



Modelling the Short- T_2^* Signal in the Liver with Ultrashort Echo Time Magnetic Resonance Imaging (UTE- MRI)

by

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A thesis submitted for the degree of Doctor of Philosophy

Michaelmas Term 2019

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Abstract

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Thesis submitted for the degree of Doctor of Philosophy

Liver fibrosis, a consequence of most chronic liver diseases, is characterised by collagen deposition. Collagen has a short- T_2^* signal that is undetectable with conventional magnetic resonance (MR) sequences. However, collagen signal is measurable with the ultrashort echo time (UTE) sequence, which can achieve an echo time of less than 1 ms. This thesis explored the modelling of the short- T_2^* signal in the liver with UTE.

Given the implication of collagen in liver disease, MR signal properties, including the R_2^* and resonant frequency shift, were investigated in oriented collagen. *Ex vivo* measurements were performed at 3 T in tendon, an example of oriented collagen. Furthermore, the R_2^* of collagen was investigated in phantoms *in vitro*.

The UTE sequence was optimised for *in vivo* imaging on the Siemens Trio 3-T and Prisma 3-T scanners. On the Trio, optimisations included reduced gradient spoiling, the use of a double-echo vs. a single-echo acquisition and the use of spatial saturation. Calibrations were performed on the Prisma for the bandwidth, radiofrequency coil and minimum TE.

From the R_2^* and resonant frequency shift of tendon, an R_2^* model was developed for describing isotropic collagen and free water in the liver. Other exponential models were also considered, accounting for short- T_2^* signal. UTE acquisitions were performed to measure R_2^* signal in the liver from healthy subjects and liver-disease patients *in vivo*. The models were used to fit the R_2^* signal and the most appropriate model was selected. Subsequently, the repeatability and reproducibility of the short- T_2^* signal fraction were evaluated. Comparisons of the short- T_2^* signal fraction were made between healthy subjects and patients.

Given the selected model for the short- T_2^* signal in the liver, potential sources of short- T_2^* signal were discussed. Future work involves the identification of the constituent(s) giving rise to the short- T_2^* signal in the liver.

Acknowledgements

I am indebted to my supervisors, Prof. Matthew D. Robson and Dr. Luca Biasioli, for their supervision and support throughout this academic journey. Thank you to Matt for accepting me as a student and giving me the opportunity to come to Oxford. Thank you to Luca for always being willing to discuss the small details of my project.

I would like to thank Dr. Michael Pavlides, Dr. Ferenc E. Mózes, Arjun N. Jayaswal and the liver MR group at OCMR for their collaboration on the liver fibrosis study. This includes help with recruiting patients and conducting the study.

It has been a pleasure working with everyone at OCMR for the past few years. I am grateful to my colleagues and friends, especially Dr. Justin Y.C. Lau and Prof. Angus Z. Lau, for their advice on scientific matters and camaraderie. Thank you particularly to those who were willing to be scanned.

I would like to acknowledge my college adviser, Prof. Stephen Oppenheimer, for meeting with me every term and for his interest in my project.

Finally, I would like to thank my parents, godparents and godsister for their love and encouragement.

List of Publications

1. Siu AG, Biasioli L, Robson MD. Analysis of the Orientation-Dependent Frequency of Tendon via Ultrashort Echo Time (UTE) MRI. Proc. Intl. Soc. Mag. Reson. Med. 2018;26:1414. Accepted for a poster presentation.
2. Siu AG, Mózes FE, Biasioli L, Robson MD. Quantification and Repeatability of the Collagen Signal Fraction in the Healthy Liver Using Ultrashort Echo Time (UTE) MRI. Proc. Intl. Soc. Mag. Reson. Med. 2019;27:3554. Accepted for an oral presentation.

List of Abbreviations

ADC	analogue-to-digital conversion
AIC _c	corrected Akaike information criterion
ALD	alcoholic liver disease
ASH	alcoholic steatohepatitis
CPA	collagen proportionate area
CR	coefficient of repeatability or coefficient of reproducibility
BV	blood volume
ECV	extracellular volume
FA	flip angle
FOV	field of view
FID	free induction decay
GRE	gradient echo
HCV	Hepatitis C virus
IR-UTE	inversion recovery ultrashort echo time
MAS	magic angle spinning
MR	magnetic resonance

MRI	magnetic resonance imaging
MT	magnetisation transfer
NAFLD	non-alcoholic fatty liver disease
PDFF	proton density fat fraction
ROI	region of interest
RF	radiofrequency
STEAM	stimulated echo acquisition mode
TE	echo time
TR	repetition time
UTE	ultrashort echo time

1. Introduction

1.1. Motivation

Chronic liver diseases are a global health problem, with a prevalence of 844 million people worldwide and a mortality rate of 2 million deaths per year (1,2). Such diseases, including Hepatitis C, fatty liver disease and non-alcoholic fatty liver disease (NAFLD), are accompanied by liver fibrosis. Liver fibrosis is the accumulation of extracellular matrix proteins, including collagen (3–6), eventually leading to cirrhosis and hepatocellular carcinoma (4–7). As a consequence of most diseases, liver fibrosis is assessed via the gold-standard method of biopsy, aiding in the staging of the disease (5,7–9). However, biopsy is unideal due to the risk of complications (9,10) and sampling error (5,7–9). This has motivated the development of non-invasive imaging techniques for detecting liver fibrosis, including ultrasound and magnetic resonance (MR) elastography.

The purpose of this thesis was to investigate the short- T_2^* signal in the liver with ultrashort echo time (UTE) MR imaging. UTE can potentially be sensitive to changes in tissue properties or disease, as it uses TEs < 1 ms to measure species that are undetectable with conventional MR techniques. For instance, collagen has a short T_2^* that has been characterised in tendon (11,12). Collagen was the focus of this thesis, due to its implication in liver fibrosis.

1.2. Objectives

The objective of this thesis was to investigate the short- T_2^* signal in the liver with UTE. This involved:

1. Characterising the short- T_2^* signal of collagen, as collagen is associated with liver fibrosis
2. Modelling the short- T_2^* signal in the liver
3. Evaluating if the short- T_2^* signal in the liver can be described by isotropic collagen

1.3. Outline of Chapters

Chapter 2 describes the background MR principles that underlie this thesis. It specifies how MR signal is generated, particularly in the context of T_2^* relaxation. The UTE acquisition method is outlined.

Chapter 3 provides a literature review on the liver and short- T_2^* signal. The structure and diseases of the liver are briefly discussed, along with the implication of collagen in disease. Current literature on short- T_2^* signal in the liver is presented. Short- T_2^* properties in collagen and tendon (oriented collagen) are explained.

Chapter 4 describes *ex vivo* experiments in tendon and collagen. This includes measuring the $R_2^* = \frac{1}{T_2^*}$ and resonant frequency shift of tendon, as well as the R_2^* of collagen phantoms. The R_2^* and resonant frequency shift of tendon were subsequently used for modelling isotropic collagen signal, described in Chapter 6.

Chapter 5 delineates the optimisation of the UTE technique for *in vivo* imaging. The optimised pulse sequences for the Trio 3-T and Prisma 3-T scanners are presented and compared with the original Trio 3-T sequence. Changes on the Trio included the reduction of gradient spoiling, the use of a double-echo vs. a single-echo acquisition and

the use of spatial saturation. On the Prisma, the bandwidth, RF coil and minimum TE were optimised.

Chapter 6 is the main chapter describing *in vivo* experiments for characterising the short- T_2^* signal in the liver, in both healthy subjects and patients. Different models for fitting the short- T_2^* signal were used, including the model of isotropic collagen and free water, and the selection of the best model. The repeatability and reproducibility of the short- T_2^* signal fraction were assessed, and the short- T_2^* signal fraction was compared between healthy subjects and patients.

Chapter 7 concludes the thesis, summarising the findings for the short- T_2^* signal in the liver and relating to collagen. Potential origins of the short- T_2^* signal are discussed. Methods for further delineating the source of the short- T_2^* signal in the liver are proposed.

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2. Theory of Magnetic Resonance (MR)

This chapter describes the theory of magnetic resonance (MR) that underpins this thesis. Concepts include how MR signal is generated and the origins of T_2^* relaxation. Image formation is illustrated with an example gradient-echo sequence. The ultrashort echo time (UTE) sequence is presented along with exchange theory for the modelling of T_2^* signal. Particularly, textbooks by Nishimura (1), McRobbie et al. (2), Gore et al. (3) and Haacke et al. (4) were used in the writing of this chapter.

2.1. MR Signal Generation

2.1.1. Spins

The phenomenon of magnetic resonance occurs in nuclei with a spin angular momentum. Mainly, magnetic resonance is used to study nuclei with a spin of $\frac{1}{2}$, i.e. with an odd number of nucleons, with nucleons consisting of protons and neutrons. Examples of atoms with nuclei of spin $\frac{1}{2}$ include ^1H , ^{31}P and ^{13}C , of which ^1H is the most naturally abundant, due to its presence in water. A ^1H (hydrogen-1) nucleus is referred to as a proton. Classically, one can think of the nucleus as a rotating charged sphere. The magnetic field generated from the moving charge is the magnetic moment.

2.1.2. Resonance: Quantum Mechanics Model

The quantum mechanics model considers the behaviour of individual spins. A spin can either be in the spin-up (parallel) state, or spin-down (anti-parallel) state, as shown in Fig. 2.1.

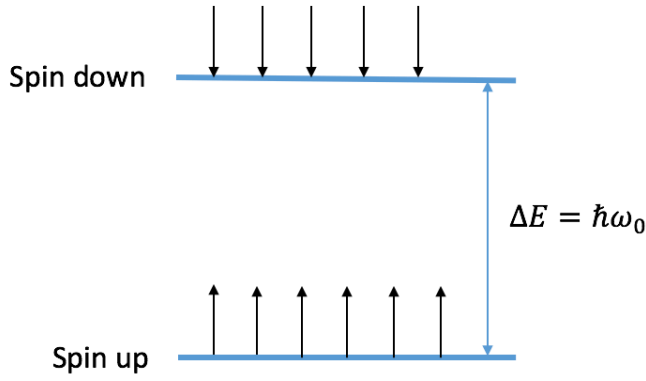


Fig. 2.1. Spin-up and spin-down states. The Larmor frequency (ω_0) is required to transition from a spin-up to a spin-down state.

From the quantum description, the magnetic moment ($\vec{\mu}$) is related to the angular momentum ($\vec{J}$) by:

$$\vec{\mu} = \gamma \vec{J} = \gamma \hbar I. \quad [2.1]$$

γ is the gyromagnetic ratio, with a proton value of $267.522 \times 10^6 \text{ rad}\cdot\text{s}^{-1}\cdot\text{T}^{-1}$. $\hbar$ is Planck's constant, divided by 2π . I is the spin angular momentum quantum number, which equals $\pm 1/2$ for protons.

The potential energy associated with a magnetic moment in a magnetic field ($\vec{B}$) is:

$$E = \vec{\mu} \cdot \vec{B} = \gamma \hbar I B. \quad [2.2]$$

Hence, the difference in potential energy between a spin-up state ($I = 1/2$) and a spin-down state ($I = -1/2$) is:

$$\Delta E = \gamma \hbar B. \quad [2.3]$$

From the De Broglie wave equation, one has:

$$\Delta E = \hbar\omega = \gamma\hbar B. \quad [2.4]$$

Hence, the frequency of precession or Larmor frequency (ω_0) is:

$$\omega_0 = \gamma B_0, \quad [2.5]$$

where B_0 refers to the external magnetic field. This is the frequency required for a spin to transition from the spin-up to the spin-down state, resulting in energy absorption of ΔE . Transferring from spin down to spin up results in energy release.

The ratio of the spin-up population (N_{up}) to the spin-down population (N_{down}) is:

$$\frac{N_{up}}{N_{down}} = e^{-\frac{\Delta E}{kT}}, \quad [2.6]$$

where k is the Boltzmann constant and T is the absolute temperature. When imaging at body temperature at a clinical field strength, the difference in spin populations is:

$$N_{up} - N_{down} \approx \frac{N_{up+down}}{2} \frac{\gamma\hbar B_0}{kT}, \quad [2.7]$$

giving rise to a net magnetisation (M) per unit volume of:

$$M = \frac{\rho\gamma^2\hbar^2 B_0}{4kT}. \quad [2.8]$$

ρ is the proton density, which has replaced the total spin population ($N_{up+down}$). Hence, the M is proportional to the external magnetic field (B_0).

2.1.3. Resonance: Classical Mechanics Model

Contrary to the quantum mechanics model, the classical mechanics model considers the net magnetisation of an ensemble of spins, illustrated in Fig. 2.2.

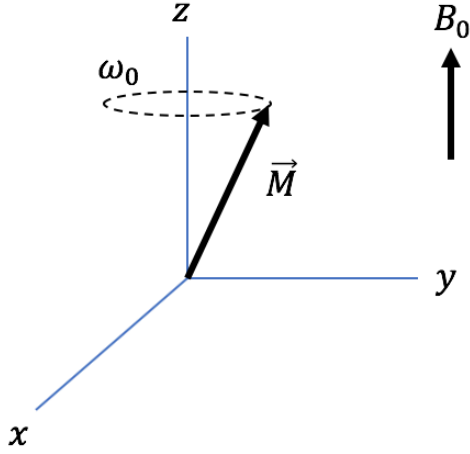


Fig. 2.2. Net magnetisation ($\vec{M}$) in the classical mechanics model, precessing at the Larmor frequency (ω_0).

One can consider the torque experienced by a spin with a magnetic moment ($\vec{\mu}$) in an external magnetic field ($\vec{B}$). The torque is given by the derivative of the angular momentum ($\vec{J}$):

$$\frac{d\vec{J}}{dt} = \vec{\mu} \times \vec{B}. \quad [2.9]$$

As $\vec{\mu} = \gamma \vec{J}$ from Eq. [2.1], the following results when one multiplies both sides of Eq. [2.9] by γ :

$$\frac{d\vec{\mu}}{dt} = \vec{\mu} \times \gamma \vec{B}. \quad [2.10]$$

Considering that the net magnetisation moment ($\vec{M}$) is a summation over all magnetic moments, one has:

$$\frac{d\vec{M}}{dt} = \vec{M} \times \gamma \vec{B}, \quad [2.11]$$

where $\vec{M}$ precesses around $\vec{B}$ at the Larmor frequency (ω_0).

Combining the quantum and classical models, the Larmor frequency is both a frequency allowing for transitions between spin-up and spin-down states and a precessional frequency, describing the rotation of the net magnetisation of the spins.

Behaviour of the net magnetisation is governed by the Bloch equation, which expands on Eq. [2.11] and includes the relaxation time constants T_1 and T_2 . The Bloch equation is as follows:

$$\frac{d\vec{M}}{dt} = \vec{M} \times \gamma \vec{B} - \frac{M_x \hat{i} + M_y \hat{j}}{T_2} - \frac{(M_z - M_0) \hat{k}}{T_1}. \quad [2.12]$$

With the time evolution of the net magnetisation, one can detect the net magnetisation via the process of excitation.

2.1.4. Excitation

The net magnetisation of the spins ($\vec{M}$) can be detected through radiofrequency (RF) excitation, which excites the spins and tips the magnetisation towards the transverse plane. Along the transverse plane is located a detector that can measure the net magnetisation. When discussing excitation, it is helpful to use a frame of reference that rotates at the Larmor frequency. In the classical mechanics model mentioned, a stationary frame of reference has been used. Fig. 2.3 illustrates the behaviour of the magnetisation in the stationary frame, during RF excitation (denoted by the applied field B_1).

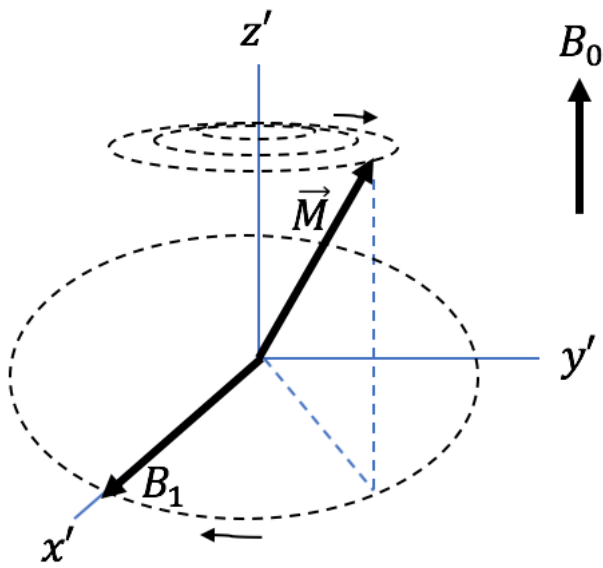


Fig. 2.3. Behaviour of the magnetisation in the stationary frame of reference. $\vec{M}$ spirals towards the transverse plane, moving away from the z -axis.

In a frame of reference that rotates at the Larmor frequency, B_1 is static, as the RF field is also rotating at the Larmor frequency and located in the transverse plane. This is shown in Fig. 2.4.

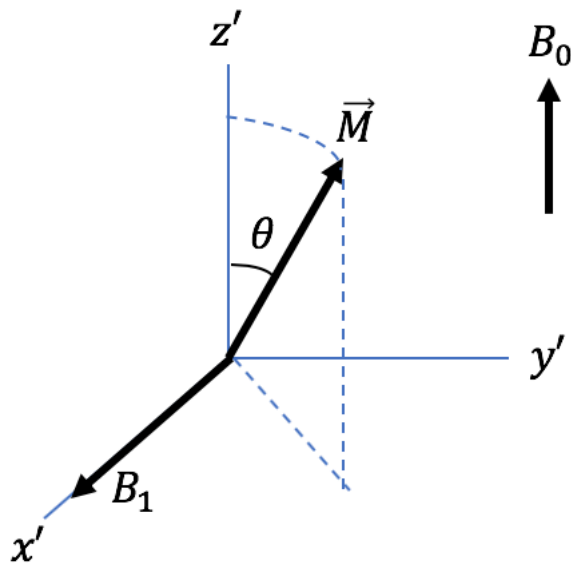


Fig. 2.4. Behaviour of the magnetisation in a frame of reference rotating at the Larmor frequency. $\vec{M}$ is tipped away from the z' -axis, towards the transverse plane, with a flip angle of θ .

The flip angle (θ) describes the angle at which $\vec{M}$ is tipped due to the B_1 field, relative to the z -axis. It is given by:

$$\theta = \gamma B_1 \tau, \text{ for constant } B_1,$$

$$\theta = \gamma \int_0^\tau B_1(t) dt, \text{ for time-varying } B_1(t), \quad [2.13]$$

for a B_1 duration of time τ . As such, a flip angle of 90° will tip $\vec{M}$ onto the transverse plane. An RF pulse inducing a flip angle of 90° is referred to as a 90° pulse.

$\vec{M}$ can be detected in the transverse plane by a receiver coil, which is usually the same as the RF transmit coil, but is instead in receiving mode. In the stationary frame, the receiver coil is static and $\vec{M}$ tipped in the transverse plane oscillates at the Larmor frequency. The oscillating magnetic field induces a voltage in the receiver coil. After the application of B_1 , the voltage decays as a function of time, known as the free induction decay. The free induction decay, illustrated in Fig. 2.5, is the MR signal.

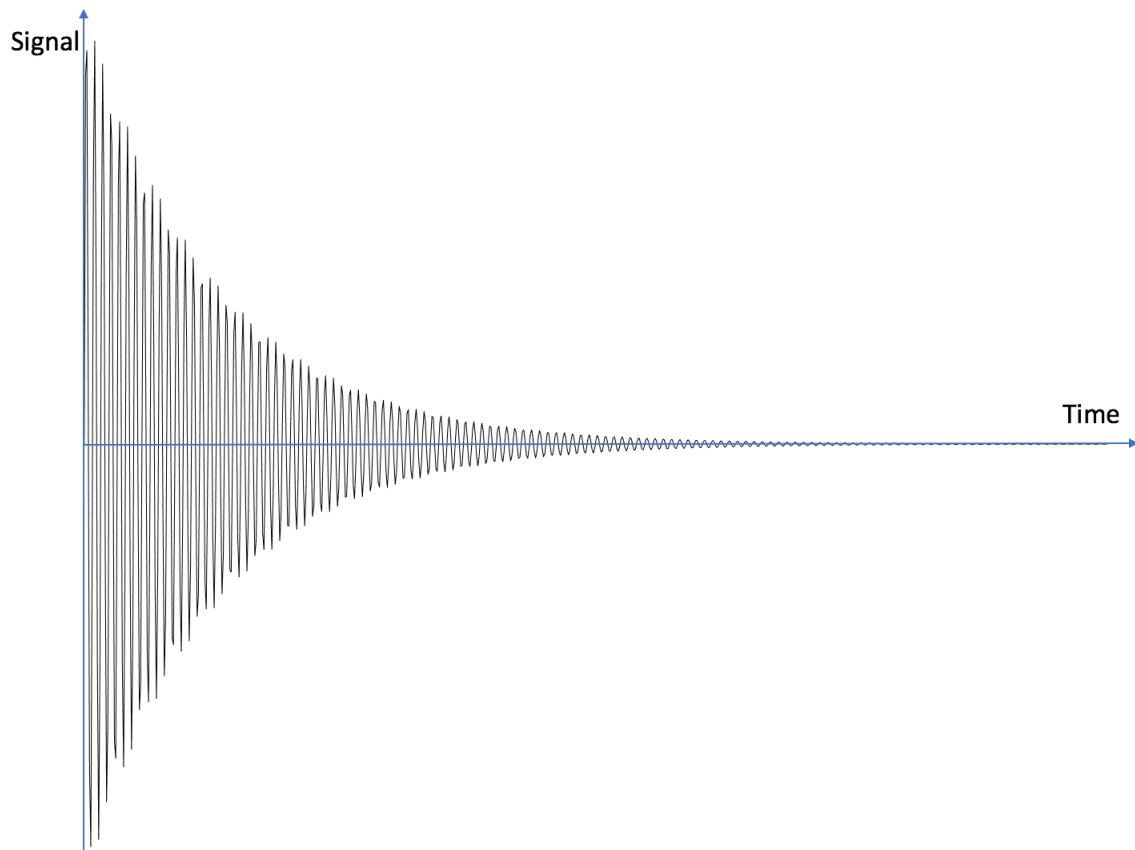


Fig. 2.5. Free induction decay. The MR signal decays as a result of T_2^* relaxation (Section 2.1.7).

2.1.5. T_1 Relaxation

As mentioned previously, the net magnetisation of the spins undergoes relaxation. From the Bloch equation in Eq. [2.12], the longitudinal component of the magnetisation over time is characterised by:

$$\frac{dM_z}{dt} = - \frac{(M_z - M_0)}{T_1}. \quad [2.14]$$

After an excitation of 90° , the longitudinal magnetisation is:

$$M_z = M_0(1 - e^{-\frac{t}{T_1}}). \quad [2.15]$$

T_1 is the spin-lattice time constant describing the return of the longitudinal magnetisation to equilibrium. Mathematically, this is the time required for M_z to return to 63 % of its original value (M_0). From the quantum description, spins will absorb RF energy in order to transition from a low-energy state (spin up) to a high-energy state (spin down). With an increased population of spins in the high-energy state compared to the low-energy state, spins in the high-energy state will return to the low-energy state, losing energy to the surrounding lattice. This process is known as spin-lattice relaxation. The lattice usually includes neighbouring spins or protons from molecules, which have magnetic moments. The main relaxation mechanism is dipole-dipole interactions between protons. Here, the magnetic moment experienced by one proton is affected by the magnetic moment of another proton, as the protons tumble in space.

Spin-lattice relaxation is most effective when the tumbling rate is at the Larmor frequency. The tumbling rate is dependent on the extent to which the motion of the proton is restricted. For instance, protons from pure water have a high tumbling rate and hence high T_1 ; these are known as “free protons”. Protons that are restricted to structural compartments or tightly bonded to other atoms (i.e. in macromolecules) have a tumbling rate that is lower than the Larmor frequency and hence T_1 is still high; these are known as “bound protons”. Fig. 2.6 illustrates the T_1 as a function of the correlation time, which is the average time it takes for the local magnetic field experienced by a proton to change.

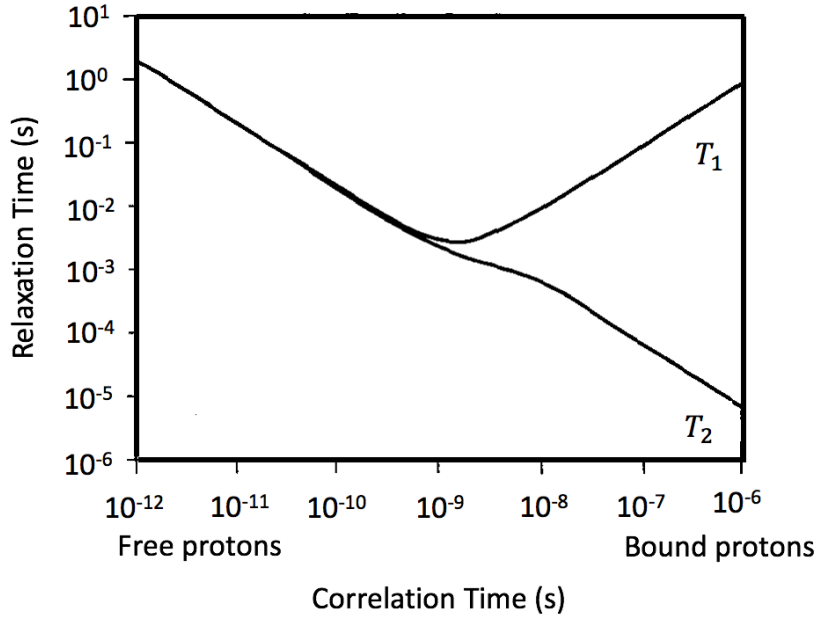


Fig. 2.6. T_1 and T_2 relaxation times as functions of the correlation time, adapted from Gore et al. 1999 (3). Copyright Elsevier 1999. Used with permission. $T_2 \leq T_1$, as T_1 is only sensitive to tumbling at or near the Larmor frequency.

2.1.6. T_2 Relaxation

Fig. 2.6 introduces the concept of T_2 relaxation, which differs from T_1 at high correlation times. The transverse component of the magnetisation behaves as follows:

$$\frac{dM_{xy}}{dt} = -\frac{M_{xy}}{T_2}. \quad [2.16]$$

After an excitation of 90° , the transverse magnetisation is given by:

$$M_{xy} = M_0 e^{-\frac{t}{T_2}}. \quad [2.17]$$

T_2 is the spin-spin time constant, describing the time required for the transverse magnetisation (M_{xy}) to decay to 37 % of its original value (M_0). This is caused by the exchange of energy amongst spins, as a transition from spin-up to spin-down is complemented by a transition from spin-down to spin-up. The decay in transverse

magnetisation results from the dephasing (loss of phase coherence) of spins. This arises from local inhomogeneities in the magnetic field due to molecular tumbling.

Although T_1 is affected by tumbling at the Larmor frequency, T_2 is affected by tumbling at low frequencies and the Larmor frequency. This causes $T_2 \leq T_1$, as observed in Fig. 2.6. A proton that tumbles quickly tends to experience a local magnetic field that fluctuates rapidly. Hence, the field is relatively homogenous, resulting in less dephasing and a longer T_2 . A proton that tumbles slowly experiences a slowly-fluctuating magnetic field. The relatively constant inhomogeneity in the field results in more dephasing and a shorter T_2 . Free protons tend to have a long T_2 , whereas bound protons (such as those in collagen) tend to have a short T_2 .

2.1.7. T_2^* Relaxation

T_2^* is a time constant characterised by T_2 relaxation mechanisms, as well as relaxation mechanisms due to inhomogeneities in the external magnetic field. Such external inhomogeneities result in spins that precess at frequencies other than the Larmor frequency, while spins experiencing no inhomogeneities precess at the Larmor frequency. This leads to more dephasing and hence $T_2^* \leq T_2$. The T_2^* relaxation rate ($R_2^* = \frac{1}{T_2^*}$, in s^{-1}) is given by:

$$\frac{1}{T_2^*} = \frac{1}{T_2} + \gamma \Delta B_0, \quad [2.18]$$

where ΔB_0 is the term for external magnetic field inhomogeneities. Examples of inhomogeneities include those arising from the scanner or the presence of paramagnetic substances, including iron.

2.1.8. Chemical Shift

Apart from relaxation mechanisms, MR signal can be affected by differences in resonant frequency, or chemical shift, between molecular environments. The Larmor frequency can be expressed in Hz (f_0), as opposed to rad/s, as follows:

$$f_0 = \frac{\gamma}{2\pi} B_0. \quad [2.19]$$

This form of the gyromagnetic ratio ($\frac{\gamma}{2\pi}$) has a value of 42.577 MHz/T in protons.

The resonant frequency of a nucleus can be shifted from the Larmor frequency due to shielding induced by the orbital motion of neighbouring electrons in response to B_0 . Shielding can be characterised by a shielding constant (σ). The shifted or effective frequency (f_{eff}) is:

$$f_{eff} = f_0(1 - \sigma). \quad [2.20]$$

This gives rise to a chemical shift (δ) in the sample, expressed relative to a reference:

$$\delta = \frac{(f_{sample} - f_{scanner}) \times 10^6}{f_{scanner}}. \quad [2.21]$$

Resonant frequencies of the sample (f_{sample}) and the scanner ($f_{scanner}$) are indicated. The frequency of the scanner is usually tuned to the resonant frequency of water, as the dominant source of proton signal. Hence, resonant frequency shifts are expressed relative to water. A tissue that demonstrates a resonant frequency shift is tendon, which consists of bound water associated with collagen.

2.2. MR Image Formation

2.2.1. Gradients and Pulse Sequences

This section describes how MR images (hence the term MRI) are formed, based on the concept of spatial encoding. The free induction decay detected with RF transmission and reception is useful, but does not differentiate between spins at different locations in the sample. Namely, all spins within the sample precess at the Larmor frequency. For spatial localisation in MR, linear magnetic-field gradients are applied, in units of mT/m. For each dimension $i = x, y, z$, the gradient amplitude is:

$$G_i = \frac{dB_z}{di}. \quad [2.22]$$

G_x and G_y are the frequency- and phase-encoding gradients, respectively. G_z is the slice-selection gradient. The gradient along each dimension changes the magnetic field ($B(i)$), and consequently the frequency ($\omega(i)$) of the spin according to its location, as follows:

$$B(i) = B_0 + G_i i,$$

$$\omega(i) = \gamma B(i) = \omega_0 + \gamma G_i i. \quad [2.23]$$

Fig. 2.7 is a gradient-echo pulse sequence, illustrating the timings of the RF pulse and gradients in an MRI acquisition. Like UTE, this sequence provides T_2^* contrast and is presented to explain concepts in image formation. Although gradients ideally possess a rectangular shape, they are trapezoidal in reality, as indicated in the figure. The slew rate (in mT/m/ms) indicates the rate at which the gradient achieves its maximal amplitude; a rectangular shape would require an infinite slew rate, which is not physically possible.

Hence, there are ramp-up and ramp-down times on either end of the gradient. The effects of the G_x , G_y and G_z gradients on spatial localisation will be described.

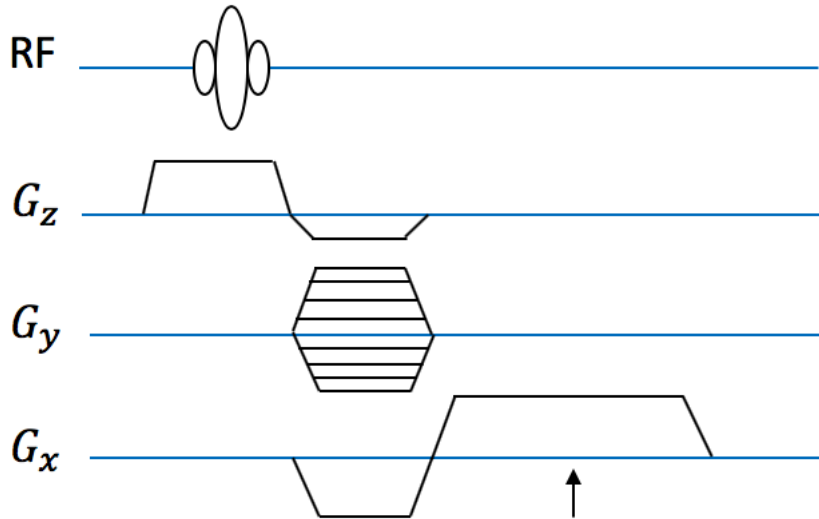


Fig. 2.7. Gradient-echo pulse sequence, adapted from McRobbie et al. 2006 (2). Copyright D. W. McRobbie, E. A. Moore, M. J. Graves and M. R. Prince 2003, 2006. Reproduced with permission of the Licensor through PLSclear. The signal acquired is highest at the point indicated by the arrow.

2.2.2. Frequency Encoding

Fig. 2.8 illustrates the magnetic field as a function of position, when applying a frequency-encoding gradient (G_x). As such, spins at $x = 0$ experience a magnetic field of B_0 and precess at the Larmor frequency. Spins at $x < 0$ experience a magnetic field that is lower than B_0 and precess slower than the Larmor frequency. Spins at $x > 0$ experience a magnetic field that is higher than B_0 and precess faster than the Larmor frequency. As the gradient amplitude is on the order of mT/m, the change in the magnetic field due to the application of the gradient is small.

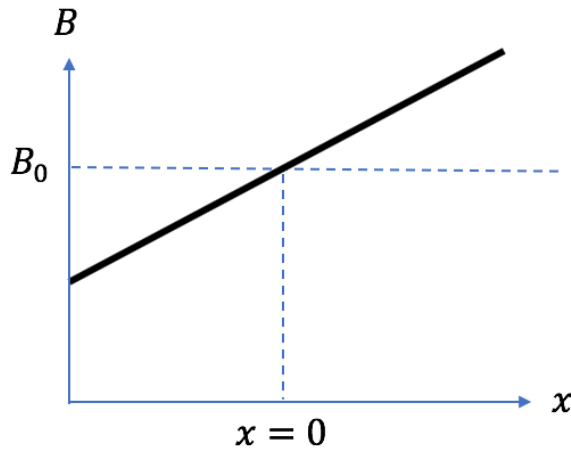


Fig. 2.8. The magnetic field as a function of position, due to the application of a gradient in the x -direction. Adapted from McRobbie et al. 2006 (2). Copyright D. W. McRobbie, E. A. Moore, M. J. Graves and M. R. Prince 2003, 2006. Reproduced with permission of the Licensor through PLSclear.

G_x is shown in the fourth row of Fig. 2.7, including a gradient with a negative amplitude for dephasing and a gradient with a positive amplitude for rephasing. Over the duration of the first G_x gradient, the spins dephase due to their varying precessional frequencies. In other words, spins at different locations in x have different phases. The spins are rephased with a second G_x gradient, which has the same amplitude as the dephasing gradient, but is of the opposite sign and applied for twice the duration. The signal is sampled at different points along this rephasing gradient. At each point, the signal has a different phase, depending on the spatial location. The signal is highest at the point where the area of the rephasing gradient is equal to the area of the dephasing gradient, referred to as the gradient echo. For an image consisting of $n_x \times n_y$ pixels, the signal is sampled at n_x points.

2.2.3. Phase Encoding

To address the y -direction, a phase-encoding gradient is used. Fig. 2.9 illustrates the concept of phase encoding. Consider spins that are in phase before G_y is applied, as in the

third row. When G_y is applied, the spins at positions $y < 0$ precess slower than the Larmor frequency and those at positions $y > 0$ precess faster than the Larmor frequency. The spins dephase over the duration of G_y , to the extent that after the end of G_y , they return to precessing at the Larmor frequency with the phases retained (as in the bottom row). This is known as phase encoding. In each repetition of the sequence (third row of Fig. 2.7), the amplitude of G_y is changed to encode for each pixel in the y -direction. For an image with $n_x \times n_y$ pixels, G_y is varied n_y times.

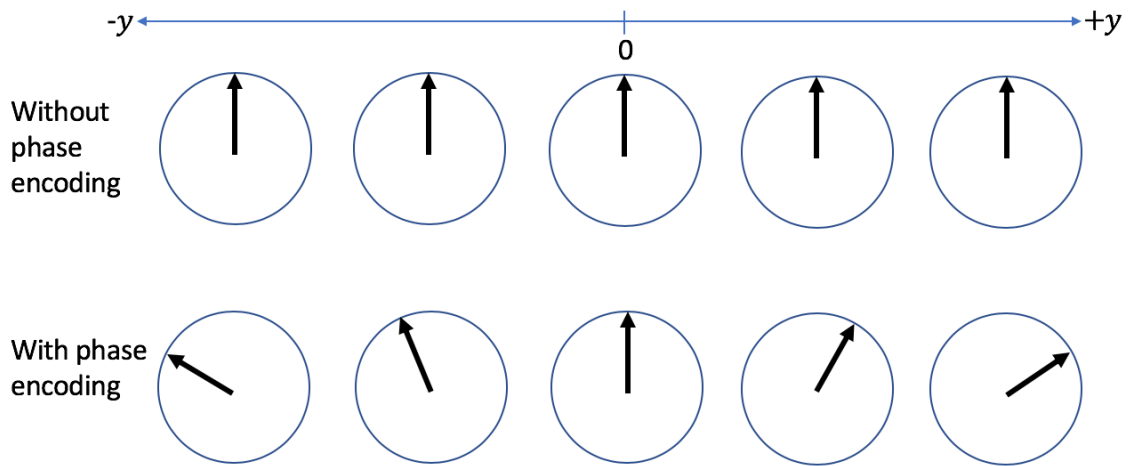


Fig. 2.9. The dephasing of spins as a result of phase encoding. Adapted from McRobbie et al. 2006 (2). Copyright D. W. McRobbie, E. A. Moore, M. J. Graves and M. R. Prince 2003, 2006. Reproduced with permission of the Licensor through PLSclear. The phase angle of each spin is preserved after the end of G_y .

2.2.4. Slice Selection

The imaging slice is selected via the RF pulse and slice-selection gradient (G_z) in the first and second rows of Fig. 2.7. The dephaser gradient is applied after the RF pulse. To specify the position of the slice, the RF pulse has a carrier frequency and bandwidth (Δf). The slice thickness (Δz) is:

$$\Delta z = \frac{\Delta f}{\frac{\gamma}{2\pi} G_z}. \quad [2.24]$$

This equation indicates that to increase the slice thickness, one can either increase the RF bandwidth or decrease the gradient amplitude. To maximise the signal obtained after the dephaser gradient, the rephaser gradient in G_z corrects the loss of phase in the spins. The rephaser gradient is of half the area of the dephaser gradient, as one considers the beginning of the RF pulse to be the centre of the RF waveform.

2.2.5. k -Space and Sampling

In MR imaging, one acquires spatial frequency information from the object of interest in k -space. k is the variable for spatial frequency, in units of cycles/mm. The MR signal ($s(t)$) is related to the net magnetisation in the transverse plane ($m(x, y)$) by a Fourier transform. This is known as the signal equation:

$$s(t) = \int_x \int_y m(x, y) e^{-i2\pi[k_x(t)x + k_y(t)y]} dy dx, \quad [2.25]$$

where:

$$k_x(t) = \frac{\gamma}{2\pi} \int_0^t G_x(\tau) d\tau, \\ k_y(t) = \frac{\gamma}{2\pi} \int_0^t G_y(\tau) d\tau. \quad [2.26]$$

$m(x, y)$ is dependent on the proton density $\rho(x, y)$ and relaxation time constants $T_2(x, y)$ and $T_1(x, y)$. The Fourier-transform relationship between $s(t)$ and $m(x, y)$ indicates that there is a direct mapping between a measured signal and a trajectory in k -space, specified

by the integrals of $G_x(t)$ and $G_y(t)$. Such integrals are the areas under the respective gradients at time t . In other words, manipulating the gradient area affects the sampling trajectory in k -space.

Fig. 2.10 illustrates a typical k -space image. From the k -space reconstruction, most of the spatial frequencies are near the centre, i.e. are low spatial frequencies. The low spatial frequencies contain information on the gross features of the imaged object. Near the edges of k -space are the high spatial frequencies, which contain information on the fine details and edges of the object.

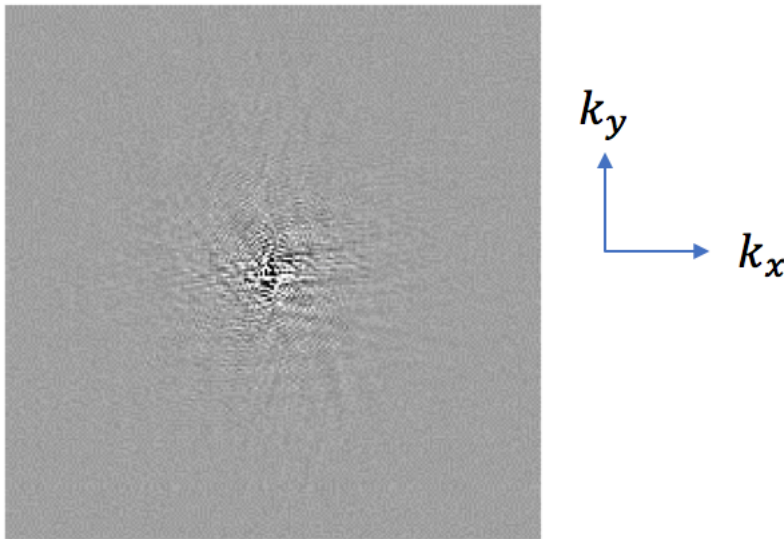


Fig. 2.10. k -space image, adapted from McRobbie et al. 2006 (2), Copyright D. W. McRobbie, E. A. Moore, M. J. Graves and M. R. Prince 2003, 2006. Reproduced with permission of the Licensor through PLSclear.

Fig. 2.11 demonstrates the k -space trajectory for the gradient-echo sequence, in which k -space is acquired on a line-by-line basis. This is known as Cartesian sampling. For an $n_x \times n_y$ matrix, there are n_x and n_y points sampled along k_x and k_y , respectively. The intervals between points are Δk_x and Δk_y for each respective direction.

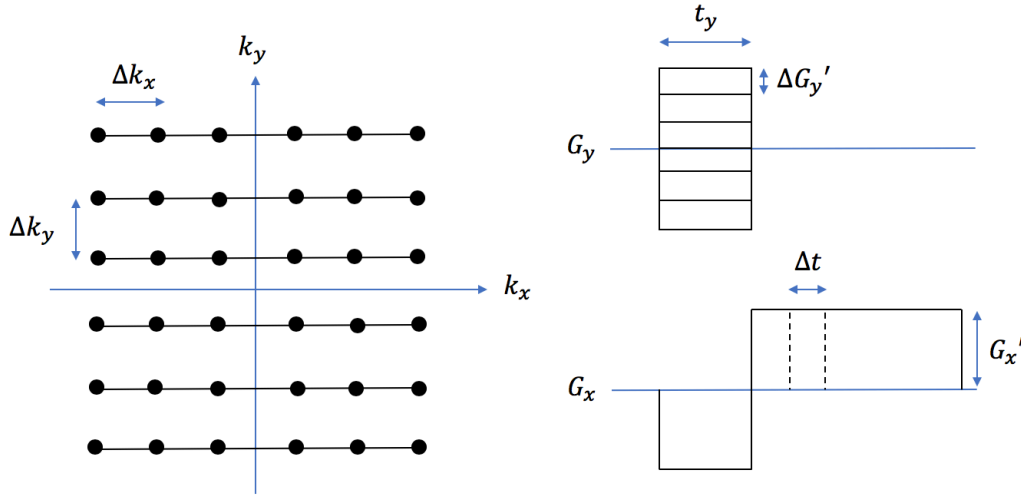


Fig. 2.11. k -space trajectory for the gradient-echo sequence and associated G_x and G_y gradients, adapted with permission from Nishimura (1). Copyright 2010 by Dwight G. Nishimura.

Referring to Fig. 2.11, one can observe that one line of k -space is sampled in one repetition of the sequence by specifying the amplitude of G_y and sampling along the G_x gradient, with a sampling interval of Δt . In the next repetition of the sequence, the next line of k -space is sampled with G_y varied by the increment of $\Delta G_y'$. The sampling continues until $n_x \times n_y$ points are obtained. The field of view (FOV, in mm) is the sampling rate along the k -space direction. It is given by:

$$FOV_x = \frac{1}{\Delta k_x} = \frac{1}{\frac{\gamma}{2\pi} G'_x \Delta t} ,$$

$$FOV_y = \frac{1}{\Delta k_y} = \frac{1}{\frac{\gamma}{2\pi} \Delta G'_y t_y} . \quad [2.27]$$

The equations for the sampling intervals Δk_x and Δk_y are similar to those for k_x and k_y , but account for increments in either the time or gradient amplitude.

The spatial resolution in each direction (in mm/pixel) is:

$$\delta_x = \frac{FOV_x}{n_x} = \frac{1}{n_x \Delta k_x} ,$$

$$\delta_y = \frac{FOV_y}{n_y} = \frac{1}{n_y \Delta k_y} . \quad [2.28]$$

To increase the FOV and worsen the spatial resolution (i.e. to increase δ_x), one decreases the sampling interval in k -space. Increasing the number of pixels improves the spatial resolution (i.e. decreases δ_x). From this, one can observe the relationships between k -space and image-domain parameters.

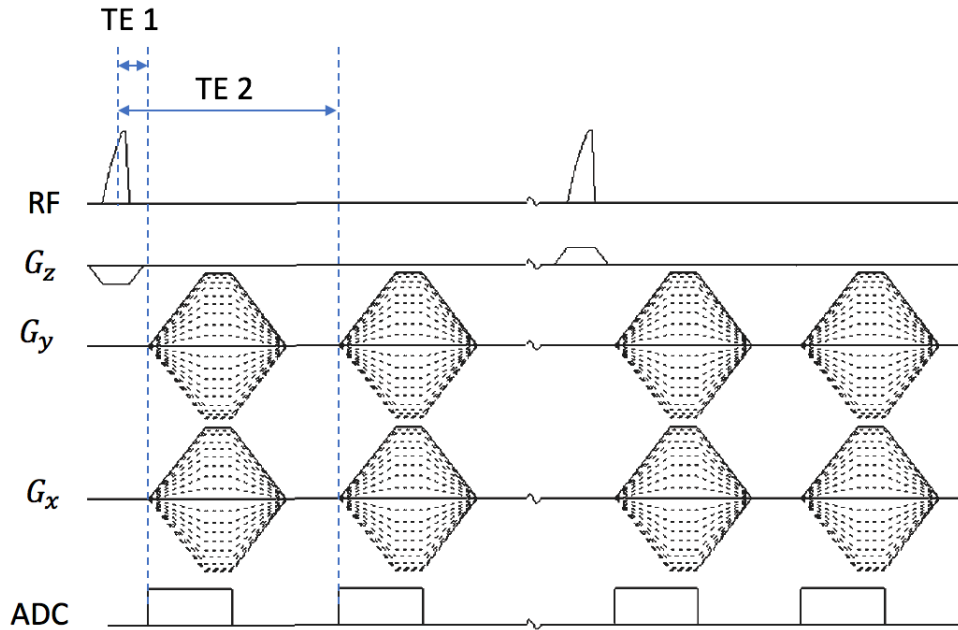
2.3. Ultrashort Echo Time (UTE)

With the gradient-echo sequence and sampling scheme explained, one can proceed to understand the ultrashort echo time (UTE) pulse sequence and k -space sampling trajectory, shown in Fig. 2.12. Like the gradient-echo sequence, UTE provides T_2^* contrast, although with echo times (TEs) < 0.5 ms, as opposed to minimum TE of ~ 0.8 ms in gradient echo. This difference is significant when measuring short- T_2^* signal with $T_2^* < 1$ ms.

The UTE pulse sequence consists of two half-pulse acquisitions. The half pulse is a half-sinc RF waveform, applied during a slice-selection gradient, which is opposite in sign in the second acquisition. The two half-pulse acquisitions are summed in the complex domain to achieve the slice profile (5–7). This RF and slice-selection design allows the TE to be minimised, as the half-sinc is a short pulse. Furthermore, the sum of the two acquisitions removes the need for rephasing after the RF pulse, which is used in the gradient-echo sequence (6,7). Unlike the gradient-echo sequence, UTE does not measure an echo from rephased gradients, but rather the free induction (T_2^*) decay.

The calculation of the TE differs between literature. Simplistically, TE has been measured from the end of the RF pulse to the beginning of k -space sampling, when the analogue-to-digital converter (ADC) is applied. However, for long- T_2 species, the actual TE should be measured from the centroid of the RF pulse to beginning of k -space sampling. For short- T_2 species, where T_2 is on the order of the RF pulse duration, the centroid should be weighted by $e^{-\frac{\Delta t}{T_2}}$, where Δt is the duration affected by T_2 decay (6,8). As the TE is dependent on T_2 , which may be unknown, the TE is difficult to quantify and compare between experiments. In this thesis, the actual TE was calculated from the centroid without the T_2 weighting term, unless otherwise stated.

In this version of the UTE sequence, two echoes are acquired in one repetition, resulting in a dual-echo acquisition. The gradient amplitude for the second echo acquisition is the same as that for the first echo acquisition. The image at each echo is reconstructed from k -space, sampled radially.



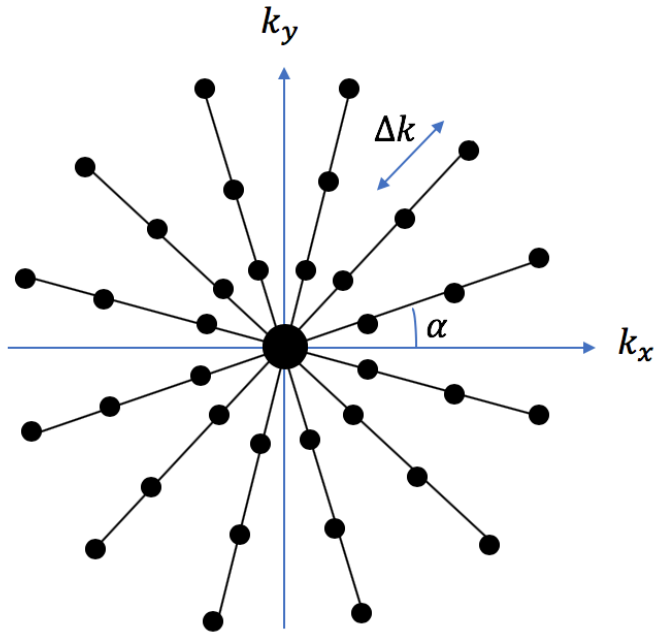


Fig. 2.12. UTE pulse sequence and k -space sampling trajectory. The sequence diagram is adapted from Robson et al. 2006 (6), with permission from Wiley; Copyright 2006 John Wiley & Sons, Ltd. It demonstrates a dual-echo acquisition, with two TEs calculated from the centroid of the half-sinc RF pulse. The slice-selection gradient is negative in the first repetition of the sequence, and positive in the second repetition. The UTE sequence produces two images (one at each TE). A representative k -space trajectory for each TE is shown. The number of points in the sampling trajectory has been reduced for clarity.

For k -space sampling, G_x and G_y are applied to form a radial trajectory. The gradient amplitudes are given by:

$$G_x = G_0 \cos(\alpha),$$

$$G_y = G_0 \sin(\alpha). \quad [2.29]$$

Here, G_0 is the gradient amplitude at $\alpha = 0$, where α is the angle of the radial spoke.

Hence, the trajectory is a variant of the Cartesian case, with the spoke characterised by α .

The FOV is:

$$FOV = \frac{1}{\Delta k} , \quad [2.30]$$

where Δk is the interval between k -space points (9). Sampling normally starts on the gradient ramp, continuing until the end of the gradient plateau. As the radial trajectory begins at the centre of k -space, dephaser gradients in G_x or phase-encoding gradients in G_y are not required to return to the centre at the end of each spoke (6,7). The k -space data are regridded onto a Cartesian coordinate system before an inverse Fourier transform is performed.

2.4. R_2^* Modelling

Following from Sections 2.1.5 - 2.1.7 on relaxation, one can model the relaxation in multi-component systems with acquisitions such as UTE, which provides T_2^* contrast. From Fig. 2.6, one can model the T_2^* relaxation of the components from bound ($T_2^* < 1$ ms) and free ($T_2^* \sim 10$ ms) protons. In doing so, one has to account for exchange, as protons may diffuse from one environment to another. The two limits are fast and slow exchange. The net exchange rate is given by:

$$\frac{1}{\tau} = \frac{1}{\tau_b} + \frac{1}{\tau_f} , \quad [2.31]$$

where τ_b and τ_f are the average residence times in the bound- and free-proton environments. For instance, the bound-proton environment may pertain to water that is bound to proteins, and the free-proton environment may pertain to bulk water that is not associated with proteins.

In the regime of fast exchange between the environments, one has $\frac{1}{\tau} \gg R_{2b}^* - R_{2f}^*$.

Here:

$$R_{2,avg}^* = P_b R_{2b}^* + P_f R_{2f}^*, \quad [2.32]$$

where R_{2b}^* and R_{2f}^* are the relaxation rates, and P_b and P_f are the population fractions in each environment. Hence, $R_{2,avg}^*$ is an average of the two environments, weighted by the population fractions, resulting in the modelling of R_2^* as a mono-exponential function:

$$|S(TE)| = |S_0 \exp(-TE \cdot R_{2,avg}^*)|. \quad [2.33]$$

In the regime of slow exchange, one has $\frac{1}{\tau} \ll R_{2b}^* - R_{2f}^*$. This results in relaxation rates that are dependent on the residence times, as follows:

$$\begin{aligned} R_{2b'}^* &= R_{2b}^* + \frac{1}{\tau_b}, \\ R_{2f'}^* &= R_{2f}^* + \frac{1}{\tau_f}. \end{aligned} \quad [2.34]$$

where $R_{2b'}^*$ and $R_{2f'}^*$ are the effective relaxation rates. This leads to the modelling of R_2^* as a bi-exponential function:

$$|S(TE)| = |S_{0b} \exp(-TE \cdot R_{2b'}^*) + S_{0f} \exp(-TE \cdot R_{2f'}^*)|. \quad [2.35]$$

A third exchange regime intermediate to the fast and slow limits also exists, where the residence times are on the same order as the relaxation rates. As this regime is complicated to model, resulting in underdetermined parameters, often only fast and slow

exchange are considered. The fast- and slow-exchange models are explored in Chapters 4 and 6 in describing short- T_2^* signal associated with collagen and the liver.

2.5. Summary

This chapter discusses MR theory, from the origin of MR signal, to relaxation and image acquisition. Theory on UTE and R_2^* modelling are introduced. The next chapter will describe literature on short- T_2^* signal associated with collagen and its relation to the liver.

2.6. References

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3. Liver and Collagen Imaging

The objective of this thesis was to model the short- T_2^* signal in the liver and to evaluate the suitability of an R_2^* model describing isotropic collagen. This chapter provides background on the liver, including the implication of collagen and fibrosis in liver diseases. Existing literature on short- T_2^* signal in the liver is presented, along with the short- T_2^* signal properties of oriented collagen.

3.1. The Liver

The liver is located inferior to the diaphragm, on the right side of the abdomen. It is the largest internal organ, weighing ~ 1.5 kg in a healthy person (1). The structure and functions of the liver will be discussed. Functions of the liver include bile production, glucose and lipid metabolism, iron storage, and protein metabolism.

3.1.1. Structure of the Liver

The liver is categorised by the left, right, caudate and quadrate lobes. The five peritoneal folds or ligaments connect the liver to the abdominal wall and diaphragm. These include the falciform ligament, left triangular ligament, right triangular ligament, coronary ligament and teres ligament (2,3). Liver anatomy is depicted in Fig. 3.1.

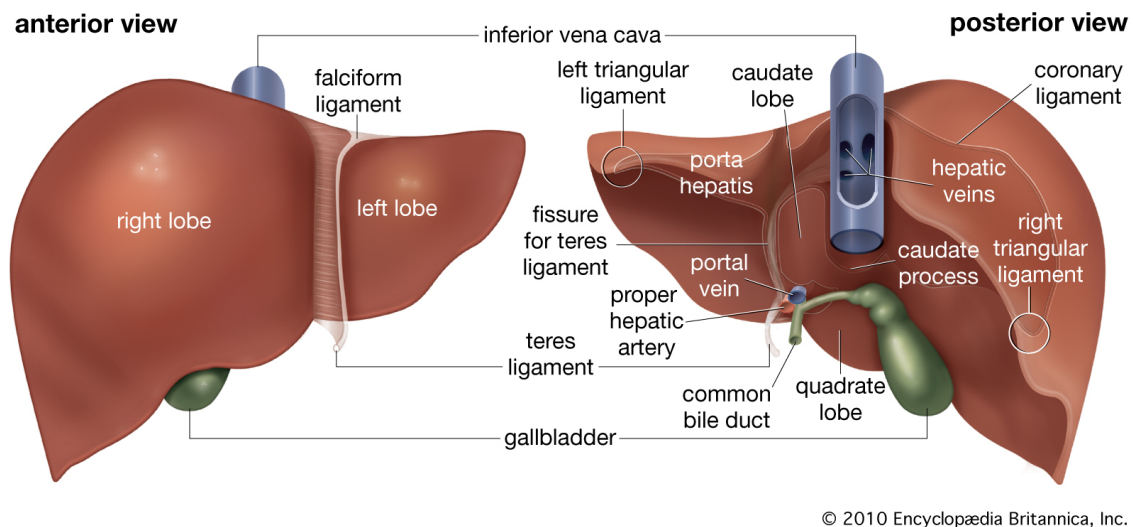


Fig. 3.1. Liver anatomy, by courtesy of Encyclopædia Britannica, Inc., copyright 2010; used with permission (4).

The liver receives ~ 75 % of its blood supply from the portal vein, and ~ 25 % from the hepatic artery (5); these two blood vessels enter the liver via the porta hepatis. The portal vein supplies partially deoxygenated blood and nutrients from the spleen and intestines. The hepatic artery supplies oxygenated blood from the heart to the liver. Partially deoxygenated blood exits from the liver to the heart via the hepatic veins (2,3).

Each lobe is divided into hexagonal functional units called lobules. A liver lobule is shown in Fig. 3.2. A lobule consists of millions of hepatocytes (liver cells). Hepatocytes form ~ 80 % of the liver's volume (3,6). They process the nutrients, toxins and waste from the blood. At each corner of the lobule is a portal triad comprising a portal vein, hepatic artery and bile duct. The hepatic sinusoids are capillary-like vessels that accept processed nutrients from hepatocytes and carry blood to the central vein, which connects to the hepatic vein (3).

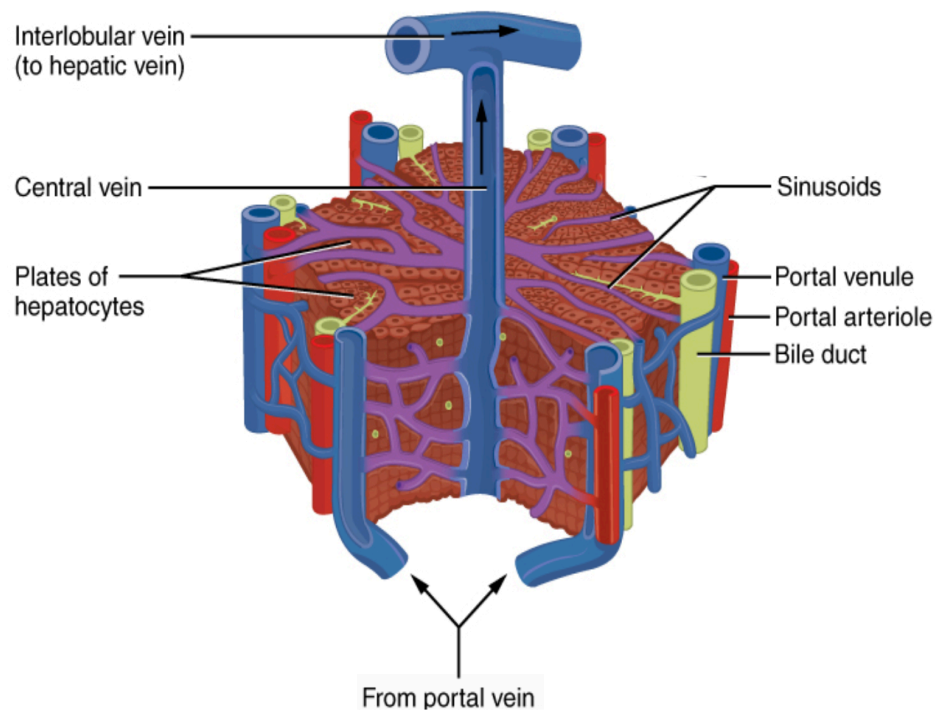


Fig. 3.2. Liver lobule, modified from *Anatomy and Physiology* by OpenStax College (3), under the Creative Commons Attribution 4.0 International License (CC BY 4.0). Copyright 2017 Rice University.

3.1.2. Functions of the Liver

The liver is responsible for the following main functions:

3.1.2.1. Bile Production

Hepatocytes produce bile, which aids in the digestion of lipids and absorption of fat-soluble vitamins (5). Bile is composed of water (~ 95 %), containing dissolved bile salts, bilirubin, phospholipids, cholesterol and inorganic salts (7). Bile exits the hepatocyte from the bile canaliculus to the bile duct in the portal triad, leaving the liver through the common hepatic duct (3).

3.1.2.2. Glycogen Storage

The liver acts as a site for glycogen storage. Glycogen is a large, branched polymer of glucose that can be broken down into glucose molecules for energy. After a meal, glucose from the blood enters the hepatocytes, where it is synthesised into glycogen (8). Glycogen forms < 2 % of MR signal in the liver, as assessed by spectroscopy (9,10).

3.1.2.3. Lipid Metabolism

Fatty acids and triglycerides are synthesised in the liver, and are transported via lipoproteins for storage in adipose tissue (11). Fatty acids can be oxidised to yield energy in the form of ATP. Furthermore, the liver synthesises cholesterol and phospholipids, which are important for maintaining the cell membrane. Lipids normally form < 5 % of the MR signal, given by the proton density fat fraction (PDFF) (12).

3.1.2.4. Iron Storage

The liver is responsible for the storage of excess iron, the production of proteins to maintain iron homeostasis and the transport of iron to the bloodstream. Hepatocytes are the main cell type that stores iron, mostly in the form of ferritin, a protein (13). Iron causes the T_2^* of the liver to shorten, with $R_2^* = 1/T_2^*$ being linearly correlated with the iron concentration (14).

3.1.2.5. Protein Metabolism

The hepatocyte is responsible for the synthesis of proteins and amino acids, with ~ 80 % of the proteins being secreted into the blood (15). Examples of such proteins include albumin, fibrinogen and transferrin. Amino acids are detectable with MR spectroscopy of the liver (16,17).

3.2. Liver Fibrosis

3.2.1. Liver Fibrosis

Collagen is implicated in liver disease through the development of fibrosis. Collagen is a protein measuring $\sim 14 \text{ \AA}$ in diameter and $\sim 3,000 \text{ \AA}$ in length, consisting of a triple-helical structure of three polypeptide chains (18). The amino acid sequence has the form of [glycine-X-Y], where X is usually proline and Y is usually 4-hydroxyproline (19,20). There are 28 types of collagen (20). The accumulation of extracellular matrix proteins in the liver, mainly consisting of collagen types I and III, is termed fibrosis (21–24). Fibrosis is a response to liver injury, progressing to cirrhosis and ending in liver failure or HCC (22–26).

Fibrosis can cause the collagen proportionate area to increase from $\sim 2 \%$ to $\sim 28 \%$ in histology (27). The most effective treatment for liver fibrosis involves removing the causal agent, which can lead to the reversal of fibrosis (22,23,25). The reversibility of fibrosis has warranted the development of antifibrotic drugs; however, these drugs have not been translated to the clinic as of yet (21,24). Hence, there is motivation to detect collagen in the liver, as a measure of disease.

3.2.2. Diseases of the Liver

Common liver diseases leading to fibrosis are summarised below:

3.2.2.1. *Hepatitis*

Hepatitis is the inflammation of the liver. Viral hepatitis includes Hepatitis A, B, C, D and E, although D and E are less prevalent. Autoimmune hepatitis is a heterogeneous

disease of unknown cause; risk factors include genetics, environmental conditions and failure in immunity (28,29).

3.2.2.2. Alcoholic Liver Disease (ALD)

ALD is caused by the chronic consumption of alcohol, typically > 40 g of pure alcohol per day, over a sustained time period (30). ALD begins with hepatic steatosis, defined by intrahepatic triglycerides > 5 % of the liver weight or 5 % of hepatocytes containing lipid vacuoles (31). The disease can progress to alcoholic steatohepatitis (ASH), characterised by inflammation and ballooning of hepatocytes (30).

3.2.2.3. Non-Alcoholic Fatty Liver Disease (NAFLD)

Similar to ALD, NAFLD begins with hepatic steatosis, progressing to non-alcoholic steatohepatitis (NASH). This disease manifests in the absence of excessive alcohol consumption and is caused by factors including obesity and diabetes (31,32).

3.2.3. Detection of Liver Fibrosis

Techniques have been developed to detect fibrosis associated with liver disease. As the techniques present benefits and limitations, there is room for direct and non-invasive detection of fibrosis.

3.2.3.1. Biopsy and Histopathology

The gold standard for the assessment of liver fibrosis is biopsy (23,25,27,33). However, biopsy is prone to sampling error (23,25,27,33), as it accounts for ~ 1/50,000 of the total liver mass (33,34). Furthermore, the risk of complications is of the order of 1 %, which includes bleeding, pain and hypotension (27,35). To evaluate the collagen proportionate area and fibrosis stage, histopathological assessment of the biopsy sample is performed.

Picrosirius red stains collagen red and other structures yellow; this stain is sensitive to collagen types I and III, which are prevalent in the liver (21–24). The collagen proportionate area is assessed by quantifying the area that is stained red as a percentage of the total area. An example is in Fig. 3.3. Histopathological features are evaluated via semi-quantitative scoring systems, the most popular of which include Ishak (scores 0 to 6) and METAVIR (scores 0 to 4) (36,37). Due to the sampling error and risk of complications associated with biopsy, alternative techniques have emerged for the assessment of fibrosis.

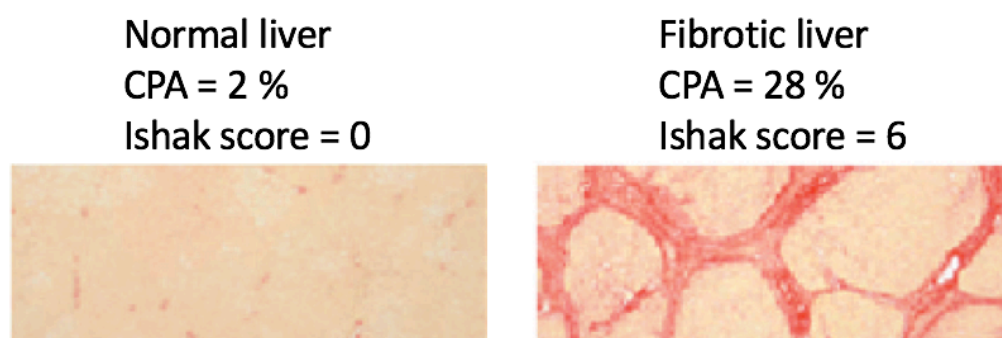


Fig. 3.3. Histopathology with Picrosirius red staining, modified from Standish 2006 (27). Adapted by permission from BMJ Publishing Group Limited. Copyright 2006 by Gut. CPA = collagen proportionate area. Collagen is stained red. At a CPA of 28 %, collagen is abundant and oriented in multiple directions.

3.2.3.2. Serum Markers

Alternatively, tests on serum markers assess levels of proteins implicated in fibrosis. Examples of such proteins are amino-terminal propeptide of type III collagen (PIIINP), tissue inhibitor of metalloproteinase 1 (TIMP-1) and hyaluronic acid (23,38,39). Tests on serum markers are helpful as an indicator of fibrosis in the body, as they can distinguish between advanced and minimal fibrosis. However, the tests are limited in distinguishing between intermediate fibrosis stages and are not organ-specific (23,38,39).

3.2.3.3. Ultrasound Elastography

Ultrasound elastography utilises ultrasound to measure liver stiffness. The most widely used ultrasound elastography technique is transient elastography, which assesses liver stiffness through shear-wave propagation. Transient elastography is non-invasive and more representative than biopsy, as the assessed volume is at least 100 times larger than a biopsy sample (40,41). Transient elastography demonstrates adequate sensitivity and specificity to cirrhosis, but is less accurate for earlier stages of fibrosis (42). Limitations include increased waist circumference, as shear and ultrasonic waves can be attenuated by lipids, and insufficient operator experience. These have resulted in failure or unreliable results in 18 % of transient elastography scans (43,44).

3.2.3.4. MR

MR techniques have been developed for assessing liver fibrosis, as alternatives to the gold standard of biopsy. This includes MR elastography, the most established MR technique, and emerging techniques including iron-corrected T_1 mapping. Similar to transient elastography, MR elastography assesses liver stiffness via shear-wave propagation. MR elastography has a high diagnostic accuracy for hepatic fibrosis, including early stages of the disease (45,46). It has been shown to have similar (47) or higher diagnostic accuracy (48,49) than ultrasound elastography methods. Advantages include feasibility in obese patients (50,51), operator-independent measurements (51,52) and a low technical failure rate of 4% (45). Disadvantages include signal confounders, such as iron and inflammation, and the need for a driver to propagate the shear waves.

An emerging technique is iron-corrected T_1 (cT_1) mapping, part of a multi-parametric MR protocol for assessing liver disease (53,54). In fibrosis, there is increased

extracellular water, which increases T_1 . The T_1 -reducing effect of iron is mitigated via correction with an algorithm (53,55). cT_1 has been shown to correlate with fibrosis stage and provide adequate diagnostic accuracy for fibrosis (53,56), although transient elastography is more robust for assessing fibrosis stage (56).

It is noted that both MR elastography and cT_1 provide indirect measures of liver fibrosis via liver stiffness and T_1 of extracellular water. A more direct approach would involve quantifying the short- T_2^* signal of the liver potentially describing collagen, which is investigated in my thesis.

3.2.4. Short- T_2^* Signal in the Liver

3.2.4.1. *Motivation for Measuring Short- T_2^* Signal*

The motivation for measuring short- T_2^* signal in the liver includes finding a specific signal that may be indicative of collagen and quantifying the signal. Conventional pulse sequences are unable to detect a $T_2^* < 1$ ms, due to limitations in minimum TE. Given that the liver is made of ~ 70 % water, most liver MR signal will originate from free water, which has a long T_2^* of 9 - 21 ms at 3 T (57,58). The difference between a short T_2^* of < 1 ms and a long T_2^* of 9 - 21 ms allows a short- T_2^* signal to be distinguishable and quantifiable.

3.2.4.2. *Literature on Short- T_2^* Signal in the Liver*

Short- T_2^* signal has been measured in the liver, as shown in Table 3.1 and Table 3.2. Fig. 3.4 provides a key to the abbreviations used in the tables. Both UTE and MR spectroscopy (FID) sequences were considered for their use of short TEs.

Hum. = human
Bov. = bovine

IV = *in vivo*
EV = *ex vivo*

T = temperature
BT = body temperature

H = healthy
C = cirrhosis
S = steatosis (but no cirrhosis)
CF = congenital fibrosis
HC = haemochromatosis
F0-1, F2, F3-4 = METAVIR F-scores 0-1, 2, 3-4
HCV = Hepatitis C virus
NP = non-pathological

est. = estimated (i.e. the value was not explicitly mentioned, but was calculated from information provided in the paper)

MAS = magic angle spinning
FID = free induction decay

SSD = statistically significant difference from healthy (yes or no)
Y = yes
N = no

Fig. 3.4. Key to abbreviations in Table 3.1 and Table 3.2.

Paper	Sample	Source	Prep.	T (°C)	B ₀ (T)	NTE	Reported TE range (ms)	Actual TE range (ms) (est.)	State	% signal		T ₂ [*] (ms)	
										Value	SSD (<i>p</i> -value)	Value	SSD (<i>p</i> -value)
Zhu et al. 2019 (59)	Liver	Hum.	IV	BT	1.5	11	0.10 – 6.0	0.16 – 6.06	H	14 ± 1	--	0.8 ± 0.2	--
					3.0	11	0.10 – 6.0	0.16 – 6.06	H	10 ± 2	--	0.21 ± 0.08	--
					3.0	11	0.10 – 7.0	0.16 – 7.06	H	10 ± 2	--	0.23 ± 0.05	--
									C	7 ± 2	N (0.08)	0.20 ± 0.05	N (0.79)
									S	7 ± 2	N (0.08)	0.10 ± 0.02	Y (0.01)
Chappell et al. 2003 (60)	Liver	Hum.	IV	BT	1.5	4	0.08 – 17.70	*	H	--	--	23 ± 2	--
									CF	--	--	52 ± 1	--
									C	--	--	40 ± 10	Y (0.04)
									HC	--	--	7 ± 3	Y (0.002)
Kim et al. 2008 (61)	Liver	Hum.	IV	BT	1.5	2	0.08, 4.4	*	F0-1	--	--	17 ± 2	--
									F2	--	--	19 ± 1	--
									F3-4	--	--	19 ± 2	--

Table 3.1. Literature on short-T₂^{*} signal in the liver with UTE. (*) indicates that neither the TE calculation method nor the RF pulse length were mentioned.

Paper	Sample	Source	State	Prep.	T (°C)	B_0 (T)	Sequence	TE (ms) (est.)	Location of broad peak (ppm) (est.)	Line width (ppm) (est.)	T_2^* (ms) (est.)
Martinez-Granados et al. 2006 (16)	Liver	Hum.	NP (meta-stasis)	EV	0	11.75	MAS + FID	0.08	0.81 – 5.31	4.50	0.14 ± 0.02
Cobbold et al. 2009 (9)	Liver	Murine	H	EV	4	11.75	MAS + FID	≤ 0.12	0.83 – 5.42	4.59	0.14 ± 0.02
Cobbold et al. 2010 (10)	Liver	Hum.	HCV	EV	4	11.75	MAS + FID	≤ 0.12	0.85 – 2.26	1.41	0.45 ± 0.05
Fathi et al. 2017 (17)	Liver	Avian	H	Extract	25	14.1	FID	0.05	3.38 – 4.09	0.71	0.75 ± 0.05

Table 3.2. Literature on short- T_2^* signal in the liver with MR spectroscopy. Data on the broadest peak are provided.

Literature demonstrates short- T_2^* signal in the liver with UTE, although the detection of the signal is dependent on the selection of TEs. The most relevant data is by Zhu et al.. They characterised the short- T_2^* signal in the livers of healthy subjects, patients with cirrhosis and patients with steatosis (but no cirrhosis), with UTE at 3.0 T (59). 11 TEs from 0.16 to 7.06 ms were acquired, allowing the short- T_2^* signal to be sampled. They found a short T_2^* of 0.23 ± 0.05 ms with a signal fraction of 10 ± 2 % in healthy subjects. The patients did not show a significant difference in short T_2^* or signal fraction, except for the short T_2^* of the steatotic patients, which was 0.10 ± 0.02 ms.

For healthy subjects across field strengths, Zhu et al. found a short- T_2^* value of 0.21 ± 0.08 ms at 3.0 T, compared to 0.8 ± 0.2 ms at 1.5 T, when acquired with the same protocol and subjects. This constituted a decrease of almost fourfold in T_2^* . Discrepancies in the T_2^* may have been contributed by B_0 field dependence and lipid signal. Literature has shown an approximately twofold decrease in liver T_2^* from 1.5 T to 3.0 T due to field-dependent effects, which account for the presence of iron (57,58). Moreover, the -3.4 ppm frequency shift between the lipid CH_2 group and water caused the T_2^* signal to be out of phase at 1.15 ms at 3.0 T, and at 2.30 ms at 1.5 T. Hence, the short- T_2^* signal (at TEs < 1 ms) was potentially confounded by off-resonant lipid signal at 3.0 T. These discrepancies could have contributed to the T_2^* being shorter at 3.0 T than at 1.5 T.

By contrast, Chappell et al. found relatively high T_2^* values (7 to 52 ms) in the livers of healthy subjects and patients at 1.5 T (60). Their acquisition method was not sensitive to T_2^* s < 6 ms, as they measured four TEs: 0.08, 5.95, 11.08 and 17.70 ms. Similarly, Kim et al. acquired two TEs at 0.08 and 4.4 ms to find a T_2^* ranging from 17 to 19 ms in liver fibrosis patients at 1.5 T (61). It is possible that the T_2^* values measured by

Chappell et al. and Kim et al. were of free water or a component intermediate to bound and free water, as free water has a T_2^* between 23 and 28 ms at 1.5 T (57).

Short- T_2^* signal in the liver has been found with MR spectroscopy, revealing resonances with broad line widths or short T_2^* s. Martinez-Granados et al. characterised the MR spectrum of a non-pathological liver of a patient with an unspecified metastasis with magic angle spinning (MAS) at 11.75 T (16). They found a broad peak from 0.81 to 5.31 ppm, corresponding to a short T_2^* of 0.14 ± 0.02 ms at 11.75 T. Due to the dependence of T_2^* on field strength, the T_2^* would be higher at 3 T. Although MAS is a different experimental setting from UTE imaging, MAS has the advantage of averaging the dipolar interactions that are orientation-dependent, providing narrow line widths (62). The presence of a broad peak in MAS suggests that it is a real entity and not an effect of exchange. Cobbold et al. identified a similar broad peak in the liver with MAS at 11.75 T (9). However, the broadest peaks observed by Cobbold et al. in 2010 and Fathi et al. had T_2^* s of 0.45 ± 0.05 ms (at 11.75 T) and 0.75 ± 0.05 ms (at 14.1 T), respectively, indicating that there is variability in the baseline signal (10,17). The variability is not surprising, considering that the baseline signal can differ between samples and individuals. It is noted that the broad peak from 3.38 to 4.09 ppm observed by Fathi et al. was similarly found by Martinez-Granados et al. and Cobbold et al. (in 2009), although the other papers had a broader baseline signal. Hence, there is potential for a broad resonance or short- T_2^* signal in the liver, although it may differ between subjects.

3.3. Tendon

Given the presence of signal with $T_2^* < 1$ ms in the liver detected with UTE and MR spectroscopy, the objective was to model this short- T_2^* signal and to test the hypothesis that it results from collagen. Collagen is a reasonable candidate, given its presence in the liver and short- T_2^* properties. Tendon serves as a biological model of collagen, as tendon is oriented collagen, with T_2^* and resonant frequency shift properties that are dependent on the orientation relative to B_0 . Properties of tendon will be investigated in order to form a T_2^* model of isotropic collagen, to be described in Chapter 6.

3.3.1. Structure

Tendon is connective tissue consisting of 90 - 100 % type I collagen (63), rendering it an appropriate model tissue for studying collagen. Fig. 3.5 from Silver et al. 2003 (64) illustrates the structure of tendon, starting from the collagen molecule. A tendon unit consists of many subunits of collagen, progressively assembled into collagen fibrils, fibres and fascicles, all parallel to the geometrical axis of the tendon. This gives rise to the oriented nature of tendon.

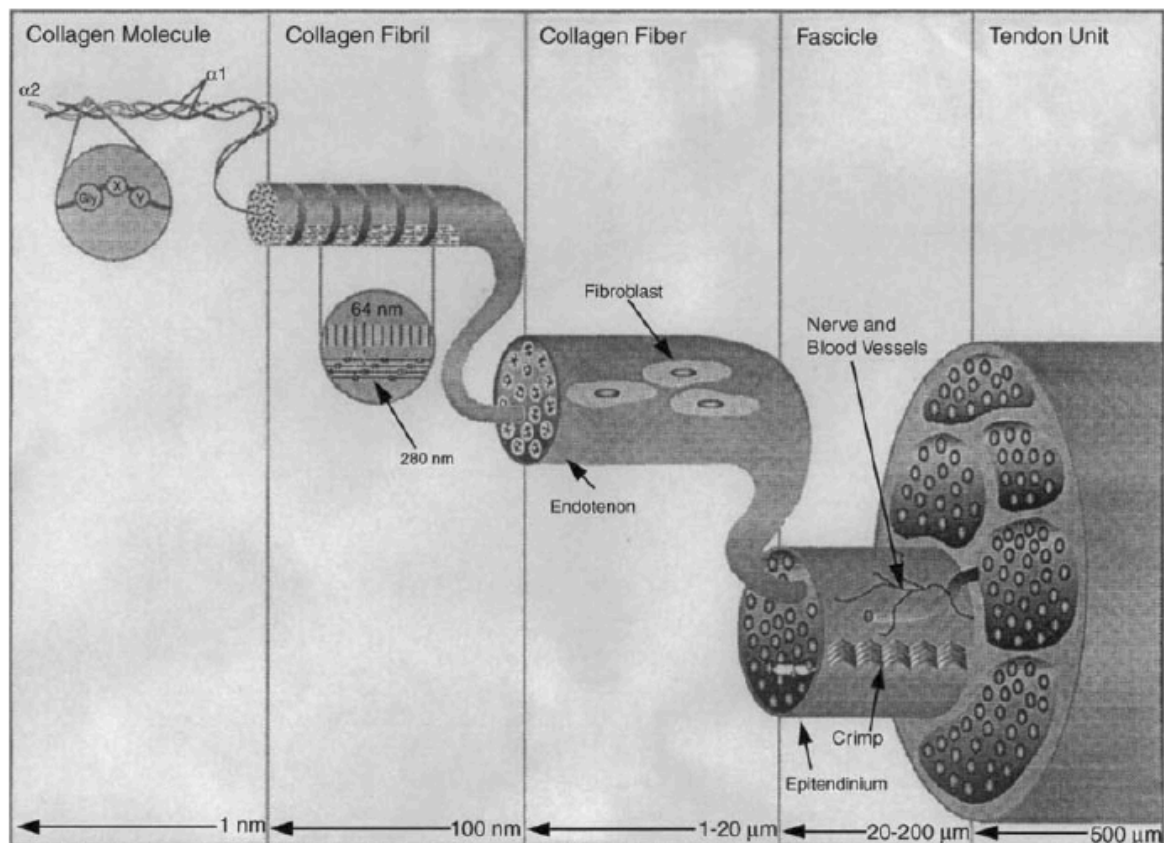


Fig. 3.5. Structure of tendon, from Silver et al. 2003 (64); Copyright 2003. Reproduced with permission from Elsevier.

3.3.2. Magic Angle Effect

The orientation of tendon makes it susceptible to the magic angle effect, arising due to the dipole-dipole interactions between protons. A schematic is shown in Fig. 3.6.

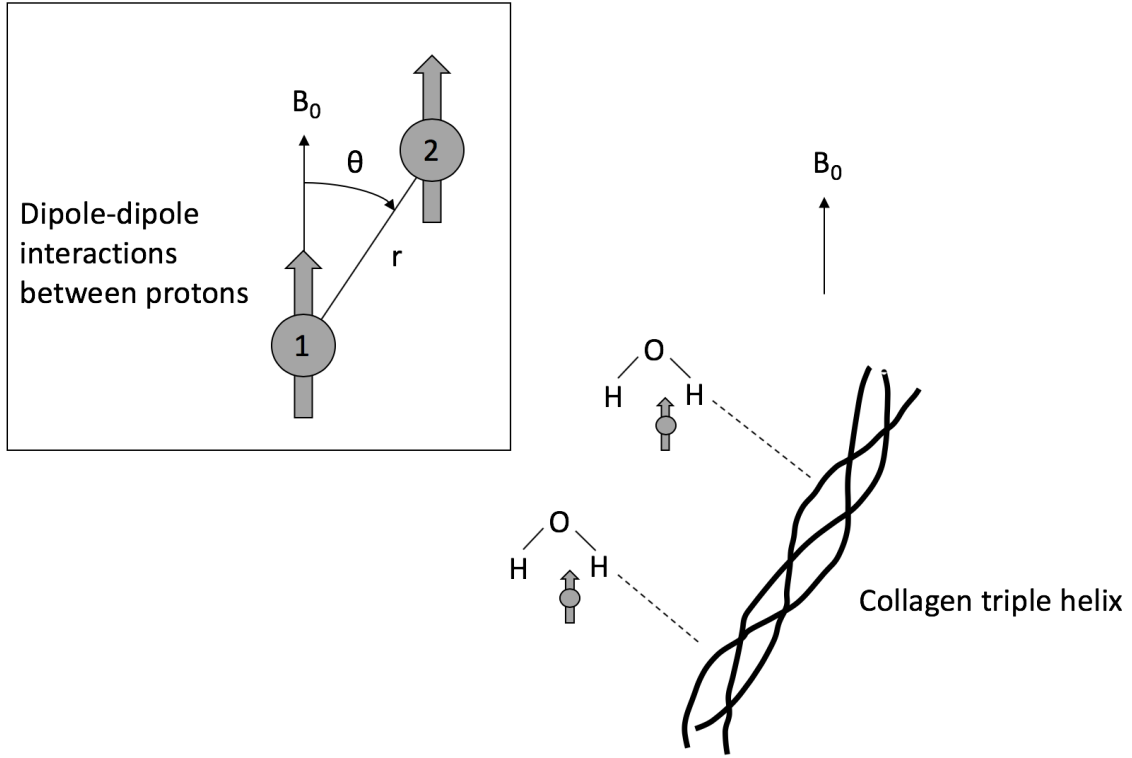


Fig. 3.6. Magic angle effect between protons associated with collagen, based on Figure 2 of Erickson et al. 1993 (65).

The change in the z-component of the magnetic field ($B_{1,z}$) at dipole 1 due to dipole 2 is given by the following equation:

$$B_{1,z} = \pm \left(\frac{\mu_0}{4\pi} \right) \mu_2 \frac{(3\cos^2\theta - 1)}{r^3}, \quad [3.1]$$

where dipole 2, having a magnetic dipole moment of μ_2 , is located a distance r away from dipole 1 (66). μ_0 is the permeability of free space ($= 4\pi \times 10^{-7} \text{ T}\cdot\text{m/A}$). Here, T_2 or T_2^* is maximised at the “magic angle” of 54.7° , i.e. when $3\cos^2\theta - 1 = 0$. This explains why, on conventional pulse sequences, tendon oriented at 0° relative to B_0 appears dark, whereas at 55° , it appears bright. With UTE, tendon is visible at all angles, and the T_2^* can be quantified as a function of the orientation relative to B_0 .

3.3.3. T_2^*

The T_2^* values of tendon are listed in Table 3.3. Fig. 3.7 is a list of abbreviations. I calculated the actual TE from the centroid of the RF pulse. In most cases, the actual TEs are higher than the reported TEs, as the reported TEs do not account for the time from the centroid to the end of the RF pulse. Consequently, the reported T_2^* s are underestimated. Nevertheless, it can be generalised that, when modelling the T_2^* of healthy tendon as a single component, the T_2^* is ~ 3 ms at 0° , ~ 13 ms at 55° and ~ 4 ms at 90° at 3 T. When modelling the T_2^* of healthy tendon as a short- T_2^* component in a bi-exponential model at 3 T, the T_2^* is ~ 1 ms at 0° , forming ~ 73 % of the signal. Hence, there is significant short- T_2^* signal in tendon, which varies according to the magic angle effect.

Differences in T_2^* may be due to *ex vivo* and *in vivo* experimental conditions, including TE selection and tensile loading. In healthy *in vivo* tendon, Juras et al. found a T_2^* of 3.4 ± 0.5 ms with 20 TEs ranging from 0.8 to 20 ms, whereas Grosse et al. reported a T_2^* of 1.2 ± 0.4 ms with four TEs ranging from 0.07 to 1.57 ms. In the latter case, the smaller range of TEs allowed the sampling of a shorter T_2^* . With cycling loading, Koff et al. found a statistically significant difference in T_2^* (4 ± 1 ms unloaded vs. 2.8 ± 0.5 ms loaded) (67). By contrast, Chang et al. found no significant difference (2 ± 2 ms unloaded vs. 3 ± 3 ms loaded) (68). Differences between the studies include the type of loading: Koff et al. applied a 45-N force on the tendon for 100 cycles at 1 Hz; imaging was performed after loading. Chang et al. applied a 1-kg load on the tendon during scanning. It is hypothesised that the loading procedure used by Koff et al. was more vigorous, resulting in the straightening of collagen fibres and restriction of motion, causing a shorter T_2^* (68).

There is a small but significant difference in T_2^* between healthy and tendinopathic tendons at 0° . Juras et al. found significantly different T_2^* s of 0.68 ± 0.05 and 0.87 ± 0.08 ms in healthy and degenerated tendons *in vivo*, respectively (69). Grosse et al. found T_2^* s of 2.0 ± 0.9 and 4 ± 3 ms in diseased non-symptomatic and symptomatic tendons, *in vivo*, respectively; both were significantly different from the T_2^* of 1.2 ± 0.4 ms in healthy controls (70). It is hypothesised that the higher T_2^* in diseased tendon is due to freer motion of the water associated with collagen. This could result from the collagen fibres becoming disrupted and less tightly bundled with tendinopathy (71). By contrast, Diaz, Chung et al. found similar short T_2^* s between healthy and diseased tendons of 0.61 ± 0.06 and 0.65 ± 0.04 ms, respectively. However, the long T_2^* decreased from 8 ± 1 to 4.8 ± 0.8 ms between health and disease. Their result may have been confounded by lipid signal, which resulted in oscillations in the T_2^* signal decay. Nevertheless, the T_2^* properties of tendon provide reference values for modelling the T_2^* of collagen.

AT = Achilles tendon
post. = posterior
ant. = anterior
T = tendon (location unspecified)
DFT = digital flexor tendon
PT = patellar tendon

Bov. = bovine
Hum. = human
Lap. = lapine

D = degenerated (tendinopathic)
H = healthy
UL = unloaded
L = loaded
S = symptomatic
NS = non-symptomatic

EV = ex vivo
IV = in vivo

T = temperature
RT = room temperature
BT = body temperature

NTE = number of TEs
 NT_2^* = number of T_2^* components

est. = estimated (i.e. the value was not explicitly mentioned, but was calculated from information provided in the paper)

Fig. 3.7. Key to abbreviations in Table 3.3 and Table 3.4.

Paper	Sample	Source	State	Prep.	T (°C)	B_0 (T)	NTE	Reported TE range (ms)	Actual TE range (ms)	NT_2^*	0°		55°	90°
											% signal	T_2^* (ms)	T_2^* (ms)	T_2^* (ms)
Gold, Pauly et al. 1995 (72)	AT	Hum.	H	IV	BT	1.5	16 (est.)	0.2 - 10.5	0.4 - 10.7 (est.)	1	100	2		
Robson, Benjamin et al. 2004 (73)	AT (post.)	Hum.	H	IV	BT	1.5	8	0.14 - 2.46	0.14 - 2.46	2	88	0.53		
											12	4.80		
	AT (ant.)	Hum.	H	IV	BT	1.5	8	0.14 - 2.46	0.14 - 2.46	2	70	0.60		
											30	4.2		
Du, Diaz et al. 2012 (74)	AT	Bov.	H	EV	RT	3	10 - 34	0.008 - 40	0.108 - 40.1 (est.)	2	71 ± 5	1.28 ± 0.08		
											29 ± 4	18 ± 5		
Juras, Apprich et al. 2013 (69)	AT	Hum.	H	IV	BT	3	20	0.8 - 20	*	1	100	3.4 ± 0.5		
										2	60 ± 10	0.68 ± 0.05		
											40 ± 10	17 ± 7		
			D	IV	BT	3	20	0.8 - 20	*	1	100	7 ± 2		
										2	60 ± 9	0.87 ± 0.08		
											40 ± 9	20 ± 7		
Chang, Du et al. 2015 (68)	T	Hum.	H; UL	EV	RT	3	16	0.008 - 30	0.108 - 30.1 (est.)	1	100	2 ± 2		
										2	79 ± 8	1.0 ± 0.3		
											21 ± 8	20 ± 5		

			H; L	EV	RT	3	16	0.008 - 30	0.108 - 30.1 (est.)	1	100	3 ± 3		
										2	80 ± 9	0.9 ± 0.3		
											21 ± 9	24 ± 6		
Diaz, Chung et al. 2012 (75)	AT	Hum.	H	IV	BT	3	108	0.008 - 17	0.108 - 17.1 (est.)	2	76 ± 6	0.61 ± 0.06		
											24 ± 5	8 ± 1		
			D (rupture)	IV	BT	3	108	0.008 - 17	0.108 - 17.1 (est.)	2	77 ± 5	0.65 ± 0.04		
											23 ± 5	4.8 ± 0.8		
Du, Chiang et al. 2010 (76)	AT	Hum.	H	EV	RT	3	45	0.008 - 5.4	0.108 - 5.5 (est.)	1	100	2	10	4
Du, Pak et al. 2009 (77)	AT	Hum.	H	EV	RT	3	8	0.1 - 32	0.22 - 32.12	1	100	1.9 ± 0.3	15 ± 2	
Koff, Pownder et al. 2014 (67)	PT	Lap.	H; UL	EV	RT	3	4	0.05 - 15	*	1	100	4 ± 1		
			H; L	EV	RT	3	4	0.05 - 15	*	1	100	2.8 ± 0.5		
Grosse, Syha et al. 2015 (78)	AT	Hum.	H; NS	IV	BT	3	4	0.07 - 1.57	0.07 - 1.57	1	100	1.5 ± 0.3		
			H; S	IV	BT	3	4	0.07 - 1.57	0.07 - 1.57	1	100	1.6 ± 0.4		
			D; NS	IV	BT	3	4	0.07 - 1.57	0.07 - 1.57	1	100	2.0 ± 0.9		

			D; S	IV	BT	3	4	0.07 - 1.57	0.07 - 1.57	1	100	4 ± 3		
			H (con -trol)	IV	BT	3	4	0.07 - 1.57	0.07 - 1.57	1	100	1.2 ± 0.4		

Table 3.3. Tendon T_2^* values from literature, mainly at 3 T. Note that T_2^* values were obtained from the reported TE ranges. (*) indicates that neither the TE calculation method nor the RF pulse length were mentioned.

3.3.4. Resonant Frequency Shift

In addition to T_2^* , the resonant frequency shift of tendon changes as a function of its orientation relative to B_0 . Generally, the magnetic field shift experienced by an object in an external magnetic field (B_0) is affected by the geometry of the object and magnetic susceptibility of surrounding objects. The internal field shift experienced by an infinite cylinder at angle θ with respect to B_0 is given by:

$$\Delta B(\theta) = \frac{\Delta\chi}{6} (3\cos^2\theta - 1)B_0 + \frac{1}{3}\chi_e B_0, \quad [3.2]$$

where χ_e is the magnetic susceptibility external to the cylinder and $\Delta\chi$ is the difference in magnetic susceptibilities internal and external to the cylinder (79). Consequently, the frequency shift inside an infinite cylinder is:

$$\Delta f(\theta) = \frac{\Delta\chi}{6} (3\cos^2\theta - 1) + \frac{1}{3}\chi_e. \quad [3.3]$$

Although the tendon is a finite, rather than an infinite cylinder, its frequency shift exhibits a similar sinusoidal relationship. Table 3.4 reports the resonant frequency shift of tendon from two studies. It is noted that Krasnosselskaia et al. modelled the frequency shift with an additional $\pi/2$ shift.

Paper	Sample	Source	State	Prep.	Medium	T (°C)	B ₀ (T)	Frequency shift (ppm)		
								0°	55°	90°
Du, Chiang et al. 2010 (76)	ATT	Hum.	H	EV	Cylindrical glass tube (in air)	RT	3	0	-1	-1.2
Krasnosselskaia, Fullerton et al. 2005 (80)	DFT	Bov.	H	EV	Cadaveric ankle	18.5 ± 0.2	2	0	-2.1	-3.1
								$f(\theta) = -2.08 \cdot \frac{1}{2} \left(3 \cos^2 \left(\frac{\pi}{2} + \theta \right) - 1 \right) + 9.45$		

Table 3.4. Tendon resonant frequency shift values from literature.

Du et al. and Krasnosselskaia et al. reported maximal frequency shifts of -1.2 and -3.1 ppm from 0°, respectively. Differences in frequency shift could be due to experimental setup, particularly since Krasnosselskaia imaged her tendon in air, in a cylindrical glass tube, whereas Du et al. imaged a cadaveric ankle. Krasnosselskaia's frequency shift could have been affected by the frequency shift of the glass tube and the presence of susceptibility effects at the air-tissue interface. By contrast, Du et al.'s frequency shift measurement could have been affected by the frequency shifts of the surrounding tissues in the ankle, although the tissues minimised susceptibility effects at interfaces. Chapter 4 will investigate the differences in the frequency shifts more closely.

3.4. Summary

This chapter reviewed the role of collagen in liver fibrosis, a consequence of liver disease. Literature on short- T_2^* signal in the liver and tendon was presented, the latter acting as a model of collagen. The next chapter will describe measurements of the R_2^* and resonant frequency shift of tendon.

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4. Modelling the Short- T_2^* Signal of Collagen with UTE

Chapter 3 described how the $R_2^* (\frac{1}{T_2^*})$ and resonant frequency shift of tendon are orientation-dependent. In this chapter, the R_2^* and resonant frequency shift of *ex vivo* tendon were measured and compared with literature values. Additionally, the R_2^* signal of isotropic collagen was investigated in phantoms.

4.1. R_2^* of Tendon

4.1.1. Introduction

The objective of this experiment was to characterise the R_2^* of tendon and to compare it with literature values. Notably, in separate studies involving UTE of cadaveric ankle specimens at 3 T, Du et al. found the R_2^* of Achilles tendon to be 500 s^{-1} (1) and $530 \pm 80 \text{ s}^{-1}$ (2) at 0° , and 100 s^{-1} (1) and $67 \pm 9 \text{ s}^{-1}$ (2) at 55° , where the values were converted from T_2^* data.

4.1.2. Methods

Bovine leg tendon was obtained from a local butcher one day post-sacrifice. Visible fat was excised from the tendon. The tendon was subsequently incubated in 80 % acetone solution at 4°C overnight for the removal of lipids (3), and washed and stored in saline at 4°C until imaging at 15 days post-sacrifice. For imaging, three strips of tendon were angled at 0° , 55° and 90° in a cylindrical container filled with Fluorinert (3M, Bracknell, United Kingdom) (refer to Fig. 4.1). Fluorinert was used to minimise magnetic susceptibility effects. The container was placed on a paper protractor and rotated such that the first tendon strip was at angles of 0° , 10° , 20° , 30° , 40° , 50° , 60° , 70° , 80° and

90° relative to B_0 . At each rotation, the short T_2^* signal was measured with UTE for TEs from 0.23 to 1.29 ms, with the parameters specified in Table 4.1. As described in Chapter 2, a dual-echo UTE sequence on the Trio 3 T (Siemens Healthineers, Erlangen, Germany) was used, with the second echo omitted from analysis.

Coil	4-channel phased-array coil (Machnet, Roden, The Netherlands)
Image orientation	Coronal
Repetition time (ms)	50
Flip angle (°)	4
Number of averages	4
Slice thickness (mm)	8
Field-of-view (mm x mm)	300 x 300
Matrix size	256 x 256
Bandwidth (Hz/pixel)	980
Number of rotations	10
Angles (°)	Tendon 1: 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 Tendon 2: 55, 65, 75, 85, 95, 105, 115, 125, 135, 145 Tendon 3: 90, 100, 110, 120, 130, 140, 150, 160, 170, 180
TEs from dual-echo acquisitions (ms)	First TE: 0.23, 0.29, 0.39, 0.54, 0.69, 0.94, 1.29 Second TE: 40.19 for all acquisitions (not analysed)
Scan time per acquisition (s)	51
Number of acquisitions per rotation	7

Table 4.1. UTE scan parameters for measuring the R_2^* of tendon.

For each angle, an ROI was drawn covering each tendon and was copied for all TEs. The complex signal intensity was averaged in the ROI and fitted to the following equation:

$$S(TE) = S_0 \exp(-TE \cdot R_2^*) \cdot \exp(i2\pi f_g TE) \cdot \exp(i\varphi_g), \quad [4.1]$$

where S_0 and R_2^* are the initial signal intensity and $R_2^* \left(= \frac{1}{T_2^*}\right)$ relating to tendon-bound water, respectively. f_g is the resonant frequency shift arising from global B_0 inhomogeneities and the inherent tendon frequency shift. φ_g is a global phase term.

From Chappell et al. (4), the transverse relaxation rate of collagen angled at θ is:

$$R_2(\theta) = R_{2base} + \alpha(3\cos^2\theta - 1)^2. \quad [4.2]$$

By solving for R_2 at $\theta = 0^\circ$ and 55° , we can find the values for R_{2base} and α . Substituting R_2^* for R_2 , we have:

$$R_2^*(\theta) = R_2^*(55^\circ) + \frac{R_2^*(0^\circ) - R_2^*(55^\circ)}{4}(3\cos^2\theta - 1)^2. \quad [4.3]$$

R_2^* as a function of the angle was fitted to Eq. [4.3]. All fitting was performed using a trust-region-reflective non-linear least-squares algorithm in MATLAB R2015b (The MathWorks, Natick, USA), with initial conditions specified in Table A4.1 and Table A4.2. The uncertainties in the fitted parameters are the standard errors.

4.1.3. Results

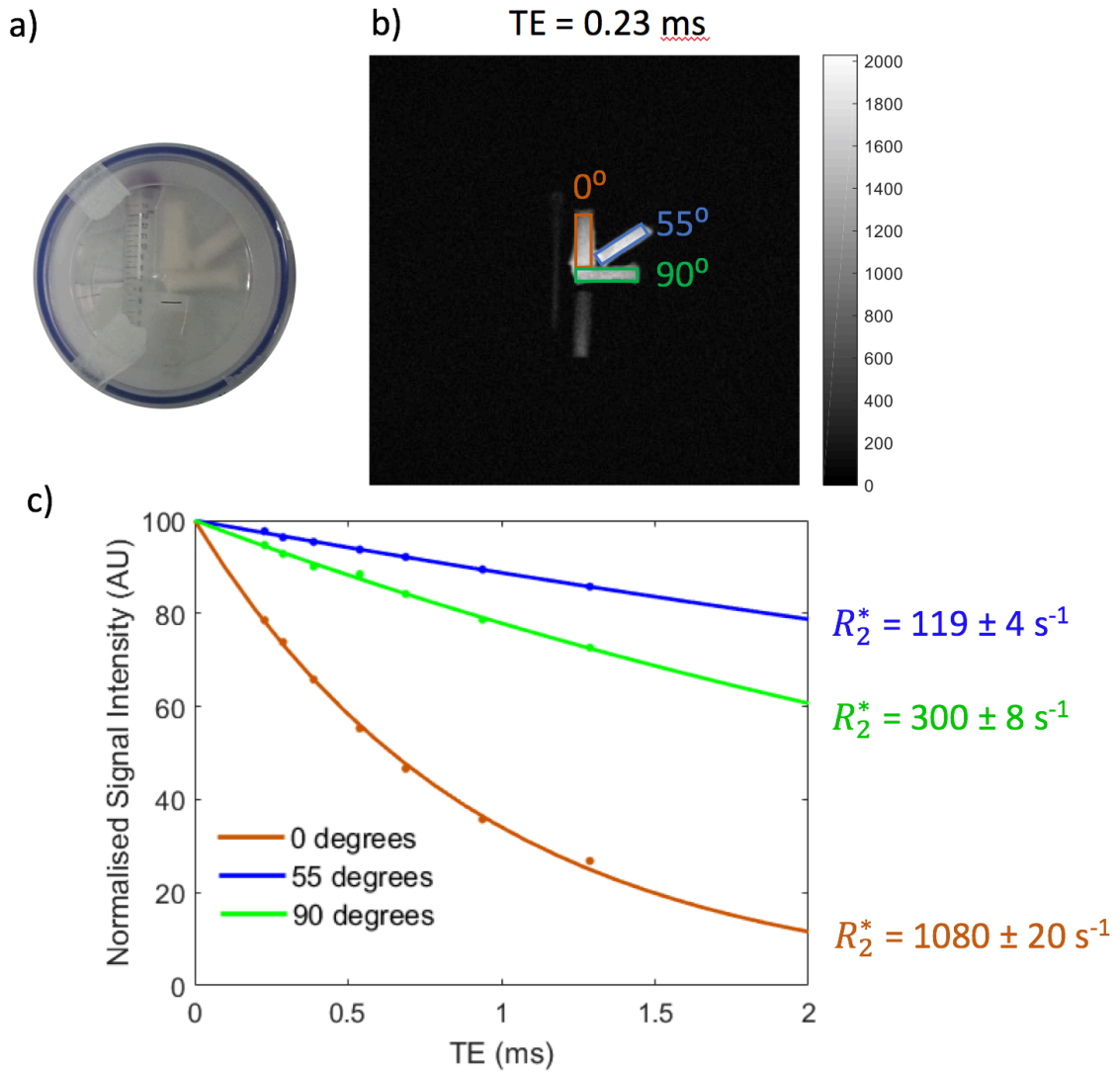


Fig. 4.1. Set-up and results for tendon experiment. a) Experimental set-up with three strips of tendon at 0°, 55° and 90°. Tubes of water and oil were included for reference. b) Corresponding UTE image at the lowest TE of 0.23 ms. c) Normalised signal decays for the three strips of tendon, fitted to Eq. [4.1].

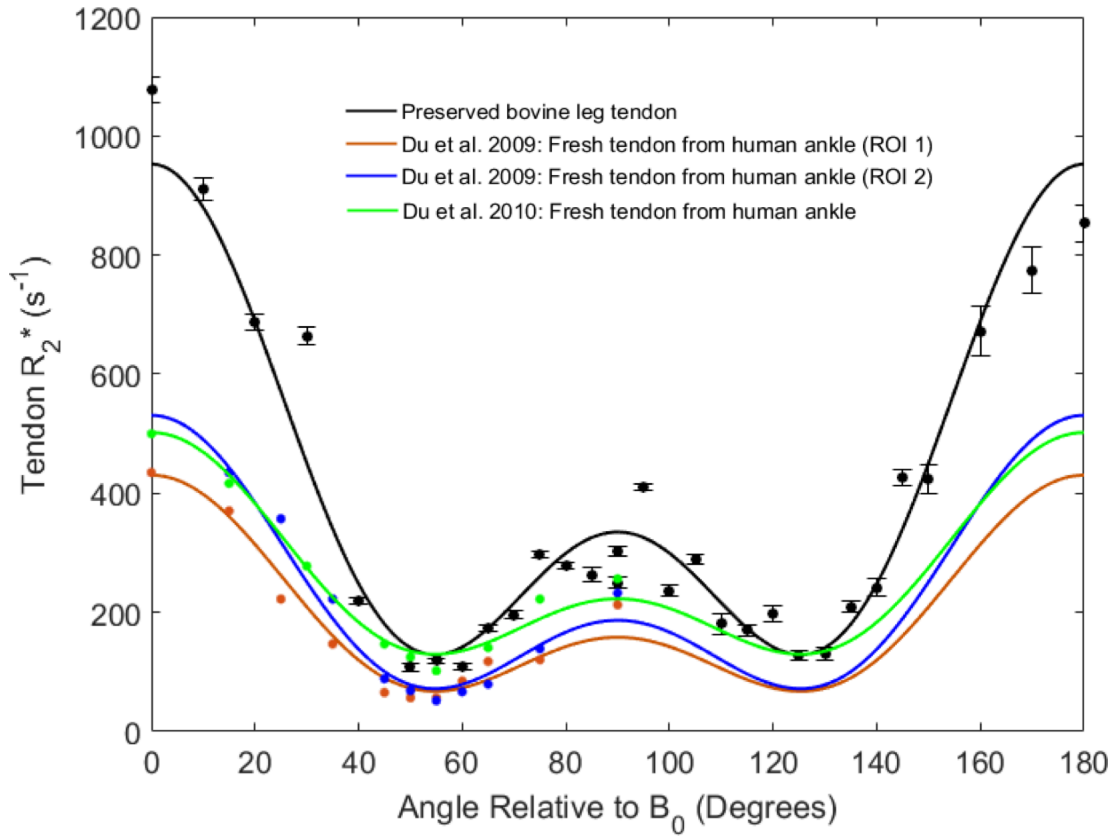


Fig. 4.2. R_2^* as a function of tendon orientation. My results (in black) are compared to data by Du et al. Data by Du et al. were obtained from their figures with a T_2^* uncertainty of ± 0.1 ms.

	$R_2^*(0^\circ)$ (s^{-1})	$R_2^*(55^\circ)$ (s^{-1})
Preserved bovine leg tendon	950 ± 30	130 ± 20
Du et al. 2009 (2): Fresh tendon from human ankle (ROI 1)	430 ± 20	70 ± 10
Du et al. 2009 (2): Fresh tendon from human ankle (ROI 2)	530 ± 20	70 ± 10
Du et al. 2010 (1): Fresh tendon from human ankle	500 ± 20	130 ± 10

Table 4.2. R_2^* data in tendon, after fitting to Eq. [4.3].

Fig. 4.1 demonstrates the experimental set-up and signal decays for the three strips of tendon at the first rotation. Note that R_2^* is lowest at 55° .

Fig. 4.2 illustrates the results in *ex vivo* tendon, as compared to literature by Du et al.. I fitted each R_2^* dataset, including Du et al.'s datasets, to Eq. [4.3]. The values for $R_2^*(0^\circ)$ and $R_2^*(55^\circ)$ are shown in Table 4.2. From my preserved bovine tendon dataset, $R_2^*(0^\circ) = 950 \pm 30 \text{ s}^{-1}$ and $R_2^*(55^\circ) = 130 \pm 20 \text{ s}^{-1}$.

4.1.4. Discussion

Eq. [4.1] modelled the R_2^* signal decays for the preserved bovine tendon reasonably well, as shown by the fits in Fig. 4.1 and the small uncertainties in R_2^* in Fig. 4.2. Furthermore, Eq. [4.3] was appropriate in modelling the $R_2^*(\theta)$ relationship for my and Du et al.'s datasets. Due to deviations from the model near 0° and 180° , the uncertainties in $R_2^*(0^\circ)$ and $R_2^*(55^\circ)$ in the preserved tendon were higher than the analogous uncertainties in Du et al.'s fresh tendon data.

It is evident that my $R_2^*(0^\circ)$ value in the preserved tendon was around twice as large as the $R_2^*(0^\circ)$ values by Du et al.. However, the overall behaviour of $R_2^*(\theta)$ was similar to that from Du et al.'s studies. Graphically, my R_2^* plot for preserved tendon rests above the R_2^* plots for Du et al.'s fresh tendon data. The freshness of the tissue (preserved vs. fresh) could have contributed to the discrepancies in R_2^* . For this experiment, the tendon was immersed in 80 % acetone solution to remove fat (3), as the fat MRI signal could confound the collagen signal. A consequence of incubation in acetone is the preservation of tissue by dehydration and protein denaturation (5,6). Although storing the tendon in saline solution after overnight acetone immersion improved tissue hydration, some changes due to tissue fixation were irreversible. Notably, dehydration renders a tissue “more solid” and hence with a shorter T_2^* (faster R_2^*) (7), in line with the faster R_2^* I observed in preserved tendon when compared to Du et al.'s fresh tendon.

The R_2^* values in preserved tendon were compared to those by Du et al.. These datasets modelled the R_2^* signal decay of tendon with a mono-exponential function, with the exception of Du et al. 2010, where the T_2^* was derived from the line-shape fitting of the magnitude spectra within an ROI (1). From the literature table in Section 3.3.1.3, the R_2^* signal decay of tendon at 0° can alternatively be modelled with a bi-exponential function to delineate the collagen-bound and free water pools. Various papers reported R_2^* components of $\sim 1000 \text{ s}^{-1}$ (signal fraction = 60 – 80 %) and $\sim 50 \text{ s}^{-1}$ (signal fraction = 20 – 40 %) in the tendon at 3 T (8–10). For instance, Du et al. found R_2^* components of $780 \pm 50 \text{ s}^{-1}$ ($71 \pm 5 \%$) and $60 \pm 20 \text{ s}^{-1}$ ($29 \pm 4 \%$) in fresh *ex vivo* bovine tendon, where the TEs ranged from 0.008 to 40 ms (8). Although modelled with different functions, the $950 \pm 30 \text{ s}^{-1}$ R_2^* value I found in preserved tendon at 0° aligned with this $\sim 1000 \text{ s}^{-1}$ R_2^* component in the literature. Note that the reported studies utilised longer TEs of up to 20 - 40 ms. Given the maximal TE of 1.29 ms considered in my experiment, modelling the signal with a mono-exponential function was reasonable.

4.1.5. Conclusion

The R_2^* of tendon was investigated, yielding $R_2^*(0^\circ) = 950 \pm 30 \text{ s}^{-1}$ and $R_2^*(55^\circ) = 130 \pm 20 \text{ s}^{-1}$. These values were comparable to the values found in the literature.

4.2. Resonant Frequency Shift of Tendon

4.2.1. Introduction

The dependence of the resonant frequency shift of tendon on its orientation relative to B_0 has been reported (1,11). Krasnosselskaia et al. found a resonant frequency shift of 3.1 ppm from 0° to 90° in *ex vivo* bovine digital flexor tendon enclosed in a cylindrical glass tube at 2 T (11). By contrast, Du et al. described a maximal frequency shift of 1.2 ppm in

human Achilles tendon within an ankle specimen at 3 T (1). The objective of this experiment was to characterise the resonant frequency shift of tendon with UTE at 3 T. Factors affecting the frequency shift were investigated, including the effects of the geometry of the tendon and the surrounding container. The results were presented at the ISMRM 2018 Scientific Sessions (12).

4.2.2. Methods

Three experiments were performed, according to Table 4.3, to investigate:

- (1) the resonant frequency shift of water,
- (2) the effect of the geometry of the tendon on the tendon frequency shift, and
- (3) the effect of the container on the tendon frequency shift.

Experiment	Sample	Sample Geometry	Time Post-Sacrifice	Container
(1): Frequency shift of water (reference)	Distilled water	N/A	N/A	Quartz tube, longitudinal axis $\parallel B_0$ (D = 2.77 cm, L = 7.04 cm)
(2): Effect of the <u>geometry of the tendon</u> on the tendon frequency shift	Bovine digital flexor tendon	(2.a) <u>Rectangular prism</u> , longitudinal axis $\parallel B_0$ (L = 4.20 cm, W = 1.73 cm, H = 0.70 cm). Collagen fibres $\parallel B_0$.	7 hours	Quartz tube, longitudinal axis $\parallel B_0$ (D = 2.77 cm, L = 7.04 cm)
		(2.b) <u>Cylinder</u> , longitudinal axis $\perp B_0$, out of plane (D = 1.70 cm, H = 0.70 cm). Collagen fibres $\parallel B_0$.	10 hours	
(3): Effect of the <u>container</u> on the tendon frequency shift	Bovine digital flexor tendon	Cylinder, longitudinal axis $\perp B_0$, out of plane (D = 1.70 cm, H = 0.70 cm). Collagen fibres $\parallel B_0$.	4 days	(3.a) <u>Quartz tube</u> , longitudinal axis $\parallel B_0$ (D = 2.77 cm, L = 7.04 cm)
				(3.b) <u>Cylindrical plastic container</u> , longitudinal axis $\perp B_0$, out of plane (base D = 7.55 cm, L = 3.68 cm)

Table 4.3. Three experiments performed to investigate the orientation-dependent frequency of water (reference) and tendon. L: length, W: width, H: height, D: diameter.

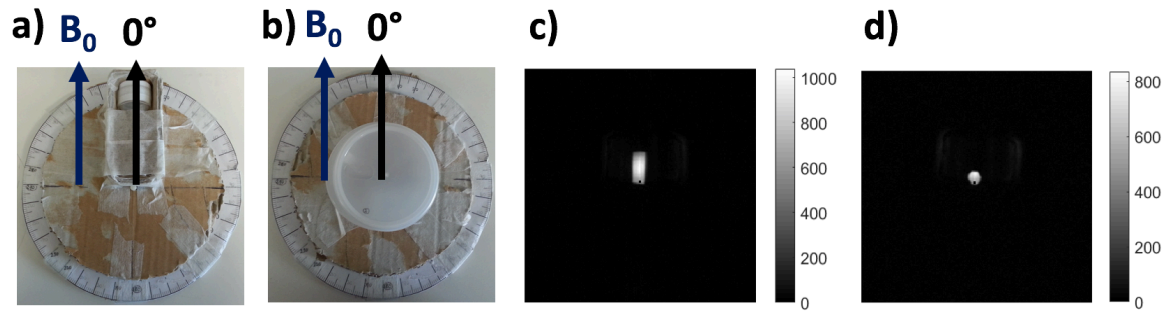


Fig. 4.3. Experimental set-up with: (a) a cylindrical quartz tube, and (b) a cylindrical plastic container, on a rotating plate. For Experiment 2, UTE images in the coronal plane at the shortest TE of 0.23 ms are shown for tendon as a: (c) rectangular prism and (d) cylinder.

Coil	4-channel phased-array coil (Machnet, Roden, The Netherlands)
Image orientation	Coronal
Repetition time (ms)	50
Flip angle (°)	4
Number of averages	4
Slice thickness (mm)	8
Field-of-view (mm x mm)	300 x 300
Matrix size	256 x 256
Bandwidth (Hz/pixel)	980
Number of angles	11
Angles (°)	0, 10, 20, 30, 40, 50, 55, 60, 70, 80, 90
TEs from dual-echo acquisitions (ms)	First TE: Water: 2.69, 3.94, 5.19, 6.44, 7.69, 8.94, 10.19 Tendon: 0.23, 0.29, 0.39, 0.54, 0.69, 0.94, 1.29 Second TE: 40.19 for all acquisitions (not analysed)
Scan time per acquisition (s)	51
Number of acquisitions per angle	7

Table 4.4. UTE scan parameters for measuring the resonant frequency shift of tendon.

For Experiments 2 – 3, bovine deep digital flexor tendon was obtained from a local abattoir within one hour of the sacrifice. The tendon originated from the front left leg of a cow of British Blue breed, aged 1.4 years. The leg was placed on ice until it was transported to the laboratory for dissection. One unrinsed sample (length = 4.20 cm, width = 1.73 cm, height = 0.70 cm) was cut along the length of the tendon and was imaged in air. The methods were designed to reproduce the experimental conditions in the paper by Krasnosselskaia et al. (11). In this paper, the tendon was similarly imaged in air, i.e. without a susceptibility-matching liquid such as Fluorinert.

The water and tendon samples were placed in a container on a rotating plate to position the sample at angles of 0° , 10° , 20° , 30° , 40° , 50° , 55° , 60° , 70° , 80° and 90° relative to B_0 (Fig. 4.3). Imaging was done on the Trio 3 T. At each angle, the sample was imaged using the parameters specified in Table 4.4. TEs were 2.69, 3.94, 5.19, 6.44, 7.69, 8.94 and 10.19 ms for the water sample (Experiment 1), and 0.24, 0.29, 0.39, 0.54, 0.69, 0.94 and 1.29 ms for the tendon (Experiments 2 – 3). The tendon sample was stored at 4°C between experiments.

The averaged signal from a 3-pixel $\times$ 3-pixel ROI at each angle was fitted to Eq. [4.1] to obtain the frequency (f_g), with the fitting procedure as outlined in Section 4.1.2. The ROIs in the tendon were chosen in the same location, regardless of the changes in geometry or the container. The frequency shift was calculated relative to the minimum frequency. The frequency shifts of water in a cylindrical tube (Experiment 1) and rectangular prismic tendon (Experiment 2) were fitted to:

$$\delta_c(\theta) = A(3\cos^2\theta - 1) + B, \quad [4.4]$$

where $\delta_c(\theta)$ is in ppm.

Specifically, the frequency shift of an infinite cylinder (in ppm) is given by:

$$\delta(\theta) = \frac{\Delta\chi}{6}(3\cos^2\theta - 1) + \delta_0. \quad [4.5]$$

Eq. [4.4] was used to describe the frequency shift of water in a cylindrical tube. Relating Eq. [4.5] to [4.4], we have $\Delta\chi = 6A$ and $\delta_0 = B$. A and B were determined by fitting via a trust-region-reflective non-linear least-squares algorithm in MATLAB R2015b, with the initial conditions described in Table A4.3. Using the “corrcoef” function in MATLAB R2015b, the coefficients of determination (R^2) and p -values for the t -statistic assessing the hypothesis of no correlation were calculated. These correlation parameters were assessed for the difference in frequency shift between the two tendon geometries (Experiment 2) and the difference in frequency shift between the two containers (Experiment 3).

4.2.3. Results and Discussion

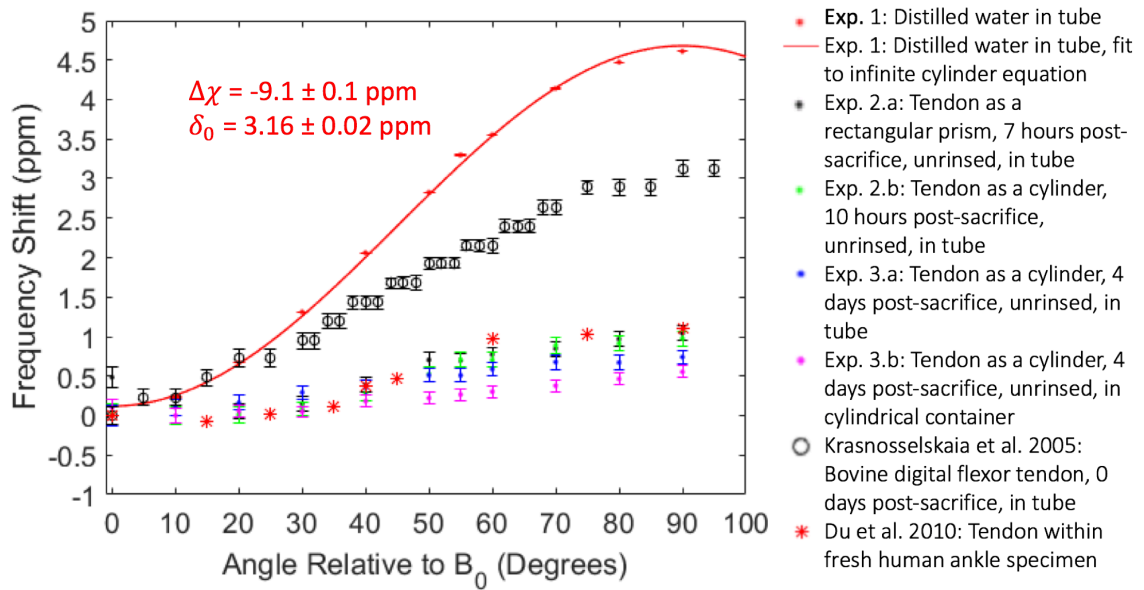


Fig. 4.4. Frequency shift as a function of the angle relative to B_0 for all experiments. Data from Du et al. 2010 and Krasnosselskaia et al. 2005 were obtained from their figures, shifted by $\pi/2$. As a result, the reading error in the frequency shift was ± 0.02 ppm for Du et al. 2010. For Krasnosselskaia et al. 2005, the reading error in the frequency shift was ± 0.04 ppm and ± 0.02 ppm for the uncertainty in the frequency shift. The frequency shift in distilled water was fitted to Eq. [4.5].

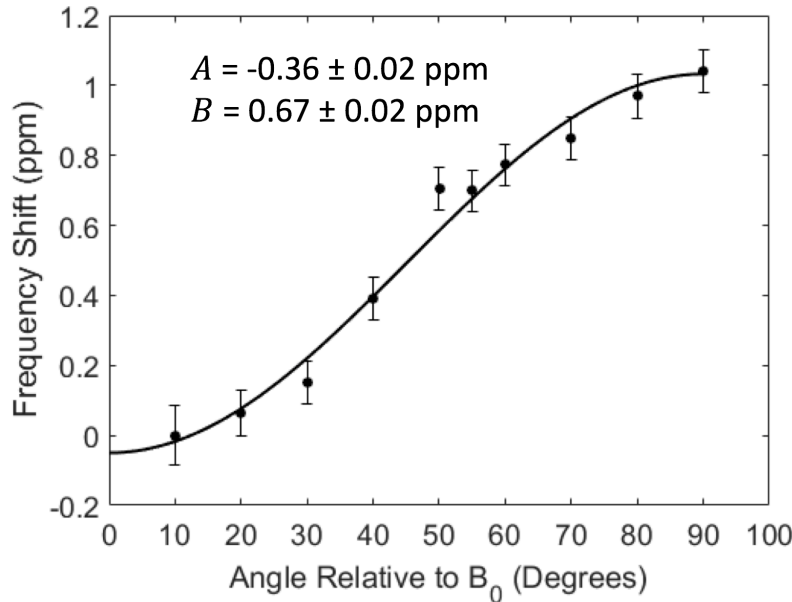


Fig. 4.5. Frequency shift of tendon as a rectangular prism (Experiment 2.a). Data from 10^0 to 90^0 were fitted to Eq. [4.4].

Fig. 4.4 and Fig. 4.5 demonstrate the frequency shift data for all experiments, zeroed at the minimum frequency. Of particular interest was the frequency shift of fresh tendon in its natural geometry (Experiment 2.a). Fitting to Eq. [4.4] yielded $A = -0.36 \pm 0.02$ ppm and $B = 0.67 \pm 0.02$ ppm. The frequency shift at 0^0 was excluded due to possible incorrect positioning of the sample. The results indicated a maximal frequency shift of 1.0 ppm in fresh tendon, which was lower than the maximal frequency shift of 3.1 ppm found by Krasnosselskaia et al. using a similar experimental set-up. The measurements were similar to those of Du et al., who found a maximal frequency change of 1.2 ppm; however, their tendon sample was in a cadaveric ankle, where the frequency shift may have been influenced by surrounding tissue in the specimen.

Differences in frequency shift obtained by myself and Krasnosselskaia et al. could have been due to discrepancies in the experiment set-up. The following lists differences between our experiments:

1. Time from tissue harvesting to imaging: Krasnosselskaia et al. imaged the tendon immediately after harvesting the sample. For Experiment 2, I imaged the rectangular prismic tendon at seven hours after harvesting, and the cylindrical tendon at 10 hours after harvesting. The delay in imaging was due to the transportation of the tendon and sample preparation. Experiment 3 was performed four days after harvesting. Delays in imaging may have caused sample drying and/or protein denaturation, resulting in an altered frequency shift.
2. Temporal sampling: Krasnosselskaia et al. utilised an FID sequence with a hard pulse and a 2.5 ms delay from the middle of the pulse to the beginning of acquisition. Hence, their minimum “TE” was 2.5 ms. By contrast, my TEs were shorter, ranging from 0.23 to 1.29 ms.
3. Angular sampling: Krasnosselskaia et al. acquired angles in increments of $2 - 5^{\circ}$, whereas my increments were of $5 - 10^{\circ}$. Hence, Krasnosselskaia et al. had 32 samples from 0° to 95° , when I had 11 samples.
4. Number of tendon samples: Krasnosselskaia et al. displayed the frequency shifts as the average of two tendon samples. I used one tendon sample.
5. Magnetic field strength: Krasnosselskaia et al. performed their experiment at 2 T in a small-animal scanner, whereas my experiment was performed at 3 T

in a clinical scanner. As the resonant frequency shift was converted to ppm, the magnetic field strength should not have affected the resonant frequency shift substantially.

The most notable difference was that of possible sample drying and/or protein denaturation associated with a delayed imaging time. Particularly, the amount of bound water associated with collagen may have decreased, causing the comparatively lower resonant frequency shift in my experiment.

As a reference, Experiment 1 was performed to determine the resonant frequency shift of water. After fitting to Eq. [4.5], the difference in magnetic susceptibility between air and water ($\Delta\chi$) was determined to be -9.1 ± 0.1 ppm. Assuming the magnetic susceptibility of air to be 0.36 ppm, the magnetic susceptibility of water derived from the experiment was -8.8 ± 0.1 ppm, which was similar to the actual value of -9.05 ppm (13). This suggested that the frequency shift of water was dependent on the geometry of the cylindrical tube containing the water.

Experiments 2 and 3 demonstrated that the frequency shift of tendon was influenced by the container, but not the geometry of the tendon. Experiment 2 indicated that the geometry of the tendon had no discernible effect on the tendon frequency shift ($R^2 = 0.23$, $p = 0.14$). Experiment 3 demonstrated a small but significant effect of the container on the tendon frequency shift ($R^2 = 0.47$, $p = 0.02$). Contributing factors may have included the container geometry and material. Krasnosselskaia et al. hypothesised that the resonant frequency shift arises from susceptibility effects due to water cavities within the collagen molecules (11). However, susceptibility effects at the air-tissue interface need to be considered, as their tendon sample as well as mine were imaged in

air, as opposed to Fluorinert. The contributions of the susceptibility effects due to water cavities and the air-tissue interface to the resonant frequency of tendon need to be further explored.

4.2.4. Conclusion

The orientation-dependent frequency of fresh tendon varied by 1.0 ppm. The frequency in ppm was modelled by $\delta_c(\theta) = A(3\cos^2\theta - 1) + B$, with $A = -0.36 \pm 0.02$ ppm and $B = 0.67 \pm 0.02$ ppm.

4.3. R_2^* Signal of Collagen Phantoms

4.3.1. Introduction

Thus far, the signal properties of oriented collagen in tendon have been measured. However, in fibrosis, collagen has a relatively isotropic distribution, which may affect its R_2^* signal properties. The objective of the following experiment was to model the R_2^* signal in isotropic collagen phantoms at varying concentrations. The exchange between collagen-bound water and free water was characterised in the phantoms.

4.3.2. Theory

The phantoms consisted of bundles of type I collagen that are isotropically oriented. A type I collagen bundle is 2 – 10 μm in diameter (14). Consider a water molecule diffusing in collagen. For three-dimensional diffusion, the mean path length of the water molecule (L) is given by:

$$L = \sqrt{6Dt}, \quad [4.6]$$

where D is the diffusion coefficient for water, which ranges from $1 \times 10^{-11} \text{ m}^2/\text{s}$ to $1 \times 10^{-9} \text{ m}^2/\text{s}$ (15–17). t is the diffusion time, which is the T_2^* value. From Section 4.1, $R_{2c}^*(0^\circ)$

$= 950 \pm 30 \text{ s}^{-1}$ and $R_{2c}^*(55^\circ) = 130 \pm 20 \text{ s}^{-1}$ in tendon. Hence, at 0° , the mean path length ranges from $0.251 \pm 0.004 \text{ }\mu\text{m}$ (at $D = 1 \times 10^{-11} \text{ m}^2/\text{s}$) to $2.51 \pm 0.04 \text{ }\mu\text{m}$ (at $D = 1 \times 10^{-9} \text{ m}^2/\text{s}$). As the mean path length is either smaller than or on the same order of magnitude as the $2 - 10 \text{ }\mu\text{m}$ diameter of the collagen bundle, it is predicted that water remains in collagen bundles at 0° and does not mix with bundles at other angles. By contrast, at 55° , the mean path length ranges from $0.68 \pm 0.04 \text{ }\mu\text{m}$ to $6.8 \pm 0.4 \text{ }\mu\text{m}$. Depending on the diameter of the collagen bundle, water can visit collagen bundles at different angles. Hence, water associated with isotropic collagen is hypothesised to be in a regime of intermediate to fast exchange. Fast exchange can be modelled via the following mono-exponential equation:

$$|S(TE)| = |S_0 \exp(-TE \cdot R_2^*)|, \quad [4.7]$$

where S_0 and R_2^* relate to the collagen phantoms. For n collagen bundles, where each bundle i is oriented at an angle, R_2^* is the weighted average of the $R_{2c,i}^*$ s of the collagen-bound (R_{2c}^*) and free water pools (R_{2f}^*):

$$R_2^* = \sum_{i=1}^n P_{c,i} R_{2c,i}^* + P_f R_{2f}^*, \quad [4.8]$$

given population fractions P_c and P_f .

The mono-exponential signal model was explored in the collagen phantoms.

4.3.3. Methods

Seven collagen phantoms were prepared by immersing 1 g of type I collagen from bovine Achilles tendon (Sigma-Aldrich, Poole, United Kingdom) in 25 g of distilled water

(Barnstead Nanopure, Thermo Fisher Scientific, Cramlington, United Kingdom) for two days at room temperature. Each phantom was blended in a homogeniser (T25 Digital, Ultra-Turrax, IKA Laboratories, Oxford, United Kingdom) and subsequently incubated at 37 °C to evaporate the water and achieve the desired collagen concentration. The final collagen concentrations were 0 (for distilled water), 5, 10, 12, 19, 31, 39 and 53 % g/g. All phantoms were scanned together using the UTE parameters specified in Table 4.5. on the Trio 3 T.

Coil	4-channel phased-array coil (Machnet, Roden, The Netherlands)
Image orientation	Coronal
Repetition time (ms)	50
Flip angle (°)	20
Number of averages	2
Slice thickness (mm)	8
Field-of-view (mm x mm)	300 x 300
Matrix size	256 x 256
Bandwidth (Hz/pixel)	980
TEs from dual-echo measurements (ms)	First TE: 0.49, 0.59, 0.69, 0.79, 0.89, 0.99, 1.09, 1.19, 1.29, 1.39, 1.49, 1.59, 1.69, 1.79, 1.89, 1.99, 2.09, 2.19, 2.44, 2.69, 2.94, 3.19, 3.69, 4.19, 4.69, 5.19, 5.69, 6.19, 6.69, 7.19, 7.69, 8.19, 8.69, 9.19, 9.69 Second TE: 40.19 for all measurements (not analysed)
Scan time per measurement (s)	26
Number of measurements per acquisition	2 (pause for 2 s after first measurement) First measurement discarded
Scan time per acquisition (s)	53
Number of acquisitions	35

Table 4.5. UTE scan parameters for the collagen phantoms.

In each phantom, the averaged magnitude signal within a 5-pixel $\times$ 5-pixel ROI as a function of TE was fitted to Eq. [4.7]. Magnitude data was fitted, in order to use a simplified model without global frequency and phase terms. As employed previously,

fitting was performed using a trust-region-reflective nonlinear least-squares algorithm in MATLAB R2015b. Initial conditions were: S_{0c} = the maximal signal for all TEs acquired and $R_{2c}^* = 100 \text{ s}^{-1}$. A linear fit was performed to determine the relationship between R_2^* and the collagen fractional density. The slope, intercept and coefficient of determination (R^2) were evaluated from a linear regression model; p -values were obtained from the t -test (MATLAB R2015b).

4.3.4. Results

Fig. 4.6 illustrates the R_2^* signal decays of all phantoms. The linear fit between the collagen R_2^* and collagen fractional density revealed a slope of $9 \pm 2 \text{ g}/(\% \text{g} \cdot \text{s})$ ($p = 0.007$) and intercept of $27 \pm 58 \text{ s}^{-1}$ ($p = 0.66$), with $R^2 = 0.73$.

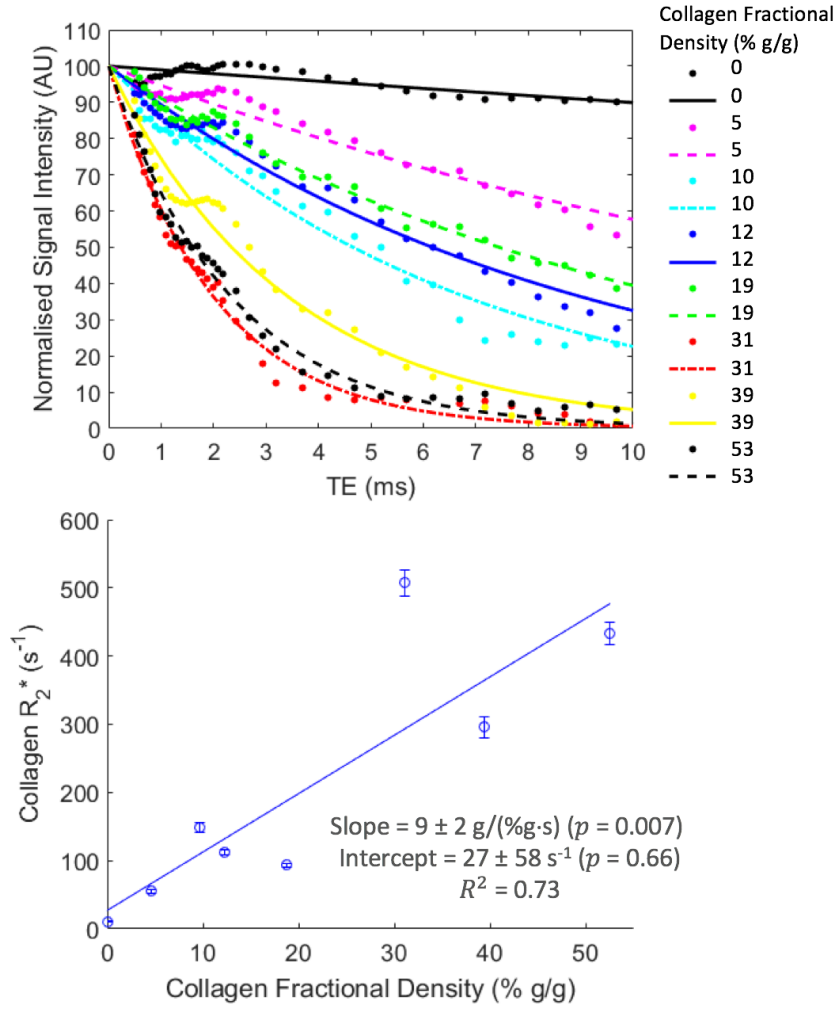


Fig. 4.6. Results for the collagen phantoms. (a) Normalised R_2^* signal decays for all phantoms. (b) Relationship between the collagen R_2^* and collagen fractional density.

4.3.5. Discussion

The R_2^* signal decays of the isotropic collagen phantoms were well described by a mono-exponential model. Deviations from the mono-exponential function, such as the decrease in signal at TE = 1.39 ms and increase in signal at TE = 2.19 ms, could have been caused by the signal not being in a steady state. Notably, similar signal deviations were observed in the water phantom (0 % g/g collagen), when the R_2^* signal decay of water is expected to be purely exponential at steady state. Due to the high flip angle of 20°, two

measurements were taken at each TE, separated by 2 s. The second measurement was reported, as it was less influenced by non-steady-state effects.

The R_2^* signal in collagen phantoms was similar to that observed by other researchers. Horch et al. modelled a 20 % g/g collagen phantom with a multi-exponential function, yielding a lowest R_2^* of $\sim 200 \text{ s}^{-1}$; two other components had R_2^* s on the order of $100,000 \text{ s}^{-1}$ (18). Comparatively, my 19 % g/g collagen phantom had an R_2^* of $87 \pm 3 \text{ s}^{-1}$; the lower R_2^* in my experiment may have been contributed by the homogenisation of the collagen, which reduced the number of water pores and consequent signal dephasing. As my minimum TE was 0.49 ms, R_2^* s on the order of $100,000 \text{ s}^{-1}$ could not have been detected. Given my range of TEs, a mono-exponential R_2^* model was reasonable.

There was a linear relationship between the collagen R_2^* and collagen fractional density. Water fractional density decreases with increasing collagen fractional density. According to Eq. [4.8], the R_2^* is weighted by the fractions of collagen-bound and free water. Hence, a decreased water fractional density would result in a higher R_2^* .

A mono-exponential R_2^* model suggests fast exchange between collagen-bound and free water. Note that in the mean path length calculations in Section 4.3.2, the environment was assumed to be *in vivo* unhomogenised collagen, whereas the type I collagen from Sigma-Aldrich used in this experiment may have different structural properties when homogenised in water. It is postulated that homogenisation would encourage the mixing of water associated with collagen bundles at different angles, as the pores between collagen bundles would be minimised. It is debatable whether homogenised isotropic collagen phantoms approximate human tissue, where collagen is not homogenised. Chapter 6 will explore the modelling of liver signal *in vivo*.

4.3.6. Conclusion

The signal from isotropic collagen phantoms was modelled with a mono-exponential R_2^* function, exhibiting fast exchange of water associated with collagen and the free pool.

4.4. Summary

This chapter produced the following results:

1. The R_2^* of tendon was characterised by $R_2^*(0^\circ) = 950 \pm 30 \text{ s}^{-1}$ and $R_2^*(55^\circ) = 130 \pm 20 \text{ s}^{-1}$.
2. The frequency of fresh tendon varied by 1.0 ppm. The frequency was described by $\delta_c(\theta) = A(3\cos^2\theta - 1) + B$, with $A = -0.36 \pm 0.02 \text{ ppm}$ and $B = 0.67 \pm 0.02 \text{ ppm}$.
3. The R_2^* signal was modelled in isotropic collagen phantoms with a mono-exponential function. This suggests a regime of fast exchange between collagen-bound water and free water.

This data was used to understand the signal properties of oriented and isotropic collagen, before proceeding with *in vivo* imaging of the liver. The next chapter will discuss optimisations performed on the UTE sequence for *in vivo* imaging.

4.5. References

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4.6. Appendix

	S_0 (AU)	$T_2^* (= 1/R_2^*)$ (ms)	f_g (Hz)	φ_g (rad)
Initial value	Maximal signal	10	-100	0
Lower bound	0	0	-1000	-2π
Upper bound	Maximal signal $\times 10$	100	1000	2π

Table A4.1. Initial conditions for non-linear least-squares fitting for the tendon R_2^* and frequency experiments. The maximal signal is the highest signal intensity of all data points acquired for a given T_2^* signal decay.

	$R_2^*(55^\circ)$ (s ⁻¹)	$R_2^*(0^\circ)$ (s ⁻¹)
Initial value	200	1100
Lower bound	0	0
Upper bound	2000	2000

Table A4.2. Initial conditions for non-linear least-squares fitting for the tendon R_2^* experiment, with parameters from Eq. [4.3].

	A (ppm)	B (ppm)
Initial value	-10	5
Lower bound	-20	-20
Upper bound	20	20

Table A4.3. Initial conditions for non-linear least-squares fitting for the tendon resonant frequency experiment, with parameters from Eq. [4.4].

5. UTE Sequence Optimisation for *In Vivo* Imaging

This chapter describes how the UTE sequence for *ex vivo* imaging was optimised for *in vivo* imaging on the Siemens Trio and Prisma 3-T scanners. On the Trio 3 T, the feasibility of the following was investigated:

1. The effect of removing gradient spoiling after the echo to minimise the acquisition length
2. The use of a double-echo sequence, as opposed to a single-echo sequence, to shorten the number of acquisitions
3. The effectiveness of fat saturation and spatial saturation in suppressing lipid and out-of-slice signal

On the Prisma 3 T, experiments examined:

1. The effect of adjusting the bandwidth to mimic gradient strengths on the Trio 3 T
2. The use of different coil set-ups for providing the lowest TE with non-artefactual signal

In vivo protocols on the Prisma and Trio were devised and validated in the healthy liver. For each optimisation, the change made in the sequence and how it affected the measurement of parameters experimentally are outlined.

5.1. UTE Optimisation on the Trio 3 T

5.1.1. Original UTE Sequence for *Ex Vivo* Experiments

Fig. 5.1 and Table 5.1 demonstrate the original UTE sequence and parameters on the Trio 3 T, as described in Chapter 2. This was the sequence used for the *ex vivo* experiments in Chapter 4. In brief, it utilised a dual-echo UTE acquisition with a half-sinc RF pulse.

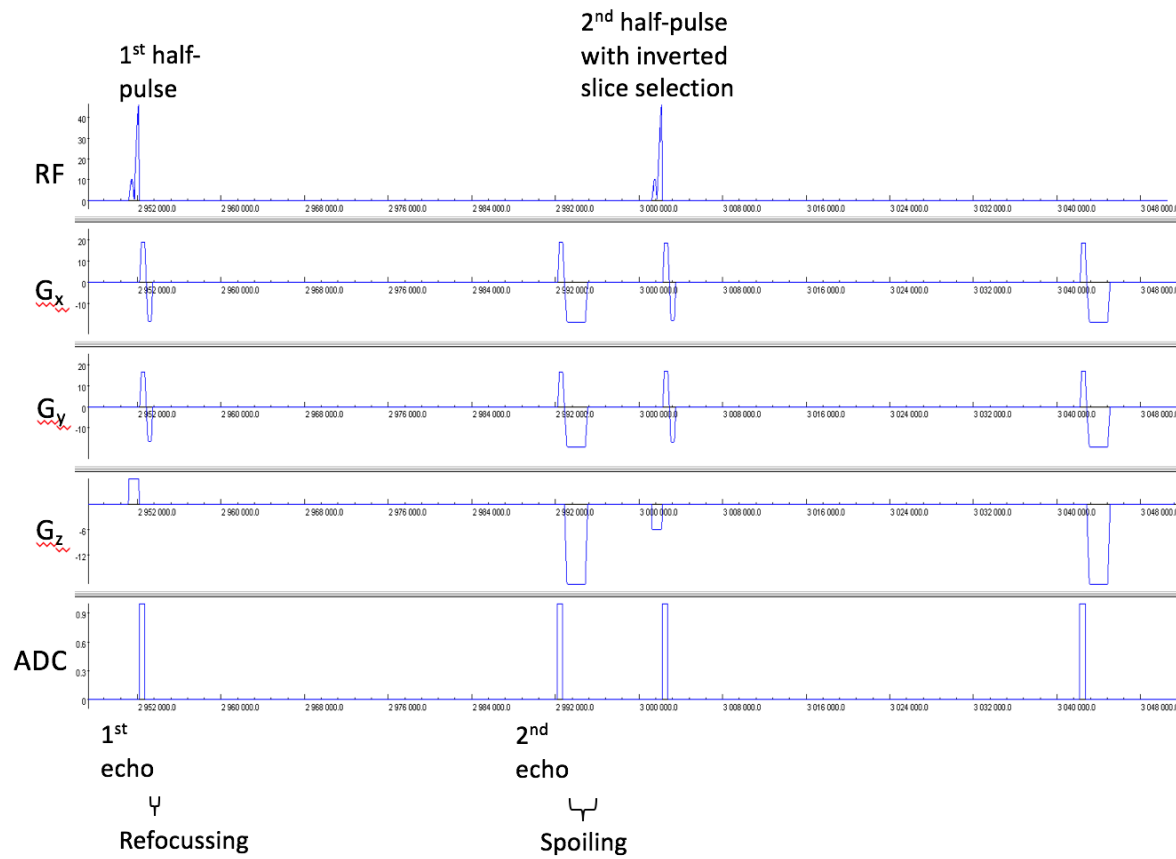


Fig. 5.1. Original dual-echo UTE pulse sequence. A TR of 50 ms is shown, with a first interleaved echo of 0.23 ms and second interleaved echo of 40.19 ms. Note that the G_x and G_y gradients change in amplitude and sign throughout the sequence to achieve radial k -space sampling. Gradient spoiling is performed at the end of the second interleaved echo. Two half-pulse acquisitions with inverted slice-selection gradients are combined to obtain the correct slice profile.

Parameter	Value	Optimisation step
TR (ms)	50	1
Flip angle (°)	4 - 20	1
Number of averages	2 - 4	2
Slice thickness (mm)	8	--
Field-of-view (mm x mm)	300 x 300	--
Matrix size	256 x 256	--
Bandwidth (Hz/pixel)	980	--
TEs from dual-echo acquisitions (ms)	First interleaf TE: 0.23 ms (minimum) Second interleaf TE: 40.19 ms	1
Fat saturation or spatial saturation	--	3

Table 5.1. Original UTE sequence parameters. Optimisation steps 1 - 3 and associated parameters are further explained below.

The *in vivo* UTE protocol was originally optimised for cardiac imaging, as an investigation of myocardial fibrosis. Subsequently, the protocol was applied to the liver in the study of liver fibrosis. The following optimisation steps were performed:

1. The length of each acquisition had to be minimised to allow a breath-hold duration of 10 - 15 seconds. Namely, the TR had to be reduced, while accounting for the TEs needed.
2. The number of acquisitions had to be optimised to minimise the examination time. To this effect, the number of averages was kept at 2, which was previously used in the collagen phantom experiments. Moreover, the double-echo UTE acquisition favoured the reduction in examination time, but the effect of the double-echo

acquisition on the signal in comparison with a single-echo acquisition had to be investigated.

3. Signal contamination from outside the slice, including lipid signal, had to be reduced. The contamination arose from imperfections in the RF slice profile. Fat saturation and spatial saturation were investigated as means of decreasing signal contamination.

The following sections will describe the pulse sequence modifications and tests for each optimisation step.

5.1.2. Reducing the Acquisition Length

5.1.2.1. Introduction

The TR was reduced to minimise the acquisition length. At a TR of 7 ms, the breath-hold duration was ~ 10 seconds, which was feasible for patients. However, this limited the second interleaf TE to a maximum of 3.29 ms, which did not allow for adequate sampling of the T_2^* signal decay. The limitation arose from gradient spoiling proceeding the second interleaf echo, with a duration of 2.23 ms, as shown in Fig. 5.1. Theoretically, spoiling may not be needed, as the radial sampling in UTE effectively drives residual magnetisation away from the centre of k -space. The removal of spoiling after the second interleaf echo would increase the maximum TE to 5.52 ms, improving the sampling of the T_2^* signal decay.

To assess the feasibility of removing the spoiling after the echo, an oil and gadoteridol phantom and a healthy subject were imaged with the UTE sequence. In the phantom, the real, imaginary and magnitude parts of the averaged signal within an ROI

were compared with and without spoiling. In the healthy subject, the T_2^* signal decay was measured in the septum, with the unspoiled sequence.

5.1.2.2. Methods

A cylindrical phantom consisting of peanut oil and 0.96 mM gadoteridol (with distilled water as the solvent) was imaged using the UTE sequence, with and without gradient spoiling following the echo. The healthy subject was imaged with the UTE sequence without spoiling as validation. Fig. 5.2 and Table 5.2 summarise the UTE sequence and parameters.

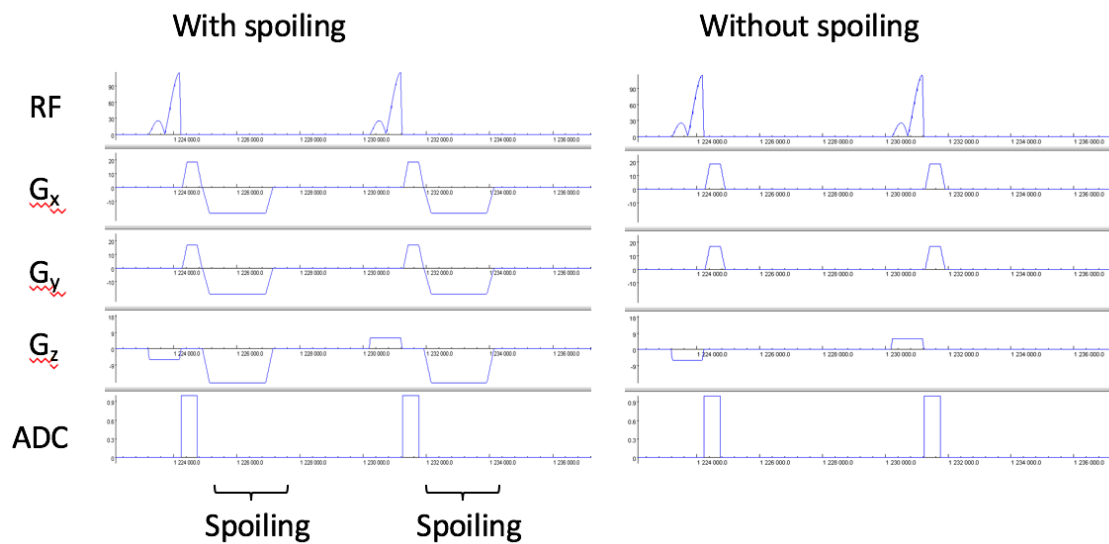


Fig. 5.2. Single-echo UTE sequence with and without gradient spoiling after the echo.

UTE scan parameters	Phantom of oil and 0.96 mM gadoteridol	Healthy subject
Gradient spoiling	With and without	Without
Single- or dual-echo acquisition	Single	Single
Coil	32-channel torso array coil (Invivo, Florida, USA)	32-channel torso array coil (Invivo, Florida, USA)
Image orientation	Coronal	Oblique (cardiac short-axis view)
Repetition time (ms)	7	7
Flip angle (°)	10	10
Number of averages	2	2
Slice thickness (mm)	8	8
Field-of-view (mm x mm)	300 x 300	300 x 300
Matrix size	256 x 256	256 x 256
Bandwidth (Hz/pixel)	980	980
Segmentation	40 readouts/heartbeat	40 readouts/heartbeat
Acquisition window (ms)	870	870
Trigger delay (ms)	550	550
TEs (ms)	0.23, 0.35, 0.55, 0.85, 1.45, 2.05, 2.54, 3.04	0.23, 0.35, 0.55, 0.85, 1.45, 2.05, 2.54, 3.04
Scan time per acquisition (s)	~ 15 (breath-hold duration)	~ 15 (breath-hold duration)

Table 5.2. UTE parameters for the phantom and healthy-subject experiments.

A single-echo UTE acquisition was chosen for the experiment, as a starting point. As flip angles of 4 - 20° were used in *ex vivo* and phantom studies (in Chapter 4), a flip angle of 10° was selected to achieve sufficient short- T_2^* signal. The Ernst angle (θ_E) is the flip angle that allows for the maximal signal intensity for a given TR and T_1 . The relationship is given by: $\theta_E = \arccos(\exp(-\frac{TR}{T_1}))$. Assuming a T_1 of 610 ms associated with the short- T_2^* signal (1), the Ernst angle would be 9° at TR = 7 ms. Hence, compared to the lowest flip angle used of 4°, a flip angle of 10° would result in a higher signal intensity due to T_1 weighting. The acquisition window and trigger delay were selected for imaging during diastole.

A 5-pixel $\times$ 5-pixel ROI was drawn in the gadoteridol phantom for analysis. For the healthy subject, cardiac images were first cropped and rigid-body image registration was performed via the “imregister” function (MATLAB R2015b). An ROI was contoured in the septum for analysis.

Fig. 5.3 illustrates the analysis for the phantom experiment. The signal within the ROI was averaged, and compared with and without spoiling. Correlation and Bland-Altman plots were generated in Microsoft Excel 2013. Using the “fitlm” regression model (MATLAB R2015b), the coefficient of determination (R^2) was calculated; the uncertainties in the slope and intercept of the correlation plot were estimated and p -values were calculated from the t -test. The coefficient of reproducibility (CR) was calculated as $1.96 \times$ standard deviation of the difference in the signal between the two experiments.

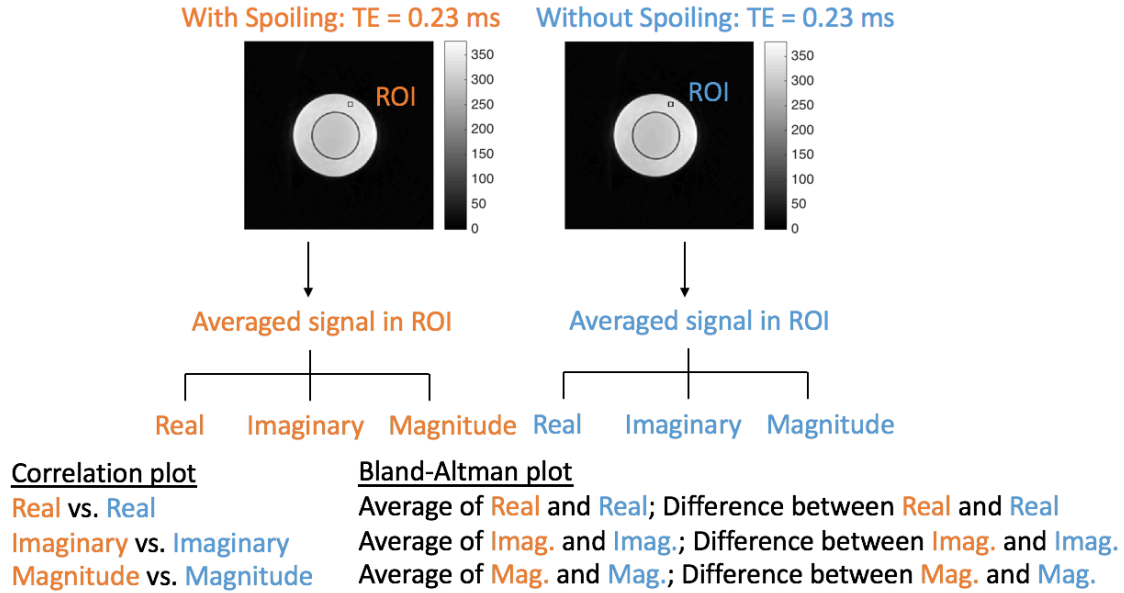


Fig. 5.3. Analysis for the phantom experiment. For each TE, the images acquired with and without spoiling were compared. A 5 x 5 ROI was drawn in the gadoteridol phantom. The signal within the ROI was averaged and divided into real, imaginary and magnitude components. This was devised, as magnitude signal would subsequently be analysed in the *in vivo* experiments of Chapter 6. Correlation and Bland-Altman plots were generated to compare the signal components at each TE.

The magnitude of the averaged signal within the septum ROI was modelled via a function accounting for isotropic collagen (short- T_2^* signal), free water and lipids. Fitting was performed via a trust-region-reflective non-linear least-squares algorithm (“lsqcurvefit” function in MATLAB R2015b). The model is summarised in the Appendix. Section 6.2.2 will provide the derivation for the model for isotropic collagen and free water. For the current section, it was investigated whether the short- T_2^* signal acquired from the UTE sequence without spoiling can be used for modelling, rather than the actual modelling and quantification of the signal.

5.1.2.3. Results and Discussion

Fig. 5.4 shows example magnitude images of the phantom and heart with and without spoiling.

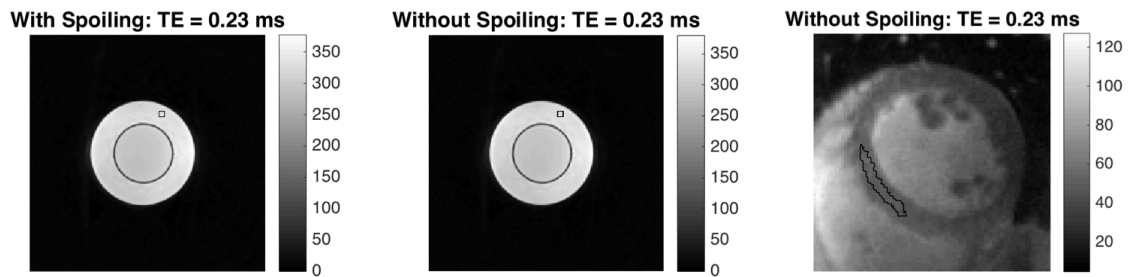


Fig. 5.4. UTE images of the phantom and heart of a healthy subject at TE = 0.23 ms, with ROIs outlined. For the phantom, the inner circle is peanut oil and the outer circle is 0.96 mM gadoteridol. The 5 x 5 ROI drawn in the gadoteridol phantom is denoted by the square.

The signal was averaged over an ROI within the 0.96 mM gadoteridol phantom. Fig. 5.5 and Fig. 5.6 show the correlation and Bland-Altman plots for the real, imaginary and magnitude averaged signal over an ROI within the 0.96 mM gadoteridol phantom. For the real signal, the slope of the correlation plot was 0.98 ± 0.09 , with $R^2 = 0.947$. The CR was 29.3. For the imaginary signal, the slope was 1.03 ± 0.02 , with $R^2 = 0.997$ and the

CR was 26.1. Finally, the magnitude signal had a slope of 0.97 ± 0.03 , with $R^2 = 0.995$ and a CR of 1.2. In the septum, the T_2^* signal did not present significant artefacts that hindered modelling.

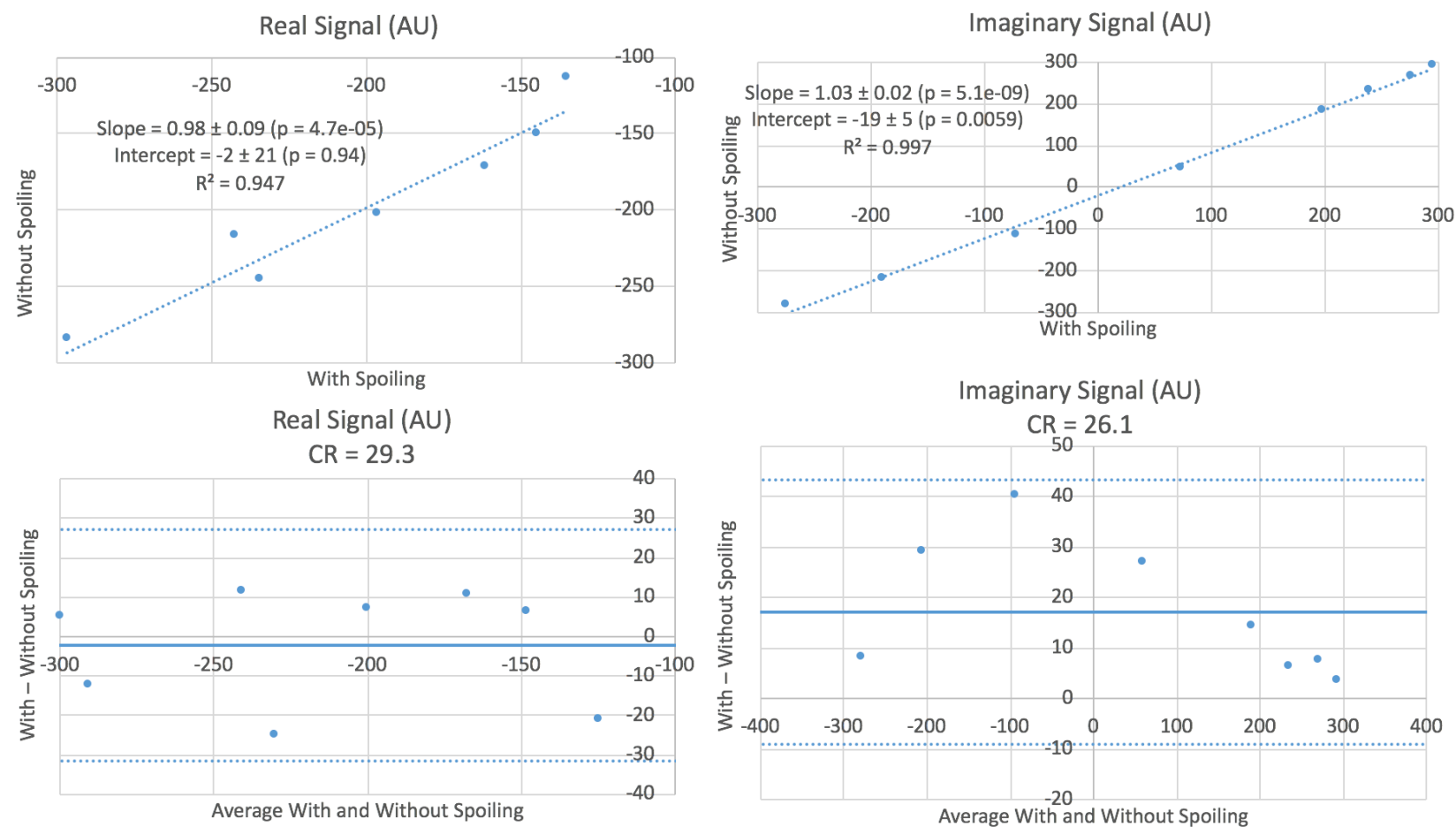


Fig. 5.5. Correlation and Bland-Altman plots for the real and imaginary parts of the averaged signal in an ROI within the 0.96 mM gadoteridol phantom.

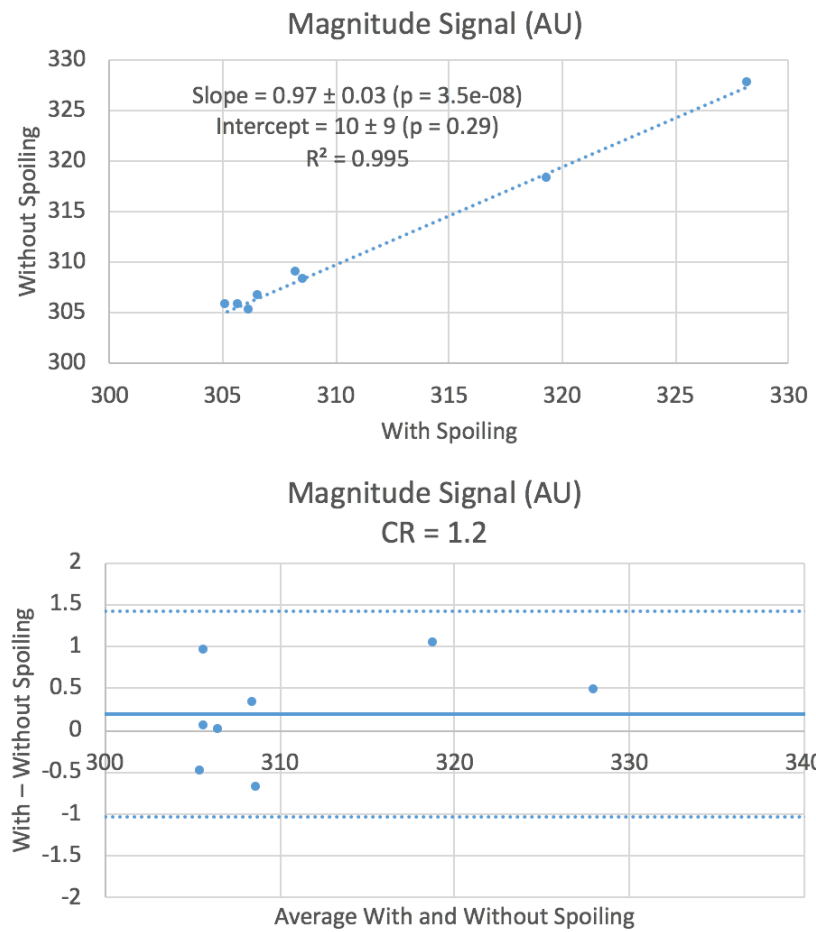


Fig. 5.6. Correlation and Bland-Altman plots for the magnitude part of the averaged signal within an ROI in the 0.96 mM gadoteridol phantom.

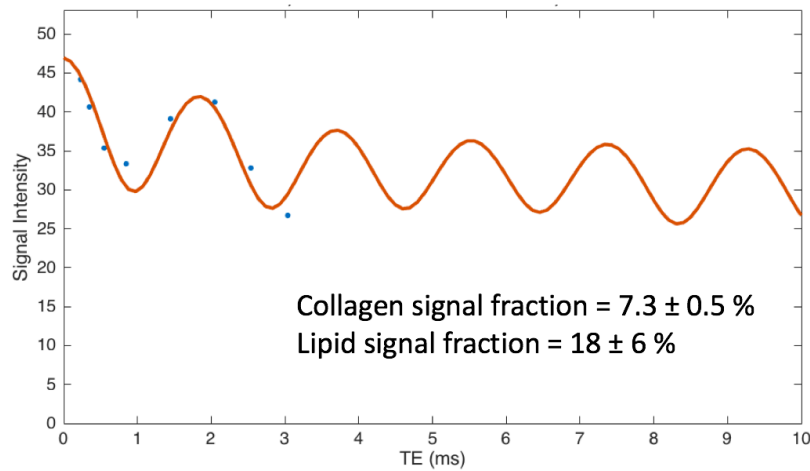


Fig. 5.7. T_2^* signal decay in an ROI in the septum of a healthy volunteer, modelled with a function accounting for isotropic collagen, free water and lipids (summarised in the Appendix).

For each signal component, there was a high correlation between the spoiled and unspoiled sequences, as the slope and R^2 of the linear regression were very close to 1. Furthermore, the unspoiled sequence did not generate a systematic bias in the signal. Data in the septum, acquired without gradient spoiling, did not produce image artefacts that affected T_2^* signal modelling. Hence, the UTE acquisition without gradient spoiling seemed usable for *in vivo* T_2^* signal analysis.

5.1.2.4. Conclusion

Given the highly correlated signal between the spoiled and unspoiled sequences, the unspoiled sequence was used for imaging the septum of a healthy subject. This allowed an increase in the maximum TE to 5.52 ms for future experiments.

5.1.3. Reducing the Number of Acquisitions

5.1.3.1. Introduction

For T_2^* signal analysis, ideally one can acquire five TEs < 1 ms for characterising the short- T_2^* signal, and nine TEs for characterising two lipid oscillations (4 – 5 TEs per

oscillation), amounting to 14 TEs. If a single-echo UTE acquisition is used, 14 breath holds are needed, which is tiring for the subject and can cause signal artefacts from breathing inconsistencies. The number of acquisitions can be halved by using a double-echo acquisition, instead of a single-echo acquisition. To determine if the double-echo acquisition is feasible, the oil and gadoteridol phantom from Section 5.1.2 was imaged; the real, imaginary and magnitude of the averaged signal in an ROI was compared between the single- and double-echo acquisitions. The T_2^* signal decay was measured in the septum of a healthy subject, with the double-echo acquisition.

5.1.3.2. Methods

As in the experiment for gradient spoiling, the same oil and gadoteridol phantom was imaged with the single- and double-echo acquisitions. The single-echo UTE acquisition without spoiling from Fig. 5.2 was used. Fig. 5.8 illustrates the double-echo acquisition. The UTE parameters for both acquisitions are reported in Table 5.3. In the double-echo acquisition for the phantom experiment, the first TE was varied between 0.35 and 4.03 ms, whereas the second TE was fixed at 5.52 ms, as signal associated with TEs < 1 ms were of interest in modelling short- T_2^* signal. For the healthy subject, five TEs from 0.23 to 0.85 ms were chosen to sample the short- T_2^* signal. TEs were selected from 1.45 to 5.45 ms for sampling two lipid CH₂ oscillations, with a gap of 0.4 to 0.6 ms between each TE to allow for variations in the CH₂ frequency shift.

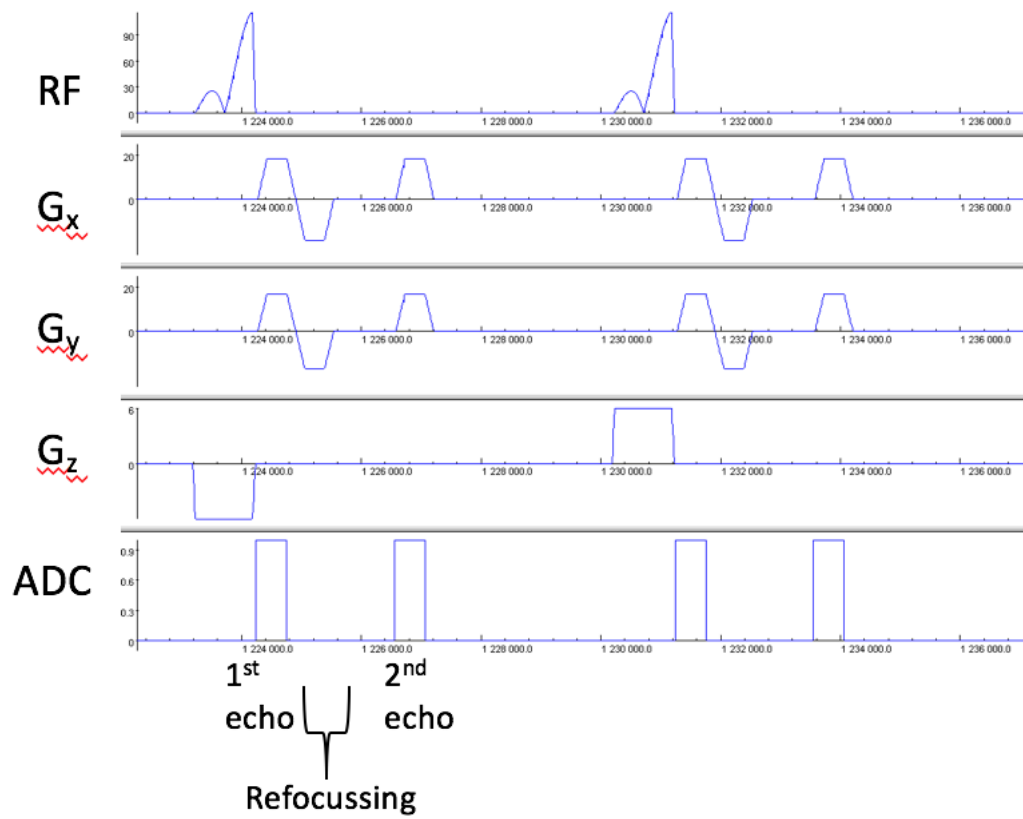


Fig. 5.8. Double-echo UTE acquisition, without the spoiling at the end of the second interleaf echo. Note that the gradient between the first and second interleaf echoes is for refocussing.

UTE scan parameters	Phantom of oil and 0.96 mM gadoteridol	Healthy subject
Single- or dual-echo acquisition	Single and dual	Dual
Gradient spoiling following the second interleaf echo	Single echo: not applicable Dual echo: No	No
Coil	32-channel torso array coil (Invivo, Florida, USA)	32-channel torso array coil (Invivo, Florida, USA)
Image orientation	Coronal	Oblique (cardiac short-axis view)
Repetition time (ms)	7	7
Flip angle (°)	10	10
Number of averages	2	2
Slice thickness (mm)	8	8
Field-of-view (mm x mm)	300 x 300	300 x 300
Matrix size	256 x 256	256 x 256
Bandwidth (Hz/pixel)	980	980
Segmentation	40 readouts/heartbeat	40 readouts/heartbeat
Acquisition window (ms)	870	870
Trigger delay (ms)	550	550
TEs (ms)	<u>Single-echo TEs:</u> 0.35, 0.44, 0.55, 0.85, 1.45, 2.05, 2.54, 3.04, 3.64, 4.03 <u>Dual-echo TEs:</u> First interleaf TE: 0.35, 0.44, 0.55, 0.85, 1.45, 2.05, 2.54, 3.04, 3.64, 4.03 Second interleaf TE: 5.52 (fixed for all acquisitions)	First interleaf TE: 0.23, 0.35, 0.44, 0.55, 0.85, 1.45, 2.05 Second interleaf TE: 2.54, 3.04, 3.64, 4.03, 4.55, 5.14, 5.45
Scan time per acquisition (s)	~ 15 (breath-hold duration)	~ 15 (breath-hold duration)

Table 5.3. UTE parameters for the phantom and healthy subject, using single-echo and double-echo acquisitions.

Analysis for the phantom and heart were as in Section 5.1.2.2. Briefly, a 5-pixel $\times$ 5-pixel ROI was drawn in the gadoteridol phantom. The ROI signal at a given TE in the single-echo acquisition was compared against the ROI signal at the same TE in the first interleaf of the double-echo acquisition. Signal from the two types of acquisitions were evaluated for correlations, considering the real, imaginary and magnitude components independently. Correlation and Bland-Altman plots were produced. Cardiac images were cropped and registered, and an ROI was contoured in the septum. The averaged signal in the ROI was modelled with an R_2^* function accounting for isotropic collagen, free water and lipids (mentioned in the Appendix). Artefacts in the short- T_2^* signal in the double-echo acquisition were investigated.

5.1.3.3. Results and Discussion

Example magnitude images of the phantom, with the single- and double-echo acquisitions, and heart are illustrated in Fig. 5.9.

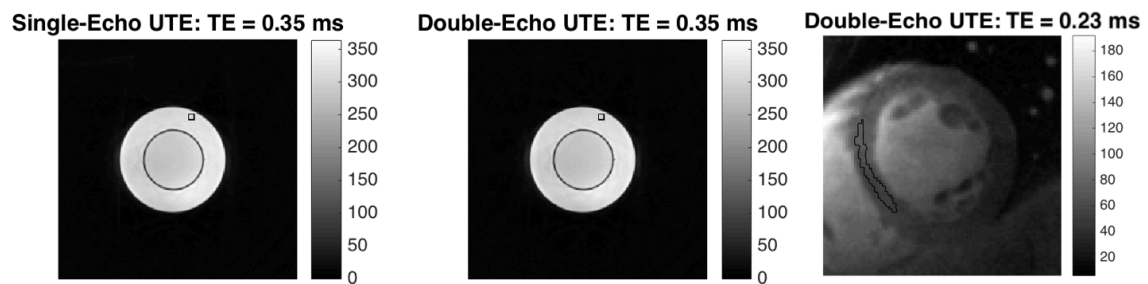


Fig. 5.9. UTE images of the phantom and heart of a healthy subject at TE = 0.35 ms and 0.23 ms, with ROIs drawn.

Fig. 5.11 and Fig. 5.10 demonstrate the correlation and Bland-Altman plots for the real, imaginary and magnitude parts of the averaged ROI signal. The slope of the correlation plot for the real signal was 1.00 ± 0.01 , with an R^2 of 0.9988 and the CR was 13.8. The slope for the imaginary signal was 0.996 ± 0.009 , with an R^2 of 0.9994 and the CR was

10.0. For the magnitude signal, the slope of the correlation plot was 1.08 ± 0.05 , R^2 was 0.9852 and the CR was 3.0. Fig. 5.12 is the T_2^* signal decay in the septum, without signal artefacts affecting the signal modelling.

As in the experiment for the spoiled and unspoiled sequences, there was a high correlation between signal from the single- and double-echo acquisitions. When each signal component was considered separately, the slope and R^2 of the linear regression were very close to 1. Deviations in signal were caused by noise, but no systematic bias was present as a result of using the double-echo acquisition. The T_2^* signal in the septum was acceptable for analysis.

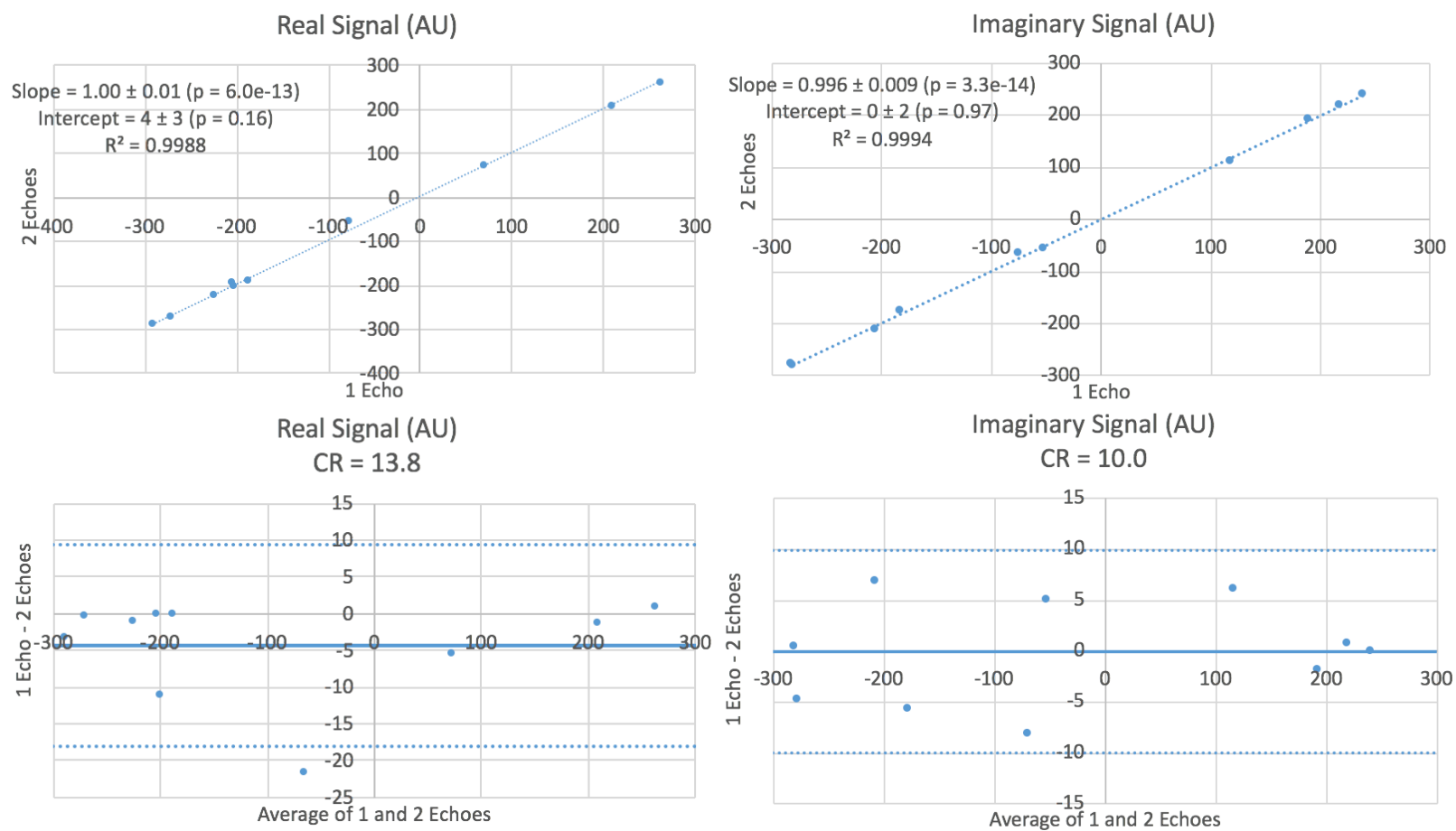


Table 5.4.

Fig. 5.10. Correlation and Bland-Altman plots for the real and imaginary parts of the averaged signal within an ROI in the 0.96 mM gadoteridol phantom. The UTE sequence with the single- and double-echo acquisitions was used.

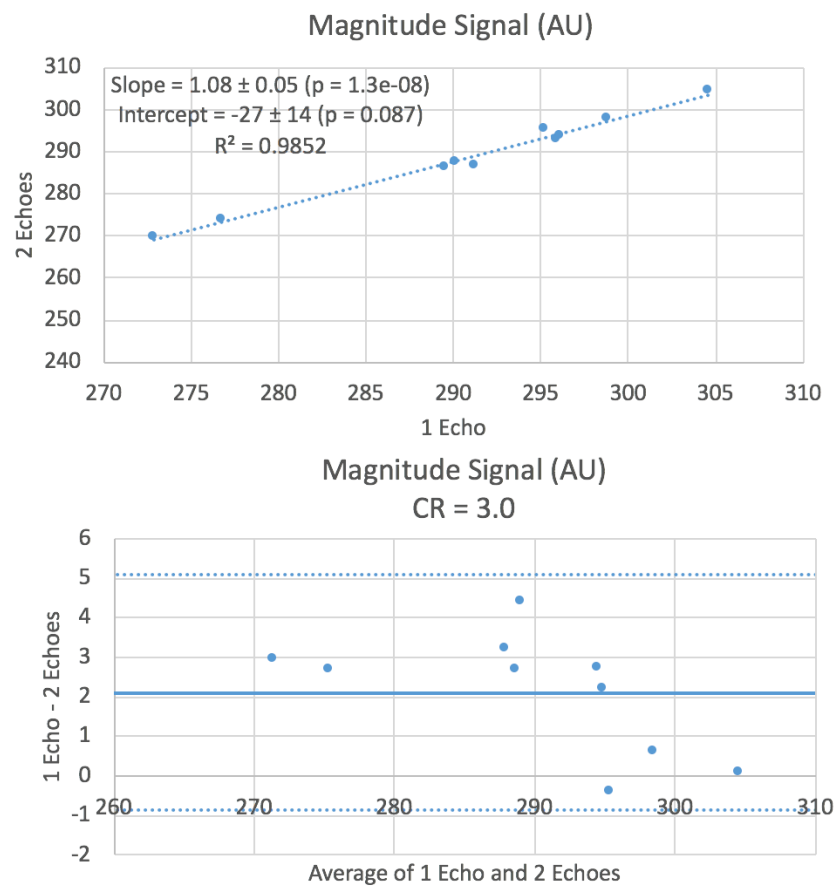


Fig. 5.11. Correlation and Bland-Altman plots for the magnitude of the averaged signal in an ROI within the 0.96 mM gadoteridol phantom.

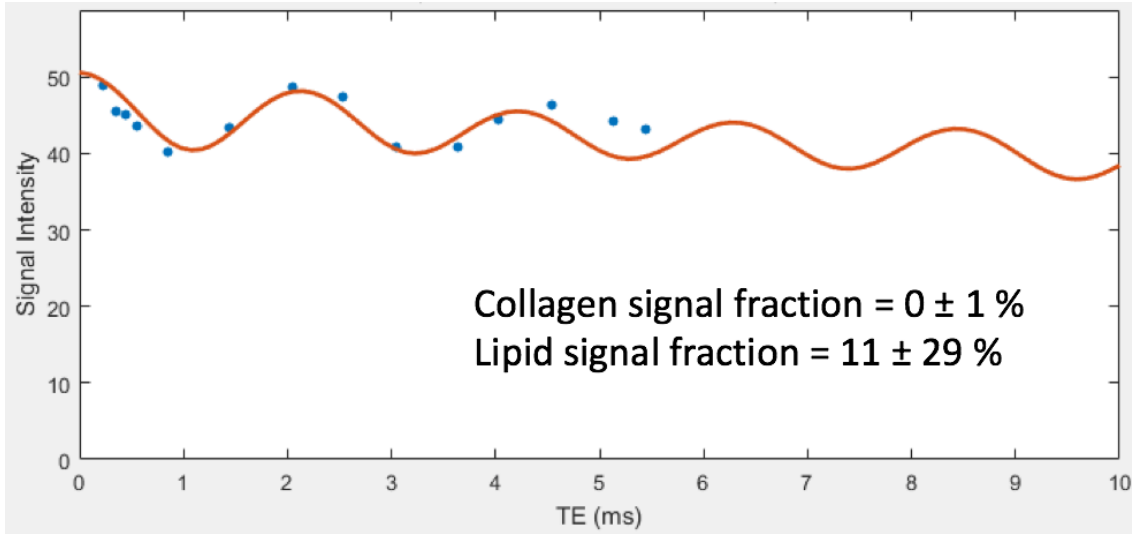


Fig. 5.12. T_2^* signal decay in an ROI in the septum of a healthy volunteer, modelled with a function accounting for isotropic collagen, free water and lipids.

5.1.3.4. Conclusion

Given the high correlation in signal between the single- and double-echo acquisitions and protocol benefits of the double-echo acquisition, the double-echo UTE acquisition was used for further studies *in vivo*.

5.1.4. Decreasing Signal Contamination

5.1.4.1. Introduction to Signal Suppression Techniques

In 2D-UTE, two half-pulse acquisitions are combined in the complex domain to obtain the correct slice profile. As the half pulses are acquired with the slice-selection gradient in opposite directions, out-of-slice signal should be cancelled out, theoretically. In reality, this method is sensitive to imperfections in the slice-selection gradient, including gradient delays and eddy currents (2,3). Hence, out-of-slice signal can contaminate the desired signal. Two methods of suppressing signal include fat saturation and spatial saturation. The original application for this optimisation was cardiac imaging. In this case, fat (lipid) signal can originate from outside the slice, especially when an oblique orientation is used

for acquiring a cardiac short-axis view. In the liver, Krafft et al. have applied fat saturation and spatial saturation to UTE acquisitions, resulting in the suppression of lipid signal associated with disease and out-of-slice signal (4). They employed fat saturation to saturate the methylene (CH_2) lipid peak. Moreover, two spatial saturation bands were applied parallel to the imaging slice, with a band thickness of 100 mm and a gap to the imaging slice of 10 mm. The aim of this section was to determine which method of suppression was optimal, out of fat saturation and spatial saturation. *In vivo* cardiac imaging and Bloch simulations were performed to evaluate the effects of the saturation schemes on short- T_2^* signal.

5.1.4.2. Introduction: Fat Saturation

Fat saturation is a chemical-shift-selective technique that employs an RF pulse centred on the main fat peak (at ~ 3.4 ppm) to saturate the magnetisation of fat, while leaving the magnetisation of water intact (5). Fig. 5.13 illustrates this preparation technique.

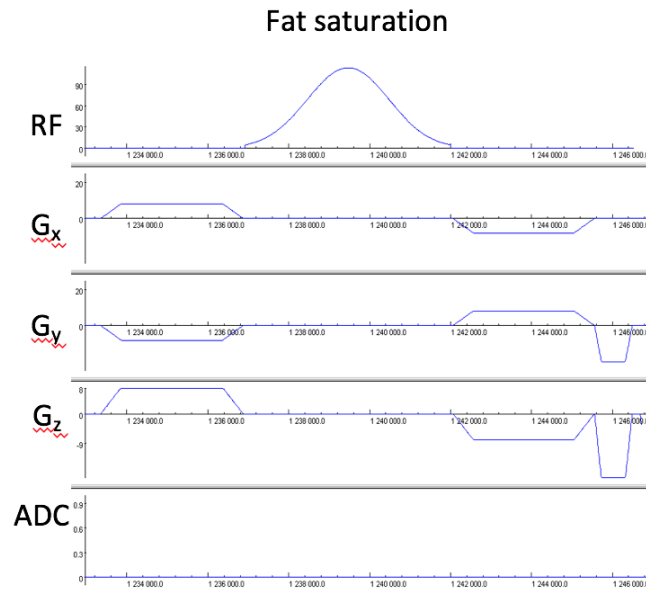


Fig. 5.13. Fat-saturation technique, characterised by an RF pulse of 400 Hz (3.25 ppm relative to water) and spoiler gradients. This technique saturates fat globally and is not location-specific.

In this case, the fat-saturation pulse is a Gaussian function, centred at 400 Hz or 3.25 ppm relative to water. In the Fourier domain, the pulse has a half-width of 190 Hz, and hence spans 210 Hz to 590 Hz. Consequently, the transverse magnetisation is saturated with a spoiler gradient. The following is the MR spectrum of lipids:

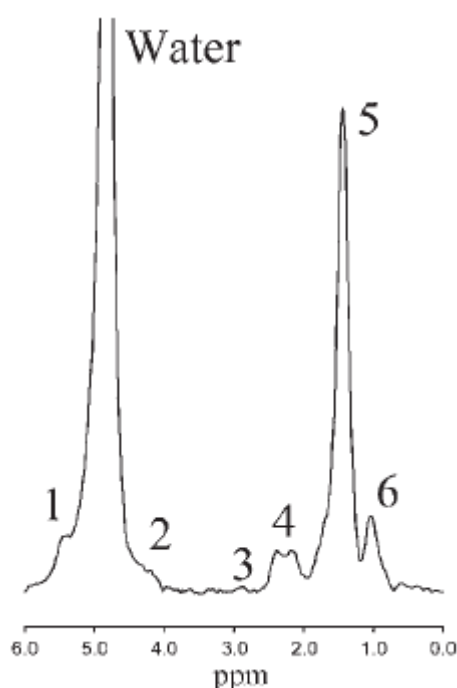


Fig. 5.14. Proton MR spectrum of a patient with fatty-liver disease at 3 T (6). Copyright 2010 John Wiley & Sons, Ltd. Adapted with permission from Wiley. The MR spectrum was measured *in vivo* with the stimulated echo acquisition mode (STEAM) sequence at TE = 10 ms.

Without fat saturation, lipid signal can be described by six peaks. With fat saturation centred on the methylene peak (number 5), peaks 3 - 6 should be saturated, with peaks 1 - 2 remaining. As such, lipid signal can be described by either a six- or two-peak model, the latter being used when fat saturation is applied. The following describes the signal from lipids, consisting of either six or two peaks:

$$S_{lipids}(TE) = S_{0l} \exp(-TE \cdot R_{2l}^*) \cdot \exp(i\varphi_l) \cdot \sum_{p=1}^{6 \text{ or } 2} S_{0p} \exp(i2\pi f_p TE). \quad [5.1]$$

Fitted parameters in the model include the initial signal intensity (S_{0l}), relaxation rate (R_{2l}^*), phase (φ_l) and frequency (f_p). Here, it is assumed:

$$\begin{aligned} R_{2l}^* &= R_{2l,ref} + R_{2l,fit}^*, \\ f_p &= f_{shift \text{ rel. to largest peak},p} + f_{fit, largest peak}, \end{aligned} \quad [5.2]$$

where $R_{2l,fit}^*$ and $f_{fit, largest peak}$ are determined through fitting. Fixed values for $R_{2l,ref}$, $f_{shift \text{ rel. to largest peak},p}$ and S_{0p} are summarised in Table 5.4. In the six-peak lipid model, lipid peak 5 is fitted and the remaining five peaks are fixed relative to peak 5. In the case of fat saturation, peak 1 is fitted and peak 2 is fixed.

Peak number	Peak name	Chemical shift relative to water (ppm)	6-peak lipid model			2-peak lipid model		
			Resonant frequency shift relative to largest peak, $f_{shift\ rel.\ to\ largest\ peak,p}$ (Hz)	Relaxation rate, $R_{2l,ref}$ (s ⁻¹)	Norm. signal intensity, S_{0p}	Resonant frequency shift relative to largest peak, $f_{shift\ rel.\ to\ largest\ peak,p}$ (Hz)	Relaxation rate, $R_{2l,ref}$ (s ⁻¹)	Norm. signal intensity, S_{0p}
1	Olefinic	0.6	492.2	20.8	0.049	0	20.8	0.557
2	Glycerol	-0.4	369.1	23.3	0.039	-123.0	23.3	0.443
3	Diacyl	-1.94	179.6	16.4	0.004	--	--	--
4	Alpha-carboxyl and alpha-olefinic	-2.6	98.4	19.2	0.128	--	--	--
5	Methylene	-3.4	0	16.4	0.693	--	--	--
6	Methyl	-3.8	-49.2	18.5	0.087	--	--	--

Table 5.5. Fixed parameters for the six- and two-peak lipid models, derived from Mózes et al. 2018 (7).

As a major source of signal contamination is fat, fat saturation was investigated. The six- and two-peak lipid models were used in this chapter to determine the lipid signal fraction, or proton density fat fraction.

5.1.4.3. Introduction: Spatial Saturation

Spatial saturation utilises a spatially selective 90° pulse and spoiler gradients to saturate signal at given locations (8,9). The technique is illustrated in Fig. 5.15.

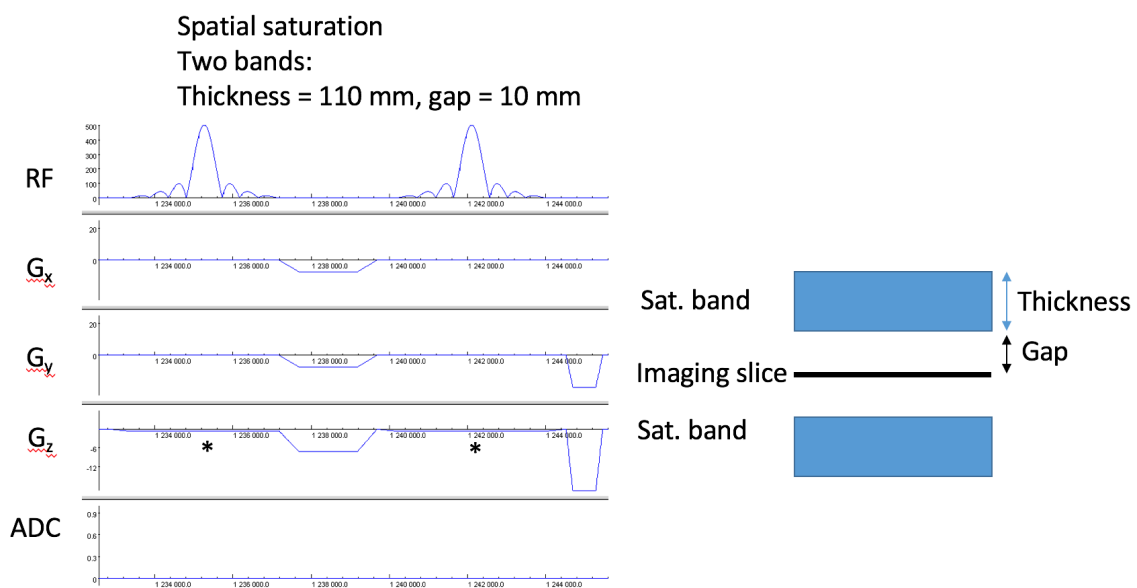


Fig. 5.15. Spatial-saturation technique. Here, the two spatial saturation pulses generate saturation bands with thickness of 110 mm and gap to imaging slice of 10 mm. G_z gradients are played out at starred (*) locations during the saturation pulses according to the locations of the bands.

In this case, two saturation bands are imposed parallel to the imaging slice (above and below). Spoiler gradients are used to further disperse phase after each saturation pulse. Saturation bands are advantageous for their specificity to out-of-slice signal, motivating their investigation in this section.

5.1.4.4. Methods: Experiments

Cardiac UTE images of two healthy subjects (one subject for each experiment) were acquired using the sequence from Fig. 5.8. For the fat-saturation experiment, the acquisition was performed with and without fat saturation. In the spatial-saturation experiment, the thickness and gap of the two saturation bands were varied. Table 5.5 summarises the UTE parameters. Both fat saturation and spatial saturation were pre-programmed by Siemens and used without modification.

UTE scan parameters	Fat saturation	Spatial saturation
Single- or dual-echo acquisition	Dual	Dual
Gradient spoiling following the second interleaved echo	No	No
Signal suppression	<u>Fat saturation:</u> with and without	<u>Spatial saturation:</u> Thickness/gap (mm): 0/0, 27/5, 55/5, 110/5, 110/10, 110/20
Frequency of suppression pulse (Hz)	400	1832
Coil	32-channel torso array coil (Invivo, Florida, USA)	32-channel torso array coil (Invivo, Florida, USA)
Image orientation	Coronal	Oblique (cardiac short-axis view)
Repetition time (ms)	7	7
Flip angle (°)	10	10
Number of averages	2	2
Slice thickness (mm)	8	8
Field-of-view (mm x mm)	300 x 300	300 x 300
Matrix size	256 x 256	256 x 256
Bandwidth (Hz/pixel)	980	980
Segmentation	40 readouts/heartbeat	40 readouts/heartbeat
Acquisition window (ms)	870	870
Trigger delay (ms)	550	550
TEs (ms)	First interleaved TE: 0.23, 0.35, 0.44, 0.55, 0.85, 1.45, 2.05, 2.54, 3.04, 3.64, 4.03	First interleaved TE: 0.23, 0.35, 0.44, 0.55, 0.85, 1.45, 2.05

	Second interleaf TE: 5.52 (fixed for all acquisitions)	Second interleaf TE: 2.54, 3.04, 3.64, 4.03, 4.55, 5.14, 5.45
Scan time per acquisition (s)	~ 15 (breath-hold duration)	~ 15 (breath-hold duration)

Table 5.6. UTE parameters for the fat-saturation and spatial-saturation experiments.

For phantom validation, the 0.96 mM gadoteridol and peanut oil phantom was acquired without saturation, with fat saturation (400 Hz) and with saturation bands (thickness = 100 mm, gap = 10 mm). UTE phantom parameters from Table 5.2 were used.

Cardiac images were cropped to include the left ventricle and rigid-body image registration was performed. ROIs were drawn in the septum. The averaged signal within an ROI was analysed with the R_2^* model of isotropic collagen, free water and lipids, as used in previous sections. The collagen and lipid signal fractions were obtained. Details are in the Appendix. For the acquisition with fat saturation, a two-peak lipid model was used (Eq. [5.1]). Otherwise, the six-peak lipid model was used. Fitting was performed with a trust-region-reflective non-linear least-squares algorithm (MATLAB R2015b).

5.1.4.5. Methods: Bloch Simulations

Bloch simulations were performed to determine the effects of fat and spatial saturation on short- T_2^* signal. The Bloch simulator provided by Brian Hargreaves was used (10). Characteristics included: $T_1 = 600$ ms; $T_2 = 0.5, 0.8, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0$ ms; pulse frequency = 400 Hz (fat saturation), 1832 Hz (spatial saturation). T_1 and T_2 were estimated from the values for tendon (11,12). A range of T_2 values were used, as the exact T_2 was unknown. The residual magnetisation after application of each saturation technique was calculated from the simulation.

5.1.4.6. Results and Discussion

Fig. 5.16 shows the effect of the saturation techniques on the 0.96 mM gadoteridol and peanut oil phantom. Fat saturation effectively saturated lipid signal. Spatial saturation was less effective at suppressing lipid signal, due to the off-resonance property of the methylene lipid peak.

The T_2^* signal decays without and with fat saturation are illustrated in Fig. 5.17. Without fat saturation, the six-peak lipid model fitted the signal well, with a lipid signal fraction of 11 ± 3 %. With the two-peak lipid model, there were still oscillations, probably due to the methylene peak, with a lipid signal fraction of 60 ± 60 %. The high lipid signal fraction may have been contributed by the lack of discernment between lipids and water, due to close proximity in chemical shift. Hence, fat saturation was imperfect and rendered the modelling of the lipid signal more challenging.

Fig. 5.18 demonstrates the T_2^* signal decays without and with two spatial saturation bands (thickness = 110 mm, gap = 10 mm). With saturation bands, the lipid signal fraction decreased from 15 ± 6 % to 10 ± 5 %, with the collagen signal fraction relatively constant. Table 5.6 summarises the collagen and lipid signal fractions with changing thickness and gap of the saturation bands. At a thickness of 110 mm and gap of 10 mm, the collagen signal fraction was sufficiently preserved, with a 33 % decrease in lipid signal fraction. Band thicknesses of 27 and 55 mm did not decrease the lipid signal fraction. A band thickness of 110 mm with gaps of 5 mm and 20 mm resulted in decreased collagen signal fractions.

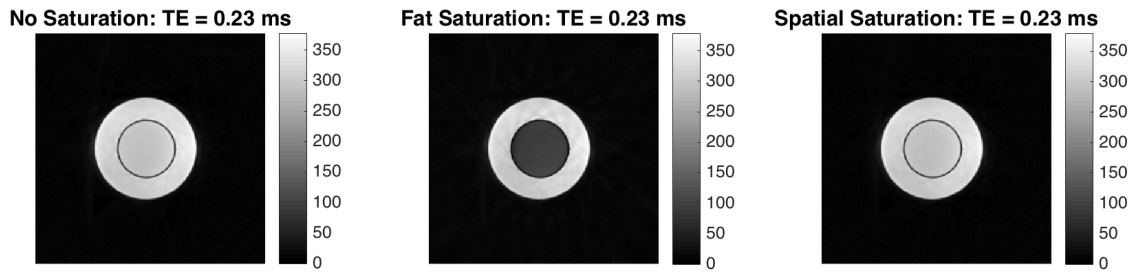


Fig. 5.16. Saturation techniques applied in a 0.96 mM gadoteridol (outer ring) and peanut oil (inner circle) phantom. Fat saturation effectively saturated lipid signal. Spatial saturation (thickness = 100 mm, gap = 10 mm) did not saturate lipid signal as well, due to the off-resonance of the main lipid peak with water.

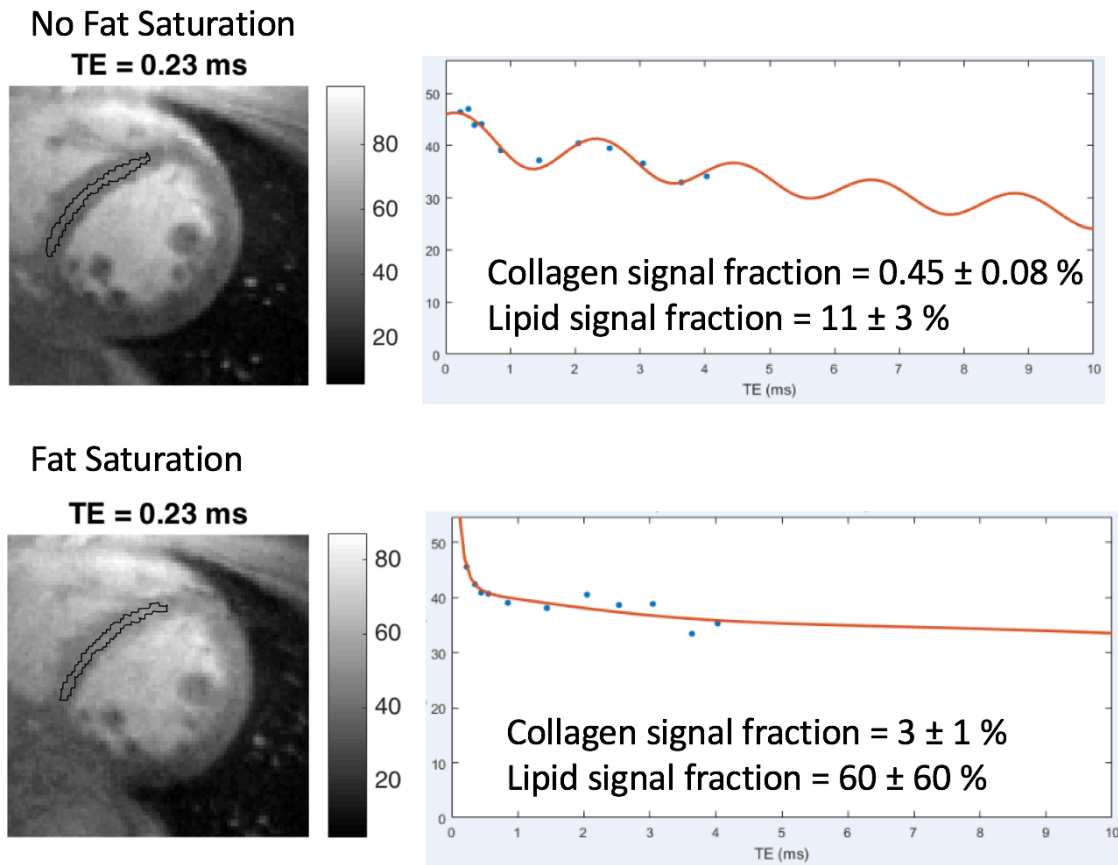
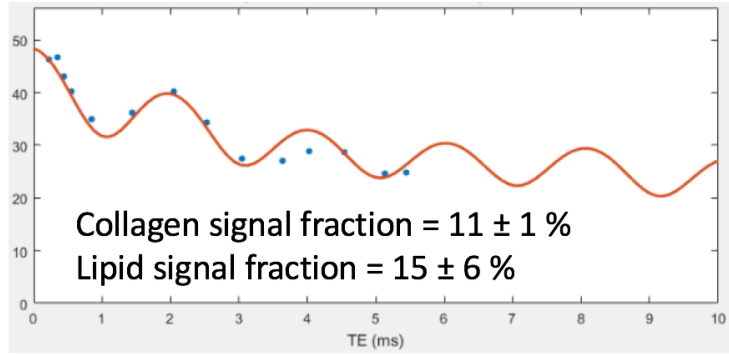
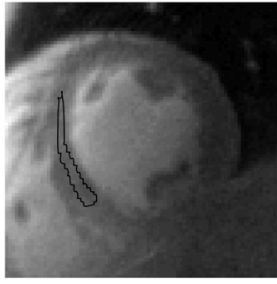


Fig. 5.17. T_2^* signal decays without and with fat saturation. The lipid signal fraction was determined from a six-peak fat model when fat saturation was not applied, and a two-peak fat model when fat saturation was applied.

No Saturation Bands

TE = 0.23 ms



Saturation Bands: Thickness = 110 mm, Gap = 10 mm

TE = 0.23 ms

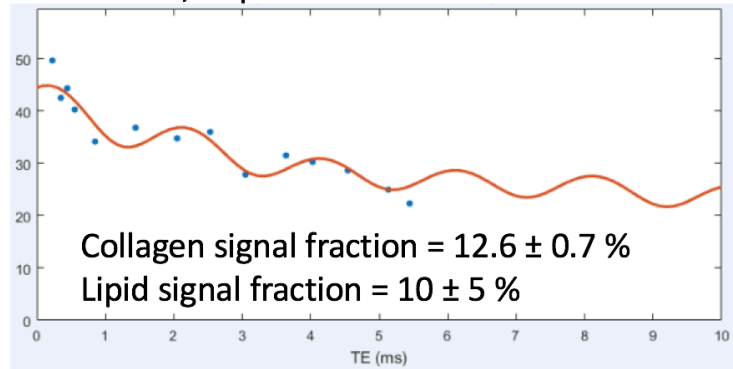
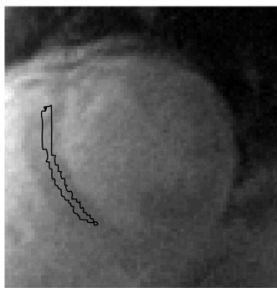


Fig. 5.18. T_2^* signal decays without and with spatial saturation. In this example, the spatial saturation bands had a thickness of 110 mm and gap of 10 mm.

Thickness (mm)	Gap (mm)	Collagen signal fraction (%)	Lipid signal fraction (%)
0	0	11.5 ± 0.7	15 ± 6
27	5	12.4 ± 0.9	19 ± 6
55	5	13.3 ± 0.7	16 ± 5
110	5	8.1 ± 0.3	8 ± 4
110	10	12.6 ± 0.7	10 ± 5
110	20	8.7 ± 0.4	9 ± 4

Table 5.7. Optimisation of the thickness and gap of the spatial saturation bands. Collagen and lipid signal fractions were derived from an R_2^* model of isotropic collagen, free water and lipids (refer to the Appendix).

Fig. 5.19 and Fig. 5.20 demonstrate the Bloch simulations for the magnetisation loss of the short- T_2^* component after application of the fat saturation and spatial saturation pulses. Assuming a T_2 of 1.0 ms (where $T_2^* \leq T_2$), the lost magnetisation was 0.11 and

0.05 with the two saturation schemes, respectively. From literature, fat saturation may reduce the fraction of short- T_2^* signal by up to 20 % (2,3,13). Hence, less magnetisation was lost with the spatial saturation bands with a thickness of 110 mm and gap of 10 mm.

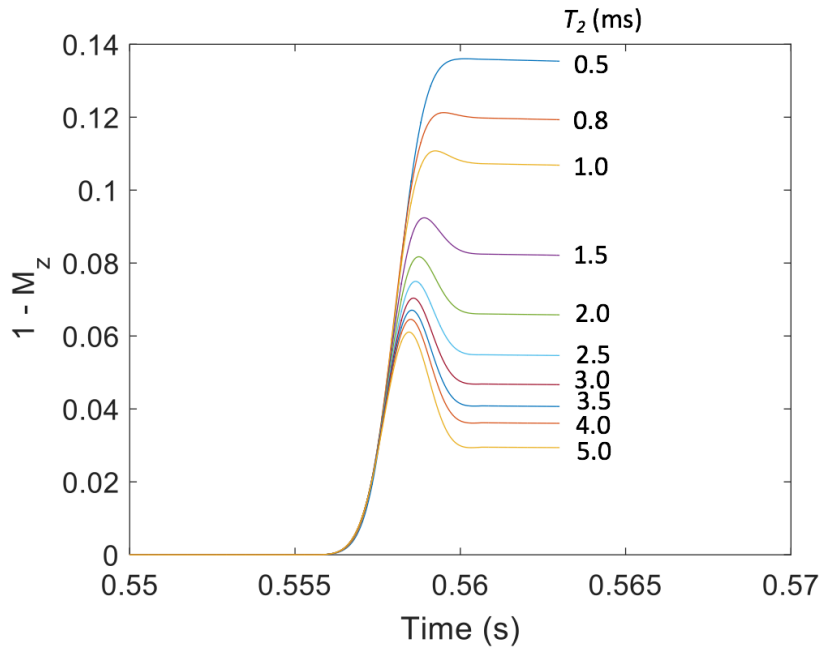


Fig. 5.19. Magnetisation loss of the short- T_2^* signal after the application of a 400-Hz fat saturation pulse.

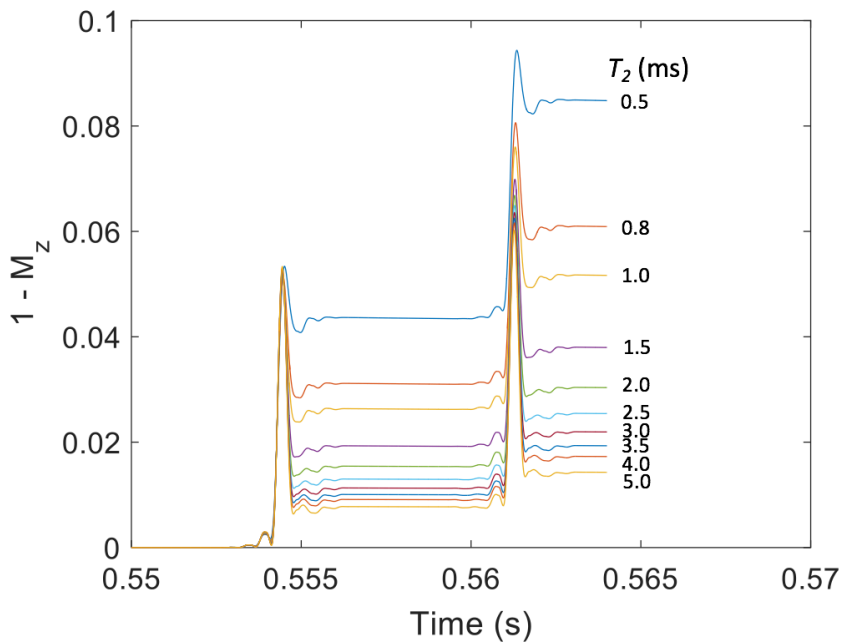


Fig. 5.20. Magnetisation loss of the short- T_2^* signal after the application of two 1832-Hz spatial saturation pulses.

5.1.4.7. Conclusion

Overall, using spatial saturation bands with a thickness of 110 mm and gap of 10 mm seemed favourable, given that:

1. They decreased the lipid signal fraction by 33 %.
2. They would result in a 5 % signal loss of the short- T_2^* component ($T_2 = 1.0$ ms), as opposed to an 11 % signal loss with fat saturation.
3. Krafft et al. used sat bands with thickness 100 mm and gap 10 mm for UTE of the liver (4).

5.1.4.8. Final UTE Sequence for In Vivo Experiments

Fig. 5.21 and Table 5.7 summarise the final UTE sequence and parameters used for *in vivo* experiments on the Trio 3 T. The sequence and parameters were applied directly for liver imaging, with example T_2^* signal decays shown in Section 6.4.

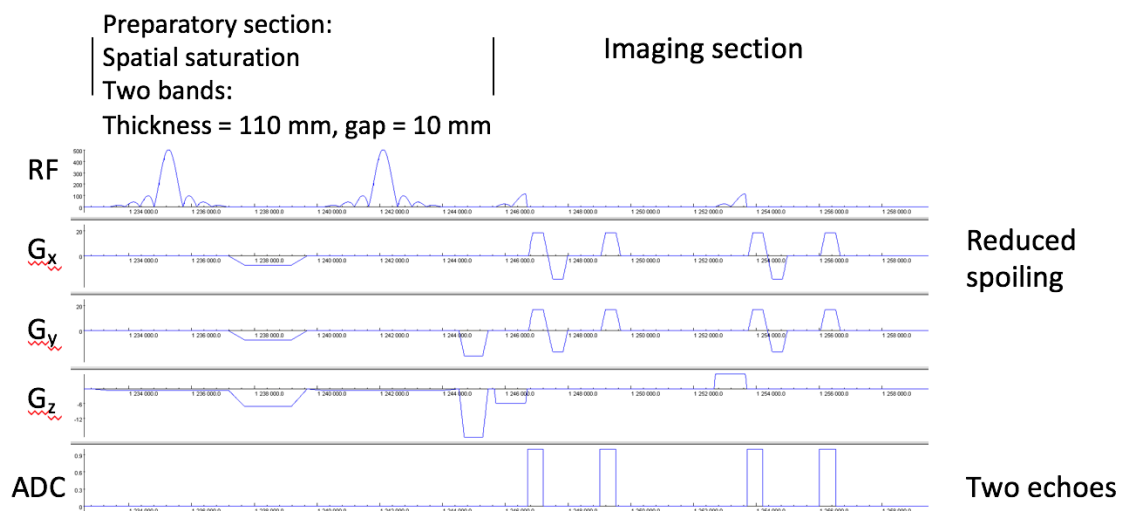


Fig. 5.21. Final UTE sequence for *in vivo* experiments on the Trio 3 T.

UTE scan parameters	Prisma 3 T
Single- or dual-echo acquisition	Dual
Gradient spoiling following the second interleaf echo	No
Signal suppression	<u>Spatial saturation:</u> Thickness = 110 mm Gap = 10 mm
Frequency of suppression pulse (Hz)	1832
Coil	32-channel torso array coil (Invivo, Florida, USA)
Repetition time (ms)	7
Flip angle (°)	10
Number of averages	2
Slice thickness (mm)	8
Field-of-view (mm x mm)	300 x 300
Matrix size	256 x 256
Bandwidth (Hz/pixel)	980
Segmentation	60 readouts/heartbeat
Acquisition window (ms)	820
Trigger delay (ms)	400
TEs (ms)	First interleaf TE: 0.23, 0.35, 0.44, 0.55, 0.85, 1.45, 2.05 Second interleaf TE: 2.54, 3.04, 3.64, 4.03, 4.55, 5.14, 5.45
Scan time per acquisition (s)	~ 10 (breath-hold duration)

Table 5.8. Final UTE parameters for *in vivo* experiments on the Trio 3 T. The acquisition window and trigger delay were adjusted for imaging during diastole. Segmentation was 60 readouts/heartbeat for a breath-hold duration of ~ 10 s.

5.2. UTE Optimisation on the Prisma 3 T

5.2.1. UTE Sequence on the Prisma 3 T

Sequence optimisation was performed on the Prisma 3 T, as the study on liver-disease patients was performed on this scanner. Additionally, the peak gradient strength on the Prisma 3 T was 80 mT/m, compared to 40 mT/m on the Trio 3 T, amounting to better gradient performance. Fig. 5.22 and Fig. 5.23 illustrate the given UTE sequence on the

Prisma 3 T, compared against the full sequence on the Trio 3 T (Fig. 5.24). The parameters on the Prisma are listed in Table 5.8.

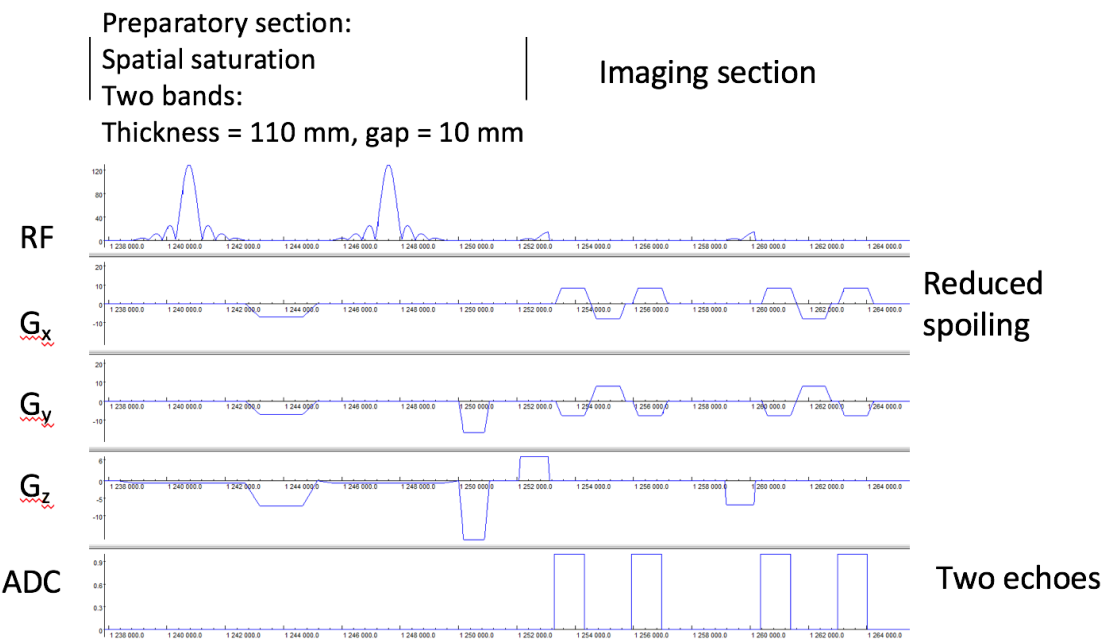


Fig. 5.22. UTE sequence on the Prisma 3 T. Gradient amplitudes are around half of those on the Trio 3 T.

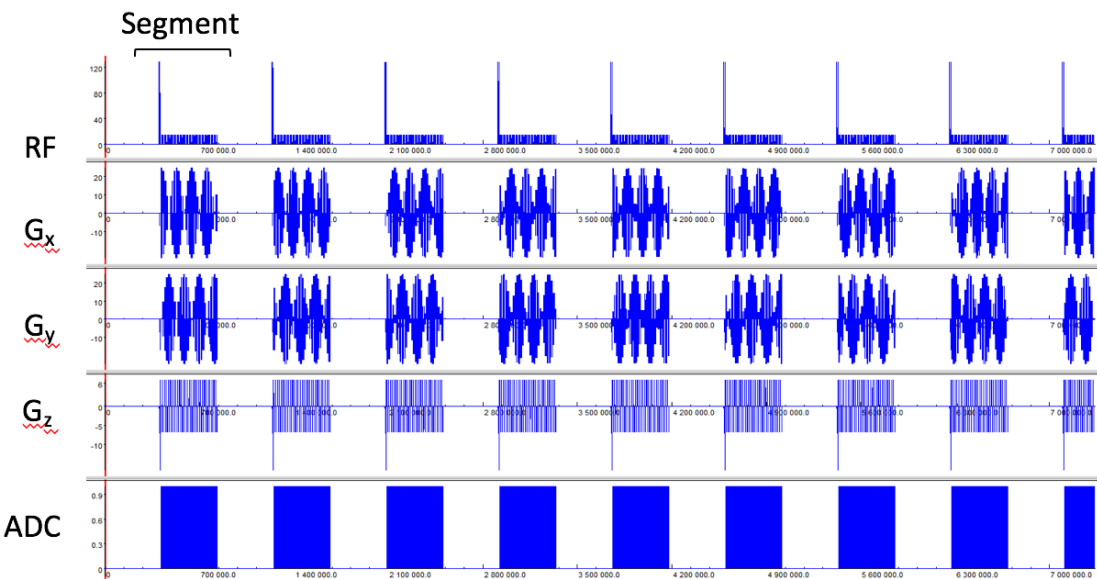


Table 5.9.

Fig. 5.23. UTE sequence on the Prisma 3 T (entire acquisition interval). Note that k -space is traversed within one cardiac segment, via the G_x and G_y gradients.

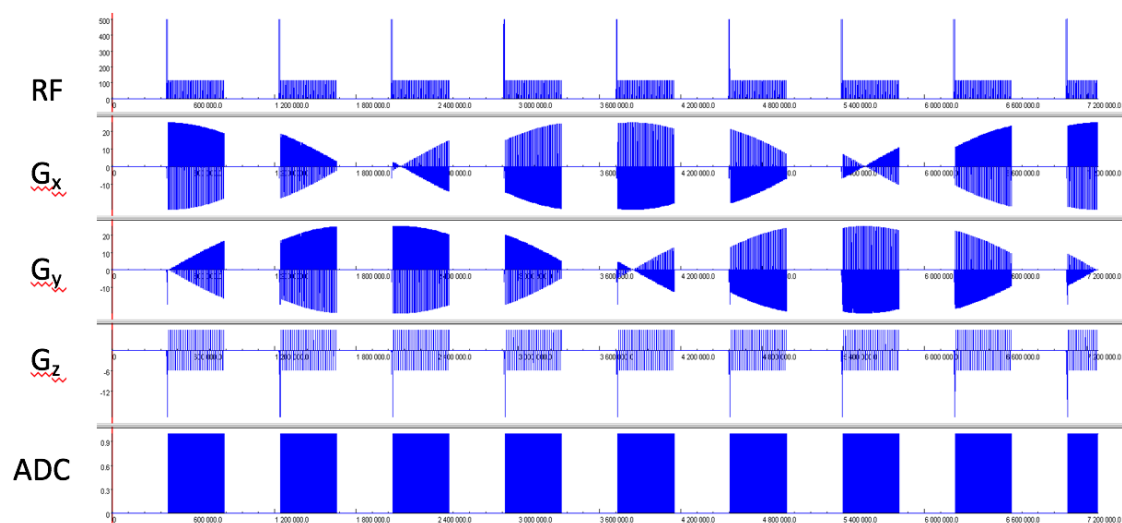


Fig. 5.24. UTE sequence on the Trio 3 T (entire acquisition interval). Note that k -space is traversed over all cardiac segments, via the G_x and G_y gradients.

UTE scan parameters	Prisma 3 T	Optimisation step
Single- or dual-echo acquisition	Dual	--
Gradient spoiling following the second interleaf echo	No	--
Signal suppression	<u>Spatial saturation:</u> Thickness = 110 mm Gap = 10 mm	--
Coil	To be determined	2
Repetition time (ms)	7	--
Flip angle (°)	10	--
Number of averages	2	--
Slice thickness (mm)	8	--
Field-of-view (mm x mm)	300 x 300	--
Matrix size	256 x 256	--
Bandwidth (Hz/pixel)	980	1
Segmentation	60 readouts/heartbeat	--
Acquisition window (ms)	820	--
Trigger delay (ms)	400	--
TEs (ms)	First interleaf TE: 0.23, 0.35, 0.44, 0.55, 0.85, 1.45, 2.05 Second interleaf TE: 2.54, 3.04, 3.64, 4.03, 4.55, 5.14, 5.45	2
Scan time per acquisition (s)	~ 12 (breath-hold duration)	Not optimised
k -space coverage	Per cardiac segment	Not optimised

Table 5.10. UTE parameters on the Prisma 3 T, with optimisation steps.

Differences between the Trio and Prisma sequences included the gradient strength (due to a difference in oversampling factor), breath-hold duration (~ 12 s vs. ~ 10 s) and k -space trajectory. Moreover, the coil set-up had to be optimised.

The breath-hold duration and k -space trajectory were left unchanged. The breath-hold duration was estimated from the time the scan was started on the console. This duration was longer on the Prisma, despite using the same segmentation parameters as on the Trio, due to a longer pause after the scan was started. The k -space trajectory on the Prisma was more robust than on the Trio, as complete k -space sampling was achieved within one cardiac segment, as opposed to spread over all cardiac segments, as on the Trio. Hence, the k -space trajectory was not changed.

Optimisation was performed for the bandwidth and minimum TE, as follows:

1. In order to replicate the gradient strengths on the Trio, the bandwidth on the Prisma sequence was made almost twice the bandwidth on the Trio sequence, to account for a halved oversampling factor. The effect of adjusting the bandwidth was investigated in a long- T_2 phantom.
2. Due to hardware limitations, the signal at the minimum TE may be artefactual. The minimum TE in the protocol was determined by measuring the signal intensity in the short-TE range in a long- T_2 phantom, using several coils.

The optimisation steps are detailed in the proceeding sections.

5.2.2. Bandwidth Optimisation

5.2.2.1. Introduction

The Trio sequence utilised an oversampling factor of 2 to reduce aliasing artefacts. In the image reconstruction, the FOV was truncated to the original size. On the Prisma sequence, the oversampling factor was 1. The sampling rate is given by:

$$\text{Sampling rate} = \frac{1}{BW \cdot OSF \cdot N} , \quad [5.3]$$

where BW is the bandwidth, OSF is the oversampling factor and N is the number of points. The gradient amplitude (G) is:

$$G = \frac{2\pi}{\gamma} \cdot \frac{BW \cdot OSF \cdot N}{FOV} . \quad [5.4]$$

Hence, with the oversampling factor halved on the Prisma, the gradient amplitude was also halved. As the objective was to reproduce the gradient amplitudes on the Trio, the bandwidth was doubled to achieve the same gradient amplitude. Other options included doubling the number of points or halving the FOV, which were not viable, as these would change the spatial resolution.

This section investigated the feasibility of doubling the bandwidth on the Prisma and Trio UTE sequences, as tested in a long- T_2 phantom by Siemens. The T_2^* signal intensity in the phantom was analysed for unexpected behaviour, including short- T_2^* decay and inconsistencies at the shortest TEs.

5.2.2.2. Methods

A long- T_2 phantom by Siemens was used, consisting of nickel sulphate hexahydrate (per 1,000 g of distilled H_2O : 3.75 g $NiSO_4 \cdot 6H_2O$ + 5 g NaCl). This phantom was selected for its lack of short- T_2^* signal. Signal in the phantom was compared between bandwidths

of 490 and 890 Hz/pixel on the Trio, and between bandwidths of 980 and 1780 Hz/pixel on the Prisma. Note that the bandwidths on the Prisma were exactly twice those on the Trio. UTE parameters are summarised in Table 5.9. A 10-pixel x 10-pixel ROI was drawn in the phantom for signal analysis.

UTE scan parameters	Prisma 3 T	Trio 3 T
Single- or dual-echo acquisition	Dual	Dual
Gradient spoiling following the second interleaf echo	No	No
Signal suppression	<u>Spatial saturation:</u> Thickness = 110 mm Gap = 10 mm	<u>Spatial saturation:</u> Thickness = 110 mm Gap = 10 mm
Coil	18-channel torso array coil and 32-channel spinal array coil (Siemens Healthineers, Erlangen, Germany)	32-channel torso array coil (Invivo, Florida, USA)
Image orientation	Transversal	Transversal
Repetition time (ms)	7	7
Flip angle (°)	10	10
Number of averages	2	2
Slice thickness (mm)	8	8
Field-of-view (mm x mm)	300 x 300	300 x 300
Matrix size	256 x 256	256 x 256
Bandwidth (Hz/pixel)	980, 1780	490, 890
Segmentation	60 readouts/heartbeat	60 readouts/heartbeat
Acquisition window (ms)	820	820
Trigger delay (ms)	400	400
TEs (ms)	First interleaf TE: 0.24, 0.35, 0.44, 0.55, 0.85, 1.45, 2.05 Second interleaf TE: 2.66, 3.04, 3.64, 4.03, 4.55, 5.00, 5.00	First interleaf TE: 0.24, 0.35, 0.44, 0.55, 0.85, 1.45, 2.05 Second interleaf TE: 2.66, 3.04, 3.64, 4.03, 4.55, 5.00, 5.00
Scan time per acquisition (s)	~ 12 (breath-hold duration)	~ 10 (breath-hold duration)
<i>k</i> -space coverage	Per cardiac segment	Over all cardiac segments

Table 5.11. UTE parameters on the Prisma and Trio for the investigation of bandwidth.

5.2.2.3. Results and Discussion

Fig. 5.25 illustrates UTE magnitude images of the phantom at TE = 0.24 ms using different bandwidths on the two scanners. The signal was averaged within the ROI and plotted as a function of TE in Fig. 5.26.

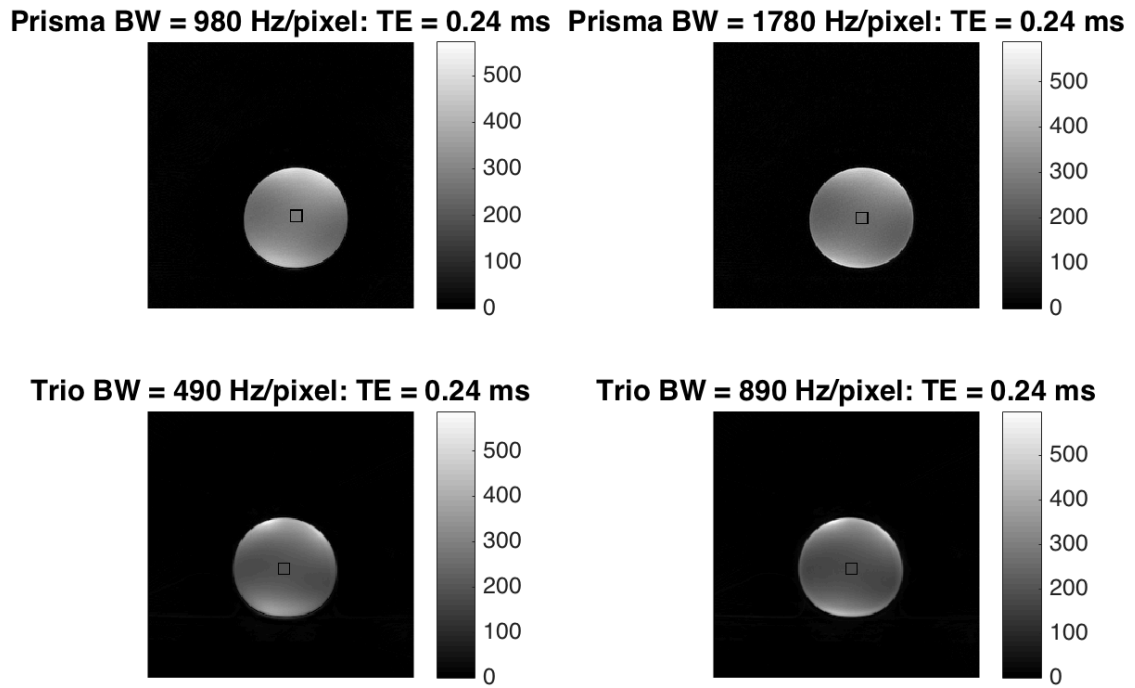


Fig. 5.25. Example UTE images in the long- T_2 phantom, acquired at different bandwidths. Note that the bandwidths on the Trio are half the bandwidths on the Prisma. The 10 x 10 ROI is outlined.

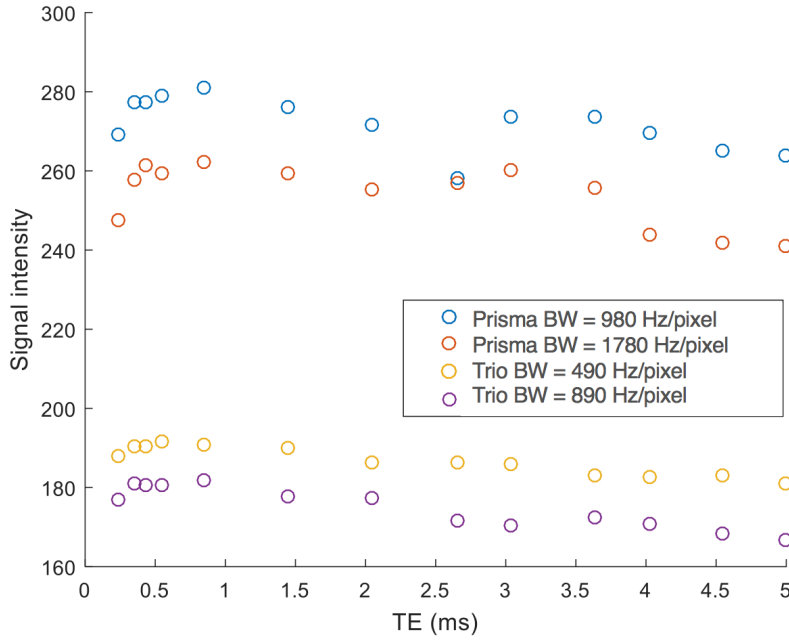


Fig. 5.26. Averaged signal intensity within the 10 x 10 ROI in the long- T_2 phantom, acquired with different bandwidths.

As the signal intensity as a function of TE was virtually flat, the long- T_2 phantom did not exhibit short- T_2^* decay, as expected. The signal intensity at the first three TEs of 0.23, 0.35 and 0.44 ms appeared inconsistent, which was more prominent on the Prisma than on the Trio. This could have been due to hardware or limitations. On the coil, the minimum TE was limited by the switching between transmit and receive, causing potential signal inconsistencies at the shortest TEs. Furthermore, the signal intensity at TE = 2.66 ms at 980 Hz/pixel was unexpected. In the next section, the signal intensity in the phantom was investigated in a variety of coils on the Prisma.

5.2.2.4. Conclusion

Doubling the bandwidth did not cause evident changes in signal intensity in the long- T_2 phantom. Investigation of the signal intensity behaviour at short TEs in different coils is needed.

5.2.3. Coil and Minimum-TE Optimisation

5.2.3.1. Introduction

Given the previous experiment, the most appropriate coil for providing the shortest TE with consistent signal was assessed on the Prisma. Namely, transmit-receive switching and coil performance can affect the minimum TE and signal consistency at short TEs. Several coils on the Prisma were investigated, including the head coil, body coil and two torso array coils.

5.2.3.2. Methods

Table 5.10 summarises the acquisition parameters for the experiment, including the use of four coil combinations. Note that eight averages were acquired for an increased signal-to-noise ratio. For the body coil, both eight and 32 averages were performed. The bandwidth was increased to 1860 Hz/pixel. A 40-pixel x 40-pixel ROI was drawn in the phantom and the signal in the ROI was averaged.

UTE scan parameters	Prisma 3 T
Single- or dual-echo acquisition	Dual
Gradient spoiling following the second interleaved echo	No
Signal suppression	<u>Spatial saturation:</u> Thickness = 110 mm Gap = 10 mm
Coils (Siemens Healthineers, Erlangen, Germany)	1. 20-channel head/neck coil 2. Body coil 3. 18-channel torso array coil and 32-channel spinal array coil 4. 30-channel torso array coil and 32-channel spinal array coil
Image orientation	Transversal
Repetition time (ms)	7
Flip angle (°)	10
Number of averages	8 (8 and 32 for body coil)

Slice thickness (mm)	8
Field-of-view (mm x mm)	300 x 300
Matrix size	256 x 256
Bandwidth (Hz/pixel)	1860
Segmentation	60 readouts/heartbeat
Acquisition window (ms)	820
Trigger delay (ms)	400
TEs (ms)	First interleaf TE: 0.24, 0.25, 0.26, 0.27, 0.28, 0.29, 0.35, 0.44, 0.55, 0.85, 1.45, 2.05 Second interleaf TE: 2.54, 2.54, 2.54, 2.54, 2.54, 2.54, 3.04, 3.64, 4.03, 4.55, 5.14, 5.45
Scan time per acquisition (s)	~ 12 (breath-hold duration)
<i>k</i> -space coverage	Per cardiac segment

Table 5.12. UTE parameters on the Prisma for the coil and minimum-TE optimisation.

5.2.3.3. Results and Discussion

Fig. 5.27 illustrates the UTE images acquired with different coils, at the shortest TE of 0.24 ms. Fig. 5.28 and Fig. 5.29 demonstrate the averaged magnitude signal intensity in the 40 x 40 ROI. In all coil set-ups, there was an artefactual increase in signal intensity from TEs 0.24 to 0.35 ms. Hence, the signal artefact was not due to the coil. The 18-channel torso array coil and 32-channel spinal array coil were suitable.

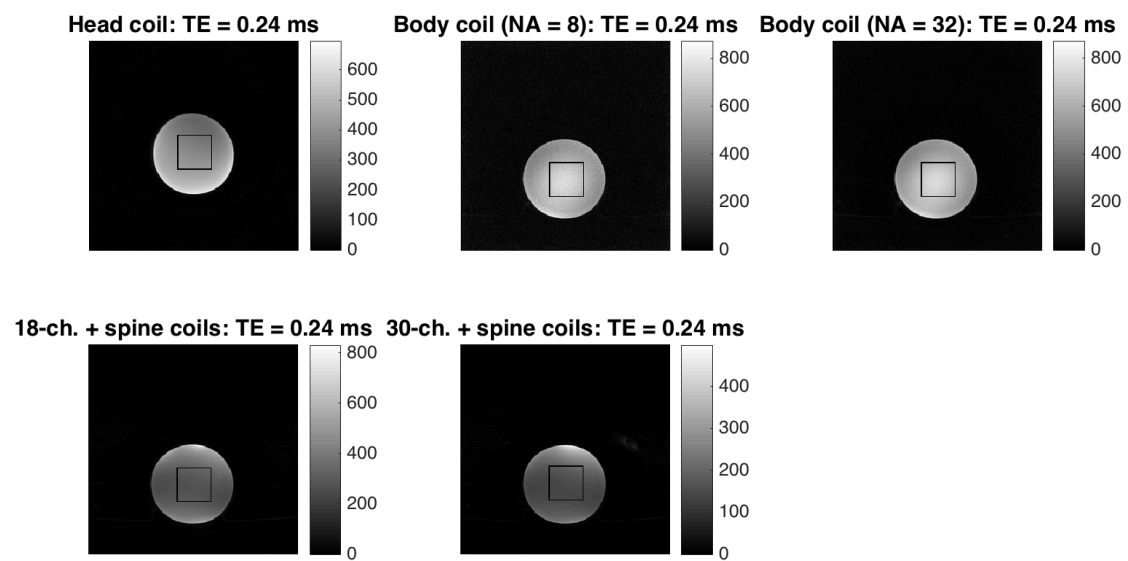


Fig. 5.27. UTE images acquired with different coils, with the 40 x 40 ROI outlined, in the long- T_2 phantom. Note the body coil was acquired with both eight and 32 averages.

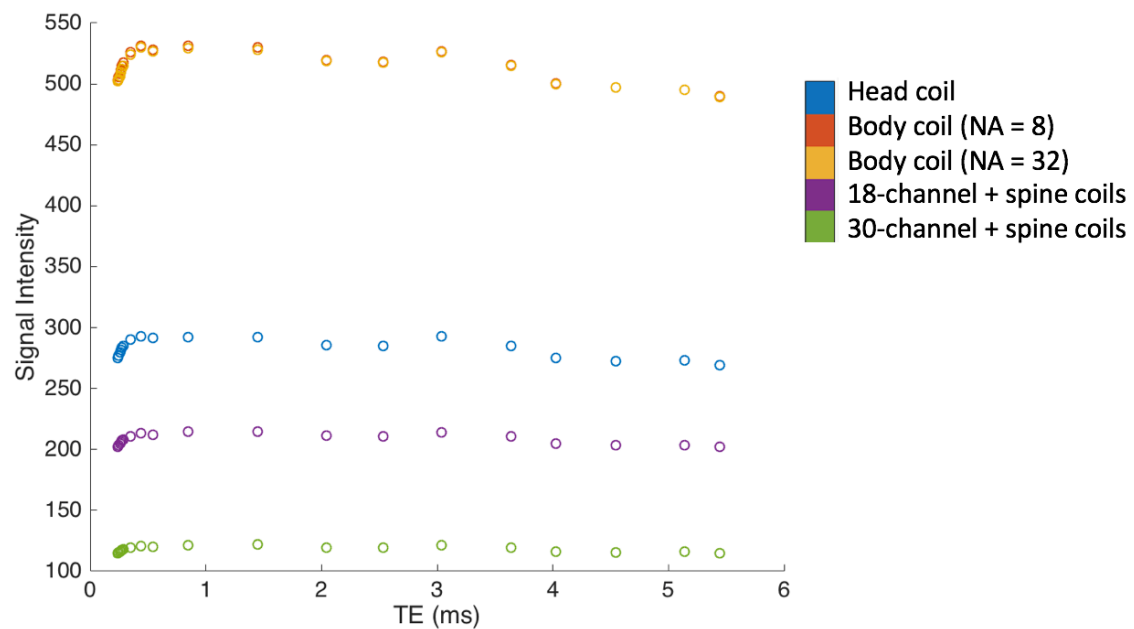


Fig. 5.28. Signal intensity in the 40 x 40 ROI in the long- T_2 phantom.

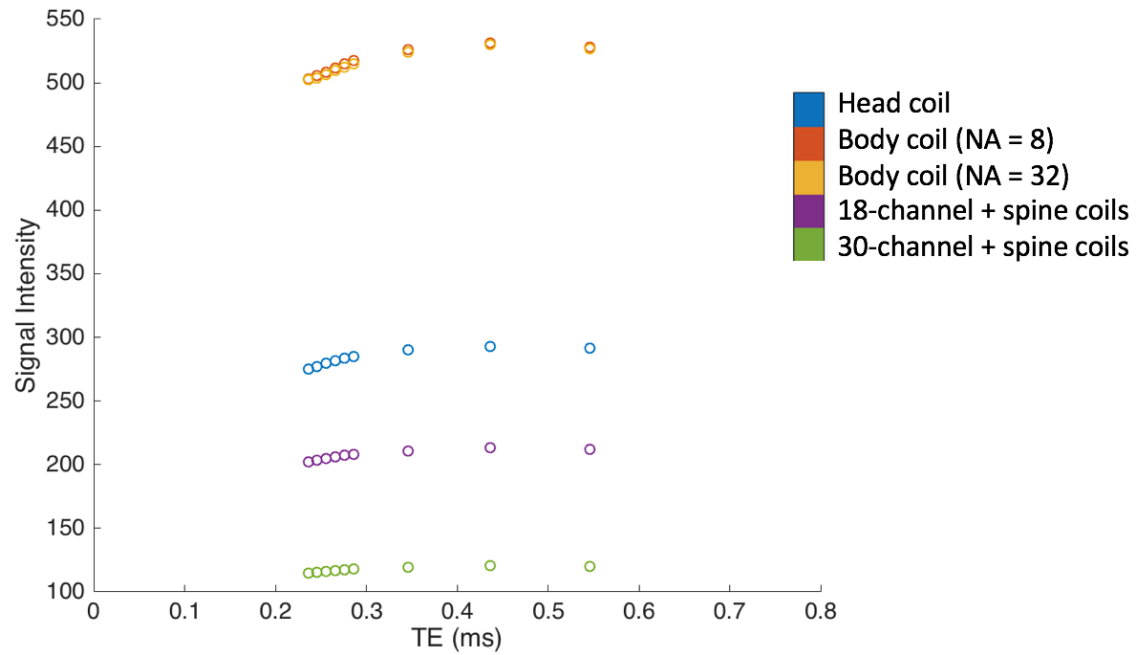


Fig. 5.29. Magnified view of the signal intensity in the 40 x 40 ROI at short TEs.

5.2.3.4. Conclusion

Signal inconsistencies at TEs < 0.35 ms in the long- T_2 phantom led to the conclusion that the minimum TE should be 0.35 ms in the *in vivo* Prisma protocol. The current coil setup of the 18-channel torso array coil and 32-channel spinal array coil was sufficient.

5.2.4. Final UTE Parameters

Fig. 5.30 and Table 5.11 summarise the final UTE sequence and parameters for *in vivo* imaging on the Prisma 3 T. The maximum bandwidth achievable on the Prisma sequence was 1950 Hz/pixel, which was closest to the desired bandwidth of 1960 Hz/pixel (twice the bandwidth of 980 Hz/pixel on the Trio sequence). A 1950 Hz/pixel bandwidth produced the same gradient amplitudes as on the Trio sequence, to within ~ 5 %.

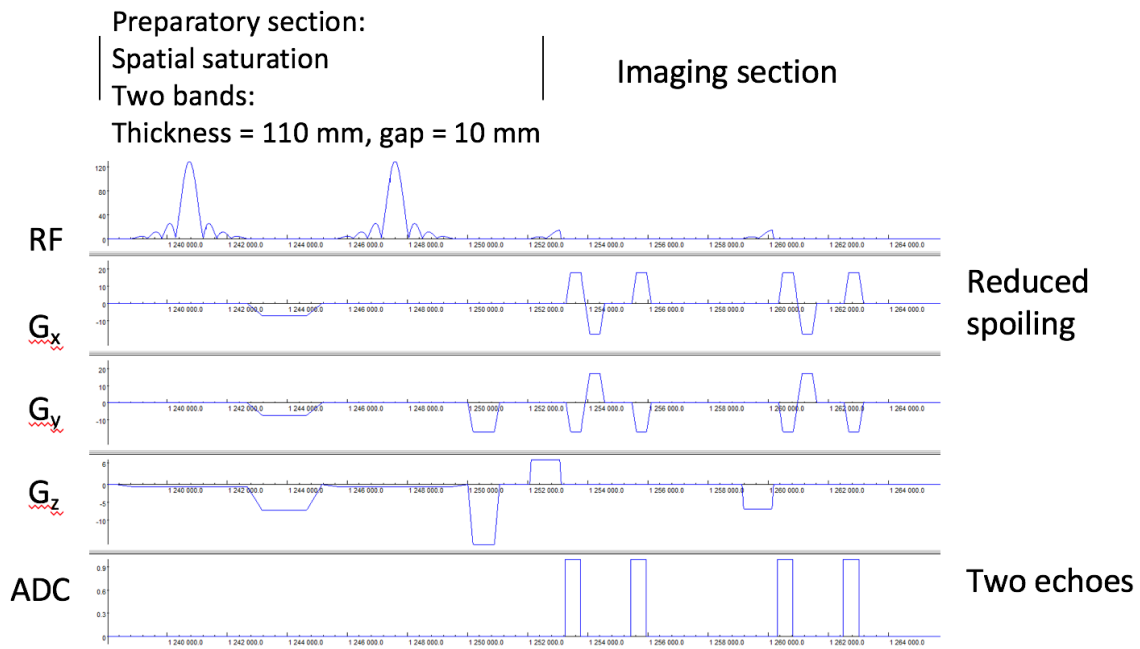


Fig. 5.30. Final UTE sequence on the Prisma 3 T.

UTE scan parameters	
Single- or dual-echo acquisition	Dual
Gradient spoiling following the second interleaf echo	No
Signal suppression	<u>Spatial saturation:</u> Thickness = 110 mm Gap = 10 mm
Coil	18-channel array coil and 32-channel spinal array coil (Siemens Healthineers, Erlangen, Germany)
Repetition time (ms)	7
Flip angle (°)	10
Number of averages	2
Slice thickness (mm)	8
Field-of-view (mm x mm)	300 x 300
Matrix size	256 x 256
Bandwidth (Hz/pixel)	1950
Segmentation	60 readouts/heartbeat
Acquisition window (ms)	820
Trigger delay (ms)	400
TEs (ms)	First interleaf TE: 0.35, 0.39, 0.44, 0.55, 0.85, 1.45, 2.05

	Second interleaf TE: 2.54, 3.04, 3.64, 4.03, 4.55, 5.14, 5.45
Scan time per acquisition (s)	~ 12 (breath-hold duration)
<i>k</i> -space coverage	Per cardiac segment

Table 5.13. Final UTE parameters for *in vivo* imaging on the Prisma 3 T.

5.3. *In Vivo* Protocol Validation on the Trio and Prisma 3 T

5.3.1. Introduction

Given the minimum-TE optimisation on the Prisma 3 T, the *in vivo* protocols on the Prisma and Trio were tested for signal inconsistencies at the shortest TEs. Particularly, the minimum TE on the Trio protocol was 0.23 ms, as opposed to 0.35 ms on the Prisma. Optimisation of the minimum TE was performed in the long- T_2 phantom. *In vivo* acquisitions in the healthy liver were performed for validation on both scanners.

5.3.2. Methods

The UTE parameters for the Prisma and Trio were as specified in Table 5.11 and Table 5.7, respectively, for the *in vivo* liver data. For the long- T_2 phantom data, the same parameters on the respective scanners were kept, except more TEs were acquired. The interleaf TEs are listed in Table 5.12. The final UTE parameters for the Prisma and Trio are denoted by the “Prisma reproducibility protocol” and “Trio repeatability protocol”, respectively. These protocol names refer to reproducibility and repeatability studies in the liver that will be discussed in Chapter 6. Additionally, the “Trio reproducibility protocol” was included, with the first two acquisitions differing from the “Trio repeatability protocol”.

First interleaf TE (ms)	Second interleaf TE (ms)	Long- T_2 phantom		<i>In vivo</i> liver		
		Prisma	Trio	Prisma reproducibility protocol	Trio repeatability protocol	Trio reproducibility protocol
0.23	2.54					
0.24	2.54					
0.25	2.54					
0.26	2.54					
0.27	2.54					
0.28	2.54					
0.29	2.54					
0.30	2.54					
0.31	2.54					
0.32	2.54					
0.33	2.54					
0.34	2.54					
0.35	2.54					
0.35	2.54	(repeat)				
0.35	3.04					
0.39	3.04					
0.44	3.64					
0.55	4.03					
0.85	4.55					
1.45	5.14					
2.05	5.45					

Table 5.14. TEs used for the phantom and *in vivo* experiments. The *in vivo* liver protocols pertain to reproducibility and repeatability studies that will be discussed in Chapter 6.

For analysis of the phantom, signal was averaged in a 40-pixel x 40-pixel ROI.

Liver images were cropped and rigid-body image registration was performed. 20-pixel x 20-pixel ROIs were drawn in the posterior region of the liver.

5.3.3. Results and Discussion

Magnitude UTE images of the long- T_2 phantom are shown in Fig. 5.31. Fig. 5.32 and Fig. 5.33 graph the signal intensity in the phantom against the TE. There were inconsistencies in the signal intensity for TEs < 0.35 ms on both scanners, where the signal intensities increased. The effect was more pronounced on the Prisma, with an increase in signal of ~ 10 on the Prisma vs. an increase of ~ 5 on the Trio, from the first TE of 0.23 or 0.24 ms. Liver images are presented in Fig. 5.34, with signal intensity graphs in Fig. 5.35 for

the three protocols. The signal intensities did not exhibit the artefacts observed in the phantom. To account for the potentially inconsistent signal at a TE of 0.23 ms, the Prisma and Trio reproducibility protocols were devised with a higher minimum TE of 0.35 ms. The minimum TE for the Trio repeatability protocol was 0.23 ms, as the data for this study had already been acquired. The *in vivo* protocols were used for repeatability and reproducibility studies in the liver, described in Chapter 6.

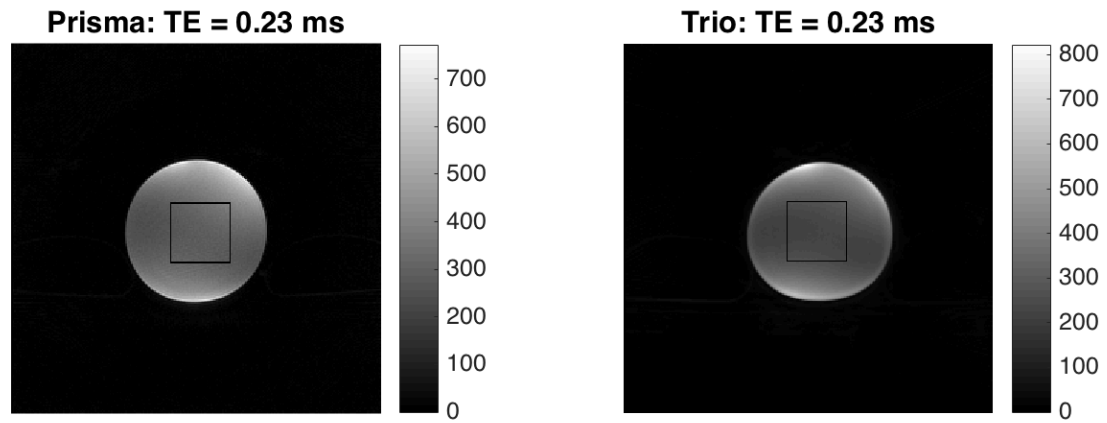


Fig. 5.31. UTE images at TE = 0.23 ms in the long- T_2 phantom, with 40 x 40 ROIs.

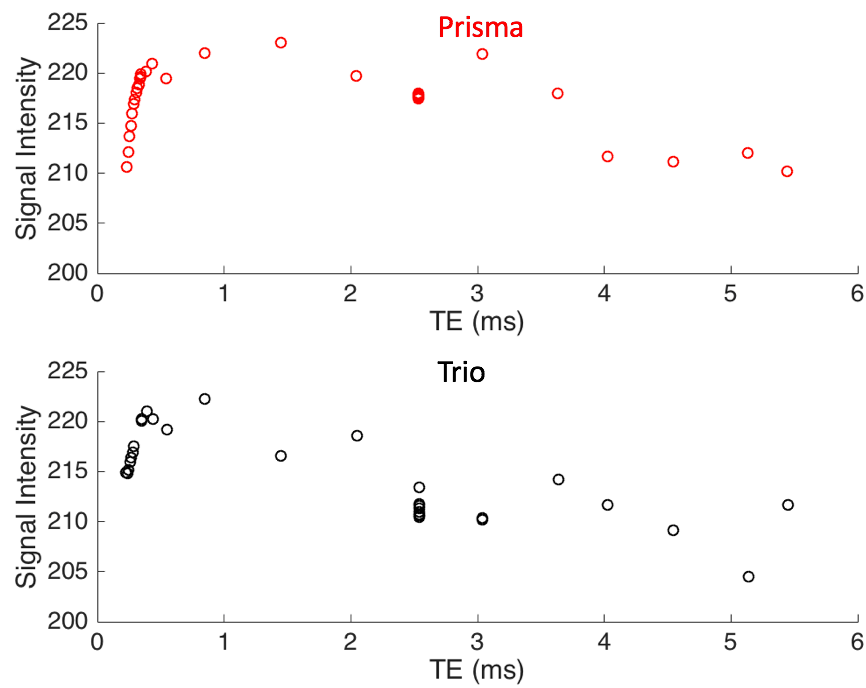


Fig. 5.32. Signal intensity in the long- T_2 phantom vs. TE.

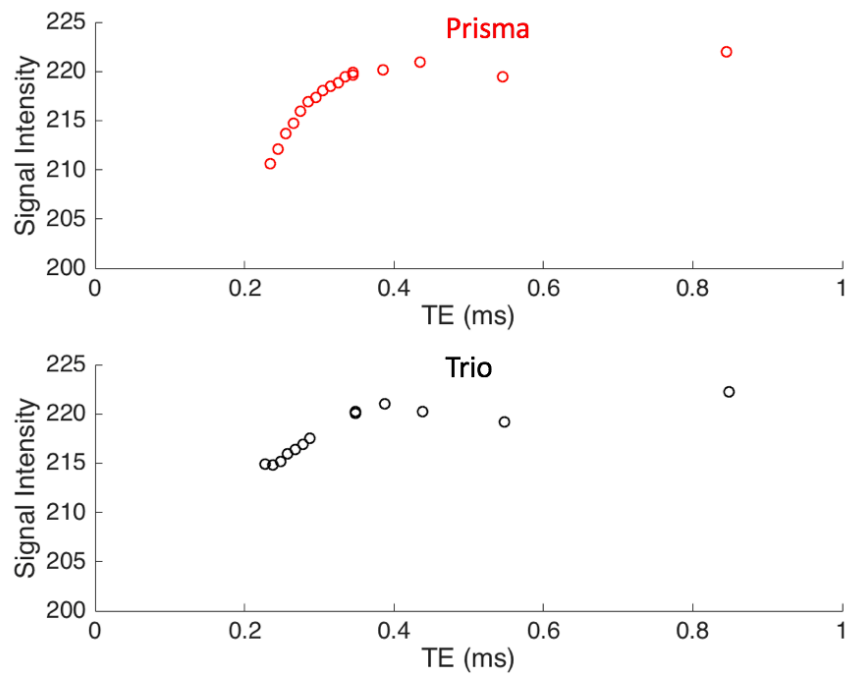


Fig. 5.33. Signal intensity in the long- T_2 phantom vs. TE, showing the shortest TEs.

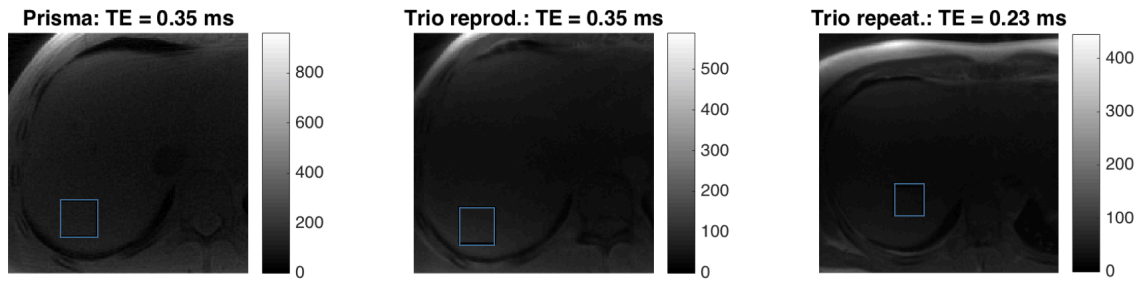


Fig. 5.34. UTE images in the *in vivo* liver using three different protocols. 20 x 20 ROIs are outlined in blue. Note the rightmost image appears smaller because the image was cropped to a larger size for image registration.

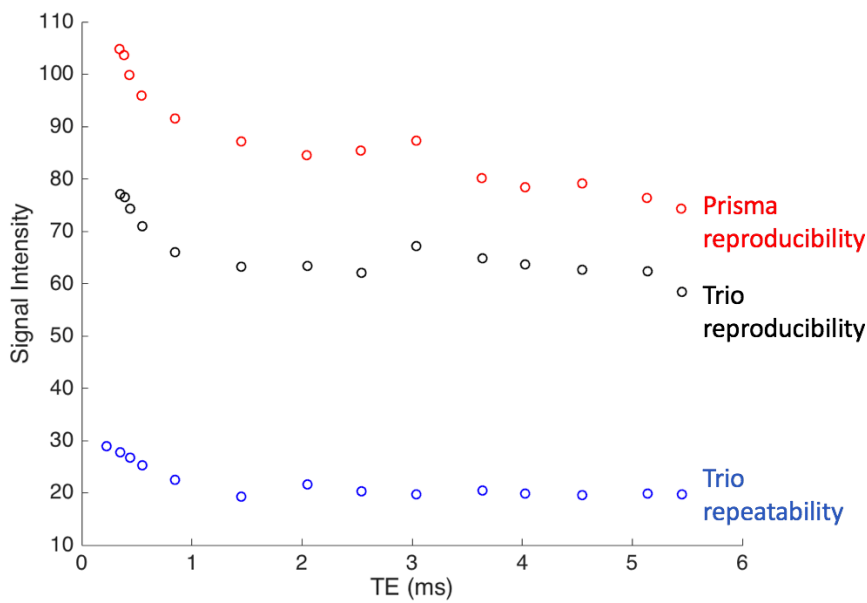


Fig. 5.35. Signal intensity in the liver ROI with the three different protocols.

5.3.4. Conclusion

Based on the validation in the long- T_2 phantom, the minimum TE required for non-artefactual signal was 0.35 ms. This was applied to *in vivo* protocols in the liver, to be described in Chapter 6.

5.4. Summary

In summary, this chapter devised the following optimisations on the Trio 3 T:

1. Gradient spoiling was removed to include TEs up to 5.52 ms, while using a short TR of 7 ms to minimise the acquisition length.
2. A double-echo sequence was used to halve the number of acquisitions from a single-echo sequence. For 14 TEs, this resulted in seven acquisitions, as opposed to 14 acquisitions.
3. Spatial saturation was used for suppression of out-of-slice signal, including that arising from lipids. For cardiac imaging, spatial saturation consisting of two bands (thickness = 110 mm, gap to imaging slice = 10 mm) resulted in a 33 % decrease in lipid signal, while preserving the collagen signal.

On the Prisma 3 T, optimisation was as follows:

1. The bandwidth on the Prisma sequence was increased to approximately twice the bandwidth on the Trio sequence, to match the gradient strengths on the Trio sequence. The final bandwidth on the Prisma sequence was 1950 Hz/pixel, compared to 980 Hz/pixel on the Trio sequence.
2. The minimum TE for obtaining non-artefactual signal was determined to be 0.35 ms on the Prisma sequence. The use of different coil set-ups did not improve signal consistency at TEs < 0.35 ms. The protocol on the Prisma was updated to reflect this TE and the Trio protocol was revised; these were named the Prisma reproducibility and Trio reproducibility protocols after studies in Chapter 6. The Trio protocol with a minimum of TE of 0.23 ms was kept as the Trio repeatability protocol for a repeatability study.

The protocols on the Trio and Prisma 3 T were validated in *in vivo* imaging of the healthy liver. These protocols were applied in Chapter 6 for assessing the repeatability and reproducibility of the short- T_2^* signal fraction in the healthy liver.

5.5. References

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5.6. Appendix

For the modelling of the short- T_2^* signal in the septum, the following equation was used, accounting for complex signal from isotropic collagen, free water, and six or two lipid peaks:

$$S(TE) = \{ \exp [i(2\pi f_g TE + \varphi_g)] \} \left(\begin{aligned} & S_{0c} \int_0^\pi \sin \theta \exp [-TE \cdot R_{2c}^*(\theta)] \cdot \exp [i2\pi f_c(\theta)TE] d\theta \\ & + S_{0l} \exp(-TE \cdot R_{2l}^*) \cdot \exp(i\varphi_l) \cdot \sum_{p=1}^{6 \text{ or } 2} S_{0p} \exp(i2\pi f_p TE) \\ & + S_{0w} \exp(-TE \cdot R_{2w}^*) \end{aligned} \right)$$

For the purposes of this chapter, suffice it to say that an R_2^* signal model was used to fit cardiac data, yielding the collagen and lipid signal fractions as the important parameters. The collagen and lipid signal fractions were calculated as follows:

$$\text{Collagen signal fraction} = \frac{S_{0c}}{S_{0c} + S_{0l} + S_{0w}} \times 100 \%$$

$$\text{Lipid signal fraction} = \frac{S_{0l}}{S_{0c} + S_{0l} + S_{0w}} \times 100 \%$$

Justifications for the model of isotropic collagen and free water and the model of six lipid peaks are provided in Sections 6.2.2 and 5.1.4.2. As this chapter addresses cardiac R_2^* data with significant lipid contamination, the lipid peaks had to be modelled. Table A5.1 lists the four fixed parameters and eight fitted parameters in the function.

Fixed or fitted parameter	Parameter symbol	Parameter name	Fixed parameter value
Fixed (4 parameters)	$R_{2c}^*(\theta)$	Relaxation rate of collagen	$R_{2c}^*(0^\circ) = 1256 \text{ s}^{-1}$ $R_{2c}^*(55^\circ) = 100 \text{ s}^{-1}$ See Eq. [4.3]
	$f_c(\theta)$	Resonant frequency shift of collagen	$A = -0.36 \text{ ppm}$ $B = 0.67 \text{ ppm}$ See Eq. [6.4]
	S_{0p}	Initial signal intensity of p^{th} lipid peak	See Table 5.4.
	R_{2w}^*	Relaxation rate of free water	14 s^{-1}
Fitted (8 parameters)	S_{0c}	Initial signal intensity of collagen	--
	S_{0l}	Initial signal intensity of lipids	--
	R_{2l}^*	Relaxation rate of lipids	$R_{2l}^* = R_{2l,ref} + R_{2l,fit}$ See Table 5.4 for $R_{2l,ref}$ values.
	φ_l	Phase of lipids	--
	f_p	Resonant frequency shift of p^{th} lipid peak	$f_p = f_{shift \text{ rel. to largest peak},p}$ $+ f_{fit, largest \text{ peak}}$ See Table 5.4 for $f_{shift \text{ rel. to largest peak},p}$ values.
	S_{0w}	Initial signal intensity of free water	--
	f_g	Global resonant frequency shift	--
	φ_g	Global phase	--

Table A5.1. Fixed and fitted parameters in the model.

6. Modelling the Short- T_2^* Signal in the Liver with UTE

6.1. Introduction

The objective of this chapter was to model the short- T_2^* (fast- R_2^*) signal in the liver. Particularly, the suitability of an R_2^* model of isotropic collagen and free water was investigated. Scanning was performed in healthy subjects and liver-disease patients, with the UTE pulse sequence modified for *in vivo* imaging described in Chapter 5. The efficacy of various R_2^* signal models in fitting the R_2^* data was evaluated. With the most suitable model, the signal fraction and R_2^* of the fast- R_2^* component were characterised. In healthy subjects, the intra-scanner repeatability and inter-scanner reproducibility of the fast- R_2^* signal fraction were assessed. Differences in R_2^* signal properties between healthy subjects and patients were evaluated.

6.2. Models

6.2.1. Introduction to Signal Models

It was hypothesised that the R_2^* signal in the liver can be described by one of the following models:

- A. Mono-exponential
- B. Bi-exponential
- C. Bi-exponential with lipids
- D. Isotropic collagen and free water

The constituents giving rise to fast- R_2^* signal may either be in fast exchange, representing one signal exponential component (Model A), or in slow exchange with one or several slow- R_2^* components, representing distinct exponential components (Models B - D). In the case of the fast- R_2^* signal originating from isotropic collagen, the R_2^* signal can be described by a model of isotropic collagen and free water in the healthy liver. The theory on isotropic collagen will be presented, followed by the theory on other exponential models.

6.2.2. Model of Isotropic Collagen and Free Water

An R_2^* model of isotropic collagen and free water was developed, assuming that the short- T_2^* signal in the liver originates from isotropic collagen in the intermediate to slow exchange regime. Section 4.3.2 described the theory on the diffusion and R_2^* of water associated with collagen in phantoms. The R_2^* signal observed supported the hypothesis that the water in collagen phantoms is in a regime of fast exchange. However, it is unknown whether water associated with collagen in the liver is also in fast exchange, exhibiting one signal component decaying exponentially. With intermediate to slow exchange, the distinct signal components can be modelled as isotropic collagen and free water.

Section 3.2.3.1 described the histopathology of liver fibrosis, where the collagen fibres are not oriented. Isotropic distributions of collagen fibres have previously been described in muscle tissue (1,2) and used in the modelling of mechanical properties of collagen (3). This isotropic distribution was applied to collagen in the liver.

Consider an isotropic distribution of collagen fibres starting from the centre and oriented uniformly around the surface of a sphere.

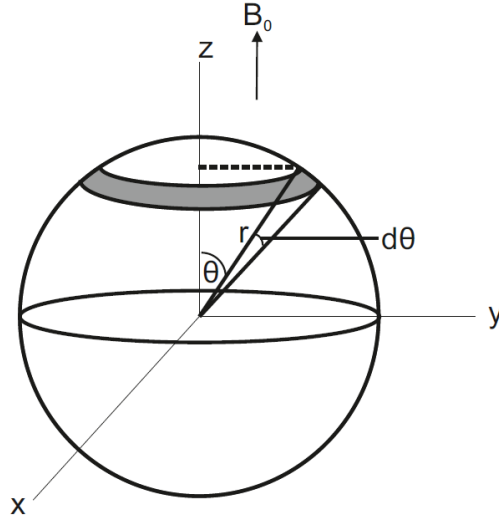


Fig. 6.1. Diagram of isotropic collagen. 3D representation of isotropic collagen along a sphere of radius r , where a collagen fibre is angled at θ relative to B_0 .

Following from Fig. 6.1, let a collagen fibre be angled at θ relative to the static magnetic field (B_0). As described in Chapter 3, oriented collagen (tendon) obeys the magic angle effect, whereby the R_2^* and resonant frequency shift are dependent on the orientation of the tendon with respect to B_0 . The total signal from all collagen fibres is weighted by the number of collagen fibres at angle θ . The area of the region occupied by the collagen fibres is given by:

$$Area = 2\pi \cdot r \sin \theta \cdot r d\theta. \quad [6.1]$$

One can verify that $2\pi \int_0^\pi r^2 \sin \theta d\theta = 4\pi r^2$, which is the surface area of a sphere.

The normalised density of the fibres is:

$$\rho(\theta) = \frac{\sin \theta}{2}. \quad [6.2]$$

Hence, the isotropic collagen signal is:

$$S_{c,isotropic}(TE) = S_{0c} \int_0^\pi \frac{\sin \theta}{2} \exp[-TE \cdot R_{2c}^*(\theta)] \cdot \exp[i2\pi f_c(\theta)TE] d\theta, \quad [6.3]$$

where $f_c(\theta)$ and $R_{2c}^*(\theta)$ denote the resonant frequency and R_2^* relaxation rate associated with the collagen fibres at angle θ . From (4), the resonant frequency shift of collagen (in Hz) is:

$$f_c(\theta) = \left(\frac{\gamma B_0}{2\pi}\right) [A(3\cos^2\theta - 1) + B], \quad [6.4]$$

where A is the change in magnetic susceptibility (in ppm) and B is the offset frequency (in ppm). Eq. [6.4] is equivalent to Eq. [4.4]. $R_{2c}^*(\theta)$ is given by Eq. [4.3]. S_{0c} is the signal intensity due to all collagen fibres at $TE = 0$. The isotropic collagen signal is similar to the signal of a powder spectrum, describing the aggregate signals of crystallites at various orientations relative to B_0 (5).

Furthermore, there is a signal component from free water, i.e. water that is associated with hepatocytes but not with isotropic collagen.

Hence, the MRI signal in the liver, derived from magnitude data, can be modelled by:

$$|S(TE)| = \left| S_{0c} \int_0^\pi \frac{\sin \theta}{2} \exp[-TE \cdot R_{2c}^*(\theta)] \cdot \exp[i2\pi f_c(\theta)TE] d\theta + S_{0w} \exp(-TE \cdot R_{2w}^*) \right|. \quad [6.5]$$

The equation accounts for signal intensities due to isotropic collagen (S_{0c}) and free water (S_{0w}). Here, the liver is assumed to have low fat signal, which applies to healthy subjects. A magnitude signal model was preferred over a complex signal model, to reduce the number of parameters to be determined through fitting.

The parameters determined by fitting the MRI signal in the liver to Eq. [6.5] are: S_{0c} , S_{0w} and R_{2w}^* .

The fixed parameters in Eq. [6.5], which are in bold, are: $f_c(\theta)$ and $R_{2c}^*(\theta)$. $f_c(\theta)$ is given by Eq. [6.4], with $A = -0.36 \pm 0.02$ ppm and $B = 0.67 \pm 0.02$ ppm. $R_{2c}^*(\theta)$ is given by Eq. [4.3], with $R_{2c}^*(0^\circ) = 950 \pm 30$ s⁻¹ and $R_{2c}^*(55^\circ) = 130 \pm 20$ s⁻¹. These parameters were determined through measurements in *ex vivo* tendon, as described in Chapter 4.

The collagen signal fraction is calculated as follows:

$$\text{Collagen signal fraction} = \frac{S_{0c}}{S_{0c} + S_{0w}} \times 100 \%. \quad [6.6]$$

This provides a measure of the amount of collagen, based on the proton densities of the signal components of the liver.

6.2.3. Other Signal Models in the Liver

Considering that the short- T_2^* signal in the liver can be derived from constituents other than isotropic collagen, there are various signal models for magnitude data that are feasible. Furthermore, lipid content increases with disease, which can be modelled. For instance, there is ~ 4 % fat in the healthy liver (6), increasing to ~ 12 % in non-alcoholic fatty liver disease (NAFLD) (7). To account for the possible constituents in the liver, the following signal models were considered:

- A. Assume that all liver constituents are in fast exchange, including the short- T_2^* signal component. This is the R_2^* signal model used in the collagen phantoms from

Section 4.3, although the source of the short- T_2^* signal in the liver may not necessarily be collagen. One can model the signal as a mono-exponential function:

$$|S(TE)| = |S_0 \exp(-TE \cdot R_2^*)|. \quad [6.7]$$

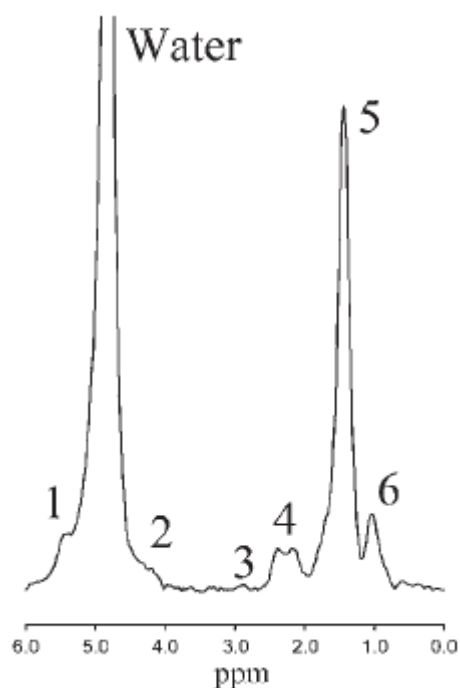
B. Alternatively, assume that two main liver constituents are in slow exchange, giving rise to distinct signal components. This consists of a fast-decaying R_2^* (f) or short- T_2^* component, of which the signal source is to be determined. There is also a slow-decaying R_2^* (s) or long- T_2^* component, arising from free water associated with hepatocytes. One can model the signal as a bi-exponential function:

$$|S(TE)| = |S_{0f} \exp(-TE \cdot R_{2f}^*) + S_{0s} \exp(-TE \cdot R_{2s}^*)|. \quad [6.8]$$

C. Assuming slow exchange, one can further introduce signal components due to lipids. Adding lipids (l) to Eq. [6.8], one has:

$$|S(TE)| = |S_{0f} \exp(-TE \cdot R_{2f}^*) + S_{0s} \exp(-TE \cdot R_{2s}^*) + S_{0l} \exp(-TE \cdot R_{2s}^*) \cdot \sum_{p=1}^6 S_{0p} \exp(i2\pi f_p TE)|. \quad [6.9]$$

The R_2^* of fat is set to the R_2^* of the slow component (R_{2s}^*) for higher fitting accuracy (8). Moreover, the frequency shift (f_p) and signal intensity (S_{0p}) of each lipid peak p are taken from *in vivo* measurements in human liver (9). Fig. 6.2 demonstrates the *in vivo* lipid model used, from Hamilton et al. 2011 (9).



Peak number	Peak name	Chemical shift relative to tetramethylsilane (ppm)	T_2 (ms)	% of total triglyceride signal (S_{0p})
1	Olefinic and glycerol	5.3	--	4.7
--	Water	4.7	23	--
2	Glycerol	4.2	--	3.9
3	Diacyl	2.75	51	0.6
4	Alpha-carboxyl and alpha-olefinic	2.1	52	12.0
5	Beta-carboxyl and methylene	1.3	62	70.0
6	Methyl	0.9	83	8.8

Fig. 6.2. Proton MR spectrum of a patient with fatty-liver disease at 3 T (9). Copyright 2010 John Wiley & Sons, Ltd. Adapted with permission from Wiley. The MR spectrum was measured *in vivo* with the stimulated echo acquisition mode (STEAM) sequence at TE = 10 ms. Triglyceride peaks 1 - 6 are described in the table.

The MR spectrum of lipids consists of six triglyceride peaks, of which 70 % of the signal originates from the beta-carboxyl and methylene groups. The frequency shift (f_p) is converted from the chemical shift (in ppm) using the actual imaging frequency. The T_2 is not used in the model.

6.2.4. Summary of the Signal Models

The suitability of the above signal models for describing the liver R_2^* signal was assessed. Fig. 6.3 graphically illustrates the signal models discussed so far, with arbitrary values assigned in Table 6.1. Essentially, the mono-exponential function (Model A) demonstrates the behaviour of a signal with a low R_2^* of 20 s^{-1} , without a distinct fast- R_2^* component. The bi-exponential function (Model B) illustrates the signal when there is a fast R_2^* of $2,500 \text{ s}^{-1}$ ($T_2^* = 0.4 \text{ ms}$) at 30 % of the total signal, in addition to the slow R_2^* of 20 s^{-1} ($T_2^* = 50 \text{ ms}$) at 70 % of the total signal. Lipids cause oscillations in the signal decay, as evidenced in the bi-exponential function with lipids (Model C), with 4 % lipid signal. Collagen has a lower relaxation rate of $R_2^*(0^\circ) = 950 \text{ s}^{-1}$ and $R_2^*(55^\circ) = 130 \text{ s}^{-1}$, causing slower signal decay in the short-TE regime in the isotropic collagen and free water function (Model D). Models A - D were subsequently applied to R_2^* signal data in healthy subjects.

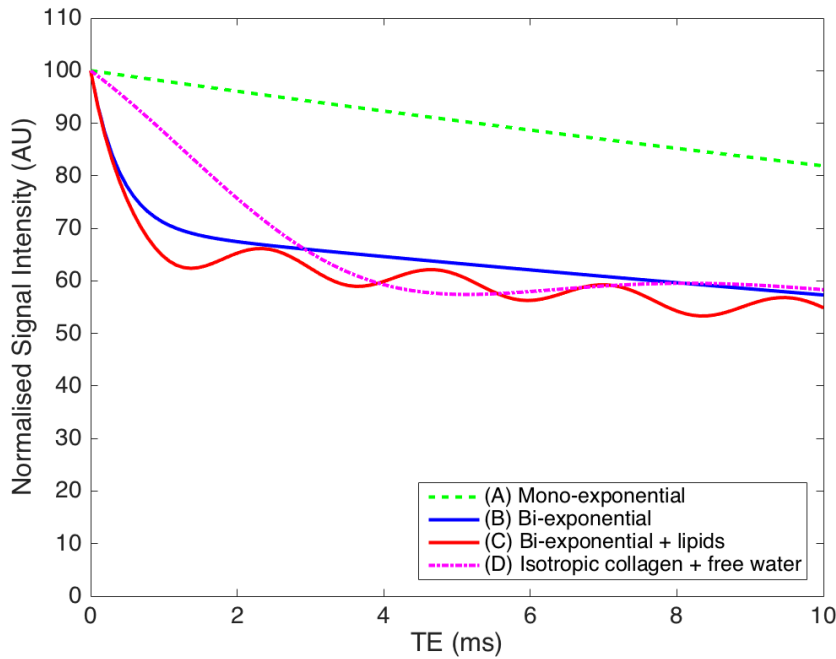


Fig. 6.3. R_2^* signal models in the liver.

	$S_0 = S_{0f} = S_{0c}$ (AU)	R_{2f}^* (s ⁻¹)	$S_{0s} = S_{0w}$ (AU)	S_{0l} (AU)	$R_2^* = R_{2s}^* = R_{2w}^*$ (s ⁻¹)
(A) Mono-exponential	100	--	--	--	20
(B) Bi-exponential	30	2,500	70	--	20
(C) Bi-exponential + lipids	30	2,500	66	4	20
(D) Isotropic collagen + free water	30	--	70	--	20

Table 6.1. Assumptions for signal models, based on normalised signal intensities. (--) denote parameters that are not applicable to the model.

6.3. Methods

6.3.1. Data Acquisition

Three studies were performed to measure liver R_2^* signal in healthy subjects (Studies 1 - 2) and liver-disease patients (Study 3). The suitability of Models A - D was assessed; the most appropriate model was used to evaluate the intra-scanner repeatability and inter-scanner reproducibility of the fast- R_2^* signal fraction. Finally, the fast- R_2^* signal was compared between healthy subjects and liver-disease patients.

6.3.1.1. Study 1: Repeatability of the Fast- R_2^* Signal Fraction in Healthy Subjects on the Trio 3 T

Ten healthy subjects (seven males, age = 29 ± 5 years) consented to the study and underwent two UTE experiments (“test” and “retest”) on the Trio 3 T (Siemens Healthineers, Erlangen, Germany), as demonstrated in Fig. 6.4. Each session consisted of eight dual-echo UTE acquisitions, with the sequence parameters specified in Table 6.2. A transversal slice was localised along the portal vein. The first seven acquisitions were used for signal decay analysis; saturation bands were applied for the suppression of signal outside of the slice, arising from poor slice selection. The last acquisition was performed

without saturation pulses. This was done to increase contrast between the liver tissue and vessels, facilitating the drawing of an ROI at TE = 13.49 ms in a non-vascular area. The UTE pulse sequence modified for *in vivo* imaging on the Trio was specified in Chapter 5. Between each test and retest, the scanner bed was brought to the home position, the coil was removed and the volunteer was allowed to move freely. Volunteers were instructed to refrain from alcohol consumption and strenuous exercise starting from the day before the scan, to refrain from caffeine consumption on the day of the scan, to fast for at least four hours before the scan, and to not drink liquids starting from at least one hour before the scan. Such requirements were specified to ensure that the livers of the healthy subjects were in similar metabolic states.

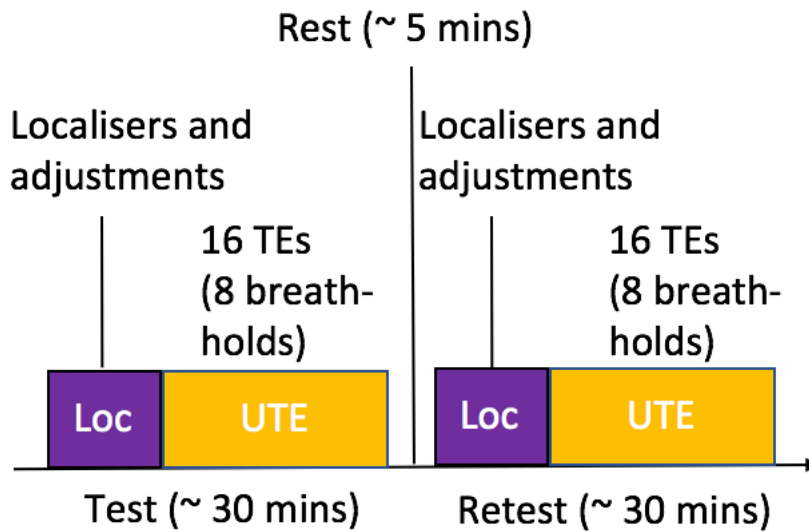


Fig. 6.4. Design for the Trio repeatability study (Study 1).

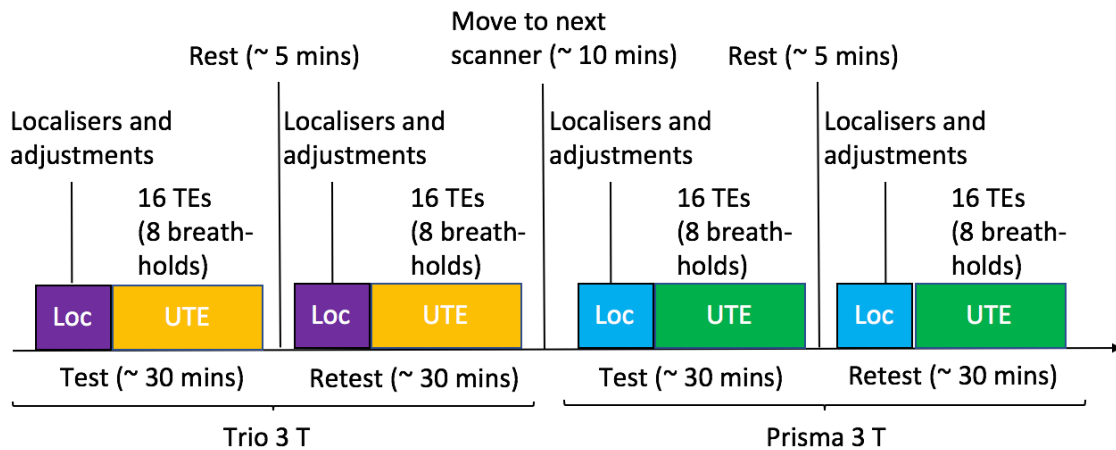
UTE scan parameters		Trio 3 T							
Coil		32-channel torso array coil (In-vivo, Florida, USA)							
Image orientation		Transversal							
Repetition time (ms)		7							
Flip angle (°)		10							
Number of averages		2							
Slice thickness (mm)		8							
Field-of-view (mm x mm)		300 x 300							
Matrix size		256 x 256							
Bandwidth (Hz/pixel)		980							
Segmentation		60 readouts/heartbeat							
Saturation bands		2 saturation bands; 10 mm gap; 110 mm thickness							
Single- or dual-echo acquisition		Dual							
Number of TEs		16 (8 dual-echo acquisitions)							
TEs (ms)	Acquisition number	1	2	3	4	5	6	7	8: No sat. bands (**)
	First interleaf TE (ms)	0.23	0.35	0.44	0.55	0.85	1.45	2.05	0.23
	Second interleaf TE (ms)	2.54	3.04	3.64	4.03	4.55	5.14	5.45	13.49
Scan time per acquisition (s)		~ 10 (breath-hold duration)							

Table 6.2. UTE scan parameters for the Trio repeatability study (Study 1). Note that the last acquisition without saturation bands (**) was at a TR of 15 ms, which was a ~19-second breath-hold.

6.3.1.2. Study 2: Reproducibility of the Fast- R_2^* Signal Fraction in Healthy Subjects on the Trio and Prisma 3 T

Ten healthy subjects (six males, age = 31 ± 5 years) consented to the study and were placed in one of two groups. The groups were conceived to minimise bias from the order the experiments were performed. Group 1 (three males, two females, age = 31 ± 5 years) underwent the UTE test and retest experiments on the Trio 3 T, followed by the UTE test and retest experiments on the Prisma 3 T (Siemens Healthineers, Erlangen, Germany). Group 2 (three males, two females, age = 31 ± 7 years) underwent the test and retest on the Prisma, followed by the test and retest on the Trio. Fig. 6.5 demonstrates the timelines for the two groups. In accordance with the guidelines for patients, volunteers were instructed to fast for at least four hours before the scan and to not drink more than 0.5 L of water in the hour preceding the scan. Transverse slices were localised at approximately the level of T10/11 along the vertebrae.

Group 1



Group 2

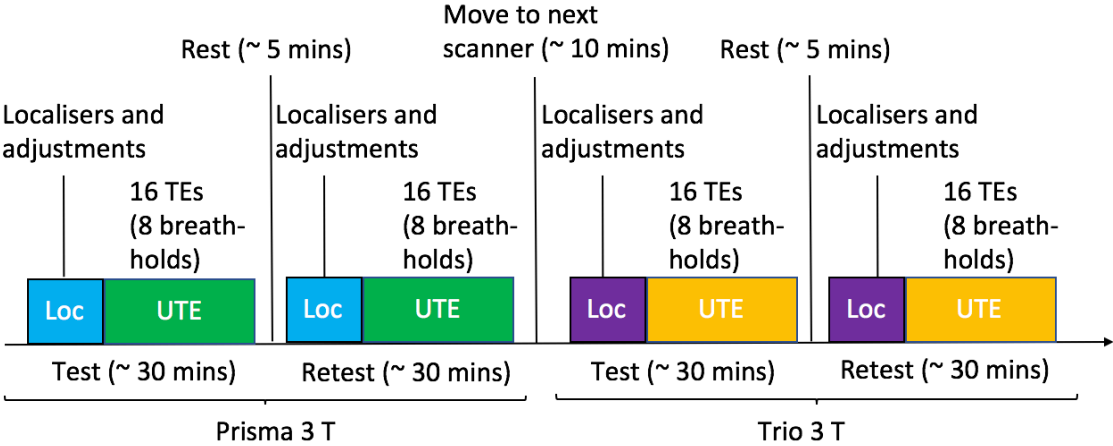


Fig. 6.5. Design for the Trio-Prisma reproducibility study (Study 2).

The UTE sequences on the Trio 3 T and Prisma 3 T were unchanged from Chapter 5. The parameters for both scanners are described in Table 6.3, involving eight dual-echo acquisitions.

UTE scan parameters		Trio 3 T								Prisma 3 T							
Study		Reproducibility study								Reproducibility study and patient study							
Coil		32-channel torso array coil (Invivo, Florida, USA)								18-channel torso array coil and 32-channel spinal array coil (Siemens Healthineers, Erlangen, Germany)							
Image orientation		Transversal								Transversal							
Repetition time (ms)		7								7							
Flip angle (°)		10								10							
Number of averages		2								2							
Slice thickness (mm)		8								8							
Field-of-view (mm x mm)		300 x 300								300 x 300							
Matrix size		256 x 256								256 x 256							
Bandwidth (Hz/pixel)		980								1950							
Segmentation		60 readouts/heartbeat								60 readouts/heartbeat							
Saturation bands		2 saturation bands; 10 mm gap; 110 mm thickness								2 saturation bands; 10 mm gap; 110 mm thickness							
Single- or dual-echo acquisition		Dual								Dual							
Number of TEs		16 (8 dual-echo acquisitions)								16 (8 dual-echo acquisitions)							
TEs (ms)	Acquisition number	1	2	3	4	5	6	7	8: No sat. bands (**)	1	2	3	4	5	6	7	8: No sat. bands (**)
	First TE (ms)	0.23	0.35	0.44	0.55	0.85	1.45	2.05	0.23	0.23	0.35	0.44	0.55	0.85	1.45	2.05	0.23
	Second TE (ms)	2.54	3.04	3.64	4.03	4.55	5.14	5.45	13.49	2.54	3.04	3.64	4.03	4.55	5.14	5.45	13.49
Scan time per acquisition (s)		~ 10 (breath-hold duration)								~ 12 (breath-hold duration)							

Table 6.3. UTE scan parameters for the Trio-Prisma reproducibility study (Study 2) and Prisma patient study (Study 3). Note that the acquisition without saturation bands () was a ~ 20-second breath-hold, with a TR of 15 ms.**

6.3.1.3. Study 3: Patients with Liver Disease on the Prisma 3 T

Seven patients with liver disease were recruited from the John Radcliffe Hospital (Oxford, UK) from February to August 2019 and gave written informed consent. The study was approved by the institutional review board and the UK National Research Ethics Service. The ethical guidelines of the 1975 Declaration of Helsinki were followed. Table 6.4 summarises the patient characteristics, of which four patients had liver cirrhosis. The fasting and water instructions, coil set-up and scan protocol were followed from the reproducibility study. The flow diagram is in Fig. 6.6 and the UTE sequence parameters are in Table 6.3.

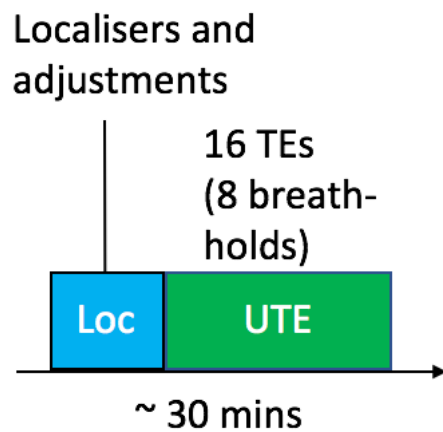


Fig. 6.6. Design for the Prisma patient study (Study 3).

Total number of patients	7	
Number of male patients	3	
Age (mean $\pm$ standard deviation; years)	63 $\pm$ 11	
BMI (mean $\pm$ standard deviation; kg/m ²)	30 $\pm$ 4	
Diagnosis (number of patients)	Liver cirrhosis Relating to:	4
	• Autoimmune hepatitis	1
	• Alcohol consumption	2
	• Non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH)/alcoholic steatohepatitis (ASH)	1
	Wilson's disease and steatosis	1
	NAFLD	1
	Unknown	1

Table 6.4. Patient characteristics for the Prisma patient study (Study 3).

6.3.2. Analysis Workflow

6.3.2.1. Overview of the Analysis Workflow

Fig. 6.7 summarises the workflow for the selection of the most appropriate R_2^* signal model and the respective analyses in healthy subjects and liver-disease patients. Firstly, Models A - C were used to fit all healthy-subject and patient datasets using a fixed initial-condition approach. Using the corrected Akaike Information Criterion (AIC_c), the most appropriate signal model was determined. To further improve the AIC_c , the R_2^* of the fast component was fixed to the value that minimises the AIC_c . Secondly, the most suitable signal model (with fixed R_{2f}^*) was compared to Model D in healthy subjects; the initial conditions of the fitted parameters were randomised according to a Monte Carlo approach. This two-step process of model selection was devised, as the Monte Carlo approach was lengthy and calculation-intensive. Hence, the Monte Carlo approach was reserved for exhaustive testing of the model of isotropic collagen and free water against the most suitable of Models A - C, to be satisfied that the solutions were independent of the initial conditions of the fitted parameters.

The preferred signal model was used to evaluate the intra-scanner repeatability and inter-scanner reproducibility of the fast- R_2^* signal fraction in healthy livers. The R_2^* signal properties of the liver were compared between the healthy subjects and liver-disease patients.

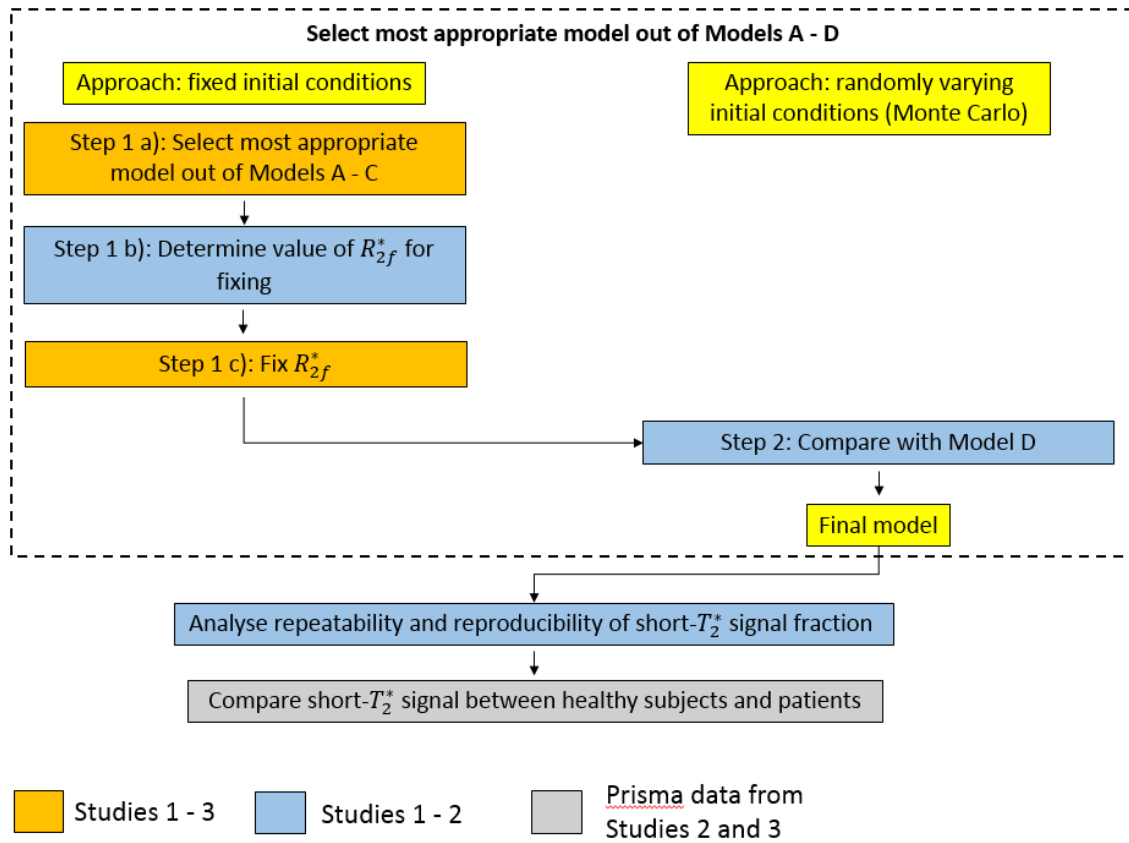


Fig. 6.7. Analysis workflow.

6.3.2.2. Data Processing

Images were cropped around the liver to facilitate image registration. Rigid body image registration was performed to correct motion between TEs via the “imregister” function in MATLAB R2015b. The registration was configured with the one-plus-one evolutionary optimizer and Mattes’ mutual information metric. For each session, the reference image was the image at the lowest TE, acquired with saturation pulses. The first echo in each of the remaining seven acquisitions was registered to the reference image and the same transformation matrix was applied to the second echo. For each subject dataset, a 20-pixel $\times$ 20-pixel ROI was drawn on the image at TE = 13.49 ms, in the posterior region of the liver, avoiding vascular regions and image artefacts. The ROI was cloned for the seven acquisitions to be used in the signal decay analysis.

6.3.2.3. Model Selection: Step 1

For all subject datasets, the averaged signal within the ROI was graphed as a function of TE and modelled using functions A - C, via a trust-region-reflective nonlinear least-squares algorithm (MATLAB R2015b). The initial conditions are included in Section 6.8.1 of the Appendix. The AIC_c allows one to compare the performance of models in fitting a given dataset, by balancing the goodness of fit with the number of parameters in each model. The model with the lowest AIC_c is preferred. The AIC_c was calculated as follows (10–12):

$$AIC_c = n \cdot \ln \left(\frac{RSS}{n} \right) + 2k + \frac{2k(k+1)}{n-k-1}, \quad [6.10]$$

where n is the sample size, RSS is the residual sum of squares and k is the total number of estimated parameters in the model, including the RSS .

The likelihood of the i^{th} model being the best model is given by the Akaike weight w_i (12):

$$w_i = \frac{\exp \left(-\frac{1}{2} \Delta_i \right)}{\sum_{r=1}^R \exp \left(-\frac{1}{2} \Delta_r \right)}, \quad [6.11]$$

for R models. The AIC_c difference is (12):

$$\Delta_i = AIC_{c,i} - AIC_{c,min}, \quad [6.12]$$

where $AIC_{c,min}$ pertains to the AIC_c of the best model. Note that the relative value of the AIC_c is important, in particular the AIC_c difference.

After determining the best model via the AIC_c , it was investigated whether the AIC_c improves when the R_2^* of the fast component is fixed. As the origin of the short- T_2^* signal is unknown, the most appropriate R_2^* value was found by minimising the AIC_c via a fitting approach. Only the healthy-subject datasets were considered for analysis, to minimise changes in the R_2^* of the fast component (R_{2f}^*) between healthy and diseased groups and to reduce confounds due to lipid signal. Lipid signal was avoided, to prevent the model from allocating the short- T_2^* signal to the six-component lipid model. In total, six healthy-subject experiments were performed: the Trio repeatability test, Trio repeatability retest, Trio reproducibility test, Trio reproducibility retest, Prisma reproducibility test and Prisma reproducibility retest. For each experiment, the R_{2f}^* value to be fixed was determined by calculating the AIC_c averaged over all subjects (denoted the mean AIC_c) as a function of the fixed R_{2f}^* value for each experiment. The mean AIC_c vs. fixed R_{2f}^* curves were summed over all six experiments and the R_{2f}^* associated with the minimum mean AIC_c was taken. The best model with fixed R_{2f}^* was applied to the healthy-subject and patient datasets.

6.3.2.4. Model Selection: Step 2

The objective of this step was to determine if the short- T_2^* signal in the liver can be described by isotropic collagen. The most suitable model from Step 1 was compared against the model of isotropic collagen and free water. Healthy-subject data from Studies 1 - 2 were used, given the low lipid signal. Each model was fitted to the signal data according to a Monte Carlo approach. For each subject dataset, $n = 50$ iterations were performed to fit the R_2^* signal decay. At each iteration, the initial conditions of the fitting parameters were varied randomly according to a uniform distribution with a specified

range. This ensured that the solution found by the fitting algorithm was the global minimum, rather than a local minimum dependent on the initial conditions. The initial conditions are provided in Section 6.8.2 of the Appendix. The residual sum of squares was calculated at the end of each iteration. After all 50 iterations were complete, the solution with the lowest residual sum of squares was taken, as this is the objective of least-squares minimisation. The goodness of fit of each model was evaluated with the AIC_c and Akaike weights, as described above.

6.3.2.5. *Analysis with the Appropriate Models*

The intra-scanner repeatability and inter-scanner reproducibility of the fast- R_2^* signal fraction were assessed in the healthy subjects from the Trio repeatability and Trio-Prisma reproducibility studies. The analysis included:

- (1) The Trio repeatability study: test vs. retest experiments
- (2) The Trio-Prisma reproducibility study: Trio test vs. Trio retest experiments
- (3) The Trio-Prisma reproducibility study: Prisma test vs. Prisma retest experiments
- (4) The Trio-Prisma reproducibility study: Trio test vs. Prisma test experiments

The most suitable model for the healthy subjects was used for analysis, i.e. the bi-exponential function with fixed $R_{2f}^* = 2130 \text{ s}^{-1}$, as determined from the “Model Selection” steps. Bland-Altman and correlation plots were generated. The coefficient of repeatability or coefficient of reproducibility (CR) was calculated as $1.96 \times$ standard deviation of the difference in the fast- R_2^* signal fraction between two experiments. The 95 % confidence intervals were determined as the mean difference $\pm$ CR, referring to the mean difference in the fast- R_2^* signal fraction between two experiments. The correlations of the fast- R_2^*

signal fraction between two experiments were investigated; using the “fitlm” function, the slope, intercept and coefficient of determination (R^2) were calculated from a linear regression model and p -values were generated from the t -test (MATLAB R2015b).

Signal properties between healthy subjects and patients was compared using two models: the bi-exponential function with fixed $R_{2f}^* = 2130 \text{ s}^{-1}$ and lipids, and the bi-exponential function with unfixed R_{2f}^* and lipids. The former model was the most suitable model for the patients, as assessed in the “Model Selection” steps. The latter model was used by Zhu et al. (13) and enabled comparison with their results. The Prisma retest experiment from the Trio-Prisma reproducibility study was used for the healthy-subject data ($n = 10$), due to its low mean AIC_c . For patient data ($n = 7$), the Prisma patient study was used. A two-tailed Wilcoxon rank-sum test was performed to evaluate the differences between the two groups, with $\alpha = 0.05$ (MATLAB R2015b).

6.4. Results

6.4.1. Model Selection: Step 1

Fig. 6.8 illustrates example UTE images at the lowest TE for Studies 1 - 3. The signal intensities within the ROIs were averaged for R_2^* signal analysis.

Fig. 6.9 demonstrates how the models performed in a healthy subject and patient. Overall, the most appropriate models were the bi-exponential function for the healthy subject, and the bi-exponential function with lipids for the patient. In the healthy subject, the bi-exponential function with lipids performed similarly to the bi-exponential function in the healthy subject. However, the inclusion of lipids increased the number of parameters in the model, rendering the model with lipids less appropriate. The Appendix

includes the fits for all subjects and values for the slow component, where appropriate. The sections in the Appendix are grouped by model.

From Fig. 6.9, the mono-exponential function did not fit the first three TEs (≤ 0.44 ms), which give rise to the fast- R_2^* signal. By contrast, the bi-exponential function accounted for signal from the fast- R_2^* component. Results for the fast component are shown in Fig. 6.10. For the bi-exponential function with lipids, the lipid signal fractions for patients were higher than for healthy subjects ($\leq 15\%$ vs. $\leq 3\%$); this resulted in the oscillations in the R_2^* signal decays in Fig. 6.9. Values for the fast and lipid components are shown in Fig. 6.11 and Fig. 6.12, respectively.

Table 6.5 reports the mean AIC_c s and Akaike weights for Studies 1 - 3, fitted with the three models. For each healthy-subject experiment, the lowest mean AIC_c was obtained with the bi-exponential model, with a mean Akaike weight ranging from 0.69 to 0.90. For the patient experiment, the bi-exponential model with lipids had the lowest mean AIC_c of 35.19, with a mean Akaike weight of 0.71. Hence, the bi-exponential model was most suitable for the healthy subjects, and the bi-exponential model with lipids was most suitable for the patients, out of the models tested.

The mean AIC_c as a function of the fixed R_{2f}^* in the bi-exponential model was plotted for the six experiments in healthy subjects (Fig. A6.13). The summative mean AIC_c over the six experiments is in Fig. 6.13. R_{2f}^* was fixed to the minimum R_{2f}^* of 2132.50 s^{-1} (rounded = 2130 s^{-1}) in subsequent analyses involving the bi-exponential model and the bi-exponential model with lipids.

Fig. 6.14 shows how the models with fixed $R_{2f}^* = 2130 \text{ s}^{-1}$ performed in a healthy subject and patient. The fits in the healthy subject were virtually identical. In the patient, the model with lipids accounted for the oscillations in signal.

As observed in Fig. 6.15, fixing R_{2f}^* resulted in lower uncertainties in the signal fraction and R_2^* . The results for the fast and lipid components are in Fig. 6.16 and Fig. 6.17, respectively. Lower uncertainties in each fitted parameter were achieved by fixing R_{2f}^* .

The mean AIC_c s and Akaike weights for the models with fixed R_{2f}^* are summarised in Table 6.6. For the healthy-subject experiments, the bi-exponential model with fixed R_{2f}^* had the lowest mean AIC_c . The mean Akaike weights ranged from 0.87 to 0.93. For the patient experiment, the bi-exponential model with lipids and fixed R_{2f}^* had the lowest mean AIC_c of 29.24. The mean Akaike weight was 0.74. Hence, the most suitable models for the healthy subjects and patients were the bi-exponential model with fixed R_{2f}^* , and the bi-exponential model with lipids and fixed R_{2f}^* , respectively. For further information, the per-subject mean AIC_c s and Akaike weights are reported in Section 6.8.1.7 of the Appendix.

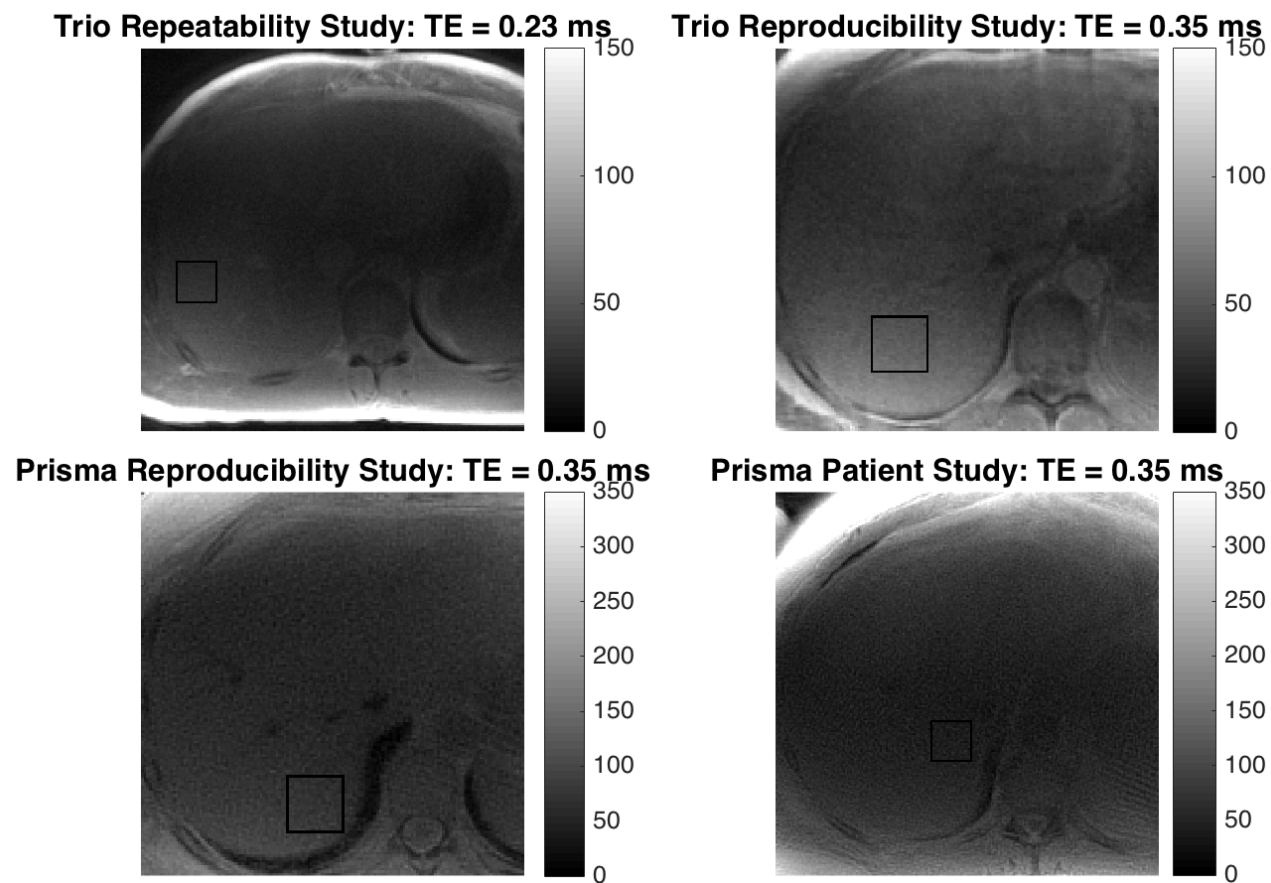


Fig. 6.8. Example UTE images at the lowest TE for the three studies. The two images from the reproducibility study are from the same subject. The 20-pixel $\times$ 20-pixel ROI in the posterior region of the liver is outlined. Each image has been cropped around the liver, resulting in different image dimensions and ROI magnifications.

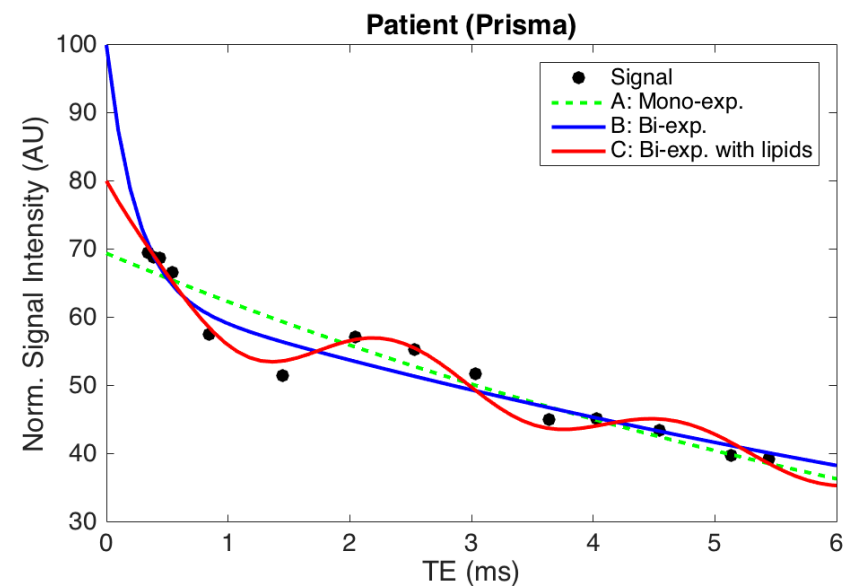
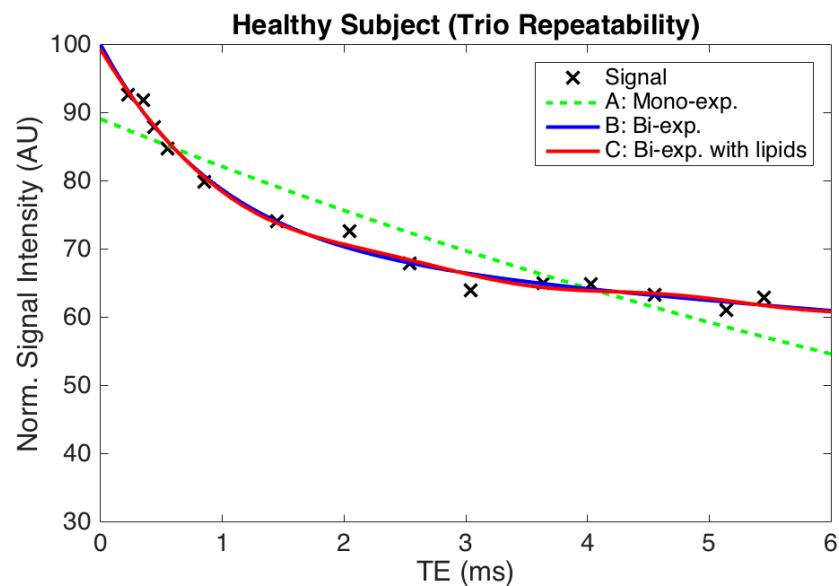


Fig. 6.9. Example fits in a healthy subject and patient. The healthy subject was Subject 8 from the retest in the Trio repeatability study. The patient was Subject 3 from the Prisma patient study. Refer to the Appendix for the fits for all subjects and models.

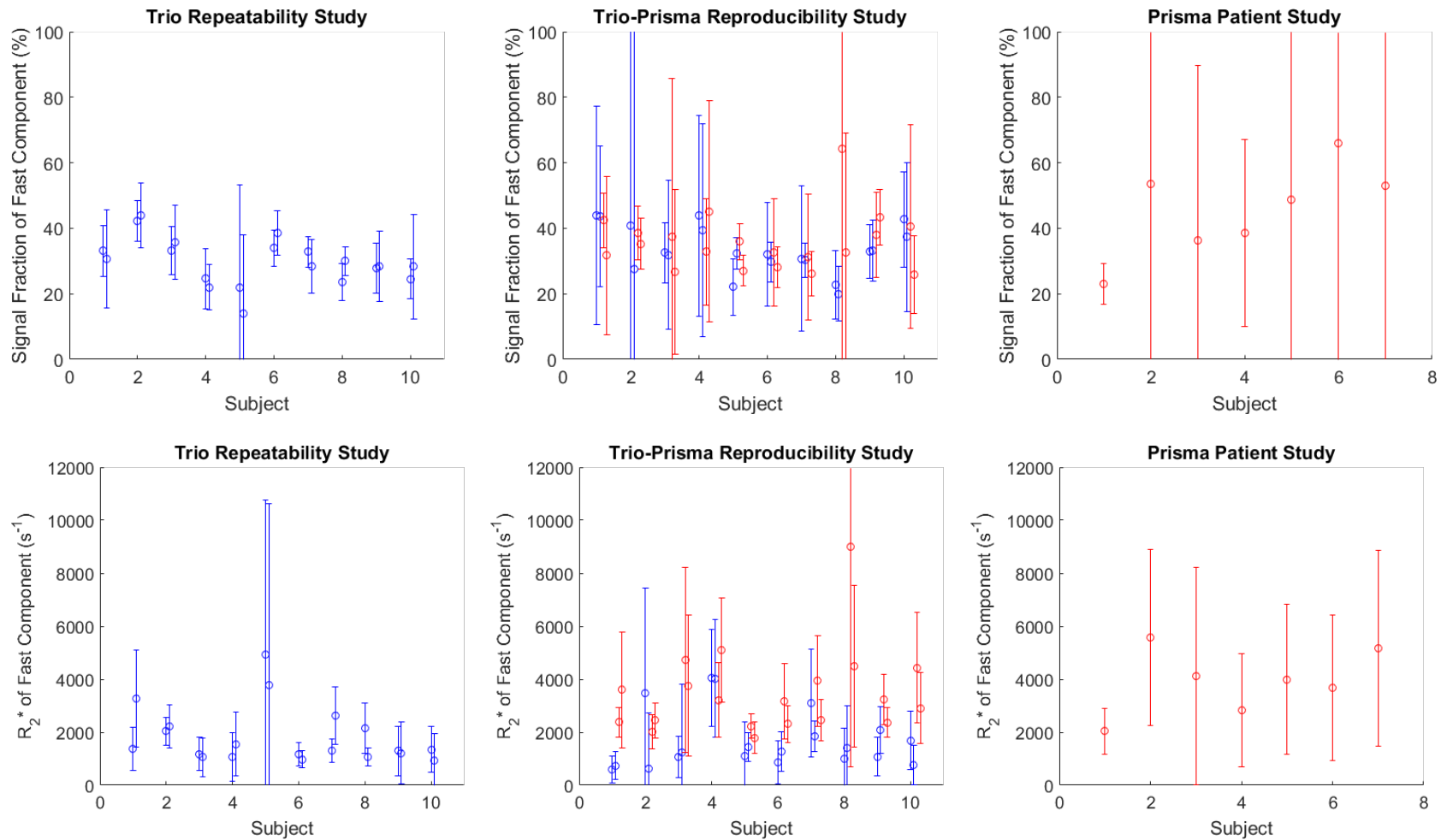


Fig. 6.10. Fast-component results for signal decays fitted with a bi-exponential function (Model B: Eq. [6.8]). For the Trio repeatability study, the data for each subject are ordered: Trio test, Trio retest. For the Trio-Prisma reproducibility study, the data for each subject are ordered: Trio test, Trio retest, Prisma test, Prisma retest. The Trio data are in blue and the Prisma data are in red.

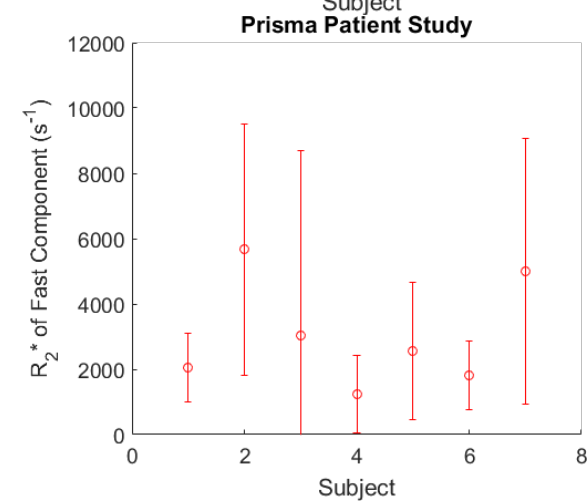
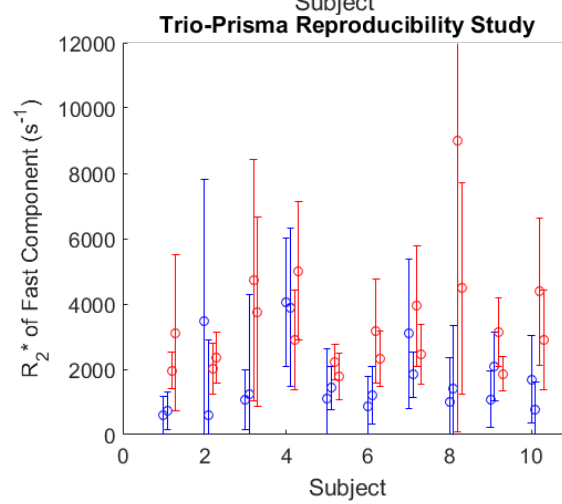
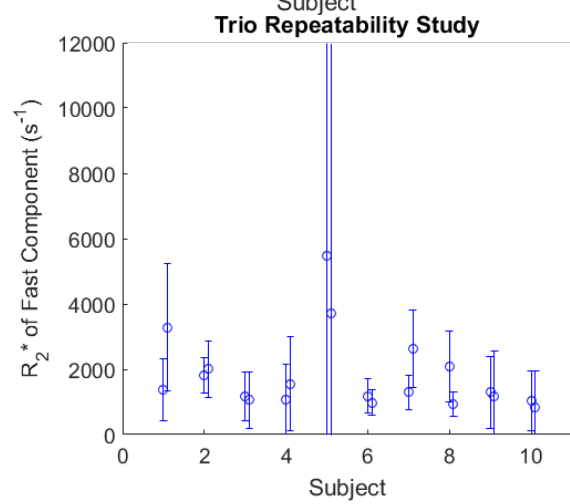
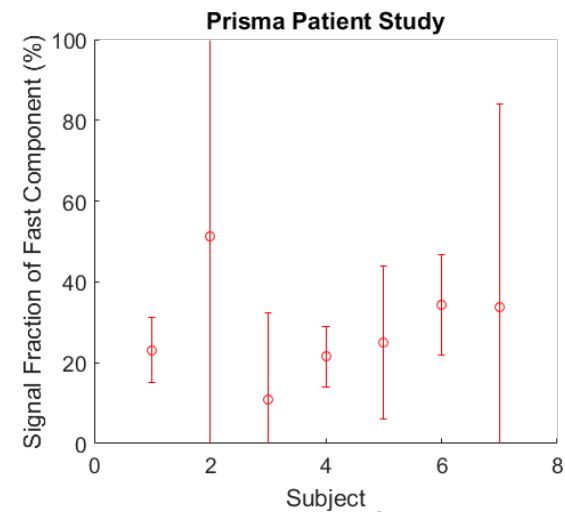
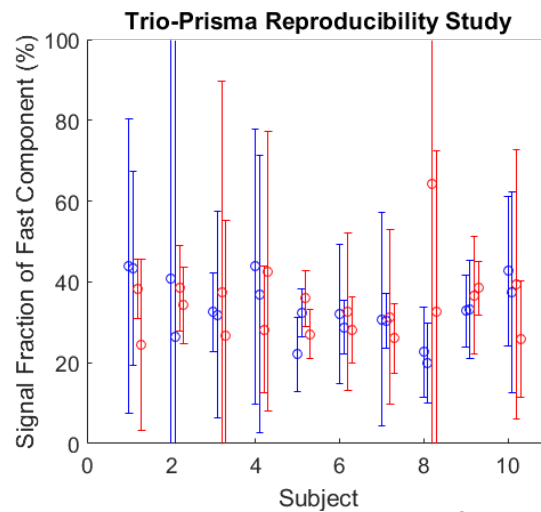
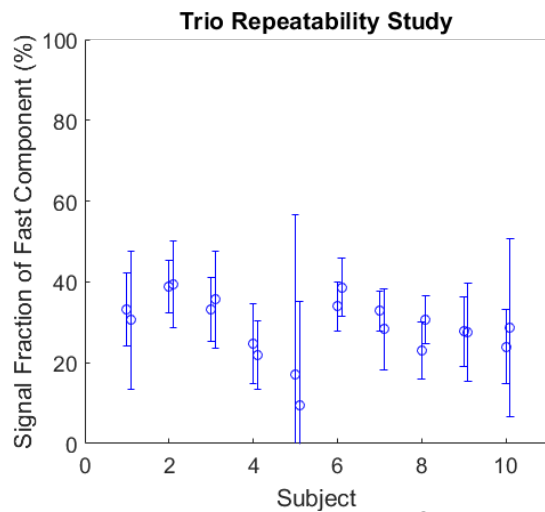


Fig. 6.11. Fast-component results for signal decays fitted with a bi-exponential function with lipids (Model C: Eq. [6.9]). For the Trio repeatability study, the data for each subject are ordered: Trio test, Trio retest. For the Trio-Prisma reproducibility study, the data for each subject are ordered: Trio test, Trio retest, Prisma test, Prisma retest. The Trio data are in blue and the Prisma data are in red.

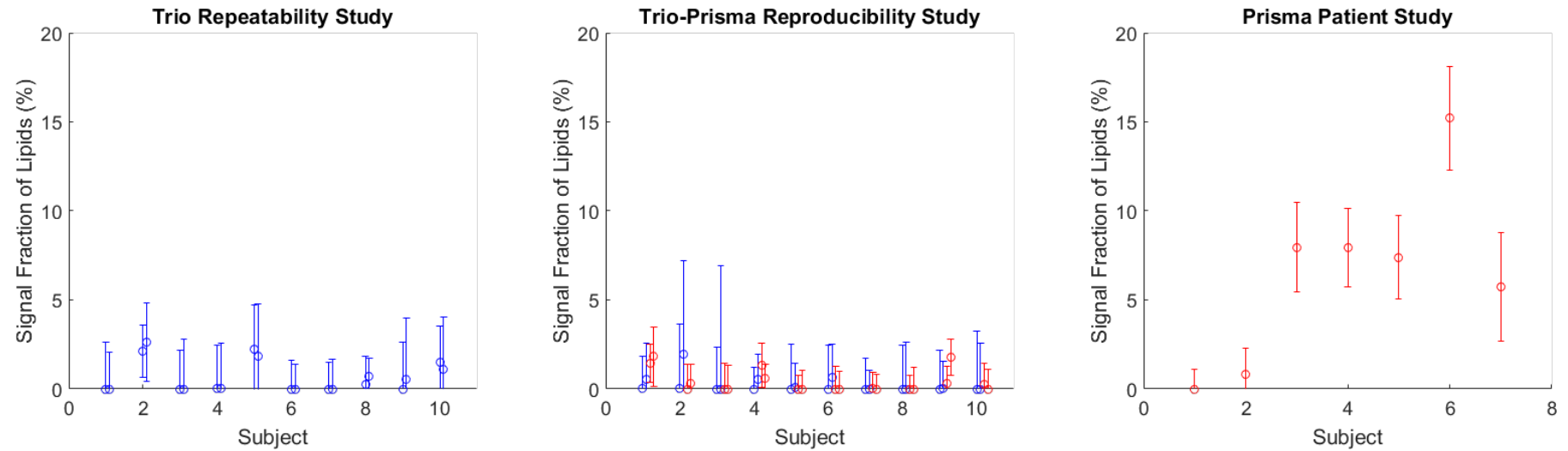


Fig. 6.12. Lipid results for signal decays fitted with a bi-exponential function with lipids (Model C: Eq. [6.9]).

Study	Exp't	Mean AIC _c			Mean Akaike weight		
		A: Mono-exp.	B: Bi-exp.	C: Bi-exp. with lipids	A: Mono-exp.	B: Bi-exp.	C: Bi-exp. with lipids
1: Trio repeat.	Test	28.68	19.68	25.65	0.14	0.82	0.05
	Retest	28.50	21.34	27.46	0.24	0.72	0.04
2: Trio-Prisma reprod.	Trio test	39.82	35.50	42.00	0.22	0.75	0.03
	Trio retest	39.85	32.80	39.21	0.28	0.69	0.03
	Prisma test	45.26	30.37	36.41	0.10	0.85	0.05
	Prisma retest	41.53	26.54	32.38	0.04	0.90	0.06
3: Prisma patients	N/A	44.78	41.19	35.19	0.02	0.27	0.71

Table 6.5. Goodness-of-fit values for all experiments. The mean AIC_c was taken over the R_2^* signal decays for all subjects in a given experiment. The AIC_cs and Akaike weights were derived from fitting the signal decay for each subject; these were calculated according to Eqs. [6.10] and [6.11].

Trio Repeatability and Trio-Prisma Reproducibility Studies

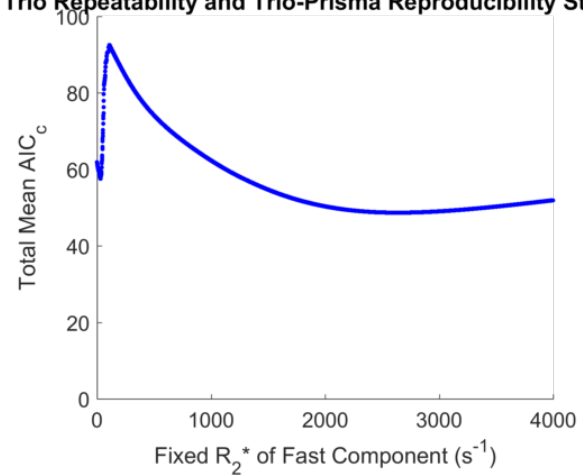


Fig. 6.13. Mean AIC_c curves summed over the six experiments from Fig. A6.13. The minimum of the curve was reached at $R_{2f}^* = 2132.50 \text{ s}^{-1}$ (rounded = 2130 s^{-1}).

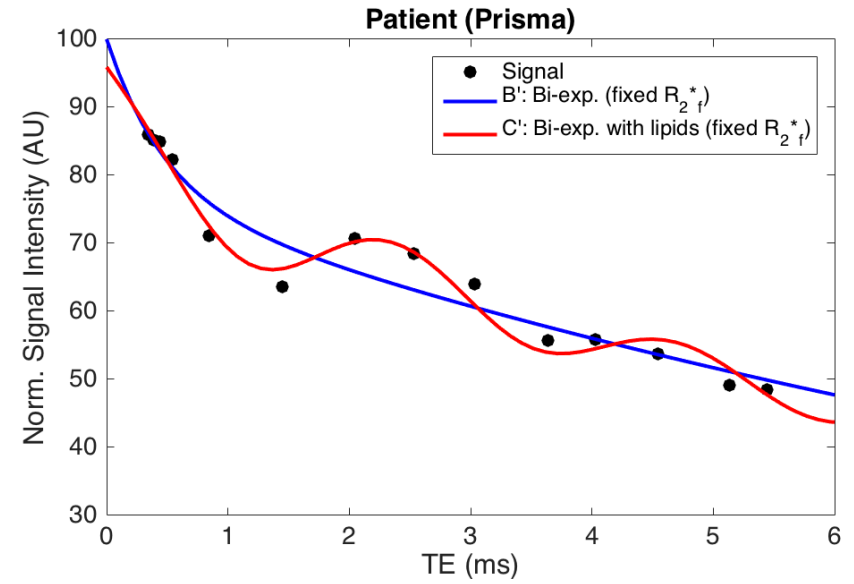
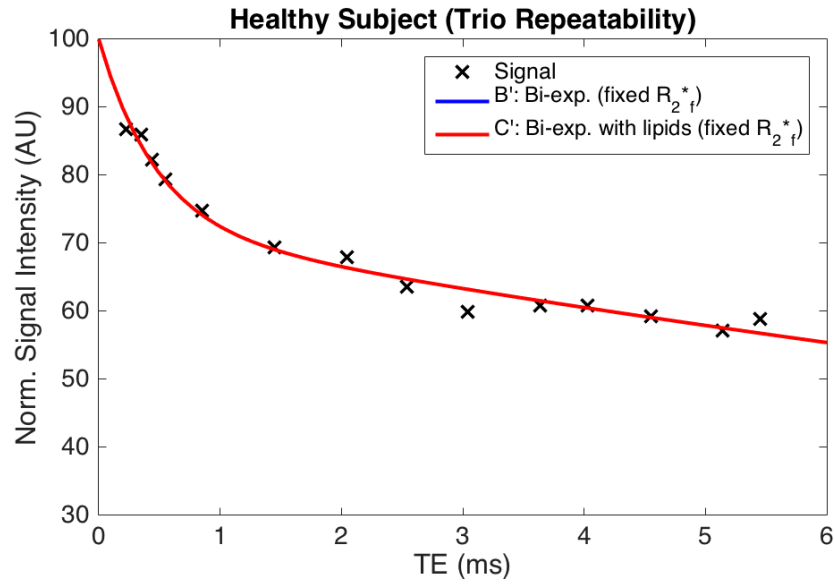


Fig. 6.14. Example fits in a healthy subject and patient. Models with fixed $R_{2f}^* = 2130 \text{ s}^{-1}$ were used. Note that the models overlap for the healthy subject. The healthy subject was Subject 8 from the retest in the Trio repeatability study. The patient was Subject 3 from the Prisma patient study. Refer to the Appendix for the fits for all subjects.

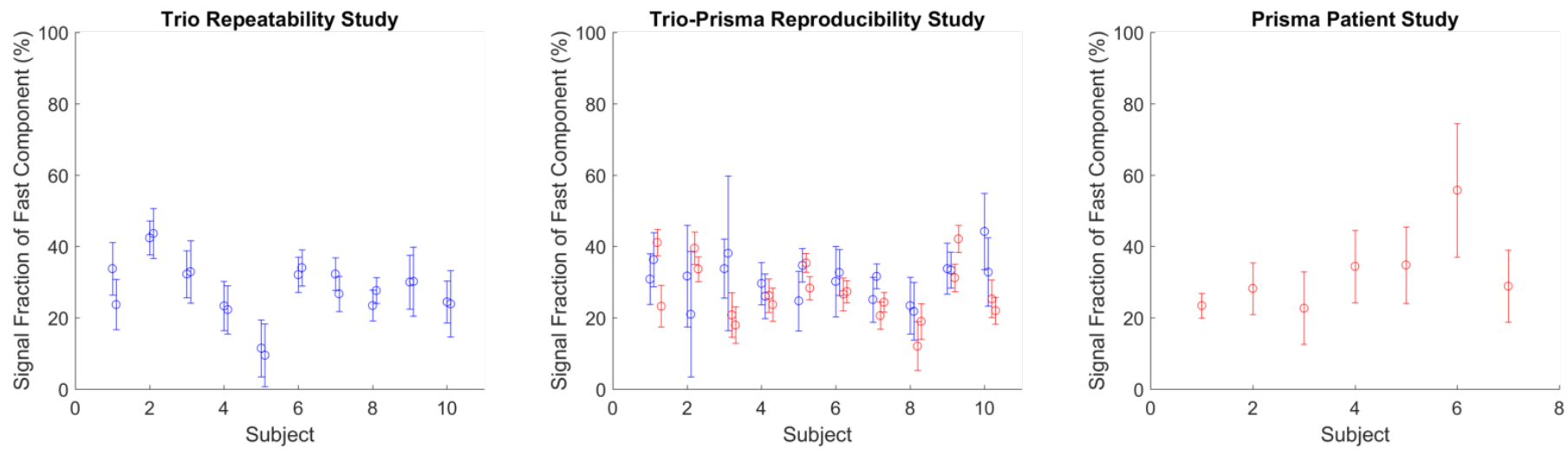


Fig. 6.15. Fast-component results for signal decays fitted with a bi-exponential function (Model B: Eq. [6.8]) with $R_{2f}^* = 2130 \text{ s}^{-1}$.

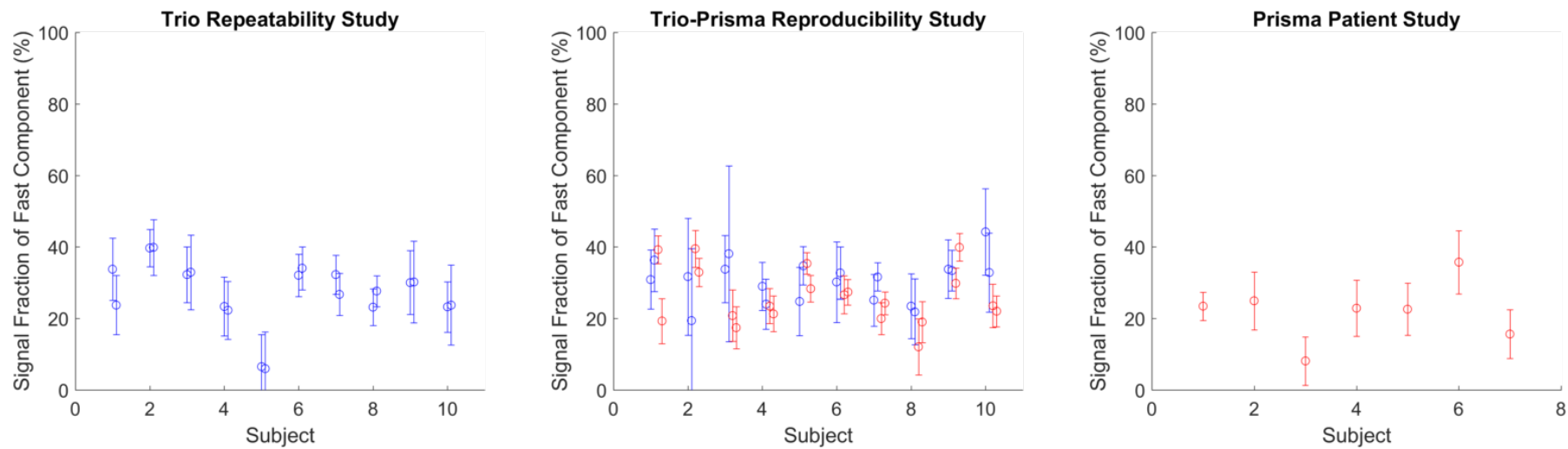


Fig. 6.16. Fast-component results for signal decays fitted with a bi-exponential function with lipids (Model C: Eq. [6.9]), with $R_{2f}^* = 2130 \text{ s}^{-1}$.

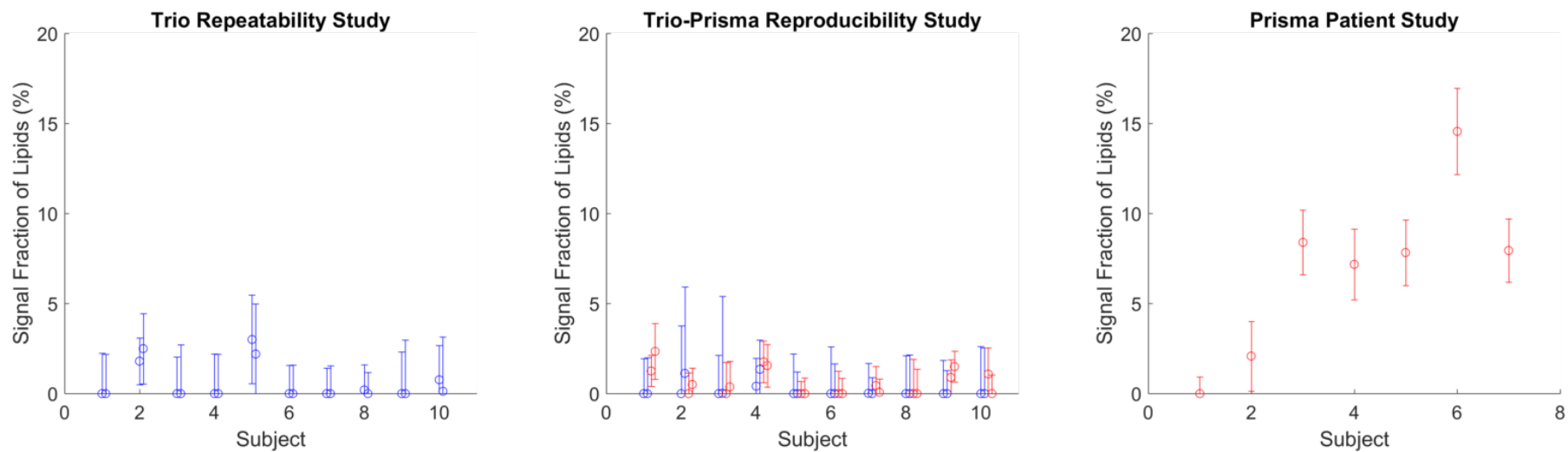


Fig. 6.17. Lipid results for signal decays fitted with a bi-exponential function with lipids (Model C: Eq. [6.9]), with $R_{2f}^* = 2130 \text{ s}^{-1}$.

Study	Exp't	Mean AIC _c		Mean Akaike weight	
		B': Bi-exp. (fixed R_{2f}^*)	C': Bi-exp. (fixed R_{2f}^*) with lipids	B': Bi-exp. (fixed R_{2f}^*)	C': Bi-exp. (fixed R_{2f}^*) with lipids
1: Trio repeat.	Test	16.01	20.60	0.90	0.10
	Retest	18.29	23.05	0.91	0.09
2: Trio-Prisma reprod.	Trio test	32.02	37.06	0.93	0.07
	Trio retest	28.90	33.85	0.92	0.08
	Prisma test	27.03	31.32	0.88	0.12
	Prisma retest	22.65	26.76	0.87	0.13
3: Prisma patients	N/A	37.55	29.24	0.26	0.74

Table 6.6. Goodness-of-fit values for experiments with models with fixed $R_{2f}^* = 2130 \text{ s}^{-1}$.

6.4.2. Model Selection: Step 2

Fig. 6.18 illustrates the performance of the bi-exponential function with fixed $R_{2f}^* = 2130 \text{ s}^{-1}$ and the function for isotropic collagen and free water. A Monte Carlo approach was used to randomly vary the initial conditions of the fit. The isotropic collagen and free water function did not fit the fast- R_2^* signal, characterised by TEs $< 1 \text{ ms}$. The fits for all healthy subjects and results for the slow/free-water components are found in Section 6.8.2 of the Appendix.

The results for the fast component, as fitted with the bi-exponential function with fixed R_{2f}^* , are displayed in Fig. 6.19. The results were identical to those when a fixed initial-condition approach to fitting was used (Fig. 6.11). The collagen signal fraction, associated with the model of isotropic collagen and free water, did not account for the fast- R_2^* signal (Fig. A6.27).

The bi-exponential R_2^* model with fixed $R_{2f}^* = 2130 \text{ s}^{-1}$ was more suitable than the model of isotropic collagen and free water. The mean AIC_cs and Akaike weights for the two models are summarised in Table 6.7. The per-subject mean AIC_cs and Akaike weights are reported in Table A6.12, for further information. For each experiment, the bi-exponential R_2^* model with fixed $R_{2f}^* = 2130 \text{ s}^{-1}$ had the lowest mean AIC_c, with a mean Akaike weight ranging from 0.80 to 0.99. Furthermore, the bi-exponential R_2^* model with fixed $R_{2f}^* = 2130 \text{ s}^{-1}$, when fitted with a Monte Carlo approach, yielded the same mean AIC_c values as in Step 1, when a fixed initial-condition approach was used (Table 6.6). Hence, the fixed initial-condition approach was sufficient.

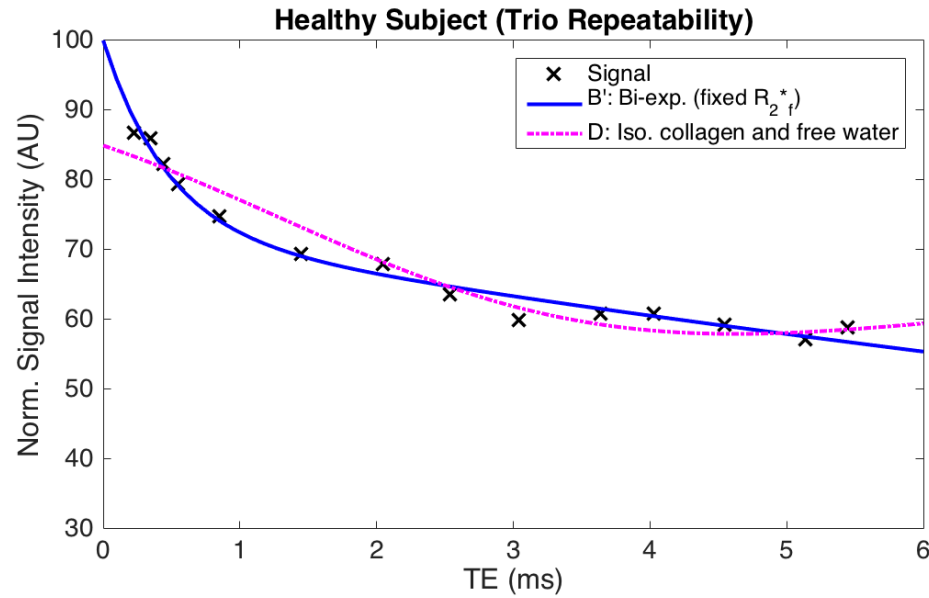


Fig. 6.18. Example fits in a healthy subject and patient, with the Monte Carlo approach. For the bi-exponential function, R_{2f}^* was fixed to 2130 s^{-1} . The healthy subject was Subject 8 from the retest in the Trio repeatability study. Refer to the Appendix for the fits for all subjects.

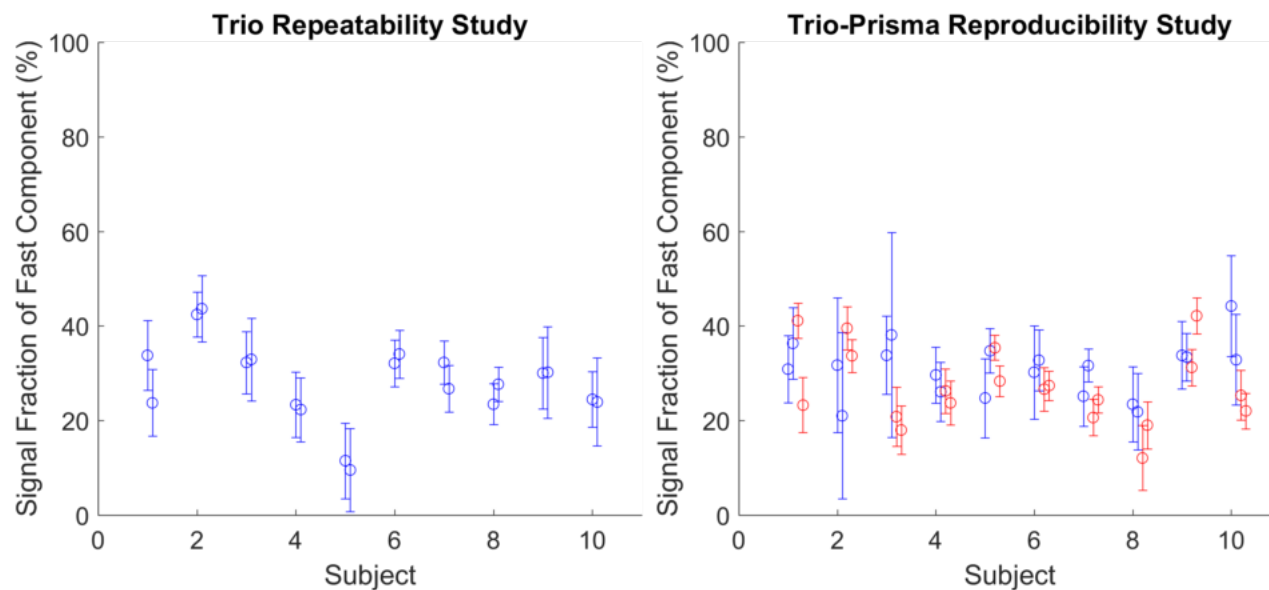


Fig. 6.19. Fast-component results for signal decays fitted with a bi-exponential function (Model B: Eq. [6.8]) with $R_{2f}^* = 2130 \text{ s}^{-1}$. A Monte Carlo approach was used for fitting.

Study	Exp't	Mean AIC _c		Mean Akaike weight	
		B': Bi-exp. (fixed)	D: Iso. coll. and water	B': Bi-exp. (fixed)	D: Iso. coll. and water
1: Trio repeat.	Test	16.01	25.91	0.95	0.05
	Retest	18.29	27.07	0.90	0.10
2: Trio-Prisma reprod.	Trio test	32.02	37.46	0.81	0.19
	Trio retest	28.90	36.79	0.80	0.20
	Prisma test	27.03	44.37	0.98	0.02
	Prisma retest	22.65	39.62	0.99	0.01

Table 6.7. Goodness-of-fit values for the healthy-subject experiments, fitted with a Monte Carlo approach.

6.4.3. Analysis with the Appropriate Models

The Bland-Altman and correlation plots relating to the intra-scanner repeatability and inter-scanner reproducibility of the fast- R_2^* signal fraction in healthy subjects are shown in Fig. 6.20 to Fig. 6.23. The repeatability of the fast component fraction was highest in the Trio repeatability study (CR = 8 %, slope of the correlation plot = 1.0 ± 0.2). The repeatability and reproducibility of the fast component fraction were lower in the Trio-Prisma reproducibility study. The CR and the slope of the correlation plot were, respectively: 14 % and 0.3 ± 0.3 for the Trio experiments, 16 % and 0.4 ± 0.2 for the Prisma experiments, and 20 % and 0.2 ± 0.5 for the Trio and Prisma test experiments.

Table 6.8 summarises the results for the fast- R_2^* signal, indicating that the two models with and without fixing R_{2f}^* were comparable. For further information, comparisons of the slow component between healthy subjects and patients are shown in the Appendix.

Comparisons between healthy subjects and patients using the bi-exponential function with lipids and fixed $R_{2f}^* = 2130 \text{ s}^{-1}$ are shown in Fig. 6.24. No statistically significant difference was found in the fast component. The fast component had a signal fraction of $25 \pm 7 \%$ in healthy subjects and $22 \pm 9 \%$ in patients ($p = 0.67$). There was a statistically significant difference in the lipid signal fraction, which was $0.6 \pm 0.8 \%$ in healthy subjects and $7 \pm 5 \%$ in patients ($p = 0.01$).

Fig. 6.25 and Fig. 6.26 illustrate the comparisons between healthy subjects and patients using the bi-exponential function with lipids and unfixed R_{2f}^* . Apart from a lipid signal fraction of $0.5 \pm 0.7 \%$ in healthy subjects and $6 \pm 5 \%$ in patients ($p = 0.02$), no statistically significant difference was found in the parameters. The fast component had

a signal fraction of 31 ± 6 % and T_2^* of 0.4 ± 0.1 ms in healthy subjects, and a signal fraction of 29 ± 13 % and T_2^* of 0.4 ± 0.2 ms in patients. The p -values were 0.42 for the signal fraction and 0.89 for the T_2^* .

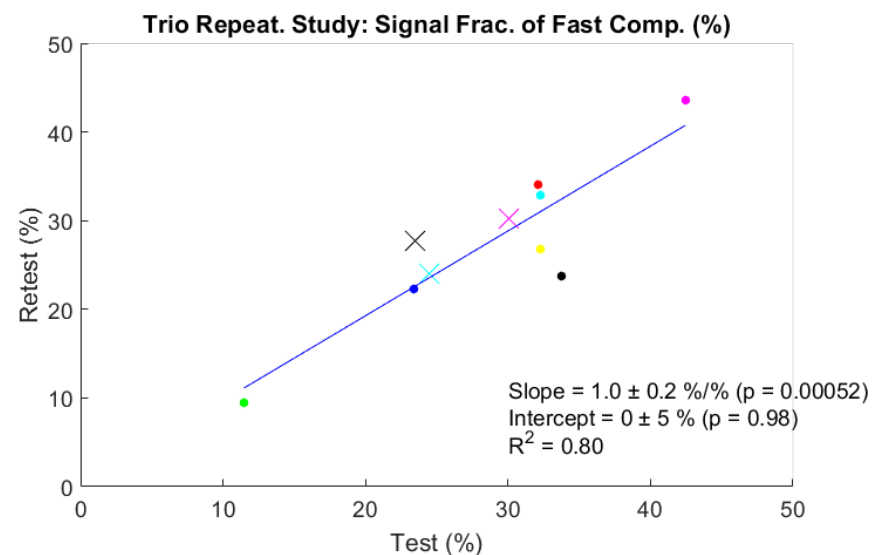
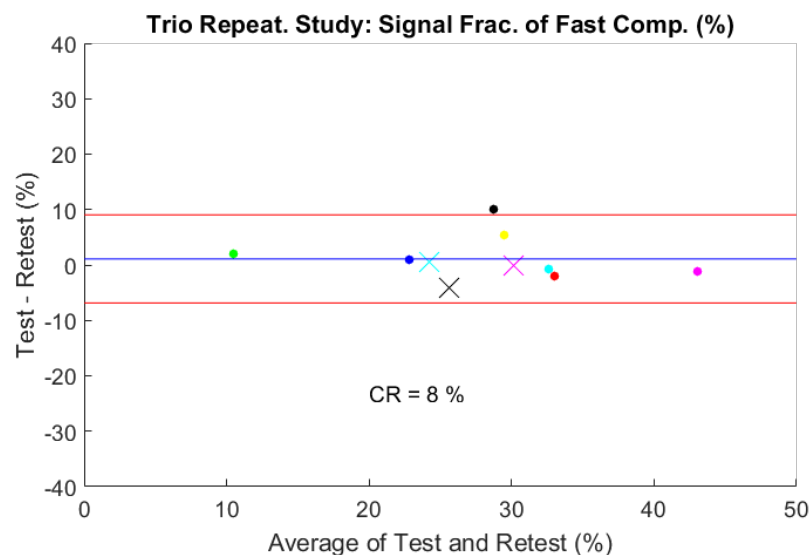
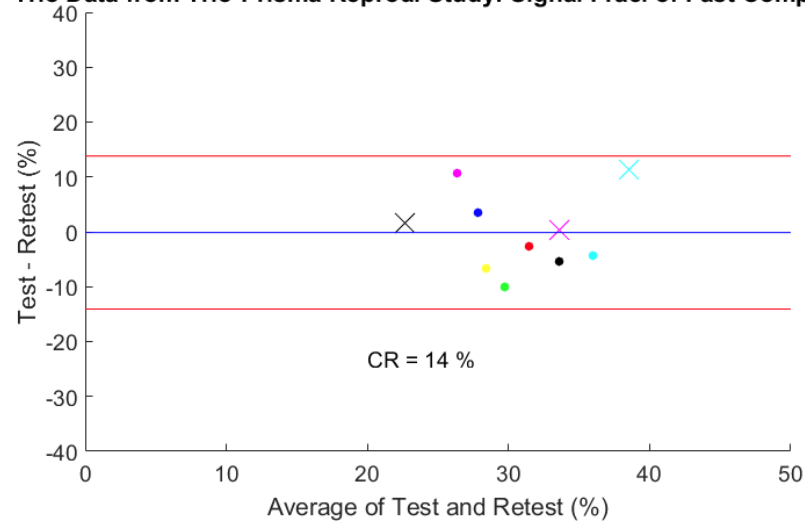
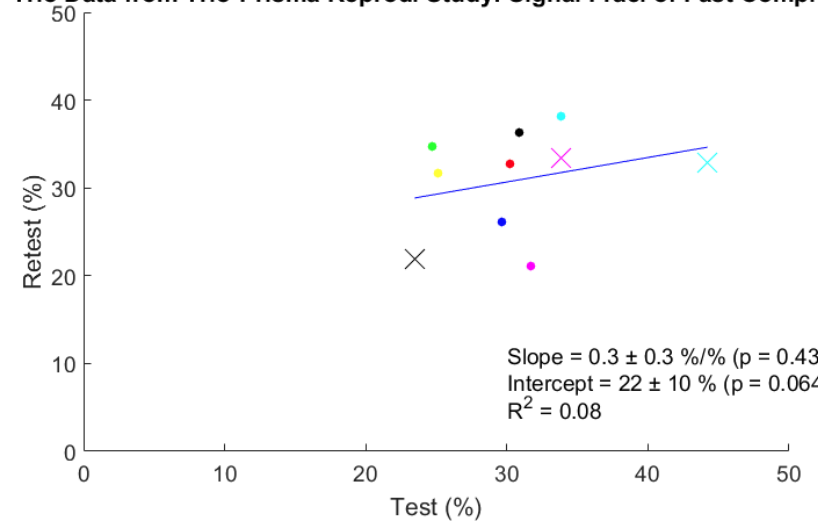


Fig. 6.20. Repeatability of the signal fraction of the fast component for the Trio repeatability study. The bi-exponential function with fixed $R_{2f}^* = 2130 \text{ s}^{-1}$ was used for modelling the fast component. On the left is the Bland-Altman plot, with the coefficient of repeatability (CR). The blue line is the mean of the difference between the test and retest experiments. The red lines are the mean $\pm$ CR. On the right is the correlation plot for the test and retest experiments.

Trio Data from Trio-Prisma Reprod. Study: Signal Frac. of Fast Comp. (%)



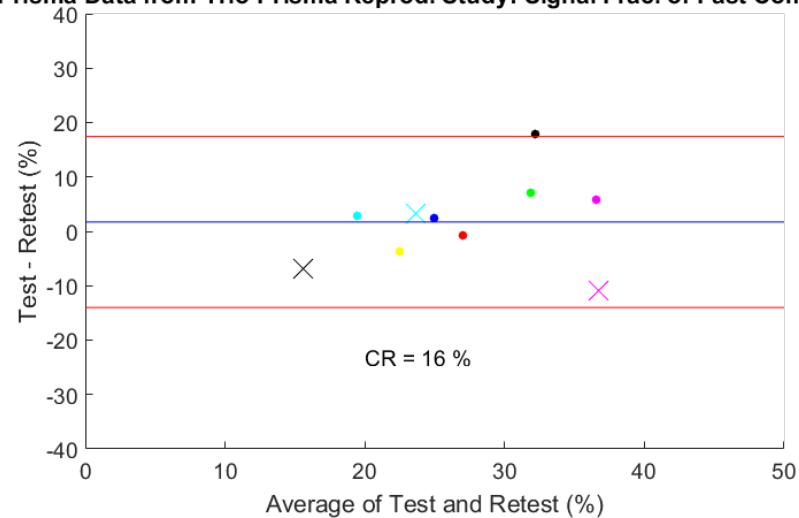
Trio Data from Trio-Prisma Reprod. Study: Signal Frac. of Fast Comp. (%)



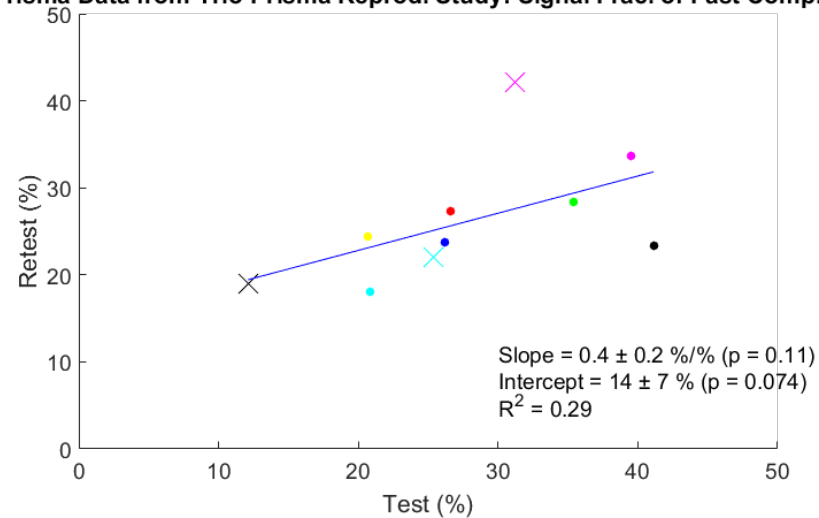
• Subject 1 • Subject 2 • Subject 3 • Subject 4 • Subject 5 • Subject 6 • Subject 7 × Subject 8 × Subject 9 × Subject 10

Fig. 6.21. Repeatability of the signal fraction of the fast component for the Trio data from the Trio-Prisma reproducibility study. The bi-exponential function with fixed $R_{2f}^* = 2130 \text{ s}^{-1}$ was used for modelling the fast component.

Prisma Data from Trio-Prisma Reprod. Study: Signal Frac. of Fast Comp. (%)

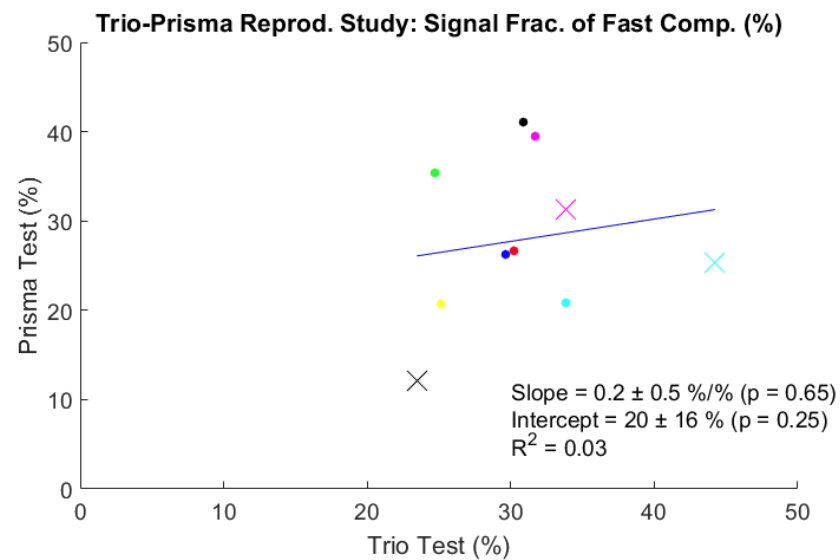
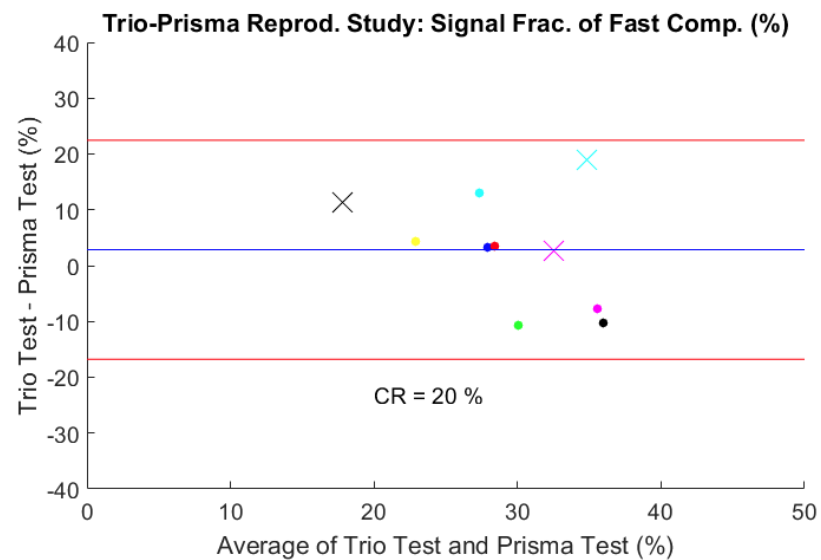


Prisma Data from Trio-Prisma Reprod. Study: Signal Frac. of Fast Comp. (%)



• Subject 1 • Subject 2 • Subject 3 • Subject 4 • Subject 5 • Subject 6 • Subject 7 × Subject 8 × Subject 9 × Subject 10

Fig. 6.22. Repeatability of the signal fraction of the fast component for the Prisma data from the Trio-Prisma reproducibility study. The bi-exponential function with fixed $R_{2f}^* = 2130 \text{ s}^{-1}$ was used for modelling the fast component.



• Subject 1 • Subject 2 • Subject 3 • Subject 4 • Subject 5 • Subject 6 • Subject 7 × Subject 8 × Subject 9 × Subject 10

Fig. 6.23. Reproducibility of the signal fraction of the fast component for the Trio and Prisma test experiments from the Trio-Prisma reproducibility study. The bi-exponential function with fixed $R_{2f}^* = 2130 \text{ s}^{-1}$ was used for modelling the fast component.

Study	Bi-exp. (fixed R_{2f}^*) with lipids		Bi-exp. (unfixed R_{2f}^*) with lipids		
	Fraction of fast comp. (%)	Fraction of lipids (%)	Fraction of fast comp. (%)	T_2^* of fast comp. (ms)	Fraction of lipids (%)
2: Trio- Prisma reprod. (Prisma retest)	25 ± 7	0.6 ± 0.8	31 ± 6	0.4 ± 0.1	0.4 ± 0.7
3: Prisma patients	22 ± 9	7 ± 5	29 ± 13	0.4 ± 0.2	6 ± 5

Table 6.8. Results for signal in the livers of healthy subjects and liver-disease patients, using two models.

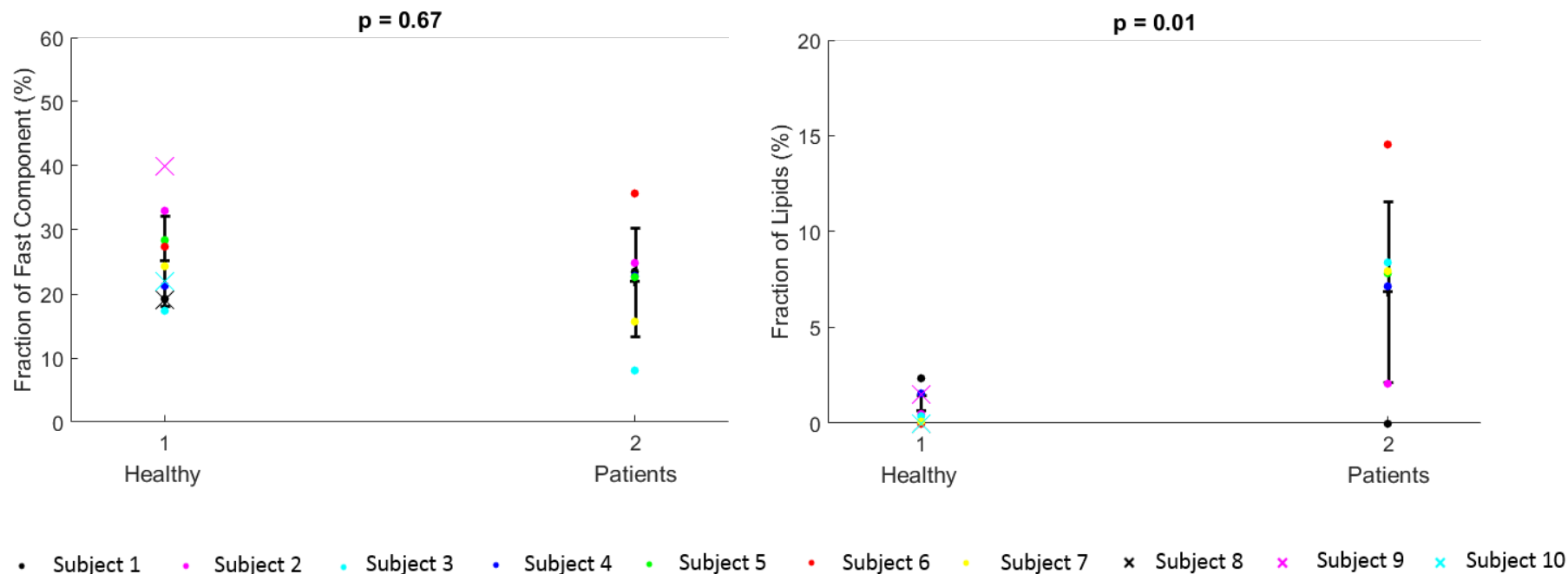
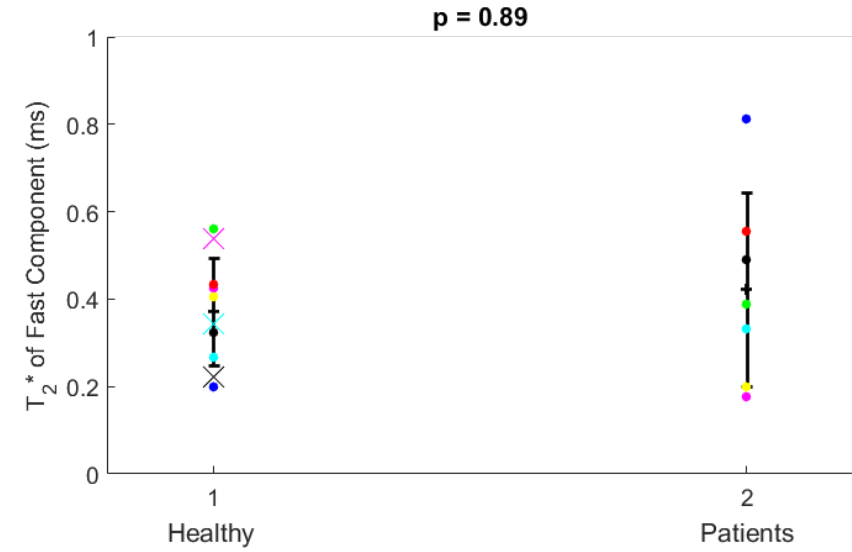
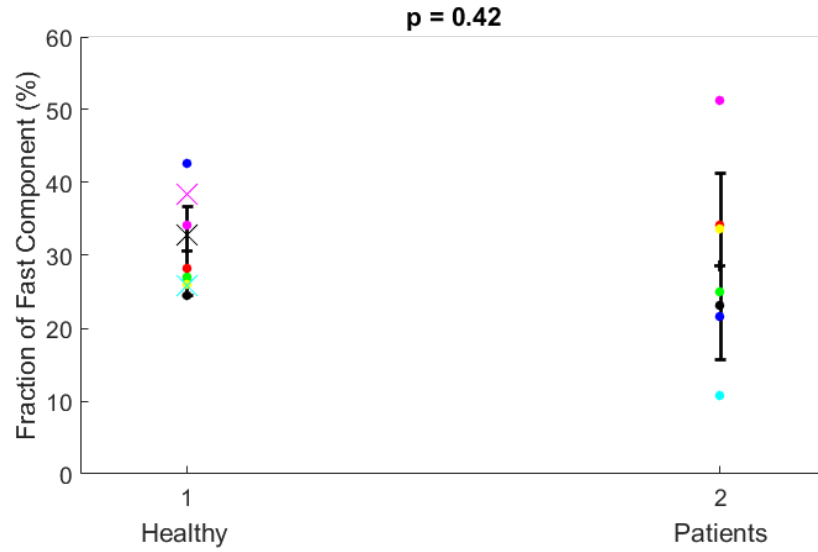


Fig. 6.24. Comparison of the signal fractions of the fast component and lipids between healthy subjects and patients. The bi-exponential function with lipids and fixed $R_{2f}^* = 2130 \text{ s}^{-1}$ was used for modelling the fast component. The healthy-subject data ($n = 10$) were taken from the Prisma retest experiment from the Trio-Prisma reproducibility study. The patient data ($n = 7$) were taken from the Prisma patient study. The p -value was calculated from a two-tailed Wilcoxon rank-sum test. The error bars indicate the mean $\pm$ standard deviation.



• Subject 1 • Subject 2 • Subject 3 • Subject 4 • Subject 5 • Subject 6 • Subject 7 × Subject 8 × Subject 9 × Subject 10

Fig. 6.25. Comparison of the signal fraction and T_2^* of the fast component between healthy subjects and patients. The bi-exponential function with lipids and unfixed R_{2f}^* was used for modelling the fast component, as in (13).

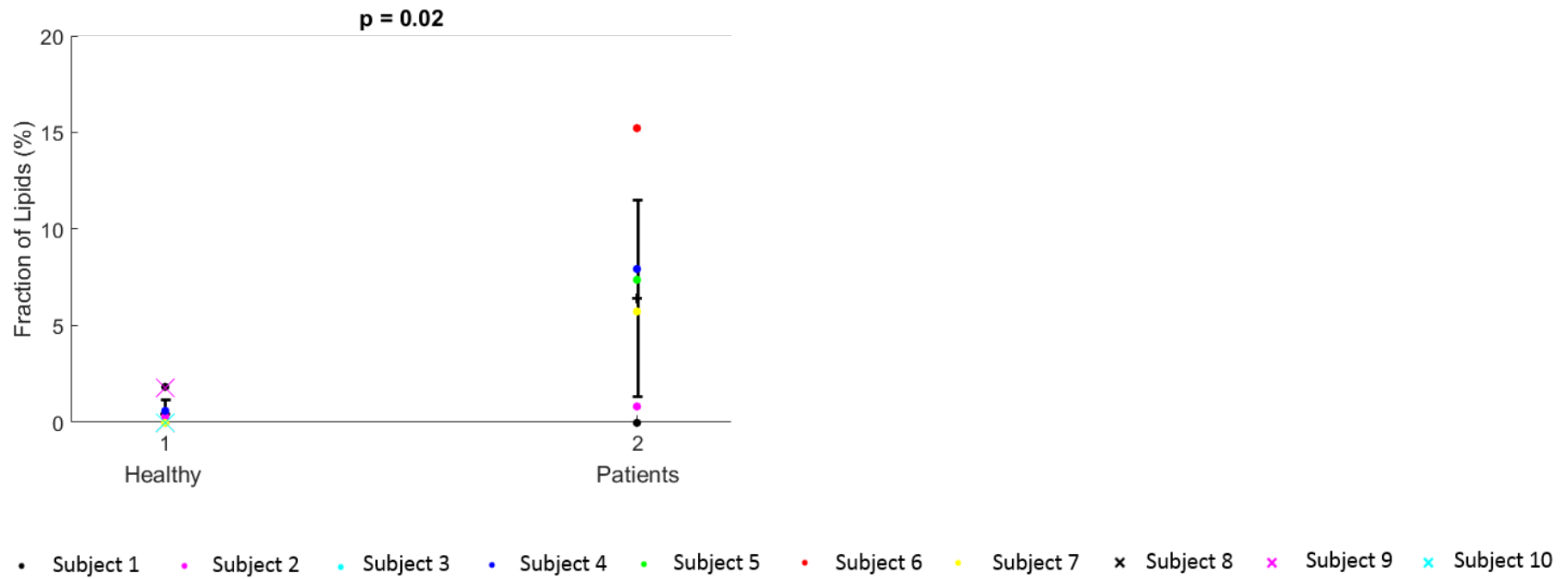


Fig. 6.26. Comparison of the signal fraction of lipids between healthy subjects and patients. The bi-exponential function with lipids and unfixed R_{2f}^* was used for modelling the signal fraction of lipids, as in (13).

6.5. Discussion

6.5.1. Model Selection: Step 1

The optimal model for the healthy subjects was the bi-exponential model with fixed $R_{2f}^* = 2130 \text{ s}^{-1}$, with mean Akaike weights ranging from 0.62 to 0.74. The bi-exponential model with lipids and fixed $R_{2f}^* = 2130 \text{ s}^{-1}$ was most suitable for the patients, with a mean Akaike weight of 0.70. The high values of the Akaike weights indicated a high likelihood that these models were most appropriate, out of the models tested. The additional modelling of lipids in patients was reasonable, considering that these patients with liver disease were expected to have higher lipid content than the young healthy subjects. More generally, the bi-exponential model with lipids and fixed $R_{2f}^* = 2130 \text{ s}^{-1}$ should be used for subjects with noticeable lipid R_2^* signal, characterised by off-resonance. This applies to most subjects, including older healthy subjects, who have higher lipid content than the young healthy subjects studied here. Hence, one would expect the bi-exponential model with fixed $R_{2f}^* = 2130 \text{ s}^{-1}$ to suffice only when analysing data from young healthy subjects. When comparing R_2^* signal from healthy low-lipid subjects and high-lipid patients, the bi-exponential model with lipids and fixed $R_{2f}^* = 2130 \text{ s}^{-1}$ should be used for all participants.

For a given model, the test and retest experiments from the Trio repeatability study yielded the lowest mean AIC_c values. This reflected how the data from the Trio repeatability study had less noise than data from the other studies, subsequently resulting in a more repeatable fast- R_2^* signal fraction. Sources of noise included signal contamination and breathing artefact, to be discussed in Section 6.5.3.

As expected, fixing the R_{2f}^* value to 2130 s^{-1} in the bi-exponential model and the bi-exponential model with lipids decreased the uncertainties in the fitting parameters, as well as the mean AIC_cs. Large uncertainties in the fitting parameters were caused by:

1. More degrees of freedom in the fit: A higher number of parameters in the model caused more variation in each parameter value.
2. Longer minimum TE: The minimum TE was 0.23 ms for the Trio repeatability study, and 0.35 ms for the other two studies. Having a minimum TE on the order of R_{2f}^* decreased the accuracy of R_{2f}^* .
3. Low TE sampling density: Being an *in vivo* experiment, fewer acquisitions were performed to reduce scan time. As a result, the sampling density of the TEs was lower, affecting the accuracy of the fitting parameters, especially in the presence of noise.
4. Noise from signal contamination and breathing artefact: Refer to Section 6.5.3.

Hence, it was favourable to fix the R_{2f}^* value in the bi-exponential models with and without lipids; this was done in subsequent analyses.

6.5.2. Model Selection: Step 2

Using a Monte Carlo approach for randomly varying the initial conditions of the fit, the bi-exponential model with fixed $R_{2f}^* = 2130 \text{ s}^{-1}$ was deemed more suitable than the model of isotropic collagen and free water. For the bi-exponential model with fixed $R_{2f}^* = 2130$

s⁻¹, the Monte Carlo approach yielded the same results as the fixed initial-condition approach. Hence, the fixed initial-condition approach was sufficient for analysis.

For analysis with the Monte Carlo approach, only healthy-subject experiments were considered, to minimise confounds from lipid signal. The model of isotropic collagen and free water was not appropriate for fitting the short- T_2^* signal in healthy subjects. Reasons why the model did not fit the short- T_2^* signal include: (1) collagen in the liver is not truly isotropic and (2) collagen is not the source of short- T_2^* signal in the liver. As mentioned in Section 6.2.2, assumptions for the R_2^* of collagen were: $R_{2c}^*(0^\circ) = 950 \pm 30 \text{ s}^{-1}$ and $R_{2c}^*(55^\circ) = 130 \pm 20 \text{ s}^{-1}$. Given that the short- T_2^* signal was characterised by $R_{2f}^* = 2130 \text{ s}^{-1}$, which was higher than $R_{2c}^*(0^\circ)$ and $R_{2c}^*(55^\circ)$, it is not surprising that the model did not account for the short- T_2^* signal. Although it is possible that collagen in the liver is not truly isotropic, the short- T_2^* signal equally does not describe oriented collagen. Notably, $R_2^*(0^\circ)$ in tendon found by other researchers also exceeded $R_{2f}^* = 2130 \text{ s}^{-1}$ (Section 3.3.1.3), where collagen is oriented. The highest $R_2^*(0^\circ)$ at 3 T was $1640 \pm 160 \text{ s}^{-1}$, found by Diaz et al. (14). Hence, even if the R_2^* model in the liver accounted for oriented collagen, the model would not fit the $R_{2f}^* = 2130 \text{ s}^{-1}$ component. Therefore, it is unlikely that the short- T_2^* signal originated from collagen, independent of the orientation.

The bi-exponential model indicated a short- T_2^* component, and a long- T_2^* component due to free water. This was a reasonable model for *in vivo* imaging; given the short TEs and relatively low TE sampling density, modelling of additional T_2^* components would have been difficult. The R_2^* of the long- T_2^* component (R_{2s}^*) varied from close to 0 to 90 s^{-1} . This was consistent with literature, reporting differences in R_{2s}^* between subjects

(8). As the signal decay was sampled at $TEs < (\frac{1}{90\ s^{-1}} \sim 11\ ms)$, R_{2s}^* was relatively inaccurate. Given that the short and long components were distinct, this did not hinder the quantification of the short- T_2^* component.

6.5.3. Analysis with the Appropriate Models

6.5.3.1. Repeatability and Reproducibility Studies

The fast- R_2^* signal fraction in healthy subjects was repeatable in the Trio repeatability study, but not as repeatable or reproducible in the Trio-Prisma reproducibility study. Discrepancies in repeatability between the two studies existed for the Trio test and retest experiments (Fig. 6.20 and Fig. 6.21), suggesting that differences in protocol may have been the cause. Differences between the studies included:

1. TEs: The first two acquisitions in the Trio repeatability study were different from the other two studies. For the Trio repeatability study, the first two dual-echo acquisitions consisted of TEs 0.23 and 2.54 ms, and TEs 0.35 and 3.04 ms. For the other studies, the first two acquisitions consisted of TEs 0.35 and 2.54 ms, and TEs 0.39 and 3.04 ms.
2. Sequences: The Trio utilised a different sequence from the Prisma. Refer to the discussion on signal contamination below.
3. Diet and exercise restrictions: Subjects in the Trio repeatability study had restrictions on alcohol and caffeine consumption and exercise, and stricter restrictions on liquid consumption before the scan.
4. Slice localisation: Slices were at a lower position in the liver (i.e. in the inferior direction) in the Trio repeatability study; these transversal slices localised on

the portal vein. In the other studies, transversal slices were localised at approximately the level of T10/11.

5. Subjects: Different sets of subjects were used for each study. Four subjects (two males) overlapped between the Trio repeatability study and the Trio-Prisma reproducibility study. Otherwise, there were no repeats of subjects between studies.

The main sources of artefacts were:

1. Signal contamination: The Prisma sequence did not sample the data at twice the field of view, and hence was susceptible to contamination from aliased signal. At the same time, the Prisma sequence had less contamination from background lipid signal, believed to be due to a superior gradient system.
2. Breathing motion: Seven dual-echo UTE acquisitions were performed to gather the R_2^* signal from one subject. Rigid-body image registration was done to minimise motion between UTE acquisitions. However, rigid-body image registration does not account for changes in liver shape as a result of differences in breathing patterns between acquisitions. Hence, motion due to breathing could have caused the ROI to cover slightly different parts of the liver between TEs, resulting in small changes in signal intensity. Breathing consistency differed between subjects.

Differences in TE between studies contributed minimally to the differences in mean AIC_c . When analysed with the same TEs, the Trio repeatability study still had the lowest mean AIC_c values and the slope of the correlation plot closest to 1 (not shown).

In this case, the combination of the lower slice position and better breathing consistency in subjects in the Trio repeatability study may have contributed to the higher repeatability of the fast- R_2^* signal fraction and lower mean AIC_c. During expiration, the posterior region of the liver in a superior slice exhibits more displacement in the superior-inferior direction (9 ± 3 mm) than the posterior region of the liver in an inferior slice (7 ± 4 mm) (15). Although imaging was performed after expiration, the data suggests that more displacement in the liver can occur in a superior slice if the subject does not hold their breath at the same position. Anatomically, this is reasonable because the superior part of the liver is closest to the diaphragm and chest, which are responsible for breathing. Hence, future studies on the Trio and Prisma will include localisation along the portal vein instead of the T10/11 vertebrae. Additionally, the Prisma UTE sequence will be optimised to minimise aliasing, namely by oversampling the data and truncating to obtain the original field of view.

6.5.3.2. Patient Study

The short- T_2^* value in the liver was comparable to that of Zhu et al. (13); their results were introduced in Chapter 3. Table 6.9 summarises the differences between our studies. The most prominent difference was that Zhu et al. used shorter TEs, resulting in a more accurate short- T_2^* value. I found a T_2^* of 0.4 ± 0.1 ms in healthy subjects and 0.4 ± 0.2 ms in patients, whereas Zhu et al. found a T_2^* of 0.23 ± 0.05 ms in healthy subjects and 0.20 ± 0.05 ms in patients. My minimum TE was 0.35 ms, rendering it difficult to identify a T_2^* of ~ 0.2 ms. On the contrary, Zhu et al. sampled the short- T_2^* signal with shorter TEs of 0.10, 0.15, 0.20, and 0.25 ms, enabling them to obtain their lower T_2^* value. As described in Chapter 2, the TE in the UTE sequence is measured from the centroid of the RF pulse. The relatively high RF pulse duration of 1 ms resulted in a minimum TE of

0.35 ms. Decreasing the minimum TE would be possible with a shorter RF pulse, e.g. with the SLR pulse used by Zhu et al. of 112 μ s duration, or a hard pulse. This would, however, require the UTE sequence to be 3D instead of 2D. A 3D-UTE sequence with a short RF pulse can be considered in the future. Otherwise, the existing half-sinc pulse can be shortened to a duration of 80 μ s (35), which can potentially decrease the minimum TE to ~ 0.20 ms.

Table 6.10 compares my results with those obtained by Zhu et al.. The short- T_2^* signal fraction that I found was higher than that of Zhu et al., likely caused by a lower minimum TE and higher T_1 weighting in the acquisition. I had a signal fraction of 31 ± 6 % in healthy subjects and 29 ± 13 % in patients, acquired with TEs ranging from 0.35 to 5.45 ms. Zhu et al. had a signal fraction of 10 ± 2 % in healthy subjects and 7 ± 2 % in patients, acquired with TEs ranging from 0.10 to 7.0 ms. As Zhu et al. acquired four TEs before my minimum TE, they obtained a more accurate signal fraction. Fig. 6.29 illustrates data in a healthy subject taken from Zhu et al.'s paper, fitted with the bi-exponential function with lipids and unfixed R_{2f}^* ; signal data with and without TEs 0.10, 0.15 and 0.20 ms are shown. Without the first three TEs, the signal fraction is overestimated by almost threefold (13 % vs. 34 %), similar to the 31 ± 6 % fraction I observed in healthy subjects. The effect of the high minimum TE on the signal fraction accuracy is larger than the effect of the higher T_1 weighting in my acquisition. Namely, I utilised a flip angle of 10° and a TR of 7 ms, whereas Zhu et al. had a flip angle of 4° and a TR of 9.6 ms. As water and lipids were modelled with the same T_2^* , there were effectively two signal components: short T_2^* and water. The intensity of each signal component is given by:

$$S = S_0 e^{-TE/T_2^*}, \quad [6.13]$$

where S_0 and T_2^* were measured. Here,

$$S_0 = kW = k \frac{\sin \alpha (1 - e^{-TR/T_1})}{(1 - (\cos \alpha) e^{-TR/T_1})} \quad [6.14]$$

where k is the proton density, W is the T_1 weighting factor, and α the flip angle (16). Assume a T_1 of 720 ms for water (17). The proton density fraction for the short- T_2^* component, calculated from S_0 and W , should agree for my and Zhu et al.'s results, assuming that the difference in signal (S_0) fraction is due primarily to T_1 weighting. However, this was not the case, as my proton density fraction was still higher than Zhu et al.'s, regardless of the T_1 of the short- T_2^* component. As an extreme case, for a T_1 of the short- T_2^* component of 0.1 ms, my proton density fraction was 15 % vs. 9 % for Zhu et al.. This supports the minimum TE as being the dominant cause for the discrepancy in the short- T_2^* signal fraction.

Both Zhu et al. and I found no statistically significant difference in short- T_2^* value or signal fraction between healthy subjects and patients. However, corroboration of a non-statistically-significant finding does not necessarily indicate that the short- T_2^* component is the same between healthy subjects and patients. Additional studies with higher numbers of subjects will need to be performed to assess changes in the short- T_2^* component between these two groups. That being said, Zhu et al. found a statistically significant difference in short- T_2^* value between healthy subjects (0.23 ± 0.05 ms) and steatotic (but non-cirrhotic) patients (0.10 ± 0.02 ms) ($p = 0.01$). As I only had one steatotic but non-cirrhotic patient, I could not make this comparison.

	Parameter	Siu 2019	Zhu et al. 2019
Acquisition	TE (ms)	0.35, 0.38, 0.44, 0.55, 0.85, 1.45, 2.05, 2.54, 3.04, 3.64, 4.03, 4.55, 5.14, 5.45	0.10, 0.15, 0.20, 0.25, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0
	TE randomisation	No	Yes
	TR (ms)	7	9.6
	Flip angle (°)	10	4
	Single- or dual-echo acquisition	Dual	Single
	2D or 3D	2D	3D
	Scanner	Siemens Prisma 3 T ($B_0 = 2.89$ T)	GE Discovery MR 750, 3.0 T
	RF excitation	Half-sinc pulse (duration = 1 ms)	Minimum-phase Shinnar-Le Roux pulse (duration = 112 μ s)
Experiment	Subject restrictions	Fast for at least 4 hours and no more than 0.5 L of water during hour before scan	No restrictions
	Healthy subjects	n = 10	n = 6
	Patients	n = 7 in total (n = 4 with cirrhosis)	n = 9 in total (n = 5 with cirrhosis; n = 4 with steatosis but no cirrhosis)

Table 6.9. Comparison with Zhu et al.'s experimental design for measuring the short- T_2^* signal in the liver.

	Siu 2019		Zhu et al. 2019	
	Fraction of fast comp. (%)	T_2^* of fast comp. (ms)	Fraction of fast comp. (%)	T_2^* of fast comp. (ms)
Healthy	31 ± 6	0.4 ± 0.1	10 ± 2	0.23 ± 0.05
Cirrhotic	29 ± 13	0.4 ± 0.2	7 ± 2	0.20 ± 0.05
Steatotic			7 ± 2	0.10 ± 0.02

Table 6.10. Comparison with Zhu et al.'s results for the short- T_2^* signal in the liver.

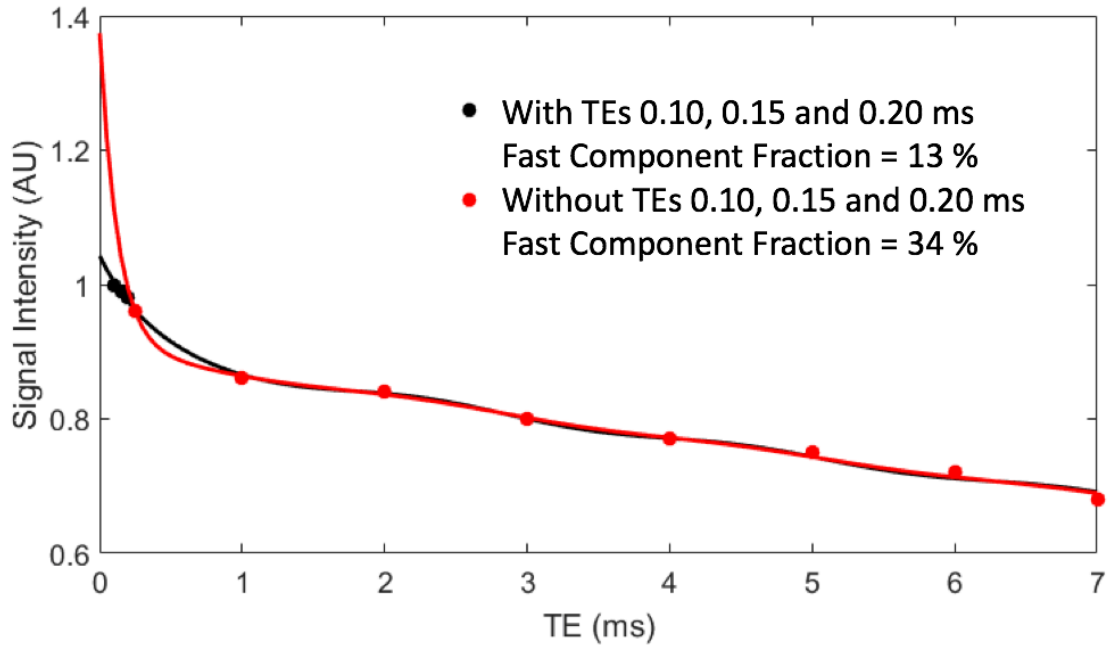


Fig. 6.27. Signal data from a healthy subject from Zhu et al.'s paper (reading error = ± 0.01), including all reported TEs, are shown as black points. These were fitted with the bi-exponential function with lipids and unfixed R_{2f}^* , yielding a fraction of 13 % for the fast component. Subsequently, data at TEs 0.10, 0.15 and 0.20 ms were removed and re-fitted with the bi-exponential function with lipids and unfixed R_{2f}^* , yielding an increased fraction of 34 % for the fast component. Hence, without the first three TEs, the fraction of the fast component is overestimated by almost threefold.

6.6. Conclusions

This chapter modelled the short- T_2^* signal in the liver, with the following results:

1. A model of isotropic collagen and free water was inappropriate, as it did not fit TEs < 1 ms that were characteristic of the short- T_2^* signal. This suggests that the short- T_2^* signal may not originate from isotropic collagen.
2. A bi-exponential model with fixed $R_{2f}^* = 2130 \text{ s}^{-1}$ ($T_{2f}^* = 0.5 \text{ ms}$) was deemed most suitable for describing liver signal in the healthy subjects. A bi-exponential model with lipids and fixed $R_{2f}^* = 2130 \text{ s}^{-1}$ was most appropriate for the liver-

disease patients, given the higher lipid content. This latter model was generalised as an appropriate model for healthy subjects and patients, with varying lipid content.

3. Repeatability of the short- T_2^* signal fraction was established in a Trio 3-T UTE protocol, although repeatability and reproducibility were inferior in a subsequent study, due to noise and altered slice localisation.
4. No statistically significant difference was found in the short- T_2^* signal between healthy subjects and patients. The short- T_2^* signal was comparable to that found by Zhu et al.; differences included a less accurate T_2^* value of ~ 0.4 ms and a higher short- T_2^* signal fraction of $\sim 30\%$, due to a higher minimum TE of 0.35 ms.

6.7. References

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6.8. Appendix

6.8.1. Model Selection: Step 1

6.8.1.1. A: Mono-Exponential Function

	S_0 (AU)	R_2^* (s ⁻¹)
Initial value	$0.9 \times \text{MS}$	50
Lower bound	0	0
Upper bound	$10 \times \text{MS}$	1,000,000

Table A6.1. Initial conditions for signal decays fitted with a mono-exponential function (Model A: Eq. [6.7]). MS stands for the maximal signal out of the data collected.

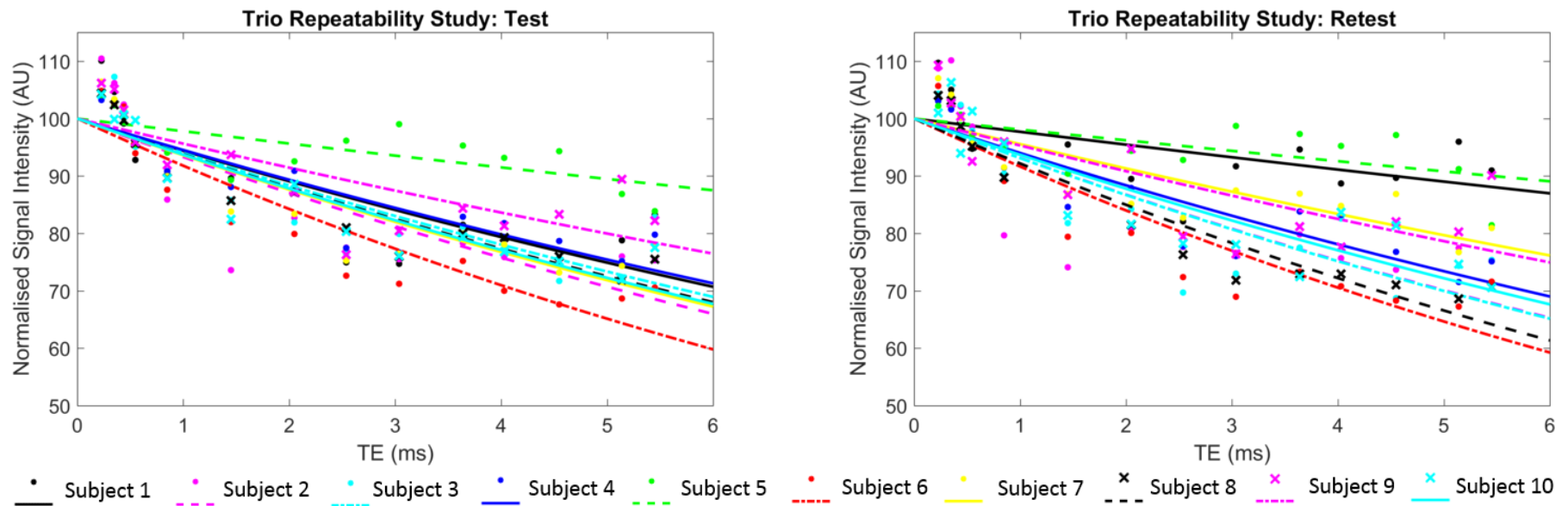


Fig. A6.1. Signal decays from the Trio repeatability study (Study 1), fitted with a mono-exponential function (Model A: Eq. [6.7]).

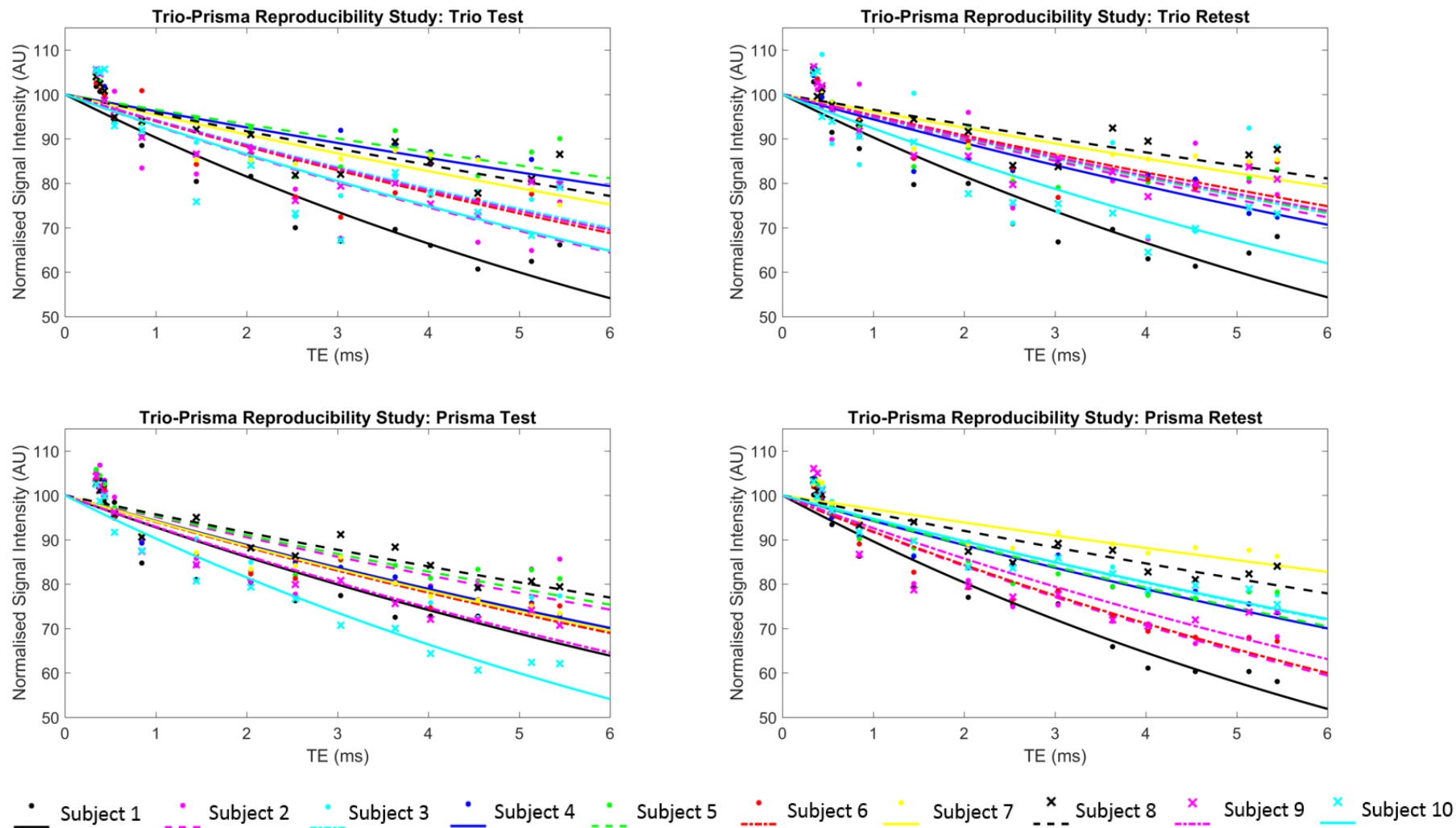


Fig. A6.2. Signal decays from the Trio-Prisma reproducibility study (Study 2), fitted with a mono-exponential function (Model A: Eq. [6.7]).

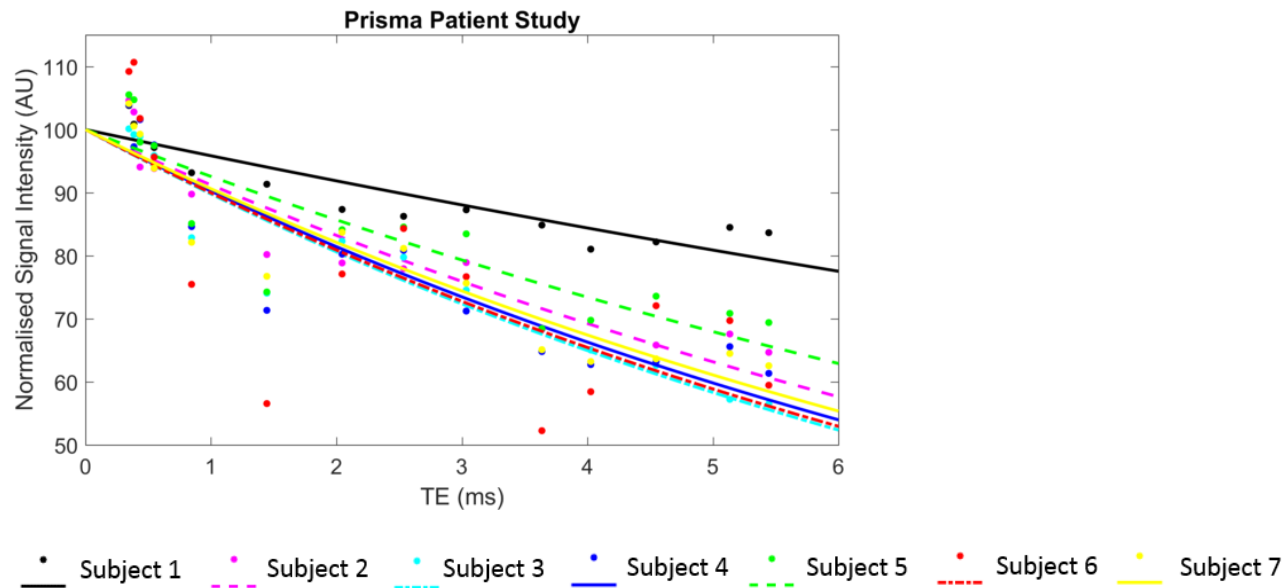


Fig. A6.3. Signal decays from the Prisma patient study (Study 3), fitted with a mono-exponential function (Model A: Eq. [6.7]).

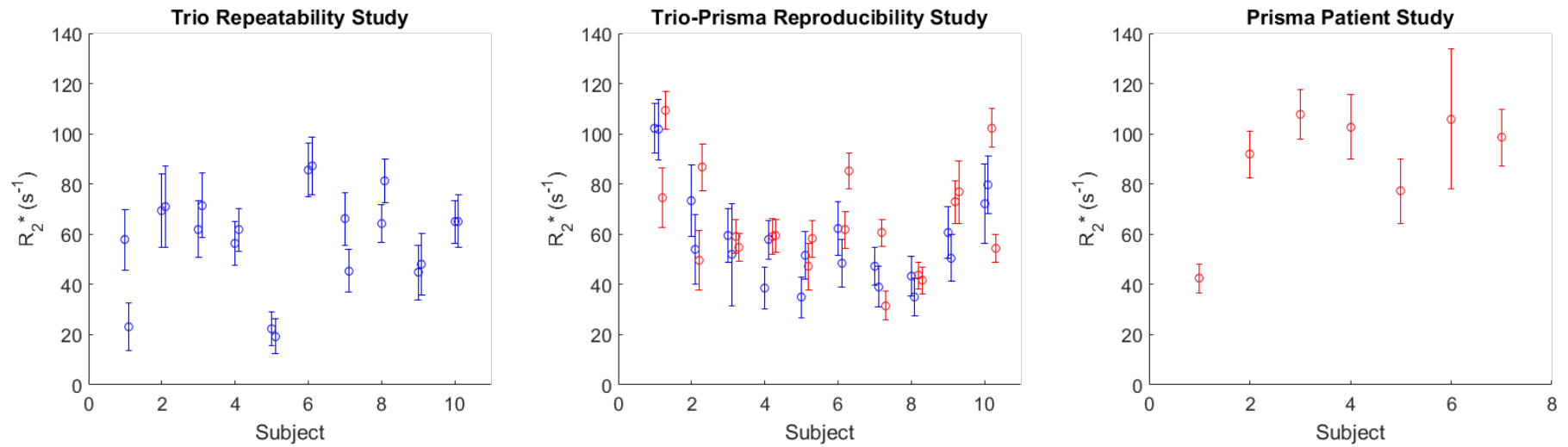


Fig. A6.4. Results for signal decays fitted with a mono-exponential function (Model A: Eq. [6.7]). For the Trio repeatability study, the data for each subject are ordered: Trio test, Trio retest. For the Trio-Prisma reproducibility study, the data for each subject are ordered: Trio test, Trio retest, Prisma test, Prisma retest. The Trio data are in blue and the Prisma data are in red.

6.8.1.2. B: Bi-Exponential Function

	S_{0f} (AU)	R_{2f}^* (s ⁻¹)	S_{0s} (AU)	R_{2s}^* (s ⁻¹)
Initial value	$0.2 \times \text{MS}$	1,500	$0.7 \times \text{MS}$	15
Lower bound	0	0	0	0
Upper bound	$10 \times \text{MS}$	1,000,000	$10 \times \text{MS}$	1,000,000

Table A6.2. Initial conditions for signal decays fitted with a bi-exponential function (Model B: Eq. [6.8]).

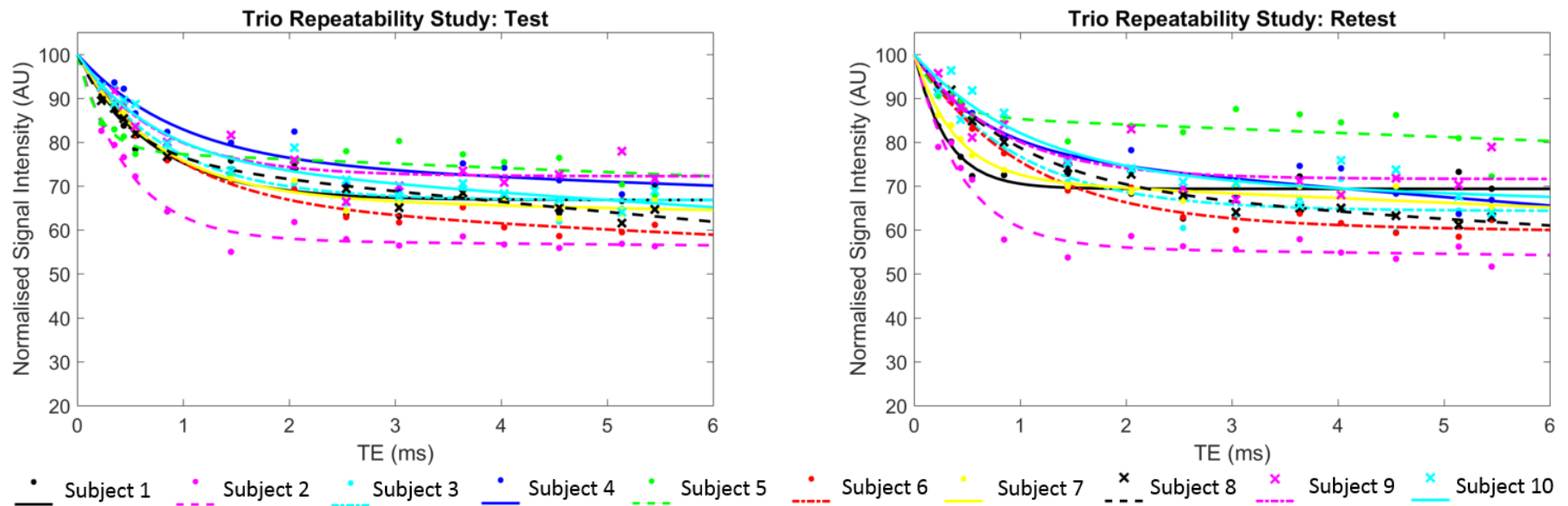
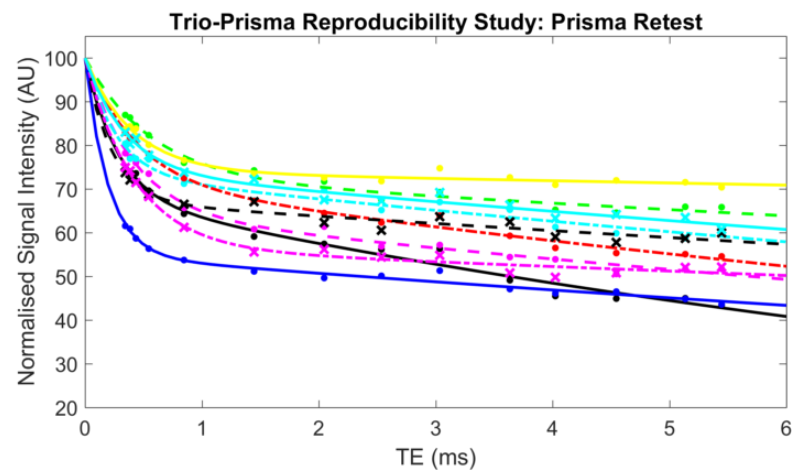
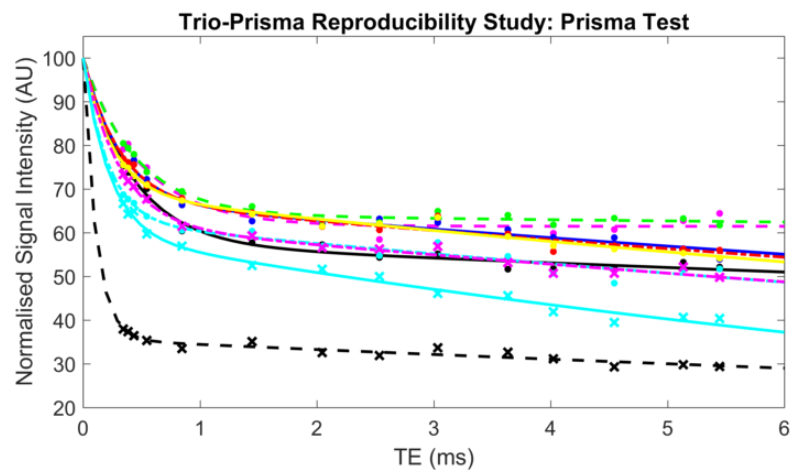
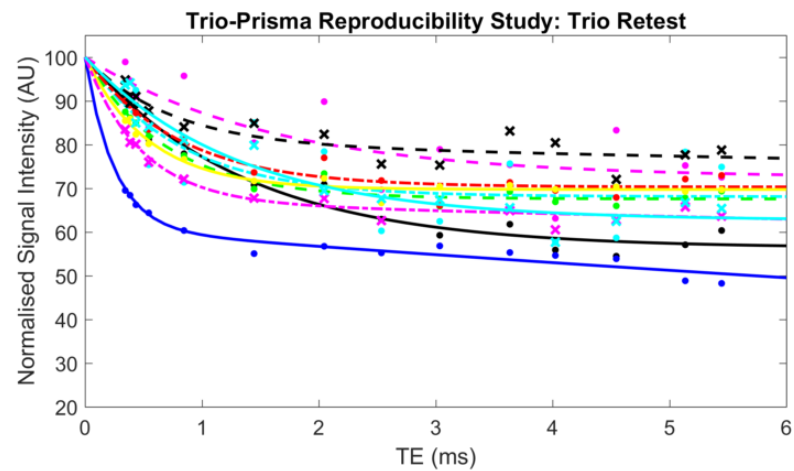
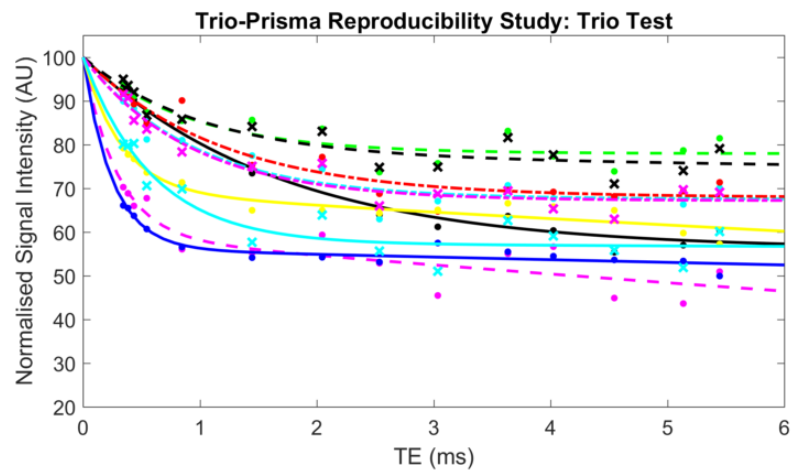


Fig. A6.5. Signal decays from the Trio repeatability study (Study 1), fitted with a bi-exponential function (Model B: Eq. [6.8]).



• Subject 1 • Subject 2 • Subject 3 • Subject 4 • Subject 5 • Subject 6 • Subject 7 x Subject 8 x Subject 9 x Subject 10
 — Subject 1 - - Subject 2 - - Subject 3 - - Subject 4 - - Subject 5 - - Subject 6 - - Subject 7 - - Subject 8 - - Subject 9 - - Subject 10

Fig. A6.6. Signal decays from the Trio-Prisma reproducibility study (Study 2), fitted with a bi-exponential function (Model B: Eq. [6.8]).

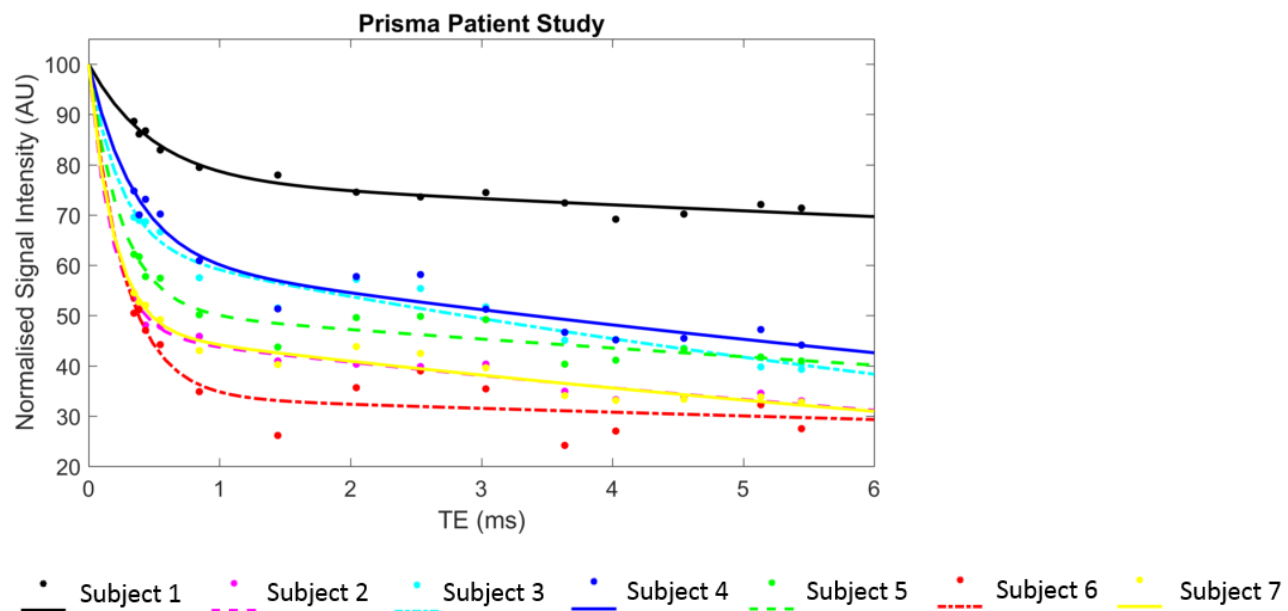


Fig. A6.7. Signal decays from the Prisma patient study (Study 3), fitted with a bi-exponential function (Model B: Eq. [6.8]).

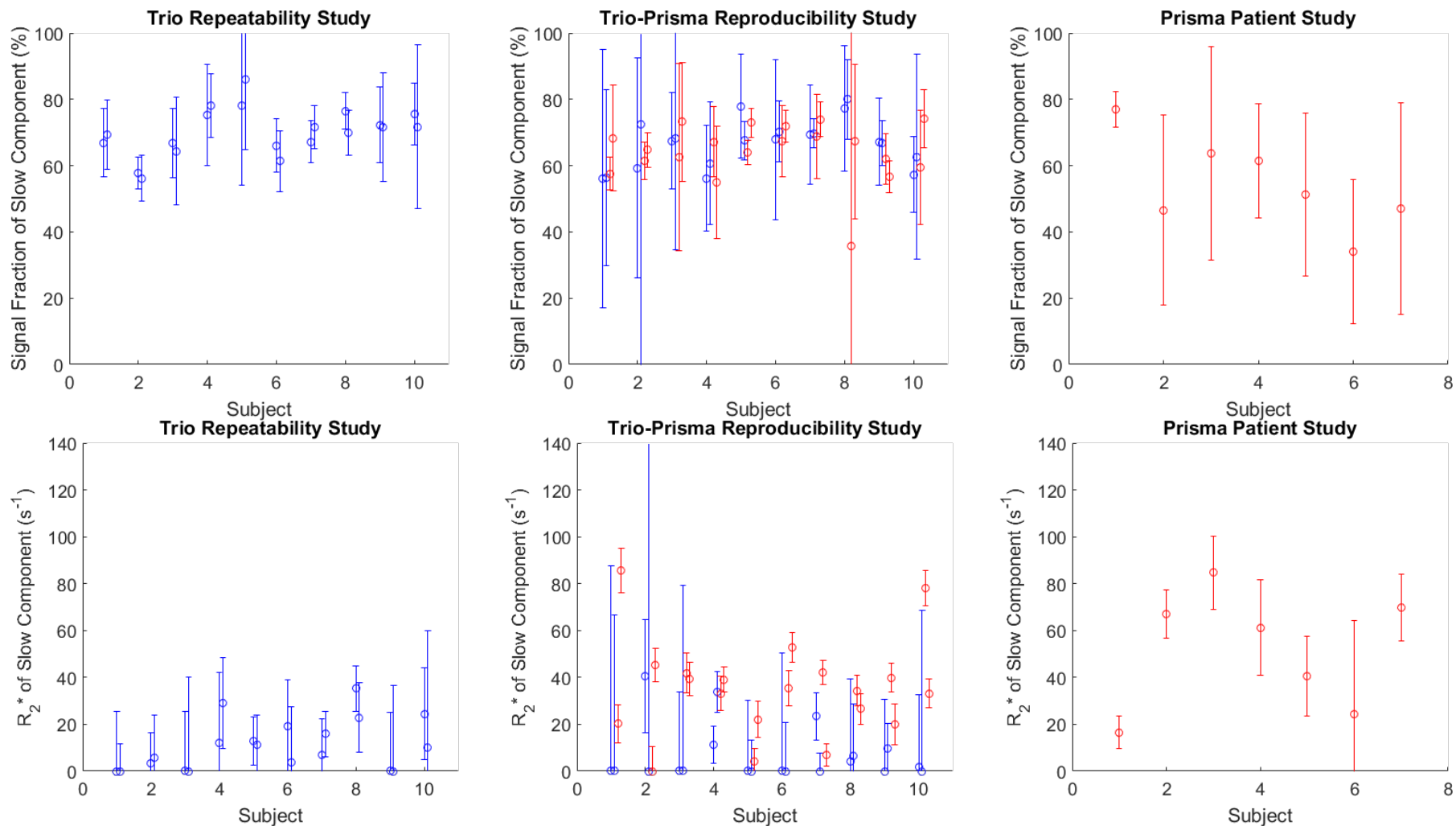
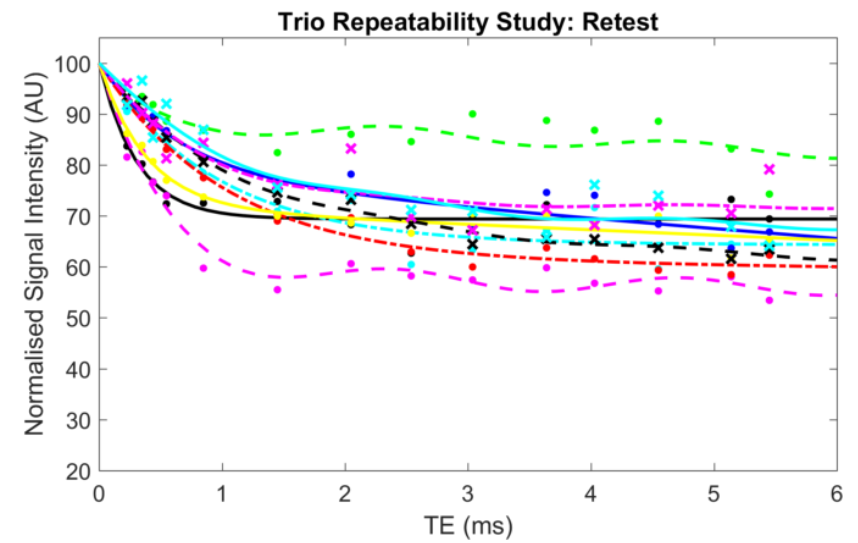
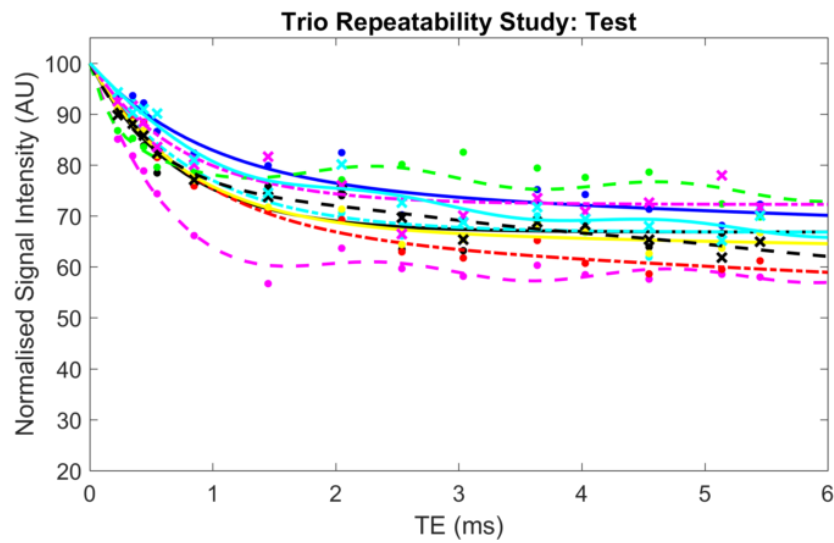


Fig. A6.8. Slow-component results for signal decays fitted with a bi-exponential function (Model B: Eq. [6.8]).

6.8.1.3. C: Bi-Exponential Function With Lipids

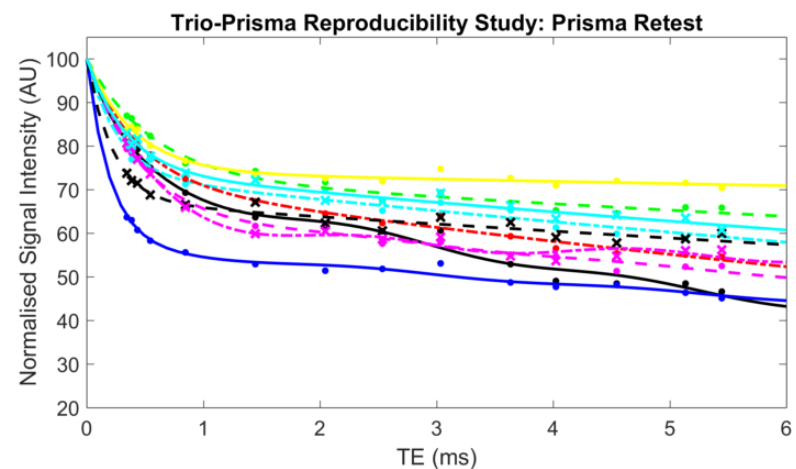
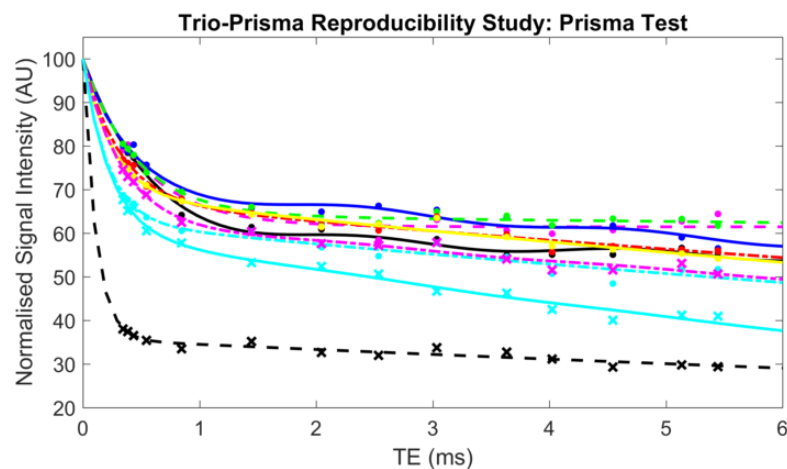
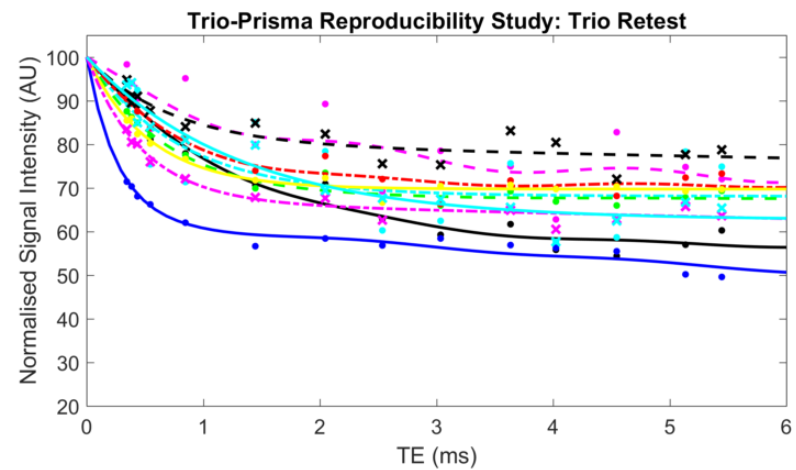
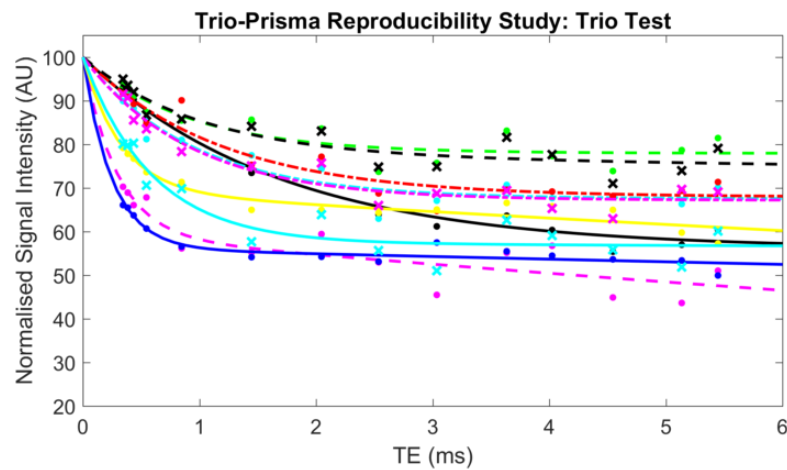
	S_{0f} (AU)	R_{2f}^* (s ⁻¹)	S_{0s} (AU)	S_{0l} (AU)	R_{2s}^* (s ⁻¹)
Initial value	$0.2 \times \text{MS}$	1,500	$0.7 \times \text{MS}$	$0.005 \times \text{MS}$	15
Lower bound	0	0	0	0	0
Upper bound	$10 \times \text{MS}$	1,000,000	$10 \times \text{MS}$	$10 \times \text{MS}$	1,000,000

Table A6.3. Initial conditions for signal decays fitted with a bi-exponential function with lipids (Model C: Eq. [6.9]).



• Subject 1 • Subject 2 • Subject 3 • Subject 4 • Subject 5 • Subject 6 • Subject 7 • Subject 8 • Subject 9 • Subject 10
 — Subject 1 - - Subject 2 - - Subject 3 - - Subject 4 - - Subject 5 - - Subject 6 - - Subject 7 - - Subject 8 - - Subject 9 - - Subject 10

Fig. A6.9. Signal decays from the Trio repeatability study (Study 1), fitted with a bi-exponential function with lipids (Model C: Eq. [6.9]).



• Subject 1 • Subject 2 • Subject 3 • Subject 4 • Subject 5 • Subject 6 • Subject 7 x Subject 8 x Subject 9 x Subject 10
 — Subject 1 - - Subject 2 - - Subject 3 - - Subject 4 - - Subject 5 - - Subject 6 - - Subject 7 - - Subject 8 - - Subject 9 - - Subject 10

Fig. A6.10. Signal decays from the Trio-Prisma reproducibility study (Study 2), fitted with a bi-exponential function with lipids (Model C: Eq. [6.9]).

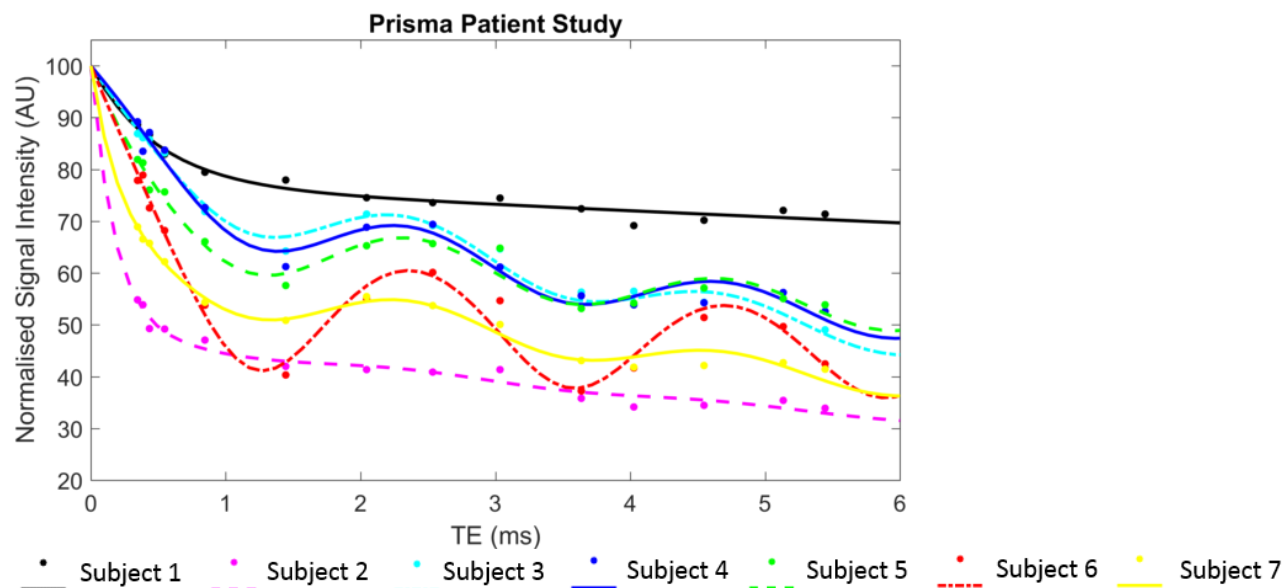


Fig. A6.11. Signal decays from the Prisma patient study (Study 3), fitted with a bi-exponential function with lipids (Model C: Eq. [6.9]).

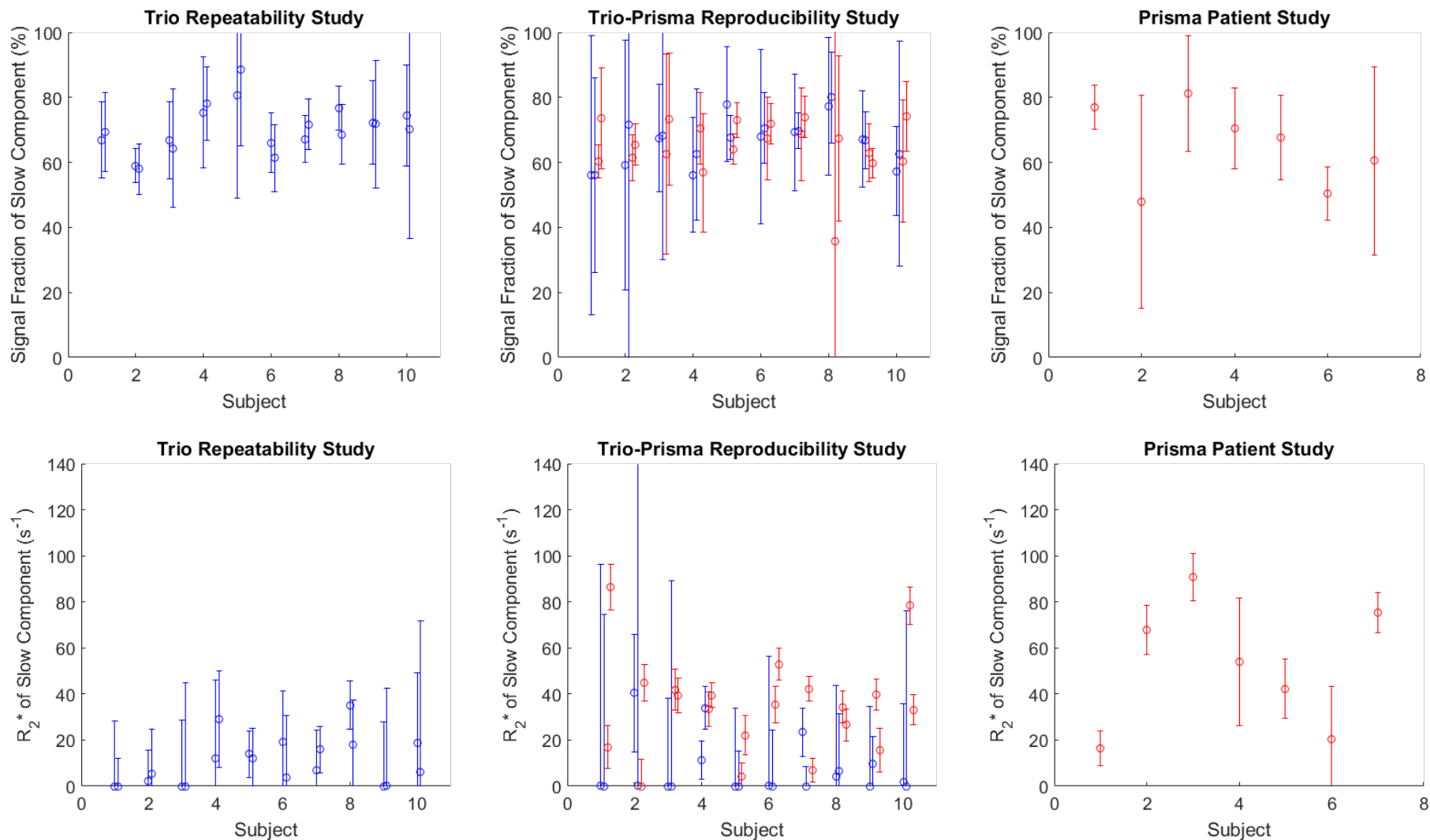


Fig. A6.12. Slow-component results for signal decays fitted with a bi-exponential function with lipids (Model C: Eq. [6.9]).

6.8.1.4. Fixing R_{2f}^*

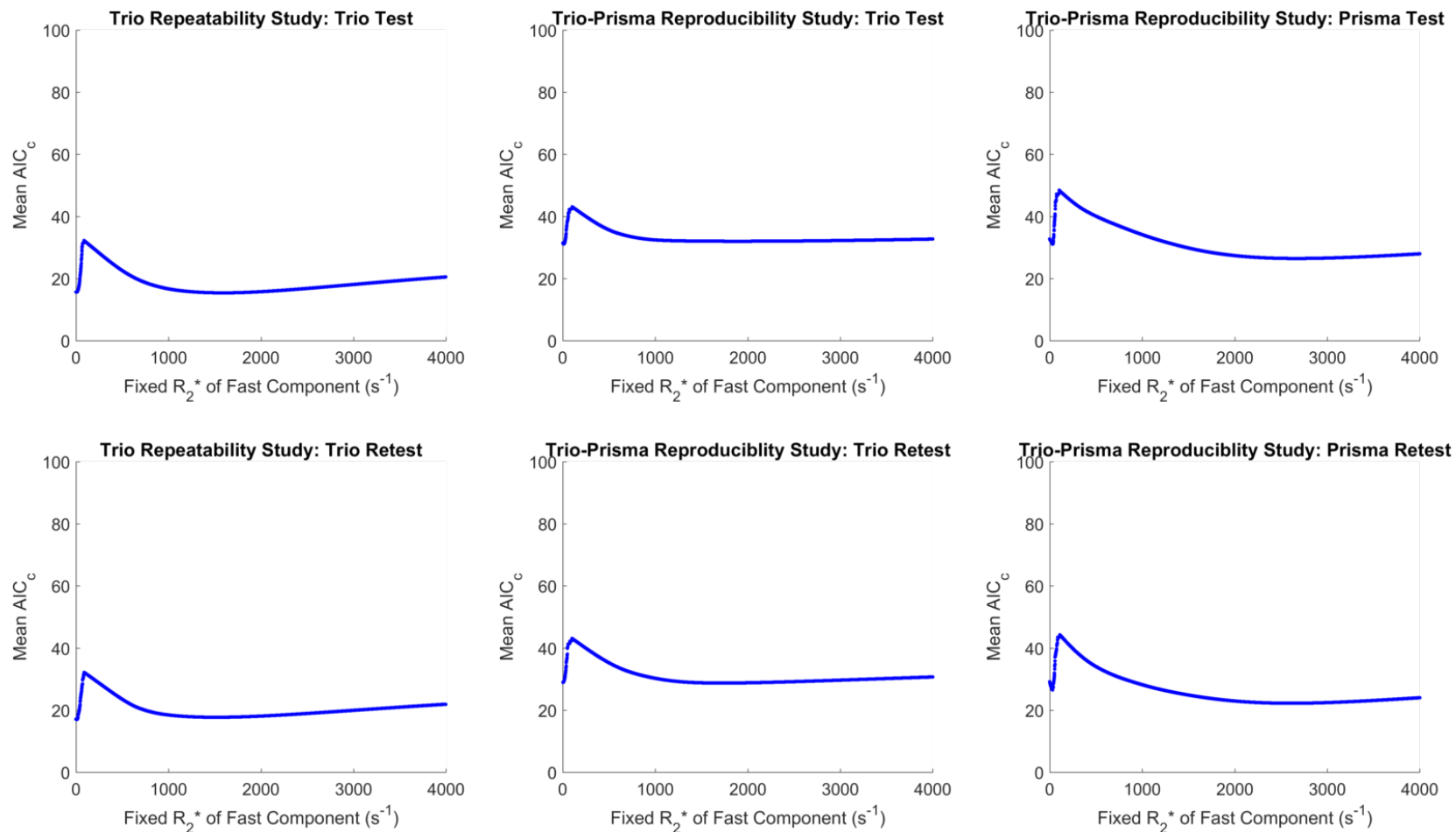


Fig. A6.13. Mean AIC_c according to the value of the R_{2f}^* of the fast component, when fixed in the bi-exponential model.

6.8.1.5. *B'*: Bi-Exponential Function with Fixed R_{2f}^*

	S_{0f} (AU)	S_{0s} (AU)	R_{2s}^* (s ⁻¹)
Initial value	$0.2 \times \text{MS}$	$0.7 \times \text{MS}$	15
Lower bound	0	0	0
Upper bound	$10 \times \text{MS}$	$10 \times \text{MS}$	1,000,000

Table A6.4. Initial conditions for signal decays fitted with a bi-exponential function with fixed $R_{2f}^* = 2130 \text{ s}^{-1}$.

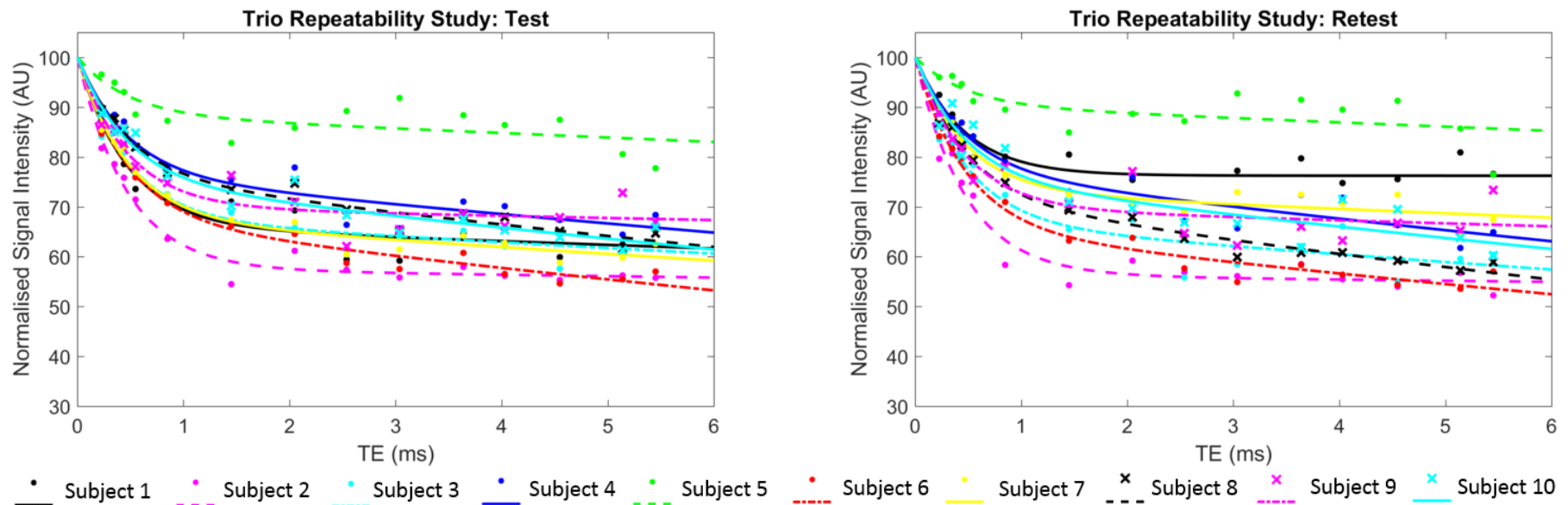


Fig. A6.14. Signal decays from the Trio repeatability study (Study 1), fitted with a bi-exponential function (Model B: Eq. [6.8]) with $R_{2f}^* = 2130 \text{ s}^{-1}$.

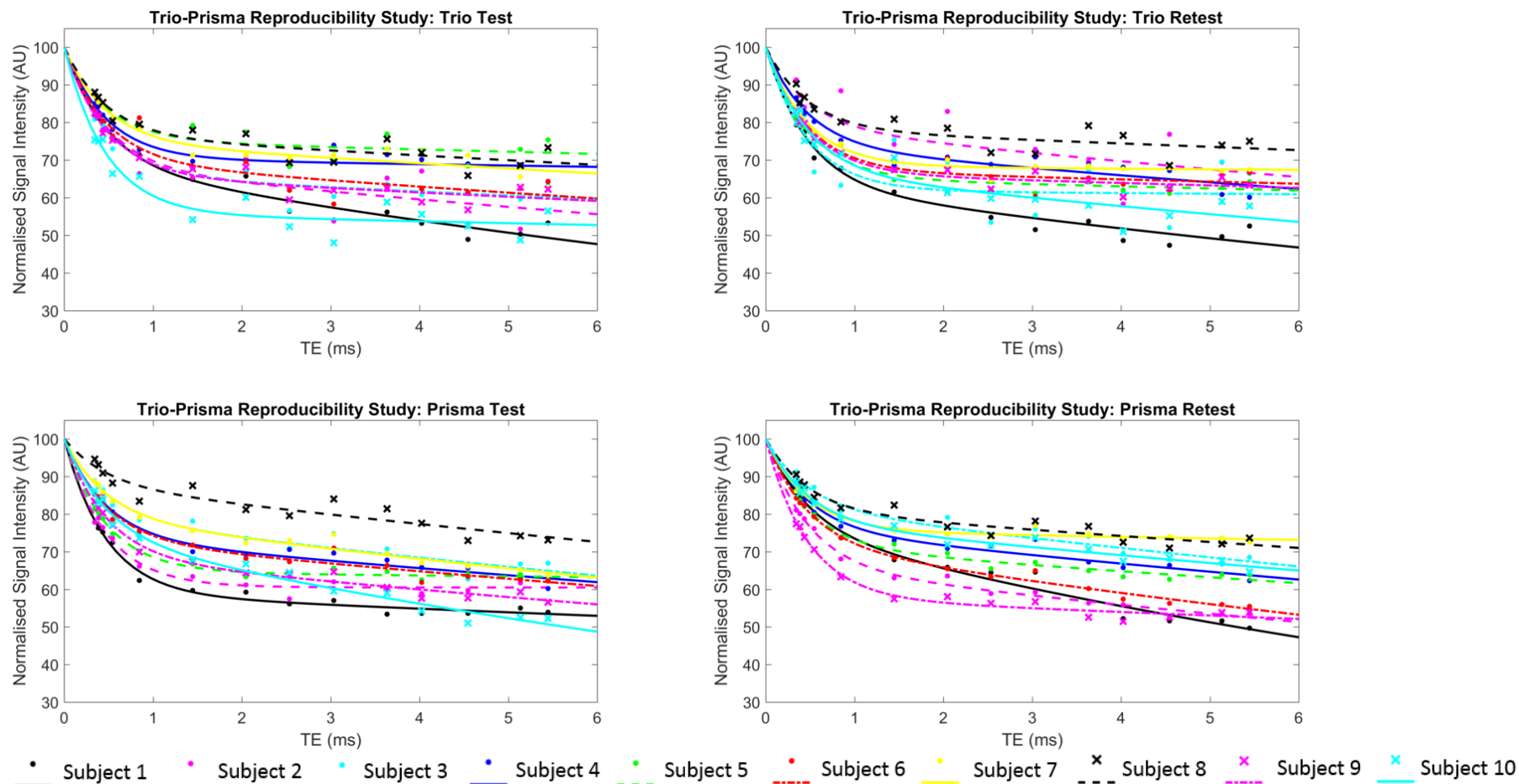


Fig. A6.15. Signal decays from the Trio-Prisma reproducibility study (Study 2), fitted with a bi-exponential function (Model B: Eq. [6.8]) with $R_{2f}^* = 2130 \text{ s}^{-1}$.

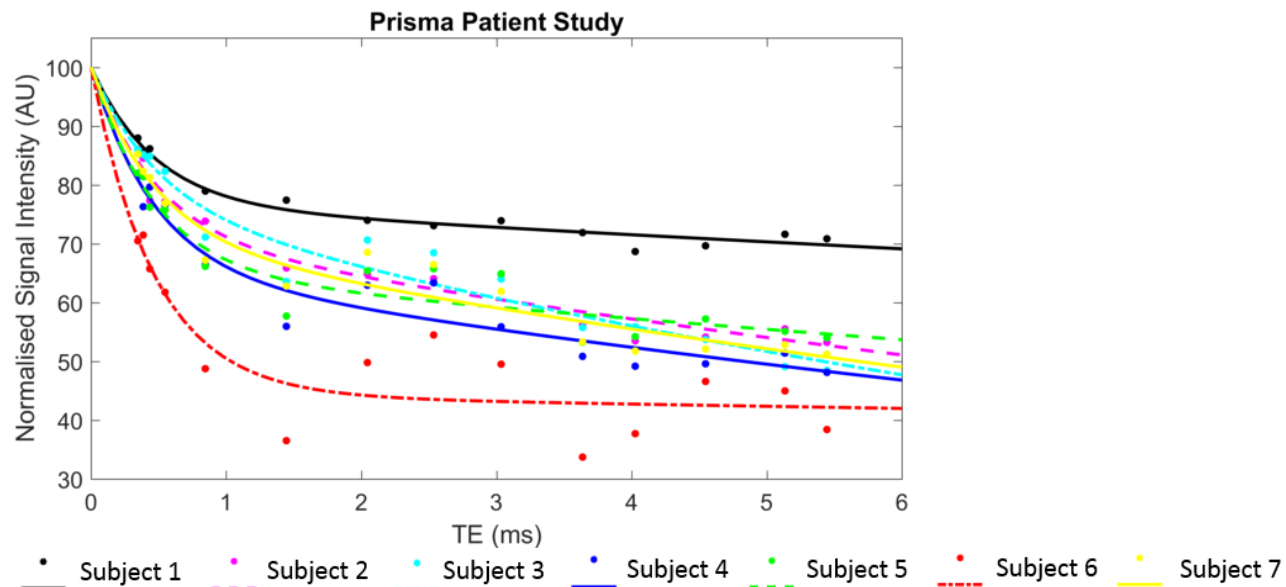


Fig. A6.16. Signal decays from the Prisma patient study (Study 3), fitted with a bi-exponential function (Model B: Eq. [6.8]) with $R_{2f}^* = 2130 \text{ s}^{-1}$.

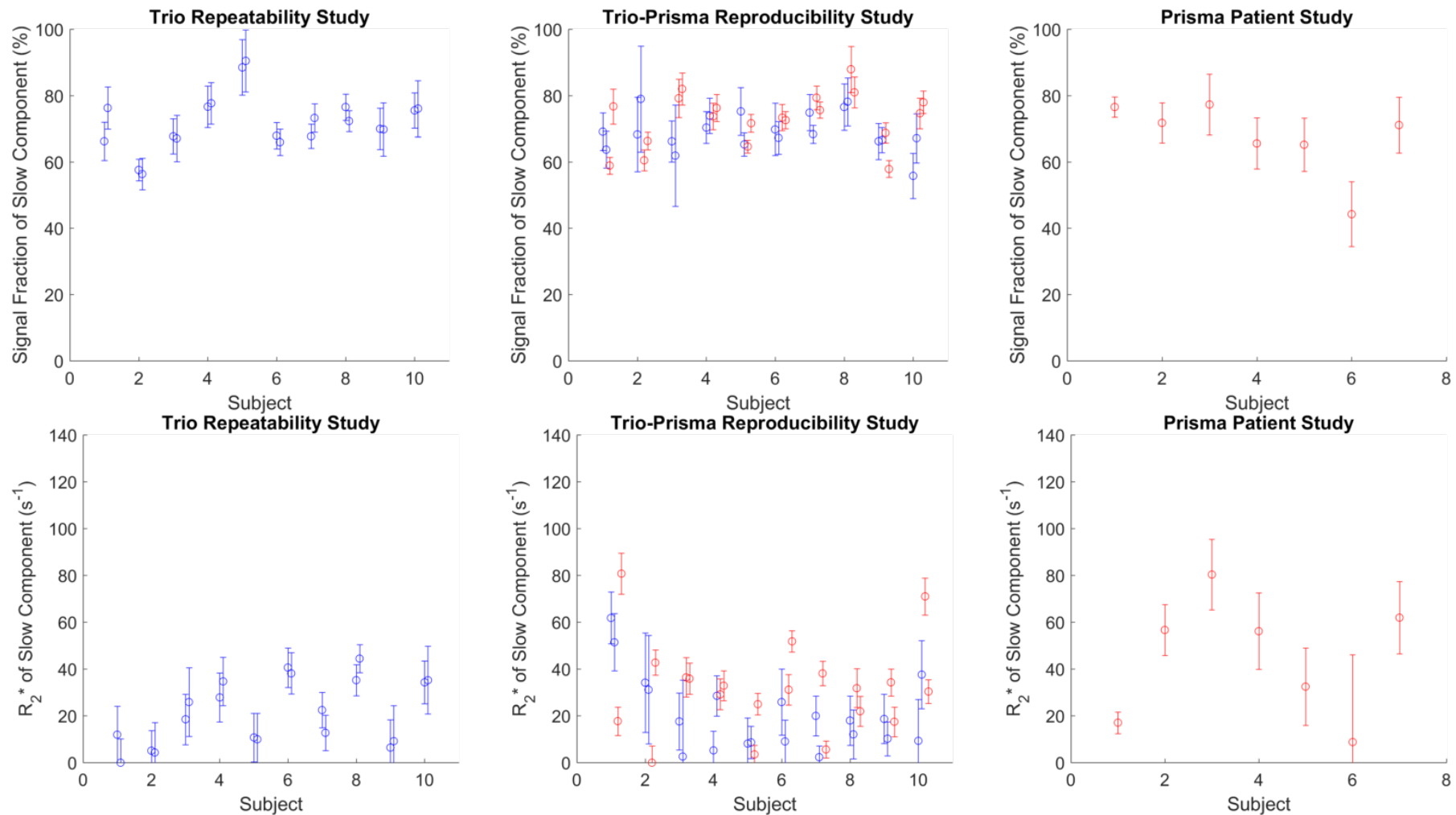


Fig. A6.17. Slow-component results for signal decays fitted with a bi-exponential function (Model B: Eq. [6.8]) with $R_{2f}^* = 2130 \text{ s}^{-1}$.

6.8.1.6. *C'*: Bi-Exponential Function with Lipids, with Fixed R_{2f}^*

	S_{0f} (AU)	S_{0s} (AU)	S_{0l} (AU)	R_{2s}^* (s ⁻¹)
Initial value	$0.2 \times \text{MS}$	$0.7 \times \text{MS}$	$0.005 \times \text{MS}$	15
Lower bound	0	0	0	0
Upper bound	$10 \times \text{MS}$	$10 \times \text{MS}$	$10 \times \text{MS}$	1,000,000

Table A6.5. Initial conditions for signal decays fitted with a bi-exponential function with lipids, with fixed $R_{2f}^* = 2130 \text{ s}^{-1}$.

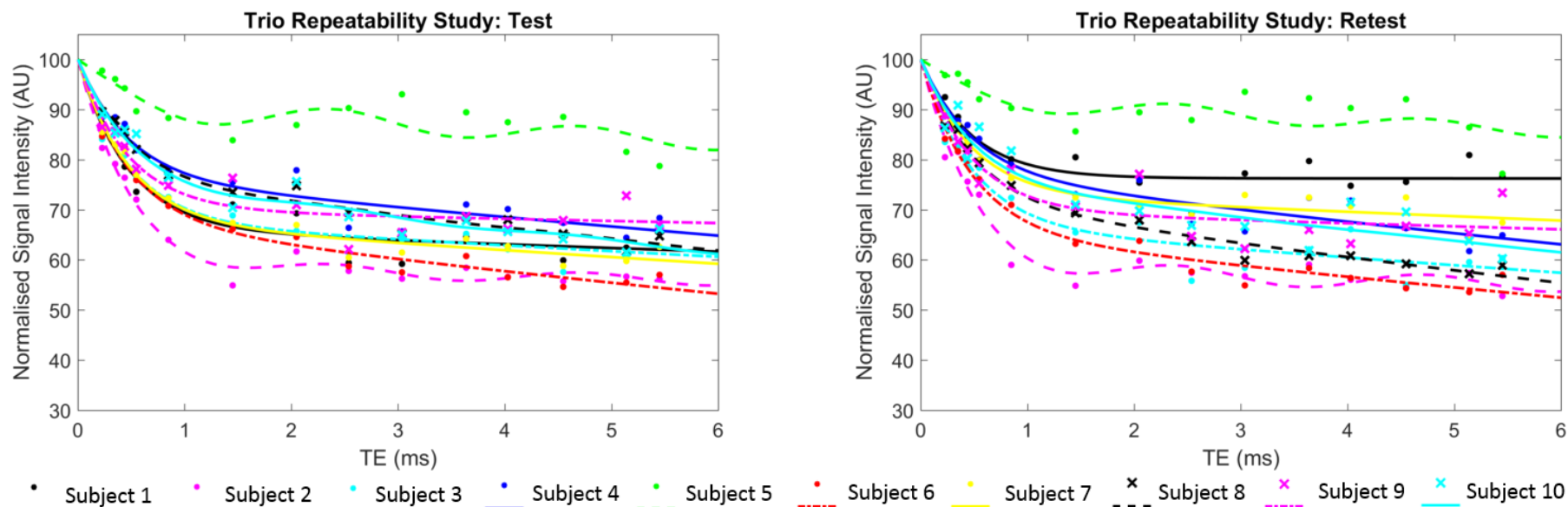
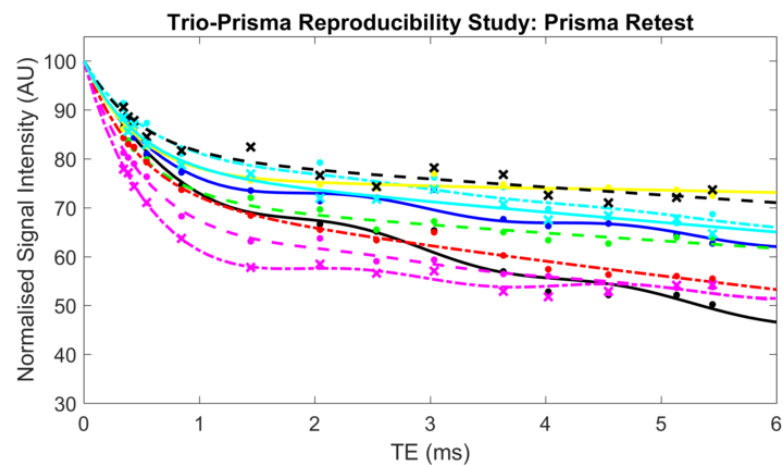
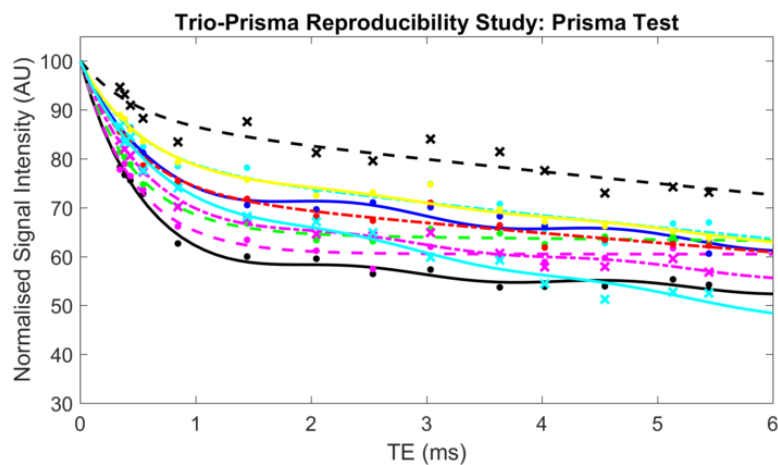
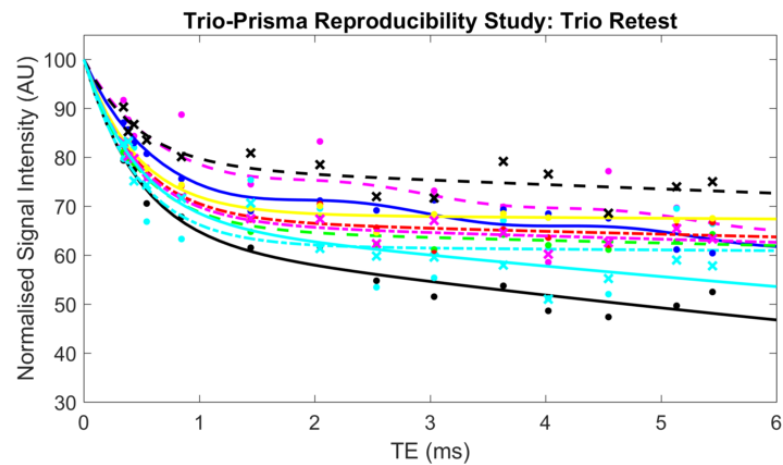
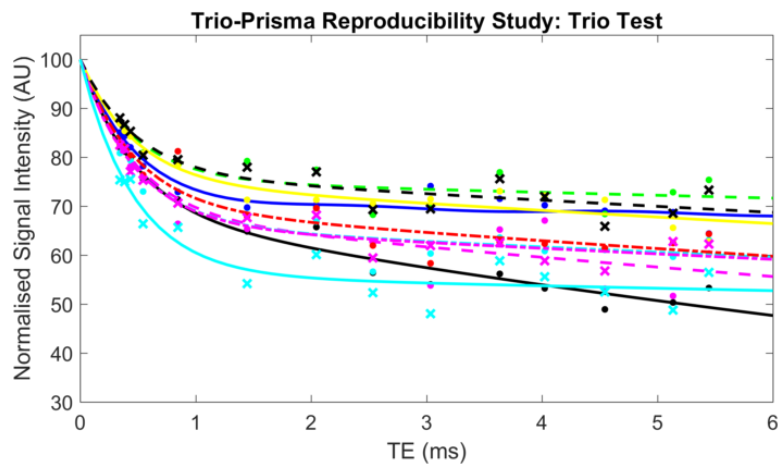


Fig. A6.18. Signal decays from the Trio repeatability study (Study 1), fitted with a bi-exponential function with lipids (Model C: Eq. [6.9]), with $R_{2f}^* = 2130 \text{ s}^{-1}$.



• Subject 1 • Subject 2 • Subject 3 • Subject 4 • Subject 5 • Subject 6 • Subject 7 • Subject 8 • Subject 9 • Subject 10
 --- Subject 1 --- Subject 2 --- Subject 3 --- Subject 4 --- Subject 5 --- Subject 6 --- Subject 7 --- Subject 8 --- Subject 9 --- Subject 10

Fig. A6.19. Signal decays from the Trio-Prisma reproducibility study (Study 2), fitted with a bi-exponential function with lipids (Model C: Eq. [6.9]), with $R_{2f}^* = 2130 \text{ s}^{-1}$.

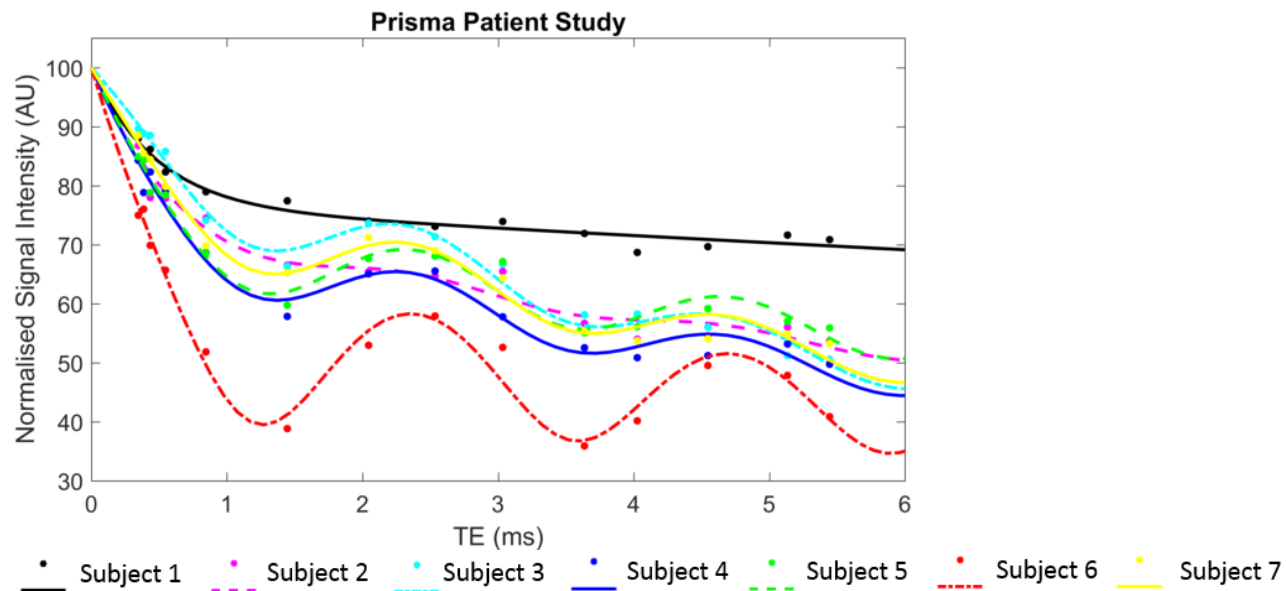


Fig. A6.20. Signal decays from the Prisma patient study (Study 3), fitted with a bi-exponential function with lipids (Model C: Eq. [6.9]), with $R_{2f}^* = 2130 \text{ s}^{-1}$.

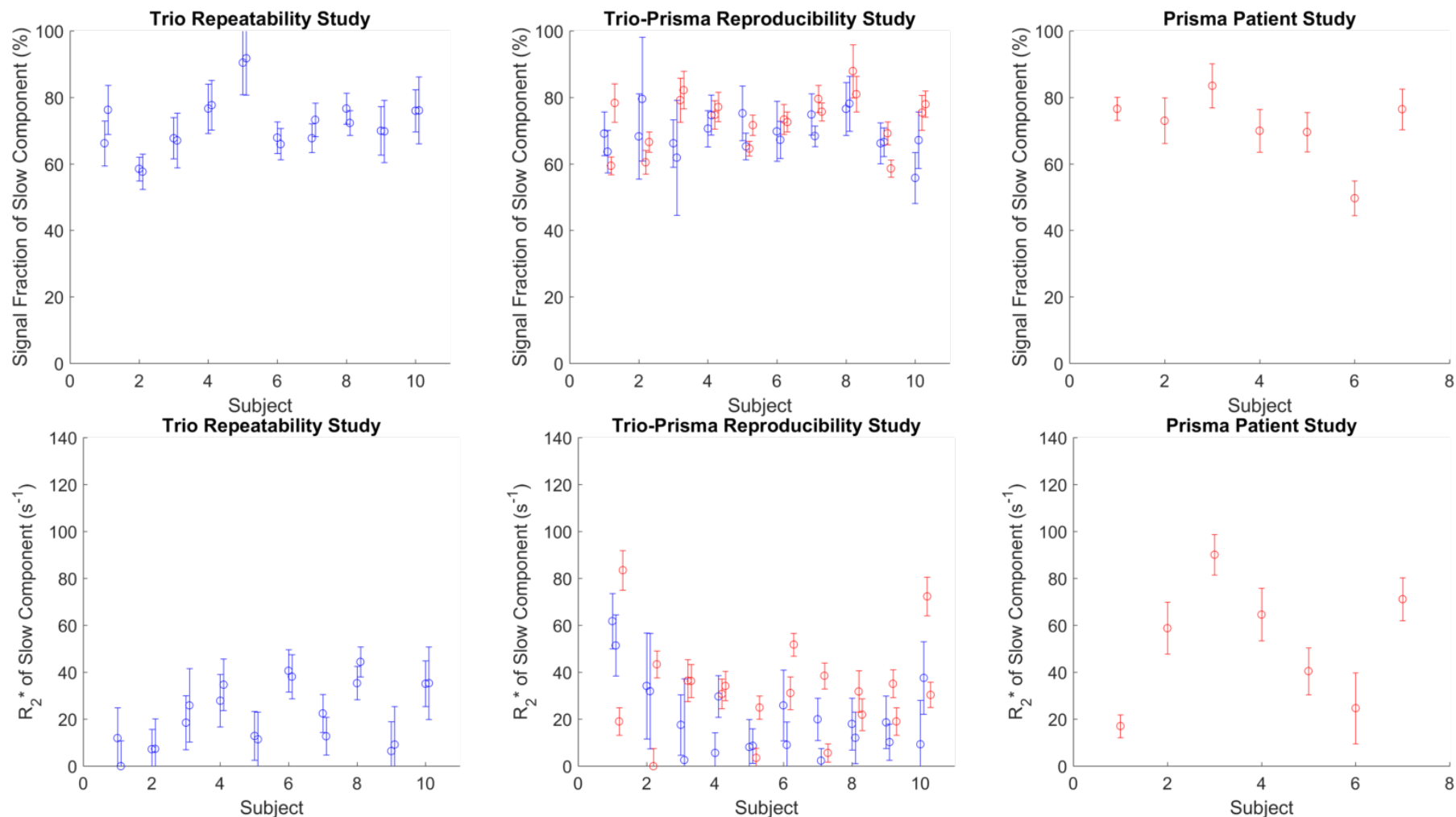


Fig. A6.21. Slow-component results for signal decays fitted with a bi-exponential function with lipids (Model C: Eq. [6.9]), with $R_{2f}^* = 2130 \text{ s}^{-1}$.

6.8.1.7. Per-Subject AIC_c Values

Subject	Test AIC_c					Retest AIC_c				
	A: Mono- exp.	B': Bi- exp. (fixed R_{2f}^*)	B: Bi- exp. (fixed R_{2f}^*) with lipids	C': Bi- exp	C: Bi- exp. with lipids	A: Mono- exp.	B': Bi- exp. (fixed R_{2f}^*)	B: Bi- exp.	C': Bi- exp	C: Bi- exp. with lipids
1	34.08	22.73	27.37	27.79	33.87	35.25	26.19	30.34	31.24	36.84
2	26.97	0.23	5.23	2.88	8.72	26.69	8.35	13.38	11.29	17.73
3	32.73	20.11	23.01	25.16	29.51	37.00	29.12	31.33	34.18	37.83
4	28.12	22.03	26.24	27.08	32.74	22.70	16.98	21.66	22.03	28.16
5	21.33	22.92	26.55	25.97	31.61	17.57	20.17	24.82	24.38	30.69
6	39.27	20.72	22.41	25.77	28.91	33.48	14.12	12.63	19.17	19.13
7	42.94	22.50	24.68	27.55	31.18	34.96	20.39	24.98	25.44	31.48
8	12.84	-1.71	3.35	3.32	9.82	13.07	-9.07	-9.81	-4.01	-4.02
9	28.34	19.69	22.87	24.75	29.37	34.99	30.00	34.24	35.06	40.69
10	20.19	10.87	15.12	15.70	20.79	29.29	26.68	29.80	31.73	36.09
Mean	28.68	16.01	19.68	20.60	25.65	28.50	18.29	21.34	23.05	27.46

Table A6.6. Per-subject goodness-of-fit values for the Trio repeatability study.

Subject	Test AIC _c					Retest AIC _c				
	A: Mono- exp.	B': Bi- exp. (fixed R_{2f}^*)	B: Bi- exp. (fixed R_{2f}^*) with lipids	C': Bi- exp	C: Bi- exp. with lipids	A: Mono- exp.	B': Bi- exp. (fixed R_{2f}^*)	B: Bi-exp.	C': Bi- exp	C: Bi- exp. with lipids
1	34.08	23.86	27.07	28.92	33.57	37.52	25.22	29.06	30.27	35.44
2	47.48	46.07	50.77	51.12	57.27	49.96	52.29	56.69	57.27	62.91
3	33.12	23.76	26.54	28.82	33.04	48.76	49.19	54.10	54.24	60.60
4	42.49	29.46	31.03	34.42	37.53	33.66	23.98	26.38	28.12	32.65
5	34.11	29.87	33.98	34.93	40.48	33.79	12.25	15.50	17.30	22.00
6	41.70	36.98	36.71	42.03	43.21	38.33	25.02	28.12	30.08	34.40
7	33.02	24.25	28.61	29.31	35.11	35.73	9.07	13.73	14.12	20.23
8	40.39	36.16	41.11	41.21	47.61	41.05	37.91	42.92	42.97	49.42
9	36.76	24.81	29.36	29.86	35.86	35.43	16.16	21.21	21.21	27.71
10	55.00	44.94	49.82	49.99	56.32	44.24	37.89	40.28	42.94	46.78
Mean	39.82	32.02	35.50	37.06	42.00	39.85	28.90	32.80	33.85	39.21

Table A6.7. Per-subject goodness-of-fit values for the Trio experiments from the Trio-Prisma reproducibility study.

Subject	Test AIC _c					Retest AIC _c				
	A: Mono- exp.	B': Bi- exp. (fixed R_{2f}^*)	B: Bi- exp. (fixed R_{2f}^*) with lipids	C': Bi- exp	C: Bi- exp. with lipids	A: Mono- exp.	B': Bi-exp. (fixed R_{2f}^*)	B: Bi- exp.	C': Bi- exp	C: Bi- exp. with lipids
1	47.91	16.08	20.87	18.48	24.86	39.50	30.85	34.36	32.96	38.98
2	50.97	23.48	28.45	28.53	34.95	44.66	16.47	21.15	21.14	27.50
3	39.32	33.57	37.34	38.62	43.84	24.71	18.10	21.85	23.06	28.35
4	46.87	31.65	34.64	33.74	39.27	42.87	29.48	28.41	32.28	34.15
5	45.33	8.41	13.40	13.46	19.90	40.37	15.11	19.95	20.17	26.45
6	44.89	29.16	33.15	34.21	39.65	45.85	20.00	24.95	25.05	31.45
7	42.38	28.35	30.35	33.17	36.85	40.34	15.03	19.68	20.07	26.18
8	40.41	40.92	42.17	45.97	48.67	42.08	34.28	38.63	39.34	45.13
9	47.90	24.20	26.64	28.19	32.97	53.05	21.04	25.85	22.36	28.55
10	46.60	34.52	36.69	38.82	43.12	41.90	26.11	30.54	31.17	37.04
Mean	45.26	27.03	30.37	31.32	36.41	41.53	22.65	26.54	26.76	32.38

Table A6.8. Per-subject goodness-of-fit values for the Prisma experiments from the Trio-Prisma reproducibility study.

Subject	AIC _c				
	A: Mono- exp.	B': Bi- exp. (fixed R_{2f}^*)	B: Bi- exp. (fixed R_{2f}^*) with lipids	C': Bi- exp	C: Bi- exp. with lipids
1	35.91	16.83	21.88	21.88	28.38
2	54.27	45.87	49.20	49.38	55.13
3	26.95	25.56	29.02	12.68	18.95
4	32.86	26.47	31.18	18.55	24.23
5	50.25	44.32	47.61	33.25	39.62
6	68.23	62.78	66.60	40.01	46.31
7	45.00	40.99	42.83	28.89	33.66
Mean	44.78	37.55	41.19	29.24	35.19

Table A6.9. Per-subject goodness-of-fit values for the Prisma patient study.

6.8.2. Model Selection: Step 2

6.8.2.1. B' : Bi-Exponential Function with Fixed R_{2f}^*

		S_{0f} (AU)	S_{0s} (AU)	R_{2s}^* (s^{-1})
Initial value	Lower bound	0	0	0
	Upper bound	$1.3 \times MS$	$1.3 \times MS$	100
Fitting solution	Lower bound	0	0	0
	Upper bound	$10 \times MS$	$10 \times MS$	1,000,000

Table A6.10. Initial conditions for Step 2 of the model selection with a Monte Carlo fitting approach. The model was the bi-exponential function with fixed $R_{2f}^* = 2130 \text{ s}^{-1}$, selected from Step 1.

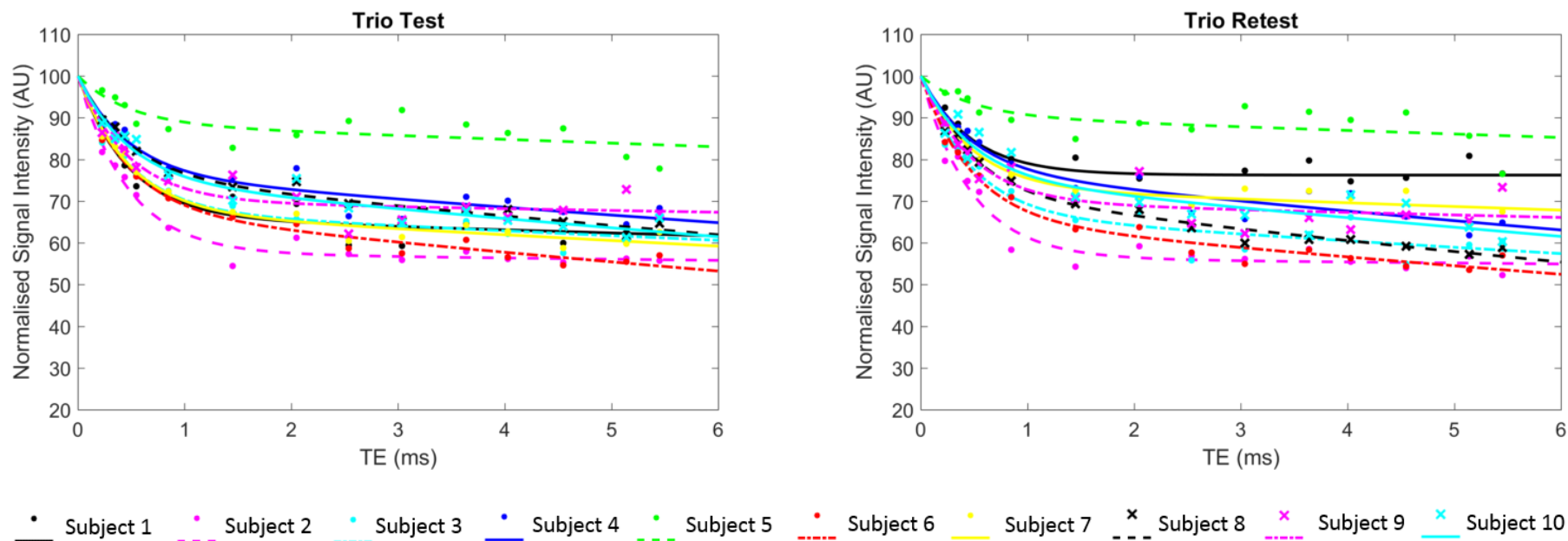


Fig. A6.22. Signal decays from the Trio repeatability study (Study 1), fitted with a bi-exponential function (Model B: Eq. [6.8]) with $R_{2f}^* = 2130 \text{ s}^{-1}$. A Monte Carlo approach was used for fitting.

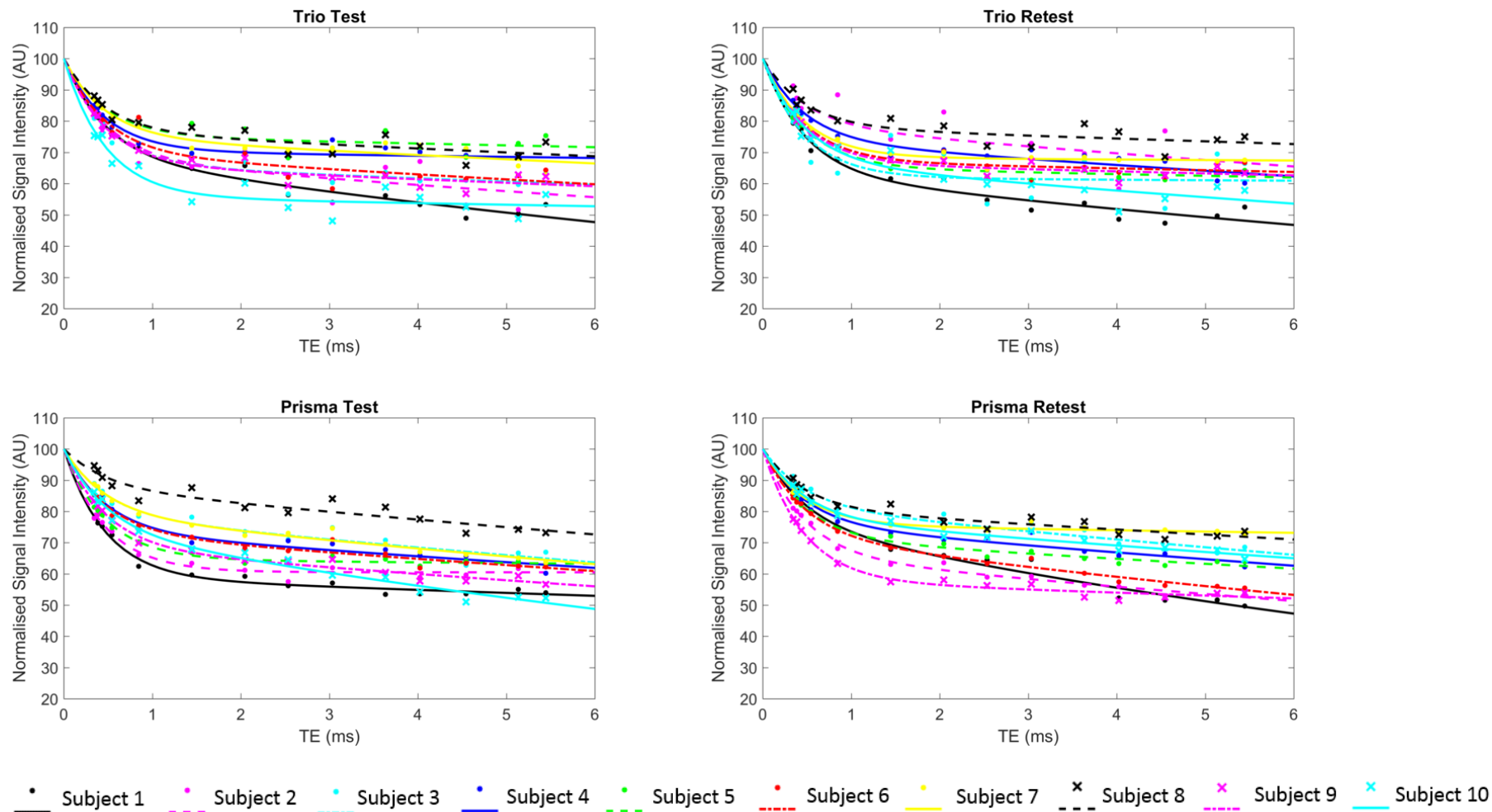


Fig. A6.23. Signal decays from the Trio-Prisma reproducibility study (Study 2), fitted with a bi-exponential function (Model B: Eq. [6.8]) with $R_{2f}^* = 2130 \text{ s}^{-1}$. A Monte Carlo approach was used for fitting.

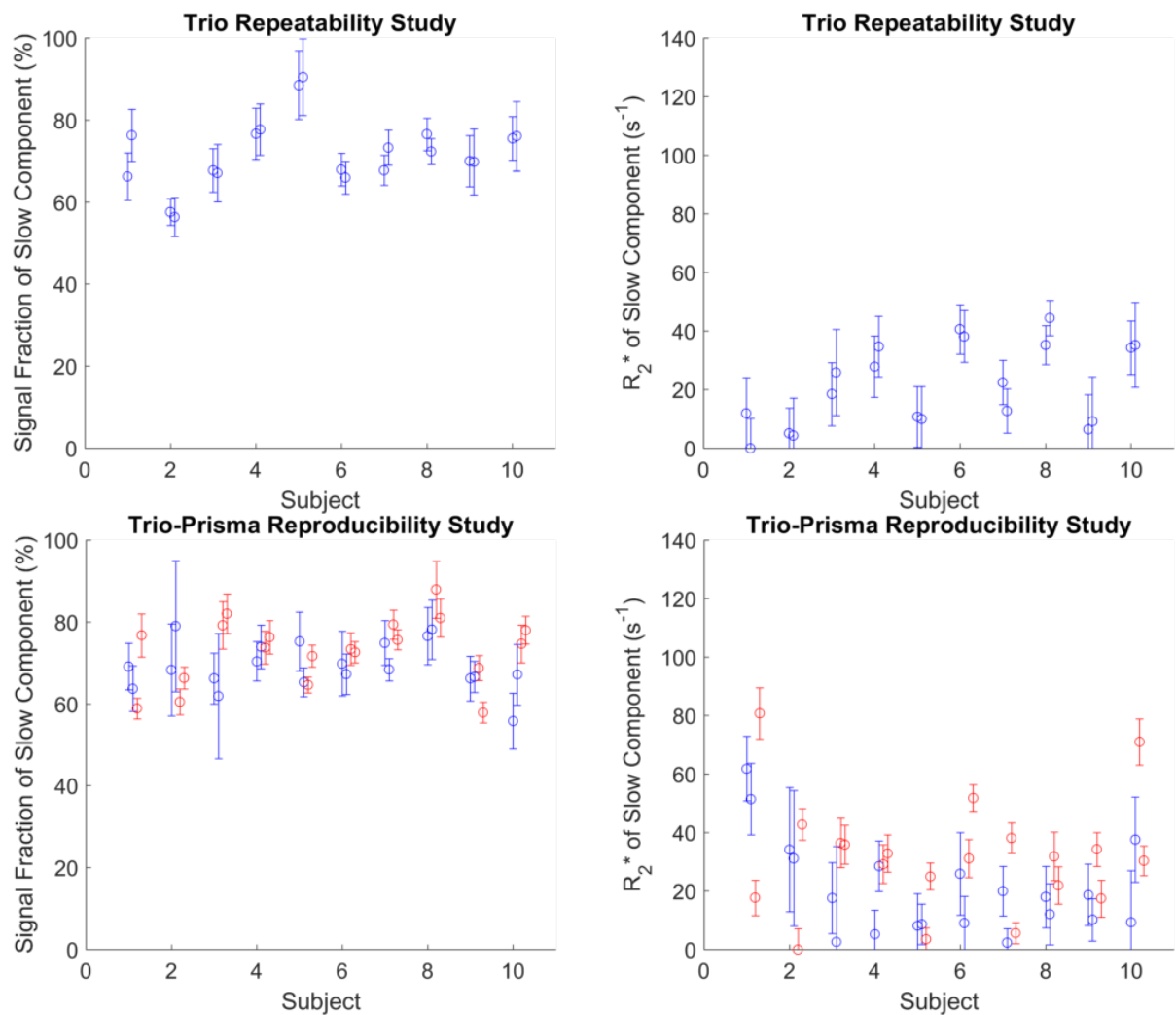


Fig. A6.24. Slow-component results for signal decays fitted with a bi-exponential function (Model B: Eq. [6.8]) with $R_{2f}^* = 2130 \text{ s}^{-1}$. A Monte Carlo approach was used for fitting.

6.8.2.2. *D: Function for Isotropic Collagen and Free Water*

		S_{0c} (AU)	S_{0s} (AU)	R_{2s}^* (s ⁻¹)
Initial value	Lower bound	0	0	0
	Upper bound	$1.3 \times \text{MS}$	$1.3 \times \text{MS}$	100
Fitting solution	Lower bound	0	0	0
	Upper bound	$10 \times \text{MS}$	$10 \times \text{MS}$	1,000,000

Table A6.11. Initial conditions for Step 2 of the model selection with a Monte Carlo fitting approach. The model was isotropic collagen and free water.

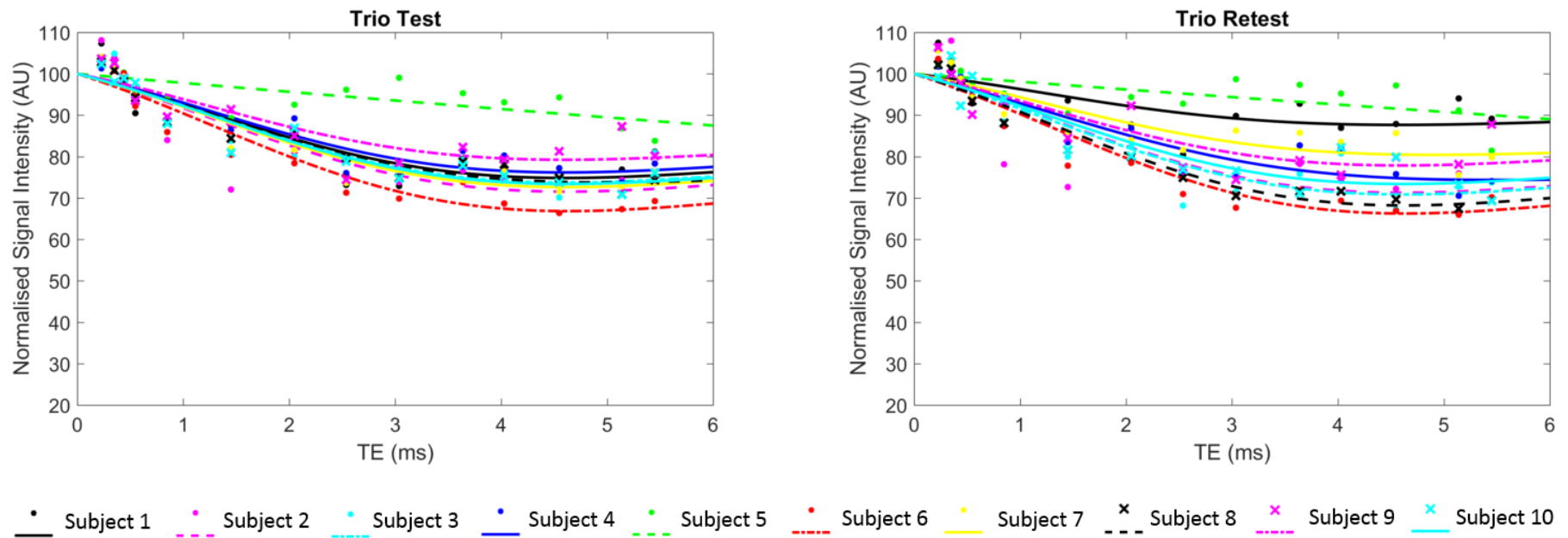


Fig. A6.25. Signal decays from the Trio repeatability study (Study 1), fitted with a model for isotropic collagen and free water (Model D: Eq. [6.5]). A Monte Carlo approach was used for fitting.

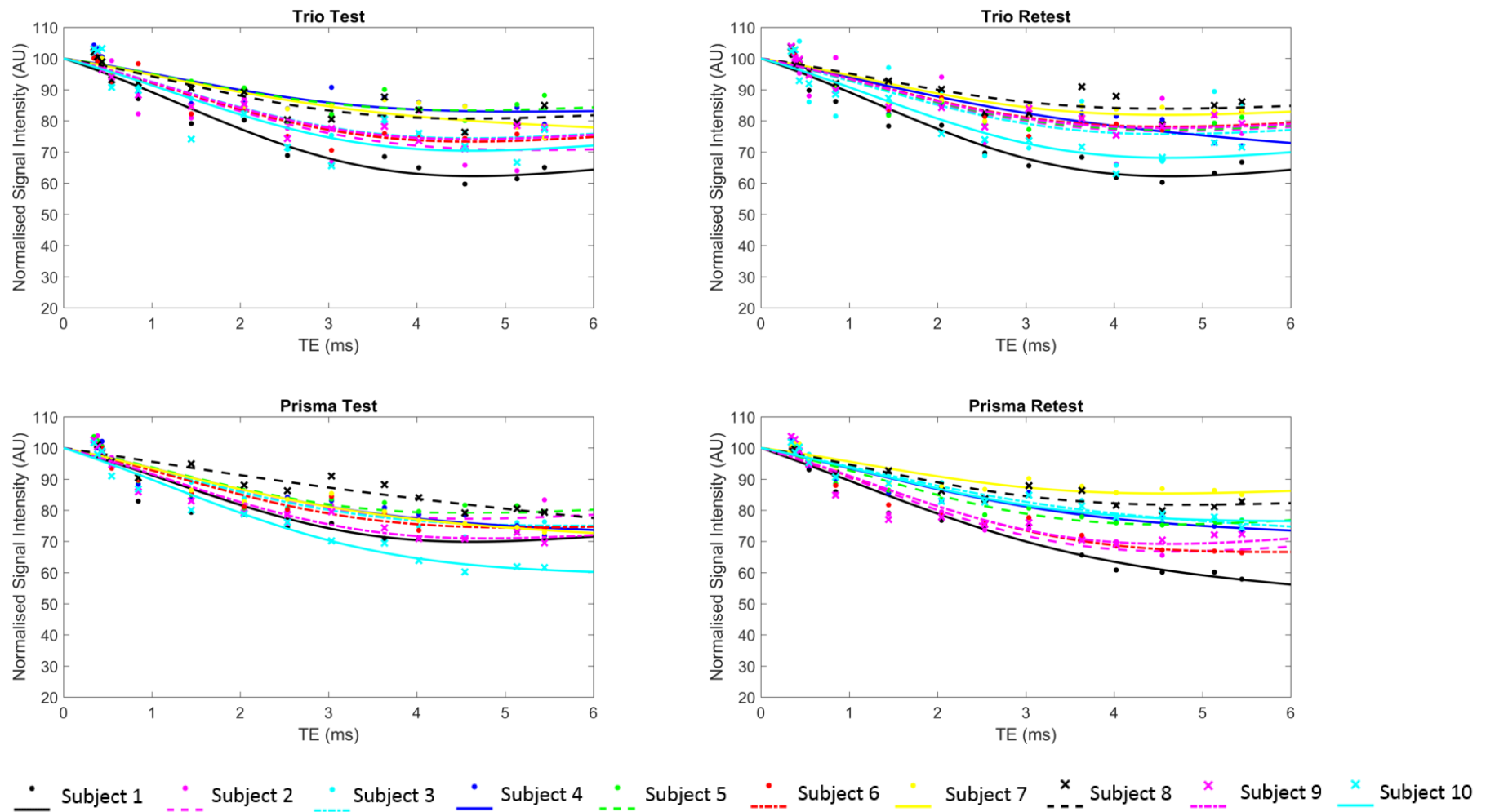


Fig. A6.26. Signal decays from the Trio-Prisma reproducibility study (Study 2), fitted with a model for isotropic collagen and free water (Model D: Eq. [6.5]). A Monte Carlo approach was used for fitting.

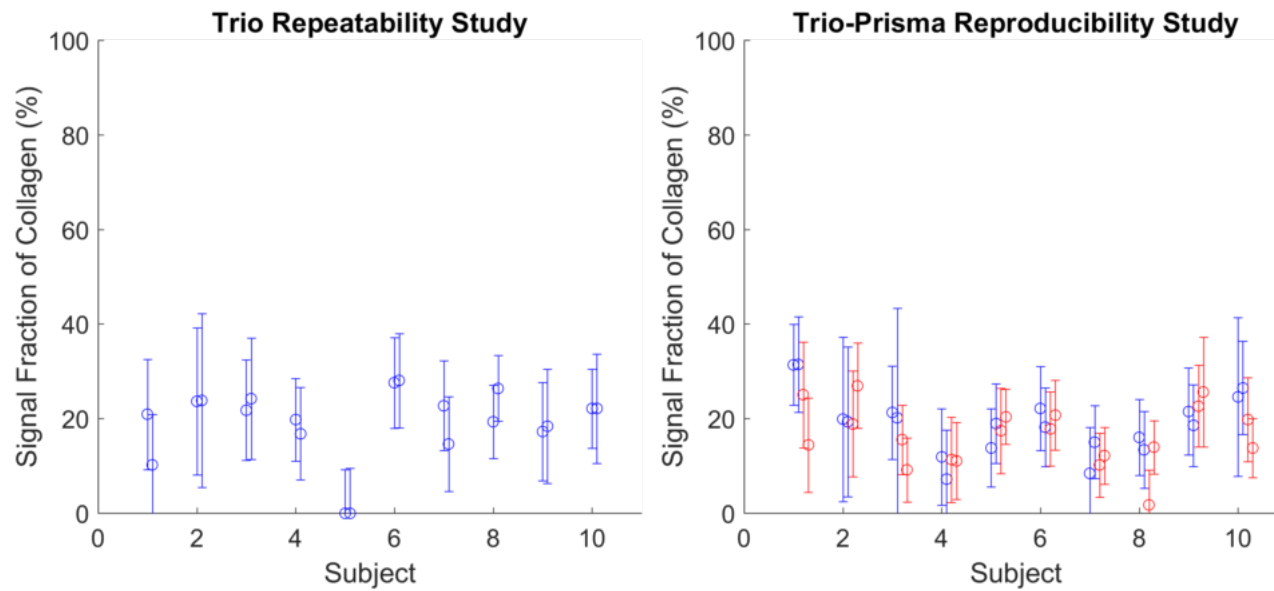


Fig. A6.27. Collagen results for signal decays fitted with a model for isotropic collagen and free water (Model D: Eq. [6.5]). A Monte Carlo approach was used for fitting.

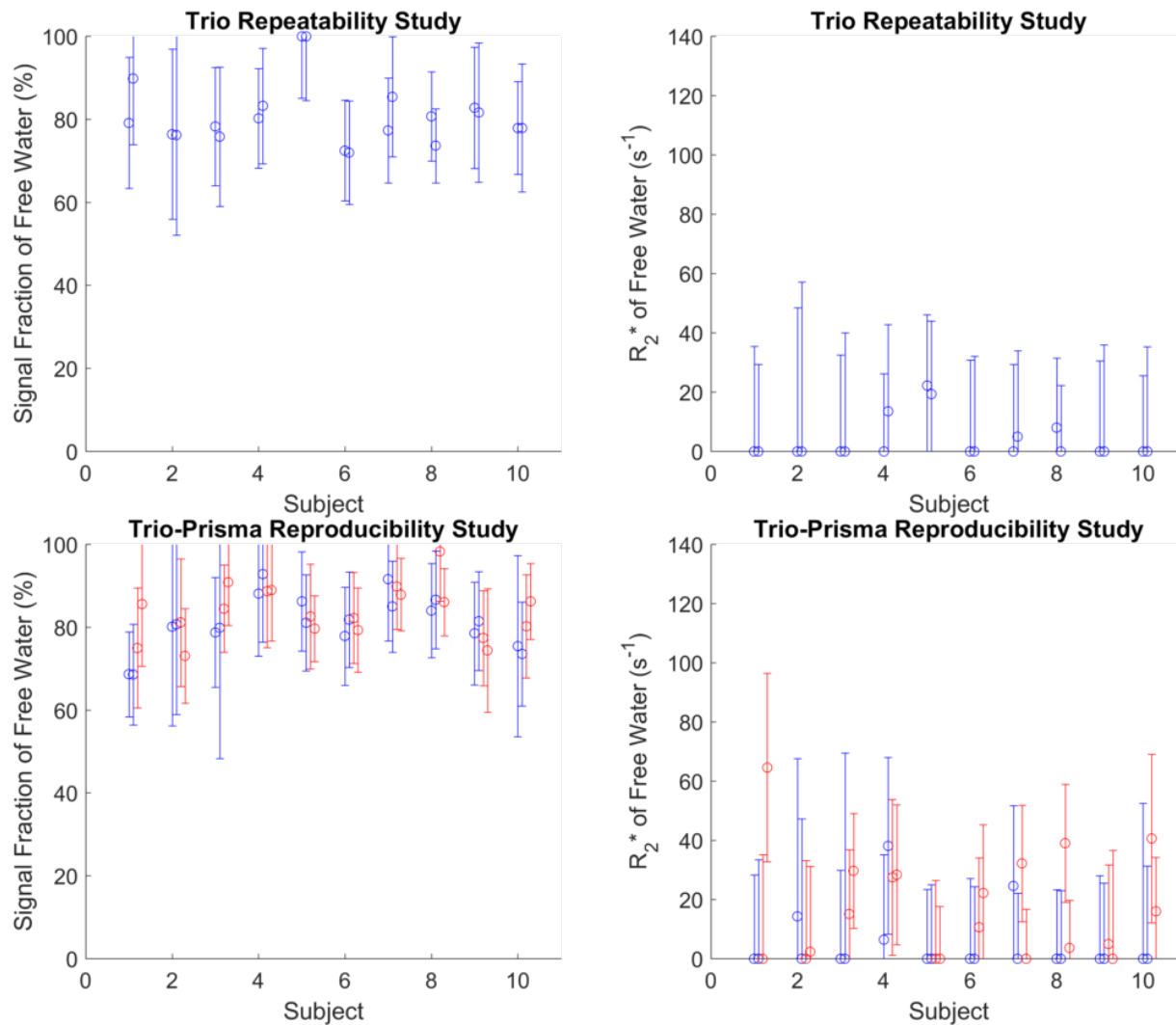


Fig. A6.28. Free-water results for signal decays fitted with a model for isotropic collagen and free water (Model D: Eq. [6.5]). A Monte Carlo approach was used for fitting.

6.8.2.3. Per-Subject AIC_c Values

Subject	Trio repeat. study		Trio-Prisma reprod. study									
	Test AIC_c		Retest AIC_c		Trio test AIC_c		Trio retest AIC_c		Prisma test AIC_c		Prisma retest AIC_c	
	B': Bi-exp. (fixed R_{2f}^*)	D: Iso. coll. and water	B': Bi-exp. (fixed R_{2f}^*)	D: Iso. coll. and water	B': Bi-exp. (fixed R_{2f}^*)	D: Iso. coll. and water	B': Bi-exp. (fixed R_{2f}^*)	D: Iso. coll. and water	B': Bi-exp. (fixed R_{2f}^*)	D: Iso. coll. and water	B': Bi-exp. (fixed R_{2f}^*)	D: Iso. coll. and water
1	22.73	30.33	26.19	35.42	23.86	26.77	25.22	30.04	16.08	43.56	30.85	41.38
2	0.23	25.97	8.35	27.48	46.07	50.03	52.29	51.43	23.48	46.61	16.47	40.87
3	20.11	28.45	29.12	34.21	23.76	27.95	49.19	49.52	33.57	38.88	18.10	26.67
4	22.03	25.56	16.98	23.49	29.46	44.89	23.98	37.11	31.65	49.07	29.48	44.79
5	22.92	25.37	20.17	21.62	29.87	31.40	12.25	27.73	8.41	42.03	15.11	31.72
6	20.72	33.25	14.12	26.47	36.98	33.93	25.02	31.82	29.16	43.79	20.00	42.76
7	22.50	37.79	20.39	36.56	24.25	36.09	9.07	31.72	28.35	43.90	15.03	38.19
8	-1.71	10.96	-9.07	4.33	36.16	38.37	37.91	40.39	40.92	44.38	34.28	40.16
9	19.69	24.58	30.00	32.10	24.81	31.09	16.16	30.96	24.20	45.51	21.04	48.64
10	10.87	16.88	26.68	28.97	44.94	54.08	37.89	37.19	34.52	45.95	26.11	41.00
Mean	16.01	25.91	18.29	27.07	32.02	37.46	28.90	36.79	27.03	44.37	22.65	39.62

Table A6.12. Per-subject AIC_c values for all studies, fitted with a Monte Carlo approach.

6.8.2.4. Analysis with the Appropriate Models

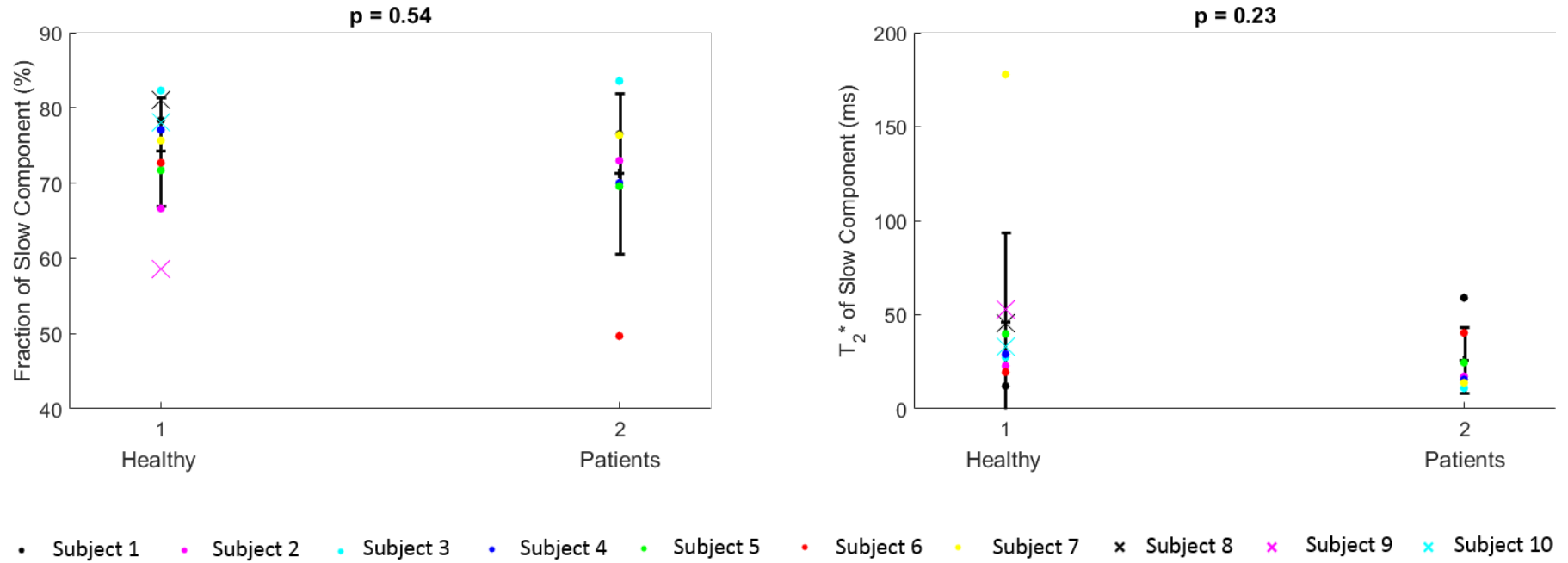


Fig. A6.29. Comparison of the signal fraction and T_2^* of the slow component between healthy subjects and patients. The bi-exponential function with lipids and fixed $R_{2f}^* = 2130 \text{ s}^{-1}$ was used for modelling the slow component.

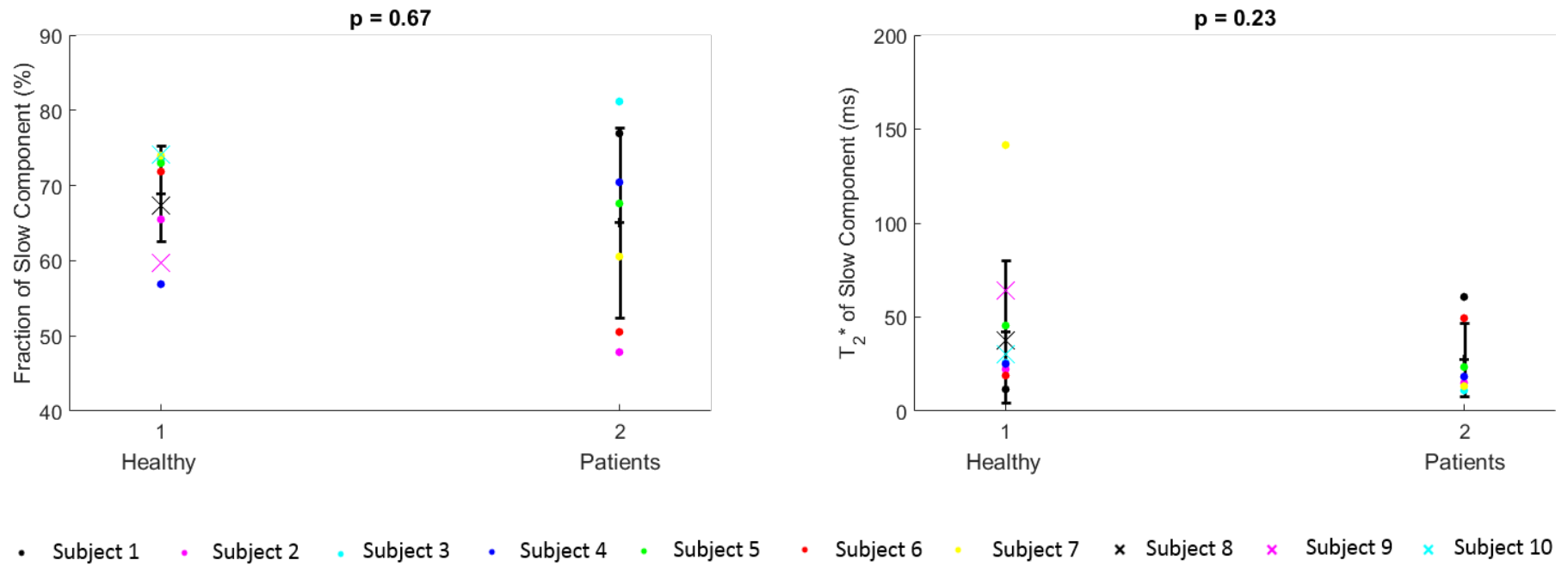


Fig. A6.30. Comparison of the signal fraction and T_2^* of the slow component between healthy subjects and patients. The bi-exponential function with lipids and unfixed R_{2f}^* was used for modelling the slow component, as in (13).

Study	Bi-exp. (fixed R_{2f}^*) with lipids		Bi-exp. (unfixed R_{2f}^*) with lipids	
	Fraction of slow comp. (%)	T_2^* of slow comp. (ms)	Fraction of slow comp. (%)	T_2^* of slow comp. (ms)
2: Trio- Prisma reprod. (Prisma retest)	74 ± 7	46 ± 48	69 ± 6	42 ± 38
3: Prisma patients	71 ± 11	26 ± 18	65 ± 13	27 ± 20

Table A6.13. **Results for the slow- R_2^* (long- T_2^*) signal in the livers of healthy subjects and liver-disease patients, using two models.**

7. Summary and Potential Sources of Short- T_2^* Signal in the Liver

This chapter summarises the work in this thesis. Furthermore, it reviews the potential sources of short- T_2^* signal, by discussing current literature and the composition of the liver. Future work on identifying the origin of the short- T_2^* signal is proposed.

7.1. Summary

This thesis investigated the short- T_2^* signal in the liver with UTE. According to the objectives outlined in Chapter 1, the following was achieved:

1. The R_2^* and resonant frequency shift of tendon (oriented collagen) were characterised. Namely, R_2^* s of $950 \pm 30 \text{ s}^{-1}$ and $130 \pm 20 \text{ s}^{-1}$ at 0° and 55° relative to B_0 were found, respectively, with a maximal frequency shift of 1.0 ppm. Furthermore, the R_2^* signal in isotropic collagen phantoms was described by a model of fast exchange between collagen-bound water and free water.
2. An R_2^* model of isotropic collagen and free water was developed based on measurements of the R_2^* and resonant frequency shift in tendon. The suitability of the model describing isotropic collagen, as well as other models with varying signal components, was assessed in the liver. A bi-exponential model with fixed $R_{2f}^* = 2130 \text{ s}^{-1}$ ($T_{2f}^* = 0.5 \text{ ms}$) was most appropriate for the healthy subjects, whereas a bi-exponential model with lipids and fixed $R_{2f}^* = 2130 \text{ s}^{-1}$ was best for the liver-disease patients. For studies with both healthy subjects and patients, the

bi-exponential model with lipids and fixed $R_{2f}^* = 2130 \text{ s}^{-1}$ was generalised to be the most suitable model.

3. The R_2^* model of isotropic collagen and free water was inferior to the bi-exponential model with fixed $R_{2f}^* = 2130 \text{ s}^{-1}$ ($T_{2f}^* = 0.5 \text{ ms}$) in healthy subjects. Namely, the assumptions for isotropic collagen signal, based on the measurements in tendon, did not account for the short- T_2^* signal observed in the liver. The findings did not disprove the presence of isotropic collagen in the liver. However, as a bi-exponential model was better able to describe the R_2^* signal in the liver, it raises the possibility of the short- T_2^* signal originating from sources other than isotropic collagen.

Characterisation of the short- T_2^* signal in the liver can be improved in future work. I found a less accurate short T_2^* of 0.4 - 0.5 ms at $\sim 30 \%$ of the total signal. By contrast, Zhu et al. observed signal with a short T_2^* of $\sim 0.2 \text{ ms}$ and $\sim 10 \%$ signal fraction. Accuracy can be improved with reductions in minimum TE, signal contamination and breathing motion. The minimum TE of 0.35 ms can be lowered by shortening the existing half-sinc pulse or optimising the sequence to 3D with a short RF pulse. Aliased signal can be reduced by oversampling the data at twice the field of view and truncating. Finally, localising a slice that is lower in the liver (e.g. on the portal vein) can reduce artefacts from breathing motion.

Having modelled the short- T_2^* signal in the liver, the following section discusses potential sources of the signal and future investigations.

7.2. Potential Sources of Short- T_2^* Signal in the Liver

7.2.1. Literature on Short- T_2^* Signal in White Matter

Overall, one would like to identify a constituent with 10 - 30 % signal in the liver and a short T_2^* of 0.2 - 0.5 ms. Interestingly, a similar signal with a short T_2^* of 0.3 - 0.7 ms and signal fraction < 15 % has been found in white matter at 3 T, suggested to be due to methylene protons in phospholipid membranes and/or protons and water bound to macromolecules. Table 7.1 summarises the literature in the brain. A key to the abbreviations used in the tables of this chapter is provided in Fig. 7.1.

comp. = component

WM = white matter

CH = cholesterol

PC = phosphatidylcholine

GC = galactocerebroside

SM = sphingomyelin

PI = phosphatidylinositol

PS = phosphatidylserine

Bov. = bovine

Hum. = human

US = unspecified

IVT = *in vitro*

IV = *in vivo*

T = temperature

BT = body temperature

RT = room temperature

NTE = number of TEs

FID = free induction decay

SPGR = spoiled gradient echo

MOLLI = modified Look-Locker inversion recovery

ULSTIR-EPI = ultrashort short- T_1 inversion recovery echo planar imaging

GRE = gradient echo

MT = magnetisation transfer
MAS = magic angle spinning
pre-sat. = pre-saturation
IR-UTE = inversion recovery UTE

IO = iron overload
H = healthy
D = diseased
CVD = cardiovascular disease
C = cirrhosis
S = steatosis (but no cirrhosis)
F0, F1, F2, F3, F4 = METAVIR F-scores
HCV = Hepatitis C virus
NP = non-pathological

PDFF = proton density fat fraction
ECV = extracellular volume
BV = blood volume

SSD = statistically significant difference from healthy (yes or no)
Y = yes
N = no

Seq. = sequence
Peak loc. = peak location
LW = line width

est. = estimated from data in paper

Fig. 7.1. Key to abbreviations in the tables.

Paper	Sample	Source	State	Prep.	T (°C)	B_0 (T)	NTE	Reported TE range (ms)	Actual TE range (ms)	% signal	T_2^* (ms)	Other
Horch et al. 2011 (1)	Myelin extract	Bov.	H	IVT	20	4.7	FID	0.01 – 0.50	0.01 – 0.50	32 (est.)	~ 0.05	--
										21 (est.)	~ 0.33	--
	Myelin lipids	--	H	IVT	20	4.7	FID	0.01 – 0.50	0.01 – 0.50	--	~ 0.05	--
										--	~ 1.5	--
Boucneau et al. 2018 (2)	Brain (WM)	Hum.	H	IV	BT	3	32	0.056 – 5.03	Min: 0.08 Max: 5.05 – 5.06	7 - 15	0.5 - 0.7	Freq. shift = -3 ppm
Du, Ma et al. 2014 (3)	Brain (WM)	Hum.	H	IV	BT	3	4	0.008 – 4.4	0.24 – 4.64	4.1 ± 0.9	0.42 ± 0.08	--
Du, Sheth et al. 2014 (4)	Brain (WM)	Hum.	H	IV	BT	3	5	0.008 – 4.4	0.24 – 4.64	--	0.32 ± 0.09	$T_1 = 230 \pm 50$ ms

Table 7.1. Literature on short- T_2^* signal in the brain with FID or UTE acquisitions. The Horch et al. study utilised an FID acquisition, otherwise the other studies used UTE acquisitions.

The brain and liver have similar constituents. This includes lipids such as phosphatidylcholine, phosphatidylethanolamine, cholesterol and sphingomyelin, proteins and polysaccharides (1). At 4.7 T, Horch et al. suggested that the short- T_2^* signal in *in vitro* myelin extract originates from the methylene protons associated with myelin lipids. Myelin extract had T_2^* components of ~ 0.05 and ~ 0.33 ms, whereas myelin lipids had T_2^* components of ~ 0.05 and ~ 1.5 ms (1). However, as the ~ 0.33 ms component was not observed in myelin lipids, other sources of short- T_2^* signal should be considered. *In vivo* measurements yielded similar T_2^* values (2–4), with the source of the signal hypothesised. Notably, Boucneau et al. found a ~ -3 ppm frequency shift from water associated with the short T_2^* of 0.5 - 0.7 ms with a signal fraction of 7 - 15 % at 3 T; the frequency shift suggested that the short T_2^* arises mainly from methylene protons. Other possible sources (although not investigated) include protons and water bound to macromolecules, such as proteins. Finally, Du et al. found similar T_2^* values in white matter (0.42 ± 0.08 ms and 0.32 ± 0.09 ms) at 3 T (3,4). The lower T_2^* found by Du et al. was likely due to the exclusion of a frequency offset in their signal model. Nevertheless, the short- T_2^* signal in the brain is comparable to short- T_2^* signal I observed in the liver. As white matter has a different composition to the liver, the origins of the short- T_2^* signal need to be explored in order to be applied to the liver.

7.2.2. Liver Composition

The liver is comprised of various components, including water, lipids, proteins, polysaccharides and minerals. Table 7.2 describes the components by percentage mass of the liver, in normal, diseased and fasted states; components in myelin are presented for comparison. Table 7.3 describes the proton density fat fraction (PDFF), extracellular volume (ECV) fraction and blood volume (BV) fraction as measured with MRI.

Paper	Sample	Source	State	% mass							
				Water	Lipids		Protein		Polysaccharides		Ash ("minerals")
					Total	Fatty acids	Total	Nitrogen	Total	Glycogen	
Woodard et al. 1986 (5)	Liver	Hum.	H	72.8 - 75.6	1.5 - 7.8	--	16.1 - 19.6	--	2.2	--	1.2 (1.1)
Kaplan et al. 1936 (6)	Liver	Canine	H and D	28 - 72	3 - 66	--	*5.63 - 20.13	0.90 - 3.22	--	0.05 - 10.5	--
den Otter et al. 1971 (7)	Liver	Murine	H (fed)	69	--	2	14	--	--	14	--
			H (24-hour fast)	69	--	2	24	--	--	0	--
Berg et al. 2002 (8)	Liver	Not specified	H	--	--	--	--	--	--	10	--
Laule et al. 2007 (9)	Myelin	Not specified	H	~ 40	~ 48	--	~ 12	--	--	--	--

Table 7.2. Components of the liver and myelin, by percentage mass. In Woodard et al. 1986, the ranges were taken from three liver datasets. The protein content in (*) was calculated according to the approximation that protein is 16 % nitrogen by mass (10). Kaplan et al. utilised 59 adult dogs under a range of conditions, including normal, thyroid-fed, fasted and with the pancreas and/or pituitary gland removed. In den Otter et al. 1971, each result is the mean of six rats.

Paper	Sample	Source	B_0 (T)	Seq.	State	% fraction (from MRI)					
						PDFF			ECV		BV
						Mean $\pm$ standard deviation	Median (interquartile range)	95 th percentile	Mean $\pm$ standard deviation	Median (interquartile range)	Mean $\pm$ standard deviation
Wilman et al. 2017 (11)	Liver	Hum.	1.5	SPGR (min. TE: 2.38 ms)	H	2.0 ± 2.3	1.3 (1.0 - 2.1)	5.2	--	--	--
					H and D	3.9 ± 4.6	2.1 (1.3 - 4.4)	14.0	--	--	--
Wang et al. 2019 (12)	Liver	Hum.	1.5	GRE	F0	--	--	--	--	20.4 (17.4 - 24.9)	--
					F1	--	--	--	--	26.2 (20.6 - 30.0)	--
					F2	--	--	--	--	23.9 (21.1 - 27.1)	--
					F3	--	--	--	--	27.9 (24.3 - 30.8)	--
					F4	--	--	--	--	33.5 (28.6 - 37.3)	--
Ostovaneh et al. 2018 (13)	Liver	Hum.	1.5	MOLLI	H	--	--	--	29.2 ± 7.9	--	--
					CVD	--	--	--	32.2 ± 12.1	--	--
Schwickert et al. 1995 (14)	Liver	Murine	2	ULSTIR-EPI	H	--	--	--	--	--	33 ± 3

Table 7.3. *In vivo* MRI data in the liver, providing estimates of PDFF, ECV and BV.

If one is looking for short- T_2^* signal with signal fraction of 10 - 30 %, likely candidates would appear to be lipids, proteins and glycogen, based on their composition in the liver. These three constituents also possess methylene groups and are termed macromolecules. Macromolecules are large molecules, including lipids, proteins and polysaccharides. The lipid content is < 8 % in the healthy liver, increasing to 66 % as a result of disease (5). Although the dogs investigated were not claimed to have steatosis, a 66 % lipid content is in line with grade 3 severe (> 66 %) steatosis associated with NAFLD (15,16). Protein varies from 6 - 24 %, with a healthy range of 14 - 20 %. Glycogen (a polysaccharide) varies from 0 - 14 %, with the lower and upper limits due to fasting and feeding, respectively. It is noted that the healthy liver has a lower composition of lipids (< 8 % vs. ~ 48 %), but similar composition of proteins (14 - 20 % vs. ~ 12 %), compared to myelin. Based on composition, the source of the short- T_2^* signal in the liver is more likely to be proteins and glycogen than lipids.

Woodard et al. referred to components with elements of atomic number > 8 as “minerals”, e.g. the lipid sphingomyelin, proteins and iron, accounting for 1 % of the liver weight. High hepatic iron concentrations associated with iron overload (> 12.5 mg Fe/g dry weight) can cause the liver T_2^* to decrease to 0.31 - 0.7 ms, as measured with UTE at 3 T (17). Furthermore, the T_2^* due to iron decreases with field strength (18,19). As subjects with low/normal iron concentrations were investigated in this thesis, iron is unlikely to be a source of short- T_2^* signal.

From MRI measurements, the PDFF is < 5 % in healthy subjects only (according to a BMI of < 25 kg/m² and a lack of diabetes), and < 14 % in healthy and unhealthy subjects. The PDFF for healthy subjects is of the same order of the liver content by mass.

However, this fraction does not necessarily include short- T_2^* signal from lipids (if present), given the minimum TE of 2.38 ms used. The ECV (20 - 34 %) and BV (~ 33 %) fractions provide indications of the sizes of extracellular space and blood, respectively. Although these fractions may not pertain to short- T_2^* signal, it is possible for short- T_2^* signal to originate from the extracellular space or blood. Potential sources include macromolecules, such as proteins.

Table 7.4 summarises the literature on potential sources of short- T_2^* signal in the liver. Liver water and iron are provided as references. Unless otherwise indicated, only studies that were able to detect short- T_2^* signal were selected.

Liver comp.	Sub-comp.	Paper	Sample	Source	State	Prep.	T (°C)	B ₀ (T)	Seq.	Peak loc. (ppm) (est.)	LW (ppm) (est.)	% signal	T ₂ [*] (ms)
Water (reference)	--	Alam et al. 2016 (18)	Liver	Hum.	H	IV	BT	3	GRE	--	--	100	15 - 21*
		Storey et al. 2007 (19)	Liver	Hum.	H	IV	BT	3	GRE	--	--	100	14 ± 5*
Iron	--	Krafft et al. 2017 (17)	Liver	Hum.	IO (> 25mg Fe/g dry wt.)	IV	BT	3	UTE	--	--	100	Min: 0.31 ± 0.08 Max: 0.7 ± 0.1
Macro-molecules (US)	Macro-molecular pool of blood	Stanisz et al. 2005 (20)	Blood	Hum.	H	IVT	37	3	MT	--	--	2.8 ± 0.7	T ₂ = 0.28 ± 0.05
	Macro-molecular pool of liver	Stanisz et al. 2005 (20)	Liver	Murine	H	EV	37	3	MT	--	--	6.9 ± 0.7	T ₂ = 0.0077 ± 0.0002
Glycogen	Glycogen	Chen et al. 1994 (21)	Glycogen in D ₂ O	Oyster	H	IVT	37	8.5	FID	3.60 – 4.00	0.40	--	2.2 ± 0.1 (est.)
		Bollard et al. 2000 (22)	Glycogen in D ₂ O	Bov. liver	H	IVT	RT	11.75	FID	3.64 - 4.03	0.39	--	1.6 ± 0.1 (est.)
	Glucose, glycogen, amino acids	Martinez-Granados et al. 2006 (23)	Liver	Hum.	NP (meta-stasis)	EV	0	11.75	MAS + FID (water pre-sat.)	3.35 – 3.96	0.61	--	1.0 ± 0.1 (est.)

		Cobbold et al. 2009 (24)	Liver	Murine	H	EV	4	11.75	MAS + FID	3.36 – 3.88	0.52	< 2	1.2 ± 0.1 (est.)
		Fathi et al. 2017 (25)	Liver	Avian	H	Ex-tract	25	14.1	FID (water pre-sat.)	3.38 – 3.90	0.52	--	1.0 ± 0.1 (est.)
	Glucose, glycogen	Cobbold et al. 2010 (26)	Liver	Hum.	HCV	EV	4	11.75	MAS + FID	3.50 – 3.95	0.45	< 1	1.4 ± 0.1 (est.)
Lipids	CH, PC, SM	Horch et al. 2011 (1)	13.5 % m/m CH, 13 % GC, 19.3 % PC, 4.2 % SM	US	H	IVT	20	4.7	FID (water not shown)	--	--	--	~ 0.05, ~ 1.5
		He et al. 2017 (27)	CH, GC, PC, SM in D ₂ O	US	H	IVT	RT	3	IR-UTE	--	--	100	0.31 ± 0.02
		Sheth et al. 2016 (28)	CH, GC, PC, SM in D ₂ O	US	H	IVT	RT	3	IR-UTE	--	--	100	0.29 ± 0.01
			CH, GC, PC, SM (in H ₂ O)	US	H	IVT	RT	3	IR-UTE	--	--	100	0.32 ± 0.01
		Sheth et al. 2017 (29)	13.5 % m/m CH, 13 % GC, 19.3 % PC, 4.2 % SM in D ₂ O	US	H	IVT	RT	3	UTE	--	--	81, 19	0.29 ± 0.02, 1.2 ± 0.2
Proteins	Serum albumin	Sadler et al. 1992 (30)	Serum albumin	Bov.	H	IVT	37	11.7		6.75 - 7.55	0.80	--	0.8 ± 0.1 (est.)

			(2mM in 0.1 M phosphate)						FID (water pre-sat.)	3.17 - 4.50	1.33	--	0.5 ± 0.1 (est.)
		Gallier et al. 1987 (31)	Serum albumin in D ₂ O (13.2 % m/m)	Bov.	H	IVT	37	1.1	FID	--	--	--	4 - 5

Table 7.4. Literature on potential sources of short- T_2^* signal in the liver. (*) indicates the TEs were not short enough to measure a $T_2^* < 1$ ms. Liver water and iron T_2^* are provided as references. *In vitro* samples were prepared in water, unless otherwise stated.

7.2.2.1. Macromolecules

As a first step, it is helpful to investigate if short- T_2^* signal associated with macromolecules in the liver has been found. As explained in Chapter 2, water can be compartmentalised into free and bound pools, the latter pool originating from macromolecules and possessing a short T_2^* . Magnetisation transfer has been used to detect the macromolecular pool in the liver.

Stanisz et al. characterised the T_2 of the macromolecular pools of blood and liver to be 0.28 ± 0.05 ms (at 2.8 ± 0.7 % of the signal) and 0.0077 ± 0.0002 ms (at 6.9 ± 0.7 % of the signal), respectively (20). From these results, it appears that macromolecules in the liver are unlikely to be detected with TEs on the order of ms. Here, the T_2 was calculated based on a two-pool MT model, instead of measured directly. Hence, the T_2 may have been affected by model assumptions concerning the exchange between the two pools. For instance, the results of Stanisz et al. do not eliminate the possibility of intermediate pools that exchange with water, which may produce less of an MT effect but are observable in T_2^* signal decays. Assuming the ~ 3 % macromolecular pool of blood to originate from proteins, it is not unreasonable for a similar macromolecular pool to be found in the liver, considering that the liver produces most plasma proteins. Namely, plasma proteins form ~ 4 % of the blood (32,33), which is similar to the size of the macromolecular pool of blood found by Stanisz et al.

In a separate study, Rosenkrantz et al. found no difference in magnetisation transfer ratio between healthy and cirrhotic subjects at 1.5 T, using eight frequency offsets ranging from 200 to 2,500 Hz (34). In this case, saturation of water and changes in water due to disease may have obscured the ability of detecting the macromolecules associated

with fibrosis. Alternatively, magnetisation transfer may not be specific to macromolecules that develop with fibrosis.

Overall, the magnetisation transfer literature suggests a short- T_2^* signal of macromolecules that is clinically undetectable and does not correlate with fibrosis. This is in contrast to the short- T_2^* signal that Zhu et al. and I detected with UTE, although none of us showed an association of this signal with disease. Namely, UTE and MR spectroscopy have detected short- T_2^* signal on the order of ms. In the following sections, the short- T_2^* signal of different macromolecules in the liver will be discussed, with these techniques.

7.2.2.2. *Glycogen*

Glycogen is a polysaccharide for glucose storage, which has been detected with MR spectroscopy. From Table 7.4, it can be deduced that glycogen/glucose has a T_2^* of 1 - 2 ms at 8.5 - 14.1 T, characterised by a broad peak from ~ 3.4 to 4.0 ppm. Considering that T_2^* decreases with field strength, one would expect a longer T_2^* at 3 T. *In vitro* studies of glycogen in D₂O yielded a T_2^* of ~ 2 ms, whereas *in vivo* liver glycogen studies yielded a T_2^* of ~ 1 ms at high field. The discrepancy in T_2^* is reasonable, considering that signal from glycogen in D₂O is not confounded by other compounds and originates from non-exchangeable protons, resulting in narrower resonances and a longer T_2^* . Furthermore, the intracellular environment of glycogen may result in the motion of glycogen being more restricted than when it is in solution, which would shorten T_2^* . From the studies by Cobbold et al., the glycogen signal fraction was $< 2\%$, associated with a T_2^* of 1.2 - 1.4 ms at 11.75 T (24,26). This amount of signal is small, compared to the 10 - 30 % short- T_2^* signal fraction that is being investigated.

7.2.2.3. Lipids

Lipids can be divided into lipid classes, each being distinguishable with MR spectroscopy. The following table illustrates the composition of each lipid class by mass, as well as its detectability with MR spectroscopy:

Lipid class	Mass (%) in rat liver	Detectable with MR spectroscopy (as a defined resonance)	Signal (%) from spectroscopy (est. TE $\leq$ 0.12 ms)
Cholesterol esters	2	(25)	--
Triglycerides (triacylglycerols)	7	(23), (24), (26), (25)	(24): 2 (26): 2.5
Cholesterol	5	(25)	--
Free fatty acids	trace	See triglycerides	--
Cardiolipin	5	Not found	--
Phosphatidylethanolamine	20	Ethanolamine: (25)	--
Phosphatidylinositol	4	Not found	--
Phosphatidylcholine	55	Choline-containing compounds: (23), (24), (26), (25)	(24): 1.6 (26): 0.4
Sphingomyelin	2	See phosphatidylcholine	--
Total	100		

Table 7.5. Composition of lipids in rat liver by mass. Mass percentages were taken from Christie 1985 (35). Percentage signal from spectroscopy is indicated, where water pre-saturation was not applied. Note that the percentage signal from triglycerides is equivalent to the PDFF.

As previously discussed, the PDFF is $< 5\%$ in healthy subjects, which mainly pertains to triglyceride signal (11). This suggests that the signal contribution due to lipids is small, although it does not exclude the possibility of lipid signal caused by broad, unidentified line widths in spectroscopy. If lipids are responsible for short- T_2^* signal, the most abundant lipid classes are the most likely candidates. These include phosphatidylcholine (55 %), phosphatidylethanolamine (20 %) and triglycerides (7 %); they form 82 % of all lipid mass in the liver, or 1.2 - 6.4 % of the total liver mass in a healthy person (based on

Table 7.2). Hence, T_2^* signal from these three lipid classes, as well as a combination of the lipid classes, are worth considering:

1. Triglycerides: Triglycerides contribute to the increase in lipid content in steatosis (15), and are well-characterised with MR spectroscopy. This six-peak model of triglycerides by Hamilton et al. was introduced and used for analysis in Chapter 6 (Section 6.2.3) (36). Using a seven-peak lipid model of triglycerides, Cobbold et al. found a lipid content of 2 - 2.5 %, at an estimated TE of ≤ 0.12 ms (24,26). In a separate study by Leporq et al., the composition of triglyceride fatty acids, including the number of double bonds, number of methylene-interrupted double bonds and chain length, was determined from spectroscopy at TE > 1 ms and verified against the known composition (37). This suggests a low abundance of lipids, based on identified resonances. Hence, the chemical composition of fatty acids in triglycerides appears to be well-described, although the possibility of a short- T_2^* component has not been disproven.
2. Phosphatidylcholine and phosphatidylethanolamine: These are phospholipids residing in the membranes of hepatocytes, and are relatively constant in composition, despite physiological and dietary changes (35). The percentage of MR signal originating from phosphatidylcholine and phosphatidylethanolamine is not easily determined with spectroscopy, due to close agreement with other resonances and low signal. Choline-containing compounds, including choline, phosphatidylcholine, phosphorylcholine, phosphocholine, glycerophosphocholine and sphingomyelin, can be identified with MR spectroscopy with a ~ 0.1 ppm line width; the resonance is close to that of trimethylamine N-oxide (TMAO) and betaine, complicating peak

separation (24,26). Cobbold et al. identified a total choline content of 0.4 - 1.6 %, indicating that the choline contribution to MR signal is small relative to its mass (24,26). Ethanolamine, a precursor to phosphatidylethanolamine, was identified in Fathi et al. 2017, although the peak was similar in intensity to the baseline (25). Given the high abundance of these phospholipids in the liver, is possible that there is more MR signal from phospholipids than attributed in MR spectroscopy, raising the possibility of short- T_2^* signal.

3. Combinations of lipid classes: UTE studies have been performed on lipid phantoms mimicking the composition of lipids in myelin. Three papers by He et al. and Sheth et al. identified a short T_2^* of ~ 0.3 ms in a phantom of cholesterol, galactocerebroside, phosphatidylcholine and sphingomyelin, either in D_2O or H_2O , at 3 T (27–29). It is noted that although galactocerebroside is not found in the liver, the other components in the phantom are. The short T_2^* value was found in both the normal UTE sequence and the UTE sequence preceded by an adiabatic inversion pulse. In a similar phantom, Horch et al. identified T_2^* values of ~ 0.05 ms and ~ 1.5 ms at 4.7 T (1). Differences may have been due to the field strength and acquisition (FID). Nevertheless, short- T_2^* signal may arise due to a combination of cholesterol, phosphatidylcholine and sphingomyelin, which are found in the liver.

Overall, there is potential for short- T_2^* signal in the liver due to lipids, although more investigation is needed to identify the lipid classes responsible with UTE.

7.2.2.4. *Proteins*

Proteins are responsible for functions including signal transduction, metabolism, transportation and cellular organisation (38). MR spectroscopy provides information on

which metabolites yield MR signal. With MR spectroscopy, amino acids, the building blocks of proteins, have been discerned in the liver (23–25). For instance, Fathi et al. identified 19 out of the 21 amino acids in *ex vivo* avian liver with an FID spectroscopy acquisition at 14.1 T; cysteine and selenocysteine were missing (25). However, the identification of proteins from a tissue sample is difficult with MR spectroscopy, as the amino acids may belong to more than one protein. Normally, purified proteins are studied with MR spectroscopy; the protein structure is determined through the assignment of resonances, exchange spectroscopy and structural calculations (39). Hence, proteins could not be identified from the literature on MR spectroscopy of liver tissue.

Given the challenges of protein identification with MR spectroscopy, it is reasonable to investigate the most abundant proteins in the liver. Table 7.6 and Table 7.7 identify the 20 most abundant proteins in hepatocytes and hepatic stellate cells, reported by Azimifar et al. with mass spectrometry-based proteomics (40). Hepatic stellate cells are mentioned, as they are implicated in fibrosis, producing extracellular matrix proteins. Additionally, the protein copy numbers for albumin and the most abundant collagens are listed. The copy number is the number of copies of the protein in the given cell type. For more information, the 100 most abundant proteins in hepatocytes and hepatic stellate cells are reported in Tables A7.1 and A7.2.

Copy number rank	Copy number	Protein ID	Protein name
1	56777854	P12710	Fatty acid-binding protein, liver
2	49595426	P19157;F8WJD9;P46425	Glutathione S-transferase P 1;Glutathione S-transferase P 2
3	34793014	Q8C196	Carbamoyl-phosphate synthase [ammonia], mitochondrial
4	33535184	A2CEK7	Major urinary protein 12
5	25482051	Q8BWT1	3-ketoacyl-CoA thiolase, mitochondrial
6	23413179	O35490;A2ALP9	Betaine--homocysteine S-methyltransferase 1
7	21636508	Q91Y97	Fructose-bisphosphate aldolase B
8	21285854	P35700;B1AXW5;B1AXW6;B1AXW4	Peroxisomal acyl-CoA oxidase 1
9	20937639	P52760	Ribonuclease UK114
10	20486170	P16460	Argininosuccinate synthase
11	20162755	Q64433	10 kDa heat shock protein, mitochondrial
12	19981819	P00329	Alcohol dehydrogenase 1
13	18951442	P16015	Carbonic anhydrase 3
14	18610696	P08228	Superoxide dismutase [Cu-Zn]
15	18264777	P62806	Histone H4
16	16402541	Q61176	Arginase-1
17	15988716	P16858;E9PX42;E9PX72;E9Q9E5;E9Q1S8;F8WJL5;F6ZQM8;E9Q5U3;E9PXL6;E9Q0E3;D3YV03;D3YUV0;D3YYI5;E9PYX4;D3Z1B7;E9PZP4;F6UT49;E9PX99;Q64467;E9QP42	Glyceraldehyde-3-phosphate dehydrogenase
18	15884744	P56480	ATP synthase subunit beta, mitochondrial
19	15670785	Q63836;G3UYY2	Selenium-binding protein 2
20	15300085	P10649	Glutathione S-transferase Mu 1
...	...	...	...

207	3356883	P07724	Serum albumin
4884	12515	P39061;E9QPX1;P39061-1;P39061-2;D3Z5C2;D3Z556	Collagen alpha-1(XVIII) chain;Endostatin
6879	3261	P11087;P11087-2;F8WGB7;CON_Q862S4	Collagen alpha-1(I) chain

Table 7.6. The most abundant proteins in hepatocytes of healthy murine liver, as identified by mass spectrometry-based proteomics (40). The 20 top-ranked proteins by copy number are listed, along with albumin and the most abundant collagens. The copy number is the number of copies of the given protein in the hepatocyte.

Copy number rank	Copy number	Protein ID	Protein name
1	142474585	P62806	Histone H4
2	98485647	Q6ZWY9;Q64478;Q64525;P10854;G3X9D5	Histone H2B type 1-C/E/G;Histone H2B type 1-H;Histone H2B type 2-B;Histone H2B type 1-M
3	40821402	P20065-2	Thymosin beta-4
4	35131523	Q8CGP6;Q8CGP5;Q8BFU2;P22752;Q8R1M2;Q8CGP7;Q8CGP4	Histone H2A type 1-H;Histone H2A type 1-F;Histone H2A type 3;Histone H2A type 1;Histone H2A.J;Histone H2A type 1-K;Histone H2A
5	34192879	P84228	Histone H3.2
6	32504366	P15864	Histone H1.2
7	27626766	P27661	Histone H2A.x
8	26907576	P17742;E9Q1E3;F8VPN3	Peptidyl-prolyl cis-trans isomerase A
9	22457696	P04117;P24526	Fatty acid-binding protein, adipocyte
10	20337233	Q9CPX4;P29391;P49945	Ferritin;Ferritin light chain 1;Ferritin light chain 2
11	20097794	P62204;Q3UKW2;G3UX57;G3UZ90	Calmodulin
12	19966431	P16045	Galectin-1
13	19267425	P35700;B1AXW5;B1AXW6;B1AXW4	Peroxisomal protein 4
14	18862094	P20152;E9PZV5;A2AKJ2	Vimentin
15	17983609	P18760;F8WGL3;E9Q1T2	Cofilin-1
16	16594546	P62983	Ubiquitin-40S ribosomal protein S27a;Ubiquitin;40S ribosomal protein S27a
17	16358623	P62962;Q5SX49	Profilin-1
18	14942996	P48036	Annexin A5
19	14277258	P08228	Superoxide dismutase [Cu-Zn]
20	14102558	P10126;D3YZ68;D3Z3I8	Elongation factor 1-alpha 1
...	...	...	...
187	3084481	P07724	Serum albumin

1588	271236	P11087;P11087-2;F8WGB7;CON_Q862S4	Collagen alpha-1(I) chain
2302	143070	E9Q6U9;Q01149;E0CXI2	Collagen alpha-2(I) chain

Table 7.7. The most abundant proteins in hepatic stellate cells of healthy murine liver, as identified by mass spectrometry-based proteomics (40). The 20 top-ranked proteins by copy number are listed, along with albumin and the most abundant collagens. The copy number is the number of copies of the given protein in the hepatic stellate cell.

Azimifar et al. quantified 11,520 proteins, of which 8,338 (72 %) were detected in all hepatic cell types. The 100 most abundant proteins in hepatocytes form ~ 43 % of the total protein mass. Assuming a total protein mass of 24 % in the liver (7), the 100 most abundant proteins form ~ 10 % of the liver by mass. Although this mass proportionality may not directly translate to an MR signal fraction, it is on the order of the short- T_2^* fraction expected.

As hepatocytes form 90 % of the liver volume, the 100 most abundant proteins in hepatocytes are almost identical to the most abundant proteins in the liver. 59 % of the 11,520 quantified proteins show differential expressions among hepatic cell types (40). For instance, the 100 most abundant proteins differ between hepatocytes and hepatic stellate cells.

Although the proteins are ranked by copy number, it is noted that the ratio of the copy numbers of the most abundant and 100th most abundant protein are small. For hepatocytes, the ratio of the copy numbers of fatty acid-binding protein (ranked first) and indolethylamine N-methyltransferase (ranked 100th) is $56777854/5326858 = 11$. For hepatic stellate cells, the ratio of the copy numbers of histone H4 (ranked first) and actin (ranked 100th) is $142474585/4554152 = 31$. Hence, the top 100 proteins are abundant; however, the relative ranking of a protein is not important and may vary between experiments.

If the short- T_2^* signal originates from proteins, likely sources are the top 100 proteins in hepatocytes. Given their role in fibrogenesis, the top 100 proteins in hepatic stellate cells may be an indication of proteins implicated in fibrosis, although the murine samples studied were healthy. Given that all proteins are made of amino acids and have

similar chemical groups, it would not be surprising for many different proteins to contribute to the short- T_2^* signal.

T_2^* properties of albumin and collagen have been investigated with MR, and hence the rankings of these proteins are included. Albumin is the most abundant plasma protein, with a concentration of 35 - 50 g/L in serum (41). Albumin and the most abundant collagens are ranked lower than the top 100 most abundant proteins in hepatocytes and hepatic stellate cells. Albumin is relatively abundant in these cells, as the copy numbers are < 50 times lower than the top-ranked proteins. As healthy murine liver was measured, collagen copy numbers are > 500 times lower than the top-ranked proteins, and hence are significantly less abundant than the most abundant proteins.

There is a possibility of short- T_2^* signal originating from albumin, as demonstrated in the literature. Although the macromolecular pool of blood found by Stanisiz et al. may be indicative of blood proteins, literature in albumin suggests a T_2^* on the order of ms at 3 T. Sadler et al. found two broad peaks in bovine serum albumin with a T_2^* of 0.5 - 0.8 ms at 11.7 T (30); presumably, the T_2^* would be longer at 3 T. Moreover, as the spectrum was taken at 37 °C, it is possible that the broad peak could be an effect of exchange rather than a real entity. For instance, Gallier et al. found a T_2^* of 4 - 5 ms in bovine serum albumin in D₂O at 37 °C at 1.1 T (31), which would be decreased at 3 T. UTE studies at 3 T would need to be done to investigate the presence of short- T_2^* signal associated with albumin and other proteins.

7.2.2.5. *Conclusions*

In macromolecules of the liver, including glycogen, lipids and proteins, the presence of short- T_2^* signal has not been disproven. In the case of lipids, short- T_2^* signal has been

found, but in a phantom containing galactocerebroside, a lipid that is not present in the liver. Literature has identified signal fractions of < 2 % for glycogen at a T_2^* of 1.2 - 1.4 ms at 11.75 T (24,26), where the T_2^* is expected to increase at lower field strengths. A PDF of < 5 % has been detected in healthy subjects (11,24,26), which may not include short- T_2^* signal. Finally, the 100 most abundant proteins in the liver form ~ 10 % of the liver mass, which may be potential sources of short- T_2^* signal. Glycogen, lipids and proteins may be responsible for the 10 - 30 % signal at a short- T_2^* of 0.2 - 0.5 ms observed with UTE. Additional experiments are needed to identify the origin of the short- T_2^* signal in the liver with UTE.

7.2.2.6. Future Work

Future experiments involve determining the source(s) of the short- T_2^* signal in the liver with a T_2^* of 0.2 - 0.5 ms and signal fraction of 10 - 30 % with UTE. T_2^* measurements with UTE are suggested, as a short- T_2^* signal found with another pulse sequence, e.g. magnetisation transfer or spectroscopy, may not necessarily be detected with UTE. As it may be challenging to identify the exact source(s) of the short- T_2^* signal, especially if it involves proteins, one can take the approach of eliminating glycogen and lipids as sources of short- T_2^* signal. The following phantoms are suggested:

1. Glycogen: Phantom of glycogen
2. Lipids:
 - Phantom of lipids with composition mimicking that in the liver (Table 7.5)
 - Phantoms for each lipid class (e.g. phantom of pure triglycerides, phantom of pure phosphatidylcholine)

3. Proteins:

- Sample of blood plasma (for the investigation of plasma proteins)
- Phantoms for each plasma protein (e.g. albumin, fibrinogen, globulin)
- Phantom of extracellular matrix proteins with composition mimicking that in the liver
- Phantoms for each extracellular matrix protein (e.g. proteoglycans, glycoproteins)

Controlled phantom data would aid in explaining the short- T_2^* signal in the liver *in vivo*. Once glycogen and lipids are eliminated, one can verify the presence of short- T_2^* signal from proteins in certain locations, e.g. plasma vs. extracellular matrix proteins. It is noted that this method does not exclude proteins from other locations providing short- T_2^* signal, given their ubiquity. Although MR does not appear to have the specificity for protein identification, phantoms of glycogen and lipids would enable a better understanding of the constituent(s) responsible for the short- T_2^* signal.

7.3. References

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7.4. Appendix

Copy number rank	Copy number	Protein ID	Protein name
1	56777854	P12710	Fatty acid-binding protein, liver
2	49595426	P19157;F8WJD9;P46425	Glutathione S-transferase P 1;Glutathione S-transferase P 2
3	34793014	Q8C196	Carbamoyl-phosphate synthase [ammonia], mitochondrial
4	33535184	A2CEK7	Major urinary protein 12
5	25482051	Q8BWT1	3-ketoacyl-CoA thiolase, mitochondrial
6	23413179	O35490;A2ALP9	Betaine--homocysteine S-methyltransferase 1
7	21636508	Q91Y97	Fructose-bisphosphate aldolase B
8	21285854	P35700;B1AXW5;B1AXW6;B1AXW4	Peroxisomal acyl-CoA oxidase 1
9	20937639	P52760	Ribonuclease UK114
10	20486170	P16460	Argininosuccinate synthase
11	20162755	Q64433	10 kDa heat shock protein, mitochondrial
12	19981819	P00329	Alcohol dehydrogenase 1
13	18951442	P16015	Carbonic anhydrase 3
14	18610696	P08228	Superoxide dismutase [Cu-Zn]
15	18264777	P62806	Histone H4
16	16402541	Q61176	Arginase-1
17	15988716	P16858;E9PX42;E9PX72;E9Q9E5;E9Q1S8;F8WJL5;F6ZQM8;E9Q5U3;E9PXL6;E9Q0E3;D3YV03;D3YUV0;D3YYI5;E9PYX4;D3Z1B7;E9PZP4;F6UT49;E9PX99;Q64467;E9QP42	Glyceraldehyde-3-phosphate dehydrogenase
18	15884744	P56480	ATP synthase subunit beta, mitochondrial
19	15670785	Q63836;G3UYY2	Selenium-binding protein 2

20	15300085	P10649	Glutathione S-transferase Mu 1
21	14648058	Q99LC5	Electron transfer flavoprotein subunit alpha, mitochondrial
22	14476491	P10126;D3YZ68;D3Z3I8	Elongation factor 1-alpha 1
23	14106011	Q03265;D6RJ16	ATP synthase subunit alpha, mitochondrial
24	13914562	O35215;G3UZN1	D-dopachrome decarboxylase
25	13586297	Q6GT24;D3Z0Y2	Peroxioredoxin-6
26	13455506	P17742;E9Q1E3;F8VPN3	Peptidyl-prolyl cis-trans isomerase A
27	13278849	P30115	Glutathione S-transferase A3
28	13068282	Q6ZWY9;Q64478;Q64525;P10854;G3X9D5	Histone H2B type 1-C/E/G;Histone H2B type 1-H;Histone H2B type 2-B;Histone H2B type 1-M
29	13047980	Q9QXD6;E9Q0T7;P97323	Fructose-1,6-bisphosphatase 1
30	13011247	P47738;D3YYF3	Aldehyde dehydrogenase, mitochondrial
31	12934501	P25688;D3Z4U1	Uricase
32	12587546	G5E850;P56395;E0CY88	Cytochrome b5
33	12485021	P50247;A2ALT5	Adenosylhomocysteinase
34	11878987	Q8R1A8;P11725	Ornithine carbamoyltransferase, mitochondrial
35	11562412	P63038;D3Z2F2;D3Z7J9	60 kDa heat shock protein, mitochondrial
36	11551256	Q64374	Regucalcin
37	11541219	P05202	Aspartate aminotransferase, mitochondrial
38	11452445	P54869	Hydroxymethylglutaryl-CoA synthase, mitochondrial
39	11425926	P08249	Malate dehydrogenase, mitochondrial
40	11315131	P99027;G3UW69	60S acidic ribosomal protein P2
41	11239142	P24270;A2AL20	Catalase
42	11054572	P24549	Retinal dehydrogenase 1
43	10362685	Q9QXF8	Glycine N-methyltransferase
44	10242466	P26443	Glutamate dehydrogenase 1, mitochondrial
45	10229281	P11352	Glutathione peroxidase 1
46	9977534	Q64442	Sorbitol dehydrogenase

47	9859658	P99029-2;P99029;E9Q9H9;G3UZJ4	Peroxioredoxin-5, mitochondrial
48	9727757	P32020;A2APS4;A2APS5	Non-specific lipid-transfer protein
49	9701747	Q9DCW4	Electron transfer flavoprotein subunit beta
50	9545473	P51881	ADP/ATP translocase 2
51	9316656	Q9WVL0	Maleylacetoacetate isomerase
52	9298361	P06151;G5E8N5;D3Z736;D3YZQ9; Q6NXW3;D3YVR7;D3YZE4;P00342	L-lactate dehydrogenase A chain;L-lactate dehydrogenase
53	9296805	Q8R0Y6	Cytosolic 10-formyltetrahydrofolate dehydrogenase
54	9165431	P14152;B1ATQ3	Malate dehydrogenase, cytoplasmic
55	8980481	P17182;Q6PHC1;B0QZL1;B1ARR7;B1ARR6	Alpha-enolase;Enolase
56	8825952	Q9DCX2;G3X9L6;B1ASE2;D3Z1B2	ATP synthase subunit d, mitochondrial
57	8746776	Q91VS7;D3YU60;E9QJW0	Microsomal glutathione S-transferase 1
58	8424641	P20029	78 kDa glucose-regulated protein
59	8418562	P09103;E9Q8G8	Protein disulfide-isomerase
60	8293865	P31786;F6WUQ1;Q4VWZ5;F6ZM12	Acyl-CoA-binding protein
61	8187082	P09411	Phosphoglycerate kinase 1
62	8093890	Q62425	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 4
63	7948223	P10639	Thioredoxin
64	7789712	P62983	Ubiquitin-40S ribosomal protein S27a;Ubiquitin;40S ribosomal protein S27a
65	7717517	Q06185;Q8BTB6	ATP synthase subunit e, mitochondrial
66	7282667	Q9DB20	ATP synthase subunit O, mitochondrial
67	7188199	Q8QZT1	Acetyl-CoA acetyltransferase, mitochondrial
68	7178476	P11679;Q9CPR6	Keratin, type II cytoskeletal 8
69	7080191	Q9CPQ1;D3Z6E1	Cytochrome c oxidase subunit 6C
70	6875369	P17751	Triosephosphate isomerase
71	6545975	P70694;D3Z3P8;E0CYR9;D3YWM3; Q91WR5	Estradiol 17 beta-dehydrogenase 5

72	6492333	Q9CPQ8;F2Z452	ATP synthase subunit g, mitochondrial
73	6374201	Q8VC30;Q3UTZ4	Bifunctional ATP-dependent dihydroxyacetone kinase/FAD-AMP lyase (cyclizing);ATP-dependent dihydroxyacetone kinase;FAD-AMP lyase (cyclizing)
74	6351907	P49429;D3Z1U3;F6X9Z6	4-hydroxyphenylpyruvate dioxygenase
75	6126076	O88844;F8WIY0;D3YVY3	Isocitrate dehydrogenase [NADP] cytoplasmic
76	6093405	P14211	Calreticulin
77	6079986	Q9EQ20;Q8K0L1	Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial
78	6058206	Q8CGP6;Q8CGP5;Q8BFU2; P22752;Q8R1M2; Q8CGP7;Q8CGP4	Histone H2A type 1-H;Histone H2A type 1-F;Histone H2A type 3;Histone H2A type 1;Histone H2A.J;Histone H2A type 1-K;Histone H2A
79	5973358	P34914;P34914-2	Bifunctional epoxide hydrolase 2;Cytosolic epoxide hydrolase 2;Lipid-phosphate phosphatase
80	5955902	P62204;Q3UKW2;G3UX57;G3UZ90	Calmodulin
81	5955888	O88451;F6U1Q7	Retinol dehydrogenase 7
82	5955344	P27773	Protein disulfide-isomerase A3
83	5880469	P35505;D3Z2R9	Fumarylacetoacetase
84	5846925	P56391	Cytochrome c oxidase subunit 6B1
85	5787370	P62908;D3YV43	40S ribosomal protein S3
86	5731588	P28474	Alcohol dehydrogenase class-3
87	5653299	Q9CRB3	5-hydroxyisourate hydrolase
88	5651523	Q8VCT4;E9PYP1	Carboxylesterase 1D
89	5622745	P34884	Macrophage migration inhibitory factor
90	5593310	Q91YI0;E0CXM2;E0CY49;F7D439; E0CYV3	Argininosuccinate lyase
91	5561751	Q99N15;A2AFQ2;O08756	3-hydroxyacyl-CoA dehydrogenase type-2
92	5535246	P24369	Peptidyl-prolyl cis-trans isomerase B
93	5493965	Q9R0H0-2;A2A848	Peroxisomal straight-chain acyl-CoA oxidase

94	5484816	P11499;E9Q3D6;E9PX27;D3Z1R1	Heat shock protein HSP 90-beta
95	5469722	P63017;Q504P4;D3Z5E2;D3YW43	Heat shock cognate 71 kDa protein
96	5459599	P41216;D3Z041;D3Z457;D3YVF6; F6WNZ2;F6WYN6	Long-chain-fatty-acid--CoA ligase 1
97	5405344	P13707;E0CXN5	Glycerol-3-phosphate dehydrogenase [NAD(+)], cytoplasmic
98	5390167	Q63880;Q63880-2	Carboxylesterase 3A
99	5345123	Q7TSZ0;P38647	Stress-70 protein, mitochondrial
100	5326858	P40936	Indolethylamine N-methyltransferase
...	...	...	...
207	3356883	P07724	Serum albumin
4884	12515	P39061;E9QPX1;P39061-1;P39061- 2;D3Z5C2;D3Z556	Collagen alpha-1(XVIII) chain;Endostatin
6879	3261	P11087;P11087-2;F8WGB7;CON_Q862S4	Collagen alpha-1(I) chain

Table A7.1. The most abundant proteins in hepatocytes of murine liver, as identified by mass spectrometry-based proteomics (40). The 100 top-ranked proteins by copy number are listed, along with albumin and the most abundant collagens. The copy number is the number of copies of the given protein in the hepatocyte.

Copy number rank	Copy number	Protein ID	Protein name
1	142474585	P62806	Histone H4
2	98485647	Q6ZWY9;Q64478;Q64525;P10854;G3X9D5	Histone H2B type 1-C/E/G;Histone H2B type 1-H;Histone H2B type 2-B;Histone H2B type 1-M
3	40821402	P20065-2	Thymosin beta-4
4	35131523	Q8CGP6;Q8CGP5;Q8BFU2;P22752;Q8R1M2;Q8CGP7;Q8CGP4	Histone H2A type 1-H;Histone H2A type 1-F;Histone H2A type 3;Histone H2A type 1;Histone H2A.J;Histone H2A type 1-K;Histone H2A
5	34192879	P84228	Histone H3.2
6	32504366	P15864	Histone H1.2
7	27626766	P27661	Histone H2A.x
8	26907576	P17742;E9Q1E3;F8VPN3	Peptidyl-prolyl cis-trans isomerase A
9	22457696	P04117;P24526	Fatty acid-binding protein, adipocyte
10	20337233	Q9CPX4;P29391;P49945	Ferritin;Ferritin light chain 1;Ferritin light chain 2
11	20097794	P62204;Q3UKW2;G3UX57;G3UZ90	Calmodulin
12	19966431	P16045	Galectin-1
13	19267425	P35700;B1AXW5;B1AXW6;B1AXW4	Peroxisomal protein 4
14	18862094	P20152;E9PZV5;A2AKJ2	Vimentin
15	17983609	P18760;F8WGL3;E9Q1T2	Cofilin-1
16	16594546	P62983	Ubiquitin-40S ribosomal protein S27a;Ubiquitin;40S ribosomal protein S27a
17	16358623	P62962;Q5SX49	Profilin-1
18	14942996	P48036	Annexin A5
19	14277258	P08228	Superoxide dismutase [Cu-Zn]
20	14102558	P10126;D3YZ68;D3Z3I8	Elongation factor 1-alpha 1
21	14046681	Q60605-2	Myosin light polypeptide 6
22	13940991	P27773	Protein disulfide-isomerase A3

23	13171266	P07356;B0V2N7;B0V2N5;B0V2N8;REV__F6 WNG1;REV_Q8BND4	Annexin A2
24	12500295	P63101;D3YXN6;D3YXF4;D3YW45	14-3-3 protein zeta/delta
25	12463489	P43274	Histone H1.4
26	12213749	P20029	78 kDa glucose-regulated protein
27	11691729	P16858;E9PX42;E9PX72;E9Q9E5;E9Q1S8;F8 WJL5;F6ZQM8;E9Q5U3;E9PXL6;E9Q0E3;D3 YV03;D3YUV0;D3YYI5;E9PYX4;D3Z1B7;E9 PZP4;F6UT49;E9PX99;Q64467;E9QP42	Glyceraldehyde-3-phosphate dehydrogenase
28	11584044	P62259;D6REF3;F6WA09	14-3-3 protein epsilon
29	11230966	Q99PT1	Rho GDP-dissociation inhibitor 1
30	10914987	P10639	Thioredoxin
31	10599617	P43276	Histone H1.5
32	10430770	Q9QXY6	EH domain-containing protein 3
33	10309281	P21107-2;D3Z2H9	Tropomyosin alpha-3 chain
34	10020307	P99024	Tubulin beta-5 chain
35	10003802	P99027;G3UW69	60S acidic ribosomal protein P2
36	9870218	Q9WVA4	Transgelin-2
37	9382811	P11499;E9Q3D6;E9PX27;D3Z1R1	Heat shock protein HSP 90-beta
38	9299325	P14211	Calreticulin
39	8949549	P47738;D3YYF3	Aldehyde dehydrogenase, mitochondrial
40	8866694	P63017;Q504P4;D3Z5E2;D3YW43	Heat shock cognate 71 kDa protein
41	8857467	P52480	Pyruvate kinase isozymes M1/M2
42	8568037	P99029-2;P99029;E9Q9H9;G3UZJ4	Peroxisomal protein 5, mitochondrial
43	7870345	P08249	Malate dehydrogenase, mitochondrial
44	7727866	Q3THW5;P0C0S6;Q8R029;Q5NC91;Q3UA95; G3UWL7	Histone H2A.V;Histone H2A.Z;Histone H2A
45	7688019	D3YTN4;D3YWB5;Q99JY3;D6RGS2;D3Z7Q4 ;D3Z3F3;D3Z7N6	GTPase IMAP family member 4

46	7456053	P10922	Histone H1.0
47	7186988	P08113;F7C312	Endoplasmin
48	7018762	P56480	ATP synthase subunit beta, mitochondrial
49	6979725	P26041	Moesin
50	6872006	Q05816;E9Q964;REV P70696	Fatty acid-binding protein, epidermal
51	6841083	Q9CQV8-2;Q9CQV8;A2A5N1	14-3-3 protein beta/alpha;14-3-3 protein beta/alpha, N-terminally processed
52	6831431	P17182;Q6PHC1;B0QZL1;B1ARR7;B1ARR6	Alpha-enolase;Enolase
53	6814615	G5E850;P56395;E0CY88	Cytochrome b5
54	6770120	P24369	Peptidyl-prolyl cis-trans isomerase B
55	6695715	Q9CQI6	Coactosin-like protein
56	6642373	Q64433	10 kDa heat shock protein, mitochondrial
57	6606812	Q9D8U8	Sorting nexin-5
58	6465171	P43277	Histone H1.3
59	6436040	P63001;Q3TLP8	Ras-related C3 botulinum toxin substrate 1
60	6431766	P10649	Glutathione S-transferase Mu 1
61	6211589	Q99JI6	Ras-related protein Rap-1b
62	6167186	P51881	ADP/ATP translocase 2
63	6139495	Q6IRU2	Tropomyosin alpha-4 chain
64	6137365	Q3THE2	Myosin regulatory light chain 12B
65	6136772	P09103;E9Q8G8	Protein disulfide-isomerase
66	6020077	P06151;G5E8N5;D3Z736;D3YZQ9;Q6NXW3;D3YVR7;D3YZE4;P00342	L-lactate dehydrogenase A chain;L-lactate dehydrogenase
67	6002111	Q9R0P5	Destrin
68	5936857	Q61598;Q61598-2	Rab GDP dissociation inhibitor beta
69	5886230	P51150	Ras-related protein Rab-7a
70	5863767	P21981;G3UXE8	Protein-glutamine gamma-glutamyltransferase 2
71	5804311	Q9WUU7;E9Q5W3	Cathepsin Z
72	5803266	Q03265;D6RJ16	ATP synthase subunit alpha, mitochondrial

73	5778366	P84244;E0CZ27	Histone H3.3;Histone H3
74	5703282	P06330;P01757;P01756;P06329;P03980	Ig heavy chain V region AC38 205.12;Ig heavy chain V region J558;Ig heavy chain V region MOPC 104E
75	5619565	Q8VDD5;F7AUR3;E9Q3T2;F2Z494	Myosin-9
76	5526430	E9PZ00;Q8BFQ1;Q61207	Sulfated glycoprotein 1
77	5484360	P26883;Q3UKJ3;F6X9I3;Q1JUQ8;Q1JUQ6	Peptidyl-prolyl cis-trans isomerase FKBP1A;Peptidyl-prolyl cis-trans isomerase
78	5452197	P47955	60S acidic ribosomal protein P1
79	5404293	P62880;E9QKR0;D3Z1M1;D3YZX3;D3Z1T4	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2
80	5312415	P47757-2;A2AMW0;A2AMV7;F7CAZ6;F6YHZ8	F-actin-capping protein subunit beta
81	5298088	P14069	Protein S100-A6
82	5277173	P09411	Phosphoglycerate kinase 1
83	5230988	Q9QYB1	Chloride intracellular channel protein 4
84	5207339	Q3TML0	Protein disulfide-isomerase A6
85	5170882	Q61171;D3Z4A4	Peroxisomal protein 2
86	5104703	P62908;D3YV43	40S ribosomal protein S3
87	5078624	P57780;E9Q2W9	Alpha-actinin-4
88	5064338	P68510;E9Q4V8	14-3-3 protein eta
89	5021074	Q9Z1Q5	Chloride intracellular channel protein 1
90	4985163	P61979-2;P61979;D3YWG1	Heterogeneous nuclear ribonucleoprotein K
91	4981515	P40124;B1ARS0	Adenylyl cyclase-associated protein 1
92	4795520	Q5SW88;Q5SW87;Q5SW86	RAB1A, member RAS oncogene family
93	4722977	Q78ZM0;O70492;D3Z789;D3Z6Z0	Sorting nexin-3
94	4711760	P46638;E9Q3P9;G3UZD3;G3UY29;F8WGS1;G3UZL4;F6R2Z5	Ras-related protein Rab-11B

95	4707873	P62075	Mitochondrial import inner membrane translocase subunit Tim13
96	4697850	P97352	Protein S100-A13
97	4690062	P01837	Ig kappa chain C region
98	4651546	Q68FD5;Q5SXR6;E9Q434;F6Z1R4	Clathrin heavy chain 1
99	4589075	P28843	Dipeptidyl peptidase 4;Dipeptidyl peptidase 4 membrane form;Dipeptidyl peptidase 4 soluble form
100	4554152	P62737	Actin, aortic smooth muscle
...	...	...	...
187	3084481	P07724	Serum albumin
1588	271236	P11087;P11087-2;F8WGB7;CON Q862S4	Collagen alpha-1(I) chain
2302	143070	E9Q6U9;Q01149;E0CXI2	Collagen alpha-2(I) chain

Table A7.2. The most abundant proteins in hepatic stellate cells of murine liver, as identified by mass spectrometry-based proteomics (40). The 100 top-ranked proteins by copy number are listed, along with albumin and the most abundant collagens. The copy number is the number of copies of the given protein in the hepatic stellate cell.