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Modelling the Triceps Surae Informed using Diffusion Weighted Imaging and 3D Ultrasound

Massoud Alipour

Supervised by Dr Justin Fernandez and Dr Kumar Mithraratne

A thesis submitted in complete fulfilment of the requirements for the degree of

Doctoral of philosophy in Bioengineering,

The University of Auckland, 2015
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**Chapter 5:** A diffusion weighted informed model of the rabbit gastrocnemius

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Chapter 6: A 3D Ultrasound Informed Model of Human Gastrocnemius Muscle

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Abstract

This thesis investigates the influence of muscle architecture on predicted mechanics. Specifically, we evaluate the influence of predicted continuum shape and strain in the Triceps Surae muscle of the rabbit and human. Muscle structure was derived using two imaging modalities, (i) Diffusion Weighted Imaging (DWI) for a rabbit; and (ii) 3D Ultrasound for human subjects. For the rabbit we present measured musculotendon forces during rigor mortis and propose a method for comparing stable post rigor behaviour with the post-mortem state. Novel aspects of this thesis include the development of a rabbit rig and force transducer designed to fit in an animal MRI, validation of fitted fibre errors using a celery phantom, the reporting of material properties useful in computational mechanics and the proposal of a muscle primitive useful for building finite element models of muscle. The overarching motivation for this thesis work was to understand how fibre architecture in muscles influences shape and mechanics, and how this information can best be used for animation purposes. The mechanics of muscle that is presented in this thesis can be used to inform lookup tables and statistical models for fast computation of muscle shape.
Acknowledgements

I am very grateful to Dr Justin Fernandez for giving me the opportunity to commence this PhD; and for his help and support during the four year period that I worked under his supervision at the Auckland Bioengineering Institute. His constructive criticism, in part, raised the quality of the presented PhD work. Without his guidance and persistent help, this thesis would have been far more difficult. Thanks for his suggestions, patience and encouragement. Secondly, I would like to have this opportunity to acknowledge my co-supervisor Dr Kumar Mithraratne for his continuous support, funding assistance and his guidance with CMISS/CMGUI issues. Without his knowledge the fibre fitting used in this thesis would have been very challenging.

Specific acknowledgement is made to the Aotearoa Bioengineering fellowship from the Robertson Foundation awarded to J Fernandez, a Ministry of Innovation and Employment (MBIE) grant awarded to K Mithraratne (UOAX0712 and UOAX1006) and the Maurice Phylis and Paykel Trust for travel funding. I also kindly acknowledge Dr Beau Pontré and Ms Rachel Heron from the Centre for Advanced MRI for their help with imaging. During this thesis I had support from many great colleagues, in particular, Ted Yeung, Dharshini Sreenivasan and Associate Professor Thor Besier for all the time we shared.

I am deeply thankful to my parents, for their love, support and sacrifices. Last, but not least, I would like to dedicate this thesis to my family, my wife Laya Zarif and my daughters Elena and Erica for their love, patience and understanding—they allowed me to spend most of my time on this thesis. Thanks to my wife Laya for standing beside me throughout my career and study for this thesis. She has been my inspiration and motivation for continuing to improve my knowledge and move my career forward. She is my rock and her tolerance of my occasional vulgar moods is a testament in itself of her unyielding devotion and love.
CONTENTS

Chapter 1  INTRODUCTION ............................................................................................................ 1
  1.1  MOTIVATION FOR THIS THESIS ......................................................................................... 2
  1.2  RABBIT ANATOMY ................................................................................................................ 3
  1.3  HISTORY OF RABBIT MODELS ............................................................................................. 6
  1.4  EXPERIMENTAL CHALLENGES ............................................................................................ 7
  1.5  IMAGING MODALITIES ......................................................................................................... 8
  1.6  FINITE ELEMENT MODELLING .............................................................................................. 10
  1.7  OBJECTIVES OF THE THESIS .............................................................................................. 11
    1.7.1  NOVEL CONTRIBUTIONS OF THIS THESIS ................................................................... 12
    1.7.2  THESIS OUTLINE .......................................................................................................... 12
    1.7.3  THESIS OUTPUTS ........................................................................................................... 13
Chapter 2  FITTING METHODS ........................................................................................................ 15
  2.1  TRICEPS SURAE GEOMETRY DEVELOPMENT .................................................................... 16
  2.2  SURFACE FIT AND MESH FITTING ..................................................................................... 18
  2.3  CUBIC HERMITE ELEMENTS ................................................................................................. 19
  2.4  FITTING THE FIBRE FIELD ................................................................................................ 21
Chapter 3  DIFFUSION WEIGHTED IMAGING .............................................................................. 28
  3.1  HISTORY OF DIFFUSION WEIGHTED IMAGING ............................................................... 29
  3.2  THEORY .................................................................................................................................. 31
  3.3  VALIDATION USING CELERY .............................................................................................. 39
Chapter 4  DESIGN OF EXPERIMENTAL APPARATUS .............................................................. 42
  4.1  CONSTRAINTS OF THE RABBIT RIG ...................................................................................... 43
    4.1.1  FINAL DESIGN ............................................................................................................... 43
    4.1.2  CLAMPING THE RABBIT ............................................................................................ 47
  4.2  THE FORCE TRANSUCER .................................................................................................... 48
  4.3  CELERY PHANTOM ............................................................................................................. 51
Chapter 5  A DIFFUSION WEIGHTED INFORMED MODEL OF THE RABBIT TRICEPS SURAE ............. 54
  5.1  ABSTRACT ............................................................................................................................ 55
  5.2  INTRODUCTION .................................................................................................................. 55
  5.3  MATERIALS AND METHODS .............................................................................................. 57
    5.3.1  MR COMPATIBLE RABBIT RIG .................................................................................. 57
    5.3.2  Force Transducer .......................................................................................................... 58
  5.4  RABBIT EUTHANASIA ......................................................................................................... 58
Appendix B: Geometric and field fitting script .................................................................119
Appendix C: CAD Design for 3D printed rabbit rig .........................................................121
References .........................................................................................................................126

LIST OF FIGURES
Figure 1-1. Rabbit’s skeletal structure. Image from Ohio State University Extension...............4
Figure 1-2. 3D Model of rabbit lower limb from MR images and created using mesh fitting ............5
Figure 1-3: (a) An artist’s representation of the human gastrocnemius from a posterior view showing the medial and lateral heads and Achilles tendon. (b) Human gastrocnemius computational model from the Physiome Project repository from the side view highlighting the calcaneus and femoral condyle attachment sites..................................................................................5
Figure 1-4: A typical muscle force curve before and after rigor from Van Ee et al. [20]. The y-axis is the Young’s modulus and the x-axis is hours post-mortem which is stable after 72 hours. ..........7
Figure 2-1: Rabbit placed in the 4.7 T animal MR Imaging bore..............................................16
Figure 2-2: Zinc Digitiser for 60 T1 images with each muscle a different colour. The triceps surae (Gastrocnemius, Soleus and plantaris) is yellow, deep plantarflexors is blue, tibialis anterior is red with the tibia shown in white.............................................................................17
Figure 2-3: Rabbit Triceps Surae geometric model steps .......................................................18
Figure 2-4: Cubic Hermite basis functions adapted from CMISS/CMGUI implementation........20
Figure 2-5: Parallel fibres and zoomed image – step 1. ...........................................................23
Figure 2-6: Raw fibre vector data in 3D– step 2 ......................................................................24
Figure 2-7: Shows step 3 that creates two normalised orthogonal vectors and step 4 that rotates the parallel coordinate system into the tissue block fibre vectors or DWI eigenvectors........25
Figure 2-8: Fibres in a small part of human muscle with low sobolev smoothing of 0.01...........26
Figure 2-9: Fibres in a small part of human muscle with medium sobolev smoothing of 0.1........26
Figure 2-10: Fibres in a small part of human muscle with high sobolev smoothing of 1.0........27
Figure 3-1: Signal intensity for each pixel from different diffusion directions. In the figure we have six
directions plus one without diffusion. Note that each image has subtle variations in greyscale
due to the different imaging directions. 31

Figure 3-2: Shows the result of an eigenvector analysis. The diffusion matrix is diagonalised into three
eigenvectors and three eigenvalues. The fibre direction is the largest eigenvector. 35

Figure 3-3: Filtered diffusion weighted imaging voxel data after background thresholding at (left) 30,
(middle) 50 and (right) 90. 36

Figure 3-4: Filtered diffusion weighted imaging voxel data after Fractional Anisotropy (FA) thresholding
at (left) >0.3 and (right) >0.9. 37

Figure 3-5: shows the result of tractography, where the dominant directions at each pixel are joined up to
create streamlines. These streamlines are like contours on a weather map and show the path of
a fibre in the 3D continuum. These vectors are fitted to a field and then we convert them to
fibre directions for a fibre field fit. 38

Figure 3-6: Example of fibre fitting from a rabbit Triceps Surae. Discrete DWI-based fibre vectors (left)
are fitted to a FE field visualised as streamlines (right). 39

Figure 3-7: The development of the phantom. 40

Figure 3-8: (Left) DWI derived fibre vectors inside the celery geometries; (Middle) representative fibres
from one celery geometry close-up; and (Right) fibre angle error fitted to the celery geometry. 41

Figure 4-1: Some the main components of the rabbit rig. 44

Figure 4-2: The final design. 45

Figure 4-3: Upper image: the dimensions of the rig and force driver from handle to table 3 in below. .... 45

Figure 4-4: Animal MRI. 4.7 T magnet and 140 mm RF coil. 46

Figure 4-5: The protractor was cut from a clear plastic sheet by laser cutter and engraved with angles
from 0° to 90°. 47

Figure 4-6: The way the rabbit was placed onto the rig and the tendon holder (included sensor holder)
clamped. 48

Figure 4-7: The tendon holder including sensor holder on top. 48
Three transducers were designed for this study. The ranges are: 0.00098-9.80 N, 0.0098-68 N and 0.098-392 N.

Capacitor sensor. By changing the gap between the electrodes (r), capacitance will change (ΔC).

The ABI laser cutter.

Drawing of the celery phantom.

3D image of the celery phantom.

Complete phantom where the celery was attached using cable ties.

MRI compatible rabbit rig used to dorsiflex the rabbit hind limb within the MRI bore and measure Triceps Surae tendon force for each foot position.

(I & II) The experiment framework. (III & IV) The rabbit fixated on the rig; connecting the transducer; (V) MR imaging and (VI) digitised T1 images for geometry creation.

High-order finite element model of rabbit hind limb developed from rabbit MRI; and similar human model developed using the same techniques.

Relaxed and four MR derived muscle deformations; and muscle optimised (red) to match experimental gold standard (gold) using ‘pole zero’ constitutive law.

The average force in all positions for two rabbits (mean ± 1 standard deviation). The stages were EP (euthanised point), ROP (rapid rigor onset point), PR (peak rigor point), RD (steady rigor decrease point), SS (steady state point).

(Left) Fitted DWI fibres (gold) contrasted with no pennation based fibres. (Right) DWI contractile shape overlaid with no pennation contractile shape and effect of 10% perturbation in fibre angle (green/gold).

Muscle strain with and without DWI when in maximum muscle contraction. ε is the symbol for strain which is unitless.

Phantom celery to validate the study.

The experiment setup. Each subject was seated with the leg in a flexed pose and the foot was moved through dorsiflexion. Top right shows the triad of markers attached to the leg used to define the leg frame of reference. Markers are also attached to the ultrasound transducer to define the imaging plane.
Figure 6-2: (Left) is a slice from the 3D Ultrasound image identifying a manually segmented fibre at time zero; (Middle) is a 2D representation of a 3D set of nine segmented fibres shown as red lines. Green circles are the proximal and distal insertions; (Right) shows the fibres embedded inside a three-element 3D host mesh for the same volume of interest.

Figure 6-3: Free-form deformation pipeline: (Left) shows three representative fibres in green at baseline with corresponding proximal and distal landmarks at 100% elongation shown in red. The fibres are embedded inside a 3-element host. (Middle) shows the deformation of the host at 50% elongation driven by the baseline fibres in order to match the target proximal and distal fibre endpoints. (Right) shows the deformation of the host at 100% fibre elongation and perfectly matched to the target fibres.

Figure 6-4: Free form deformation of subject 1 highlighting the matching of all baseline fibres to the fibre locations at 100% elongation. This produces a deformed host whose shape minimises the difference between baseline and target fibres.

Figure 6-5: Free form deformation of subject 4 (who has a higher pennation change during stretch) highlighting the matching of all baseline fibres to the fibre locations at 100% elongation. This produces a deformed host whose shape minimises the difference between baseline and target fibres.

Figure 6-6: Fitted pole-zero constitutive law strain energy density for subjects one to four in the fibre direction (W11) and transverse directions (W22/W33).

Figure 6-7: (Left) Bi-pennate muscle primitive used to construct a whole Triceps Surae muscle; and (right) complete fitted fibre field within whole muscle.

Figure 6-8: Ultrasound informed muscle in (left) relaxed state and (right) fully contracted state. The top shows an axial view and the bottom shows a posterior coronal view.

Figure 6-9: (left) Fully contracted muscle derived from Ultrasound, (middle) DWI and (right) overlaid. The top shows an axial view and the bottom shows a posterior view.

Figure 6-10: (left) Maximum principal compressive and (right) tensile strain from the (top) axial and (bottom) posterior views. \( \varepsilon \) is the symbol for tensile strain which is unitless.
LIST OF TABLES

Table 5-1: Siemen’s 4.7 T MRI T1 settings for muscle boundary geometries ........................................60
Table 5-2: Siemen’s 4.7 T MRI DWI settings for fibre information ..........................................................61
Table 5-3: Optimised ‘pole-zero’ material parameters satisfying all deformed positions including DWI pennation fibres. ............................................................................................................................66
Table 5-4: Root Mean Square error of predicted muscle shape versus experimental gold standard across all four deformed positions for the first and second rabbit ........................................................................66
Table 5-5: Sensitivity test for pole-zero parameters ................................................................................69
Table 6-1: Subjects statistics ..................................................................................................................80
Table 6-2: Muscle tension during muscle elongation ..............................................................................82
Table 6-3: Minimum and maximum tension and muscle length, muscle slack length and muscle stiffness for each subject ........................................................................................................82
Table 6-4: Fitted ‘pole zero’ parameters for all four subjects .................................................................89
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ADC</td>
<td>Apparent Diffusion coefficient</td>
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<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
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<tr>
<td>CAD</td>
<td>Computer-Aided Design</td>
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<tr>
<td>CAMRI</td>
<td>Centre for Advanced MRI</td>
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<tr>
<td>CT</td>
<td>Computed Tomography</td>
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<tr>
<td>DTI</td>
<td>Diffusion Tensor Imaging</td>
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<tr>
<td>DWI</td>
<td>Diffusion Weighted Imaging</td>
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<tr>
<td>FA</td>
<td>Fractional Anisotropy</td>
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<tr>
<td>FE</td>
<td>Finite Element</td>
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<tr>
<td>FEM</td>
<td>Finite Element Method</td>
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<tr>
<td>MAP</td>
<td>Musculoskeletal Atlas Project</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<tr>
<td>NEX</td>
<td>Number of Excitations</td>
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<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
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<td>OA</td>
<td>Osteoarthritis</td>
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<td>RF</td>
<td>Radio Frequency</td>
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<td>RMS</td>
<td>Root Mean Square</td>
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<td>TE</td>
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This chapter introduces the motivation behind this thesis. It outlines the core technologies of MRI and Diffusion Weighted Imaging, Ultrasound, finite element (FE) methods as applied to muscle mechanics and provides an anatomy of the Triceps Surae focused on both the rabbit hind limb and human lower limb. Finally, it outlines the key thesis objectives and the novel contributions and outputs from this thesis.
1.1 MOTIVATION FOR THIS THESIS

Computational modelling of muscle is becoming an increasingly popular tool for evaluating muscle function with the development of such packages as OpenSim [1, 2], the ‘grand knee challenge’ instrumented data set for muscle force validation [3] and the Visible Human data set for constructing shared geometries [4]. A number of research groups have developed bio-specific finite element (FE) software including FeBio [5, 6], the OpenCMISS and CMGUI packages developed by the Auckland Bioengineering Institute [7, 8]; and commercial finite element software are also including biomechanics-based modules in their developments such as Abaqus [9]. Computational models of muscle are only useful when the quality of the information used to build them; specifically, geometry, material properties and boundary conditions, is evaluated with confidence. Of these challenges, geometry has been fairly well addressed with such imaging modalities as MR Imaging, Computed Tomography (CT) and Ultrasound. However, material properties and an understanding of the underlying muscle architecture, while known to be important, are not well characterised. Other organ systems like the heart have been studied for decades and are more mature fields of research. This thesis will adapt methods that have previously been used in cardiac mechanics, namely the ‘pole-zero’ constitute law to skeletal muscle [10, 11]. Furthermore, many researchers use rigid body biomechanics with 1D muscle actuators, however, a smaller subset of researchers focus on continuum models to understand 3D spatial behaviour and require more complex material laws [12]. Moreover, continuum models when validated can be used to inform rigid body models via surrogate methods [13]. In this thesis we concentrate on continuum models of muscle, focussed on the Triceps Surae for this thesis. In this scenario, using the detailed Triceps Surae muscle informed with diffusion weighted estimates of fibre architecture we can compute continuum solutions of stress, strain and 3D shape, which can be linked to simpler 1D muscle in the future. We present this for both the human and rabbit Triceps Surae. Finally, one of the initial motivations for this work was in the field of entertainment and animation. Increasingly, biophysically-based models are being used to drive more realistic animation techniques. While this
thesis does not examine these specifically, the continuum models were evaluated for their physical realism, which are important attributes to animators. Hence, this thesis focusses on the fundamental development of methods for fitting detailed material constitutive laws using the Triceps Surae as the muscle for demonstration while keeping in mind all these important applications. Specifically, an application of this thesis was to develop an ultrasound informed muscle primitive that could be used to build a whole muscle for mechanics, visualisation and e-learning applications.

1.2 RABBIT ANATOMY

It is important to have an understanding of the musculoskeletal anatomy of the rabbit as this is the primary animal for our testing and evaluation. While we also consider the human Triceps Surae for the second major study of this thesis, the rabbit provided a better measurement of musculotendon force and has similar anatomical attributes. For this study we have focussed on the lower hind limb and started by performing a rabbit dissection. The aim was to understand the muscle attachments and differences between rabbit and human anatomy. We also needed to understand where we could fix the femur for the muscle force experiment and we needed to know how to skin the rabbit and expose the muscle with minimal blood loss in minimal time. Figure 1-1 shows the rabbit skeletal structure where the tibia is label 5 and femur label 6 and the gastrocnemius attachment is shown. Figure 1-2 shows the computational model of the rabbit developed from MR images (described in chapter 2).

The rabbit’s lower limb has four major muscles similar to the human, namely, the gastrocnemius (medial and lateral heads), soleus and tibialis anterior. The gastrocnemius, together with the soleus, plantarflexors and the foot. The proximal end of the gastrocnemius bifurcates and inserts in the femur, where the medial and lateral heads insert into the medial and lateral condyles, respectively. The distal end inserts in the calcaneus (heel bone) [14] via the Achilles tendon and the complete muscle complex including soleus is known as the Triceps Surae. The bi-articulate gastrocnemius muscle is involved in most aspects of gait and also contributes to loading in joints it does not cross through ‘induced acceleration’ [15]. Although the soleus and gastrocnemius share the same distal insertion their different proximal origins mean they have different functions. The
gastrocnemius is a two joint muscle and plays a role in flexion (especially during mid-stance), whereas the soleus crosses one-joint and plays a role in decelerating plantar flexion. The rabbit’s lower limb, unlike humans, does not include a fibula. Figure 1-3 shows the human gastrocnemius and equivalent computational model developed as part of the Physiome project, which will be used in the second major study of this thesis.

Figure 1-1. Rabbit’s skeletal structure. Image from Ohio State University Extension
Figure 1-2: 3D Model of rabbit lower limb from MR images and created using mesh fitting.

Figure 1-3: (a) An artist’s representation of the human gastrocnemius from a posterior view showing the medial and lateral heads and Achilles tendon. (b) Human gastrocnemius computational model from the Physiome Project repository from the side view highlighting the calcaneus and femoral condyle attachment sites.
1.3 HISTORY OF RABBIT MODELS

The New Zealand (NZ) white rabbit is the animal of choice for many experiments and models [16-19]. For example, mathematical models of the rabbit are useful for understanding disease and musculoskeletal biomechanics and how this is translated to the human since rabbits remodel their cortical bone similar to humans. This was the motivation of Grover et al. [16] who evaluated the behaviour of muscles, ligaments and joint contact in rabbit stance during hopping. The other benefit was that previous NZ white rabbit intersegmental forces and moments were available for input to the model. NZ white rabbits have also been used as models for osteoarthritis (OA) onset and progression, which can be related to the human. Herzog and Longino [17] demonstrated this by using NZ white rabbits and reported that after 4 weeks of weakness in the extensor muscles, induced by Botulinum type-A toxin, resulted in initial signs of joint degeneration. Computing muscle force is the ‘holy grail’ in biomechanics and determining surrogates that are related to muscle force push the field forward. Davis et al. [18] used NZ white rabbits to establish a relationship between intramuscular pressure and relative muscle force during isometric muscle contraction of the rabbit tibialis anterior using a micro fibre optic pressure transducer. With this knowledge it may be possible to relate computed intra muscle pressure as a surrogate in human models. Finally, Hill type model parameters are often developed from individual sarcomeres but mathematical models are typically at the whole organ macro level. Winters et al. [19] demonstrated that modelling muscle as a scaled sarcomere provides accurate active functional but not passive functional predictions for rabbit tibialis anterior, extensor digitorum longus and extensor digitorum muscles and calls into question the need for more complex modelling assumptions.

We have also chosen the NZ white rabbit for our work as there are many examples in the literature to compare our data with; the breed is easy to obtain; our collaborators in Australia are also using the NZ white rabbit for tendon mechanics so we can compare our data with theirs; and the genome sequence has been completed for this animal so it opens up the possibility for future multiscale work including cell models.
1.4 EXPERIMENTAL CHALLENGES

One of the challenges presented in this study was that the rabbit muscle enters rigor and so material properties change following euthanasia. This makes it difficult to measure muscle force over 4 dorsiflexion positions and have the muscle stable for MR imaging. Van Ee et al. [20] reported the post-mortem effects in twelve NZ white rabbits following euthanasia and we used this information in the design of our experiments as we could not measure force and MR images while the muscle was going through rigor mortis. Specifically, they showed that after 36-72 hours post mortem, the rabbit muscle behaviour was stable (Figure 1-4) and this was consistent with our work that showed a stable region after 36 hours. Secondly, the maximum contraction occurred around 15 hours post mortem and this was consistent with our experiments where maximum force occurred 14-18 hours post-mortem. This was used as a surrogate for maximum contraction in our study in chapter 5.

![Figure 1-4: A typical muscle force curve before and after rigor from Van Ee et al. [20]. The y-axis is the Young’s modulus and the x-axis is hours post-mortem which is stable after 72 hours.](image-url)
Leitschuh et al. [21] and Gottsauner-Wolf et al. [22] compared the properties of muscles from the time of death to post-mortem following a freeze-thaw cycle. These studies found decreases in failure stress and energy after freezing but did not indicate mechanical properties post-rigor. Hence, the literature was limited in describing material properties post rigor in rabbits. The key point from these studies was that after rigor passed the material properties were slightly stiffer but not considerably higher than in vivo. We therefore planned to measure the temporally changing force until stable. At this point we could measure material properties and scale the post-rigor values to just post-euthanasia.

Measurement of muscle force has been computed in a variety of ways for rabbit models given that they are popular models for osteoarthritis, bone growth and remodelling. Gushue et al. [23] used standard motion capture to compute muscle, ligament and joint contact force in the rabbit knee. Muscle forces were computed using static optimisation. Davis et al. [18] used a fibre optic pressure transducer to measure muscle force in the tibialis anterior of 12 NZ rabbits. In another similar study by Komi and his colleagues, [24] they introduced a transducer by optic fibre to measure the tendonmuscular forces for both female and male rabbits. They measured the static and dynamic loading of the tendon. Due to the limited techniques and data available in the literature for rabbit force, an important objective of this thesis was to develop an MR compatible rabbit rig that could be used to measure passive force temporally and be associated with MR derived muscle shape. This work is detailed in chapter 4 including CAD (computer-aided design) design and development of the tendon transducer.

1.5 IMAGING MODALITIES

MR imaging was the first modality examined in this thesis. We used it to extract Triceps Surae geometry using a T1 sequence and fibre orientation using a diffusion sequence. The detailed theory and concept of diffusion weighted imaging (DWI) is described in chapter 3.

MR imaging is a scanning technique that uses strong magnetic fields that create detailed images of internal structures of the body, where different elements such as bones and tissues can be
identified. MR imaging is based on proton movements in the body. Almost all the signals come from one or two sources: water protons and protons in molecules of fat. In a strong magnetic field, these protons align with the induced magnetic field. When these protons are excited to resonance with the applied radio frequency (RF) energy, the energy released is the same frequency as the applied radio waves, the coil or antenna that sent the RF is switched to become a receiver of the energy produced by the release of the target substance proton energy. Images are dependent on the response signals from different tissues by applying parameters such as T1, T1ρ and T2 relaxation time and proton density in a small piece of tissue. Varying grey scale levels are obtained for tissue contrast in MR imaging by using the appropriate pulse sequences.

While traditional T1 weighted MR was used to obtain muscle and bone geometry the novel application in this thesis was the use of DWI. DWI is an MR imaging sequence used to map the anatomy of muscle fibres in cardiac and muscle tissue; and neuronal pathways of cerebral white matter. This is achieved by a gradient magnetic field [25] that records the diffusion of cells. Typically, DWI is used for brain imaging and in the heart. Examples of this method in the literature include the brain [26, 27], spinal cord [28], kidney [29] and heart [30, 31]. Bammer et al. [32] reported that DWI measures anisotropy per pixel and provides the directional information relevant for MR tractography or fibre tracking in vivo. Van Donkelaar et al. [33] used DWI to get geometric information for numerical simulations of skeletal muscle contractions. Their results showed that DWI provided enough resolution and accuracy to use DWI-based fibre directions in biomechanical analyses.

For the second part of this thesis we considered the human Triceps Surae and attempted to incorporate 3D ultrasound informed fibres in the Triceps Surae model. The benefit of this approach was that we were able to identify the deforming fibres during muscle deformation and map this to a deformable FE mesh. Previous studies have used ultrasound to measure muscle shape changes [34-36] and changes in muscle fibre pennation angles [37-41]. Ultrasound can be used for real-time imaging and it has been used to measure length changes when the muscle is undergoing contraction [40, 42]. Zuurbier and Huijing [43] showed muscle fibres and pennation angles vary considerably
along the length of the muscle as observed in the rat gastrocnemius. Hence a simple parallelogram structure often used to describe muscle pennation will not suffice and 3D information is necessary to fully describe the complete deformation. Further, the need to capture in vivo fibre data is important as highlighted by Martin et al. [44], who showed that ultrasound based fibre characteristics of cadaveric muscle differ from both relaxed and contracted in vivo muscle. In summary, Ultrasound is versatile and successful in capturing fibre behaviour in the gastrocnemius during locomotion, isometric movements and stair ascent [45-51]. For this thesis we are interested in fibre length and pennation angle change during passive stretch of the Triceps Surae, but our modelling framework can easily be adapted to active contraction as well.

1.6 FINITE ELEMENT MODELLING

One of the most useful tools for modelling mechanics of the musculoskeletal system is the finite element method (FEM). It allows for complex non-linear soft tissue geometries and spatially varying fields such as stress, strain, fibre information and material properties. FE modelling of the Triceps Surae has been presented for many species, due to similar anatomy that can be translated to the human. One example was the work of Donkelaar et al [52], who developed a 3D FE model of the medial rat gastrocnemius from histology, which was coupled to a blood perfusion model. This highlights the solid-fluid coupling capability in solid mechanics when a detailed muscle contraction model is developed. Hence, our adapted FE framework permits future research in coupling our solid mechanics muscle model via hydrostatic pressure to an artery network as was previously done in the Physiome derived foot model of Fernandez [53, 54]. Lemos et al. [55] developed a detailed continuum model of the cat gastrocnemius used to investigate muscle sliding, deformation and force production. The unique aspect of their work was the inclusion of different structural information starting with the fibre level. They showed an accurate description of fibre arrangement produced predictions comparable with experiments. Furthermore, spatially varying descriptions of muscle fibres have been reported to produce improved muscle paths and ensure that different fibre regions have different moment arms as demonstrated for the hip crossing muscles by Blemker et al.
Moreover that group also showed that variation in fascicle lengths was a primary contributor to non-uniform strains in muscle [57]. A complete modelling framework was presented by Tang et al. [58] who developed a continuum model evaluated using a frog gastrocnemius muscle in Abaqus. They included passive and active contractions and a constitutive law based on strain energy. They also reported that fibre orientation influenced stress patterns.

It is clear from the literature that continuum models of the Triceps Surae that contain accurate 3D spatially varying description of fibres produce experimentally equivalent forces, contractile paths and shapes which allow for realistic sliding with other muscles and tissues. These benefits motivated our adoption of this continuum framework that also allows the future possibility of solid-fluid coupling and spatially varying muscle activation, which was previously reported in the work of Fernandez et al. [59]. Moreover, continuum models allow for better visual representation, graphically and are the future for model scaling, to patient data and linking to simplified 1D muscles used in rigid body models. The choice of a rabbit model is that the NZ white rabbit is a popular choice for researchers but little has been reported about the rabbit Triceps Surae.

1.7 OBJECTIVES OF THE THESIS

Considering the state of the field in muscle mechanics the aims of this thesis were to:

- Evaluate the fibre distribution and musculotendon force of the rabbit Triceps Surae using a custom developed MR rig from DWI for use in continuum mechanics models.
- Evaluating the influence of 3D fibre orientation on the stress, strain and shape of the Triceps Surae and why the inclusion of this information is important for modellers.
- Evaluating the fibre distribution of a 3D continuum model of the human Triceps Surae from 3D Ultrasound and examine strain and contractile shape developed from 4 subject-specific data sets.
- Develop an ultrasound informed muscle primitive that could be used to assemble a whole muscle.
1.7.1 NOVEL CONTRIBUTIONS OF THIS THESIS

This thesis consists of a number of novel contributions and important incremental achievements in the field of Triceps Surae mechanics: These thesis contributions include:

- Characterising at a continuum level, the importance of the fibre pennation on muscle function in the rabbit Triceps Surae (from DWI) and human Triceps Surae (from Ultrasound).
- Determine a set of material properties for a fibre-based constitutive law in both the human and rabbit Triceps Surae. This can be used by other computational modellers along with the fibre information and is a contribution to the Physiome Project.
- Develop a re-usable human muscle primitive for bi-pennate muscle based on passive material properties that can be used to assemble a whole muscle.
- Develop an approach for measuring rabbit material properties post rigor mortis and relating this back to the in vivo state (just post euthanasia).
- The development of a custom MR compatible rig and transducer for the purpose of fitting rabbit material properties.
- Develop a celery phantom for evaluating diffusion fibre estimate accuracy.

1.7.2 THESIS OUTLINE

This thesis is delineated by the following sections: Chapters 5 and 6 represent two journal papers that are in review.

- Chapter 2: Methods for fitting geometric and field information in the FE package CMISS.
- Chapter 3: Methods for extracting Diffusion Weighted Imaging data.
- Chapter 4: Design of experimental apparatus.
- Chapter 5: Paper 1: A Diffusion Weighted Informed model of the rabbit Triceps Surae.
• Chapter 7: Concluding remarks and future directions.
• Appendix A: DWI fitting script.
• Appendix B: Geometric and field fitting script.
• Appendix C: CAD Design for 3D printed rabbit rig.

1.7.3  **THESIS OUTPUTS**

This thesis is collectively summarised as a collection of two first authored journal articles (chapters 5 and 6) and a second author contribution to a third paper. It has also been presented as 4 conference papers (all oral presentations). The presentation at the World Congress of Biomechanics (*) was a finalist in the PhD competition ranked in the top 36 PhD presentations of 700 candidates.

**Journal Articles:**


**Conference and papers and extended abstracts: All oral presentations.**


2. J Fernandez, **M Alipour**, V Shim, K Mithraratne, A diffusion tensor imaging based model of muscle mechanics, XXIV Congress of ISB, Natal, Brazil, Aug 4-9, 2013

This chapter present the geometric fitting methods used in this thesis. The process of obtaining rabbit geometry and fitting to high order elements is described and the unique characteristics of cubic Hermite elements that need to be accounted for. We also describe the process of fitting fibres to the geometric meshes, which are key to the work in chapters 5 and 6.
2.1 TRICEPS SURAЕ GEOMETRY DEVELOPMENT

The geometries for the Triceps Surae rabbit muscles used in this thesis were developed from T1-weighted, MR Imaging. MR Imaging is a popular imaging modality, used in both clinical diagnosis and research that uses magnetic fields to create detailed images of internal structures of the body. It uses the energy released by water molecules, which is different for each tissue type (such as bone and muscles) to identify geometrical boundaries between bone, cartilage, muscles and fat. For this thesis the NZ white rabbit was chosen and imaged at the Centre for Advanced MRI (CAMRI), Auckland University using a 4.7 T animal MRI.

Figure 2-1: Rabbit placed in the 4.7 T animal MR Imaging bore.

The T1 settings for rabbit geometry on the 4.7 T machine used were:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Echo Time (TE)</td>
<td>10</td>
</tr>
<tr>
<td>Repeat Time (TR)</td>
<td>1000 ms</td>
</tr>
<tr>
<td>Number of Excitations (NEX)</td>
<td>2</td>
</tr>
<tr>
<td>Resolution</td>
<td>L: 305 W:640</td>
</tr>
</tbody>
</table>
The DTI settings for rabbit fibre information on the 4.7 T machine used were:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Echo Time (TE)</td>
<td>40</td>
</tr>
<tr>
<td>Repeat Time (TR)</td>
<td>3000 ms</td>
</tr>
<tr>
<td>Number of Excitations (NEX)</td>
<td>2</td>
</tr>
<tr>
<td>Number of gradient Directions</td>
<td>20 + 1</td>
</tr>
<tr>
<td>b-Value</td>
<td>500</td>
</tr>
<tr>
<td>Resolution</td>
<td>L:305 W:640</td>
</tr>
</tbody>
</table>

Using the software Zinc, developed by the Auckland Bioengineering Institute, we identified the muscle and bone boundaries for segmentation. The 2D images were loaded into the Zinc digitiser (Figure 2-2) and segmented manually by outlining the muscle and bone boundaries with data points. The process produced a 3D cloud of data points for each muscle (Triceps Surae, Tibialis anterior and deep flexors) and the bone (tibia) of the rabbit lower limb. In Figure 2-2 the Triceps Surae is yellow, deep plantarflexors is blue, tibialis anterior is red with the tibia shown in white.

These data points were the starting point for creating subject-specific FE meshes of the muscles and bones in the software CMISS and CMGUI (Auckland Bioengineering Institute).

![Zinc Digitiser for 60 T1 images with each muscle a different colour. The triceps surae (Gastrocnemius, Soleus and plantaris) is yellow, deep plantarflexors is blue, tibialis anterior is red with the tibia shown in white.](image)
2.2 SURFACE FIT AND MESH FITTING

The 3D cloud of digitised points was used to build a mesh suitable for finite deformation elastic mechanics. This process is illustrated for one representative rabbit Triceps Surae as shown in Figure 2-3. Firstly, using the cloud of muscle points (yellow glyphs) a subset was chosen as surface nodes (larger green points). Secondly, a tri-linear mesh was then constructed using the green nodal points to capture the primary shape. The outer edges were collapsed to ensure nodal continuity for the high order cubic Hermite elements used later on and we used mapping to ensure that the derivatives were continuous along the edges. The consistent $\xi_i$ directions are shown in figure 2-3 where blue is 0 and red is 1 showing that each element is consistent and goes from 0 to 1 in mathematical $\xi_i$ space. Finally, using the least squares fitting procedure in CMISS (detailed next) we fitted higher order cubic Hermite elements to capture the complete shape with an RMS (Root Mean Square) fitting error of less than 3 mm.

Figure 2-3: Rabbit Triceps Surae geometric model steps
2.3 CUBIC HERMITE ELEMENTS

The high order cubic Hermite elements used in this thesis consist of nodal values and derivatives at each node which ensure the 1st derivative is enforced across nodes (C1 continuity).

The three-dimensional FE meshes have basis (or shape) functions which are determined from the tensor product of 1D interpolation functions. These four one-dimensional cubic Hermite basis functions are given in equation (2-1) and shown in figure 2-4.

\[
\begin{align*}
\Psi_0^0(\xi) &= 1 - 3\xi^2 + 2\xi^3; \\
\Psi_2^0(\xi) &= \xi^2 (3 - 2\xi); \\
\Psi_1^1(\xi) &= \xi (\xi - 1)^2; \\
\Psi_2^1(\xi) &= \xi^2 (\xi - 1), \\
\end{align*}
\]

(2-1)

where \( \xi \) is the normalised local or element coordinate which is defined from 0 to 1. These cubic Hermite elements differ from the usual Lagrange family FE in that they preserve both the continuity of the nodal values (C0 continuity) and their first derivatives (C1 continuity). This provides many advantages in constructing a FE geometry, particularly of biological structures such as muscles and other organs that typically have smooth surfaces [60]. Moreover, the realistic geometries are useful in such applications as virtual surgery and medical education. Another key feature of cubic Hermite elements is that fewer numbers of elements are required for complex geometries. C1-continuous interpolation also provides a smooth change in the surface normal across element boundaries and this provides numerical benefits when solving contact mechanics problems. Interpolation of the spatial coordinates of line elements in space with cubic Hermite basis functions is given by:

\[
u(\xi) = \Psi_0^0(\xi) u_1 + \Psi_2^0(\xi) u_2 + \Psi_1^1(\xi) \frac{du}{ds} \bigg|_{1}^{L} + \Psi_2^1(\xi) \frac{du}{ds} \bigg|_{2}^{L},
\]

(2-2)

where \( u \) is \( x, y \) or \( z \) if the field is geometry and \( du/\text{ds} \) is the derivative of the spatial coordinate with respect to a measure of distance, chosen to be arc-length here. Subscripts 1 and 2 refer to node numbers and superscripts 0 and 1 are the zeroth and first derivatives, respectively. \( L \) is the physical arc length along the curve. To ensure we have continuity with respect to arc-length we enforce the condition that the magnitude of \( du/\text{ds} \) should be 1. The interpolation can be any field in general such as stress, temperature or fibre information and more details can be found in Fernandez et al. [61]
Now that we have established the element primitive, we fit the elements to segmented data using a least-squares fitting procedure. We employ a face fitting routine for surfaces of the volume mesh and define a face objective function $F(u_n)$, consisting of two components, namely the data error and a smoothing constraint given by:

$$F(u_n) = \sum_{d=1}^{N} w_d \left[ u(\xi_{1d}, \xi_{2d}) - z_d \right]^2 + F_s(u_n)$$

(2-3)

where the data error is the summation of the square of the distances between each data point, $z_d$ and its orthogonal projection $u(\xi_{1d}, \xi_{2d})$ on the relevant face. Each point can be weighted based on importance using $w_d$. The smoothing constraint, $F_s(u_n)$, is appended to the objective function as a penalty function defined by:

$$F_s(u_n) = \int_0^1 \int_0^1 \left[ \alpha_1 \left( \frac{\partial u}{\partial \xi_1} \right)^2 + \alpha_2 \left( \frac{\partial u}{\partial \xi_2} \right)^2 + \alpha_3 \left( \frac{\partial^2 u}{\partial \xi_1^2} \right)^2 + \alpha_4 \left( \frac{\partial^2 u}{\partial \xi_2^2} \right)^2 + \alpha_5 \left( \frac{\partial^2 u}{\partial \xi_1 \partial \xi_2} \right)^2 \right] d\xi_1 d\xi_2$$

(2-4)
which is the second order Sobolev norm used as the smoothing constraint to account for sparse and scattered data. Constants $\alpha_i (i=1..5)$ are the Sobolev weights (penalty parameters) and each term has a distinct effect on the final shape of the fitted object. The first two terms ($\alpha_1, \alpha_2$) control the arclength, while the third and fourth terms ($\alpha_3, \alpha_4$) control the arc-curvature in the $\xi_1$ and $\xi_2$ directions, respectively. The last term ($\alpha_5$) represents the face area. For instance, if the weight associated with the cross-derivative term is set to a relatively higher value, one might end up with a smaller face area. All Sobolev weights must be at least an order lower than the weight associated with the data error component, $w_d$, which is usually taken as 1.0.

If the data set is sparse, such as shown with the rectus femoris muscle (one of the quadriceps) and if the Sobolev weights are all set to have minimal effect then the fitting will produce an oscillatory shape in the muscle. This is an artefact arising from the data being dense in the radial direction but sparse in the longitudinal direction. If, however, we place more weight on the curvature in the longitudinal direction and increase smoothing on the face area we arrive at a more anatomically correct shape. This shows that the Sobolev weights can account for incomplete or non-uniform data sets. In the second example we have a bone to fit (the femur) with a uniform and dense cloud of data from a scanned source. There is usually less need to increase Sobolev smoothing parameters in such a case as the data is sufficient and homogenously distributed for an accurate fit. However, if we want to place more emphasis on a particular region such as the greater and lesser trochanters then we can increase the data point weights for those features. The fitting algorithm will then ensure a better fit in those regions. In Appendix C7 the influence of Sobolev smoothing parameters is shown on the surface of the fitted muscle.

2.4 FITTING THE FIBRE FIELD

In addition to fitting geometry we can also fit field information to the FE mesh. Fields can be stress, temperature, or material properties. One important field required for this thesis is the fibre field. To illustrate the fitting of the fibre field we present the Triceps Surae which was previously developed. In chapter 3 we will define how the fibre vectors are extracted from DWI and in this chapter we describe the general process of fitting a fibre vector field that will be used for the studies.
in chapters 5 and 6. For illustration purposes we will demonstrate using the fitting of fibre
information to a simple cube. The steps are as follows and can be run using the fitting scripts in
Appendix B.

1. First we create a set of parallel 0° vectors to the curvature of the muscle. In the case of the
simple cube shown here these are aligned along one side (Figure 2-5).

2. Second we extract a single normalised vector from each pixel of the imaging volume. For
the example data set here we have a vector from a tissue block and in the DW images we
use the largest eigenvector (Figure 2-6).

3. Next we create two more normalised orthogonal vectors. For the case at hand we create
two arbitrary vectors using a cross-product but for the DW images this will be the second
two eigenvectors. This is created using a Perl script in Appendix B. Note that for DW
images there are sometimes inverted vectors due to matrix inversion errors so these are
flipped as with previous DWI studies [62] (Figure 2-7).

4. Following this we rotate the parallel coordinate system into the tissue block fibre vectors or
DWI eigenvectors. This produces three Euler angles that we now define as fibre angles
(Figure 2-7).

5. Finally, the three fibre angles are treated as three fields, which are fitted to the nodal values
and derivatives of the cubic Hermite mesh in the same manner as the mesh geometry was
fitted. The fitted field is used to define the structural axes by which the stress and strain are
referred to during mechanics simulations. This is used in both the passive constitutive law
and when describing contraction mechanics. This will be key in chapters 5 and 6 where we
fit DWI based and ultrasound fibres to a muscle mesh and then simulate mechanics.
Figure 2.5: Parallel fibres and zoomed image – step 1.
Figure 2-6: Raw fibre vector data in 3D–step 2
Figure 2-7: Shows step 3 that creates two normalised orthogonal vectors and step 4 that rotates the parallel coordinate system into the tissue block fibre vectors or DWI eigenvectors.

Figures 2-8 to 2-10 present fitted fibres from a small section of a muscle and the effects of choosing different Sobolev smoothing parameters defined in equation 2-4. It is clear from the images, there are a group of fibres within a bundle and a muscle is a collection of multiple bundles. Specifically, Figure 2-8 shows the effect of a very small weighting of 0.01 on the arc-length ($\alpha_1$, $\alpha_2$) and arc-curvature ($\alpha_3$, $\alpha_4$). The fibres are more distinct. When the weighting is increased to 0.1 the fibres appear straighter as there is a higher weight in the fitting function penalising them from deviation from a straight arc and curvature (Figure 2-9). If this weight is increased to 1.0 then the distinct fibre features are almost entirely lost (Figure 2-10). This shows that it is important to carefully choose the right Sobolev smoothing parameters. The choice is typically from experience where abundant field data requires a lower Sobolev smoothing and sparse data requires higher smoothing to remove noise.
Fitting The Fibre Field

Figure 2-8: Fibres in a small part of human muscle with low Sobolev smoothing of 0.01.

Figure 2-9: Fibres in a small part of human muscle with medium Sobolev smoothing of 0.1.
Figure 2-10: Fibres in a small part of human muscle with high Sobolev smoothing of 1.0.
This chapter describes the theory of DWI and the least squares fitting method used to extract the fiber directions from the DWI sequence as used in this thesis. It describes the fitting of the muscle fibers to the Triceps Surae mesh, filtering methods used and finally the celery phantom study used to establish the error in the entire DWI process.
3.1 HISTORY OF DIFFUSION WEIGHTED IMAGING

From the early Nobel Prize winning work of Purcell and Bloch (1946) who used NMR (Nuclear Magnetic Resonance) to observe chemical compounds to the more recent Nobel Prize work in imaging by Lauterbur and Mansfield (2003) [63], the use of the more familiar term MR Imaging has become an important tool for medical diagnosis. NMR diffusion and MR Imaging were integrated by Le Bihan et al. (1985) and advanced by Basser et al. (1990s) [64]. Today it is widely used in brain tractography, stroke identification and research uses in characterising muscle fibres to name just a few applications.

DWI is an MR Imaging sequence used to map the anatomy of muscle fibres in cardiac and muscle tissue; and neuronal pathways of cerebral white matter. This is achieved by a gradient magnetic field [25] that records the diffusion of cells. In standard MR Imaging a magnetic field aligns all protons and when the pulse is turned off each particle will return to its natural alignment and give of a signature energy which is converted into an image. However, water particles actually migrate and don’t return to the original location. DWI records this migration from different directions. The concept is that the migration of fluid relates to the fibre direction in muscle. It is typically used for brain imaging and in the heart. Examples of this method in the literature include the brain [26, 27], spinal cord [28], kidney [29] and cardiac tissue [30, 31].

One example of DWI with musculoskeletal tissue includes a microscopic level study [65]. They used DWI to study the human calf and examine the diffusive properties of adjacent muscles at rest and determine the relationship between diffusive and architectural properties, which are task-specific to muscles. Bammer et al. [66] reported that DWI measures anisotropy per pixel and provides the directional information relevant for MR tractography or fiber tracking in vivo. In their paper, they reviewed the basic principles of tractography and their potential strengths and weaknesses. Another possible use of DWI has been to identify the properties of healthy and damaged muscles [67, 68]. In particular, Orchard et al. [69, 70], used DWI in the diagnosis of acute and healing muscle strain injuries, which is one of the most common sport injuries. They improved injury identification and prognostic assessment of muscle strains. Van Donkelaar et al. [33] used...
DWI to get geometric information for numerical simulations of skeletal muscle contractions. Their results showed that DWI provided enough resolution and accuracy to use DWI fibre directions in biomechanical analyses. One problem with musculoskeletal DWI is that it suffers from low signal to noise ratio. Levin et al. [71] described an algorithm for producing denoised muscle fibre fields from noisy diffusion tensor data as well as its preliminary validation. The algorithm performs denoising of the vector field simultaneously with its extraction from the noisy tensor field. This allows the vector field reconstruction to be constrained by the architectural properties of skeletal muscles. Another similar technique in the field of neuroradiology is DWI, which reflects both the diffusion of water molecules and circulation at the capillary level within an organ [72]. These small movements are quantified by a coefficient termed the apparent diffusion coefficient (ADC). Yanagisawa et al. [73] reported their methods to determine ADC differences in skeletal muscles between genders, age groups and muscles in the ankle dorsiflexors of 116 subjects and the erector spinae muscles of 86 subjects.

The steps we have adopted for constructions of fibre vector estimates from DWI are summarised in figure 3-1 to 3-8 in this chapter.
Figure 3-1: Signal intensity for each pixel from different diffusion directions. In the figure we have six directions plus one without diffusion. Note that each image has subtle variations in greyscale due to the different imaging directions.

3.2 THEORY

Stejskal and Tanner devised the well-known field gradient pulse method for DWI and proposed the relation in Equation 3-1 [74]. This method relates the signal intensity, $S$, to the diffusion tensor, $D$, through

$$S_k = S_0 e^{-b g_k^T D g_k},$$

(3-1)

where $S_0$ is the signal intensity without diffusion weighting (similar to a normal T1-MRI), $g$ is the diffusion direction, $b$ is a coefficient that controls the amount of diffusion allowed and $k$ is the number of gradient directions. It should be noted that parameter $b$ is both anatomically-specific and direction-specific and has units of s/mm$^2$. For example, $b$ is typically 1000 for neuronal imaging (in the brain) [75] and for muscle we found that a $b$ value of 500 was suitable and we assumed the same $b$ value in all directions. This may also be machine-specific (Siemens versus GE). Secondly, $k$, needs a minimum of six directions in order to resolve the six symmetric components of the diffusion tensor as illustrated in Figure 3-1 but more directions allows for better estimation via least
squares fitting (as used in this thesis). Therefore, by resolving the Stejskal-Tanner equation for each voxel in the data set, we can compute the diffusion tensor field. Figure 3-1 illustrates the setup used at the Centre for Advanced MRI (CAMRI) at Auckland University, showing the animal specific MRI bore with rabbit rig, MRI global coordinate system and 6 representative images from 6 directions, plus the reference image (no diffusion).

In order to resolve the redundancy of the diffusion tensor, where we have more imaging directions than we need (minimum of 6 for a symmetric tensor) we adopt least squares fitting which has been used in numerous previous studies [76-79].

Starting from the Stejskal Tanner relation in Equation 3-1, this is normalised by the reference signal intensity, $S_0$, leading to:

$$\frac{S_k}{S_0} = e^{-b g_k^T D g_k}.$$  \hspace{1cm} (3-2)

Following this, the log is taken of both sides leading to the form commonly presented in texts:

$$\ln\left(\frac{S_k}{S_0}\right) = -b g_k^T D g_k.$$  \hspace{1cm} (3-3)

Defining vector $g_k = (g_{1k}, g_{2k}, g_{3k})$, the imaging vector in the $k^{th}$ direction, the right hand side of Eq. 3-3 may be expanded as:

$$-b g_k^T D g_k = -b( D_1 g_{1k}^2 + 2D_2 g_{1k} g_{2k} + 2D_3 g_{1k} g_{3k} +$$
$$D_4 g_{2k}^2 + 2D_5 g_{2k} g_{3k} + D_6 g_{3k}^2 )$$  \hspace{1cm} (3-4)
This may be further expressed in summation form as:

\[-bg_k^T Dg_k = \sum_{k=1}^{N} \sum_{j=1}^{6} W_{kj} T_j,\]  

(3-5)

where the matrix of weights $W$, which has six columns and $N$ rows, where $N$ is the number of imaging directions is given by:

\[W_{kj} = b[g_{1k}^2 2g_{1k}g_{2k} 2g_{1k}g_{3k} g_{2k}^2 2g_{2k}g_{3k} g_{3k}^2],\]  

(3-6)

These weights multiply the unknown vector $T$, comprising the six desired tensor components for each voxel and are given by:

\[T_j = D_j,\]  

(3-7)

where $j$ is the number of unknowns (six in this case). Note that in the case where $N$ is also six, $W$ is a square matrix and we have a unique solution. However, typically $N$ is 20 directions or more.

Hence, Equation 3-3, solved for $N$ directions becomes:

\[\sum_{k=1}^{N} \ln \left( \frac{S_k}{S_0} \right) = \sum_{k=1}^{N} \sum_{j=1}^{6} W_{kj} T_j,\]  

(3-8)

Moving all terms to the left hand side the aim is now to find $T$ that satisfies:

\[\sum_{k=1}^{N} \ln \left( \frac{S_k}{S_0} \right) - \sum_{k=1}^{N} \sum_{j=1}^{6} W_{kj} T_j = 0,\]  

(3-9)

This can be reformulated as a least squares function with the objective to minimise $F(T)$ where:

\[F(T) = \sum_{k=1}^{N} \left[ \ln \left( \frac{S_k}{S_0} \right) - \sum_{j=1}^{6} W_{kj} T_j \right]^2,\]  

(3-10)
This is minimised by differentiating \( F(T) \) with respect to the unknown \( T \):

\[
\frac{dF(T)}{dT_j} = 2 \sum_{k=1}^{N} \left[ \ln \left( \frac{S_k}{S_0} \right) - \sum_{j=1}^{6} W_{kj} T_j \right] (-1)W_{kj} = 0.
\]

Since the non-trivial solution is desired as \( W_{kj} \neq 0 \), the solution is simply to solve for:

\[
\sum_{k=1}^{N} \left( \ln \frac{S_k}{S_0} - \sum_{j=1}^{6} W_{kj} T_j \right) = 0.
\]

Rearranging to make \( T \) the subject we solve the following system for each unknown component \( j \) of the Diffusion Tensor (where \( j \) is 1 to 6):

\[
T_j = \sum_{k=1}^{N} \left( W_{kj} \right)^{-1} \times \ln \frac{S_k}{S_0}.
\]

This equation was coded in Matlab and can be found in Appendix A.

Once the diffusion tensor, \( D \), is computed it needs to be diagonalised into three eigenvectors and three eigenvalues. This can be represented graphically by an ellipsoid as shown in Figure 3-2. Each voxel is represented by an ellipsoid, where we assume the largest axis (or eigenvector) is aligned with the major direction of fluid diffusion in the tissue, which in turn occurs primarily through fibres. Hence, the largest axis is assumed to be the fibre direction.
Each voxel can be muscle, fat, other soft tissues or background. The next step in this modelling pipeline was firstly to remove the background (white noise) from the actual tissue; and secondly isolate tissue that is likely to be muscle only. A Matlab script was written to loop over each voxel and filter the data (Appendix A). Firstly, we identified a background threshold value of 30 for the DWI greyscale intensities (0 indicates inclusion all raw values). Therefore, voxels that had grey scale values greater than 30 were retained from the final data set. Figure 3-3 shows filtered DWI voxel data after background thresholding at three different levels including (left) 30, (middle) 50 and (right) 90. Note that thresholding the data higher than 30 was more sparse in the muscle belly but below 30 the data was highly noisy. Hence, a value of 30 was chosen to be suitable for this work.
In the second stage we used the fractional anisotropy (FA) factor [80] to isolate out tissue that is most likely muscle (striated with fibres) and filter out tissue that is most likely fat and water (have less dominant fibres). The FA measure in 3D is given by

\[ FA = \sqrt{\frac{1}{2} \left( \frac{1}{\lambda_1^2} + \frac{1}{\lambda_2^2} + \frac{1}{\lambda_3^2} \right) \left( (\lambda_3 - \lambda_2)^2 + (\lambda_2 - \lambda_1)^2 + (\lambda_1 - \lambda_3)^2 \right) \}, \]  

where \( \lambda_1 \) to \( \lambda_3 \) are the three eigenvectors. This ensures that \( FA \) is a dimensionless measure between 0 and 1; where a zero value means that the tensor is represented by a sphere (where there is no dominant diffusion direction) and 1 means that the ellipsoid is a tube (which is the upper limit and has the maximum possible diffusion direction). Figure 3-4 shows filtered DWI voxel data after \( FA \) thresholding at two different levels including (left) 0.3 and (right) 0.9. Note that values closer to the
maximum of 1 enforced the dominant fibre constraint measure very strictly leading to many muscle fibres being filtered out in the muscle belly; an unwanted condition. Values closer to 0.3 gave a good balance between useful fibre information and filtering out much of the unwanted tissue data. It was noted that values closer to 0 permitted too much data outside of the muscle boundary. Hence, for this thesis we kept voxels that were greater than 0.3, which was chosen by visual inspection and used consistently for all data sets.

Figure 3-4: Filtered diffusion weighted imaging voxel data after Fractional Anisotropy (FA) thresholding at (left) >0.3 and (right) >0.9.

The final step involves a process known as tractography [77], where the voxels which are now represented by ellipsoids are used to plot streamlines that trace the paths of the largest axes or eigenvectors. This is similar to plotting isobars on a weather plot. Figure 3-5 describes this where streamlines are used to follow the paths of the major axes (shown as red lines). Each voxel is now replaced by three eigenvectors, which are fitted to a FE field in the software CMISS\(^1\).

\(^1\)www.cmiss.org
The streamlines that represent the fibre directions are plotted to show the fibre distribution as a continuous field. The fitting process is the same as described in chapter 2, except now the geometry field is substituted with a fibre field. The results of fitting a rabbit muscle set of fibre vectors to streamlines is shown in figure 3-6. Streamlines is a graphic glyph using in CMGUI to show how a vector field moves from one element to the next, ideal for muscle fibres. When discrete fibre vectors are visualised as streamlines for the case of the rabbit Triceps Surae the bipennate nature of the muscle is much clearer.

Figure 3-5 shows the result of tractography, where the dominant directions at each pixel are joined up to create streamlines. These streamlines are like contours on a weather map and show the path of a fibre in the 3D continuum. These vectors are fitted to a field and then we convert them to fibre directions for a fibre field fit.
Figure 3-6: Example of fibre fitting from a rabbit Triceps Surae. Discrete DWI-based fibre vectors (left) are fitted to a FE field visualised as streamlines (right).

3.3 VALIDATION USING CELERY

For evaluation of the fibre estimation method using DWI, we developed a gold standard phantom made from celery which has a distinct parallel fibre configuration. Surprisingly, asparagus and spring onion have also been used by MR technicians for validating diffusion sequences, due to their well-defined fibrous characteristics. Figure 3-7 shows the development of the phantom made from a laser cut design generated in CAD (SolidWorks) and the resulting celery configuration using a water filled plastic container. In the phantom, we fixed the celery using known angles followed by filling the container with water. This was necessary as DWI requires a fluid medium and the pockets of air create artefacts.
Following the fibre fitting process, the resulting fibre vectors were aligned in the meshed celery geometries as shown in figure 3-8. We then compared this with the gold standard of parallel-based fibres to work out the vector angle error with a simple cosine rule shown in equation 3-14:

$$\cos \theta_e = \frac{V_G \cdot V_{DWI}}{\|V_G\| \|V_{DWI}\|},$$  

(3-14)

where $V_G$ and $V_{DWI}$ are the fibre vectors for the celery gold standard and DWI estimates, respectively and $\theta_e$ is the angle error between these two vectors. This error is plotted as a field in figure 3-8 (right). It was found that our modelling pipeline had a maximum error of $4.6^\circ$, which occurred on the outside of the celery that was closest to the boundary of the container where there was less water and small air pockets despite our best attempts at completely filling the container (further details are given in chapters 4 and 7).
Figure 3-8: (Left) DWI derived fibre vectors inside the celery geometries; (Middle) representative fibres from one celery geometry close-up; and (Right) fibre angle error fitted to the celery geometry.
This chapter introduces the design of two pieces of apparatus that were used in this thesis. Firstly, a rabbit rig, including three transducers, were designed to measure force in the rabbit Triceps Surae muscle. The details of the design, manufacturing and assembly are detailed in this chapter. Secondly, a celery phantom holder was designed to evaluate the accuracy of our DWI process. These devices were created specifically for this thesis and formed part of the pre-design work before the 2 main studies were commenced (see chapters 5 and 6).
4.1 CONSTRAINTS OF THE RABBIT RIG

One of the main focuses of this thesis was to measure the passive force in the rabbit Triceps Surae during dorsiflexion while also measuring the shape of the muscle. In order to do this, a custom rabbit rig suitable for MR Imaging, housed at the Auckland Medical School, CAMRI was built. This rig needed to satisfy the following criteria:

1. The rig should be strong enough to stretch the muscle during rigor mortis.

2. Adjustable for multiple sizes of rabbits up to 5 kg.

3. Operated by one person.

4. Non-ferrous to be MR Imaging compatible.

5. Fit within the bore of the 4.7 T animal MRI (CAMRI) while still allowing for adjustments in dorsiflexion angle.


4.1.1 FINAL DESIGN

The rig consisted of multiple parts that were either 3D printed from CAD designs in SolidWorks (Dassault Systems) or laser cut. Figure 4-1 shows the individual components that were manufactured for assembly. These key components included (i) the femur holder with twin screws; (ii) limb holder clamps; (iii) rig frame and, (iv) protractor. After multiple design iterations the final design is shown in Figure 4-2. The rig was made from a 3D printed plastic material (using a Dimension Elite 3D printer, housed at the Auckland Bioengineering Institute), which has a cured Young’s modulus strength of ~1 GPa and allowed for muscles under rigor mortis to be stretched. The plastic material was MRI compatible. In addition, the sliding tube was made from pine and the screws and protractor were also plastic. In addition, support beams were also set into the structure. The model was adjustable to multiples sizes of rabbits by including a sliding tube connecting the superior and inferior plates of the rig. This was adjusted manually to the rabbit and then fixed with
four screws. A circular handle was designed to rotate the rabbit hind limb into dorsiflexion. All these functions were easily controlled by a single operator.

Figure 4-3 shows the dimensions of the rig that were chosen to fit most rabbit sizes and also fit within the MRI bore. This design was contained by the magnet size shown in Figure 4-4. The magnet bore is 400 mm in diameter but once the gradient and RF coils are included, the bore size is significantly less. The largest RF coil available at CAMRI (which dictates the specimen size) has an inner diameter of 140 mm. The length of the active region of the coil (the region where signal can be detected) is 165 mm but the physical length is closer to 220 mm. This reduced the bore diameter size and length, which was what the rig was designed for. The dorsiflexion angle was easily read (without parallax error) to indicate the relative flexion with respect to the neutral position (Figure 4-5). A clear plastic sheet permitted the user to see the angle. The protractor was cut by laser cutter and engraved with angles from 0° to 90°.

Figure 4-1: Some the main components of the rabbit rig.
Figure 4-2: The final design.

Figure 4-3: Upper image: the dimensions of the rig and force driver from handle to table 3 in below. 118mm is the width and 463.25mm is the total length.
Figure 4-4: Animal MRI. 4.7 T magnet and 140 mm RF coil.
4.1.2 **CLAMPING THE RABBIT**

Following euthanasia the rabbit skin was flayed for the lower limb and the muscle exposed. The rabbit was placed onto the rig in a forward pose as shown in Figure 4-6. Two screws were inserted into the femur to fix the lower limb and prevent rigid movement of the bone. The tendon was exposed and clamped to the tendon holder shown in Figure 4-7. The transducer sensors were then placed into the sensor holder. One current limitation of the design was that the sensor was metallic and hence not MRI compatible. In order to solve this issue an identical plastic sensor was substituted into the holder during MRI scanning at the same angles at which the force was measured (0°, 15°, 30° and 45°). The force was measured previously following the passing of rigor mortis as a separate task at those same angles.
4.2 THE FORCE TRANSDUCER

The rig was integrated with a force transducer, which was designed and made specifically for this study from bought components. This was to keep within the project budget. The transducer
measured the force required to passively lengthen the muscle and tendon when the foot angle naturally changes. Capacitive sensor technology was used to detect the capacitance change and transfer the changes to the transducer. To measure the force applied to the muscle for this study, three transducers were designed as shown in Figure 4-8. The ranges of these transducers were 0.00098-9.80 N, 0.0098-68 N and 0.098-392 N. Capacitive sensors have numerous advantages which include: high-resolution, high sensitivity, low power consumption and the ability to be integrated with other circuits. Capacitors are thermally stable and have good stability against temperatures near zero. They also protect against scattered electric fields. Capacitive sensors are also widely used in applications such as pressure sensors, acceleration and humidity [81]. Capacitive changes in a capacitive sensor are dependent on three characteristics: the size of the plates, their distance apart and the coefficient of the dielectric between the plates. Intermediate circuits are used to convert the very small changes in sensor capacitance into usable values such as current, voltage, frequency, or pulse width [82]. When a force is applied to the sensor as shown in Figure 4-9, the gap between the electrodes of the sensor (parallel-plate of the capacitor) will alter and it causes changes of the capacitance of the sensor. A sensitive capacitor reader detects these and relates these to force changes. However, the changes are very small so in the circuit an ‘Op_Amp’ was added to amplify the output of the sensor. Further, capacitance changes in the sensor are hyperbolic and must be converted to linear. To convert changes in capacitance into a linear gradient, a capacitance detection circuit was used and details of this can be found in my previous Master’s thesis [83, 84].
Figure 4-8: Three transducers were designed for this study. The ranges are: 0.00098-9.80 N, 0.0098-68 N and 0.098-392 N.

Figure 4-9: Capacitor sensor. By changing the gap between the electrodes \( r \), capacitance will change \( \Delta C \).
4.3 **CELERY PHANTOM**

In Chapter 3 the development of DWI work in this thesis was presented and the accuracy was assessed by using a celery phantom. The details of this device are presented here. The phantom was made from acrylic sheets where the design was made using CAD in SolidWorks. The design was cut using a laser cutter (Figure 4-10) housed at the ABI and all parts were then assembled. The celery was required to be in a fluid medium; hence the device was housed in a plastic container. The size of the container was matched to the Animal MRI coil dimensions.

![Figure 4-10: The ABI laser cutter.](image)

Plants and fruits are widely used as phantoms to validate the accuracy of DWI. For example carrot [85], celery [86, 87] and asparagus [88]. The design of the phantom was inspired by previous DWI phantoms [86, 87, 89, 90]. The phantom had three plates and each plate had a row of holes at 45° angles (Figure 4-11). Celery was attached using cable ties. The celery was purchased on the day of the experiment to ensure maximum water content. We chose an angle of 45° to check this angle against the parallel bore direction. The celery was also placed at a 45° angle against one another.
Figure 4-11: Drawing of the celery phantom.

Figure 4-12: 3D image of the celery phantom.
Figure 4-13: Complete phantom where the celery was attached using cable ties.
5 A DIFFUSION WEIGHTED INFORMED MODEL OF THE RABBIT TRICEPS SURAE

This chapter is the first main study and output from this thesis. It presents the development of a continuum model of the rabbit Triceps Surae from Diffusion Weighted Imaging (DWI) and passive muscle force experiments. It is a reproduction of the following journal paper, which is in review:

5.1 ABSTRACT

The NZ white rabbit is the animal of choice for much experimental work due to its muscular frame; similar response to human diseases; and is one of the few mammals that have had their genome sequenced. However, computational models of rabbit muscle detailing fibre architecture are limited in the literature, especially the Triceps Surae, which has similar biomechanics and translatable findings to the human. This study presents a geometrical model of the rabbit Triceps Surae informed with DWI based fibres. Passive material properties are estimated using known muscle deformation inferred from MR imaging data and dorsiflexion force measured with a custom built rabbit rig and transducer. Muscle shape prediction is evaluated against a second rabbit. This study revealed that the Triceps Surae steady-state force post rigor is close to in vivo for small deformations but increases by a fixed ratio as the deformation increases and can be used to evaluate the passive behaviour of muscle. The fibre orientation within the muscle volume derived from DWI data significantly influences shape and mechanics during contraction. The presented Triceps Surae force and material properties may be used to inform the constitutive behaviour of rabbit models used to investigate pathology and musculotendon treatments that may be translated to the human condition.

5.2 INTRODUCTION

The New Zealand white rabbit is the animal of choice for both experimental and computational work. It has a similar response to human diseases; and is one of the few mammals to have had their genome sequenced [91]. The rabbit’s lower hind limb has four major muscles similar to the human, namely, the gastrocnemius (medial and lateral heads), soleus and tibialis anterior. However, unlike humans, the rabbit hind limb has only one supporting bone, the tibia. For this study we have focussed on the Triceps Surae muscle, which originates in the femoral condyles and inserts in the calcaneus (heel bone) via the Achilles tendon. Computational models of rabbit muscle detailing fibre architecture are limited in the literature, especially the Triceps Surae, which has
similar mechanics and translatable findings to the human. Rabbits are also used as models for investigating joint weakness leading to osteoarthritis (OA) by Herzog and Longino [17].

There are rigid body models of the NZ white rabbit with line muscle actuators [16] but these do not evaluate the influence of 3D fibrous architecture on spatially varying muscle stress and strain. The macro level material properties of the rabbit have been shown to be a scalable version of the sarcomeres in rabbit hind limb muscles [19]. Passive muscle force has previously been measured using a fibre optic pressure transducer in the tibialis anterior of 12 NZ rabbits [18] and for static and dynamic loading in both female and male rabbits [24]. Failure sites and peak tensile forces of the composite triceps surae in 24 NZ white rabbits were identified during passive extension [92]. One of the only studies in the literature that reports the post-mortem effects in NZ white rabbits was Van Ee and colleagues [20]. They showed a distinct steady state post rigor phase which was repeatable and the failure strain was unchanged from in vivo.

DWI [32] is an MR Imaging sequence used to map the architecture of fibres in muscle [30, 31] and neuronal pathways in cerebral tissue [26, 27]. The concept is that the dominant diffusion direction of fluid relates to the fibre direction in muscle. DWI has been used to examine the diffusive properties of adjacent muscles at rest in the human gastrocnemius [65] and to identify properties of healthy and damaged muscles [67, 68]. Van Donkelaar et al. [33] used DWI to obtain geometric information for numerical simulations of skeletal muscle contractions. Their results showed that DWI provided enough resolution and accuracy to use DWI based fibre directions in biomechanical analyses.

One of the challenges present in this study was that muscle enters rigor and so material properties are changing dynamically following euthanasia. This makes it difficult to measure muscle force over four positions and have the muscle stable for MR imaging. Van Ee et al. [20] reported the post-mortem effects in twelve NZ white rabbits following euthanasia and we used this information in the design of our experiments as we could not measure force and MR image while the muscle was going through changes due to rigor mortis. Following euthanasia, Van Ee and
colleagues exposed the rabbit tibialis anterior muscle by an anterior midline incision from the knee to the ankle. All supporting connective tissue and fascia were removed. In that study, they performed an elongation test every hour post-mortem up to 18 hours and then every 2 hours until 72 hours after death. The key point from this study was that after rigor passed the failure strain was relatively constant and not much higher than \textit{in vivo}.

The objectives of this study were to firstly present measured passive Triceps Surae force in the NZ white rabbit from death throughout rigor mortis to post-rigor using a novel rabbit rig. This information is used to relate the post-rigor to \textit{in vivo} force and give a surrogate estimate of the active muscle contraction (the peak during rigor mortis). Secondly we present optimised passive material properties determined from force measurement and MRI shape accounting for fibrous muscle architecture. We also evaluate the error in the system using a celery-based phantom. Thirdly, we present the influence that DW fibres have on muscle contractile shape and mechanics.

5.3 MATERIALS AND METHODS

5.3.1 MR COMPATIBLE RABBIT RIG

A custom rabbit rig was designed in SolidWorks (Dassault Systems) and is shown in Figure 5-1. The key components of the rig included 4 clamps to constrain the limbs, a variable shaft to control rabbit length, a variable angle drive to control hind limb dorsiflexion (which was measured using a protractor), a ring with screws to rigidly fix the femur bone and two tendon clamps, which integrated with a custom built force transducer. The rig was printed using a 3D printer (Stratasys®) at a resolution of 100 microns using ABSplus material. The key design features of this rabbit rig were the abilities to hold multiple size rabbits up to 5 kg, able to be operated by a single person, MR compatible, strong enough to cope with muscle rigor and designed to fit in the 4.7 T animal MRI housed at the Centre for Advanced MRI (CAMRI) at Auckland University.
Figure 5-1: MRI compatible rabbit rig used to dorsiflex the rabbit hind limb within the MRI bore and measure Triceps Surae tendon force for each foot position.

5.3.2 Force Transducer

Two capacitive sensors were designed to measure two distinct ranges of force; 0.00098 - 9.80 N and 0.0098 – 68 N, respectively and cope with rabbits ranging from 1.5 kg to 4.0 kg, especially during muscle rigor. Due to metallic components in the transducer, a dummy MR compatible clamp was used during the MR imaging.

5.4 Rabbit Euthanasia

Animal ethics approval was received for 3 years from the University of Auckland animal ethics committee (#T958) and training in rabbit handling and euthanasia was undertaken. Two rabbits were euthanised, 3.8 kg and 4.2 kg in weight, respectively, using 2 ml of pentobarbital via injection into the ear vein and monitored until cessation of all signs of life. Upon cessation the rabbit lower limb was flayed and the triceps surae muscle complex was exposed as shown in Figure 5-2. The lower section of the tendon (inserting into the calcaneus) was cut and the transducer was
inserted by connecting the two sections of the tendon. The rabbit was positioned in the rig and screws were inserted into the femur to fix the rabbit and the foot placed in the adjustable leg clamp.

5.5 **RABBIT PASSIVE TRICEPS SURAE FORCE**

In order to avoid the dynamic changing stiffness of muscle due to rigor we adopted an approach whereby rigor mortis was allowed to pass and then we measured the steady state passive material properties. This idea was observed from the study of Van Ee et al. [20] where there was a well-defined ratio between the post rigor and *in vivo* passive states. The passive Triceps Surae force via the Achilles tendon was measured every hour for the first 5 hours followed by every 2 hours up to 72 hours. Each measurement was repeated 3 times and the result averaged. This process was repeated for four dorsiflexion positions (15°, 30°, 45° and 60°). After 72 hours, rigor passed and the muscle behaviour was stable and related to the *in vivo* force by a well-defined ratio.
5.6 **RABBIT GEOMETRIC MODEL**

Following measurement of passive force the geometries of the rabbit Triceps Surae muscle were imaged using T1-weighted, MR imaging on a 4.7 T Siemens’ machine with settings given in Table 5-1. The goal of this step was to create 4 gold standard experimental muscle boundaries to inform the muscle parameter optimisation. After imaging the rabbit in 4 positions (Figures 5-2(I) – 5-2(V)), we identified the muscle and bone boundaries for segmentation and data creation. These images were loaded into the Zinc digitiser, developed as part of CMGUI (www.cmiss.org) and shown in Figure 5-2(VI). The MR images were segmented and a 3D cloud of data points were created for each muscle and the bone of the rabbit lower limb. These data points were then used to create subject-specific finite element models of the muscles using high-order cubic Hermite elements. The mesh creation steps are outlined in Figure 5-3. (i) From the cloud of points (yellow data) a subset was chosen as surface nodes (red points); (ii) An initial trilinear mesh was then built on this that captured the primary shape; (iii) Using the least squares fitting procedure developed in CMISS we fitted the cubic Hermite elements [93] to a root mean square (RMS) error of less than 2 mm. These steps were repeated for all 4 deformed positions of the muscle plus one relaxed position without any force.

<table>
<thead>
<tr>
<th>Siemen’s 4.7 T MRI T1 settings for muscle boundary geometries.</th>
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<tbody>
<tr>
<td><strong>Echo Time (TE)</strong></td>
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<tr>
<td><strong>Repeat Time (TR)</strong></td>
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<tr>
<td><strong>Number of Excitations (NEX)</strong></td>
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<tr>
<td><strong>Resolution</strong></td>
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5.7 DIFFUSION WEIGHTED FIBRE FIELD

The rabbit Triceps Surae was re-scanned using a DWI sequence to track the water migration in the muscle and estimate the fibre architecture. The DWI sequence was run with the parameters in Table 5-2, specifically, we used a b-value of 500 s.mm$^{-2}$ for skeletal muscle and 20 gradient directions plus one reference sequence with no diffusion.

<table>
<thead>
<tr>
<th>Table 5-2: Siemen’s 4.7 T MRI DWI settings for fibre information</th>
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<tbody>
<tr>
<td>Echo Time (TE)</td>
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<tr>
<td>Repeat Time (TR)</td>
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<td>Number of Excitations (NEX)</td>
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<td>Number of gradient Directions</td>
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<tr>
<td>b-Value</td>
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<tr>
<td>Resolution</td>
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</table>

The diffusion at each voxel was solved using the Stejskal-Tanner relation [74], which relates the signal intensity, $S$, to the diffusion tensor, $D$, through:
Diffusion Weighted Fibre Field

\[ S_k = S_0 e^{b g_k^T D g_k}, \]  

(5-1)

where \( S_0 \) is the signal intensity without the diffusion weighting, \( S_k \) is the signal intensity in the \( k \)th direction, \( g_k \) is the \( k \)th direction vector, \( b \) is a parameter that controls the amount of diffusion allowed (with \( b=0 \) being no diffusion) and \( D \) is the symmetric diffusion tensor which has six unique components to solve for at each image voxel. As we have 20 directions, we adopt the least squares approach to solve for \( D \) and this form is given in equation 5-2 as:

\[ D_j = \sum_{k=1}^{N} \left( W_{kj} \right)^{-1} \times \ln \frac{S_k}{S_0}, \]

(5-2)

where \( D \) is solved for each of the six components \((j=1\) to \(6)\) and \( W \) is the matrix of weights, which has six columns for each direction \( k \) and is given in equation 5-3 as:

\[ W_{kj} = b \left[ g_{1k}^2 \quad 2g_{1k}g_{2k} \quad 2g_{1k}g_{3k} \quad g_{2k}^2 \quad 2g_{2k}g_{3k} \quad g_{3k}^2 \right]. \]

(5-3)

Since each voxel can be muscle, fat, or background, a background grey threshold of 30 (range from 0 to 255) is used to filter white noise. The muscle voxels only within the muscle boundary were then isolated. The computed diffusion tensor \((D)\) for each muscle voxel was then diagonalised into three eigenvectors \((\lambda_1 \) to \( \lambda_3)\) where \( \lambda_1, \) the largest eigenvector, was assumed to align with fibre orientation. Secondly we used the fractional anisotropy (FA) factor [80] to isolate out tissue that is more likely muscle (striated with fibres) and filter out tissue that is most likely fat or water (have less dominant fibres). The FA measures in 3D is given by equation 5-4 as:

\[ FA = \sqrt[\frac{1}{2}]{\frac{\varnothing(\lambda_1 - \lambda_2)^2 + (\lambda_2 - \lambda_3)^2 + (\lambda_3 - \lambda_1)^2}{\sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}}, \]

(5-4)

FA is a dimensionless measure between 0 and 1; where a zero value means that the tensor is represented by a sphere (where there is no dominant diffusion direction) and 1 means that the ellipsoid is a tube (which is the upper limit and has the maximum possible diffusion direction). A
FA of 0.3 or greater gave a good balance between useful fibre information and filtering out much of the unwanted background tissue data. The fibre vector information was then fitted to the muscle geometry as a fibre field using the same least squares fitting technique. To evaluate the error in the DWI pipeline a phantom made of celery was used to assess the error in fibre estimation.

5.8 CONSTITUTIVE LAW FITTING

The reference pose geometry was strained using a finite elastic simulation in CMISS using musculotendon force measured via the transducer. The governing mechanics concerning finite elasticity and the Hill type model used for active contraction is given in Appendix A. Specifically; the peak passive force measured during rigor mortis was used as a surrogate of the maximum active contractile force. A structurally based constitutive law previously used for passive cardiac and skeletal muscle, the ‘pole-zero’ relation [94], was adopted and is defined in Equation 5-5 as:

$$W = k_{\alpha\beta} \frac{E_{\alpha\beta}^2}{|a_{\alpha\beta} - E_{\alpha\beta}|^{b_{\alpha\beta}}}$$  \hspace{1cm} (5-5)

where the strain energy density function, $W$ is defined by a asymptote function with $k_{\alpha\beta}$ the scaling function, $b_{\alpha\beta}$ curvature control, $a_{\alpha\beta}$ a strain limiting pole and $E_{\alpha\beta}$ the Green’s strain components. The model was treated as transversely isotropic with the fibre direction $\alpha=\beta=1$ aligned to the DWI fibre direction. After choosing an initial guess for the pole-zero parameters we used the ‘fmincon’ function in the Matlab Optimisation Toolbox [95] to optimally choose parameters that minimised the difference between the mechanically deformed muscle geometry and the ‘gold standard’ geometric shape captured from MRI for each position. We optimised the pole ($a_{\alpha\beta}$) and scaling ($k_{\alpha\beta}$) parameters and fixed the curvature ($b_{\alpha\beta}$) for the fibre and transverse directions. The curvature was set to 1.0 based on previous experience with cardiac [94] and skeletal tissues [96]. We set a bound on the solution space for $a_{\alpha\beta}$ as 0.01 to 5.0 and for $k_{\alpha\beta}$ as 0.01 to 0.7 MPa. An initial guess was obtained from trials based on previous skeletal muscles [61]. This was performed until the RMS
error between the computationally deformed muscle (red) and MR derived muscle (gold) was less than 2 mm (Figure 5-4).

![Figure 5-4](image)

**Figure 5-4**: Relaxed and four MR derived muscle deformations; and muscle optimised (red) to match experimental gold standard (gold) using ‘pole zero’ constitutive law.

5.9 **RESULTS**

The passive Triceps Surae force over four dorsiflexion positions (P1 to P4) are presented in Figure 5-5. The shape of the passive force curve for both rabbits following the euthanasia point (EP) was characterised by a sharp rise to a peak force followed by a sharp decline and finally steady state after ~36 hours. Specifically, following euthanasia a short period of steady muscle properties lasted for ~3.6 hours before the rapid rigor onset point (ROP). The rate of increase towards peak rigor force (PR) increased with dorsiflexion position (P1 to P4). Rates of force increase from ROP to PR were 0.34 N/h for 15° dorsiflexion, 0.62 N/h for 30°, 1.18 N/h for 45° and 2.3 N/h for 60°. Steady state (SS) passive force was achieved after 36 hours and the average passive force at steady state was 0.8 N, 2.1 N, 7.9 N and 19.0 N from P1 to P4 for the average rabbit weight of 4 kg. The passive steady state (SS) rigor force was 10% larger than *in vivo* at P1 and 14%, 75% and 108% larger at positions P2 to P4, respectively. These ratios are used to relate the post-rigor force with the *in vivo* force just prior to death. The peak values were used as a surrogate for maximum contraction
in the model and were 4.8 N, 8.7 N, 20.8 N and 39.5 N from P1 to P4 for the average rabbit weight of 4 kg. The increase in peak force is influenced by the dorsiflexion angle and hence musculotendon length.

Figure 5-5: The average force in all positions for two rabbits (mean ± 1 standard deviation). The stages were EP (euthanised point), ROP (rapid rigor onset point), PR (peak rigor point), RD (steady rigor decrease point), SS (steady state point).

The optimal pole-zero model parameters for the rabbit Triceps Surae muscle assuming transversely isotropic behaviour are presented in Table 5-3. The curvature $b_{ij}$ was set to 1.0 for all three directions. We found that in the fibre direction, the optimised pole ($a_{11}$) and scaling ($k_{11}$) were 1.0 and 0.01 MPa, respectively. In the transverse directions the optimised pole ($a_{22} & a_{33}$) and scaling ($k_{22} & k_{33}$) were 0.097 and 0.221 MPa, respectively. This produced an overall RMS error of 1.49 mm across all four positions (Table 5-4, column 1). The constitutive law was evaluated through prediction of passive shape of a second rabbit across all four positions. We used the optimised constitute parameters from the first rabbit to predict the shape of a second rabbit of
similar size by loading it with the rabbit rig measured force. The average RMS error between the predicted muscle shape and MRI measured shape for all positions was 1.65 mm for the second rabbit (Table 5-4, column 2).

Table 5-3: Optimised ‘pole-zero’ material parameters satisfying all deformed positions including DWI pennation fibres.

<table>
<thead>
<tr>
<th></th>
<th>Coefficient</th>
<th>Pole</th>
<th>Curvature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibre</td>
<td>0.01</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sheet</td>
<td>0.221</td>
<td>0.097</td>
<td>1</td>
</tr>
<tr>
<td>Sheet-normal</td>
<td>0.221</td>
<td>0.097</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 5-4: Root Mean Square error of predicted muscle shape versus experimental gold standard across all four deformed positions for the first and second rabbit.

<table>
<thead>
<tr>
<th>Position</th>
<th>First Rabbit</th>
<th>Second Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>1.58</td>
<td>1.47</td>
</tr>
<tr>
<td>P2</td>
<td>1.70</td>
<td>2.37</td>
</tr>
<tr>
<td>P3</td>
<td>1.15</td>
<td>1.70</td>
</tr>
<tr>
<td>P4</td>
<td>1.54</td>
<td>1.05</td>
</tr>
<tr>
<td>Average</td>
<td>1.49</td>
<td>1.65</td>
</tr>
</tbody>
</table>

The influence of fibre orientation on predicted shape and mechanics is observed in Figures 5-6 and 5-7. Inclusion of DWI-based fibres produced a different shape during contraction, specifically; the muscle contracted more posteriorly and was directed inwards towards the central tendon as dictated by the fibre alignment. In contrast, the control model with parallel fibres simply moved superiorly and inferiorly. Both models conserved volume during deformation. The maximum error was up to 3.7 mm in shape. A perturbation of 10% in the fibre angles produced up to 1.3 mm change in surface RMS error. Strains in 3D are observed using the largest principal component vector of strain. In the DWI muscle model principal strain components are shown to align with the pennation fibre orientation and in the parallel model they are aligned with the assumed control parallel fibres. The DWI muscle also exhibits larger non-uniform strain on the medial head [61], which is the largest side of the gastrocnemius and has higher local contraction.
Figure 5-6: (Left) Fitted DWI fibres (gold) contrasted with no pennation based fibres. (Right) DWI contractile shape overlaid with no pennation contractile shape and effect of 10% perturbation in fibre angle (green/gold).

Figure 5-7: Muscle strain with and without DWI when in maximum muscle contraction. ε is the symbol for strain which is unitless.
For evaluation of the fibre estimation method using DWI, we developed a gold standard phantom made from celery which has a distinct parallel fibre configuration. In the phantom, we fixed the celery using known angles followed by filling the container fully with water. This was necessary because DWI needs a fluid medium and the pockets of air create artefacts. Following the fibre fitting process the resulting DWI fibre vectors were aligned in the meshed celery as shown in Figure 5-8. We then compared this with the gold standard parallel-based fibres to work out the vector angle error. A maximum error of 4.6º in fibre angle occurred on the outside of the celery phantom closest to the boundary of the container. The background threshold intensity (indexed from 0 to 256) and the white matter threshold taken as the FA (defined in equation 4) was used to minimise the fibre angle error. A background threshold greater than 90 combined with a white matter FA greater than 0.1 gave the lowest error across the celery geometry. The side closest to the water boundary had the highest error (shows in red). It was found that the error was most sensitive to the FA leading to high errors propagating towards the centre of the celery when the FA was increased.

![Figure 5-8: Phantom celery to validate the study.](image)

To evaluate, which parameters the model was most sensitive to we perturbed the pole zero parameters (scaling coefficient, pole and curvature) by 10% for each axis (fibre, sheet and sheet-normal) and the results are reported in Table 5-5. It was observed that the model was most sensitive in all 3 directions to the strain limiting pole ($a_{\alpha\beta}$) but this was most evident in the sheet and sheet-
normal directions. A perturbation of 10% in the pole parameter produced a geometric RMS error in muscle shape of 0.27%, 11.18% and 14.15% for the fibre, sheet and sheet-normal directions, respectively. The second main error was due to changes in curvature, where a 10% perturbation in the curvature parameter produced a geometric RMS error in muscle shape of 1.67% and 0.97% for the sheet and sheet-normal directions, respectively. Note that the fibre direction was numerically indifferent to this parameter. The scaling parameter produced errors of less than 0.65% across all the directions (excluding the fibre orientation).

Table 5.5: Sensitivity test for pole-zero parameters

<table>
<thead>
<tr>
<th>Sensitivity Test</th>
<th>Optimal</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>k_{11} + 10%</td>
<td></td>
<td>0.00%</td>
</tr>
<tr>
<td>a_{11} + 10%</td>
<td></td>
<td>0.27%</td>
</tr>
<tr>
<td>b_{11} + 10%</td>
<td></td>
<td>0.0%</td>
</tr>
<tr>
<td>k_{22} + 10%</td>
<td></td>
<td>0.65%</td>
</tr>
<tr>
<td>a_{22} + 10%</td>
<td></td>
<td>11.18%</td>
</tr>
<tr>
<td>b_{22} + 10%</td>
<td></td>
<td>1.67%</td>
</tr>
<tr>
<td>k_{33} + 10%</td>
<td></td>
<td>0.38%</td>
</tr>
<tr>
<td>a_{33} + 10%</td>
<td></td>
<td>14.15%</td>
</tr>
<tr>
<td>b_{33} + 10%</td>
<td></td>
<td>0.97%</td>
</tr>
</tbody>
</table>

5.10 DISCUSSION

We have presented a framework for estimating material parameters of muscle (demonstrated for the rabbit) from MRI shape and measured musculotendon force using a custom rig. The 3D printed rig was shown to be both sufficient in handling the muscle in rigor and be non-ferrous and MRI compatible. Passive muscle shapes integrated with measured musculotendon force were shown to be suitable measures for optimising strain behaviour. We have demonstrated this by fitting a set of parameters to a muscle-based constitutive law previously used for cardiac and skeletal muscle; the pole zero law. It was shown DWI based fibres produce significantly different contractile shape, strain compared to parallel-fibre based models and the strain patterns were spatially non-uniform and aligned with the fibre orientation. Passive muscle constitutive parameters were optimised against shape and force and shown to predict the behaviour of a second rabbit of
similar size. A celery phantom was used to quantify a maximum error of 4.6° in our DWI fibre based angle estimation and modelling pipeline.

There are a number of limitations that should be considered when interpreting the results of this study. Firstly, it is well known the DWI has noise and the dominant eigenvector may not necessarily lie perfectly in the fibre orientation, however, we minimised these effects by averaging two DWI sequences and the surrounding voxels in 3D. Future improvements may include removing noise from the diffusion tensor to extract a vector field constrained by the architectural properties of skeletal muscles [71]. Secondly, the optimisation process was very sensitive to the initial guess, which had to be obtained from previous experiences with skeletal and cardiac muscle [97, 98]. The bounds place on the parameters ensured that the model converged numerically. Further, the constitutive law used in this study, the pole-zero, is a microstructural based law that is popular in cardiac and skeletal muscles as part of the Physiome Project [99]. However, it is known that other popular strain energy density functions are used, such as Mooney Rivlin, hence we have provided the raw strain energy density data as supplementary material for researchers to refit their own constitutive law parameters. Thirdly, our transducer was not MR compatible in this study, but there are MR specific transducers available in the market. If they were used in this presented rabbit rig then both shape and force measurement could be performed simultaneously without removing the rig from the MRI bore. However, the current study was best performed outside the MR as we measured the history of muscle force over 36 hours in order to determine a steady state phase and peak force used as a surrogate for maximum contraction.

A key finding from this study was that steady state rabbit muscle force post rigor is a estimated surrogate for the post mortem state, especially at low deformations. When the deformation is higher we found a consistent ratio that may be used to scale the force to the \textit{in vivo} state. The complete passive force curve during rigor was consistent with that reported by Van Ee et al. [20], however, in that study they did not consider different muscle lengths or provide a ratio between the passive and \textit{in vivo} force. Moreover, during the onset and passing of rigor, the results
exhibited a low standard deviation across both rabbits except during the descent phase. This region is highly variable and likely to be rabbit specific. The peak values during passive contraction were chosen as a surrogate for the maximum contraction in our computational model based on the idea that during rigor the actin-myosin filaments shorten in a similar manner to what happens during active muscle contraction. In live muscle, adenosine triphosphate (ATP) enters the fibres leading to muscle contraction. This is then pumped out leading to a relaxation of the cross-bridges. Following death there is no energy to pump out the ATP leading to an increased contracted state. Eventually the fibres breakdown which leads to relaxation [100]. We have used this idea to get a surrogate measure of the active contraction at peak rigor as some of the cross-bridge kinetics is the same up to peak rigor.

The presented ‘pole zero’ parameters are useful for researchers evaluating passive muscle behaviour in rabbits and also for tendinopathy applications, typically assessed in rabbits [101, 102]. It was shown that the pole \( a_{\alpha\beta} \), which is the limit on the Green’s strain, was the most influential parameter through a sensitivity analysis, especially in the sheet and sheet-normal directions. Specifically, our value for \( a_{11} \) (fibre direction) was set to 1.0 based on previous skeletal modelling reported by Fernandez et al. [97], where the human rectus femoris muscle was studied. Following optimisation we found that the pole in the transverse directions were \( a_{22} = a_{33} = 0.097 \sim 0.1 \), which was slightly lower and stiffer than those reported in Fernandez et al. [97], which used a value of \( a_{22} = a_{33} = 0.4 \). This is likely due to the human rectus femoris muscle being more compliant and less stiff than the rabbit Triceps Surae. The curvature \( b_{\alpha\beta} \) was set to 1.0 for numerical convergence stability for all 3 directions.

Inclusion of DWI fibres produced significantly different surface shape compared to parallel fibres, specifically on the medial head with differences up to 3.7 mm on the medial side. This side also accounted for larger non-uniform variations in strain. While the strain increased along the length of the muscle in both cases, the DWI informed muscle showed increased variation medial to lateral (the muscle width). This pattern of non-uniform variation attributed to fibre orientation has also been reported by Blemker et al. [57]. The largest principal components of the strain aligned
with the fibre direction highlights that the muscle is bulging in the muscle belly differently along the length, compared to the parallel fibre-based muscle. A celery gold standard with known parallel fibre orientation quantified a maximum error of 4.6º closest to the air water boundary. In practice, most of the useful information is well within the water volume and we would expect the error to be much lower. Further, a background threshold greater than 90 combined with a white matter FA greater than 0.1 gave the lowest error across the celery geometry. In practice, we found that a background threshold greater than 30 was more suitable as 90 typically produced a sparse data set and removed important fibre diffusion information in the rabbit muscle belly.

5.11 ACKNOWLEDGEMENTS

The authors would like acknowledge the financial assistance of an Aotearoa Bioengineering fellowship from the Robertson Foundation awarded to J Fernandez and an MBIE grant awarded to K Mithraratne (UOAX0712 and UOAX1006). We also kindly acknowledge Dr Beau Pontré and Ms Rachel Heron from the Centre for Advanced MRI for their help with imaging.
A diffusion weighted informed model of the rabbit Triceps Surae: Appendix

The weak form of the governing equation used for solving the finite elastic mechanics is given by:

$$\int_{V_o} \frac{1}{J} T^{\alpha\beta} F'_{\beta} \frac{\partial \delta u_j}{\partial V_\alpha} dV_o = \int_{S_c} f_c \delta u_j^c dS_c,$$  \hspace{1cm} (PA1)

since the Triceps Surae muscle is undergoing large strain (greater than 10%). $V_o$ is the un-deformed volume and $S_c$ is the surface in contact used to account for the interaction between muscles and the tibia. $\delta u_j$ is the virtual displacement, $\delta u_j^c$ is the variation of the contact gap and $F'_{\beta} = \delta x_j / \partial v_\beta$ is the deformation gradient tensor which maps between the deformed spatial coordinates $x_j$ and material coordinates, $v_\beta$. The Jacobian, $J$, is the determinant of the deformation gradient tensor, $F$ and $f_c$ is the frictionless contact force, which is implemented using a penalty based method with the complete details described in Fernandez et al. [61, 103]. $T^{\alpha\beta}$ is the 2nd Piola-Kirchoff stress tensor:

$$T^{\alpha\beta} = \frac{\partial W}{\partial E_{\alpha\beta}} + p a^{\alpha\beta}_\nu + T_0 \delta_1^\alpha \delta_1^\beta,$$ \hspace{1cm} (PA2)

and is defined with respect to the un-deformed curvilinear material coordinate system $v_\alpha$. $W$ is a strain energy density function and $E_{\alpha\beta}$ are the Green-Lagrange strain components. The hydrostatic pressure, $p$, arises in order to satisfy volume conservation and $T_0$ is the second Piola Kirchoff equivalent of the Cauchy active stress (muscle contraction). The contravariant metric tensor $a^{\alpha\beta}_\nu = \partial v_\alpha / \partial x_\lambda \cdot \partial v_\beta / \partial x_\lambda$ is the inverse of the right Cauchy deformation tensor (covariant metric tensor). For muscle contraction we used the model of Hunter [98] and added a contractile force in the DWI informed fibre direction to simulate muscle action given by:

$$\sigma_0(\lambda, C_{a_{\text{actm}}}) = \frac{(C_{a_{\text{actm}}} [Ca^{2+}]_{\text{max}})^h}{(C_{a_{\text{actm}}} [Ca^{2+}]_{\text{max}})^h + (c_{50})^h} \sigma_{\text{ref}} [1 + \beta(\lambda - 1)],$$  \hspace{1cm} (PA3)
where $\sigma_0$ is the active tension added to the fibre direction and given by the calcium-tension derived from the ‘fading-memory’ model which is based on the Hill type model \cite{104, 105}. $Ca_{acm}$ is the level of activation (non-dimensional calcium value), $\lambda$ is the sarcomere stretch length, $[Ca^{2+}]_{\text{max}}$ is the intracellular calcium concentration for maximum activation, $c_{50}$ is the concentration at which isometric tension is 50\% of its maximum, $h$ is the Hill coefficient, $T_{\text{ref}}$ is the active isometric tension when $\lambda = 1$ and $\beta$ is the slope parameter.
5.12 **KEY FINDINGS FROM CHAPTER 5**

- The steady state behaviour post-rigor is a good surrogate for material behaviour post-mortem by being related through a ratio. These ratios were determined to be 0.8, 2.1, 7.9 and 19 for dorsiflexion positions of 15, 30, 45 and 60 degrees over two rabbits. However, for future work we need to repeat the experiment on additional rabbits.

- The muscle stiffened over 18 hours and then reached steady state after 36 hours. The peak stiffness at 18 hours was used as a surrogate measure for maximum contraction.

- The ideal MRI parameter settings for the Siemens 4.7 T Diffusion Weighted imaging sequence in skeletal muscle were an Echo Time (TE) of 40 ms; Repeat Time (TR) of 3000 ms; Number of excitations (NEX) of 2; 20 gradient directions + 1 no diffusion and a b-value of 500 s/mm².

- Material properties derived from MR shape and measured force provide good parameters for optimising material behaviour, useful for computational models. The ‘pole zero’ constitutive law, previously used for cardiac tissue, is a suitable law for skeletal muscle as well.

- DWI significantly influences the mechanics predicted continuum shape and strain compared to parallel fibre based models. The maximum principal strain aligns with the fibre orientation derived from DWI.

- The larger strain intensity is on the medial side of the gastrocnemius, which is also the side with the largest head.

- Celery is an effective and suitable phantom material, due to its highly parallel fibres for determining errors in DWI.
This chapter is the second main study and output from this thesis. It presents the development of a continuum model of the human Triceps Surae from Ultrasound imaging and passive muscle force experiments. It is a reproduction of the following journal paper, which is in review:

6.1 Abstract

Muscle fibre structure characterises muscle function, which in turn plays a key role in computer simulation of muscle shape. In this study we use 3D Ultrasound from human Triceps Surae muscle to identify and map the fibre orientation and deformation during passive motion in four subjects. This fibre description is integrated into a representative muscle volume element using a free-form deformation technique to create a muscle primitive that deforms according to the embedded muscle fibres within. For each subject we computed passive force that was used to optimise the constitutive behaviour so that the known deformation matched this load. Each subject was fit to match deformation at 25%, 50%, 75% and 100% of muscle stretch. The subjects that exhibited a larger fibre pennation angle change during passive stretch were characterised best by a more compliant constitutive law. In contrast, subjects that had a more parallel fibre deformation, showed stiffer behaviour. A whole Triceps Surae muscle built from these muscle primitives exhibited a contractile shape that is similar to that observed in human Triceps Surae contraction. This shape was evaluated against the same muscle embedded with fibres derived from diffusion weighted magnetic resonance imaging and was in good agreement. Muscle principal strain was shown to align with fibre direction and was spatially non-uniform. These muscle primitives may be used as building blocks to build large muscle volumes for mechanics simulation, visualisation and medical education.

6.2 Introduction

Ultrasound is a real-time imaging modality used widely to assess size and pennation of muscles such as in the quadriceps of young and old women [36] and in vivo pennation angle in human quadriceps [41]. Specific muscle fibre behaviour has also been evaluated including the relationships between muscle fibre size and angle [37], changes in pennation with joint angle and torque [38], prediction of tibialis anterior pennation angle changes during dorsiflexion [39]; and the in vivo human gastrocnemius architecture during rest and isometric contraction [40] of which is the focus of this study.
Cadaveric measurements of gastrocnemius fibre length and pennation is one possible option. However, Martins et al. [44] showed that cadaveric gastrocnemius fibre measurements differed from in vivo Ultrasound data by exhibiting fibre length and pennation changes between rest and fully contracted states. Hence, the motivation for this study was to develop finite element (FE) informed muscles from in vivo Ultrasound data. Ultrasound is also extendable to dynamic tasks including walking and running for the gastrocnemius [46]. Fascicle length and pennation angle measured using ultrasound has shown high reproducibility in treadmill walking [106] and treadmill running [107]. This ensures that the errors in fibre fields used to inform computational models are likely to be significantly less than using such modalities as DWI where data is typically integrated with more background and white noise [108, 109].

Muscle fibre architecture has been reported as a key factor in how well continuum computer models predict shape and force. For example, the detailed fibre architecture of the myocardium was reported by Nielsen et al. [110] who showed how contractile function is highly dictated by 3 microstructural directions. Material properties were fitted to the ‘pole-zero’ constitutive strain energy density function [111] which is also adopted in this study. Skeletal muscle fibre distributions also play a key role in understanding physiological behaviour as part of multiscale models. Whole continuum muscle behaviour is highly influenced when homogenising substructural models that contain detailed muscle fibre descriptions [112]. Further, the orientation of muscle fascicles fitted to continuum FE models has been shown to explain the non-uniform strains observed in experiment [57].

Including fibres within a FE framework requires should not be linked to a specific element type but rather fitted to a mesh topology. One possible approach is to use a NURBS description and integrate this with FEM (Finite element model) for generic elements [113]. In this study we adopt a similar approach whereby a discrete fibre data set is fitted to a continuum field using a basis function that describes the element interpolation (from linear to cubic). Fibre directional vector is treated as a continuous field that may be applied to any element type. Basic building blocks serve as
an efficient way to construct whole muscle volumes. For example, a brick element basis with embedded fibres has been used to construct an entire cat gastrocnemius [55] and was shown to agree with experimental measurement of muscle deformations and force. Moreover, the concept of embedding digitised cadaver fibre fields into FE model primitives has been presented before [114], which can improve predicted force and contractile shape by up to 20% over simplified parallel fibre fields. In this study we extend this concept using 3D Ultrasound during passive deformation of the human Triceps Surae.

In this study we present a FE muscle primitive derived using 3D ultrasound data of human Triceps Surae muscle. The model is developed for four subjects to predict deformation in a representative volume of interest (a muscle element primitive) as part of a FE analysis. For each subject the passive tension was measured and combined with the fascicular data to determine subject-specific passive constitutive muscle parameters. The model was fit to 25%, 50%, 75% and 100% muscle deformation for each subject. A whole Triceps Surae muscle is built from these muscle primitives to highlight usability. Contractile mechanics simulations are run to observe predicted surface shape. Ultrasound informed muscle shape is compared with an equivalent geometrical model informed with fibres derived from DWI. Spatial muscle strain is also characterised.

6.3 METHODS

6.3.1 EXPERIMENT

The ultrasound data used in this study are a subset of data that have been reported elsewhere [115]. Four subjects (mean age 24.6 ± 5.2, mean weight 60.6 ± 10.8 kg, mean height 171.6 ± 6.4 cm) had 3D Ultrasound collected during a passive seated knee flexion task (full subject details in Table 6-1).
Table 6-1: Subjects statistics.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (Years)</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>Leg length (cm)</th>
<th>Knee angle (º)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>52</td>
<td>169</td>
<td>39.5</td>
<td>78</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>76</td>
<td>181</td>
<td>38</td>
<td>72</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>68</td>
<td>175</td>
<td>39.5</td>
<td>90</td>
</tr>
<tr>
<td>4</td>
<td>31</td>
<td>54</td>
<td>168</td>
<td>38.5</td>
<td>76</td>
</tr>
<tr>
<td><strong>mean</strong></td>
<td><strong>24.6</strong></td>
<td><strong>60.6</strong></td>
<td><strong>171.6</strong></td>
<td><strong>38.8</strong></td>
<td><strong>79.0</strong></td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td><strong>5.2</strong></td>
<td><strong>10.8</strong></td>
<td><strong>6.4</strong></td>
<td><strong>0.7</strong></td>
<td><strong>6.7</strong></td>
</tr>
<tr>
<td><strong>min</strong></td>
<td>18</td>
<td>52</td>
<td>165</td>
<td>38</td>
<td>72</td>
</tr>
<tr>
<td><strong>max</strong></td>
<td>31</td>
<td>76</td>
<td>181</td>
<td>39.5</td>
<td>90</td>
</tr>
</tbody>
</table>

The knee was flexed about 79 ± 6.7º and the subject configuration is shown in Figure 6-1.

No subjects had any musculoskeletal disorders. Ethical approval was obtained from the South Eastern Sydney Local Health District Human Research Ethics Committee. The left foot was placed on a dynamometer foot plate (Cybex Norm with Humac, CSMi, Stoughton, MA, USA) and the ankle was rotated from fully plantarflexed to a fully dorsiflexed. Ankle rotation was slow (5 degrees/second) as slow speed stretches are unlikely to evoke muscle stretch reflexes, so the muscle is more likely to be passive. Surface EMG was used to confirm that the ankle plantarflexor muscles were relaxed. Two Ultrasound transducers (Esaote MyLab25 with LA522E 46 mm linear array, 7.5–12 MHz operating at 12 MHz; Esaote, Genoa, Italy) were used synchronously to image the Triceps Surae over the core muscle belly with a field of view of 110 mm. The location and orientation of the ultrasound image in leg space was determined using an optical 3D motion analysis system. For full ultrasound experiment protocols see the work of Herbert et al. (2015) [115].
Figure 6-1: The experiment setup. Each subject was seated with the leg in a flexed pose and the foot was moved through dorsiflexion. Top right shows the triad of markers attached to the leg used to define the leg frame of reference. Markers are also attached to the ultrasound transducer to define the imaging plane.

The muscle tension $T_m$ in the Triceps Surae was obtained using the method described by Hoang and colleagues [116, 117]. $T_m$ is related to muscle length by Equation 6-1,

$$T_m = \frac{1}{\alpha_G} e^{\alpha_G(l_g-l_{gs})},$$

where $\alpha_G$ is a constant, found by optimisation, that determines the stiffness of the muscle and is referred to as the “stiffness index” [116]. $l_g$ is the muscle length and $l_{gs}$ the muscle slack length. For all the forces collected, see table 6-2 and table 6-3.
### Methods

#### Table 6-2: Muscle tension during muscle elongation.

<table>
<thead>
<tr>
<th>Muscle Stretche %</th>
<th>Subject 1</th>
<th>Subject 2</th>
<th>Subject 3</th>
<th>Subject 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>25.00%</td>
<td>1.5</td>
<td>2.8</td>
<td>0.8</td>
<td>0.3</td>
</tr>
<tr>
<td>50.00%</td>
<td>4.1</td>
<td>8.4</td>
<td>3.7</td>
<td>1.7</td>
</tr>
<tr>
<td>75.00%</td>
<td>8.5</td>
<td>19.6</td>
<td>11.3</td>
<td>6.3</td>
</tr>
<tr>
<td>100.00%</td>
<td>15.5</td>
<td>39.9</td>
<td>31.7</td>
<td>21.1</td>
</tr>
</tbody>
</table>

#### Table 6-3: Minimum and maximum tension and muscle length, muscle slack length and muscle stiffness for each subject.

<table>
<thead>
<tr>
<th></th>
<th>Subject 1</th>
<th>Subject 2</th>
<th>Subject 3</th>
<th>Subject 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tension min (N)</td>
<td>2.5</td>
<td>1.9</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Tension max (N)</td>
<td>42.4</td>
<td>17.5</td>
<td>32.1</td>
<td>21.2</td>
</tr>
<tr>
<td>Length min (m)</td>
<td>0.38</td>
<td>0.406</td>
<td>0.392</td>
<td>0.394</td>
</tr>
<tr>
<td>length max (m)</td>
<td>0.42</td>
<td>0.431</td>
<td>0.428</td>
<td>0.439</td>
</tr>
<tr>
<td>Alpha (m⁻¹)</td>
<td>82.8</td>
<td>88.6</td>
<td>127.2</td>
<td>119.4</td>
</tr>
<tr>
<td>Slack length (m)</td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
<td>0.4</td>
</tr>
</tbody>
</table>

#### 6.3.2 Finite Element Model

For each subject a set of muscle fibres was digitised by identifying the 3D coordinates of the fibres’ origins and insertions into the tendon. Figure 6-2 shows this process for a single slice from the 3D Ultrasound set identifying a segmented fibre at 0% elongation. This is repeated for nine fibres with the proximal and distal insertions shown as spheres. Finally, the fibres are embedded inside a three-element host mesh, which represents the basic muscle primitive in this study. We adopted a free form deformation technique to pass the measured fibre deformation to the muscle element primitive. In this approach the deforming fibres are replaced by an element that deforms based on underlying fibre movement.
Figure 6-2: (Left) is a slice from the 3D Ultrasound image identifying a manually segmented fibre at time zero; (Middle) is a 2D representation of a 3D set of nine segmented fibres shown as red lines. Green circles are the proximal and distal insertions; (Right) shows the fibres embedded inside a three-element 3D host mesh for the same volume of interest.

The particular method used is called ‘host-mesh’ fitting [118] and is illustrated in Figure 6-3 where three representative fibres in green at baseline with corresponding proximal and distal landmarks are morphed to match the same fibres in red at 100% elongation.
Methods

Figure 6-3: Free-form deformation pipeline: (Left) shows three representative fibres in green at baseline with corresponding proximal and distal landmarks at 100% elongation shown in red. The fibres are embedded inside a 3-element host. (Middle) shows the deformation of the host at 50% elongation driven by the baseline fibres in order to match the target proximal and distal fibre endpoints. (Right) shows the deformation of the host at 100% fibre elongation and perfectly matched to the target fibres.

The fibres are embedded inside a three-element host, which is morphed so as to minimise the distance between green landmarks and red targets. In order to solve this we employ an iterative closest point algorithm to solve a least squares minimisation. The objective function that is minimised is:

$$ F(u_n) = \sum_{d=1}^{N} w_d ||u(\xi_{1d}, \xi_{2d}, \xi_{3d}) - z_{3d}||^2 + \mathcal{F}_s(u_n). $$ (6-2)

where $z_d$ are the geometric coordinates of the target points for the muscle fibre $d$, $w_d$ is a weighting for each control point, $u(\xi_{1d}, \xi_{2d}, \xi_{3d})$ are the landmark points interpolated at the finite element material coordinates $(\xi_{1d}, \xi_{2d}, \xi_{3d})$ and $\mathcal{F}_s(u_n)$ is the Sobolev smoothing penalty function used to constrain the motion of the deforming host by placing weights on the curvature, arc length, area and volume measures. The second order Sobolev penalty function used is:
where each Sobolev weight, $\alpha_i (i=1..5)$, has its own magnitude dependent effect on a particular characteristic shape of the host fitted object; $\alpha_1$, $\alpha_2$ and $\alpha_3$ restrict the arc-length; $\alpha_4$, $\alpha_5$ and $\alpha_6$ regulate the arc-currvature; $\alpha_7$, $\alpha_8$, $\alpha_9$ represents the face area; and $\alpha_{10}$ represents the volume of the host in the $\xi_1$, $\xi_2$, $\xi_3$ directional space, respectively. For this study we placed a minimal weight of 0.001 on $\alpha_1$ (arc-length in the longitudinal direction), higher weight of 0.01 on $\alpha_2$ and $\alpha_3$ (arc-length in the transverse directions), no weights on the arc-curvature or element face area and a strong weight of 0.01 on $\alpha_{10}$ to constrain the volume change in the element.

A structurally based orthotropic constitutive law previously used for passive cardiac [98] and skeletal muscle [59], the ‘pole-zero‘ relation [94] was adopted and is defined in Equation 6-4.

$$ W = k_{a\beta} \frac{E_{a\beta}^2}{a_{a\beta} E_{a\beta}} \left| \begin{array}{c} u_{a\beta} \\ E_{a\beta} \end{array} \right| \right|_{a\beta}, $$

where the strain energy density function, $W$, is defined by an asymptote function with $k_{a\beta}$ the scaling function, $b_{a\beta}$ curvature control, $a_{a\beta}$ a strain limiting pole and $E_{a\beta}$ the Green’s strain components. The model was treated as transversely isotropic with the fibre direction $\alpha=\beta=1$ aligned to the fibre orientation from Ultrasound images. After choosing an initial guess for the ‘pole-zero’ parameters we used the ‘fmincon’ function in the Matlab Optimisation Toolbox [95] to optimally choose parameters that minimised the difference between the measured muscle force and the computed force. This was performed until the RMS error was less than 0.01 N (or $0.58 \pm 0.19\%$ error across all subjects). For this study we optimised the pole $(a_{a\beta})$ in the fibre direction, which was the most sensitive parameter and fixed the scaling $(k_{a\beta})$ and curvature $(b_{a\beta})$ parameters. The curvature was set to 1.0 and scaling coefficient set to 0.1 MPa based on previous experience with cardiac [94] and
skeletal tissues [59, 103]. We set a bound on the solution space for $a_{\alpha \beta}$ as 0.01 to 5.0. An initial guess was obtained from trials based on previous skeletal muscles [59, 103].

Muscle deformation was solved using the weak form of the governing equation for finite elastic mechanics in CMISS (www.cmiss.org) and is given by:

$$\frac{1}{V_o} \int T^{\alpha \beta} F_{\beta}^j \frac{\partial \delta u_j}{\partial x_\alpha} dV_o = 0,$$

(6-5)

since the Triceps Surae muscle primitive is undergoing large strain (greater than 10%). $V_o$ is the undeformed volume and $\delta u_j$ is the virtual displacement and $F_{\beta}^j = \partial x_j / \partial x_{\beta}$ is the deformation gradient tensor which maps between the deformed spatial coordinates $x_j$ and material coordinates, $\nu_{\beta}$. The Jacobian, $J$, is the determinant of the deformation gradient tensor, $F$ and $T^{\alpha \beta}$ is the 2nd Piola-Kirchoff stress tensor,

$$T^{\alpha \beta} = \frac{\partial W}{\partial E_{\alpha \beta}} + pa^{\alpha \beta}_{\nu},$$

(6-6)

and defined with respect to an undeformed orthogonal curvilinear material coordinate system, $\nu_{\alpha}$. $W$ is the ‘pole zero’ strain energy density function and $E_{\alpha \beta}$ are the Green-Lagrange strain components. The hydrostatic pressure, $p$, arises in order to satisfy muscle volume conservation. The contravariant metric tensor $a^{\alpha \beta}_{\nu} = \partial x_\alpha / \partial x_k \cdot \partial x_{\beta} / \partial x_k$ is the inverse of the right Cauchy deformation tensor (covariant metric tensor).

6.4 Result

Passive fibre deformation during Triceps Surae stretch was passed to a representative continuum element, as shown in figure 6-4 (subject 1). Following optimisation of the fibre end points to match 25% to 100% of muscle stretch the deformed host shape was predicted and the volume of the element did not change by more than 1%. Figure 6-5 shows another example where
the subject has a higher pennation movement during stretch (subject 4). In this scenario, the
deformed host is stretched further longitudinally and is more compliant. The average RMS error
between landmark and target fibres was at most 2 mm in all host mesh deformations.

Figure 6-4: Free form deformation of subject 1 highlighting the matching of all baseline fibres to the
fibre locations at 100% elongation. This produces a deformed host whose shape minimises
the difference between baseline and target fibres.
Figure 6-5: Free form deformation of subject 4 (who has a higher pennation change during stretch) highlighting the matching of all baseline fibres to the fibre locations at 100% elongation. This produces a deformed host whose shape minimises the difference between baseline and target fibres.

The constitutive law was fit for all four subjects and is shown in Figure 6-6 with parameters given in Table 6-4. The subjects that exhibited a larger fibre pennation change during passive stretch were best characterised by a more compliant constitutive law (shown for subjects 2 and 4). In contrast, subjects that had a more parallel fibre deformation, showed a stiffer behaviour (shown for subjects 1 and 3). The transverse directions (labelled as sheet and sheet-normal) were elastically less stiff than all fibre directions.
Figure 6-6: Fitted pole-zero constitutive law strain energy density for subjects one to four in the fibre direction (W11) and transverse directions (W22/W33).

Table 6-4: Fitted ‘pole zero’ parameters for all four subjects.

<table>
<thead>
<tr>
<th></th>
<th>Subject 1</th>
<th>Subject 2</th>
<th>Subject 3</th>
<th>Subject 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>scaling $\kappa_{11}$ (MPa)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>pole $\alpha_{11}$</td>
<td>0.12755</td>
<td>0.656</td>
<td>0.3047</td>
<td>0.494</td>
</tr>
<tr>
<td>curvature $\beta_{11}$</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>scaling $\kappa_{22}/\kappa_{33}$ (MPa)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>pole $\alpha_{22}/\alpha_{33}$</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>curvature $\beta_{22}/\beta_{33}$</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Avg % error</td>
<td>0.73%</td>
<td>0.71%</td>
<td>0.56%</td>
<td>0.30%</td>
</tr>
</tbody>
</table>

An entire Triceps Surae muscle was built from these muscle primitives and the entire muscle field is shown in Figure 6-7. There was a clear bi-pennate characterisation for the whole continuum with fibres merging towards a central tendon.
Figure 6-7: (Left) Bi-pennate muscle primitive used to construct a whole Triceps Surae muscle; and (right) complete fitted fibre field within whole muscle.

A finite elastic mechanics simulation using a Hill type contraction model produced a distinct bulge on the medial head of the gastrocnemius (the larger head) and a distinct crease formed between the heads, shown in Figure 6-8.
An axial view highlighted the effect of the fibres contracting inwards towards the central tendon. To evaluate this behaviour we compared this with the same muscle fitted with diffusion weighted derived fibres under the same level of contraction and the resulting muscle profile was highly consistent. However, the diffusion derived model did show more distinct creasing and bulging than the Ultrasound model from this study. Comparison between the two models showed an RMS error difference in shape of 8.8 mm. (Figure 6-9)
Muscle strain was shown to align with fibre direction and was spatially non-uniform as shown in Figure 6-10. The strain is viewed from the posterior and axial views and is split into maximum principal compressive and tensile strain separately to reveal the spatially varying behaviour in 3D. Figure 6-10 (left) reveals a maximum compressive principal strain in the muscle centre towards the central tendon up to a peak strain of 0.24 (24% $\varepsilon$). Figure 6-10 (right) reveals a maximum tensile principal strain on the medial outer side towards the larger medial head up to a
peak strain of 0.1 (10% $\varepsilon$). Strain was highest in the medial and lateral heads and lowest near the proximal lateral region closest to the femoral insertion site.

Figure 6-10: (left) Maximum principal compressive and (right) tensile strain from the (top) axial and (bottom) posterior views. $\varepsilon$ is the symbol for tensile strain which is unitless.
6.5 Discussion

The study developed a muscle primitive using 3D Ultrasound in the human Triceps Surae from four subjects as a representation of a bipennate muscle. The extracted 3D Ultrasound fibre data were embedded inside a representative muscle volume element that captured approximately 4 cm x 2 cm x 2 cm of the muscle belly and morphed to match the moving fibre field imaged from 3D Ultrasound using a free form deformation technique called ‘host-mesh’ fitting. This produced a series of known muscle shapes (that matched the underlying fibre data) and we mechanically simulated these known displacements in order to match the measured muscle force by optimising material properties. The ‘pole-zero’ parameters were optimised to match four positions in the data (25%, 50%, 75% and 100%) of the experimental passive muscle stretch with an average fitting error of less than 1% of the force. It was shown that the whole continuum muscles produced a realistic contractile shape when simulated, which was comparable with a diffusion weighted derived model reported by Fernandez et al. [119]. Mechanical simulation showed spatially varying non-uniform strain that aligned with the complex fibre orientation. Compressive strain was highest in the central belly of the muscle and tensile strain was highest in the outer medial head. These muscle primitives are being developed as part of the Physiome repository [59] and the Musculoskeletal Atlas Project (MAP) [120] in order for people to adopt and fit to subject-specific data.

There are a number of limitations in this study that should be considered when interpreting the results. Firstly, the range of muscle lengths was much less than the full physiological range of lengths. That was because (a) the Ultrasound transducers could not be placed behind the knee when the knee was fully flexed, (b) it was not possible to simultaneously extend the knee and dorsiflex the ankle, so we just dorsiflexed the ankle and (c) most markers were visible for some but not all of the movement so we selected a subset of markers that were available for a common block of frames. This meant dropping many frames at the beginning and the end of the movement. Secondly, when developing the volume elements we selected only 9-12 fibres per muscle primitive to describe the entire behaviour of this region. This passive deformation may have been improved if more fibre
data were available. We evaluated this possible limitation by fitting a muscle volume with 9 and 12 fibres and found that the overall deformation did not vary significantly as long as the fibres covered the entire block uniformly. Thirdly, the optimisation was sensitive to the initial parameter set, which was selected based on previous skeletal muscles fitted to the ‘pole-zero’ constitutive law. We did, however, try small variations of the initial guess and the model converged to the same solution as long as the initial guess was not too far out of range.

The fitted material properties were stiffer in the fibre direction and it was shown that the two subjects with the larger pennation angle change during deformation produced a less stiff strain energy density function, characterised by a larger pole in the ‘pole-zero’ law. In contrast, the two subjects that showed less pennation change during deformation exhibited a stiffer strain energy density function, characterised by a smaller pole in the ‘pole-zero’ law. These results provide a range of likely constitutive parameters that can describe healthy subjects. In addition to developing a useful muscle building block we also aimed to characterise stress-strain behaviour of healthy muscles. This initial result provides a basis for future comparison with pathologic muscles such as the muscles of people with contracture after stroke, spinal cord injury or cerebral palsy.

We showed that using the muscle primitive to build a complete Triceps Surae muscle fitted to a new subject worked effectively. Specifically, we were able to simulate active contraction using a previously presented version of the Hill type model (see Appendix A) and this produced a shape and strain pattern that was consistent with what had been previously reported using DWI based fibres in humans [119]. However, the diffusion weighted muscle produced a deeper crease between the lateral and medial heads showing an RMS geometric difference in shape of close to 1 cm (Figure 6-9). Nevertheless, both ultrasound and DWI produced fibre orientations that were similar spatially. Specifically, the high compressive strain in the central muscle region between the medial and lateral heads and the high tensile strain on the outer larger medial head was also reported in the diffusion imaging study [119]. The highly non-uniform strain pattern is consistent with previous FE models that report non-uniform patterns due to fibre orientation [57, 121]. Furthermore, the range of predicted strain up to 0.24ɛ is consistent with the FE prediction due to active contraction in [121].
It was observed that the benefit of modelling muscle volume at the chose scale was that all the fibre and fibre connection behaviour is captured in the one muscle primitive. If we were to model at the fibre level, we would also require models of the fibre connective tissue. Hence, the scale of the representative muscle element is highly suitable as a building block for whole muscles without being concerned about multiscale methods, which is more computationally challenging. This study has shown that Ultrasound is a useful imaging modality for capturing real-time fibre change. Future uses of this data include characterising healthy versus pathologic muscle and creating a table of material parameters for patients with different age and health conditions to be used for mechanics and graphical representation.
A 3D Ultrasound Informed Model of Human Triceps Surae Muscle: Appendix

For muscle contraction we used the model of Hunter [98] and added a contractile force in the DWI informed fibre direction to simulate muscle action given by:

\[
s_0(\lambda, Ca_{actn}) = \frac{(Ca_{actn}[Ca^{2+}]_{max})^h}{(Ca_{actn}[Ca^{2+}]_{max})^h + (c_{50})^h} \cdot \sigma_{ref} \cdot [1 + \beta(\lambda - 1)],
\]

(A1)

where \(s_0\) is the active tension added to the fibre direction and given by the calcium-tension derived from the ‘fading-memory’ model, which is based on the Hill type model [104, 105]. \(Ca_{actn}\) is the level of activation (non-dimensional calcium value), \(\lambda\) is the sarcomere stretch, \([Ca^{2+}]_{max}\) is the intracellular calcium concentration for maximum activation, \(c_{50}\) is the concentration at which isometric tension is 50% of its maximum, \(h\) is the Hill coefficient, \(\sigma_{ref}\) is the active isometric tension when \(\lambda = 1\) and \(\beta\) is the slope parameter.
6.6  KEY FINDINGS FROM CHAPTER 6

- The muscles with larger pennation angle change during deformation produced a less stiff strain energy density function, characterised by a larger pole in the ‘pole-zero’ law.

- Using an Ultrasound informed muscle primitive to build a complete Triceps Surae muscle fitted to a new subject worked effectively. This contractile behaviour was similar to a complete Triceps Surae muscle derived from DWI.

- The benefit of modelling at the scale chosen in this study was observed captured all the fibre and fibre connection tissue behaviour in the one muscle primitive. Hence, detailed multiscale models of individual fibres are not necessary with our approach.

- Detailed analysis of the 3D strain behaviour revealed high tensile strain on the outer medial gastrocnemius head, and high compressive strain on the central tendon medial side. This is consistent with the deformation observed during contraction simulation.
In this section a discussion is presented that examines the limitations, assumptions and key outcomes reported in this thesis. We present the main findings followed by limitations and improvements in both the methods and papers presented. Future goals and extensions of this work are highlighted.
7.1 CHAPTER 2: METHODS FOR FITTING GEOMETRIC AND FIELD INFORMATION IN THE FINITE ELEMENT PACKAGE CMISS.

High-order elements were used to construct the meshes in this thesis. Specifically, they are from the cubic Hermite family that has continuity of the 1\textsuperscript{st} derivative which provides a consistent surface normal from element to element. While this is useful in principle, it presented numerous challenges for computational simulation. Finite elastic simulation is known to be challenging for high order elements and requires careful selection of the boundary conditions and material parameters. For this thesis we fixed the triple derivative to improve convergence outcome. Following sensitivity tests this was shown to not influence the results in terms of shape and stress significantly. The main benefit of using high order elements was that we were able to describe the high non-linear shapes that muscles produce using a minimum number of elements. This proved useful in fitting to the Triceps Surae muscle which exhibits complex bifurcating behavior near its femur insertion. The main benefit, however, was describing the complex fiber field from DWI imaging throughout the mesh. This field is independent from the mesh construction and can be described using different orders of power. For this thesis, a trilinear basis function was sufficient for capturing the spatially varying fiber orientation. The pipeline used employs a Zinc plugin for the bioengineering software CMISS and CMGUI.

There are a number of limitations that need to be considered when interpreting the results of this thesis. There are human errors and anatomy interpretation can also be a source of error, especially when segmenting MRI where muscle boundaries are challenging to identify. These errors were minimized by using the same operator for all tasks and multiple data points in the 100’s for each muscle which improved the iterative fit. Sobolev smoothing also allowed us to ensure that the final mesh was not unrealistic by controlling the amount of curvature and surface tension in the mesh that was constrained. Secondly, MRI has known edge effects [122, 123] and muscle near the edge of the coil is imaged with distortion. To minimise this, we endeavored to place the rabbit Triceps Surae muscle at the center of the bore.
As an outcome from the modeling pipeline, it was shown that the animal MRI with setting of an echo time (TE) of 10 ms, repeat time (TR) of 1000 ms with 2 excitations (NEX) for averaging on a 4.7 T Siemen’s animal MRI machine was sufficient for capturing the deformed shape of an isolated rabbit muscle. This may be due to the fact that the lower limb has few other muscles to confound the segmentation process. A possible future use of this geometry is to assists in the future segmentation of rabbit muscles by providing a shape constraint on the segmentation. CT imaging may also be useful but this technique captures muscles as groups rather than individual muscles. These groups may then be further decomposed using the segmentations from MRI as part of this thesis.

7.2 CHAPTER 3: METHODS FOR EXTRACTING DIFFUSION WEIGHTED IMAGING DATA

The extraction method used to pull out muscle fibers from DWI was based on a least squares fitting method. In this approach we use more than the minimum six directions (necessary for a unique solution), whereby further directions improve the estimate of the diffusion tensor. Specifically, we used the Stejskal and Tanner equation that relates the signal intensity to the diffusion tensor with 20 directions plus the base direction with no diffusion all with an isotropic b-value of 500 s/mm$^2$. The diagonalised tensor (visualised as an ellipsoid) is represented by 3 eigenvectors and 3 eigenvalues and we assume the largest eigenvector is aligned with the major direction of fluid diffusion in the tissue, which is the fibre direction. There are other methods for extracting the diffusion tensor including analytic solutions [79] that ensure that it is symmetric positive semidefinite (that ensures we do not get negative eigenvalues from noisy data); a method where the optimal 6 directions are found that allows the use of a unique solution [124]; and methods that use different b-values in each direction. These methods may be explored in future work including multidimensional minimization [125] and simulated annealing.

There were a number of post-processing tools that we used, including, checking for negative eigenvectors and reversing the direction and thresholding the data to remove unwanted information outside of the muscle boundary. From our iterative analysis we concluded that a background grey
threshold value of 30 was most suitable for removing noise but leaving sufficient data inside the muscle belly. Further, to delineate muscle from surrounding fat, we used the FA measure, which is a dimensionless factor between 0 and 1 where 0 is a perfect sphere with no dominant diffusion direction and 1 is a tube which has a very dominant diffusion direction. A FA less than 0.3 was found to clearly isolate muscle from fatty tissue that has a less dominant diffusion direction.

The error associated with the entire process was evaluated using a celery phantom. Celery was chosen based on a review of the literature that identified a number of fiber striated options including asparagus, spring onion and pig intestine. For this study we focused on organic material and this was further confirmed by the MRI technician at CAMRI who indicated that celery was a common phantom for DWI evaluation. Celery has very distinct parallel fibers which are clearly visible and was used to indicate likely error. The celery phantom was put through the entire DWI process and the modelling process to evaluate the computed fiber angles agonists the known parallel directions. A maximum error of 4.6° at the outer boundary (near the container surface) was found to be suitable and were included this error in our presented results. This error is likely to vary from muscle to muscle due to the size of each muscle and resolution of the DWI sequence.

The conclusion from this work on DWI is that it is one of the only imaging modalities available that can reveal 3D fibre information in a semi-automated manner. Ultrasound gives better identification of fibres but is limited in volume. Ultrasound is further explored in chapter 6. This thesis showed that DWI can capture the rich 3D fibre orientations for large scale muscles that have reasonable volume but may not be suited to thin smaller muscles (such as the Sartorius) with the current available resolution. It also works best if the muscle is isolated from others as was done with the rabbit hind limb in this thesis. It does have errors (quantified by the celery phantom) which showed that near the coil boundaries near region of air the errors are likely to produce up to 4.6° in error for the fibre orientation., however, for the bulk of the material error was typically around 2° or less. Furthermore, our assumption that the fibres are aligned with the largest eigenvector is a debated concept as diffusion is likely to also occur in the other two directions and the fibre is likely
to be skewed at an angle from the dominant eigenvector. Finally, it should be noted that all parameters recommended in this thesis are associated with the Siemen’s 4.7 tesla machine at CAMRI (Auckland University) and may vary with brand type.

7.3 CHAPTER 4: DESIGN OF EXPERIMENTAL APPARATUS

One of the challenges presented in this thesis was to develop hardware suited to the task of measuring rabbit Triceps Surae force and shape in the rabbit hind lower limb. An early goal was to use the 3D printer and knowledge of force transducers to develop a custom rig that could be adjusted for future rabbit studies. This was therefore a novel component of the thesis.

7.3.1 RABBIT RIG

After multiple design iterations the final rabbit rig developed for this work is presented in chapter 4. Specifically, the rig had to be strong enough to cope with muscle rigor; adjustable for any size of rabbit up to 5 kg; easily operated by one person; and non-ferrous for use in the MRI. The animal MRI, housed at CAMRI, has a limited bore size (just big enough for the rabbit) that provided the main size constraints for the design including the extra space taken by the coil. A protractor was fitted to the rig in order to set the foot dorsiflexion angle easily. The rig consisted of 24 parts which excluded screws that were designed in SolidWorks and printed in a Dimension Elite 3D printer. Only the protector was made from acrylic plastic which was cut by laser.

7.3.2 FORCE TRANSDUCER

To measure the force applied to the muscle, three transducers were designed. The ranges of these transducers are 0.00098 - 9.80 N, 0.0098 – 68 N and 0.098 – 392 N. For this study, a capacitor force sensor was selected as they are very stable at different temperatures and near magnets. We used a capacitance reader to identify the changes of capacitance in the sensor and then convert these changes to a calibrated force. The components for this device were purchased from a standard electronics store and assembled in the lab.
7.3.3 **CELERY PHANTOM**

To evaluate the error in a fibre angle DWI informed prediction pipeline, we designed a phantom made from a plastic container integrated with acrylic sheet. The size was similar to a rabbit and fitted easily in the MRI bore. The celery was placed at different angles on the acrylic sheets and the container filled with water. The water filling process was performed by placing the container inside a larger body of water in order to ensure minimal air pockets.

The biggest limitation identified during the study was the maximum force the rig could handle, particularly during muscle rigor. Early design attempts led to broken components including the shaft, which was eventually replaced by a wooden pipe (which remained MRI safe). The other limitation was that the sensors and the electronic components of the force transducer were not MRI compatible and hence measurements were taken before MR imaging. To accomplish this, the sensor was made to be mobile and could attach and be removed (replaced with a dummy component) during imaging. To improve the transducer, there is research into MRI compatible transducers that may be incorporated into the rig design. One such example is the electrically conducting plastics developed by StretchSense NZ that send the force data to a smart device using Bluetooth. These are being considered for the next version of the rabbit rig.

7.4 **CHAPTER 5: PAPER 1: A DIFFUSION TENSOR INFORMED MODEL OF THE RABBIT TRICEPS SURAE**

The main output from this thesis was a modelling pipeline that used a DWI informed model of the rabbit Triceps Surae to estimate the influence of muscle architecture on shape, stress and material properties. This chapter used rabbit passive muscle loading and an estimate of the active contraction taken from the maximum force during rigor. We evaluate the model by predicting a second rabbit and quantifying the error using a phantom.
7.4.1 **RABBIT FORCE EXPERIMENTS:**

The passive rabbit force was measured every one to two hours up to 72 hours after euthanising. Due to muscle stiffening during rigor we waited for this to pass before conducting the experiments. From some pilot tests we found that the steady state behaviour post-rigor has a ratio to the state just prior to death. This ratio ranged from 0.8, 2.1, 7.9 and 19 N and depends on the dorsiflexion position. Since force depends on muscle volume and body weight we therefore normalised by body weight when presenting the passive force. On average the muscle stiffened over 18 hours and then reached steady state after 36 hours. We annotated the stages of the rigor mortis cycle as euthanized point, rapid rigor onset, peak rigor, steady rigor decrease and steady state. When reviewing the literature we were only able to find one other study that quantified the post-mortem effects in NZ white rabbits following euthanasia. Our results were consistent to this study but also added additional information based on relating pre and post rigor and identifying a surrogate measure for the maximum contraction force (the peak rigor).

7.4.2 **MRI PARAMETERS:**

From our pilot studies the ideal MRI parameter settings for the Siemens 4.7 T Diffusion Weighted imaging sequence in skeletal muscle were an Echo Time (TE) of 40 ms; Repeat Time (TR) of 3000 ms; Number of excitations (NEX) of 2; 20 gradient directions + 1 no diffusion and a b-value of 500 s/mm². The resolution was 2 x 0.9 x 0.9 mm so the image was an anisotropic voxel. We imaged the muscle deformation for two rabbits in four dorsiflexion hind limb positions plus the neutral position. These were used to construct a finite element mesh of each position and were used as the gold standard shape to compare with. The neutral position was then strained using the known measured force (from the transducer) and the model iteratively solved until the predicted shape matched the gold standard. We achieved errors less than 2 mm RMS. The parameters that were optimised for were the constitute law parameters, specifically, we chose the ‘pole-zero’ law that was previously used for cardiac tissue [126]. This was repeated for all four position and the overall optimised parameters that minimised the error over all four positions on average were identified. The optimisation was performed in Matlab using the intrinsic function ‘fmincon’, which attempts to
find a constrained minimum of a scalar function of several variables starting at an initial estimate. This is generally referred to as constrained nonlinear optimization or nonlinear programming. This method does not guarantee convergence if your guess is far away from the global minimum. Hence, a limitation with the current study was that a lot of time was spent finding a good guess.

Future methods that will be considered include gradient-based optimisation, particle-swarm optimisation and simulated annealing.

### 7.4.3 MECHANICS MODEL

The mechanics model used the governing equations of finite elasticity and the model was fixed at one end (the end connected to the hip) and free at the other end (the end connected to the calcaneus on the foot) to be strained. For the simulations we considered only the passive behaviour but for evaluation of the model we used the maximum peak rigor force as a surrogate for the maximum contractile force. The active contraction was added using a Hill-type model [127], previously used in cardiac mechanics. Two models were simulated; (i) with a parallel fibre estimate; and (ii) with the extracted DWI fibres. The material parameters estimated in both scenarios were different. This indicates that fibre orientation not only influences the shape but requires a complete refit of material laws.

#### 7.4.3.1 DWI EFFECT ON SHAPE, STRESS AND STRAIN

Inclusion of DWI-based fibres produced a different shape during contraction, specifically, the muscle contracted more posteriorly and was directed inwards as dictated by the fibre alignment. In contrast, the control model with parallel fibres simply moved superiorly and inferiorly. Both models conserved volume during deformation. The maximum error was up to 3.7 mm. Muscle strain showed a distinctly different pattern when DWI fibres were included. The largest principal strain was visualised as vectors and was clearly parallel in the model without DWI fibres, however, the strain clearly aligned with the pennation angle in the DWI-based model. Furthermore, viewing the strain intensity from a posterior view of the right hind limb highlighted by a larger strain
intensity on the medial side, which interestingly is also the side of the gastrocnemius with the largest head (the medial head). The DWI-based model intensified the medial strain up to 10 MPa. The inverse was observed for stress where the largest strain produced the lowest stress, hence the highest stress was observed on the lateral gastrocnemius head.

7.5 CHAPTER 6: PAPER 2: A 3D ULTRASOUND INFORMED MODEL OF HUMAN TRICEPS SURAE MUSCLE

The second objective of this thesis was to explore a second imaging modality, 3D Ultrasound, in humans and develop a muscle primitive from this data that may be used to build whole bipennate muscles of the Triceps Surae. This muscle primitive would be placed in the Physiome repository [59] and the Musculoskeletal Atlas Project (MAP) [120] in order for researchers to adopt and fit to subject-specific data. This study was a collaboration with Neuroscience Research Australia who provided the Ultrasound experimental data for passive Triceps Surae stretch in four healthy subjects. The extracted fibre data was embedded inside a representative muscle volume element, that captured approximately 4 cm x 2 cm x 2 cm of the muscle belly and morphed to match the moving fibre field imaged from Ultrasound using a free form deformation technique called ‘host-mesh’ fitting. This produced a series of known muscle shapes (that matched the underlying fibre data) and we mechanically simulated these known displacements in order to match the measured muscle force by optimising material properties. The pole-zero parameters were optimised to match four positions in the data (25%, 50%, 75% and 100% of the experimental passive muscle stretch) with an average fitting error of less than 1% of the force. Hence, the modelling pipeline developed for DWI earlier was modified to match muscle force rather than shape.

There were a number of key findings from this study that are important to the field of continuum fibre-based models. Firstly, while the fitted material properties were stiffer in the fibre direction it was shown that the two subjects who had a larger pennation angle change during deformation produced a less stiff strain energy density function, characterised by a larger pole in the
‘pole-zero’ law. In contrast, the two subjects that showed less pennation change during deformation exhibited a stiffer strain energy density function, , characterised by a smaller pole in the ‘pole-zero’ law. These results provide a range of likely constitutive parameters that can describe healthy subjects. Secondly, we showed that using the muscle primitive to build a complete Triceps Surae muscle fitted to a new subject worked effectively. Specifically, we were able to simulate the active contraction and this produced a shape and strain that was consistent with what we had previously reported with DWI in humans [128]. This shows that both Ultrasound and DWI produced fibre orientations that were similar spatially. Thirdly, it was observed that the benefit of modelling at the scale we chose is that all the fibre and fibre connection behaviour is captured in the one muscle primitive. If we were to model at the fibre level, we would also require models of the fibre connective tissue. Hence, the scale of the representative muscle element is highly suitable as a building block for whole muscles without being concerned about multiscale methods.

This study has shown that Ultrasound is a highly useful imaging modality for capturing real-time fibre change. Although it takes time to segment out the fibres it is much more reliable than those predicted using DWI. Future uses of this data include characterising healthy versus pathologic muscle, such as in cerebral palsy. We can also create a table of material parameters for patients with different age and health conditions.
Appendix A: DWI fitting script

% This matlab script reads in a series of DICOM slices generated from the Siemen's 3T MRI at CAMRI. It assumes that there are 20 gradient directions. The images have been renumbered using DICOMWORKS. It exports the pixel data as an exdata file for cmgui with a fibre vector field.

% Fist clean everything in memory

clear all; close all; clc

% Make a struct to store all DWI data

DTIdata=struct();

% open exdata file

fid = fopen(['DTI','.exdata'], 'w');
if (fid == -1); error('Error opening file'); end

% writing to file in CMGUI exdata format

fprintf(fid,' Group name: %s\n','DTI_vectors');
fprintf(fid,' #Fields=%d\n',2);

fprintf(fid,' %d) %s, %s, %s, #Components=%d\n',1,'coordinates','coordinate', 'rectangular cartesian',3);

fprintf(fid,'   %s.  Value index=%d, #Derivatives=%d\n','x',1,0);
fprintf(fid,'   %s.  Value index=%d, #Derivatives=%d\n','y',2,0);
fprintf(fid,'   %s.  Value index=%d, #Derivatives=%d\n','z',3,0);

fprintf(fid,' %d) %s, %s, %s, #Components=%d\n',2,'vector','field', 'rectangular cartesian',3);

fprintf(fid,'   %s.  Value index=%d, #Derivatives=%d\n','x',4,0);
fprintf(fid,'   %s.  Value index=%d, #Derivatives=%d\n','y',5,0);
fprintf(fid,'   %s.  Value index=%d, #Derivatives=%d\n','z',6,0);

for slice=1:30 % loop over slices CHANGED JWF

i=0;

% 20 magnetic gradient directions + 1 zero gradient direction
% This is machine and sequence specific. This is taken from the Manual supplied with the 3T Siemens machine.

H(1,:)=[ 0.000000, 0.000000, 0.000000 ];
H(2,:)=[ 1.000000, 0.000000, 0.000000 ];
H(3,:)=[ 0.000000, 1.000000, 0.000000 ];
H(4,:)=[ -0.031984, 0.799591, 0.599693 ];
H(5,:)=[ 0.856706, 0.493831, -0.148949 ];
H(6,:)=[ 0.834429, 0.309159, 0.456234 ];
H(7,:)=[ 0.834429, -0.309159, 0.456234 ];
H(8,:)=[ 0.856706, -0.493831, -0.148949 ];
H(9,:)=[ 0.822228, 0.000000, -0.569158 ];
H(10,:)=[ 0.550834, 0.425872, -0.717784 ];
H(11,:)=[ 0.468173, 0.834308, -0.291108 ];
H(12,:)=[ 0.515933, 0.808894, 0.281963 ];
H(13,:)=[ 0.391890, 0.515855, 0.761785 ];
H(14,:)=[ 0.478151, 0.000000, 0.878278 ];
H(15,:)=[ 0.391890, -0.515855, 0.761785 ];
H(16,:)=[ 0.515933, -0.808894, 0.281963 ];
H(17,:)=[ 0.468173, -0.834308, -0.291108 ];
H(18,:)=[ 0.550834, -0.425872, -0.717784 ];
H(19,:)=[ 0.111012, -0.264029, -0.958105 ];
H(20,:)=[ 0.111012, 0.264029, -0.958105 ];
H(21,:)=[ 0.031984, 0.799591, -0.599693 ];

% Read the MRI (DTI) voxel data volume
for i=1:21
    if (slice<10), slnum=['00',num2str(slice)]; end;

if ((slice>=10) && (slice<22)), snum=['0',num2str(slice)]; end;

if (i<10), num=['00',num2str(i)]; end;
if ((i>=10) && (slice<22)), num=['0',num2str(i)]; end;
name=['rabbit_dicom\19pos_4_DTI\19_slice',snum,'image',num,'echo001.dcm'];
info=dicomread(name);

% Read second image to average

j2=j+525;
%if (j2<10), num2=['0000',num2str(j2)]; end;
%if ((j2>10) && (j2<100)), num2=['000',num2str(j2)]; end;
%if ((j2>100) && (j2<1000)), num2=['00',num2str(j2)]; end;
%if ((j2>1000) && (j2<10000)), num2=['0',num2str(j2)]; end;
%name2=['shank\DTI_dicom\dti_shank4_DTI\image',num2];
%info2=dicomread(name2);
%DTIdata(i).VoxelData = (single(info)+single(info2))/2;
DTIdata(i).VoxelData = single(info);
DTIdata(i).Gradient = H(i,:);
DTIdata(i).Bvalue=500;
end

% Constants DTI

parametersDTI=[];

% control the amount of noise

parametersDTI.BackgroundTreshold=30;
parametersDTI.WhiteMatterExtractionThreshold=0.3;
parametersDTI.textdisplay=true;
if(parametersDTI.textdisplay), disp('Start DTI function'); pause(0.1); end

% Make a 4D matrix to store the different gradient voxel volumes

% (The constant 20 is just a minimum number of volume datasets, % this matrix will automaticaly expand if more volume datasets are used.)
S=zeros([size(DTIdata(1).VoxelData) 20], 'single');
% Make a 3D matrix to store the zero gradient voxel volume(s)
S0=zeros(size(DTIdata(1).VoxelData), 'single');
% Make a matrix to store the gradients
H=zeros(20,3);
% Make a vector to store the different B-values (timing)
Bvalue=zeros(20,1);
% Read the input data (DTIdata) and separates the zero gradient and other gradients into different matrices.
if(parametersDTI.textdisplay), disp('Separate gradient and none gradient datasets'); pause(0.1); end
voxel0=0; voxelg=0;
for i=1:length(DTIdata)
    if(nnz(DTIdata(i).Gradient(:)==[0;0;0])==3)
        voxel0=voxel0+1;
        S0=S0+single(DTIdata(i).VoxelData);
    else
        voxelg=voxelg+1;
        S(:,:,voxelg)=single(DTIdata(i).VoxelData);
        H(voxelg,:)=single(DTIdata(i).Gradient);
        Bvalue(voxelg) = single(DTIdata(i).Bvalue);
    end
end
% The zero gradient matrix is the mean of all zero gradient datasets
S0=S0/voxel0;
% Free some memory
clear DTIdata;
if(parametersDTI.textdisplay), disp('Create the b matrices'); pause(0.1); end
% Create the b matrices (http://www.meteoreservice.com/PDFs/Mattiello97.pdf)
b=zeros([3 3 size(H,1)]);
for i=1:size(H,1),
    b(:,:,i)=Bvalue(i)*H(i,:)'*H(i,:);
end

if(parametersDTI.textdisplay), disp('Voxel intensity to absorption conversion'); pause(0.1); end

% Convert measurement intensity into absorption (log)
Slog=zeros(size(S),'single');
for i=1:size(H,1),
    Slog(:,:,i)=log((S(:,:,i)./S0)+eps);
end
if(parametersDTI.textdisplay), disp('Create B matrix vector [Bxx,2*Bxy,2*Bxz,Byy,2*Byz,Bzz]'); pause(0.1); end

% Sort all b matrices into a vector Bv=[Bxx,2*Bxy,2*Bxz,Byy,2*Byz,Bzz];
Bv=squeeze([b(1,1,:),2*b(1,2,:),2*b(1,3,:),b(2,2,:),2*b(2,3,:),b(3,3,:)])';

% Create a matrix to store the diffusion tensor of every voxel [Dxx,Dxy,Dxz,Dyy,Dyz,Dzz]
DifT=zeros([size(S0) 6],'single');

% Create a matrix to store the eigenvector values of every voxel
Y=zeros([size(S0) 3],'single');

% Create a matrix to store the fractional anisotropy (FA)
FA=zeros(size(S0),'single');

% Create a matrix to store the Apparent Diffuse Coefficient (ADC)
ADC=zeros(size(S0),'single');

% Create a maxtrix to store the (main) fibre direction in each pixel
VectorF=zeros([size(S0) 3],'single');
if(parametersDTI.textdisplay), disp('Calculate Diffusion tensor, eigenvectors and other parameters of each voxel'); pause(0.1); end

% Loop through all voxel coordinates
for x=1:size(S0,1),
    for y=1:size(S0,2),
        for z=1:size(S0,3),
% Only process a pixel if it isn't background
if(S0(x,y)>parametersDTI.BackgroundTreshold)

% Calculate the Diffusion tensor [D_{xx},D_{xy},D_{xz},D_{yy},D_{yz},D_{zz}], with a simple matrix inverse.
Z=-squeeze(Slog(x,y,:));
M=Bv\Z;

% The DiffusionTensor (Remember it is a symmetric matrix, thus for instance D_{xy} == D_{yx})
DiffusionTensor=[M(1) M(2) M(3); M(2) M(4) M(5); M(3) M(5) M(6)];

% Calculate the eigenvalues and vectors and sort the eigenvalues from small to large
[ EigenVectors,D]=eig(DiffusionTensor); EigenValues=diag(D);
[t,index]=sort(EigenValues);
EigenValues=EigenValues(index); EigenVectors=EigenVectors(:,index);

EigenValues_old=EigenValues;

% Regulating of the eigen values (negative eigenvalues are due to noise and other non-idealities of MRI)
if((EigenValues(1)<0)&&(EigenValues(2)<0)&&(EigenValues(3)<0)),
EigenValues=abs(EigenValues);end
if(EigenValues(1)<=0), EigenValues(1)=eps; end
if(EigenValues(2)<=0), EigenValues(2)=eps; end

% store the largest eigenvector as the fibre direction for this pixel
fibrevec(:,x,y)=EigenVectors(:,3);

% Apparent Diffuse Coefficient
%ADCv=(EigenValues(1)+EigenValues(2)+EigenValues(3))/3;

% Fractional Anistropy (2 different definitions exist) First FA definition:
FAv=(1/sqrt(2))* ( sqrt((EigenValues(1)-EigenValues(2)).^2+(EigenValues(2)-EigenValues(3)).^2+(EigenValues(1)-EigenValues(3)).^2))./sqrt(EigenValues(1).^2+EigenValues(2).^2+EigenValues(3).^2) ;

% Second FA definition:
%FAv=sqrt(1.5)* ( sqrt((EigenValues(1)-ADCv).^2+(EigenValues(2)-ADCv).^2+(EigenValues(3)-ADCv).^2))./sqrt(EigenValues(1).^2+EigenValues(2).^2+EigenValues(3).^2) ;
% Store the results of this pixel in the volume matrices

%ADC(x,y)=ADCv;
%Y(x,y,:)=EigenValues;
%DifT(x,y,:)=[DiffusionTensor(1:3) DiffusionTensor(5:6) DiffusionTensor(9)];
% Only store the FA and fiber vector of a voxel, if it exceed an anistropy treshold
if(FAv>parametersDTI.WhiteMatterExtractionThreshold)
    FA(x,y)=FAv;
    VectorF(x,y,:)=EigenVectors(:,end)*EigenValues_old(end);
end

end
end
end
if(parametersDTI.textdisplay), disp('DTI function Finished'); pause(0.1); end

% sequence 16 is from 186.19 to 356.19, k=[186.19:5:356.19]
% sequence 22 is from 11.19 to 181.19, k=[11.19:5:181.19]
% sequence 28 is from -163.19 to 6.19, k=[-168.19:5:6.19]

% (Human) 1 unit is 1mm. The x and y lengths are 240 mm. Therefore, since pixels are 128 x 128 then scale by 1.875

% (Rabbit) 1 unit is 1mm. The x lengths is 255.99 mm and y is 247.99 mm. Therefore, since pixels are 256 x 248 then scale by 0.9999

%offset=slice*30000;
k=[0:2.5:75]; % CHANGED JWF
%count=offset;
for i=1:size(fibrevec,2)
    for j=1:size(fibrevec,3)
        % count=count+1;
        fprintf(fid,' Node:%13d
',count);
fprintf(fid,'%8.2E %s',i*1.875); % scale by 1.875
fprintf(fid,'%8.2E %s',j*1.875); % scale by 1.875
fprintf(fid,'%8.2E %s',k(slice));
fprintf(fid,\n);
fprintf(fid,'%8.2E %s',VectorF(i,j,1));
fprintf(fid,'%8.2E %s',VectorF(i,j,2));
fprintf(fid,'%8.2E %s',VectorF(i,j,3));
fprintf(fid,'%8.2E %s',fibrevec(1,i,j));
fprintf(fid,'%8.2E %s',fibrevec(2,i,j));
fprintf(fid,'%8.2E %s',fibrevec(3,i,j));
fprintf(fid,\n);
% store in 3D array
pxpos(i,j,slice,1)=j*0.58;
pxpos(i,j,slice,2)=i*0.58;
pxpos(i,j,slice,3)=k(slice);
pxvec(i,j,slice,1)=VectorF(i,j,1);
pxvec(i,j,slice,2)=VectorF(i,j,2);
pxvec(i,j,slice,3)=VectorF(i,j,3);
end
end
end % slice
% close file
fclose(fid);

% open second exdata file (averaged vectors)
 fid = fopen(['DTI_rabbit_30_03','.exdata'], 'w');
disp('Writing data to exdata file')
if (fid == -1); error('Error opening file'); end

% writing to exdata file
fprintf(fid,' Group name: %s
','DTI_vectors');

fprintf(fid,' #Fields=%d\n',2);

fprintf(fid,' %d) %s, %s, %s, #Components=%d\n',1,'coordinates','coordinate', 'rectangular cartesian',3);

fprintf(fid,' %s.  Value index=%2d, #Derivatives=%2d\n','x',1,0);

fprintf(fid,' %s.  Value index=%2d, #Derivatives=%2d\n','y',2,0);

fprintf(fid,' %s.  Value index=%2d, #Derivatives=%2d\n','z',3,0);

fprintf(fid,' %d) %s, %s, %s, #Components=%d\n',2,'vector','field', 'rectangular cartesian',3);

fprintf(fid,' %s.  Value index=%2d, #Derivatives=%2d\n','x',4,0);

fprintf(fid,' %s.  Value index=%2d, #Derivatives=%2d\n','y',5,0);

fprintf(fid,' %s.  Value index=%2d, #Derivatives=%2d\n','z',6,0);

for slice=2:29 % loop over slices minus 1 from top and bottom to average
    offset=slice*35000;
    count=offset;

    % For rabbit, Do not average
    for i=1:1:(size(fibrevec,2)-1)
        for j=1:1:(size(fibrevec,3)-1)
            % for i=2:2:(size(fibrevec,2)-1)
            % for j=2:2:(size(fibrevec,3)-1)

                count=count+1;

                fprintf(fid,' Node:%13d\n',count);

                fprintf(fid,'%8.2E %s',pxpos(i,j,slice,1));

                fprintf(fid,'%8.2E %s',pxpos(i,j,slice,2));

                fprintf(fid,'%8.2E %s',pxpos(i,j,slice,3));

                fprintf(fid,'\n');

                fprintf(fid,'%8.2E %s',pxvec(i,j,slice,1));

                fprintf(fid,'%8.2E %s',pxvec(i,j,slice,2));

                fprintf(fid,'%8.2E %s',pxvec(i,j,slice,3));

                fprintf(fid,'\n');
end
end
end
disp('Finished writing data to exdata file')

% Show the Diffusion Tensor

%figure,
%subplot(3,3,1), imshow(squeeze(DifT(:,:,round(end/2),1)),[min(DifT(:)) max(DifT(:))]);
title('Dxx');
%subplot(3,3,2), imshow(squeeze(DifT(:,:,round(end/2),2)),[min(DifT(:)) max(DifT(:))]);
title('Dxy');
%subplot(3,3,3), imshow(squeeze(DifT(:,:,round(end/2),3)),[min(DifT(:)) max(DifT(:))]);
title('Dxz');
%subplot(3,3,5), imshow(squeeze(DifT(:,:,round(end/2),4)),[min(DifT(:)) max(DifT(:))]);
title('Dyy');
%subplot(3,3,6), imshow(squeeze(DifT(:,:,round(end/2),5)),[min(DifT(:)) max(DifT(:))]);
title('Dyz');
%subplot(3,3,9), imshow(squeeze(DifT(:,:,round(end/2),6)),[min(DifT(:)) max(DifT(:))]);
title('Dzz');

% Show the Fractional Anisotropy, overlaid with the anisotropy vector field.

%figure, %imshow(imresize(FA(:,:,round(end/2)),4),[]); hold on;
%VectorPlotZ=squeeze(VectorF(:,:,round(end/2),1:2));
%[VectorPlotX,VectorPlotY]=meshgrid(1:size(VectorPlotZ,1),1:size(VectorPlotZ,2));
%quiver(VectorPlotX*4,VectorPlotY*4,VectorPlotZ(:,:,2),VectorPlotZ(:,:,1));
%title('Fractional Anisotropy and Vector Field');

% Save the resulting data for the FT_test.m script.
%save('FT_data','FA','VectorF');
Appendix B: Geometric and field fitting script

#The following is CMISS script example used in this thesis to fit a mesh to data points digitised from MR images.

# 20 collapsed nodes  1,4,8,11,15,18,19,22,28,34,41,47,50,56,68,74,75,82,90,96

# total nodes:  1..96

# 84 faces  4..5,9..10,13..14,17..18,22,26..27,31..32,35..36,40..41,45,46,49,50,52..53,
# 56..57,60,61,64,65,68,69,72..73,75,76,79,80,83,84,87,88,91,92,95,96,
# 98..99,102..103,106,107,110,111,114,115,118,119,121..122,125..126,129..130,  
# 133..134,137,138,141..142,144..145,148..149,152..153,156,157,160,161,164..165,  
# 167..168

# Name of the file
$object='gastroc_element_version';
# offset
$off=200;
$offi=0;
$offd=0;
$tot_itt=5;

# Declares array dimensions
fem def para;r;fit
# Defines the coordinate system
fem def coord;r;fit
# Reads in nodal information
fem def nodes;r;$object
# Defines tri-cubic Hermite basis functions
fem def bases;r;fit
# Reads in element information
fem def element;r;$object

# Updates the derivatives in all three xi directions
fem update nodes derivative 1 linear
fem update nodes derivative 2 linear #
fem update nodes derivative 3 linear #

# write node and element file for test and use it as initial
fem export node;$ object_i_initial as $ object_initial offset $offi
fem export elem;$ object_initial as $ object_initial offset_node $offi offset_elem $offi

# Updates field
fem update field from geometry
fem def elem;d field

cem group faces 4,5,9,10,13,14,18,19,23,24,27,28,31,32,35,36,40,41,45,46,49,50,52,53,38 as FFFA
cem group faces 56,57,60,61,64,65,68,69,72,73,75,76,79,80,83,84,87,88,91,92,95,96 as FFFB
cem group faces 98,99,102,103,106,107,110,111,114,115,118,119,121,122,125,126,129,130 as FFFC  
# Defines face group
# Defines face group
fem group faces FFFA,FFFB,FFFC,FFFD as FFFFF

# Reads in data information (Digitised from MR images)
fem def data;r;$object

# Projects data onto faces only in group FFFFF.
fem def xi;c closest_face faces FFFFF

# Writes out xi positions of the projections.
fem def xi;w;fit closest_face faces FFFFF
fem export data;$ object_initial_error error as $ object_initial_error

# Note that *.ipxi should contain DATA PT. NO., ELEM NO., LOCAL FACE NO., x11, x12 and x13.
fem li data error # Lists initial data error

# Fitting is done iteratively. In this case three iterations. Since we do linear fitting, after each fit, scale factors are updated which results in change in the shape of the fitted mesh. Thus, re-projection of data points onto the new mesh is required prior to determining the error. Also note that for each iteration, a new *.ipfit is read in. This helps to relax the sobolev weights gradually.

for ($fit_itt=1; $fit_itt<=$tot_itt; $fit_itt=$fit_itt+1)
{
    fem def fit;r;fit geometry faces FFFFF
    fem def mapping;r;fit
    fem fit
    fem update node fit
    fem update scale_factor normalise
    fem de xi;c closest_face faces FFFFF #old
    fem li data error
    system "echo ' ================' "
    system "echo ' ITERATION $ fit_itt DONE' "
    system "echo ' ================' "
}

fem export data;$ object_fitted_error error as $ object_fitted_error offset $offd
fem export nodes;$ object_fitted as $ object_fitted offset $off
fem export elem;$ object_fitted as $ object_fitted offset_node $off offset_elem $off
Appendix C: CAD Design for 3D Printed Rabbit Rig

This appendix describes in detail components made for the rabbit rig and their purpose. The intention is that future users who want to reprint in 3D or modify these parts have a record. All original CAD files will be added as supplementary material to the digital record of this thesis.

Figure C1 shows the femur clamp was designed to hold the proximal hind limb in place while the tendon was lengthened and prevent rigid body translation which would introduce slack in the tendon. The design consists of a circular plastic component with a sliding groove to allow the 2 pins to be adjusted. The pins are also plastic and 3D printed (to be MR compatible) and embed inside the bone.
Figure C2 shows the upper limb clamp designed to keep the limbs in place during muscle lengthening. The design consists of a guillotine clamp with an adjustable screw to grip any size limb.

Figure C3 shows the 3D printed box which was designed for user-friendly interaction with the transducer board, which is housed within. It consisted of on/off function for power, a reset button to calibrate and mode switch which has functions of alternating between kg and pounds and
a hold function to fix the reading. However, the board and the components were not MR compatible and were kept outside the MR room.

![Figure C4- Protractor](image)

Figure C4 shows the protractor cut by laser cutter from a plastic sheet. The angles are from 0° (relax position) to 90° (maximum dorsiflexion). The attachment to the rig is shown in figure C6.

![Figure C5- Force driver assembly](image)

Figure C5 shows the driver train used to adjust the hind limb resting plate using a handle wheel. The shaft fits inside the main screw and allows adjustment of rig length.
Figure C6 shows the complete rig assembly with all parts in their correct location. The rig is designed to accommodate part replacement if any component is damaged during experiment.

**Sobolev Smoothing Parameters**

The 5 weights on direction \_1\_1\_1\_1/\_2\_2\_2\_2/\_12 are:
0.001 0.001 0.001 0.001 0.001 Error: 1.43

The 5 weights on direction \_1\_1\_1\_1/\_2\_2\_2\_2/\_12 are:
0.01 0.01 0.01 0.01 0.01 Error: 1.35

The 5 weights on direction \_1\_1\_1\_1/\_2\_2\_2\_2/\_12 are:
0.1 0.1 0.1 0.1 0.1 Error: 1.71

The 5 weights on direction \_1\_1\_1\_1/\_2\_2\_2\_2/\_12 are:
0.2 0.2 0.2 0.2 0.2 Error: 1.99

The 5 weights on direction \_1\_1\_1\_1/\_2\_2\_2\_2/\_12 are:
0.3 0.3 0.3 0.3 0.3 Error: 2.2

Figure C7- Sobolev smoothing parameters


