. The results of this analysis are not only used to assess these methods, but also to validate of the tools themselves.

§5.2 briefly introduces different methods available for the correction of EC induced distortions. §5.3 describes the ICC phantom calibration, FLIRT and the DSE acquisition correction methods. The remaining sections present and discuss the results of the quantitative assessment tools applied to these three methods.

5.2 Correction methods for eddy current induced distortions

5.2.1 Introduction

MRI acquisition sequences contain pulsed field gradients which are switched on and off very rapidly. Varying magnetic fields generate EC in the electrically conductive structures of the MRI scanner such as the cryostat, as described by Faraday’s law of electromagnetic induction (182). The intensity of the induced currents is a function of the pulse amplitude and duration of the gradient ramps and for relatively short gradient pulses, such as those used in the EPI readout train, the EC will tend to cancel out. However, for the strong diffusion-sensitising gradients, the long duration electrical currents created do not cancel completely and induce additional decaying magnetic fields that remain during the EPI readout period. The low bandwidth per voxel in the phase-encode direction of the EPI sequence makes it very sensitive to small field changes, and this produces geometric distortions in the DW images. A symbolic representation of these distortions is shown in Figure 5.1.
A residual field in the slice-selection direction (z) will cause a uniform translation (T) of all voxels in the image along the phase-encoding direction (y); a residual field in the read-out direction (x) will cause a shift of the voxels in y that varies linearly with x, introducing a shear (S) in the image parallel to y; and a residual field in the phase-encoding direction will cause a shift in y that varies linearly with y resulting in a magnification (M) of the image in this direction (183).

EC induced image distortions principally occur in the presence of strong diffusion sensitising gradients, and therefore affect the registration between $T_2$- and DW images. This will cause errors in the voxel-by-voxel calculation of $D$ and in the calculated quantitative diffusion parametric maps. To avoid these effects, methods including modification of the acquisition sequence, correction of data during processing or post-processing of images have been developed. These are reviewed below.

### 5.2.2 Compensation of eddy current effects at the acquisition level

Modifications to the original Stejskal and Tanner DW sequence have been proposed to avoid residual EC during the read-out period (174,184-186). The basis for this is that pulsed gradients of the same amplitude, but opposite sign, generate the same EC in opposite directions. Diffusion sensitisation can be introduced into the imaging sequence using bipolar gradients, where the diffusion gradient is split up in two halves with opposite sign, instead of single pulsed gradients. The EC generated...
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during both half gradients will cancel out reducing the residual gradients during the readout period (184). However, the effective diffusion sensitisation achieved by bipolar gradients is reduced by 25 % relative to the standard acquisition, so the duration of the gradients need to be increased by 59 % to obtain the same level of diffusion weighting (184). This involves increasing the echo time (TE) of the imaging sequence with a concomitant loss of signal by $T_2$ and $T_2^*$ relaxation effects.

A second modification of the Stejskal and Tanner sequence uses a dual spin echo (DSE) acquisition as well as bipolar gradients. This was introduced by Wider et al. (186) in diffusion measurements using NMR and later developed by Koch and Norris (174) and Reese et al. (185) in diffusion MRI. The advantage of using bipolar gradients with DSE acquisition, rather than conventional spin echo, is that EC can be compensated without increasing the TE while achieving same diffusion weighting as the Stejskal-Tanner spin echo sequence (174,185).

These EC reduction schemes involve modification of the pulse sequence that may not be possible on all clinical scanners. However, some of these sequences, in particular the DSE acquisition, are available on most commercial scanners. They have been shown to reduce significantly distortions and improve the quality of the quantitative parametric maps. In practice, the TE of the DSE acquisition is slightly longer than conventional diffusion MRI, causing a small decrease in SNR. In addition, care must be taken with the introduction of stimulated echoes that might appear due to the use of two consecutive 180° RF pulses.

Another approach suggested for compensation of EC is the adjustment of the preemphasis unit (187). A calibration scan is performed using the phase difference information from two DW images acquired with and without diffusion gradients to determine the time constants and amplitudes to input to the standard preemphasis unit. This technique requires access to the preemphasis settings and the efficient EC elimination is limited by the design of the preemphasis system to compensate for the character of the EC induced field.

EC effects can also be eliminated from the raw data before processing without the need to modify the diffusion gradients. Only some modifications to the imaging portion of the sequence are necessary to obtain 1D field maps of the EC in the phase-encode and read-out directions as described by Jezzard et al. (173). The EC map in
the read-out direction is acquired by setting the phase encoding gradients to zero; the map in the phase-encode direction is acquired by setting the read-out gradient to zero and collecting a set of echoes in the phase-encode direction; and the bulk shift caused by EC in the slice-selection direction can be estimated from the band-width. The EC induced magnetic gradients are then calculated using a model of the field profile and correcting magnification, translation and shear from each image in the Fourier domain before reconstruction. Another method developed by Calamante et al. (172) uses a non phase-encoded (nPE) reference scan acquired in a spherical water phantom to calculate the frequency shifts caused by the EC and correct the phase of each echo in the echo train. However, this study only addressed the elimination of shifts in the phase-encode direction.

The major disadvantage of these methods is that the acquisition of the calibration field maps requires a threefold increase in the length of the experiment. Calibration scans could, however, be acquired in separated phantoms so patient scanning times do not need to be increased (173).

5.2.3 Correction of eddy current induced distortions at the post-processing level

5.2.3.1 Image-based methods

Post-processing registration methods may also correct for EC induced distortions by considering the registration of the misaligned DW images to the reference T2-weighted image as a mathematical optimisation. The aim is to find the transformation that best aligns each image to the reference. The T2-weighted image is commonly used as the reference since EC distortions are expected to be insignificant. The optimisation consists of: selection of the space of allowed transformations; calculation of the cost-function that quantifies the quality of the alignment; minimisation of the cost-function; and interpolation, necessary to apply the optimum transformation to the distorted image since the new voxel positions after transformation will not correspond to the grid points of the image.

The most common transformations used are affine (137,188,189), with 12 degrees-of-freedom (DoF), consisting of a rigid body (RB) transformation (3 translations and 3 rotations) and a similarity part (3 scales and 3 skews). However, since the
distortions are mainly in the phase-encoding direction, some algorithms limit the parameters from the similarity part of the transformation to the variables in that direction (36,66,183,188,190-193).

Cost functions frequently used for registration of DW images are mutual information (MI) (36,66,137,188,189) and correlation ratio (CR) (137). CR and MI are similarity measures that use functional or statistical non-linear dependence between the signal intensities in the images, instead of relying on the identification of landmarks in the image (194,195). Given two images \( I \) and \( J \), CR and MI are expressed as

\[
CR(I,J) = \frac{\text{var}(I - \hat{\phi}(J))}{\text{var}(I)} \tag{5.1}
\]

where \( \hat{\phi}(J) \) is a non-linear approximation of \( I \) in terms of \( J \), and

\[
MI(I,J) = -\sum_i \sum_j p(i, j) \log \frac{p(i, j)}{p(i)p(j)} \tag{5.2}
\]

where \( p(i,j) \) is the intensity joint probability distribution of the images and \( p(i) \) and \( p(j) \) the corresponding marginal distributions (196). These cost-functions have some desirable characteristics, in particular they are suitable for registration of images with different contrast properties (multi-modality registration) (137). Thus, DW images can be registered using a \( T_2 \)-weighted image as a reference.

Other cost-functions such as cross-correlation are easier to implement, but they assume a linear relationship between the signal intensity values of the two images to be compared and are only suitable to co-register images with the same contrast properties. Unfortunately cross-correlation cannot be used to register DW to \( T_2 \)-weighted images by direct comparison using \( b \)-values higher than 300 s/mm\(^2\) (175). However, cross-correlation has been used to reduce EC distortions by comparing pairs of DW images where the diffusion gradients have been applied in opposite directions and using the symmetric behaviour of the EC generated during the acquisition of each image (192,193). In addition, registration between DW and \( T_2 \)-weighted images using cross-correlation is possible when the bright signal from CSF is suppressed from the \( T_2 \)-weighted image with FLAIR (190). A local cross-correlation cost-function can also be used for registration of two images of different modality by assuming that the relationship of grey value transfers is linear in a small neighbourhood (188).
A registration method specific for DT-MRI has also been proposed, where the optimisation minimises the residual error from fitting the data to the diffusion tensor model (191). This method introduces constraints, such as restrictions in the spatial variation of the distortions, and models the distortions as a function of the diffusion gradient strengths, thereby reducing the number of parameters needed to be estimated in the optimisation.

The main advantages of post-processing techniques are that they do not require any modification of the image acquisition sequence or increase of the patient scanning time, and most techniques correct for both patient motion between acquisitions as well as EC induced distortions.

The main limiting factor in the accuracy of the image-based registration methods is computing time spent during the optimisation process, since this is done by searching for the best transformation among all the allowed DoF. Restricting the number of DoF by introducing constraints is a common way of helping to reduce the computation time (191). Optimisation algorithms may also be susceptible to local minima of the cost-functions, thereby producing a sub-optimum solution. This can be partially addressed by using a very coarse resolution for the initial search and progressively increasing it to refine the result (137), which makes the process faster and more robust.

5.2.3.2 Calibration-based methods

The use of phantom calibration data for the correction of EC induced distortions is based in the assumption that these are constant between patient and phantom scans. The time evolution of EC has proved to be relatively slow (176), which allows the use of calibration methods without the need to repeat the calibration scan after each subject assuming the scanning parameters are the same. However, the temporal evolution of the EC generated during DW EPI should be checked for each particular scanner and the calibration should be repeated when EC may have become significantly different from the last time of calibration or after a scanner service. The calibration method suggested by Bastin (175) used the iterative cross-correlation optimisation developed by Haselgrove and Moore (183) to register slice by slice the DW images to the undistorted T$_2$-weighted images in a cylindrical water phantom. The $M$, $T$ and $S$ parameters calculated for each slice vary slowly with slice position.
and can be fitted by least squares obtaining calibration curves of distortion parameters versus slice position. Distortions in brain images acquired with the same imaging parameters can be reversed using the $M$, $T$ and $S$ calculated by interpolation of the calibration curves at the new slice positions without the need for collecting phantom data for each slice position (176). Similarly, Horsfield (197) used MI to register DW to T$_2$-weighted images to find the optimum parameters for a model of the residual fields resulting from diffusion sensitising gradient pulses of unit amplitude applied in each $x$, $y$ and $z$ directions. The final residual field at each voxel position is calculated by superposition of the component fields so the effect of the distortion can be reversed in the brain images.

These phantom calibration techniques only account for EC induced distortions; misregistration caused by bulk motion of the patient still should be corrected using a RB transformation. The major drawback of these techniques is the need to collect the calibration data separately. However, once the temporal stability of EC time has been established, it may not be necessary to repeat the calibration scan for every subject assuming the imaging parameters do not vary or the scanner undergoes a service or refilling of cryogens.

**5.3 Distortion correction methods to be assessed**

5.3.1 Introduction

One example of each, acquisition-based, image-based and calibration-based EC distortion correction techniques are implemented and available in the SBIRC for routine use during acquisition or post-processing of DT-MRI data. The quantitative and qualitative assessment methods described in Chapter 4 were used to identify advantages and disadvantages of these techniques when used in a clinical setting, such as scanning of stroke patients. Images from healthy volunteers acquired with imaging parameters similar to those used for clinical studies were used for this purpose. The sequence used is taken from the original stroke acquisition protocol, consisting of two Stejskal-Tanner diffusion sensitising trapezoidal gradient pulses of duration $\delta = 32.2$ ms, separation $\Delta = 39.1$ ms and rise time $\eta = 1.2$ ms applied in six orthogonal directions using ODG acquisition. Images were acquired with a $b$-value of 1000 s/mm$^2$. Other imaging parameters will be described in the corresponding
methods sections below. Some additional volunteer DT-MRI data were collected using a $b$-value of 3000 s/mm$^2$ to extend the assessment of the performance of these registration methods. The use of $b$-values higher than 1000 s/mm$^2$ has been suggested as a means to improve detection and estimation of the extent of acute ischaemic lesions and to study the redistribution of water between the fast and slow diffusion compartments within the lesion (198,199). It has also been used in advanced diffusion techniques able to resolve complex tissue architecture (200,201). However, to date there is no literature published about the performance of any registration method at high $b$-values.

5.3.2 Acquisition-based correction: Dual spin echo

A DSE option is available on the GE Signa LX 1.5T scanner used for these experiments. The diagram for the DSE diffusion sequence is shown in Figure 5.2. The timings of the four diffusion gradients are optimised to obtain the required diffusion weighting, while minimising the residual EC during the read-out period (§5.2.2). To avoid artifacts caused by stimulated echoes generated by the use of two consecutive 180° RF pulses, crusher gradients around these two pulses are added in the three directions (not shown in the figure).

![Figure 5.2 Dual spin echo diffusion EPI sequence for compensating residual EC (185). Each diffusion sensitisation gradient is formed by a positive and a negative gradient to compensate for the EC that build up during the switching of the gradients.](image)

5.3.3 Image-based correction: FMRIB's Linear Image Registration Tool (FLIRT)

FLIRT is the registration algorithm developed by the members of the Analysis Group in the Oxford Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB) (202). The source code of the registration tool can be downloaded from
http://www.fmrib.ox.ac.uk/fsl/flirt/ and it is currently used in Edinburgh to register patient and volunteer images of different modalities as well as to correct EC induced distortions in DT-MRI data.

FLIRT is a 3D imaged-based registration method used to correct the images after acquisition. All DW volumes acquired during a study are registered to the first T2-weighted volume to correct both bulk subject motion and EC distortions using affine transformations in 3D. All repetitions of the T2-weighted volumes are also registered to the first volume to correct for patient motion during acquisitions. Unless otherwise indicated, the default interpolation method and cost function in FLIRT, namely trilinear interpolation and correlation ratio, will have been used for the present experiments.

The number of DoF in the space of transformations searched by FLIRT can be customised. The default is affine transformation with 12 DoF (3 translations, 3 rotations, 3 magnifications and 3 shears), and is used to correct for both bulk motion and EC distortions. When six DoF are used (only translations and rotations) the transformation only corrects for RB motion. RB FLIRT can be used in combination with other EC induced distortion correction methods, which do not correct for bulk motion to achieve complete registration of the images. The chosen transformation is optimised using a global optimisation method specifically tailored for 3D registration of brain images (137).

5.3.4 Calibration-based correction: Phantom calibration

The principles of using calibration data to correct distortions in DW images were explained in §5.2.3.2. The calibration method routinely used in Edinburgh is that developed by Bastin (175) and Bastin and Armitage (176). Calibration data acquired in a cylindrical water phantom using the same imaging parameters as in the subject examination can be used to correct distortions in the latter assuming the EC are sufficiently stable over time. The temporal stability of EC distortions are investigate in Appendix 5a.
5.4 Assessment of consistency and accuracy

5.4.1 Methods

To investigate the effect of differing slice thickness and $b$-value on image registration performance, data were acquired from two volunteers. The data were collected on a GE Signa LX 1.5T scanner using a DW-EPI sequence (§5.3.1) with slice thicknesses of 2.8 and 5 mm, two $b$-values of 1000 s/mm$^2$ and 3000 s/mm$^2$ and ODG gradient sampling.

Data from Volunteer 1 was acquired for both $b$-values using 20 axial slices of 5 mm thickness, field-of-view of 240×240 mm, acquisition matrix of 96×96 (zero filled to 128×128), TR of 8 s and TE of 123.6 ms. The experiment was repeated in the same session using the DSE acquisition (§5.3.2), with the same imaging parameters except TE, which increased to 129.7 ms.

Volunteer 2 was also scanned at both $b$-values using a protocol that generated near-isotropic voxels suitable for the application of tractography methods (§3.4). To include the whole brain in Volunteer 2, 40 axial slices of 2.8 mm thickness were acquired using nearly isotropic voxels (1.9×1.9×2.8 mm$^3$) for a 240×240 mm field-of-view with a 96×96 acquisition matrix (zero filled to 128×128). Other acquisition parameters were a TR of 8 s, and a TE of 124.0 ms. The same protocol was repeated using DSE, with a TE of 131.0 ms.

Phantom calibration data were acquired using a standard DW-EPI acquisition on the same days as the volunteer scans with identical imaging parameters. The phantom was filled with water for experiments at $b = 1000$ s/mm$^2$ and with a sucrose solution for experiments at $b = 3000$ s/mm$^2$. The $M$, $T$ and $S$ distortion parameters were calculated for every slice position following the method described by Bastin (175).

To correct for EC induced distortions in the volunteer data, the three methods described above (§5.3) were applied as follows:

a) DSE: all $T_2$- and DW volumes were acquired with DSE. No further correction of these images was performed since EC effects should be minimised at acquisition.
b) Affine FLIRT: all T$_2$- and DW volumes were registered using affine FLIRT (v. 1.3), which corrects images for both bulk subject motion and EC induced distortions.

c) ICC Phantom calibration: all DW data obtained with the standard EPI acquisition were corrected for distortions slice-by-slice using the calibration data calculated from the corresponding phantom images.

Consistency tests were performed at both $b$-values to assess the robustness of the ICC algorithm used by the phantom calibration method and the optimisation algorithm used by affine FLIRT. The consistency of RB FLIRT was also assessed since this will be used to correct DT-MRI data for rigid body motion in the experiments described below. The test of the three registration methods will also demonstrate how increasing the number of DoF in the transformation affects the robustness of registration algorithms.

PCA was used to compare the overall accuracy in the image alignment given by the three methods, DSE acquisition, affine FLIRT and ICC phantom calibration. Since affine FLIRT corrects for both EC induced distortions and subject’s bulk motion, the data corrected with DSE and ICC phantom calibration were also corrected for motion with RB FLIRT to make comparison between three methods fair. In data corrected with the ICC phantom calibration method, it is important that the distortion correction is applied before the RB transformation. Since the distortions induced in the images are a function of their relative position within the scanner, phantom calibration data could not be applied directly to the brain images if they have undergone any previous transformation that may have changed their position relative to the scanner. Since subject’s motion was corrected using the same method in all cases, differences in the accuracy results should only reflect differences in performance of the methods to eliminate EC induced distortions. The % of the total variance in the 1$^{st}$ PC ($\% \text{var}\{\text{PC}_1\}$) was used as a quantitative indicator of the accuracy of the registration. Two-tailed Student's t-tests of the $\% \text{var}\{\text{PC}_1\}$ for each individual slice (null hypothesis: $\% \text{var}\{\text{PC}_1\}_{\text{data_set 1}} - \% \text{var}\{\text{PC}_1\}_{\text{data_set 2}} = 0$) were performed to assess the significance of differences in the PCA results between different methods, with $p < 0.01$ considered statistically significant.
5.4.2 Consistency tests

5.4.2.1 Results for $b = 1000 \text{s/mm}^2$

The consistency tests were run on the ICC, affine FLIRT and RB FLIRT algorithms using the standard DW-EPI acquisition data. Results from tests performed on Volunteer 1 (5 mm slice thickness) and Volunteer 2 (2.8 mm slice thickness) with $b = 1000 \text{s/mm}^2$ are shown in Figure 5.3, Figure 5.4, and Figure 5.5 for each of the methods.

A summary of the mean values (SD) over all slices for the consistency parameters, namely mean consistency error ($\varepsilon$), mean displacement ($\bar{r}$), and mean intensity error ($\sigma$) for ICC, affine FLIRT and RB FLIRT is shown in Table 5.1. As in the figures, the two first and last slices were not included in the mean value of $\sigma$ for the FLIRT methods. These slices are often chopped off by the algorithm due to the lack of data to interpolate in the $z$ direction at the top and bottom of the volume. Incomplete slices will cause very large intensity errors not directly related to the algorithm performance. As seen in the figures, $\sigma$ oscillates between positive and negative values across the slices. If the mean of $\sigma$ is calculated using the sign of the error, big intensity differences could be masked. The mean of $\sigma$ for all slices and gradient directions was therefore calculated using absolute values ($\sigma$) to obtain a more reliable measure of the mean percentage intensity error. To identify whether $\sigma$ is mainly positive or negative, the % of the total cases where $\sigma > 0$ was measured.

Table 5.1 Summary of consistency tests at 1000 s/mm².

|                | $\varepsilon$ (mm) | $\bar{r}$ (mm) | $\sigma$ (%) | $|\sigma|$ (%) | % > 0 |
|----------------|---------------------|----------------|--------------|----------------|-------|
| **Phantom**    |                     |                |              |                |       |
| Calibration    | 5mm                 | 0.24±0.17      | 6.3±2.5      | 0.0009±0.05    | 0.04±0.04 | 49    |
|                | 2.8mm               | 0.26±0.19      | 6.3±2.6      | -0.0001±0.07   | 0.05±0.05 | 45    |
| **FLIRT**      |                     |                |              |                |       |
|                | 5mm                 | 4.4±1.22       | 6.2±1.5      | 3.3±2.2        | 3.3±2.2 | 100   |
|                | 2.8mm               | 2.4±0.4        | 5.5±1.2      | 0.52±0.9       | 0.8±0.7 | 81    |
| **RB FLIRT**   |                     |                |              |                |       |
|                | 5mm                 | 2.9±1.0        | 4.1±1.1      | -0.91±0.7      | 1.3±0.8 | 20    |
|                | 2.8mm               | 1.4±0.1        | 2.9±0.1      | -1.5±1.1       | 1.6±1.0 | 11    |
Figure 5.3 Consistency tests of data acquired at $b = 1000$ s/mm$^2$ in Volunteer 1 (a) and Volunteer 2 (b) registered using ICC phantom calibration. The mean values of each parameter for all slices are indicated above each graph.
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Figure 5.4 Consistency tests of data acquired at $b = 1000$ s/mm$^2$ in Volunteer 1 (a) and Volunteer 2 (b) registered using affine FLIRT. The mean values of each parameter for all slices are indicated above each graph.
Figure 5.5 Consistency tests of data acquired at $b = 1000 \, \text{s/mm}^2$ in Volunteer 1 (a) and Volunteer 2 (b) registered using RB FLIRT. The mean values of each parameter for all slices are indicated above each graph.
There is less variation in $\varepsilon$ across slices using FLIRT or RB FLIRT than ICC phantom calibration. The reason for this is that the FLIRT methods perform a volume registration by applying the same transformation to all slices, while ICC corrects distortions slice-by-slice, and applies a different transformation to every slice. The mean value of $\bar{\varepsilon}$ was about 6 mm in both ICC phantom calibration and affine FLIRT, showing that the algorithms were applying comparable and non-negligible transformations. The mean $\bar{\varepsilon}$ is smaller for RB FLIRT showing that in these particular cases the maximum displacement of the transformation was given by the affine part of the algorithm that correct for the EC induced distortions.

As explained in Chapter 4, small consistency errors characterise robust algorithms and robustness can be affected by the number of DoF that need to be considered during the optimisation of the registration transformation. The results above corroborate this expectation and show that ICC phantom calibration (3 DoF) produces the smallest $\varepsilon$ and $\bar{\sigma}$, followed by RB FLIRT (6 DoF) and by affine FLIRT (12 DoF).

The $\varepsilon$ did not show any dependence on slice thickness for ICC phantom calibration. However, both affine and RB FLIRT performed better with thinner slices. $\bar{\sigma}$ appears to be negligible in the ICC phantom calibration method since it is positive in approximately half of the slices and negative in the other half cancelling out. The $|\bar{\sigma}|$ is also very small, indicating no major signal changes remained after applying both the forward and the reverse transformation with this methods. The relatively large and positive values of $\bar{\sigma}$ in affine FLIRT were not expected. A small change in signal intensity was anticipated, due to interpolation of the data across slices causing a 'smoothing' of the image, as shown in Figure 4.5. This change could be positive or negative, but cancelling on average. However, $\bar{\sigma}$ is consistently positive as seen in the % of cases where $\bar{\sigma} > 0$, especially with the 5 mm slice thickness. The intensity errors of RB FLIRT are small and predominantly negative. A small decrease in the signal intensity of registered DW images of the brain could be explained by interpolation due to a smoothing of the noise. Further investigation on the increase of intensity found with affine FLIRT showed that this was caused by consistency errors in the algorithm (Appendix 5b).
5.4.2.2 Results for \( b = 3000 \text{ s/mm}^2 \)

The results of the consistency tests performed at \( b = 3000 \text{ s/mm}^2 \) in Volunteers 1 and 2 are shown in Figure 5.6, Figure 5.7 and Figure 5.8.

A summary of the mean consistency parameters measured at \( b = 3000 \text{ s/mm}^2 \) for each algorithm in both volunteers is shown in Table 5.2.

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<th>Table 5.2 Summary of consistency tests at 3000 s/mm²</th>
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<td>( \varepsilon ) (mm)</td>
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The consistency errors obtained with the ICC phantom calibration method are also very small at \( b = 3000 \text{ s/mm}^2 \) with slightly larger \( \varepsilon \) and \( \bar{\sigma} \) for 2.8 mm slices as compared with 5 mm slices. This difference could be due to the larger transformation used to register the 2.8 mm slices as indicated by the \( \bar{r} \) in each case.

Both affine FLIRT and RB FLIRT produced larger consistency errors, especially at 2.8 mm slices. However, the above results at \( b = 1000 \text{ s/mm}^2 \) showed that both methods perform better with thinner slices. This suggests that the combination of high \( b \)-value and small voxel size may reduce the SNR to a level that can affect the robustness of these algorithms. Chapter 6 will investigate further how the performance of these registration algorithms is affected by the increase in \( b \)-value and the decrease of SNR. The ICC phantom calibration method is less sensitive to increased \( b \)-value since registration is performed in phantom images, which can be acquired with a SNR as high as necessary by averaging or using low diffusivity phantom materials. Note that averaging cannot be performed on brain images before the used of FLIRT since these should be registered between acquisitions beforehand to remove subject motion.
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Figure 5.6 Consistency tests of data acquired at $b = 3000 \text{ s/mm}^2$ in Volunteer 1 (a) and Volunteer 2 (b) registered using ICC phantom calibration. The mean values of each parameter for all slices are indicated above each graph.
Figure 5.7 Consistency tests of data acquired at $b = 3000 \text{ s/mm}^2$ in Volunteer 1 (a) and Volunteer 2 (b) registered using FLIRT. The mean values of each parameter for all slices are indicated above each graph.
Figure 5.8 Consistency tests of data acquired at $b = 3000 \text{s/mm}^2$ in Volunteer 1 (a) and Volunteer 2 (b) registered using RB FLIRT. The mean values of each parameter for all slices are indicated above each graph.
5.4.2.3 Conclusions

Overall, the ICC phantom calibration method showed good robustness in the consistency tests, with the results being independent of \( b \)-value or slice thicknesses. Both affine and RB FLIRT produced larger consistency errors. This could be explained by the larger number of DoF in these algorithms, which increase the difficulty in identifying the global minimum of the cost function during the optimisation of the registration transformation. The consistency errors of the FLIRT methods were also larger than ICC phantom calibration for lower \( b \)-values. Regarding slice thickness, although both FLIRT algorithms performed better for thinner slices at \( b = 1000 \) s/mm\(^2\), the consistency errors were larger for thinner slices at \( b = 3000 \) s/mm\(^2\). These results suggest that both FLIRT algorithms are more robust for thinner slices. However, the performance deteriorates at the low SNR of high \( b \)-value images. The further decrease of SNR produced by thinner slice thickness at \( b = 3000 \) s/mm\(^2\) would only deteriorate further the robustness of the algorithm.

The previous results determined the robustness of the three methods, and therefore their ability to identify the \textit{global} minimum of the cost function. This, however, is not an indicator of the accuracy to correct for EC induced distortions, or the ability to locate a minimum of the cost function, which needs to be tested by other means, such as PCA.

5.4.3 Principal component analysis

5.4.3.1 Results for \( b = 1000 \) s/mm\(^2\)

PCA was run on the DT-MRI data acquired with both standard and DSE DW-EPI acquisitions from the two volunteers described above. Five datasets were analysed in total for each volunteer at each \( b \)-value.

a) A set of unregistered standard DW-EPI acquisition data was used as the reference, to show the improvement in PCA results after applying each registration technique. This will be referred to as \textit{No Registration}.

b) A standard DW-EPI acquisition dataset registered using RB FLIRT to indicate the contribution of this component to the PCA results. This is necessary since all other correction methods are also used RB FLIRT to correct for bulk motion. This will be referred to as \textit{RB FLIRT}.
c) A DSE DW-EPI acquisition dataset registered with RB FLIRT. This will be referred to as \textit{DSE + RB FLIRT}.

d) A standard DW-EPI acquisition dataset registered with affine FLIRT. This will be referred to as \textit{affine FLIRT}.

e) A standard DW-EPI acquisition dataset registered using ICC phantom calibration data followed by RB FLIRT. This will be referred to as \textit{PHT cal + RB FLIRT}.

Figure 5.9 shows as example the first 12 PC of one slice in two of the cases. As expected the two first PC are mainly related to the $T_2^*$ and DW contrast while higher order PC are related to diffusion anisotropy and noise effects. The unregistered data (Figure 5.9 a), shows misregistration artifacts in the high order PC, indicated by the arrows. These artifacts increase the % of the total variance in those PC, therefore decreasing the share of the variance in the first PC. When a registration method such as ICC phantom calibration is applied to the data (Figure 5.9 b), most of the misregistration artifacts disappear, thus decreasing the % variance in the higher order PC and increasing it in the first PC.

Figure 5.10 and Figure 5.11 show the mean % of the total variance in the first ten PC for all slices in data acquired in Volunteers 1 and 2 at a $b$-value of 1000 s/mm$^2$. The analysis is performed for each slice, with the data points and the error bars indicating the mean ± SD for all slices. The mean (SD) values for the % variance in the 1$^{\text{st}}$ and 2$^{\text{nd}}$ PC are also shown in the figures.

The resulting distributions of the % variance are similar in both volunteers, and do not show any obvious dependence on slice thickness. As expected, the unregistered dataset has the lowest % var\{PC$_1$\} and the largest share of the total variance in the higher order PC. The distribution of the variance shifts towards the 1$^{\text{st}}$ PC and is reduced in the higher order PC when any of the registration methods are used. Values for the 1$^{\text{st}}$ PC show that the best registration is obtained for the DSE acquisition, closely followed by ICC phantom calibration.
Figure 5.9 Images of the 12 first principal components in the (a) unregistered data and the (b) data registered with ICC phantom calibration and RB FLIRT. The arrows point to misregistration artifacts.
Figure 5.10 Distribution of the percentage of the total variance over the first 10 principal components for Volunteer 1 (5 mm thick slices) at $b = 1000 \text{s/mm}^2$.

Figure 5.11 Distribution of the percentage of the total variance over the first 10 principal components for Volunteer 2 (2.8 mm slice thickness) at $b = 1000 \text{s/mm}^2$. 
The two-tailed Student's t-tests were used to compare all registration methods to the unregistered data to indicate whether they produce a significant improvement in the registration of the images ($p < 0.01$). In Volunteer 2 (2.8 mm slice thickness), the $\% \text{ var}\{\text{PC}_1\}$ of the DSE acquisition was also significantly higher than all other methods. However, in Volunteer 1 (5 mm slice thickness) $\% \text{ var}\{\text{PC}_1\}_{\text{DSE+RB}}$ was not significantly different from $\% \text{ var}\{\text{PC}_1\}_{\text{PHTcal+RB}}$ ($p = 0.04$). Therefore we cannot assume that the DSE acquisition outperforms the standard DW-EPI acquisition followed by ICC phantom calibration at a $b$-value of 1000 s/mm$^2$. Both DSE and ICC phantom calibration significantly improve the registration of the images as compared with RB FLIRT, showing that they successfully remove EC induced distortions. However, the difference between $\% \text{ var}\{\text{PC}_1\}_{\text{RB FLIRT}}$ and $\% \text{ var}\{\text{PC}_1\}_{\text{affine FLIRT}}$ not significant for 2.8 mm slice thickness ($p = 0.06$). The improvement in registration seen in affine FLIRT is therefore mainly due to the correction of bulk subject motion rather than EC induced distortion correction.

To corroborate the results of this quantitative test, an additional qualitative assessment of the images registration was carried out. The $T_2$- and DW images in each dataset were used to calculate $\mathbf{D}$ and maps of the off-diagonal elements were calculated to indicate misregistration artifacts (§4.2.1.2). Figure 5.12 shows the off-diagonal elements of $\mathbf{D}$ for the central slice in Volunteer 1.
Figure 5.12 Maps of the off-diagonal elements of $D$ in Volunteer 1 for 5 mm thick slices at $b = 1000 \text{ s/mm}^2$: (a) Unregistered, (b) DSE + RB FLIRT, (c) affine FLIRT and (d) PHTcal + RB FLIRT datasets.

Misregistration artifacts are clearly visible in the unregistered images as an artifactual correlation of water diffusion in two directions, mainly in the phase-
encoding direction at the edges of the brain (a). Registration reduces these artifacts. In particular, images corrected using the ICC phantom calibration method show very little or no misregistration artifacts (d). However, using the DSE acquisition (b) or affine FLIRT (c), some artifacts still remain as indicated by the red arrows. This qualitative test contrasts with the PCA results where the DSE acquisition showed the best performance.

Discrepancies in the results obtained between these quantitative and qualitative assessment tests are most likely cause by the fact that the DSE method requires images to be collected in a separate acquisition. Different SNR and contrast properties on DSE DW-EPI images, as compared to the standard DW-EPI images where FLIRT and ICC phantom calibration techniques are applied, prevents the use of PCA to compare directly this method with the other two. These effects will be further investigated in Appendix 5c and in §6.2.3. Different SNR and contrast properties will affect the distribution of the total variance in the PC per se and, therefore, differences seen between the DSE acquisition and registration methods which use the standard DW-EPI acquisition do not only reflect the different performance of the methods. Thus, PCA can only be used as a quantitative test to assess the performance of any image-base registration method that does not require a separate acquisition for its application, e.g. to compare FLIRT and ICC phantom calibration.

5.4.3.2 Results for $b = 3000$ s/mm$^2$

The distribution of the total variance in the experiments performed at 3000 s/mm$^2$ for both volunteers is shown in Figure 5.13 and Figure 5.14. (For consistency data obtained using the DSE acquisition is also shown.) Overall, the lower variance in the first PC and more gradual reduction in the share of the total variance of the higher order PC compared with the $b = 1000$ s/mm$^2$ graphs is related to the lower SNR of the brain images with higher diffusion weighting. This effect is more marked in data from Volunteer 2 since SNR was lower due to the use of thinner slices.

Similar to the results at $b = 1000$ s/mm$^2$, two tailed Student's t-tests show that all the registration methods significantly improve image alignment when comparing the $\% \text{ var}\{\text{PC}_1\}$ of the registered images with that of unregistered images ($p < 0.01$). The
Chapter 5 Quantitative assessment of eddy current induced distortion correction methods

ICC phantom calibration method has the highest $\% \text{ var}\{\text{PC}_1\}$, indicating the best registration. However, this value is not significantly higher than that of affine FLIRT in either of the two volunteers ($p = 0.27, 0.03$). However, ICC phantom calibration provides a significantly better registration than bulk motion correction with RB FLIRT, indicating that successful correction of EC induced distortions has been achieved ($p < 0.001$). Meanwhile, using affine FLIRT does not significantly improve the registration given by RB FLIRT in either volunteer ($p = 0.44, 0.09$).

Figure 5.15 shows the off-diagonal elements of $D$ for the central slice in Volunteer 1 at $b = 3000 \text{ s/mm}^2$. Most of the misregistration artifacts are also eliminated at this $b$-value, however some still remain as indicated by the arrows. ICC phantom calibration also appears to give the best results in this qualitative test, corroborating the results from PCA.

5.4.3.3 Conclusions

PCA of $T_2$- and DW-EP brain images acquired in DT-MRI experiments has been shown to be a practical tool for the quantitative comparison of the accuracy of image-based registration methods. PCA can also determine whether the technique being tested is significantly improving the image registration by comparing the output with that obtained from the corresponding unregistered dataset. However, due to the link between the PCA results and the SNR and contrast properties of the original images, this can only be used to compare methods that are applied to images collected using the same DT-MRI acquisition.

Amongst the three techniques tested here, the use of ICC phantom calibration data was the most successful at eliminating EC induced distortions, particularly at lower $b$-values. However, the results were obtained from phantom calibration data acquired on the same day as the volunteer scans. Calibration data obtained at a different time could decrease slightly the accuracy of this method, depending on the eddy current stability of the scanner Appendix 5a. The DSE acquisition could not be compared quantitatively with the image-based correction methods. However, qualitative tests showed that its performance was poorer than the other two methods at both $b$-values.
Chapter 5 Quantitative assessment of eddy current induced distortion correction methods

Figure 5.13 Distribution of the percentage of the total variance over the first 10 principal components for Volunteer 1 (5 mm thick slices) at $b=3000 \text{ s/mm}^2$.

Figure 5.14 Distribution of the percentage of the total variance over the first 10 principal components for Volunteer 2 (2.8 mm thick slices) at $b=3000 \text{ s/mm}^2$. 
Chapter 5 Quantitative assessment of eddy current induced distortion correction methods

Figure 5.15 Figure 5.16 Maps of the off-diagonal elements of D in Volunteer 1 for 5 mm thick slices at $b = 3000$ s/mm$^2$. (a) Unregistered, (b) DSE + RB FLIRT, (c) affine FLIRT and (d) PHTcal + RB FLIRT datasets.
5.5 ROI analysis

5.5.1 Introduction

DT-MRI data is commonly used to provide quantitative measurements of water diffusion parameters. Hence, it is important to check whether the use of a registration method is causing major changes in the signal values of the images that might affect this quantitative assessment. As described in §4.2.3, this can be done by using conventional ROI analysis to determine whether significant changes are introduced into the calculated diffusion parameters by a particular correction method, either at acquisition or at the post-processing stage.

The registration of both EC induced distortions and bulk subject motion through a 3D image-based registration method may cause "smoothing" of the image signal. The effect occurs when interpolating discrete data in the brain volume, particularly across the z-direction when thick slices are acquired. This could lead to averaging of high and low signals causing a filtering effect of the high frequencies in the image, and decreasing the mean intensity value. An artifact, probably related to this effect, was observed in Chapter 2 (§2.3.4) where a temporal change of the mean values of \( \langle D \rangle \) and FA in normal grey and white matter was observed. ROI analysis will be used here to study the effects of interpolation during the registration of serial scans.

5.5.2 Effect of eddy current induced correction methods on signal intensity

5.5.2.1 Methods

DT-MRI data was acquired in a healthy volunteer at \( b = 1000 \text{ s/mm}^2 \) using a standard DW-EPI acquisition with 15 slices of 6 mm thickness, field-of-view of 240 \( \times \) 240 mm, acquisition matrix of 98 \( \times \) 98 and TR of 8 s. Three repetitions were acquired to increase SNR. The scan was repeated in the same session using the DSE DW-EPI acquisition. Phantom calibration data was calculated using a cylindrical water phantom acquired with the same imaging parameters.

Maps of \( \langle D \rangle \) and FA were calculated from the following data:

a) Uncorrected standard DW-EPI acquisition data,

b) Standard DW-EPI acquisition data corrected using the ICC phantom calibration method,
c) Standard DW-EPI acquisition data corrected using affine FLIRT,
d) DSE DW-EPI acquisition data

![Figure 5.17 ROI used to measure diffusion parameters.](image)

ROI were placed in areas of white matter, corpus callosum and external capsule; areas of frontal and occipital cortical grey matter; and CSF within the ventricles. Figure 5.17 shows a T₂-weighted slice containing the ROI. They were then copied into the four datasets (a-d). In each case, (D) and FA were measured in all ROI and the mean values for each tissue type obtained. The % difference between the corrected and uncorrected images was calculated. Small differences in the diffusion parameters between images corrected with affine FLIRT or ICC phantom calibration and uncorrected images were expected due to transformation and interpolation effects. The significance of changes in diffusion parameters between corrected and uncorrected images were assessed using a two-tailed Student's t-test.

5.5.2.2 Results

The % difference between the values of (D) and FA measured in each set of images are shown in bar diagrams in Figure 5.18 (a) and (b) respectively.
Figure 5.18 Changes in (a) $\langle D \rangle$ and (b) FA observed between corrected and uncorrected images. * indicates $p < 0.01$.

$\langle D \rangle$ measurements detected small non-significant differences in all tissue types in images corrected with affine FLIRT or ICC phantom calibration. However, the use of DSE acquisition changes the value of $\langle D \rangle$ significantly in both grey and white matter, with differences of up to 70%. Similar results were observed in FA, where difference in the values measured were only significant with the DSE acquisition.

5.5.2.3 Conclusion

Both image-based EC induced distortion correction methods modify the values of the $\langle D \rangle$ and FA measured in the corrected images, in particular for grey matter and CSF. Changes in parameters measured in the grey matter with respect to the uncorrected images could be explained by the reduction of the 'rim' artifacts caused by the EC induced distortions at the top and bottom edges of the brain (cf. §4.2.1.3) which might affect these ROI. The same effect is expected to occur in CSF ROI, since they are very close to the boundary between tissues with very different $\langle D \rangle$ and FA properties. The transformation that eliminates the distortions might displace slightly the tissue selected by the ROI in corrected images, changing the value of the measurements.

The significant difference between diffusion parameters obtained using the standard and DSE DW-EPI acquisitions was not expected. Appendix 5c describes in more detail the reasons for this discrepancy.
5.5.3 Effect of 3D registration in serial scanning

5.5.3.1 Methods

In this section, we investigate whether the temporal changes observed in normal contralateral grey and white matter \( D \) and FA in the stroke study (§2.3.4) were mainly caused by the effects of the 3D registration. For this purpose a volunteer was scanned using the same acquisition parameters used for the stroke study (§2.3.1). Serial scanning was reproduced by imaging the volunteer several times in different conditions. The following DT-MRI datasets were acquired:

a) An initial baseline scan was performed. The positioning of the volunteer's head within the scanner was recorded by the radiographer in the same way as in the serial studies of stroke patients.

b) Following the baseline scan, and without removing the subject from the bed, a second scan was acquired. This will be referred as the still scan since the volunteer was told to stay as still as possible.

c) A third scan, again without repositioning the volunteer, was acquired. However, this time the subject was told to move during the scanning process to simulate the conditions that exist when scanning a restless patient. This scan will be referred as unquiet.

d) Finally, the volunteer was removed from the bed and set up again using the position recorded in the baseline scan, to reproduce the same location of the brain volume within the scanner as accurately as possible. This scan will be referred as back2scan.

The images of different scans were registered using affine FLIRT in the following sequence:

i) Images from still were registered using baseline as a reference.

ii) Images from baseline were registered using still as a reference

iii) Images from unquiet were registered using baseline as a reference

iv) Images from baseline were registered using unquiet as a reference

v) Images from back2scan were registered using baseline as a reference

vi) Images from baseline were registered using back2scan as a reference
In each case, all volumes from the second scan were registered to the first T$_2$-weighte
weighted volume of the reference scan. All remaining T$_2$- and DW volumes in the refe
reference scan were also registered to this, so all volumes from the two scans were ali
aligned and ROI can be copied directly and measurements compared.

ROI were placed in the same areas of white mater as in the previous section, cf. Figure 5.18. In each case (i-vi), the mean percentage difference of the FA value measured between the reference and the registered scan was calculated.

We hypothesise that the change in signal intensity (and therefore change in FA) is caused by 3D registration “smoothing” the signal intensities in the registered images due to interpolation across the 5 mm thick slices and the 1 mm slice gap. Smoothing decreases the highest signal values in the DW images acquired with different gradient directions and, as a consequence, the measured diffusion anisotropy is lower. This effect should be larger when registering the unquiet or the back2scan scans to the baseline than when registering the still scan, since bulk motion corrections would be larger, involving interpolation over larger distances.

5.5.3.2 Results

Table 5.3 shows the mean FA values measured in the images from each registration case as well as the percentage difference between the FA values measured in images from registered scan and the reference images.

Table 5.3 Mean (SD) FA ($\times 10^{-3}$) measured in each scan in each registration sequence (i-vi). The percentage difference is calculated with respect to the reference scan, hence a negative difference indicates reduction of signal.

<table>
<thead>
<tr>
<th>Case</th>
<th>REFERENCE</th>
<th>REGISTERED</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td>Baseline</td>
<td>Still</td>
<td>-2.4</td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>Back2scan</td>
<td>-4.7</td>
</tr>
<tr>
<td>ii</td>
<td>Still</td>
<td>Baseline</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>Unquiet</td>
<td>-5.7</td>
</tr>
<tr>
<td>iii</td>
<td>Baseline</td>
<td>Back2scan</td>
<td>-6.5</td>
</tr>
<tr>
<td></td>
<td>Unquiet</td>
<td>Baseline</td>
<td>-0.4</td>
</tr>
</tbody>
</table>

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The results corroborate the hypothesis. The signal in the images is believed to be smoothed due to interpolation during the registration process decreasing the diffusion anisotropy of the images. In the table it can be seen that, except in case ii where there is little change, FA is always reduced in the registered volume compared with the reference, even when the two sets are swapped. The reduction is generally larger when either the reference or the registered volume involve repositioning of the subject or when the subject is restless during the scan.

5.5.3.3 Conclusions

These findings potentially explain the unexpected temporal evolution of diffusion parameters observed in normal tissues during the stroke study (§2.3.4). It was found that the change in the \( D \) and FA measured in normal grey and white matter was significantly different at time points 2 to 5 when compared to time point 1 (Figure 2.2). True changes in these parameters possibly caused by the stroke were ruled out as they were also detected in CSF, a fluid that should not be affected by the evolution of the stroke.

Since in the stroke study all scans at later time points were registered to the first time point, it is expected that they experience interpolation effects, while the first time point scan remained unchanged. Hence all later time point scans will suffer a reduction of FA as compared with that measured at the first time point. These effects are larger due to the repositioning of the patient for each scan, and patient motion, which explains the relatively large differences in FA (-7.8 \% in normal white matter) observed in Figure 2.2.

5.6 Conclusions

The ideal image registration technique should be robust, accurate and should not cause major changes in image signal intensity, which could affect derived quantitative measures. It is difficult to assess absolutely and quantitatively these three aspects when studying the performance of one particular registration method. However, consistency tests, PCA and ROI analysis have proved to be useful tools to assess different methods.

Consistency tests check the robustness of a registration algorithm. This is the capability of the algorithm to get close to the global minimum of the cost function or,
in other words, to reach the same answer independently of the initial geometry of the image. The smaller the consistency and intensity errors, the more robust the algorithm, assuming the transformation generated is not negligible.

The distribution of the total variance between the PC gives an indication of the accuracy of the method, and its ability to reach correctly a match between images by finding a minimum of the cost function in the case of image-based methods. In addition, it permits statistical analysis to probe whether a correction method is making significant improvement to the image registration or to compare performance between two methods. However, PCA is only practical to compare registration methods that are applied to images with same SNR and contrast properties, particularly at low SNR, since the analysis is itself very sensitive to noise.

Measurement of scalar parameters in selected ROI is a simple but sensitive method to assess what effect a registration technique is having on image signal levels. This is essential when quantitative analysis of the images will be performed after correction.

These assessment tools were used to assess the performance of three EC induced distortion correction methods, namely FLIRT, ICC phantom calibration and DSE acquisition, at two slice thickness and two \( b \)-values. The consistency tests showed that robustness could depend on the number of DoF in the transformation and on the SNR of the images. Table 5.4 summarises the advantages (blue) and disadvantages (red) found for each of the three methods.

The DSE acquisition was expected to be the most advantageous method since it used a balanced diffusion sensitisation gradient that compensates the EC at source. However, the version of DSE implemented on our scanner did not produce the expected results and misregistration artifacts associated with significant EC were found in the off-diagonal elements of \( D \). In addition, the scalar diffusion parameters calculated from the DSE experiment showed large changes compared with values obtained from the standard acquisition.

Affine FLIRT is the most practical method since it corrects both EC induced distortions and RB motion without the need of increasing patient scanning time or acquiring extra data. The use of ICC phantom calibration is a more tedious process since it requires extra acquisition of phantom data to calculate the distortion
correction parameters and does not correct for RB motion. However, ICC phantom calibration performed better in the robustness and accuracy tests, particularly at the highest $b$-value tested.

ROI analysis confirmed the hypothesis form Chapter 2 that the 3D registration in FLIRT could cause reduction of the signal intensity due to smoothing, particularly when registering brain volumes acquired in different sessions or from unquiet subjects.
Table 5.4 List of advantages and (blue) and disadvantages (red) of FLIRT, phantom calibration and DSE acquisition as EC induced distortion compensation methods

<table>
<thead>
<tr>
<th>FLIRT</th>
<th>ICC phantom calibration</th>
<th>DSE acquisition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrects for EC and RB motion.</td>
<td>Needs extra RB motion registration</td>
<td>Needs extra RB motion registration</td>
</tr>
<tr>
<td>Only RB option can be selected</td>
<td>Requires collection of phantom data to calculate distortion correction factors</td>
<td>Eliminates most EC effects at acquisition</td>
</tr>
<tr>
<td>Does not require extra scanning</td>
<td>Phantom can be acquired with the SNR required at any $b$-value</td>
<td>The version of DSE tested changed SNR properties of the acquired images</td>
</tr>
<tr>
<td>Good registration at high SNR</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Robustness</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large consistency errors and unexpected large and positive intensity errors caused by the affine transformation</td>
<td>Small consistency and intensity errors</td>
<td>N/A</td>
</tr>
<tr>
<td>Performs better with thin slices</td>
<td>Consistency independent of slice thickness</td>
<td></td>
</tr>
<tr>
<td>Consistency errors larger at higher $b$-values</td>
<td>Performance independent of brain images therefore independent of $b$-value</td>
<td></td>
</tr>
<tr>
<td>Consistency sensitive to low SNR</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5.4 (contd) List of advantages and disadvantages of FLIRT, phantom calibration and DSE acquisition as EC induced distortion compensation methods

<table>
<thead>
<tr>
<th></th>
<th>FLIRT</th>
<th>ICC phantom calibration</th>
<th>DSE acquisition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Accuracy</strong></td>
<td>Improves registration of images at both $b$-values 1000 and 3000 s/mm$^2$</td>
<td>Gives the best registration results at both $b$-values 1000 and 3000 s/mm$^2$</td>
<td>Impossible to compare quantitatively to standard acquisition methods due to different SNR properties</td>
</tr>
<tr>
<td></td>
<td>Misregistration artifacts remain in off-diagonal elements of $D$</td>
<td>Eliminates all misregistration artifacts from off-diagonal elements of $D$ at 1000 s/mm$^2$ and most at 3000 s/mm$^2$</td>
<td>Misregistration artifacts remain in the off-diagonal element of $D$ at both $b$-values</td>
</tr>
<tr>
<td></td>
<td>At 3000 s/mm$^2$ affine FLIRT does not improve significantly the registration given by RB FLIRT</td>
<td>Improves significantly the RB registration at both $b$-values</td>
<td></td>
</tr>
<tr>
<td><strong>Effects on image signal</strong></td>
<td>Does not cause significant changes of the signal</td>
<td>Does not cause significant changes of the signal</td>
<td>Changes significantly image signal intensity as compared to conventional acquisition</td>
</tr>
<tr>
<td></td>
<td>Signal &quot;smoothing&quot; due to large interpolations changes slightly values of scalar diffusion parameters</td>
<td></td>
<td>Scalar diffusion parameters may differ by up to 70 %</td>
</tr>
</tbody>
</table>
Appendix 5a  Eddy current stability for the GE Signa LX 1.5T scanner

The stability of the EC induced during the EPI based DT-MRI experiments described in Chapter 5 was investigated following the method used by Bastin and Armitage (176). Sets of three different DT-MRI experiments were performed \((b = 0 \text{ and } 1000 \text{ s/mm}^2)\) using a large cylindrical doped water phantom, with slice thickness of 3, 4 and 5 mm, to check for any dependence of the EC stability on this parameter. Two sets of the DT-MRI experiments where performed initially \((\tau = 0)\) and then a set repeated each week for a month. To cover the same imaging volume in each experiment \((120 \text{ mm})\), 40, 30 and 24 slice locations were imaged for the 3, 4 and 5 mm slice thickness respectively. Since the number of slices \((n)\) is different in each experiment, if the same TR is used, the build up of the EC generated could also differ. The TR was therefore set to 0.4 s per slice location for each experiment to reduce any TR dependence. Other imaging parameters were 4 NEX, 96×96 image matrix (zero-filled to 128×128) and 24×24 cm FOV. The ICC algorithm was applied to calculate \(M, T\) and \(S\) for each DW-EP image (183). The validity of the calibration method and temporal stability of ICC derived parameters were tested using an error function (176) to compare the parameters calculated in acquisitions at different time points with the one calculated at the first time point. The error function has the form

\[
\xi_{i,j} = \frac{2L_k \left[ M_{i,j}(\tau) - M_{i,j}(0) \right] + \left[ T_{i,j}(\tau) - T_{i,j}(0) \right] + k \left[ S_{i,j}(\tau) - S_{i,j}(0) \right]}{N}, \quad \text{Eq. 5.3}
\]

where \(i = 1, \ldots, 6\) (number of gradient directions), \(j = 1, \ldots, n\) (number of slice positions), \(N\) is the number of columns in the phase-encode direction of the image, \(k\) is the column number and \(L_k\) is the number of voxels from the centre line of the image of the phantom to its edge in each column. This function, averaged over every \(i\) and \(j\) gives the average error \(\bar{\xi}\) (voxels/column) that would result if distortion parameters obtained at the first time point \((\tau = 0)\) were used to correct distortions in images collected with the same slice thickness at later time points.

The average error obtained for the experiments at the five time points is shown in Figure 5.19. The average error increases very slowly over time in a linear fashion for all the slice thickness. An error \(\bar{\xi} < 1\) voxel/column is considered acceptable (176) and hence we can establish from the figure that EC are sufficiently stable over time.
at $b = 1000 \text{ s/mm}^2$ and vary slowly in a predictable manner. The calibration data acquired in a phantom can therefore be used to correct for distortions in subject images acquired with the same imaging parameters up to a month before or after the calibration data was collected.

![Eddy current stability at $b=1000 \text{ s/mm}^2$](image)

Figure 5.19 Time evolution of the averaged error $\bar{\xi}$ at $b = 1000 \text{ s/mm}^2$. The lines show the least-squares fit of each of the data sets. The slow increase of the error over time demonstrates the stability of the EC generated during the acquisition of the DT-MRI data at this $b$-value.

The same experiment described above was repeated using a $b$-value = $3000 \text{ s/mm}^2$. Since the signal from a water phantom is completely attenuated at this diffusion weighting, sucrose was added to the water to decrease its self-diffusion coefficient and thus reduce the signal attenuation of the DW images. The concentration of sucrose in the phantom was 66.7 % weight of sucrose per weight of water. It was not necessary to dope the water since sucrose also decreases water relaxation times. Approximated $T_2$ and $\langle D \rangle$ values for the phantom were 170 ms and $630 \times 10^{-6} \text{ mm}^2/\text{s}$ at the sucrose concentration used (temperature = 21 °C). The properties of sucrose as phantom material for diffusion MRI are further investigated in Laubach et al. (203). The result for the time evolution of $\bar{\xi}$ at $b = 3000 \text{ s/mm}^2$ is shown in Figure 5.20. In this case, the average error does not increase linearly, however it is below the...
acceptable limit of 1 voxel/column, which demonstrates the stability of the EC generated during the acquisition of the DT-MRI data.

**Eddy current stability at \( b = 3000 \text{ s/mm}^2 \)**

![Graph showing eddy current stability](image)

Figure 5.20 Time evolution of the averaged error \( \bar{\xi} \) at \( b = 3000 \text{ s/mm}^2 \). There is a slow non-linear variation of the error over time. However, the error is still lower than the acceptable limit at one month.

Phantom calibration data can therefore be used to correct EC distortions of images acquired with diffusion weight of 3000 s/mm\(^2\). However, due to the less predictable evolution of \( \bar{\xi} \), it is recommended that both subject and phantom data be collected within the same week.

**Appendix 5b  ** Affine FLIRT

As explained in Chapter 4, EC induced distortions only occur in the phase-encoding direction and therefore inclusion of affine transformations in the other two directions may not be necessary. The inclusion of additional DoF may therefore complicate the registration and affect the robustness of the algorithm, as shown in the example below.

Figure 5.21 shows the subtraction image of a registered and unregistered DW brain image (normalised by the mean intensity of the unregistered image). Image (a) was corrected using affine FLIRT and image (b) using ICC phantom calibration to correct for EC induced distortion, followed by RB FLIRT to correct for subject motion. The main difference seen between these two subtraction images is that the
bright rim, which indicates the different positions of the brain, completely surrounds the brain with affine FLIRT, while is only evident at the top and bottom boundaries (phase-encoding direction) with ICC phantom calibration. This rim is also wider in image (a).

Figure 5.21 Subtraction of corrected to uncorrected images. (a) Corrected with affine FLIRT, (b) corrected with phantom calibration and RB FLIRT

This example shows that affine FLIRT might be over-correcting the DW images in some cases, since it is expanding the image in more directions than just the phase-encoding direction. The subtraction of the registered DW image from the T2-weighted image shown in Figure 5.22 confirms this. These two images have been registered to each other and their outlines should match. However, there is a dark rim around the edge of the brain with affine FLIRT (white arrows), demonstrating misregistration between the two images due to the over-magnification of the DW image (a). This dark rim is not observed when ICC phantom calibration is used to correct the EC induced distortions (b). (The reverse subtraction image of DW and T2-weighted images did not show under-magnification of the ICC phantom calibration corrected DW image.)
Figure 5.22 Subtraction of $T_2$-weighted and DW images. (a) Corrected with affine FLIRT, (b) corrected with ICC phantom calibration and RB FLIRT.

In this dataset, careful examination of the images corrected with affine FLIRT reveal this artifact in the majority of the images, which was not reversed when the inverse transformation was applied during consistency testing. This could explain the mean positive intensity error of 3.3 % in the dataset corrected with affine FLIRT (Table 5.1).

The use of 12 DoF may therefore cause errors in the correction of EC induced distortions. Affine transformation is only necessary in the phase-encoding direction and allowing more DoF increases the probability of the cost function getting trapped in local minima during the optimisation process. An effect of this could be the over-magnification of the DW images seen above. Other registration techniques where affine transformation is only allowed in the $y$ direction have been shown to correct successfully both EC induced distortions and RB motion (36,191).

**Appendix 5c  DSE acquisition**

During the above DT-MRI experiments, it was observed that the signal intensity of DW images collected with the DSE acquisition as implemented in the GE Signa LX 1.5T (software version 9.1 M4) was significantly different to that seen in DW images acquired with the standard acquisition for the same $b$-value. This artifact affects the value of $D$ and therefore the scalar parameters calculated from it (§5.5.2). This has not been reported previously and, furthermore, the DSE acquisition is becoming a common method for the elimination of EC induced distortions in DT-MRI. The
erroneous quantitative measurements could be, therefore, caused by a faulty implementation of this sequence on our scanner. This problem is investigated below.

When (D) maps from both standard and DSE acquisitions are displayed together (Figure 5.23), the difference between them is obvious.

![Figure 5.23](image)

Figure 5.23 (D) maps calculated from standard and DSE DT-MRI acquisition. Both maps are displayed with same intensity window.

To investigate the causes of this effect two experiments were performed:

1. To determine which of the two acquisitions is producing erroneous values, (D) was calculated in three test liquids and the values compared with those reported in the literature. Three small phantoms containing three different n-alkanes were scanned; these substances have been used previously as test liquids for quantitative DT-MRI methods (74). Five 5 mm thick slices were acquired in each phantom using ODG standard and DSE acquisition. The temperature of the phantoms was 20.2 °C. (D) was estimated from the images and (D) maps created. ROI were placed in the three central slices of the phantoms and the mean (SD) values of (D) measured for each phantom for both acquisitions. The results are presented in Table 5.5

<table>
<thead>
<tr>
<th>n-alkane</th>
<th>(D) measured (10^-6 mm^2/s)</th>
<th>(D) published (10^-6 mm^2/s)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard</td>
<td>DSE</td>
</tr>
<tr>
<td>n-hexadecane</td>
<td>338.7 (4.0)</td>
<td>529.6 (83.7)</td>
</tr>
<tr>
<td>n-tetradecane</td>
<td>491.3 (9.8)</td>
<td>553.4 (60.9)</td>
</tr>
<tr>
<td>n-dodecane</td>
<td>788.7 (10.7)</td>
<td>1004.5 (31.3)</td>
</tr>
</tbody>
</table>

* at 20° C
The values of \( \langle D \rangle \) measured with the standard acquisition were approximately equal to those found in the literature, therefore demonstrating the validity of this sequence. However, both the mean and SD of \( \langle D \rangle \) obtained from the DSE acquisition were systematically higher.

2. As a second test, a water phantom was scanned using both standard and DSE acquisitions at \( b = 10, 200, 400, 600, 800 \) and 1000 s/mm\(^2\) using ODG sampling. Ten 5 mm thick slices were acquired with 5 mm slice gap, and a TR of 10 s. ROI analysis was used to measure the signal intensity in each of the DW (\( S_1 \)) and \( T_2 \)-weighted (\( S_0 \)) images. The mean values of \( \ln(S_1/S_0) \) were plotted against \( b \)-value for both acquisitions (Figure 5.24).

![Figure 5.24 Plot of \( \ln(S_1/S_0) \) vs \( b \)-value for standard and DSE DT-MRI acquisitions](image)

The different slopes of the data plotted in the graph show again that the \( \langle D \rangle \) measured is different for both acquisitions. It is also noticeable that even with \( b = 0 \) s/mm\(^2\), there is still some diffusion sensitisation in the DSE acquisition. A possible explanation for this effect was found in the pulse scheme for this sequence. Figure 5.25 shows the DSE acquisition for \( b = 10 \) s/mm\(^2\) (the minimum achievable). Large crusher gradients appear in the \( z \)-direction (arrows) around the two 180\(^\circ\) RF pulses with the purpose of removing stimulated echoes derived from the two consecutive 180\(^\circ\) pulses.
These gradients may introduce diffusion sensitisation into this acquisition even when the \( b \)-value is set to zero. As a consequence, the signal intensity is reduced when compared with a conventional \( T_2 \)-weighted image. To avoid this effect, a \( b = 0 \) s/mm\(^2\) DSE DW image should be used as the baseline image, so that the residual diffusion sensitisation given by the crusher gradients is cancelled out in the ratio \( S_1/S_0 \). This will decrease the SNR of the images, but should provide accurate quantitative measurements. However, as the minimum \( b \)-value achieved by our scanner is 10 s/mm\(^2\) this is not possible. \( b = 10 \) s/mm\(^2\) could be used instead as the baseline image for DT-MRI acquisitions, but as seen in Figure 5.24, the value of \( \ln(S_1/S_0) \) given by DSE at \( b = 10 \) s/mm\(^2\) corresponds to an actual \( b \)-value of approximately 200 s/mm\(^2\). Therefore, the signal intensity obtained at that \( b \) with the DSE acquisition would be clearly dependent on the direction of measurement and data should be acquired in all the directions specified by the gradient sampling scheme. However, this would considerably increase the acquisition time of this sequence, which is why it is not used in our institution.
Chapter 6  Performance of image-based registration methods at high $b$-values

6.1 Introduction

Section §1.9 discussed the limitations of the tensor model and its inability to describe complex brain fibre structures adequately. The development of new diffusion MRI techniques, and in particular those designed for tractography, may help in understanding the changes that occur in the human brain after the onset of ischaemic stroke. In some of these new methods, high diffusion weighting is required. However, high $b$-values have two unwanted effects on the DW images, namely increased EC induced distortions, and very low SNR. In addition, the reduction of the signal intensity in brain parenchyma causes a loss of edge definition in the DW images. These effects raise doubts about the successful use of image-based registration methods to correct DW images for both bulk subject motion and EC induced distortions (66).

The aim of the present chapter is to establish the limits of (minimum) SNR and (maximum) diffusion-weighting that permit the registration of DW images within error tolerance for two image-based registration methods, namely FLIRT (137) and ICC phantom calibration (175). Synthetic and real images with different levels of SNR or diffusion weighting were used to assess the performance of each method. The error in the registration was measured using tools previously established, namely the RMS deviation for FLIRT, and the error function for ICC phantom calibration. The results were corroborated by PCA, a tool that has been shown to be a good quantitative indicator of the registration of a set of images.

6.2 FLIRT: minimum SNR

6.2.1 Methods

An initial test of the performance of the FLIRT algorithm at low SNR was carried out using synthetic $T_2$- and DW brain images generated using the Montreal Simulated Brain Database (181) as described in Bastin (175). Firstly, a noise-free $T_2$-weighted volume was created with the following parameters: 5 mm slice thickness,
2×2 mm² voxels, 30 slices, 0 % signal intensity non-uniformity. The T₂-weighted synthetic images were then segmented into grey matter, white matter and CSF based on the signal intensity in each voxel, and a single apparent diffusion tensor representative of those measured in normal human brain assigned to each of these tissues types (175). Noise-free DW images were then created from this synthetic data by applying the standard single diffusion tensor model (Eq. 1.45) to each voxel in the T₂-weighted images. To avoid large SNR fluctuations between DW images in the same set due to anisotropy effects, the B-matrix was defined with all elements equal as $B = \{b_{ij} = 1/3b\} \forall i,j = \{1,2,3\}$ with $b = 1000 \text{ s/mm}^2$. Six sets of synthetic images each one comprising one T₂- and seven DW images were generated (Table 6.1). Noise was added in quadrature to the infinite SNR synthetic brain images to simulate different SNR in the DW images in different sets. To simulate Rician noise in the synthetic image data, the noise-free DW signal intensity for each voxel was added to the real part of a set of normally distributed, complex random numbers with mean 0 and standard deviation scaled to the desired root-mean-squared noise level. The noisy amplitude signal was then obtained by taking the magnitude of this new complex number (71). The SNR for each DW image was then calculated from the signal intensity of the synthetic brain and SD of the background as previously described (71). Noise was similarly added to the T₂-weighted images to produce an SNR of approximately 30, which corresponds to the SNR measured in the T₂-weighted images of a real brain with $b = 1000 \text{ s/mm}^2$. Figure 6.1 shows the middle slice of the first DW synthetic brain volume from each set, and indicates the different levels of noise in each image set. The mean SNR achieved for the DW images in each set after adding noise is indicated under the corresponding example image in the figure. Each of the sets will be denoted by its SNR value.
Figure 6.1 Examples of the synthetic DW images used to test the performance of FLIRT at low SNR. In each set, the level of noise added was increased gradually to obtain lower SNRs. The mean SNR of the DW images in the set is indicated under each example image.

The performance of FLIRT at the different SNRs was studied as follows:

1. Distortion factors in the phase-encoding direction (y), \( M \), \( T \), and \( S \) were chosen empirically as 1.05, 5 mm and 0.02 mm/column respectively. These are representative of the EC induced distortions seen in real DW images (175). Table 1.1 shows the different combination of these factors applied to each of the seven DW images in all SNR sets to simulate distortions comprising magnification, translation or shearing of the images.

<table>
<thead>
<tr>
<th>DWI number ((i))</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distortion factors ((M, T, S))</td>
<td>((M 0 0))</td>
<td>((1 T 0))</td>
<td>((1 0 S))</td>
<td>((M T 0))</td>
<td>((M 0 S))</td>
<td>((1 T S))</td>
<td>((M T S))</td>
</tr>
</tbody>
</table>

2. The distortions were applied to each DW image using a transformation matrix \( T \) in the format used by FLIRT. Ignoring rotations, this matrix has the following form
where the sub indices indicate the direction of the transformation.

Introducing the distortion parameters selected previously, the transformation is applied voxel-by-voxel to each DW image \((i)\) as follows

\[
\begin{pmatrix}
x_i'
\end{pmatrix} = \begin{pmatrix}
1 & S_i & 0 & 0 \\
0 & M_i & 0 & T_i \\
0 & 0 & 1 & 0 \\
0 & 0 & 0 & 1
\end{pmatrix} \begin{pmatrix}
x_i \\
y_i \\
z_i \\
1
\end{pmatrix}, \tag{Eq. 6.2}
\]

where no rigid body or affine transformations in other directions have been included.

Since all the synthetic images were originally perfectly registered (all synthetic images were generated from the same data), it should only be necessary to apply the inverse of the distortion transformation \((T^{-1})\) to co-register the DW images to the \(T_2\)-weighted image in each set.

3. All the distorted DW images at each SNR were registered to the undistorted \(T_2\)-weighted image with FLIRT (v. 1.3) using the default cost-function (correlation ratio) and the resultant transformation saved.

4. The root mean square (RMS) deviation (Eq. 4.3) was used to compare the transformation \(R\) to the inverse of the distortion transformation \(T^{-1}\). In the case of perfect registration, both transformations should be identical, producing zero RMS deviation when comparing them over the image. A poor registration transformation \(R\) will be very different from the ideal transformation \(T^{-1}\) producing a large RMS deviation. This tool can therefore be used to rate the performance of FLIRT registration at each different SNR. An RMS deviation < 1 mm will be the tolerance for satisfactory registration (183).

5. A second test of the accuracy of the registration at each SNR was performed using PCA of each set of synthetic images. The quality of the registration for each SNR was assessed by comparing the distribution of the percentage of the total variance in each of the PC as shown in Figure 4.9. In particular, the value of the % of
the total variance in the first PC (\(\text{var}\{\text{PC}_1\}\)) was used to rate the accuracy of the registration. PCA of the original synthetic images with infinite SNR, both with and without distortions, and before applying FLIRT, was also performed. The distribution of variance was used to compare the results given by FLIRT at the different SNR with the ideal case, \textit{i.e.} the original images without distortions, and to test whether FLIRT improved the registration compared with the results obtained from the distorted image sets.

6.2.2 RMS difference tool

The \texttt{rmsdiff} utility provided with FLIRT was used to calculate the RMS deviation. This tool calculates Eq. 4.3 in 3D over an 80 mm sphere placed at the centre of the FOV (177). The resulting RMS deviation is expressed in mm. A test of the accuracy of this tool was performed by comparing two matrices that transformed the image by a known amount and checking that the RMS deviation is as expected. For example, comparing the following matrices over one synthetic image

\[
\begin{pmatrix}
1 & S & 0 & 0 \\
0 & M & 0 & T \\
0 & 0 & 1 & 0 \\
0 & 0 & 0 & 1
\end{pmatrix}
\quad \text{and} \quad
\begin{pmatrix}
1 & S & 0 & 0 \\
0 & M & 0 & T + 1 \\
0 & 0 & 1 & 0 \\
0 & 0 & 0 & 1
\end{pmatrix},
\]

Eq. 6.3

the RMS deviation obtained using the \texttt{rmsdiff} utility is 1 mm.

6.2.3 PCA vs SNR

Before using PCA to assess the performance of the algorithm, we must ensure that the distribution of the \(\text{var}\{\text{PC}_1\}\) resulting from the analysis is independent of the SNR of the images itself. This was achieved by applying PCA to the images in each set before registration. Since images in all sets have been distorted by the same transformation, differences in \(\text{var}\{\text{PC}_1\}\) will only be caused by the different SNR in each set. Figure 6.2 shows the distribution of the total variance in each case.
Unfortunately, the distribution of the variance of the PC depends on the SNR of the images. In particular at SNR < 10, the contribution of the noise to the image is so large that PCA encounters patterns in the image associated with noise that increase the % of the total variance in the higher order PC. This decreases the variance in the first PC and prevents the use of this method as a quantitative index for assessing the accuracy of the registration. The effect of SNR on the variance in the first PC increases dramatically as SNR decreases (Figure 6.3).

However, PCA can still be used as a quantitative objective assessment of the performance of the registration algorithm in images with the same SNR properties.
This can be achieved by using the FLIRT transformations \( \mathbf{R} \) calculated at each SNR to correct the distorted synthetic brain images with infinite SNR. This produces new sets of corrected images with equal SNR. PCA of these images will now only reflect the effects of using different \( \mathbf{R} \) and, therefore, the effect of SNR on the performance of FLIRT.

### 6.2.4 Results and discussion

The RMS deviation between the FLIRT transformation \( \mathbf{R} \) and the inverse of the corresponding distortion transformation \( \mathbf{T}^{-1} \) was calculated for each DW image at each SNR value. Table 6.2 show the results of this analysis. The mean RMS deviation for all DW images at each SNR value was also calculated.

Table 6.2 RMS deviation in mm of the registration matrix and the inverse of the distortion transformation matrix for each DW image distorted with the parameters indicated in Table 6.1. The mean ± SD for all seven DW images at each SNR is shown in the last column. Values of the RMS deviation that are greater than the error tolerance of 1 mm are shown in bold.

<table>
<thead>
<tr>
<th>Mean SNR</th>
<th>DWI(_1)</th>
<th>DWI(_2)</th>
<th>DWI(_3)</th>
<th>DWI(_4)</th>
<th>DWI(_5)</th>
<th>DWI(_6)</th>
<th>DWI(_7)</th>
<th>Mean RMS deviation (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.3 ± 2.9</td>
<td>0.57</td>
<td>0.63</td>
<td>0.63</td>
<td>0.67</td>
<td>0.63</td>
<td>0.69</td>
<td>0.63</td>
<td>0.63 ± 0.04</td>
</tr>
<tr>
<td>11.5 ± 2.1</td>
<td>0.57</td>
<td>0.63</td>
<td>0.63</td>
<td>0.95</td>
<td>0.63</td>
<td>0.69</td>
<td>0.62</td>
<td>0.67 ± 0.13</td>
</tr>
<tr>
<td>5.3 ± 1.0</td>
<td>0.55</td>
<td>0.56</td>
<td>0.59</td>
<td>0.60</td>
<td>0.59</td>
<td>0.63</td>
<td>0.59</td>
<td>0.59 ± 0.03</td>
</tr>
<tr>
<td>3.2 ± 0.5</td>
<td>0.53</td>
<td>0.67</td>
<td>0.58</td>
<td>0.61</td>
<td>0.56</td>
<td>0.59</td>
<td>0.57</td>
<td>0.58 ± 0.04</td>
</tr>
<tr>
<td>2.1 ± 0.2</td>
<td>0.57</td>
<td>0.74</td>
<td>0.63</td>
<td>0.64</td>
<td>0.65</td>
<td>0.78</td>
<td>0.59</td>
<td>0.66 ± 0.08</td>
</tr>
<tr>
<td>1.6 ± 0.1</td>
<td><strong>1.01</strong></td>
<td><strong>1.12</strong></td>
<td>0.71</td>
<td><strong>1.96</strong></td>
<td><strong>1.08</strong></td>
<td><strong>1.54</strong></td>
<td><strong>1.33</strong></td>
<td><strong>1.25 ± 0.40</strong></td>
</tr>
</tbody>
</table>

The RMS deviation results suggest that FLIRT fails to register accurately images with SNR < 2, producing errors of more than 1 mm. The error in FLIRT at higher SNR is nearly constant, but within error tolerance, at approximately 0.6 mm. However, the outlier in DWI\(_4\) at SNR of 11.5 shows that FLIRT can produce a slightly worse registration at higher SNR in some cases. This could be caused by a suboptimal optimisation of the cost function in a case where the optimised value is trapped in a local minimum during the registration of a specific image. The fact that the error does not tend to zero as SNR increases suggests that FLIRT produces a consistent error when registering DW to \(T_2\)-weighted images. This effect was explored in Chapter 5, Appendix 5b and will be further investigated in the following section.

The results of the PCA for these synthetic image datasets, corrected for the effects of different SNR, are shown in Figure 6.4.
Figure 6.4 Percentage of the total variance in each PC for sets of infinite SNR synthetic images registered with FLIRT transformation matrices calculated at different SNR. The results from the analysis in the set of original images and the set of distorted images before registration are also represented using dashed lines. The mean (SD) value of % variance for the first PC is shown next to the legend.

The dark blue dotted line shows the results of the PCA performed in the original synthetic images with infinite SNR before applying distortion or registration. Because these images were perfectly registered, % var{PC1} in this case is the maximum achievable, and thus represents the ideal case. The light green dashed line shows the % variance of the PCs calculated from the set of infinite SNR images after applying the distortions, but before registration. This line can be used as a reference to check whether the use of FLIRT is improving the alignment of the images. The continuous lines show the PCA results after applying R registration transformations calculated at the different SNRs to the infinite SNR distorted images. As seen in the graph, the use of FLIRT at SNR ≥ 5 achieves the same % variance distribution in all PC as the ideal case (overlapping lines), therefore demonstrating a good registration. The value for % var{PC1} is slightly lower than the ideal at SNR 3.2 and 2.1, indicating that the registration achieved with that noise level is slightly worse but still very close to the ideal case. The results from the RMS deviation calculations are corroborated as PCA confirms that the registration of images with matrices calculated at SNR 1.6 produces slightly poorer image alignment than at higher SNR (% var{PC1} 82.2 compared with 85.3 in the ideal case). However, although it still makes an improvement from the distorted (unregistered) case (% var{PC1} 65.0).
The effect of the consistent error found in the RMS deviation analysis is not revealed in PCA.

### 6.2.5 Consistent error of FLIRT

In the previous section, RMS deviation measurements showed that FLIRT does not achieve a perfect registration even at high SNR for synthetic brain images. Errors below 1 mm are considered difficult to eliminate and therefore considered to be acceptable (183). However, this consistent error might be explained by the fact that FLIRT is attempting to register images from different modalities, *i.e.* T$_2$- and DW images. These two types of images have different contrast properties and intensity histograms show a strong non-linear relationship (204), which might limit the accuracy of the algorithm (137). This can be confirmed by assessing whether the performance of FLIRT improves when the set of images is formed from only T$_2$-weighted images instead of a mixture of T$_2$- and DW images.

New sets of synthetic images were created, each one comprising eight T$_2$-weighted images. The first set was used as the undistorted reference, and the other seven images were distorted with same distortions factors used for the DW images and shown in Table 6.1. Rician noise was added to the distorted images in each set to achieve different SNR, while the SNR in the first image was kept constant at 30. Examples of the T$_2$-weighted images generated at different SNRs are shown in Figure 6.5. Another set of T$_2$-weighted images, all with infinite SNR, was also generated.

The FLIRT registration transformations $\mathbf{R}$ calculated at each different SNR were compared to the ideal correction transformation $\mathbf{T}^{-1}$ using the RMS deviation analysis described above. The different $\mathbf{R}$ were also used to correct the infinite SNR distorted images and the effect of each one was compared using PCA.
Figure 6.5 Examples of synthetic $T_2$-weighted images used to test the performance of FLIRT at low SNR with images of the same modality. In each set, the level of noise added was increased gradually to obtain lower SNR. The mean SNR of the $T_2$-weighted images in the set is indicated under each example image.

RMS deviation results for the sets consisting of only $T_2$-weighted images are shown in Figure 6.6. For comparison, the previous mean RMS deviation results from the registration of DW images to a $T_2$-weighted image are also plotted.

Figure 6.6 Mean RMS deviation of the FLIRT transformation $R$ to the inverse of the distortion transformation $T^{-1}$ for distorted $T_2$-weighted and DW images with different SNR. The dashed line shows the case of $T_2$-weighted images with infinite SNR.
The mean RMS deviation decreases with increasing SNR for both the distorted $T_2$- (black line) and DW (red line) images. The RMS deviation is fairly constant (~0.28 mm) except at the smallest SNR when registering $T_2$-weighted images. However, this value is lower than the one obtained when registering DW images (~0.6 mm). The minimum RMS deviation of the registration transformation obtained using FLIRT in distorted $T_2$-weighted images generated with infinite SNR was 0.27 mm.

Results from the PCA of the sets of infinite SNR $T_2$-weighted images, each registered with a FLIRT transformation $R$ calculated at different SNR, are shown in Figure 6.7. This figure also shows the percentage variance obtained from PCA in the non-distorted images and from the distorted but non-registered images.

![Figure 6.7](image)

Figure 6.7 Percentage of the total variance in each PC for sets of infinite SNR synthetic $T_2$-weighted images registered with FLIRT transformation matrices calculated at different SNR. The results from the analysis in the set of original images and the set of distorted images before registration are also represented using dashed lines. The mean (SD) value of % variance for the first PC is shown next to the legend.

The distribution of the total variance obtained from images with SNR 21.0 and infinity corrected with FLIRT show a considerable improvement in the registration compared with that obtained from the unregistered images. As seen in the figure, except at the very low SNR, 1.7, the improvement in the registration is equal at all SNRs investigated (lines overlapped in the figure). However, FLIRT never quite achieves the perfect registration of the undistorted $T_2$-weighted images indicated by the dark green dashed line. Since these images are perfectly registered, they produce
a $\% \text{var} \{PC_1\}$ with 100 $\%$ of the total variance, while the $\% \text{var} \{PC_1\}$ of registered images with infinite SNR is 99.6 $\%$.

These experiments show that even at high SNR the FLIRT algorithm generates a consistent error in the registration of images, which is significantly larger when different modality images are registered. This error is small and within tolerance, except when SNR $< 2$.

### 6.3 FLIRT: maximum $b$-value

#### 6.3.1 Introduction

The results in the previous section investigated the limits in SNR that allow registration of DT-MRI data with FLIRT within the error tolerance. However, an increase in the $b$-value in DT-MRI involves a change in the contrast properties of the DW images as well as a decrease in SNR (Figure 6.8). The aim of the present section is to simulate both effects in high $b$-value DW images to assess the performance of FLIRT.

![Figure 6.8 Change in contrast and SNR characteristics of DW images with increasing $b$-value.](image)

#### 6.3.2 Methods

The methods in §6.2 were repeated. However, now synthetic DW images were generated by increasing the $b$-value while keeping the added noise constant. The diffusion tensor model was applied to the noise free $T_2$-weighted synthetic image generating seven sets of $T_2$- and DW images with $b = 1000$, 2000, 3000, 4000, 5000, 6000 and 7000 s/mm$^2$. Rician noise was then added to the images to produce a SNR of approximately 30 in the $T_2$-weighted images. Noise factors were equal in every set of images, however, due to the attenuation of the simulated brain signal caused by
the diffusion weighting, the SNR of DW images decreased with increasing $b$ (Figure 6.8). As the $b$-value increased, the contrast of the images also changed as areas of higher diffusivity were more attenuated than regions characterised by lower diffusivity. This is therefore a more accurate synthetic model than the increasing SNR model from §6.2 to test the performance of registration of DW images acquired with high $b$-value, since most of the brain’s cortical tissue would be completely attenuated but areas of high anisotropic white matter should still be present.

![Fig 6.9 Examples of DW images used to test the performance of FLIRT at high $b$-value.](image)

In this section, the quantitative assessment of FLIRT was performed using two different similarity measures as cost functions, namely correlation ratio (CR, default) and mutual information (MI).

To simulate EC induced distortions two representative sets including stretching ($M > 1$) and shrinking ($M < 1$) of the DW images, as well as positive or negative $T$ and $S$, were used. Values for these parameters were chosen based on results from phantom experiments. The first set of distortion parameters ($P_1$) used the maximum values of $M$, $T$ and $S$ measured in phantom experiments over the range $b = 1000$,
2000, 3000 and 4000 s/mm$^2$ (maximum stretching with largest positive $T$ and $S$), while the second ($P_2$) used the minimum values of $M$, $T$ and $S$ (maximum shrinking with largest negative $T$ and $S$). The distortion parameters used were $P_1\{M, T, S\} = \{1.06, 5.6 \text{ mm}, 0.06 \text{ mm column}^{-1}\}$ and $P_2\{M, T, S\} = \{0.97, -5.8 \text{ mm}, -0.06 \text{ mm column}^{-1}\}$, which are on the same order as those used in the previous section. For both $P_1$ and $P_2$, seven DW images were created for each synthetic diffusion MRI dataset to investigate all possible combinations of $M$, $T$ and $S$ as indicated in Table 6.1.

To verify that realistic values of SNR were obtained in the simulated DW images and to corroborate the effect of high $b$ on registration, a healthy volunteer was scanned using a ODG encoding scheme at $b = 0$, 1000, 3000, 4000 6000, and 7000 s/mm$^2$. These data were collected using the following acquisition parameters: 20 axial slices of 5 mm thickness, 240x240 mm field-of-view (FOV), 128x128 image matrix, TR of 3000 ms and TE between 92.7 and 156.6 ms, depending on $b$-value.

### 6.3.3 Results and discussion

Figure 6.10 shows values of SNR measured in the synthetic and volunteer DW images as a function of $b$-value. These data show the close correspondence between SNR values produced in the simulations and those measured in real DT-MRI data.

![Figure 6.10 A comparison of SNR values measured in the simulations and human brain DW images.](image)

Table 6.3 shows the mean RMS deviation obtained when comparing the registration transformation $R$ to the ideal transformation $T^1$ for CR and MI cost.
functions as a function of \( b \)-value (SNR) averaged over the seven DW images that make up each DT-MRI dataset. The error is below the tolerance of 1 mm for \( b \)-values less than 6000 s/mm\(^2\) (SNR > 1.5) for CR and 5000 s/mm\(^2\) (SNR > 1.9) for MI, indicating that the performance of MI is slightly poorer than CR at low SNR. These SNR thresholds are lower than that established in §6.2. At high \( b \)-values and lower SNR, the error increases rapidly for both cost functions. The error is very similar for both sets of distortion parameters for each cost function, indicating that the RMS deviation may not be critically dependent on the exact form of the applied distortion parameters. Furthermore, no significant differences were found in the results for the seven individual DW images distorted with different combinations of \( M, T \) and \( S \) (data not shown).

Table 6.3 Mean (± SD) RMS deviation averaged over seven DW images for the registration transformation \( R \) calculated using correlation ratio and mutual information cost functions as a function of \( b \)-value (SNR) for two sets of distortion factors, \( P_1 \) and \( P_2 \). Values of the RMS deviation that are greater than the error tolerance of 1 mm are shown in bold.

<table>
<thead>
<tr>
<th>( b )-value (s/mm(^2))</th>
<th>SNR</th>
<th>RMS deviation (mm)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Correlation ratio</td>
<td></td>
<td>Mutual information</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>( P_1 )</td>
<td>( P_2 )</td>
<td>( P_1 )</td>
<td>( P_2 )</td>
</tr>
<tr>
<td>1000</td>
<td>11.4 ± 0.2</td>
<td>0.55 ± 0.08</td>
<td>0.61 ± 0.05</td>
<td>0.44 ± 0.08</td>
<td>0.37 ± 0.02</td>
</tr>
<tr>
<td>2000</td>
<td>6.0 ± 0.1</td>
<td>0.45 ± 0.03</td>
<td>0.48 ± 0.03</td>
<td>0.39 ± 0.10</td>
<td>0.45 ± 0.06</td>
</tr>
<tr>
<td>3000</td>
<td>4.5 ± 0.3</td>
<td>0.47 ± 0.05</td>
<td>0.47 ± 0.02</td>
<td>0.45 ± 0.12</td>
<td>0.45 ± 0.07</td>
</tr>
<tr>
<td>4000</td>
<td>2.7 ± 0.1</td>
<td>0.50 ± 0.05</td>
<td>0.54 ± 0.04</td>
<td>0.54 ± 0.10</td>
<td>0.53 ± 0.10</td>
</tr>
<tr>
<td>5000</td>
<td>1.9 ± 0.1</td>
<td>0.60 ± 0.09</td>
<td>0.65 ± 0.17</td>
<td>0.71 ± 0.09</td>
<td>0.68 ± 0.21</td>
</tr>
<tr>
<td>6000</td>
<td>1.5 ± 0.0</td>
<td>0.85 ± 0.26</td>
<td>0.89 ± 0.19</td>
<td>5.2 ± 10.9</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>7000</td>
<td>1.4 ± 0.0</td>
<td>79.4 ± 14.3</td>
<td>78.5 ± 45.3</td>
<td>81.6 ± 21.0</td>
<td>55.1 ± 18.3</td>
</tr>
</tbody>
</table>

Results from PCA corroborated these findings. Figure 6.11 shows how the total variance is shared between the first six PC for DW images generated with \( b \)-values \( \in \{1000-7000\} \) s/mm\(^2\) and distorted using \( P_1 \) for (a) CR and (b) MI. The \( \% \text{ var}[\text{PC}_1] \) is very close to the undistorted ideal case (\( \% \text{ var}[\text{PC}_1] = 99.8 ± 0.1 \% \)) for \( b \)-values up to 6000 s/mm\(^2\) for CR and 5000 s/mm\(^2\) for MI. However, at higher \( b \)-values the \( \% \text{ var}[\text{PC}_1] \) decreases dramatically, and is worse than in the unregistered distorted dataset (\( \% \text{ var}[\text{PC}_1] \approx 75 \% \)) for \( b = 7000 \) s/mm\(^2\), indicating that registration is actually worsening image alignment rather than improving it.
Chapter 6 Performance of image-based registration methods at high $b$-values

Figure 6.11 Distribution of the total variance for all slices in each set of synthetic DW images after registration with FLIRT using (a) CR and (b) MI. DWI were distorted using $P_1$. PCA of the undistorted and the unregistered images are also shown as a reference. The number next to the legend shows the mean (SD) $\% \text{var}(PC_1)$.

Figure 6.12 shows FA maps obtained from unregistered and registered DT-MRI datasets from the volunteer acquired with $b$-values of 4000, 6000 and 7000 s/mm$^2$.  

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Figure 6.12 FA maps calculated from unregistered (left column) and DW images registered with FLIRT using CR (right column) acquired with $b$-values of 4000 s/mm$^2$ (a) and (b), 6000 s/mm$^2$ (c) and (d), and 7000 s/mm$^2$ (e) and (f).

Registration of synthetic data using CR as cost function showed improvement of the registration and error below the tolerance for $b$-values up to 6000 s/mm$^2$. However, these results could not be confirmed with real DT-MRI data. For a $b$-value of 4000 s/mm$^2$ in Figure 6.12, the effect of registration is to improve the quality of the FA maps and produce white matter tracts that are better defined. However, at $b = 6000$ and 7000 s/mm$^2$, the white matter tracts are more clearly visible in the unregistered FA map, indicating that registration is making the alignment of the component $T_2$-weighted and DW images worse. These results do not completely
agree with the experiments in synthetic data, where FLIRT using CR performed within the tolerance for $b$-values up to 6000 s/mm$^2$. However, they agree with the simulations performed in §6.2 where results showed that FLIRT performed within error tolerance for SNR > 2.

Both CR and MI are multimodal similarity measures that use functional or statistical non-linear dependence between signal intensities in different images, rather than relying on the identification of landmarks (194,195). Increasing the $b$-value will change the relationship between the signal intensities of T2-weighted and DW images but there will be a dependence between them. Therefore, image-based registration methods using one of these cost functions could possibly find an optimal registration transformation even at high $b$-values. However, the decrease in SNR associated with high $b$-values makes the registration more challenging. Low SNR decreases the spatial correlation of the image intensity, which increases the number of local minima in the cost-function (194). As a result, the likelihood of the optimization being trapped in a local minimum and producing a suboptimal transformation is increased, which deteriorates the robustness of the algorithm.

The synthetic data generated for the experiments in the present section reproduced accurately the SNR of real DW images at the simulated $b$-values. However, we found difficulties to simulate the changes in DW images contrast with increasing $b$-value. The synthetic data used a single tensor model in the whole brain and characteristic single values of $\langle D \rangle$ and FA associated to grey matter, white matter and CSF. On the other hand, diffusion in real brain images might not be entirely explained by a single tensor due to the presence of more than one diffusion compartment within a voxel. In addition, the values of $\langle D \rangle$ and FA in real grey matter, white matter and CSF are far from being homogeneous in each case but they spread over a range for each tissue type. Thus, the real contrast of DW images differs from the synthetic data contrast achieved in our experiments; for instance, at high $b$-value all white matter signals are attenuated at the same level in synthetic images while there will be different levels of attenuation in real brain DW images (Figure 6.13)
There are therefore two components that may affect the performance of FLIRT at high $b$-values: the reduced SNR and the change in contrast. Both the results of FLIRT applied in real DT-MRI data (Figure 6.12), and the results from §6.2 showed that there is a SNR limit of 2 for the correct registration with FLIRT. Additional simulations could establish how the contrast component affects FLIRT's ability to register DW images acquired at high $b$-values. However, as explained above the limitations in simulating real DT-MRI data complicates this problem, as demonstrated by comparing the results from synthetic and real DT-MRI data in the present section.

In summary, we have demonstrated using quantitative methods, that FLIRT (v.1.3) registration can be used to correct EC induced distortions and bulk patient motion from low SNR DW images. This facilitates the use of high $b$-value diffusion MRI modalities where an accurate correspondence between voxels in images acquired with different degrees of diffusion weighting and different diffusion gradient directions is required. The limiting $b$-value for the use of FLIRT was 4000 s/mm$^2$ for our data. However, since there is dependence between $T_2$- and DW signals even at high $b$-values, it is possible that registration algorithms using cost-functions such as CR and MI are able to register DW images with higher $b$ providing the SNR of the images is above the minimum threshold.
6.4 **ICC Phantom calibration: minimum SNR**

### 6.4.1 Introduction

As described in §5.2.3.2, the ICC algorithm cannot be used to register brain DW images to a T₂-weighted image by direct comparison when the DW images are acquired with a \( b \)-value higher than 300 s/mm\(^2\) (175). However, the use of images from a calibration phantom allows EC induced distortions to be corrected in brain images at any \( b \)-value. Unfortunately, the decrease of SNR in phantom DW images at high \( b \)-values will also affect the performance of the ICC algorithm. The aim of this section is to establish the minimum SNR of phantom DW images that allows the calculation of the EC calibration parameters within error tolerance.

### 6.4.2 Methods

The performance of the ICC algorithm used in the phantom calibration method was assessed at low SNR using the T₂- and DW images acquired in a calibration water phantom. The cylindrical phantom was scanned using a standard ODG DT-MRI acquisition using 5 mm slice thickness, 20 slices and \( b \)-value of 1000 s/mm\(^2\). The DW images had an initial mean SNR of 26.8 ± 1.5. This was then modified by adding noise to obtain sets of images with different levels of SNR to simulate acquisition at high \( b \)-values. The T₂-weighted images were not modified, having a mean SNR of 53.1. Examples of DW images for each set are shown in Figure 6.14. The brain of a healthy volunteer was also scanned using the same acquisition parameters, giving a SNR of 30.0 and 12.6 ± 0.3 for the T₂-weighted and DW images. These brain images will be used to assess the quality of the EC induced distortion correction performed using ICC calibration parameters calculated from each set of phantom images at different SNR.
Chapter 6 Performance of image-based registration methods at high \( b \)-values

Figure 6.14 Example of DW images of the phantom with added Gaussian noise to achieve different levels of SNR. The mean SNR in each set is indicated under the example image.

The quality of the calibration factors calculated by ICC at the different SNRs was measured as follows:

1. ICC was used to calculate the calibration factors \( M, T \) and \( S \) for each DW image in the set as explained in §5.2.3.2. These calibration factors were stored, as well as those calculated from the original images of the phantom without any added noise. The latter ones were considered as the optimum calibration factors and used as a reference since they have been calculated with the highest SNR images (53.1 and 26.8 for \( T_2 \)- and DW images).

2. The calibration parameters obtained at each SNR were compared to the optimum using the error function \( \xi \) described in Eq. 5.3. Calibration parameters giving an error over the tolerance \( \xi > 0.5 \) voxel/column (approximately 1 mm) were considered as unacceptable for the correction of distortions (183).

3. As a second check, the EC induced distortions in the original phantom DW images were corrected using the calibration parameters obtained at each SNR, as well as using the optimum calibration factors. PCA was performed in the seven sets of images of the original phantom that were obtained, each one corrected with one set of calibration factors. PCA was also applied to the original images before
correction of the distortions to use as a reference. The distribution of the total variance between the PC in each case was used to assess quantitatively the registration of the images in each set and compare them. PCA is always applied to the same original images, only corrected with different calibration factors, therefore PCA results will be independent of image SNR and only represent errors due to the calibration factors (§6.2.3).

4. The same experiment as 3 above was repeated using the different sets of calibration factors to correct the volunteer images. PCA of the resulting brain images in each case was compared with results obtained using the optimum calibration factors and with the PCA of brain images before registration.

6.4.3 Results and discussion

Table 6.4 and Figure 6.15 indicate the values of the error function obtained when comparing the ICC calibration parameters calculated from the phantom DW images at different SNR with the optimum calibration parameters. The figure also represents the contribution to the error from each of the parameter $M$, $T$ and $S$.

<table>
<thead>
<tr>
<th>SNR</th>
<th>$M$</th>
<th>$T$</th>
<th>$S$</th>
<th>$\xi$ (voxel/column)</th>
</tr>
</thead>
<tbody>
<tr>
<td>26.8 ± 1.5*</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>18.4 ± 0.9</td>
<td>0.06 ± 0.06</td>
<td>0.05 ± 0.04</td>
<td>0.05 ± 0.04</td>
<td>0.09 ± 0.06</td>
</tr>
<tr>
<td>16.5 ± 0.8</td>
<td>0.07 ± 0.07</td>
<td>0.06 ± 0.05</td>
<td>0.06 ± 0.05</td>
<td>0.10 ± 0.72</td>
</tr>
<tr>
<td>10.6 ± 0.5</td>
<td>0.16 ± 0.15</td>
<td>0.11 ± 0.09</td>
<td>0.10 ± 0.08</td>
<td>0.23 ± 0.15</td>
</tr>
<tr>
<td>5.9 ± 0.3</td>
<td>0.5 ± 0.5</td>
<td>0.21 ± 0.19</td>
<td>0.17 ± 0.14</td>
<td>0.67 ± 0.60</td>
</tr>
<tr>
<td>4.1 ± 0.2</td>
<td>1.8 ± 2.1</td>
<td>0.66 ± 0.70</td>
<td>0.36 ± 0.36</td>
<td>2.2 ± 2.5</td>
</tr>
<tr>
<td>2.6 ± 0.1</td>
<td>14.1 ± 2.1</td>
<td>3.6 ± 1.6</td>
<td>1.8 ± 1.3</td>
<td>16.2 ± 2.4</td>
</tr>
</tbody>
</table>

* SNR at which the optimum calibration factors were calculated

The ICC algorithm produces errors larger than 0.5 voxel/column at SNR $\leq 8$. The error is mainly caused by the calculation of the magnification factor, affected by the ill-defined edges of the image at such low SNR.
Chapter 6 Performance of image-based registration methods at high $b$-values

Figure 6.15 Error function $\xi$ between the calibration parameters calculated for phantom DW images at different SNR compared with the optimum calibration parameters. The contribution of each parameter to the total error is also shown. The dashed line shows the error limit of 0.5 voxel/column.

The results from the PCA in the phantom images are shown in Figure 6.16

Figure 6.16 Percentage of the total variance in each of the PC of the original phantom images corrected for distortions with calibration factors calculated at different SNR. Mean (SD) % var(PC$_1$) is quoted next to the legend. The dashed lines show the result from the unregistered images and the images corrected with the optimum calibration factors.

PCA of the phantom images corroborates previous results. The light blue dotted line shows the distribution of the percentage of the total variance in the first six PC
when optimum calibration factors are used to correct for the EC induced distortions of the DW images. For SNR >10, the results of PCA are identical to this, demonstrating that the best registration is also achieved. When SNR decreases to approximately 6, the percentage variance of the first PC decreases to 99.4 %, and increases slightly in the second and third PC, showing a minor deterioration in the registration. Table 6.4 shows the error at this SNR is just over the 0.5 voxel/column tolerance. However, the use of ICC calibration factors obtained at SNR < 6 result in images with poorer registration. For example, the percentage variance in the first PC reduced to 99.0 % at SNR 4.1. We saw in Figure 6.15 that calibration factors calculated at the lowest SNR studied, 2.6, give the corrected images a very large error, which was outside the tolerance. In Figure 6.16, it is evident that these calibration factors not only do not improve the registration of the DW to the T2-weighted images, but the PCA results show that the image alignment is worse than when the images are unregistered (dashed line).

![Figure 6.17](image)

Figure 6.17 Percentage of the total variance in each of the PC for brain images corrected for EC induced distortions using ICC calibration factors calculated at different SNR. % var(PC1) is quoted next to the legend. The dashed lines show the result from the unregistered images and those corrected with the optimum calibration factors.

Similar PCA results are obtained in the brain images when the various sets of calibration parameters calculated at different SNRs are used to correct for the distortions of the DW images. Figure 6.17 shows the distribution of the percentage of
the total variance in the first six PC. Considering the variance in the first PC as an indicator of the alignment of the brain images, it can be seen that for SNR > 10 the registration achieved is equivalent to the one obtained using the optimum ICC calibration parameters. This declines at SNR 4.1, where the percentage variance of the first PC is reduced by 0.9 %. The alignment deteriorates further, when the calibration factors were obtained from phantom images acquired with SNR < 3. The % var(PC₁) obtained at the lowest SNR, 2.6, is lower than that obtained from the unregistered DW images, indicating that DW images registered using calibration factors calculated at such low SNR have a worse alignment than DW images that are not registered at all.

In summary, the limit of SNR for the phantom DW images that ensures adequate performance of ICC phantom calibration is approximately 8. This is easily achievable at intermediate b-values, e.g. SNR of the phantom containing doped water used for the previous experiments was 27. Unfortunately, due to the high diffusivity of water (⟨D⟩ ≈ 2000 × 10⁻⁶ mm²/s at 20 °C for doped water), the DW signal in this phantom decreased rapidly with increasing b-value, being almost completely attenuated at b = 3000 s/mm². However, different phantom materials with lower diffusivity can be used at high b-values to ensure that SNR > 8. For instance, solutions containing sucrose or agar have been shown to be good phantom materials for diffusion MRI (203). Our calibration phantom for high b-values is filled with a solution of 66.7 % weight of sucrose per weight of water with a ⟨D⟩ of 630 × 10⁻⁶ mm²/s at 21 °C, which provides a SNR of 16 for DW images acquired at b = 3000 s/mm². Higher SNR can also be achieved through averaging of the phantom DT-MRI data.

6.5 Conclusions

Experiments in this chapter investigated the SNR and b-value limits for the satisfactory performance of affine FLIRT and ICC phantom calibration methods.

Using affine FLIRT and a synthetic brain model where SNR was decreased, RMS deviation tests demonstrated that errors given by the registration transformation were within the tolerance of 1 mm for SNR > 2. A second simulation used to test affine FLIRT took into account the different levels of attenuation that occur in different
brain tissue types at high $b$-value DW images. It was assumed that some parts of the brain image might still be visible in DW images acquired at high $b$ and these might still be able to drive the FLIRT registration. The results from this simulation decreased the SNR threshold to 1.5 ($b = 6000 \text{ s/mm}^2$) for CR and 1.9 ($b = 5000 \text{ s/mm}^2$) for MI. Unfortunately, affine FLIRT could not register volunteer DT-MRI data with SNR < 2. However, it corroborated the SNR limit established above and demonstrated the difficulties of simulating the change in contrast caused by the increase in the $b$-value seen in real brain data.

The ICC phantom calibration method failed to produce good registration when phantom DW image SNR $\leq 8$, producing EC calibration factors that gave an error outside the tolerance when compared with the optimum calibration factors. Calibration parameters calculated at lower SNR could deteriorate the registration of brain DT-MRI data rather than improving it. It this situation, ICC phantom calibration should not be used. However, the advantage of the ICC phantom calibration method is that phantom DW images can be acquired with SNR as high as necessary by means of averaging or using a low diffusivity phantom material. In this way, correction factors can be calculated accurately at high SNR and then applied to compensate the EC distortions in DW-EP brain images, independently of the SNR of the latter and without the need to increase the scanning time of the subject. The contrast characteristics of high $b$-value DW images do not affect the performance of the ICC phantom calibration method, assuming diffusivity in the calibration phantom is homogeneous and hence the entire phantom DW image signal is attenuated equally with increasing $b$.

In conclusion, the experiments suggest that affine FLIRT could be used to register images acquired with a $b$-value of 4000 s/mm$^2$ in our scanner, and possibly at $b$-values higher than that in other scanners, providing the SNR of the brain DW images is $> 2$. The lowest SNR limit for good performance of the ICC phantom calibration method is approximately 8 for the phantom DW images. However, since the correction factors are determined from phantom data, this method could be used to correct EC distorted brain images at any $b$-value, with the condition that SNR of the phantom DW images is higher than this limit. High $b$-values should be achievable with higher gradient strengths which would allow $b$ to be increased without
extending the TE and reducing SNR. Higher main magnetic field strengths would also increase the SNR of DW images acquired at high $b$. 
Chapter 7 Conclusions

7.1 Introduction

The principal aim of this thesis was to develop optimised diffusion tensor MRI (DT-MRI) acquisition and post-processing methods that could be used in the study of acutely ill stroke patients. In this final chapter, the results of this work are summarised and discussed, while avenues for future research, such as the use of parallel imaging to speedup image acquisition time, and tractography to investigate more thoroughly how stroke affects white matter structure and integrity, are suggested.

To demonstrate the clinical application of DT-MRI, this work began by describing results from a new study in which the temporal evolution of the water diffusion parameters of grey and white matter in a group of acute stroke patients was measured. During this clinical study, issues related to the acquisition and post-processing of the DT-MRI data were identified. The thesis then focused on the development of new acquisition protocols designed to scan acutely ill stroke patients and deal with problems related to image resolution, scan time and patient motion. Post-processing methods to reduce patient motion and minimise geometric distortions created by diffusion weighting in single-shot echo-planar imaging (EPI) were then investigated using a set of tools that can quantitatively assess image registration. These tools were then used to assess three different eddy current (EC) induced distortion correction methods, as well as to clarify whether the unexpected temporal variation in contralateral water diffusion parameters seen in the stroke study was real or an artefact of the image processing methodology.

The thesis concluded by investigating what the limits on signal-to-noise ratio (SNR) and \( b \)-value are for accurate registration of diffusion-weighted (DW) images. Such an investigation is a vital precursor to future clinical studies which might use high \( b \)-value diffusion MRI to map how stroke affects regions of complex white matter structure.
7.2 DT-MRI of ischaemic stroke

The quantitative study of scalar water diffusion parameters following ischaemic stroke presented in Chapter 2 showed the temporal evolution of $\langle D \rangle$ and FA parameters within the ischaemic lesion from less than 24 hours until three months after onset. This study demonstrated that there are differences in the temporal evolution of $\langle D \rangle$ and FA of grey and white matter after stroke. These findings may explain the conflicting results reported by those previous studies that measured water diffusion parameters for the whole lesion.

The strengths of the present study, which is the largest to date investigating the temporal evolution of grey and white matter $\langle D \rangle$ and FA after stroke, are the following:

- To control for individual variability in the response to ischaemia, analysis of $\langle D \rangle$ and FA was performed separately in grey and white matter in ischaemic lesions affecting both tissue types, in the same subject scanned serially at different time points.
- A large group of stroke patients was analysed which increased the significance of the results.
- None of the patients were treated with thrombolysis or experimental drug treatment that could bias the results.

The results showed subtle but statistically significant differences in the values of $\langle D \rangle$ for grey and white matter during the first two weeks after stroke onset, which, as discussed in Chapter 2, agrees with previous findings. No significant differences in the evolution of $\langle D \rangle$ for these tissues were found at later time points. However, the statistical power of the analysis could have been decreased due to the reduction in the number of patients analysed at later time points, i.e. out of 32 subjects in total, only 24 underwent DT-MRI at 1 month and 18 at 3 months. If a larger number of patients had been scanned at these time points, there might have been further significant differences between the tissue types.

Differences in the evolution of FA between grey and white matter were significant at all time points, revealing a clear differentiation in the response of the two tissue types to ischaemia. These data also permitted the identification of two main patient subgroups that followed different patterns of white matter FA evolution (Figure 2.4),
with FA being more reduced in lesion white matter for some of the patients. Further work is required to demonstrate whether this observation has implications for predicting patient clinical outcome by measuring, for example, the evolution of white matter integrity with tractography and exploring possible correlations with clinical outcome. However, the DT-MRI data acquired in this study was not appropriate for tractography analysis due to the non-isotropic voxel size, \( i.e. \ 0.9 \times 0.9 \ mm^2 \) in-plane resolution, and 5 mm slice thickness with 1 mm slice gap.\(^3\) A new stroke DT-MRI acquisition protocol that provides near-isotropic voxels in a clinically acceptable scan time was therefore developed in Chapter 3. This protocol would permit the use of tractography in future DT-MRI studies of stroke in our institution, and could be used to investigate further the pathophysiology of stroke.

The stroke DT-MRI data were acquired with 5 NEX to increase SNR of the DW images and minimise noise bias in the FA measurements. The investigation of how FA varies with NEX (§2.4) showed that 3 NEX was sufficient to reduce FA bias to acceptable levels and that there was no clear advantage of using 5 NEX. The number of averages could, therefore, be reduced to 3, thereby reducing the scan time and increasing patient compliance. However, as an alternative, new optimised stroke protocols were developed that dealt with issues such as image resolution and patient motion during the scan. These protocols should produce more accurate measurements of \( D \) and keep scan times acceptable for neurological patients (Chapter 3).

One of the potential weaknesses of the stroke DT-MRI study was the incidence of CSF contamination in some of the ROI measured. Chapter 2 showed how a large amount of CSF signal within the ROI could significantly change the temporal

\(^3\) The reason why these slice parameters were chosen was that the patients also underwent a dynamic susceptibility contrast (DSC) perfusion MRI investigation as part of a larger over-arching study. DSC perfusion MRI requires the rapid collection of a number of gradient-echo EPI volumes, but due to restrictions on number of images than can be collected using the current GE scanning software (512), and the need to have a reasonable temporal resolution for fitting gamma-variate functions to the signal-time curves, the number of slices that can be scanned is limited (15 slices for a TR of 2.5 s). Therefore, since this over-arching study required combined measurements of diffusion and perfusion from the same slice locations covering a large region of brain tissue, the DT-MRI data could not be collected in a more optimised way.
Chapter 7 Conclusions

evolution of \langle D \rangle and FA measured, particularly in grey matter. Adequate measures, such as visual inspection of the signal histogram for each ROI, were taken to minimise this effect. However, the 5 mm slice thickness used in the DT-MRI acquisition was a limiting factor. Again, future studies could benefit from the use of thinner slices, e.g. the 2.8 mm slices used in the Stroke_B protocol described in Chapter 3.

Another issue raised during this clinical DT-MRI study was the unexpected temporal changes measured in \langle D \rangle and FA of normal contralateral brain tissues. After demonstrating that these changes also occurred in CSF – a fluid that should not be affected by the stroke – further investigation showed that registration of the data acquired at different time points could be responsible for these effects (Chapter 5). This suggested the need to develop methods to assess the image processing techniques used to register DT-MRI data. Quantitative tools for this assessment were developed in Chapter 4 and used in Chapter 5 to assess methods for the correction of EC induced distortions from DW images, and in Chapter 6, to assess the registration at high \( b \)-values/low SNR. The results from these studies will be discussed in §7.4.

7.3 Optimisation of the acquisition protocol

As discussed in §7.2, the original stroke DT-MRI protocol had a number of disadvantages, principally reduced resolution in the through-plane direction, and a scan time longer than necessary. Moreover, this protocol was based on an acquisition which used the minimum number of gradient directions required to calculate the diffusion tensor \( D \). Due to the noise present in the measured DW signals, it is generally recommended to use more than six sampling directions to improve the calculation of \( D \) using statistical regression methods for an over-determined system (32). This also provides an estimate of the error in each of the elements of \( D \). In addition, the gradient sampling scheme in the original stroke protocol was oblique double gradient (ODG) encoding, which previous studies have shown to be less optimal than other acquisition schemes, having a large \( \text{cond}(A) \) and \( TV \) (150,169).

The new optimised DT-MRI stroke protocols developed in Chapter 3 have gradient sampling schemes using orientations taken from the Icosahedral group of polyhedra with a larger number of unique diffusion sampling orientations (N) and lower
cond(A) and TV. This improves the estimation of D and, in particular, the rotational variance of the parameters calculated from it (156), and are also less sensitive to patient motion. In these optimised protocols, the use of thinner slices and near isotropic voxels has the benefit that tractography algorithms can be used on the data and CSF signal contamination should be minimised for ROI studies (§7.2).

Chapter 3 presented three novel investigations. Firstly, a study of the magnitude of bulk subject motion observed in 20 randomly selected acute stroke patients during the DT-MRI acquisition was performed. This analysis showed that the random rotations of patients during the scan could be up to 7°. Patient translational bulk motions can be corrected by registration. However, although rotational movements can also be corrected, they also have the effect that the DW signals are sampled in randomly orientated directions that are different than those specified by the acquisition scheme. It was also shown that patients are more likely to move towards the end of the scan. To avoid losing the whole dataset in cases where the patient moves excessively or the scan needs to be interrupted before completion, the new stroke acquisition protocols are composed of small blocks of fully optimised sampling directions. This permits the calculation of D from the first acquisitions without the need for completing the entire acquisition assuming the first optimised block has been acquired.

Secondly, Chapter 2 showed that increasing the SNR of the DW images reduces the noise induced overestimation of FA in low anisotropy tissues. Chapter 3 demonstrated with numerical simulations that increasing N instead of SNR also produces similar effect. Previous studies demonstrated a reduction of the rotational variance of FA with increasing N by showing a decrease in the standard deviation (156,157). However, none of them have shown improvement in the mean value of FA caused by increasing N. As discussed in §7.2, the original DT-MRI stroke protocol avoided FA bias by averaging. Conversely, the new stroke protocols improve the estimation of FA from low anisotropy tissues by increasing N from 6 to 21 and 31, which also improved the rotational variance of the diffusion parameters.

Finally, previous studies have investigated the effect on FA of the number and orientations of the diffusion gradients in DT-MRI (151,157). However, the effect of random subject motion during the scan has yet to be taken into account, a situation
likely to arise when dealing with ill patients. Chapter 3 used numerical simulations to show for first time the effect on the calculation of FA (both mean value and rotational variance) caused by different levels of patient motion. It was demonstrated that moderate motion during the scan did not significantly change the mean value but increased the rotational variance of FA. The use of larger N reduced this effect. On the other hand, mean FA could be underestimated for large motion if the $B$-matrix was not recalculated. It was also demonstrated that recalculation of the $B$-matrix did not significantly affect the optimisation of the acquisition scheme for $N = 21$ and $31$ and, therefore underestimation of FA caused by large patient rotations could be avoided.

Chapter 3, therefore, showed that the new stroke protocols not only address some of the issues raised in Chapter 2, namely giving better image resolution and permitting the use of tractography if required, but are also less affected by random patient motion. However, the acquisition scheme by itself is not sufficient to deal with patient motion and there is also the problem of geometric distortions in DW images collected using EPI. To achieve accurate voxel-by-voxel calculation of $D$, registration of the images at the post-processing stage is still needed. This topic was addressed in the remaining chapters of the thesis.

### 7.4 Assessment of image registration

It was hypothesised in Chapter 2 that the artifacts in the temporal evolution of $\langle D \rangle$ and FA measured in normal brain tissue in the stroke study were caused by the 3D registration method used to align the serial DT-MRI data. This motivated the evaluation of this and other registration methods.

A robust and accurate registration method was necessary in our DT-MRI study of stroke for two main reasons. Firstly, as demonstrated in Chapter 3, acute stroke patients are likely to move during the scan; therefore registration must be independent of the initial geometry of the images. Secondly, since this is a temporal study, DT-MRI data from subsequent scans needed to be registered accurately to the first scan. This permitted ROI created for the first time point scan to be copied directly to images acquired from later time points.

It was realised that few quantitative tools were available for the assessment of the performance of registration methods, and assessment was generally performed using
qualitative tools. These typically show misregistration artifacts by comparing the calculated parametric maps before and after registration (173,175).

With the aim of creating a set of tools that could provide a fully quantitative assessment of the performance of registration methods, Chapter 4 described a series of quantitative tests for registration techniques. Three characteristics of the registration were assessed: (i) robustness of the algorithm, (ii) accuracy of the alignment and (iii) possible image artifacts caused by the registration process.

Several authors have used quantitative tests to assess image alignment but have only checked one quantitative aspect of the registration method. Robustness is commonly measured using consistency tests, which have been used to compare the performance of different registration methods (137,178,188,205). We added a new consistency parameter, the mean intensity error, which helped to identify the source of the consistency error. Accuracy can be quantitatively tested using RMS deviation (205). However, as illustrated in Chapter 6, this method can be only applied to synthetic images where the exact registration transformation is known \textit{a priori}. Principal component analysis (PCA) was previously used as a method to check accuracy in the alignment of volunteer DT-MRI data (66). In Chapter 4, we formulated the fundamentals of PCA and illustrated its use in DT-MRI data. The use of ROI analysis to investigate possible artifacts in the image caused by registration was also described in Chapter 4. ROI, together with consistency tests and PCA, therefore form a complete set of assessment tools that permit measurement of the three quantitative aspects of registration mentioned above.

Consistency, PCA and ROI analysis were used in Chapter 5 to assess the performance of three methods used for the compensation of EC induced geometrical distortions of DW-EP images, namely dual spin echo (DSE) acquisition, iterative cross-correlation (ICC) phantom calibration and FLIRT (http://www.fmrib.ox.ac.uk). Two slice thickness (2.8 and 5 mm) and \textit{b}-values (1000 and 3000 s/mm$^2$) were tested to assess any dependence of registration on these parameters.

Consistency tests measured the mean consistency error ($\bar{\epsilon}$), the mean displacement ($\bar{r}$), and the mean intensity error ($\bar{\sigma}$). The results showed that the ICC phantom calibration was a very robust method to correct EC induced distortions, while FLIRT, in both the affine and RB modes, produced larger consistency errors. This is
most likely caused by the larger number of degrees-of-freedom (DoF) used during
the optimisation – 6 for RB and 12 for affine FLIRT – since they both correct for
subject bulk motion in 3D. Conversely, ICC phantom calibration is specifically
designed to correct for EC induced distortions using only 3 DoF. The mean
displacement was comparable for ICC phantom calibration and affine FLIRT and
larger than that of RB FLIRT. This indicates that the registration transformations that
correct for EC induced distortions were predominant over the correction of bulk
motion in the two volunteers scanned.

The PCA tests showed that the combination of ICC phantom calibration with RB
FLIRT was the most accurate method to correct for both EC induced distortions and
bulk subject motion. The poor performance of DSE acquisition in the accuracy tests
was not expected. This method should minimise the EC present during the signal
read-out of a DW-EPI sequence by using balanced diffusion sensitisation gradients.
In addition, ROI analysis showed that this method causes major signal changes in the
acquired images which affect the values of the measured DT-MRI parameters.
However, as explained in Appendix 5c, the version of DSE implemented on our
scanner was not working correctly and our results for DSE acquisition cannot be
generalised.

When selecting a method to register DT-MRI data from a large study of stroke
patients we must take into account the practical disadvantages of ICC phantom
calibration compared with FLIRT methods; that is, the need for extra phantom
calibration data, and a separate correction method to compensate for patient bulk
motion. For this reason, affine FLIRT has been commonly used to register stroke
patient images since it achieves a full 3D registration. However, the results in
Chapter 5 suggests that the combination of ICC phantom calibration and RB FLIRT
is more robust and accurate than affine FLIRT and should be considered when good
DT-MRI data registration is essential, namely for the application of tractography
techniques.

In summary, Chapter 5 demonstrated the use of quantitative assessment tools to
compare registration methods. However, ideally we want assessment tools that
provide an absolute measure of the quality of the registration by simply indicating
whether the registration is adequate or not. This is a difficult task since there is no
gold standard for the validation of a registration method. Regarding robustness, the consistency tests show the registration error in millimetres, which can be directly related to the voxel size. A tolerance threshold for the consistency error could be defined as the maximum voxel dimension, since misregistrations of less than half a voxel are very difficult to eliminate\(^4\) (183). Using this criterion, the results in Chapter 5 showed that RB and affine FLIRT and ICC phantom calibration perform satisfactorily at both slice thickness in images acquired at 1000 s/mm\(^2\). However, both FLIRT methods underperformed at 3000 s/mm\(^2\), since the error was larger than the maximum voxel dimension, particularly with 2.8 mm thick slices. As demonstrated later in Chapter 6, the increase in the error when using FLIRT was caused by changes in contrast and a decrease in SNR derived from the increase in \(b\)-value and thinner slices.

The mean displacement should be checked to ensure that small errors are not caused by negligible transformations. A tolerance threshold could be also established for the mean intensity error to keep changes in image intensity small. For instance, an absolute intensity error \(|\sigma| > 3\%\) should be investigated. This might be an indication that the registration is over or under correcting the images, which could also explain large \(\epsilon\) (Appendix 5b). However, large \(|\sigma|\) are sometimes caused by cropping of the slices at the bottom and top of the imaging volume due to lack of data to interpolate translational motions along the \(z\)-axes or rotations about \(x\) or \(y\) axis.

Similar criteria could also be applied to ROI studies by setting a limit of maximum allowed change in diffusion parameters before and after registration. However, when using ROI analysis the statistical significance of the difference in the measured diffusion parameters can be determined, and hence used as an absolute quantity of the performance in this particular aspect. For example, in lesion studies, changes in \(D\) and FA within the contralateral hemisphere could be assessed.

As far as accuracy is concerned, the distribution of the total variance between the different principal components (PC) of the DT-MRI data provided a quantity, namely

\(^4\) The consistency error is the maximum error combining the error from the forward and the reverse registrations. Therefore the real error would be approximately half of this.
the percentage of the total variance in the first PC (% var(PC1)), that can be used to compare different registration methods. However, it is difficult to determine an absolute value for % var(PC1) which indicated a satisfactory registration in terms of accuracy. There are two main reasons for this. Firstly, the distribution of the total variance in the PC is very dependent on the SNR and contrast characteristics of the DT-MRI component images, as demonstrated in Chapter 5 and Chapter 6. Therefore, even if a reference % var(PC1) value was established, a different reference would be required every time the acquisition parameters or image contrast change. Secondly, for brain DT-MRI data, it is not possible to establish a reference value for the % var(PC1) corresponding to a "perfect registration" to compare different registration methods. This is possible with synthetic data, as shown in Chapter 6, where all synthetic images were generated from the same template and were initially perfectly aligned. This permits the calculation of the "perfect" % var(PC1), which can be used as a reference for the performance of different registration techniques. That reference value, however, cannot be applied to real brain images since it is unlikely that synthetic DT-MRI data exactly reproduces the SNR and contrast properties of real data. Nevertheless, PCA permits the quantitative assessment of improvements in the alignment of DT-MRI data produced by a given registration method.

The aim of Chapter 6 was to establish the minimum SNR and maximum b-value that permitted the use of affine FLIRT and ICC phantom calibration to register adequately DT-MRI data. Synthetic brain data was used to simulate typical geometric distortions and assess the level of registration achieved by each method. The limiting SNR to achieve registration within the tolerance error was found to be 2 for the brain DW images when using affine FLIRT and 8 for the phantom DW images when using ICC phantom calibration. The limit of maximum b-value was found to be 6000 s/mm² for affine FLIRT applied to synthetic DT-MRI data when using the default cost-function. However, this result could not be reproduced in real DT-MRI data, where images with b-values up to approximately 4000 s/mm² could be registered. Nevertheless, this latter result corroborated the limit of minimum SNR of 2 given by the earlier simulations. As explained in Chapter 6, the failure to reproduce the results from real DT-MRI data with increasing b-value simulations was caused
by the difficulty of reproducing accurately the contrast properties of real DW-EP brain images.

For the ICC phantom calibration method, establishing the limit of $b$-value is not essential. The $\langle D \rangle$ of the phantom used to acquire the calibration data was constant; hence increasing the $b$-value only decreased the SNR of the phantom image homogeneously without causing contrast changes.

The minimum SNR threshold for ICC phantom calibration was higher than that of affine FLIRT. However, the main advantage of using the former method at high $b$-values is that ICC is applied to phantom images and these can be acquired with SNR as high as necessary by means of averaging or use of a low diffusivity phantom material. Unfortunately, as mentioned above, this method would not achieve complete registration and, unless subject motion can be kept to a minimum, 3D RB registration will still be necessary.

7.5 Future work

A stroke study including a larger number of patients scanned serially could clarify better the pathophysiological changes occurring with grey and white matter within the ischaemic lesion after stroke. The new stroke protocols developed in Chapter 3 would make the calculation of DT-MRI parameters less sensitive to patient motion, and would also address the issues concerning image resolution described in Chapter 2. These stroke protocols were based on the standard DT-MRI acquisition available on our scanner and are optimised taking into account that scan time is a limiting factor in examining acutely ill patients. Parallel imaging techniques such as sensitivity encoding (SENSE) (69) or generalized autocalibrating partially parallel acquisitions (GRAPPA) (206) are now commercially available on MRI scanners. A new stroke study could benefit from using these acquisition methods in many ways. The main advantage of parallel imaging is the dramatic speed up of the acquisition produced by using multiple receiver coils in parallel, which would increase the patient compliance and decrease the motion artifacts. Parallel imaging also permits acquisitions with higher resolution, allowing the acquisition of small isotropic voxels for use in tractography. In addition, EC and susceptibility induced distortions are significantly reduced in this acquisition. This may help to identify stroke lesions in areas of the brain affected by these distortions, and particularly to differentiate real
lesions from hyperintensity caused by susceptibility artifacts in DW images. However, these acquisition methods have some drawbacks. For instance they may introduce new artifacts in the images, such as spatially varying SNR with the possibility for introduction of spatial parameter variation. In addition, some parallel imaging techniques need of calibration data for coil sensitivity estimation.

Amongst the findings of the stroke study in this thesis, an important observation was that there were two groups of patients with different patterns of white matter FA evolution. As FA is thought to be a marker of white matter fibre integrity, it could be related to final patient outcome. However, as demonstrated in Chapter 2, the measurement of DT-MRI parameters with conventional ROI analysis is not straightforward, and the problem becomes more complicated if FA is to be measured reliably in small white matter fibres affected by a stroke lesion. Nevertheless, fibre integrity could be measured in a more comprehensive way by using tractography techniques, which permit the investigation of white matter fibre bundles in a non-invasive way. The development of robust metrics that permit the characterisation of white matter tracts produced by tractography algorithms, e.g. measuring tract length or volume, would help further in the understanding of white matter changes occurring after stroke and, perhaps, allow correlation with patient clinical outcome. However, much work is required to develop and test these methods for application to diseased white matter.

The registration assessment tools developed in Chapter 4 could be applied in the future to test new registration algorithms. In particular, it could be further investigated whether registration algorithms can perform adequately at high $b$-values by developing more accurate synthetic brain models. These should reproduce the changes in signal contrast and SNR occurring in real brain images acquired at high $b$ to assess the effects of registration. The use of advanced diffusion imaging techniques using high $b$-values would again help in stroke research, particularly for the study of water compartment redistribution and the effect of ischaemia on complex white matter fibre structure.
References


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### Appendix A  List of abbreviations

<table>
<thead>
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<th>Symbol</th>
<th>Description</th>
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<tr>
<td>(\gamma)</td>
<td>Gyromagnetic ratio</td>
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<tr>
<td>(\Sigma)</td>
<td>Covariance matrix</td>
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<tr>
<td>(\rho)</td>
<td>Probability density function</td>
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<tr>
<td>(\lambda_i)</td>
<td>(i)-th eigenvalue</td>
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<tr>
<td>(\delta_{ij})</td>
<td>Kronecker Delta</td>
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<tr>
<td>(\omega_0)</td>
<td>Larmor frequency</td>
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<tr>
<td>2D</td>
<td>Two dimensions</td>
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<tr>
<td>3D</td>
<td>Three dimensions</td>
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<tr>
<td>ADC</td>
<td>Apparent diffusion coefficient</td>
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<tr>
<td>(b)</td>
<td>Diffusion weight</td>
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<tr>
<td>(\tilde{B}_0)</td>
<td>Static magnetic field</td>
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<tr>
<td>CBF</td>
<td>Cerebral blood flow</td>
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<td>CR</td>
<td>Correlation ratio</td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<tr>
<td>CT</td>
<td>Computerised tomography</td>
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<tr>
<td>CU</td>
<td>Cone of uncertainty</td>
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<tr>
<td>D</td>
<td>Diffusion tensor</td>
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<tr>
<td>D</td>
<td>Diffusion coefficient</td>
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<tr>
<td>(\langle D \rangle)</td>
<td>Mean diffusivity</td>
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<tr>
<td>DAI</td>
<td>Diffusion anisotropy indices</td>
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<tr>
<td>DoF</td>
<td>Degrees-of-freedom</td>
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<td>DSE</td>
<td>Dual spin echo</td>
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<tr>
<td>DT</td>
<td>Diffusion tensor</td>
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<tr>
<td>DW</td>
<td>Diffusion weighted</td>
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<tr>
<td>EC</td>
<td>Eddy currents</td>
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<tr>
<td>EP</td>
<td>Echo planar</td>
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<td>EPI</td>
<td>Echo planar imaging</td>
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<tr>
<td>Eq.</td>
<td>Equation</td>
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<tr>
<td>FA</td>
<td>Fractional anisotropy</td>
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<tr>
<td>FID</td>
<td>Free induction decay</td>
</tr>
<tr>
<td>FLAIR</td>
<td>Fluid attenuated inversion recovery acquisition</td>
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<tr>
<td>FLIRT</td>
<td>FMRIB’s linear image registration tool</td>
</tr>
<tr>
<td>FMRIB</td>
<td>Oxford Centre for Functional Magnetic Resonance Imaging of the Brain</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full width at half maximum</td>
</tr>
<tr>
<td>GE</td>
<td>General Electrics</td>
</tr>
<tr>
<td>GRAPPA</td>
<td>Generalized autocalibrating partially parallel acquisitions</td>
</tr>
<tr>
<td>GRE</td>
<td>Gradient echo</td>
</tr>
<tr>
<td>(G_x)</td>
<td>Magnetic field gradient along the (x) axis</td>
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<tr>
<td>(\hbar)</td>
<td>Planck constant</td>
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<tr>
<td>HARDI</td>
<td>High angular resolution diffusion imaging</td>
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<td>I</td>
<td>Intrinsic spin</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
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<tr>
<td>ICC</td>
<td>Iterative cross-correlation</td>
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<tr>
<td>Icosa</td>
<td>Icosahedral</td>
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<tr>
<td>$\mathcal{M}$</td>
<td>Magnetisation</td>
</tr>
<tr>
<td>MI</td>
<td>Mutual information</td>
</tr>
<tr>
<td>MR</td>
<td>Magnetic resonance</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MS</td>
<td>Multiple sclerosis</td>
</tr>
<tr>
<td>N</td>
<td>Number of non-collinear sampling directions</td>
</tr>
<tr>
<td>NEX</td>
<td>Number of image averages</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>ODF</td>
<td>Orientation density function</td>
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<tr>
<td>ODG</td>
<td>Oblique double gradient encoding</td>
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<tr>
<td>PC</td>
<td>Principal components</td>
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<tr>
<td>PCA</td>
<td>Principal component analysis</td>
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<tr>
<td>PDF</td>
<td>Probability density function</td>
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<tr>
<td>RA</td>
<td>Relative anisotropy</td>
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<tr>
<td>RB</td>
<td>Rigid body</td>
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<tr>
<td>RF</td>
<td>Radiofrequency</td>
</tr>
<tr>
<td>RMS</td>
<td>Root mean square</td>
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<tr>
<td>ROI</td>
<td>Region-of-interest</td>
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<tr>
<td>SBIRC</td>
<td>SFC Brain Imaging Centre</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SENSE</td>
<td>Sensitivity encoding</td>
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<tr>
<td>SNR</td>
<td>Signal-to-noise ratio</td>
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<tr>
<td>STEAM</td>
<td>Stimulated-echo acquisition-mode</td>
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<tr>
<td>$t$</td>
<td>Time</td>
</tr>
<tr>
<td>TE</td>
<td>Echo time</td>
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<tr>
<td>TI</td>
<td>Inversion time</td>
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<tr>
<td>TR</td>
<td>Repetition time</td>
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<tr>
<td>$\text{Tr}(\mathbf{A})$</td>
<td>Trace of $\mathbf{A}$</td>
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Appendix D  Publications

Conference papers


- Assessment of image-based registration of diffusion-weighted images acquired at high b-value. S. Muñoz Maniega, M.E. Bastin and P.A. Armitage. Accepted for presentation in the 14th Scientific Meeting of the ISMRM.

Journal Publications

Temporal evolution of water diffusion parameters is different in grey and white matter in human ischaemic stroke

S Muñoz Maniega, M E Bastin, P A Armitage, A J Farrall, T K Carpenter, P J Hand, V Cvoro, C S Rivers, J M Wardlaw

Objectives: Our purpose was to investigate whether differences exist in the values and temporal evolution of mean diffusivity (\(<D>\)) and fractional anisotropy (FA) of grey and white matter after human ischaemic stroke.

Methods: Thirty two patients with lesions affecting both grey and white matter underwent serial diffusion tensor magnetic resonance imaging (DT-MRI) within 24 hours, and at 4–7 days, 10–14 days, 1 month, and 3 months after stroke. Multiple small circular regions of interest (ROI) were placed in the grey and white matter within the lesion and in the contralateral hemisphere. Values of \(<D>\), \(<D>\), FA(grey) and FA(white) were measured in these ROI at each time point and the ratios of ischaemic to normal contralateral values (\(<D>)_{R}$ and $FA_R$) calculated.

Results: \(<D>\) and FA showed different patterns of evolution after stroke. After an initial decline, the rate of increase of \(<D>\) was faster than \(<D>\) from 4–7 to 10–14 days. FA(white) decreased more rapidly than FA(grey) during the first week, thereafter for both tissue types the FA decreased gradually. However, FA(white) was still higher than FA(grey) at three months indicating that some organised axonal structure remained. This effect was more marked in some patients than in others. \(<D>\) was significantly higher than \(<D>\) within 24 hours and at 10–14 days ($p<0.05$), and $FA_R$ was significantly more reduced than FA(grey) at all time points ($p<0.001$).

Conclusions: The values and temporal evolution of \(<D>\) and FA are different for grey and white matter after human ischaemic stroke. The observation that there is patient-to-patient variability in the degree of white matter structure remaining within the infarct at three months may have implications for patient outcome.

D iffusion tensor magnetic resonance imaging (DT-MRI) provides quantitative measures of the mobility of water molecules in vivo. Parameters such as the mean diffusivity (\(<D>\)), or the apparent diffusion coefficient (ADC) averaged over three orthogonal directions, measure the magnitude of diffusion of the water molecules, while diffusion anisotropy indices, such as fractional anisotropy (FA), indicate the deviation from pure isotropic diffusion of water mobility in vivo. These scalar parameters are thought to be useful markers of tissue cellular integrity.

Since the introduction of diffusion-weighted (DW)-MRI, many studies have investigated the temporal evolution of the parameters of diffusion in ischaemic stroke, with most with DW-MRI rather than with DT-MRI.\(^{1-9}\) Overall these studies found that the ADC decreases immediately after stroke, then increases slowly towards normal values (pseudonormalisation). Finally, it becomes elevated, which is thought to indicate tissue necrosis. Other authors report a general reduction in diffusion anisotropy after stroke, consistent with loss of integrity of the tissue structure.\(^{10-11}\) These studies suggest that characterising the temporal evolution of the diffusion parameters may prove useful in differentiating acute from non-acute infarcts, as well as investigating how the brain structure is affected in ischaemic stroke.

There are, however, inconsistencies in the results of these studies. For example, some authors have reported a time of pseudonormalisation between five and 10 days,\(^{1-5,8,10}\) while others found that the ADC reaches normal values between 24 and 48 hours\(^{4}\) or at about one month.\(^{4,7}\) Generally, the diffusion anisotropy decreases after stroke,\(^{11}\) although some authors report an increase immediately after onset.\(^{10,12}\) Differences in imaging methodology may partly explain the conflicting results.\(^{13}\) Another possible cause could be heterogeneity of diffusion values within the lesion in the acute stage, potentially arising from the difference in the water diffusion properties of grey and white matter.\(^{14-16}\) Some investigators have considered this issue and measured the parameters of diffusion in ischaemic grey and white matter.\(^{10,17-20}\) Unfortunately not all of these studies scanned individuals serially at each time point to control for between subject variability,\(^{17,18}\) and most did not measure the full diffusion tensor.\(^{16,18,20}\) Furthermore, some studies only imaged a small number of patients,\(^{16-20}\) while not all were specifically designed to analyse differences between the two types of tissue.\(^{10-11}\) The purpose of the present study was therefore to investigate whether differences exist in the values and temporal evolution of \(<D>\) and FA of lesion grey and white matter in a group of patients imaged serially from onset to 3 months.

SUBJECTS AND METHODS

Subjects

Consecutive patients presenting with symptoms of moderate to severe cortical ischaemic stroke, who had no evidence of haemorrhage on the initial computed tomography (CT) or MR scan, were recruited during an effective period of 21 months between January 2001 and July 2003. A stroke neurologist (SWM) assessed all patients for acute stroke. Patients fulfilling the following criteria were included: age $\geq$ 18 years; diagnosis of acute ischaemic stroke with a CT or MR scan performed within 24 hours of the onset of symptoms; and a modified Rankin scale score of 0–2. The patients were excluded if they had been scanned at their local hospital or if they had received any neurovascular intervention before the initial MR scan.

Abbreviations: ADC, apparent diffusion coefficient; CT, computed tomography; \(<D>\), mean diffusivity; DT-MRI, diffusion tensor magnetic resonance imaging; DW-MRI, diffusion-weighted MRI; EP, echo planar; FA, fractional anisotropy; ROI, regions of interest
physician examined all the patients prior to scanning, and categorised the type of stroke according to the Oxfordshire Community Stroke Project (OCSP) classification.21 The inclusion criteria for the study were as follows: (a) the patient could be imaged within a maximum of 24 hours after symptom onset, (b) the patient had no contraindications to MR, and (c) the patient could tolerate the imaging protocol. For the present analysis, we identified those patients who were not receiving any thrombolytic treatment and whose visible ischaemic lesion on their first time point DW images included both grey and white matter. Patients underwent, where possible, repeat MRI at around 4–7 days, 10–14 days, 1 month, and 3 months after stroke. All patients were scanned at least twice. The local ethics committee approved the study, and in all cases informed consent was obtained from the patient or a close family member.

**Imaging methods**

All MRI data were obtained using a GE Signa LX 1.5 T (General Electric, Milwaukee, WI) clinical scanner, equipped with a self shielding gradient set (22 mT/m maximum gradient strength and 120 T/m/s slew rate) and a “birdcage” quadrature head coil supplied by the manufacturer. The MRI examination consisted of a standard fast spin-echo T2-weighted sequence, a gradient-echo T1-weighted sequence, and a previously described DT-MRI protocol based on spin-echo echo planar (EP) imaging.22 The duration of the examination was approximately 30 minutes.

To ensure that the slice locations used in the follow up scans corresponded as closely as possible to those in the first, the subject’s head position and tilt in the first scan were recorded and the patient repositioned as close as possible to this location for the follow up scans. Computational image realignment techniques were then used to realign the images in the follow up scans to the first, thereby minimising any small remaining positioning errors and removing eddy current induced artefacts.23

From these MRI data, the apparent diffusion tensor of water (D) was calculated in each voxel from the signal intensities in the component EP images.24 Maps of D, R, and FA for every patient at each time point were generated on a voxel-by-voxel basis from the sorted eigenvalues of D and converted into Analyze (Mayo Foundation, Rochester, MN) format.

**Region of interest analysis**

For each patient, the acute ischaemic lesion at less than 24 hours was outlined in each slice where the lesion was visible by tracing around the region of signal hyperintensity on the first DW-EP scan (fig 1A). The lesion outlines were copied onto the first scan T2-weighted EP images and multiple circular regions of interest (ROI) of 6 mm diameter were placed in grey and white matter within the lesion using anatomical and imaging knowledge (fig 1B). This ROI size was chosen to enable selection of regions of grey and white matter, while minimising the effects of mixed tissue and cerebrospinal fluid (CSF) contamination. ROI were also placed in comparable contralateral grey and white matter and were used as control regions for each patient. As many ROI as possible were placed in each slice of the T2-weighted EP image from the within 24 hours time point and then overlaid onto the coregistered DW-EP images, D, and FA parametric maps for the same time point (fig 1C, D) and all subsequent time points available for that patient. On average, for each patient 14 ROI were placed in grey matter and 10 in white matter. When tissue structures sampled by ROI chosen at the first time point were displaced in subsequent scans by lesion swelling or atrophy, the ROI positions were corrected using the structural T1 and T2-weighted imaging data so that the same anatomical piece of tissue was sampled at all time points. Areas of coincidental periventricular white matter disease and haemorrhagic transformation of the infarct at later time points were avoided with the aid of the structural MRI data. A neuroradiologist verified the positions of all ROI and the outlines of the ischaemic lesions.

**Statistical analysis**

Mean values of <D>grey, <D>white, FAgrey and FAwere calculated from all ROI in the lesion and contralateral tissue for every patient at each time point. However, while it is important to characterise the evolution of <D> and FA to understand how stroke affects brain tissue structure, these absolute parameters also reflect the intrinsic difference between grey and white matter (<D>grey > <D>white) and FAgrey<FAwhite for normal brain), and inter-patient variability. Therefore, to control for biological variation in <D> and FA and to allow group comparisons between individuals at each time point, the ratio of lesion to contralateral <D> and FA (<D>grey and FAgrey) were also calculated.20·22 Finally, a global mean for each time point for grey and white matter <D>, FA, <D>grey and FAgrey was determined from the individual patient mean data. Thus, all lesions were weighted equally regardless of the number of ROI in each.

Differences between <D>grey and FAgrey values of grey and white matter at each time point for each individual patient were then assessed using two tailed Student’s paired t test (null hypothesis: <D>grey <D>white = 0 and FAgrey=FAwhite = 0), with p<0.05 considered as statistically significant.17·19

![Figure 1 Examples of grey and white matter regions of interest (ROI) drawn on a representative slice from a 79 year old male patient with total right anterior circulation stroke. (A) Lesion outline drawn on the within 24 hours scan diffusion-weighted echo planar (DW-EP) image. (B) Circular ROI drawn in areas of grey and white matter in the lesion and in contralateral tissue using the coregistered T2-weighted EP images. All ROI copied onto (C) mean diffusivity <D> and (D) fractional anisotropy (FA) maps for this time point and all subsequent scans to characterise the temporal evolution of these parameters. ROI with crosses indicate grey matter while open ROI indicate white matter.](image-url)
RESULTS

From a group of 109 patients recruited within 24 hours of stroke onset during the study period, 77 were excluded from the present analysis for the following reasons: (a) in 50 patients the lesion did not affect both grey and white matter, (b) 14 patients could not tolerate the entire first time point MRI examination, (c) in 10 patients primary haemorrhage was the cause of the stroke, (d) data from two patients were corrupted, and (e) one patient presented with evidence of other concurrent neurological disease on structural MRI. The remaining 32 patients, the majority with medium to large cortical infarcts involving both grey and white matter underwent DT-MRI examinations at least twice, with 18 patients scanned at all five time points. The demographics of the recruited patient group, stroke syndromes, and number of patients scanned at each time point are summarised in table 1.

Figure 2 shows the variation in the absolute values of lesion <D> and FA after stroke for grey and white matter. After the initial reduction from normal values, both <D>{grey} and <D>{white} increased. The general trend in the evolution of <D>{grey} and <D>{white} was similar, although between time points two (4–7 days) and three (10–14 days) the rate of increase of <D>{grey} was faster than <D>{white}. FA{white} decreased rapidly during the first week, then more gradually over the next three weeks. FA{grey} decreased less rapidly than FA{white} during the first week, then more gradually over the next three weeks. Between one and three months there was little change in either FA{grey} or FA{white}. However, although FA{white} decreased more than FA{grey} it was still higher at 3 months.

Values of <D>{grey}, <D>{white}, FA_B{grey}, and FA_B{white} are shown in table 2 for all five time points. Both <D>_R{grey} and <D>_R{white} were significantly reduced during the first day after stroke onset (p<0.0001). <D>_R{grey} was significantly higher than <D>_R{white} at this time point (p=0.02), with an average reduction of 26% for grey matter and 31% for white matter. Between the first and second time points <D>_R{white} started to rise. <D>_R{grey} and <D>_R{white} were nearly identical at time point two. However, between time points two and three <D>_R{grey} increased at a faster rate and pseudonormalised earlier than <D>_R{white}. <D>_R{grey} was again significantly higher than <D>_R{white} at time point three (p=0.03). This confirms the faster recovery of <D>_R{grey} seen between time points two and three in fig 2A. Thereafter, both <D>_R{grey} and <D>_R{white} increased slowly over time becoming elevated and of the same value at 3 months. Table 2 also shows that while FA_B{grey} remained unchanged, FA_B{white} was significantly reduced in the first day after stroke (p<0.001). Between time points one and two FA_B{grey} declined less rapidly than FA_B{white}, with FA_B{grey} being reduced by 10% and FA_B{white} by 46%. At later time points both FA_B{grey} and FA_B{white} continued to decrease gradually. FA_B{grey} was significantly greater than FA_B{white} at all time points (p<0.001).

DISCUSSION

This is the largest longitudinal study to date to investigate the temporal evolution of water diffusion parameters in grey and white matter as measured by DT-MRI. Several previous studies have investigated the temporal evolution of fractional anisotropy (FA) and mean diffusivity (D) in tissue pathological states. However, none of these have focused on the temporal evolution of water diffusion parameters in stroke lesion grey and white matter.

Table 1 Demographic details, number of scans, and clinical stroke syndrome21 for the patients imaged in this study

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>No. of scans</th>
<th>M/F</th>
<th>Mean (SD) age (years)</th>
<th>Stroke syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TACS</td>
</tr>
<tr>
<td>18</td>
<td>5</td>
<td>12/6</td>
<td>67.8 (13.9)</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>4/2</td>
<td>80.5 (7.1)</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>2/3</td>
<td>77.6 (7.2)</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>2/1</td>
<td>85.3 (13.4)</td>
<td>2</td>
</tr>
<tr>
<td>32</td>
<td>135</td>
<td>20/12</td>
<td>73.9 (13.3)</td>
<td>11</td>
</tr>
</tbody>
</table>

F, female; LACS, lacunar syndrome; M, male; PACS, partial anterior circulation syndrome; POCS, posterior circulation syndrome; TACS, total anterior circulation syndrome.
white matter after stroke using DT-MRI. The above results show that there are differences in the temporal evolution of $\langle D\rangle_{\text{grey}}$ and FA of grey and white matter in ischaemic lesions affecting both tissue types. Although the overall evolution of $\langle D\rangle_{\text{grey}}$ and $\langle D\rangle_{\text{white}}$ was similar, subtle differences were observed during the first two weeks after stroke, specifically the rate of increase of $\langle D\rangle_{\text{grey}}$ was faster between 4–7 and 10–14 days. Differences in the evolution of grey and white matter FA were more marked, with the loss of FA_{\text{white}} being significantly greater than FA_{\text{grey}} during the first week after stroke.

Previous studies reporting changes in grey and white matter water diffusivity indices have shown contradictory results. As in the current study, Yang et al and Mukherjee et al have found that $\langle D\rangle_{\text{grey}}$ was significantly more reduced in white than grey matter at less than three days.\textsuperscript{18} 19 However, Sorensen et al reported that $\langle D\rangle_{\text{grey}}$ was significantly more reduced in grey matter,\textsuperscript{17} while Bastin et al and Fiebach et al did not find significant differences between grey and white matter at this stage.\textsuperscript{18} 20 In the longitudinal studies of Yang et al and Fiebach et al, it was observed that $\langle D\rangle_{\text{grey}}$ of both grey and white matter remained low during approximately the first four days, increasing thereafter, and reaching normal contralateral values at approximately day 10.\textsuperscript{18} 20 Conversely, Bastin et al only found this pattern of $\langle D\rangle_{\text{grey}}$ change in grey matter, which started to rise at approximately seven days, while no significant change was observed in white matter water $\langle D\rangle_{\text{grey}}$ during the first 14 days after the initial reduction following stroke.\textsuperscript{17} However, all these studies, as with the current work, observed a slightly earlier remodelling of grey matter water diffusivity.

Our values of FA at less than 24 hours are similar to the results of Sorensen et al\textsuperscript{17} who found that grey matter FA remained unchanged at this time point while white matter FA decreased significantly, even though only 10 of the 50 patients studied had lesions affecting both grey and white matter. Yang et al reported changes in both grey and white matter diffusion anisotropy.\textsuperscript{18} In their longitudinal study of 26 patients, most subjects showed an increase of diffusion anisotropy for both tissue types by 12 hours, after which the diffusion anisotropy declined. At approximately 90 days, the diffusion anisotropy was further reduced in all lesions. However, for most patients in their study the diffusion anisotropy was assessed by means of an orientation dependent parameter derived from ADC values, rather than a rotationally invariant index derived from $\mathbf{D}$, such as FA. Elevated $\langle D\rangle$ and reduced FA indicate destruction of membrane integrity and progression towards necrosis.\textsuperscript{21} Interestingly, fig 2A shows that grey and white matter $\langle D\rangle$ was still increasing at three months, while FA did not change significantly after one month (fig 1B). Increasing water diffusion at later time points has been observed in other studies, which have attributed this to gliosis and accumulation of CSF in the lesion secondary to encephalomalacia\textsuperscript{1} or to flow dephasing effects in regions that have undergone cystic degeneration.\textsuperscript{2} However, no measurements of diffusion anisotropy were made in these studies to compare with ours. Another important observation is that despite the greater loss of white matter FA, it was still significantly higher than grey matter FA at three months, indicating that some organised axonal structure remains. This effect was more marked in some patients than others, which contributes to the larger standard deviations measured in FA_{\text{white}} than FA_{\text{grey}} (fig 2B). To our knowledge, this has not been reported before, as such effects are not evident when measuring whole lesion diffusion parameters, and could have implications in predicting patient outcome. For instance, DT-MRI tractography methods could be used to measure how changes in tract volume and integrity following stroke relate to clinical dysfunction and outcome.

### CONCLUSIONS

By following individual patients over time with ischaemic lesions affecting both grey and white matter, we found evidence for the difference in the values and temporal evolution of $\langle D\rangle$ and FA for grey and white matter. Specifically, the rate of increase of $\langle D\rangle_{\text{grey}}$ was faster than $\langle D\rangle_{\text{white}}$ from week one to week two, while FA_{\text{white}} decreased more rapidly than FA_{\text{grey}} during the first week after stroke. Thereafter, FA decreased gradually for both tissue types. However, FA_{\text{white}} was still higher than FA_{\text{grey}} at three months indicating that some organised axonal structure remained. The observation that there is patient-to-patient variability in the degree of white matter structure remaining within the infarct at three months may have implications for predicting patients’ outcome.

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