ULTRASOUND ELASTICITY IMAGING OF HUMAN POSTERIOR TIBIAL TENDON

by

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DEDICATION

To my wife Xiaowen, and my parents, Huifang and Sui, for their steadfast love and support.
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Abstract

Posterior tibial tendon dysfunction (PTTD) is a common degenerative condition leading to a severe impairment of gait. There is currently no effective method to determine whether a patient with advanced PTTD would benefit from several months of bracing and physical therapy or ultimately require surgery. Tendon degeneration is closely associated with irreversible degradation of its collagen structure, leading to changes to its mechanical properties. If these properties could be monitored in vivo, it could be used to quantify the severity of tendonosis and help determine the appropriate treatment.

Ultrasound elasticity imaging (UEI) is a real-time, noninvasive technique to objectively measure mechanical properties in soft tissue. It consists of acquiring a sequence of ultrasound frames and applying speckle tracking to estimate displacement and strain at each pixel. The goals of my dissertation are to 1) use acoustic simulations to investigate the performance of UEI during tendon deformation with different geometries; 2) develop and validate UEI as a potentially noninvasive technique for quantifying tendon mechanical properties in human cadaver experiments; 3) design a platform for UEI to measure mechanical properties of the PTT in vivo and determine whether there are detectable and quantifiable differences between healthy and diseased tendons.

First, ultrasound simulations of tendon deformation were performed using an acoustic modeling program. The effects of different tendon geometries (cylinder and curved cylinder) on the performance of UEI were investigated. Modeling results indicated that UEI accurately estimated the strain in the cylinder geometry, but underestimated in the curved cylinder. The simulation also predicted that the out-of-plane motion of the PTT would cause a non-uniform strain pattern within incompressible homogeneous isotropic material. However, averaging within a small region of interest determined by principal component analysis (PCA) would improve the estimation.
Next, UEI was performed on five human cadaver feet mounted in a materials testing system (MTS) while the PTT was attached to a force actuator. A portable ultrasound scanner collected 2D data during loading cycles. Young’s modulus was calculated from the strain, loading force and cross sectional area of the PTT. Average Young’s modulus for the five tendons was (0.45±0.16GPa) using UEI. This was consistent with simultaneous measurements made by the MTS across the whole tendon (0.52±0.18GPa). We also calculated the scaling factor (0.12±0.01) between the load on the PTT and the inversion force at the forefoot, a measurable quantity in vivo. This study suggests that UEI could be a reliable in vivo technique for estimating the mechanical properties of the human PTT.

Finally, we built a custom ankle inversion platform for in vivo imaging of human subjects (eight healthy volunteers and nine advanced PTTD patients). We found non-linear elastic properties of the PTTD, which could be quantified by the slope between the elastic modulus (E) and the inversion force (F). This slope (E/F), or Non-linear Elasticity Parameter (NEP), was significantly different for the two groups: 0.16±0.20 MPa/N for healthy tendons and 0.45±0.43 MPa/N for PTTD tendons. A receiver operating characteristic (ROC) curve revealed an area under the curve (AUC) of 0.83±0.07, which indicated that the classifier system is valid.

In summary, the acoustic modeling, cadaver studies, and in vivo experiments together demonstrated that UEI accurately quantifies tendon mechanical properties. As a valuable clinical tool, UEI also has the potential to help guide treatment decisions for advanced PTTD and other tendinopathies.
Chapter 1

INTRODUCTION

1.1 Human posterior tibial tendon and posterior tibial tendon dysfunction

The primary function of the posterior tibial tendon (PTT) is to invert the foot and restore the arch during the stance phase of gait. It is highly susceptible to overuse injury and degeneration, leading to an acquired flatfoot deformity and difficulty with ambulation. Figure 1.1 shows how the flatfoot deformity occurs when the PTT fails to function properly. Posterior tibial tendon dysfunction (PTTD) is quite common in middle aged women, with one study reporting an incidence of 3.3% in women over age of 40.[1]

Figure 1.1: **Left:** Normal anatomy and arch as seen in this view of the left ankle from the medial side. **Right:** Abnormal anatomy and falling arch as seen in this view of the left ankle from the medial side. Compare position of navicular bone in both figures. Figures are copied from online resource [2].
The pathophysiology of PTTD involves localized tendon degeneration behind the medial malleolus, where the tendon is believed to experience maximal stress and poor blood supply.[3] It is thought that irreversible degradation of collagen structure leads to lengthening of the tendon, change in mechanical properties[4], and eventually a collapse of the medial longitudinal arch. Several studies have confirmed the link between tendinopathy and changes in the elastic properties of tendon.[5][6][7] Although no study about the change of mechanical properties of PTT can be found at the moment, one study of Achilles tendinopathy reported a 51% decrease in Young’s modulus (1.67 to 0.82 GPa).[8] Another found that strength training for the Achilles in the elderly leads to a 69% increase in Young’s modulus (1.3 to 2.2 GPa).[9]

Figure 1.2: Treatment options for PTTD. **Left:** Stage II PTTD has classically been treated with surgery. **Right:** Non-operative treatment using bracing and therapy has been demonstrated successful in many patients.

Stage II PTTD (flexible flatfoot deformity) has typically been treated with surgery using tendon transfer to restore inversion power and osteotomies (reshaping of the bones) to reconstruct the arch and restore proper alignment of the heel.[Figure 1.2 Left] However, recent studies suggest that 60-90% of these patients respond to non-operative treatment [Figure 1.2 Right] consisting of bracing and physical therapy for 3 to 6 months.[10, 11, 12, 13, 14, 15] Moreover, many of these patients (33-70%) were able to resume normal gait and become brace free. However, there is no reliable method to predict which patients will do well with conservative care and which will ultimately require surgery.[16] This has led to prolonged unsuccessful conservative treatment in
some patients and premature surgical intervention in others. Thus, there is a great need for an objective evidence-based tool to evaluate the PTT, help classify advanced-stage PTTD, determine its prognosis, and inform treatment decisions. Unfortunately, a noninvasive clinical tool for quantifying a tendon’s mechanical properties does not currently exist.

To overcome this limitation, we propose Ultrasound Elasticity Imaging as a noninvasive technique for quantifying the stiffness and elastic properties of the PTT. In the next section, I’ll briefly introduce ultrasound imaging and several technical terms that will be used in the rest of the dissertation.

1.2 Ultrasound

Ultrasound has been used to image the human body for over half a century. Today, it is one of the most widely used imaging technologies in medicine. It is real-time, portable (only recently), free of ionizing radiation, and relatively inexpensive when compared with other imaging modalities, such as magnetic resonance (MR) and computed tomography (CT).

![Figure 1.3: An ultrasound B-mode image of human PTT.](image)

Ultrasound imaging provides a cross-sectional view of anatomical structures. [Figure 1.3] The display we see on the screen of an ultrasound system is a brightness mode (B-mode) image, generated by the pulse-echo signal reflected back from target tissue.
The ultrasound pulse of energy is originally generated by an ultrasound transducer and propagates into target tissue along a straight path, like an ultrasound “beam”. The direction of ultrasound propagation along the beam is usually called the axial direction, and the direction in the image plane perpendicular to axial is called the lateral direction. However, when imaging tendons, the term “longitudinal” and “transverse” from an anatomical point of view are often used, instead of “lateral” and “axial”, respectively.

The ultrasound transducer array, or probe, contains multiple piezoelectric crystals which can vibrate in response to applied electric current. This phenomenon is called the piezoelectric effect. The sound waves generated from these vibrations are often described in terms of their frequency, wavelength and amplitude.

Medical ultrasound systems use sound waves in the range of 1-20 MHz. High frequency ultrasound waves (short wavelength) generate images of high axial resolution, but have short penetration depth. As a result, it is best to use lower frequency transducers (2-5 MHz) to image structures that are deep in bodies, and higher frequency transducers (10-15 MHz) for imaging superficial structures.

Another “frequency” often used is the pulse repetition frequency (PRF), or frame rate. It is the number of pulses emitted by the transducer per unit of time. The frame rate for medical ultrasound imaging devices ranges from 1 Hz to 10 kHz.

The wavelength of ultrasound depends on both the frequency and the speed of sound in different tissue/medium. Media with different speeds of sound have different acoustic impedances (defined as the density multiplied by the speed of sound). The difference in acoustic impedance between two media causes the reflection of the sound wave at the boundary, just like light would be reflected at the interface of two media with different refractive indices. The larger the difference, the stronger the reflection. This is the physics behind ultrasound imaging.

As mentioned before, the B-mode image is the “brightness” image. The brightness, or the intensity, at a point of interest is related to the amount of energy passing
through. It is measured in dB (decibels). The displayed B-mode image is usually a gray scale map: white represents 0 dB, which is the maximum intensity on the image and corresponds to the reference value \( p_{ref} \). The dB value \( L_p \) at another location with intensity \( p \) can be calculated by the equation

\[
L_p = 20 \log_{10} \left( \frac{p}{p_{ref}} \right) \text{ dB.}
\]  

Because the maximum intensity is used as the reference, the dB value is always equal or less than zero. For example, -6 dB is about 50% of the maximum intensity, -20 dB is 10%, -40 dB is 1%, and -60 dB is 0.1%.

### 1.3 Ultrasound elasticity imaging

Ultrasound Elasticity Imaging (UEI) is a real-time, noninvasive technique that has emerged in recent years to objectively measure mechanical properties in soft tissue. UEI consists of acquiring a sequence of ultrasound image data and applying speckle tracking using the RF data (both phase and amplitude) to estimate displacement and strain at each pixel. [17, 18, 19, 20, 21, 22, 23, 24, 25, 26]

In this section, I’ll first describe the speckle tracking algorithm we used, then what to do once displacement is obtained, and finally the clinical application for UEI.

#### 1.3.1 Speckle tracking algorithm

2D Speckle tracking is based on the cross-correlation between two ultrasound B-mode images of the same tissue taken at different times. Instead of using only the difference in intensity values in the image, the RF data (amplitude and phase) is used for speckle tracking.

For convenience, I’ll call the first image the **Source image** and the second image
the **Search image**. First, an auto-correlation is performed using the Source image: a small 2D region of interest (for example: lateral×axial = 5 mm×2 mm in our tendon experiment) is selected at the target tissue location, and cross-correlated with itself. [Figure 1.4] The full width half maximum value (FWHM) of the correlation coefficient along both directions is then used to determine the “kernel” size: if the FWHM is odd, the kernel size is the FWHM value; if the FWHM is even, the kernel size is FWHM+1. (A hanning window with size of double the FWHM is also generated to filter the B-mode data.)

![Figure 1.4: Example for auto-correlation: A small region of interest (red box) is select and cross-correlated with itself. The rightmost figure is the correlation result with maximum at the center.](image)

Before going any further, I’d like to point out that the unit we are using here is the pixel. In a typical B-mode image from our system (14 MHz ultrasound linear array), with lateral (x) extent of 25 mm and axial (z) depth of 20 mm, there are usually 192 pixels laterally and 490 pixels along the axial direction, hence each pixel is about 0.130×0.041 mm (lateral×axial). Henceforth, the coordinates will be in integer pixels.

Next, for each pixel (X, Y) of the Source image, a window with the size of the kernel centered at (X, Y) is chosen to do cross-correlation with the window of the same size at (X, Y) of the Search image. These will be called **Source kernel window** and **Search kernel window**, respectively. Normally, this is done by multiplying the value at one pixel (x, y) in Source kernel window with the complex conjugate (C.C.) of the value at each pixel in the Search kernel window and summing them up, as the 2D...
cross-correlation Equation 1.2:

\[(f \ast g)(x, y) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} f^*(x', y') \cdot g(x + x', y + y') \, dx \, dy\]  

(1.2)

where \( f(x, y) \) and \( g(x, y) \) are the ultrasound RF data within the two kernel windows. (In case of discrete data set, it should be sum \( \sum \) instead of integral \( \int \).)

To reduce the calculation task, we set a search limit based on the fact that the speckle won’t move too much between two B-mode images. In our tendon experiment, it’s usually less than 2 pixels, so the search limit is chosen to be 5 by 5 pixels (both positive and negative direction, so 5 pixels instead of 3 pixels). As a result, for one pixel \((x, y)\) in the Source kernel window, we only need to multiply it with the C.C. of each pixel in the search limit around \((x, y)\) in the Search kernel window, and sum them up.

Figure 1.5: Example for cross-correlation: For each pixel (red dot), a kernel window (red box) is select to cross-correlate with the same kernel window in a second frame. The size of the kernel window is determined by the auto-correlation in Figure 1.4. The center of the correlation result (bottom right) is not at the center but to the lower right. For simplicity, the search window is not used in the example. An enlarged kernel window is also used to show that there is visible displacement to the lower right.

For each pixel \((X, Y)\) in the source image, there is a 2D correlation value map. [Figure 1.5 Lower Right] The third step of the speckle tracking is to find the displacement of the pixel at location \((X, Y)\) in the source image, as compared to the Search
image using the 2D correlation coefficient. It turns out that the displacement is just the
shift of the peak of the correlation coefficient from the center. Figure 1.6) Furthermore, the phase information is used to improve the accuracy of locating the peak of the
correlation coefficient. A detailed description can be found in Ref [21].

The location of the maximum correlation value:

![Image showing correlation peak and displacement](image)

Figure 1.6: The peak of the correlation result is 2 pixels downward and 1 pixel to the
right. It indicates that axial displacement is +2 pixels and the lateral displacement is +1
pixel.

The whole process is repeated for every pixel in the **Source image**. The final result
from the 2D speckle tracking is stored as axial displacements and lateral displacements
from **Source frame** to **Search frame**, from which the accumulated displacement be-
tween any two frames can be calculated. Usually the spatial displacement map between
the first and each following frame is used for strain calculation. In the rest of this disser-
tation, when the term “displacement” is used, it means the accumulated displacement
started from the very first frame (t=0), unless specified otherwise.

### 1.3.2 From displacement to strain and Young’s modulus

With the displacement at each pixel calculated, we can then calculate the strain at each
pixel location. Within the imaging plane (x-z plane), the 2D strain tensor is defined as:

\[
\begin{pmatrix}
S_{xx} & S_{xz} \\
S_{zx} & S_{zz}
\end{pmatrix} = \begin{pmatrix}
\frac{\partial u}{\partial x} & \frac{\partial u}{\partial z} \\
\frac{\partial v}{\partial x} & \frac{\partial v}{\partial z}
\end{pmatrix}
\] (1.3)
where \( u \) and \( v \) are the lateral \((x)\) and axial \((z)\) displacement, respectively. In case of tendon motion, the major displacement and strain are the two principal strains called the lateral (or longitudinal) strain \( S_{xx} \) and the axial (or transverse) strain \( S_{zz} \).

Using the lateral direction as an example: For each pixel \((x_0, y_0)\), the lateral strain \( S_{xx} \) can be calculated from

\[
S_{xx} = \frac{(d_2 - d_1)}{L}
\]

where \( L \) is the length of a 1D lateral “window” centered at \((x_0, y_0)\), \( d_1 \) and \( d_2 \) are the displacements at each end of the window. This calculation is repeated across the entire image, yielding lateral strain information for the whole B-mode image. If the force and geometry information of the tissue are available, we can obtain Young’s modulus without difficulty:

\[
E_{xx} = \frac{1}{S_{xx}} \left( \sigma_x - \nu \sigma_z \right)
\]

where \( \sigma \) is the stress, and \( \nu \) is Poisson’s ratio. I’ll introduce Poisson’s ration in Chapter 2 and describe in detail how we calculate Young’s modulus in Chapter 3.

### 1.3.3 Clinical applications of ultrasound elasticity imaging

Ultrasound elasticity imaging is FDA approved for diagnostic breast imaging and classifying suspicious lesions based on the strain pattern, and has led to a reduction of unnecessary biopsies.[27] Other clinical applications include assessing contractility and strain deficiencies in the myocardium and quantifying mechanical properties of tissue-engineered implants.[28, 29, 30, 31, 32, 33] Several recent studies have implemented UEI to quantify the mechanical properties of human skeletal muscle and tendon (electrically-stimulated or under load).[5, 34, 35, 36, 37, 38, 39, 40, 41, 42] However, no studies have yet employed elasticity imaging to quantify differences in tendon me-
1.3.4 Shear wave elasticity imaging

Another ultrasound elasticity technique worth mention is Shear Wave Elasticity Imaging (SWEI). It tracks the acoustic shear waves remotely induced by an acoustic radiation force impulse. The main idea behind SWEI is that the velocity of a shear wave depends on the shear modulus of the tissue. Once the shear wave velocity is measured, the tissue shear modulus can be calculated using

\[ G = \rho \cdot v_s^2 \]  

(1.6)

where \( G \) is the shear modulus, \( \rho \) is the tissue density, and \( v_s \) is the velocity of the shear wave.

One of the advantages of SWEI is that there is no need to move the target tissue by introducing an external force or mechanical vibration. However, this is still a research technique and not yet available on all ultrasound systems, which makes it uncommon in clinic use.

1.4 Using UEI to measure human posterior tibial tendon

The long-term goal of this study is to help guide treatment decision-making by quantifying the mechanical properties of the posterior tibial tendon with PTTD. We want to identify differences in mechanical properties between PTTs which require non-operative treatment and those that need surgery.

In order to do so, we need first to verify that UEI is capable of measuring mechanical properties of the human PTT. A cadaver study was performed for this purpose. A
materials testing system was used to provide external force and validate the UEI measurement. A journal paper based on the cadaver experiment has been accepted by *IEEE Transactions on Biomedical Engineering* at the time of writing this dissertation.

The cadaver experiment also brought up a new question: Will the geometry property of the PTT affect the measurement? Previous studies were all focused on straight tendons like the Achilles tendon. PTT is curved at the imaging location, and this means more out-of-plane motion than a straight tendon. To answer this question, we simulated ultrasound images using an acoustic modeling program, and performed UEI. It turns out the out-of-plane motion indeed affects the measurement, but we can still minimize the effect by objectively selecting a “correct” region of interest.

After we tested UEI with the simulation and cadaver experiment, we decided to measure the differences in mechanical properties between healthy and diseased tendons. The differences between healthy and diseased tendons will point the way to our ultimate goal of identifying diseased tendons at different stages of degeneration. Eight healthy volunteers and nine advanced PTTD patient were recruited, and UEI was performed with PTT of both right and left side from each subject. Even though strain and elastic modulus of the PTTs were obtained by UEI *in vivo*, we could not reliably use either strain or elastic modulus to separate healthy tendons from PTTD ones, because of the large variance. However, we found the load dependence of the elastic modulus were greatly different between the two groups. A Non-linear Elasticity Parameter (NEP) was defined to describe this difference. A classifier system base one the NEP value was then built to to separate PTTD and healthy tendons.

The rest of this dissertation is organized as following: Chapter 2 includes the ultrasound simulation of tendon deformation using two models with different geometrical properties. Chapter 3 contains the UEI of human PTT in a cadaver experiment. Chapter 4 presents *in vivo* UEI of human PTT; Chapter 5 discusses the accomplishments so far and what to do next.
Chapter 2

SPECKLE TRACKING OF SIMULATED TENDON DEFORMATION

2.1 Motivation and goal of simulation

It is always a good idea to test the technique and even predict the outcomes via simulation before actual experimentation, especially when faced with objects of irregular shapes such as the heart. In many studies about the human heart, phantoms mimicking cardiac deformation have often been tested before studying a live heart; ultrasound linear arrays were also created by computer simulation to image the phantoms. In a previous study about strain rate imaging using 2D speckle tracking, a thick-walled, cylindrical, tissue-equivalent rubber model was created for both simulation and real-time ultrasound measurement. The results of both simulation and experiments showed that their strain rate imaging was capable of clearly identifying regions with different deformations.[22] In house ultrasound simulation programs were created by research groups for their studies. A recent study about 3D speckle tracking of the left ventricle
used an ultrasound imaging simulation program called FUSK (Fast Ultrasound Simulation in K-space), which was also used for 3D ultrasound imaging of muscle fiber orientation in the heart.[43, 44]

Unlike the heart, tendons often have simple geometric properties and was modeled as a combination of springs.[45] The Achilles tendon is similar to a long straight cylinder. When imaging an Achilles tendon, if the transducer can be aligned along the long axis of the tendon, most of the movement will be confined to the imaging plane. However, the posterior tibial tendon is curved at the ankle joint, like a bent cylinder. It would be hard to capture an entire tendon fiber within the imaging plane. The curved fiber will enter the imaging plane on one side and exit on the other. This kind of geometric property will inevitably introduce out-of-plane motion when the PTT is lengthening.

Out-of-plane motion is one of the major error sources for 2D ultrasound speckle tracking. It results from the movement of the imaging target or from free-hand operation of the transducer. Operators are usually trying to avoid out-of-plane motion as much as possible. The correlation coefficient is often used to determine the tracking quality. A recent study proposed a correlation-based model for out-of-plane motion estimation in freehand ultrasound.[46] However, in the case of a curved target like an arterial wall, it is important to consider the out-of-plane motion for the strain measurement.[24, 47]

My original goal of using simulation was to demonstrate that the speckle tracking technique can accurately track displacement and strain within a cylinder. A homogeneous isotropic cylinder would have uniform strain along its long axis when being pulled at the ends. If the ultrasound transducer is aligned with the long axis of the cylinder, the speckle tracking should correctly estimate the strain. However, during cadaver experiments, discontinuity of the strain pattern was noted. I suggested that it could be explained by the geometric property of the PTT, and the out-of-plane motion. In order to test this hypothesis, a curved cylinder was used to simulate the deformation of PTT.
2.1.1 A quick note for Field II

Field II$^\text{TM}$ is an acoustic pressure field simulation package written by Dr. Joergen Jensen of Technical University of Denmark.[48, 49] The program can be downloaded from http://field-ii.dk/.

With the help of Field II, one can make a “virtual” ultrasound array and image an artificial phantom with a customized distribution of scatterers. All simulations can be done using MATLAB$^\text{TM}$ and the Field II executable downloaded.

2.2 Phantom A: a straight cylinder

2.2.1 The model of phantom A

Phantom A is a $50 \times 20 \times 60$ [unit: mm] cube with total of 10,000 scatterers inside. The cube’s center is at $(x_0, y_0, z_0) = (0, 0, 60)$ [unit: mm]. The top surface of the phantom is at $y = 30$ mm. A straight cylinder (diameter = 10 mm) is placed at height $z = 60$ mm (center of the cube), with its axis along x direction (also the lateral direction of the B-mode image as defined in Chapter 1).[Figure 2.1 Left]

![Figure 2.1: Geometry structure of phantom A; Ultrasound B-mode image of phantom A simulated by Field II. The speckles inside the cylinder are brighter than the background.

The scatterers are randomly distributed within the whole cube. The ultrasound sig-
nals scattered by the scatterers inside the cylinder are 10 times stronger than the signals from the background scatterers. The speed of sound in the phantom was set to be 1540 m/s.

For the “virtual” transducer, the center frequency is 3.5 MHz, sampling frequency 100 MHz, and total of 192 elements in the array. The transducer is placed at $y = 0$ and facing down, with its long axis aligned parallel to the axis of the cylinder. Figure 2.1 also shows a simulated ultrasound B-mode image.

To deform the cylinder, the following equations were used to transfer the coordinates of each scatterer and to introduce a longitudinal strain to the cylinder.

\[
x' = (1 + s) \cdot (x - x_0) + x_0
\]
\[
y' = \frac{y - y_0}{\sqrt{1 + s}} + y_0
\]
\[
z' = \frac{z - z_0}{\sqrt{1 + s}} + z_0,
\]

where $s$ is the strain assigned. It is also under the assumption that the cylinder is incompressible, because previous study demonstrated that tendon can be considered as an incompressible material. [50] Noticing that these equation will introduces strain $s$ to the entire phantom, I reassigned the background scatterers random amplitudes after the coordinates transform, so that the background would behave like noise.

Using Field II, ultrasound B-mode data (RF data, with both amplitude and phase) of the original phantom ($s=0$), and another B-mode image of the deformed phantom ($s=2\%$) were obtained. It’s hard to see the difference when the images are placed side by side, but the movement is obvious when the images are made into an animated gif movie.
2.2.2 Tracking result between original and deformed phantom A

The speckle tracking algorithm described in section 1.3.1 was then used to calculate the displacement and strain between the two ultrasound B-mode data (RF data, with both amplitude and phase). Figure 2.2 shows the correlation coefficient between the two images.

![Correlation Coefficient](image)

Figure 2.2: The correlation coefficient between the simulated ultrasound image of original phantom A and deformed phantom A with 2% strain. Background speckles have lower correlation coefficient than those inside the cylinder. The black spot on the left side is a reference marker for 60 mm and is not part of the cylinder.

Generally, the correlation coefficient gives us the confidence for the tracking result. The correlation coefficient between two identical speckle will be 1. The scatterers outside the cylinder were reassigned with random amplitude after the deformation. As a result, the correlation coefficient of the background is very low, indicating poor correlation. The correlation coefficient of the speckles within the cylinder is much higher (near 1), indicating good correlation, and the tracking result is trustworthy.

There are many sources of error which will cause low correlation coefficient, such as noise/SNR during the generation of the image data, as well as strain decorrelation. The former is also called the white noise, which is typical for most electronic equipments. Even the correlation between two ultrasound images of a static speckle at slight different time will not have uniform correlation coefficient of 1 because of the existence of the white noise. The strain decorrelation is caused by the change of the shape of the speckle during deformation, which is non-avoidable for strain imaging. The
white noise, strain decorrelation, together with the our-of-plane motion which will be discussed in next phantom model, are the three major sources of error for ultrasound speckle tracking.

Figure 2.3: Speckle tracking results between two simulated ultrasound images, with assigned strain value of 2%. **Upper row:** The lateral and axial displacement. Hot color means positive (to the left for lateral displacement, and down for axial displacement), and cold color means negative (to the left for lateral, and up for the axial). **Lower row:** The lateral and axial strain, and the averaged strain value inside each box (black box for lateral and yellow box for axial). Hot color means positive strain (stretched), cold color means negative strain (squeezed).

Due to the poor correlation, the surrounding speckles show only noise. As a result, the displacement map clearly separates the cylinder from the background.

The lateral displacement (Figure 2.3 Upper left) at the center of the cylinder is zero because the center of the cylinder is fixed during the deformation. The cylinder was
being pulled from both left and right ends equally, so the center should not move, hence the zero displacement. The farther the sides moved away from the center, the greater the displacement. The lateral strain map (Figure 2.3 Lower left) shows an averaged value of 1.9±0.7 %, which is consistent with the assigned strain value 2 %.

The axial displacement (Figure 2.3 Upper right) and strain (Figure 2.3 Lower right) also provided very interesting results. From the axial displacement, it was noted that the center didn’t move. This was expected since the cylinder was being pulled from both ends symmetrically. The upper part of the cylinder moved down and the lower part moved up, and the further away from the center, the more displacement. The axial strain map shows an average value of -1.0±0.1 % (averaged over the yellow box in Figure 2.3 Lower right). The strain is negative because the cylinder was being squeezed along radial direction while being pulled by both ends.

When a material is under deformation, the negative ratio of transverse to longitudinal strain is defined as the Poisson’s ratio. In our case, the transverse direction of the cylinder is the axial direction of the ultrasound B-mode image, and the longitudinal direction of the cylinder is the lateral direction of the B-mode image. Under the assumption of incompressibility, the Poisson’s ratio of this cylinder should be 0.5 under small strain. This explains why the axial strain is half of the lateral strain.

The fact that the lateral strain has a larger standard deviation than the axial strain is also expected because of the nature of the ultrasound speckle tracking technique. The point spread function (PSF) of ultrasound imaging system has a much larger width along lateral direction than axial direction. It can be improved by further data processing. Reducing the width of PSF along the lateral direction and improving lateral resolution with additional processing is another popular topic of ultrasound imaging research. However, it requires large amounts of data and much longer calculation time. This was beyond the scope of the current study. The lack of phase information also caused the lateral tracking less accurate than the axial tracking.
To summarize, the Field II simulation with phantom A successfully demonstrated that the speckle tracking algorithm can accurately measure strain within a cylinder.

2.3 Phantom B: a curved cylinder

2.3.1 The model of phantom B

As mentioned earlier, ultrasound imaging shows a 2D cross-sectional view of the target tissue. When the lateral direction of the ultrasound B-mode image is along the axis of a cylinder, the cross-sectional view shown by the ultrasound image is the sagittal plane. A perfect incompressible homogeneous isotropic material, like the cylinder in phantom A, when being deformed elastically, will have uniform strain throughout, as the strain map in Figure 2.3 shows. This model might be sufficient for a straight tendon like the Achilles tendon, but is definitely not sufficient to study the posterior tibial tendon when the portion of the tendon adjacent to the medial malleolus is the imaging location.

![Figure 2.4: Upper: Change of geometrical properties will introduce out-of-plane motion. The red line denotes the imaging location of the ultrasound probe. Lower: Blue dots are the new locations of the red dots after 2% strain along the axis of the curved cylinder to the right.](image)

Figure 2.4 shows the result of bending a straight cylinder. The red lines in the upper two figures denote the imaging location of the ultrasound transducer, with the
ultrasound propagating into the paper. When bent, the scatterers would move in and out of the imaging plane. The larger the displacement, the more out-of-plane motion, and the lower the correlation coefficient. The displacement measured in the imaging plane would be the projection of the actual displacement. As a result, the strain will not be as uniform as before. Phantom B was designed to test the above hypothesis.

Phantom B is a $30 \times 30 \times 20$ [unit: mm] cube with a curved cylinder inside.[Figure 2.5] The curved cylinder has 10,000 scatters inside with enhanced scattering amplitude.

![Figure 2.5: Phantom B: a curved cylinder. Left: 3D view of the scatterers within the curved cylinder. The cylinder has a radius of 5 mm. One end of the cylinder is fixed and the other end will be pulled. The ultrasound transducer is placed 35 mm above the cylinder, facing down. There are also background scatterers outside the cylinder but not shown in the figure. Right: Top view of the scatterers within the curved cylinder. The red line denotes the imaging plane. The observer is looking at the B-mode images from $+y$ to $-y$ direction, so that the movement would be from left of the observer to the right.](image)

A similar coordinate transform was made to introduce a 2 % strain to the incompressible cylinder along its axis. Random amplitude was also reassigned to the background scatterers after the coordinate transformation. A cylindrical coordinate system was used in addition to the Cartesian coordinate system:

\[ r = \sqrt{x^2 + y^2} \]  
(2.4)

\[ \theta = \arctan \left( \frac{y}{x} \right), (\theta < 0) = \theta (\theta < 0) + \pi \]  
(2.5)

\[ z' = \frac{z}{\sqrt{1 + s}} \]  
(2.6)

\[ r' = \frac{(r - r_0)}{\sqrt{1 + s}} + r_0 \]  
(2.7)
\[ \theta' = (1 + s) \cdot \theta \]  \hspace{1cm} (2.8)

\[ x' = r' \cdot \cos (\theta'), \quad y' = r' \cdot \sin (\theta') \]  \hspace{1cm} (2.9)

Figure 2.6: Ultrasound B-mode images of phantom B: before (t0) and after (t1) 2% deformation to the right.

The same simulated transducer was used for ultrasound imaging. Figure 2.6 shows the ultrasound B-mode images of the phantom before and after 2% deformation. Again, it's hard to notice the change when the two images are placed side by side. The MATLAB™ code for generating phantom B, and coordinate transform of phantom B can be found in Appendix A.

### 2.3.2 Tracking result of phantom B

Figure 2.7 shows the correlation coefficient between the two ultrasound images. Once again, the surrounding speckles have lower correlation coefficient than the speckles within the curved cylinder. (The regions near the right and left edge are actually outside the cylinder because the cylinder is curved out of the imaging plane.) Even within the cylinder, the correlation coefficient to the right side is smaller than the left side, because the displacement at right side is larger and caused more out-of-plane motion.

Figure 2.8 shows the axial displacement between two ultrasound images before and after 2 % strain. It turns out the out-of-plane motion has only a small effect on the speckle tracking in the axial direction. The displacement map is similar to that of phantom A. The averaged strain value within the green box is \(-1.0 \pm 0.28\) %, which is half of the imposed lateral strain of 2 %. Once again, the axial strain is consistent
Figure 2.7: The correlation coefficient between the simulated ultrasound images of original phantom B and deformed phantom B.

Figure 2.8: Axial displacement and strain of phantom B. They are noisier than those of phantom A, but very similar. The averaged strain value within the green box is \(-1.0\pm0.28\%\). The negative strain indicates that the cylinder was squeezed along its radial direction.

with what we calculated from lateral strain using Poisson’s ratio. The larger variance is clearly due to the out-of-plane motion.

Figure 2.9 shows the lateral tracking result of the deformed phantom B. Unlike the straight cylinder in phantom A, the curved cylinder is fixed at one end, so the displacement at the center of the image is no longer zero. The lateral displacement map shows the displacement is positive, larger at the right side and smaller at the left side, which means the cylinder is being pulled to the right. The displacement vs x plots (left column in Figure 2.9) shows that the averaged displacement is consistent with the expected displacement only at the center region. The speckle tracking overestimated the displacement near the fixed end, and underestimated the displacement near the free end. The measured displacement curve is also very bumpy. Because the strain is calculated as the gradient of spatial distribution of the lateral displacement, the uneven distribution caused negative strain as shown in Figure 2.10.

The strain pattern is much worse than the strain pattern of phantom A. There are
Figure 2.9: Spatial distribution of the lateral displacement. **Left Column:** Lateral displacement map of phantom B after 2% deformation. The displacement map shows that the cylinder was lengthening to the right (larger displacement to the right, smaller to the left). **Upper Right:** Black line is the projection of the actual movement along the green line in the left figure. Red line is the measured displacement along the green line. Two lines are consistent with each other at center portion (labeled 2), but not for portion 1 and 3. **Lower Right:** Red line is the measured displacement averaged within the green box in the left figure. Black line is the same as in the upper right figure. The measurement is smoothed but still different from the expected value at portion 1 and 3.
Figure 2.10: Spatial distribution of the lateral strain. **Left Column:** Lateral strain map of phantom B after 2% deformation. The strain map shows larger variance than that of phantom A. There are negative (blue) area mixed in the positive (red) region. **Upper Right:** Black line is the expected strain calculated from the black line in Figure 2.9. Red line is the strain measurement along the green line in the left figure. Only the value of region 2 is close to the expected strain. **Lower Right:** Red line is the measured strain averaged within the green box in the left figure.

negative (blue) regions mixed in the positive (red) region, while the strain should be positive (2%). The strain curve clearly shows that the center region with positive strain is sandwiched by two negative strain regions. As a result the averaged strain value within the green box is much smaller (0.71±1.4%) than the expected 2%, and with very large variance. If we look at only the center red region, the strain curve is closer to the expected values.

To summarize, after considering out-of-plane motion, speckle tracking can still measure the axial strain accurately. However, it is important to find a correct region of interest for lateral strain measurement so that an accurate estimation can be obtained.

### 2.4 Conclusion

The simulation of phantom A demonstrated that the speckle tracking algorithm we were going to use was capable of measuring the strain value using ultrasound B-mode
image data (RF data, with both amplitude and phase). The mean results of both lateral and axial direction were very close to the expected value. The variance of the lateral direction was large, but it was due to the ultrasound imaging system itself and the speckle tracking technique (lack of phase information for the lateral direction). The variance of the axial direction was small, and it would be a good idea to try to use the axial strain to calculate the lateral strain (i.e., using Poisson’s ratio). However, we found later that it was not very useful when the experiment environment was not as ideal as that being simulated. This will be discussed in Chapter 3 and 4.

The simulation of phantom B showed that the geometric properties of the imaging target could cause problems! The axial tracking once again obtained very good estimation of strain along the axial direction (or transverse direction, with respect to the cylinder itself), with a small standard deviation, though larger than that of phantom A. The measured lateral displacement was consistent with the projected actual displacement only within the center region, the farther away from the center region, the larger the difference between the measured and the projected actual displacement. The bumpy displacement map also caused non-uniform strain pattern. But a close estimation could still be made if we focused on the center region.

This brings up a new challenge. How can the region of interest be picked objectively? Our answer is the principal component analysis (PCA). The method of PCA is described in detail in Appendix B.

There are many more simulations that could be carried out. First of all, I would like to quantify the size of the center region where the speckle tracking measurement is consistent with the projected actual displacement. I would call it the optimal window for speckle tracking. I would guess the central angle ($\alpha$) corresponding to the optimal window is a constant (about $25^\circ$ for phantom B here), and the optimal window size ($L$)
can be calculated by

\[ L = 2 \cdot R \cdot \tan \left( \frac{\alpha}{2} \right), \]  

(2.10)

where \( R \) is the radius of the curved cylinder. In case of a curved cylinder with curvature \( \frac{1}{10 \text{ mm}} \), the optimal window is about 4.6 mm. It can be verified by changing the curvature of the curved cylinder in phantom B.

As mentioned before, there are sources of error other than the out-of-plane motion. It is also important to find out how the other sources of error would affect the speckle tracking result. The models I used are just cylinders with simple symmetric properties and independent random scatterers. It would be more realistic to use an actual tendon model with fibers instead of small scatterers and more biology constraints. I also wasn’t able to fully replicate the ultrasound transducer we were going to use for the cadaver and in vivo experiment. Once such a transducer is simulated, and a better tendon model is used, we can compare the simulation result more accurately to any experiments directly. Lastly, it would be interesting to see how the key specifications of an ultrasound transducer, such as the center frequency and number of elements, affects the speckle tracking. Commercial ultrasound probes are expensive, but with Field II ultrasound simulation software, one should be able to model most types of transducer currently available (and even those which haven’t been built yet).
Chapter 3

ULTRASOUND ELASTICITY IMAGING OF HUMAN CADAVER POSTERIOR TIBIAL TENDON

3.1 Goals of cadaver project

As mentioned earlier, the mechanical properties of the tendon are believed to be changed by the degradation of its collagen structure. If these properties could be monitored in vivo, they could be used to quantify the severity of tendonosis and help determine the appropriate treatment. The Achilles tendon has been studied by many research groups, and its mechanical properties, such as Young’s modulus, have been reported several times, though with great variability (from 0.82 GPa to 2.2 GPa).[8] The tibialis anterior tendon has been reported to have a Young’s modulus of 1.2 GPa.[9] However, the previous studies which used ultrasound used it mainly to image the musculotendinous junction and to measure the displacement of the junction related to a fixed marker.[5] As a result, the Young’s modulus reported was for the entire tendon. Ultrasound elasticity imaging can image any location in addition to the musculotendinous junction, and
provide localized information at that specific location.

Another limitation of determining displacement by imaging only the junction with markers is that it requires the tendon to be straight. This also explains why most of the studies were focused on Achilles tendon and tibialis anterior tendon. The fact that the posterior tibial tendon is curved, and the curved location is exactly where the localized tendon degeneration is believed to occur, makes UEI the perfect tool to measure the mechanical properties of the posterior tibial tendon.

In order to utilize UEI with living subjects, a lot of preparation is required. In the previous chapter, we have demonstrated the capability of UEI using simulation and even predicted the non-uniform strain pattern in a curved cylinder model. In this chapter, a description of a cadaver experiment presents the final preparation for the \textit{in vivo} testing.

The primary goal of this project was to develop and validate the UEI technique when performed on the human PTT using a cadaver model and to obtain baseline estimates of its mechanical properties. A secondary goal was to determine the relationship between the tensile force applied to the PTT and the corresponding inversion force generated at the forefoot which is a measurable parameter for future \textit{in vivo} experiments. The long-term goal was to create a quantitative \textit{in vivo} test that can be employed clinically for grading degenerative tendon diseases, aiding in prognosis, and helping guide treatment decisions.

\subsection{3.2 Experimental design and data collection}

Five thawed human cadaver feet from male and female donors aged from 68 to 92 years were transected mid-leg, potted in a Cerrobend pot, and mounted in a materials testing system (MTS, Model 810 Systems Corporation, Eden Prairie, MN). The proximal portion of the PTT was dissected free from the muscle, whipstitched with high strength suture, and attached via a steel cable to a load cell mounted on an actuator (Figure 3.1).
The exposed portion was wrapped with gauze soaked in saline to prevent desiccation.

Figure 3.1: (Left:) Photograph of the experimental setup, including the MTS and the 14 MHz hockey stick linear array (“A”). The monitor displays a B-mode image of the PTT. The primary load cell (“LC01”) measured the load force (“F”) applied to the tendon while a secondary load cell (“LC02”) measured the inversion force. (Right:) Closer view from a reverse angle displaying imaging side of the foot. The primary load cell (“LC01”) pulled the PTT proximally via a steel cable. The yellow arrows denote the direction of pulling while loading. The foot was potted in a Cerrobend pot (“Pot”) using the fibula and tibia (“B”). The red line near the ankle marks the standard position of the ultrasound probe over the PTT.

A commercial 14 MHz linear array ultrasound probe (L14-5, Zonare Medical Systems, Mountain View, CA) was positioned over the PTT adjacent to the medial malleolus. This portion of the tendon was chosen because it is believed to be the most vulnerable to degeneration and most often affected by PTTD. The probe was connected to a portable ultrasound scanner (zOneUltra, Zonare Medical Systems, Mountain View, CA) controlled by a PC running MATLAB™ (Mathworks Inc) to acquire ultrasound frame data (both amplitude and phase) at a frame rate of 50 Hz for 6 seconds. The ultrasound probe was held by an experienced sonographer with its long axis aligned parallel to the PTT.

A preload of 10 N was applied to maintain the tendon under tension. The specimen was then subjected to three load-unload cycles. Each cycle lasted for 5 seconds, during which the axial force was gradually increased from preload to 588 N (60 kg, approx-
imating the weight of an adult female) and the tendon was then allowed to return to preload. Inversion force measurements were obtained during the trial from a second load cell that abutted the medial aspect of the first metatarsal head. Stroke of the actuator, representing the displacement of the proximal end of the PTT, was recorded by the MTS machine.

Transverse B-mode images were used to determine the cross sectional area (CSA), denoted as $A$, of each PTT. The boundary of the tendon was traced three times at three different locations (inframalleolar, retromalleolar, and supramalleolar), and the average area was used to calculate tendon stress. The total length of the PTT was measured at the conclusion of the experiment.

Frame-to-frame displacements were calculated during the loading portion of each cycle. A 2D phase sensitive cross correlation algorithm, described previously for other biomedical applications[24, 25, 26, 36], produced a displacement map along both directions at each pixel location in the ultrasound image, along with the correlation map (tractogram) for estimating tracking confidence. Strain was calculated from the displacement along the tendon axis (longitudinal, or x direction): For each pixel, the longitudinal stain $S_{xx}$ was calculated from Eq.1.4

$$S_{xx} = \frac{d_2 - d_1}{L},$$

where $d_1$ and $d_2$ are the displacements at each end of the window of length $L$. This was repeated across the entire image, yielding longitudinal strain information throughout the tendon and surrounding structures within the B-mode image. Finally, the loading force ($F$) recorded by the MTS was used to calculate stress $\sigma$ from

$$\sigma = \frac{F}{A}.$$

Young’s modulus $E_{xx}$ was then computed by dividing the estimated stress by the mea-
sured strain

\[ E_{xx} = \frac{\sigma}{S_{xx}}, \quad (3.2) \]

which is a simplified version of Eq.1.5, because transverse stress is small in our case.

To automatically isolate the tendon from background structures, and objectively choose a region of interest (ROI), Principle Component Analysis (PCA) was performed on the lateral displacement maps during the load cycle. Because the PTT has larger displacements and velocities compared to the surrounding tissue, the peak of the first principal component would always be within the PTT. The ROI was then chosen to be a rectangular region (3.9 mm \( \times \) 1.2 mm) centered at the weighted centroid of the first principal component for each trial. The mean and standard deviation (SD) of the displacement, strain and Young’s modulus of the PTT were calculated over this ROI across multiple trials. For detail discussion about PCA, please see Appendix B.

For this cadaveric study, UEI measurements were compared with the MTS as the gold standard for estimating Young’s modulus for the entire tendon. The stroke (displacement) recorded by the MTS was expected to be larger than the displacement from UEI, because the former represents the displacement of the tendon at the free end, and the latter the displacement at a region near the fixed end. Nevertheless, the two should be strongly correlated. Stroke was converted to strain for the MTS using the total length of the PTT. For comparison and validation of UEI with MTS, we expected the strain values to be similar for the two techniques. Furthermore, the average Young’s modulus determined by the MTS over the entire tendon was calculated using the same equations described previously and compared with that of UEI. The coefficient of determination \( (R^2) \) between UEI and MTS was also calculated across the specimens for displacement, strain and Young’s modulus.

Finally, to further verify the accuracy of UEI, we compared video tracking of sur-
face strain with ultrasound strain estimates in one tendon. This validation was performed in the supramalleolar portion of the PTT as it was not practical to perform video tracking in the retromalleolar position where the tendon bends around the malleolus. UEI was first performed with the skin intact and the imaging location marked. The skin at the marked location, subcutaneous tissue and tendon sheath were then dissected to expose the surface of the PTT. Fiducial ink marks were placed on the tendon surface. The same load-unload cycle was performed, and the movement of the PTT was recorded using a digital camera (Canon G12). Video strain was estimated using a motion analysis software package (Tracker, Video Analysis and Modeling Tool[51]). The coefficient of determination ($R^2$) and mean difference were then calculated to compare UEI and video tracking strain values.

### 3.3 Measurement of cross sectional area

![Ultrasound B-mode images for the cross section of a cadaver PTT at three different locations (upper row), and the boundary of the tendon was traced to calculate its cross sectional area (lower row). The green bar denotes 5 mm. Notice that the coordinates here is y-z, not x-z.](image)

Figure 3.2: Ultrasound B-mode images for the cross section of a cadaver PTT at three different locations (upper row), and the boundary of the tendon was traced to calculate its cross sectional area (lower row). The green bar denotes 5 mm. Notice that the coordinates her is y-z, not x-z.

Figure 3.2 shows the cross section of one cadaver PTT at three different locations. The boundary of the tendon was traced three times for each location and the averaged area was recorded.
3.4 Speckle tracking results

UEI displacement and strain maps for a representative PTT are displayed in Figure 3.3. The primary motion and deformation occurred along the longitudinal axis of the tendon.

Figure 3.4 presents a map of the 2D speckle tracking correlation coefficient, longitudinal displacement, strain, and Young’s modulus for one tendon at an applied load of 550 N. The displacement map (Figure 3.4b) clearly identifies the tendon from surrounding tissue. The displacement within the tendon (3.33±0.23 mm), over the ROI displayed in Figure 3.4b, is much higher than the displacement outside the tendon (0.87±0.04 mm and 0.09±0.08 mm in the malleolus and the dermal layers, respectively).
Figure 3.3: UEI displacement and strain of the PTT during a representative loading-unloading cycle. **Left column, from top:** B-mode image with PTT between two red dotted lines, longitudinal (x) displacement, longitudinal strain, transverse (y) displacement, and transverse strain over small regions (1.5 × 0.5 mm). The green scale bar in the image = 5 mm. **Right column, from top** Force measured at the proximal end of the PTT by the primary load cell, longitudinal displacement (Disp.), longitudinal strain, transverse displacement, and transverse strain in the region enclosed by the red box in the left column.
Figure 3.4: (a) 2D Map of the speckle tracking correlation coefficient (average over one cycle). Green scale bar = 5 mm, S = Skin, M = Malleolus. (b) Longitudinal displacement map. The three boxes denote the ROIs used to calculate the displacement within and outside the tendon. (c) Strain map at a near maximal load (550 N) superimposed on the B-mode image (gray). (d) Image of Young’s modulus in tendon above malleolus. The discontinuity in the strain map was due to out-of-plane motion of the PTT as it turned around the ankle joint. This was more evident in the B-mode movies. (e) Young’s modulus vs. loading force, averaged over the green box in (d).
3.5 Strain and Young’s modulus validation using MTS and video tracking

Table 3.1 lists the CSA and total tendon length, longitudinal displacement, strain, and Young’s modulus of each specimen.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>CSA [mm²]</th>
<th>Length [mm]</th>
<th>Displacement [mm]</th>
<th>Strain [%]</th>
<th>Young’s Modulus [GPa]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MTS</td>
<td>UEI</td>
<td>MTS</td>
<td>UEI</td>
<td>MTS</td>
</tr>
<tr>
<td>1</td>
<td>23.0±3.8</td>
<td>210</td>
<td>6.5±0.8</td>
<td>1.3±0.8</td>
<td>3.1±0.2</td>
</tr>
<tr>
<td>2</td>
<td>24.8±0.6</td>
<td>215</td>
<td>11.1±0.4</td>
<td>3.3±0.1</td>
<td>5.2±0.2</td>
</tr>
<tr>
<td>3</td>
<td>30.8±1.5</td>
<td>230</td>
<td>13.0±0.3</td>
<td>3.6±1.3</td>
<td>5.7±0.1</td>
</tr>
<tr>
<td>4</td>
<td>31.3±1.8</td>
<td>180</td>
<td>7.5±0.1</td>
<td>1.6±0.6</td>
<td>4.2±0.1</td>
</tr>
<tr>
<td>5</td>
<td>28.1±1.6</td>
<td>210</td>
<td>6.6±0.4</td>
<td>1.9±0.7</td>
<td>3.2±0.2</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>27.6±3.6</td>
<td>209±18</td>
<td>8.9±2.9</td>
<td>2.3±1.0</td>
<td>4.2±1.2</td>
</tr>
</tbody>
</table>

Table 3.1: Summary of tendon sizes and mechanical properties. The longitudinal displacement, strain, and Young’s modulus were at near-maximal force (550 N). The donor ages for the five specimens ranged from 68 to 92 years old. Specimen 3 and 5 were from male donors.

Figure 3.5 compares results for the MTS and UEI for measuring displacement ($R^2=0.95$), strain ($R^2=0.88$), and Young’s modulus ($R^2=0.96$). The average Young’s modulus for the five tendons calculated from UEI was 0.45±0.16 GPa, compared with 0.52±0.18 GPa for measurements from the MTS.

Figure 3.6 directly compares strain measurements obtained using UEI with those using video tracking for one tendon. Video strain at the surface of the tendon was similar to UEI measurements within the tendon ($R^2 = 0.80$). The mean difference between the two strain curves was 0.11±0.03 %.

3.6 Relationship between load and inversion force

Figure 3.7 describes the relationship between the applied load and inversion force recorded by the load cell. The slope and $R^2$ values were 0.12±0.01 and 0.999, respectively, indicating that the inversion force is approximately one eighth of the applied load. This relationship between the inversion force, a measurable quantity in vivo, and
Figure 3.5: Comparison of longitudinal displacement (Top), strain (Middle), and Young’s modulus (Bottom) using MTS (gray, entire tendon) and UEI (black, localized region). All values were calculated at 550 N. The red error bar denotes standard deviation (SD), which was calculated for each specimen over three trials. The displacement for the MTS was much larger than UEI because the measurement occurred across the entire tendon rather than just the local region above the malleolus. However, there was a high correlation between UEI and MTS for displacement, strain, and Young’s modulus for the five specimens.
Figure 3.6: Comparison between video tracking and UEI for measuring strain for the same tendon at supramalleolar location. **Top Left:** Photograph of top surface of tendon with fiducial ink markers placed for optical tracking. **Bottom Left:** Ultrasound image at the same location, green bar = 5 mm. **Right:** Strain measured at the surface of the PTT using video (black) and inside the PTT using UEI (red). The average strain for UEI was computed over the red box in the B-mode image. The mean difference between the two strain curves was $0.11 \pm 0.03 \%$.

Figure 3.7: Relationship between applied load and inversion force for five tendons. This plot includes results for three trials for each of the samples. The average slope is $0.12 \pm 0.01$ with $R^2 = 0.999$. 

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the force on the tendon is important for future clinical applications of UEI in patients with PTTD.

3.7 Discussion and conclusion

The primary goal of this study was to develop and validate the technique of UEI of the PTT. Our results indicate that UEI is capable of accurately measuring the mechanical properties of the human PTT in a cadaveric model. UEI estimates of displacement, strain, and Young’s modulus were all highly correlated with those obtained from the stroke of the MTS crosshead (Figure 3.5). There was close agreement between Young’s modulus for UEI (0.45±0.16 GPa) and MTS (0.52±0.18 GPa). Differences could be explained by regional variations in the measurements. Whereas UEI measured the mechanical properties over a small, high-stress region above the malleolus, the MTS recorded an average value across the entire length of the tendon. The tendon might also be more compliant in the imaged retromalleolar region. Moreover, dynamic video tracking of surface strain was highly consistent with ultrasound strain estimates (Figure 3.6).

Two previous studies measured Young’s modulus of the dissected PTT (0.81±0.14 GPa and 0.91±0.23 GPa).[52, 53] However, these values were at a much higher tendon load (2500 N and 3600 N) than our study (588 N). Based on the load-displacement curve provided in one of these studies, Young’s modulus was approximately 0.45 GPa at 600 N[53], which is similar to our result.

One potential limitation with this project relates to the use of thawed cadaveric specimens. There is evidence that freezing followed by thawing alters the structure and mechanical properties of the tendon.[52] That study found that frozen specimens of the human posterior tibial tendon, compared to fresh specimens, exhibited 30 % lower stress and strain than fresh specimens primarily due to a decrease in collagen
fibril density. However, Young’s modulus was not significantly different between the two groups, implying that Young’s modulus for thawed PTTs is likely similar to fresh PTTs.

Another potential limitation of the study relates to speckle tracking noise and out-of-plane motion of the tendon. For optimal imaging, it is important that the sonographer maintain the probe parallel to the long axis of the PTT during the loading phase. The speckle tracking coefficient and B-mode movie provide feedback for the quality of the images. Figure 3.4c provides evidence of out-of-plane motion of a portion of the tendon as it turned around the malleolus. This led to a non-uniform strain pattern. In addition, because the primary motion of the tendon was along its axis, transverse displacements and strain were much smaller compared to longitudinal displacements. The transverse displacement and strain would likely be more sensitive to deformation at the tissue interfaces and pressure applied by the ultrasound probe, while less sensitive to the transverse deformation of the PTT. Nonetheless, the transverse displacements were not used for calculating the mechanical properties of the tendon.

UEI is a potentially powerful, noninvasive in vivo technique to objectively quantify the mechanical properties of the PTT and other tendons. This would require estimating tensile load (or stress) noninvasively, which is a challenge in vivo. In this study, the load applied to the PTT and the resulting inversion force at the forefoot were simultaneously measured. Their relationship was linear with a slope between the inversion force and tendon load of $0.12 \pm 0.01$. This value could be used to estimate tensile load from the measured inversion force obtained in vivo. A similar approach was successfully implemented in a study of the human Achilles tendon.[54] For in vivo human testing, it would be necessary to fix the ankle in maximal plantar flexion so that other tendons would not be able to contribute to inversion of the foot. It would also be necessary to brace the lateral malleolus to prevent lateral translation of the ankle. The development of techniques for precise and accurate measurement of the inversion force is an important step.
in the development of UEI as a clinical tool for guiding treatment of tendinopathies.

In conclusion, we have demonstrated that ultrasound elastography reliably quantifies the mechanical properties of the PTT in a human cadaveric model. This is an important first step in the development of UEI as a clinical tool for objectively quantifying tendon mechanical properties in patients with tendinopathies. Such a clinical tool could aid in the prognosis, guide treatment decision-making, and monitor response to treatment for a number of degenerative tendon disorders, including PTTD.
Chapter 4

IN VIVO ULTRASOUND
ELASTICITY IMAGING OF
HUMAN POSTERIOR TIBIAL TENDON

4.1 Goal of *in vivo* project

Ultrasound imaging, like other imaging techniques, gives us the ability to look at things we normally couldn’t see. But we also want to “feel” what we are looking at. This is exactly what ultrasound elasticity imaging provides. During imaging, we can measure the mechanical properties of the target tissue. Siemens™ called one of their Acoustic Radiation Force Impulse (ARFI) systems a “Virtual Touch”. This is the perfect name for any ultrasound technique that can estimate tissue elastic properties non-invasively.

Although we demonstrated the capability of UEI using cadaver specimens, the measurement doesn’t have to be invasive. In fact, the only invasive portion of our experiment was to measure the tensile force using MTS. By obtaining the linear relationship
between inversion force and the tensile load, it is now possible to apply UEI to living subjects.

In this chapter, *in vivo* testing is described. The ultimate goal would be to find the difference between PTTD tendons so that UEI could help guide treatment decision-making for advanced tendinopathies. But first we need to determine whether there are detectable and quantifiable differences between healthy tendons and those with advanced-stage PTTD. If the degeneration of PTT is truly causing the changes in mechanical properties of the tendon, we should be able to notice the difference between healthy and diseased tendons using UEI.

### 4.2 Experimental design and data collection

Eight healthy volunteers and nine patients with PTTD were recruited for *in vivo* testing. Both left and right feet were tested, yielding 34 tendons in total. Three out of the nine PTTD patients have bilateral PTTD. As a result, there were 12 PTTD tendons, and 22 healthy tendons. From now on, I’ll use the term “subject” to refer to a volunteer or patient.

#### 4.2.1 Customized inversion platform for UEI

A customized inversion platform was made from optical post assemblies and a standard optical breadboard (Thorlabs, Inc) and mounted onto a lab bench. The subject would lie on his/her back on the bench and place his/her leg on the cast. We made sure the subject’s ankle was in maximal plantarflexion to isolate PTT and minimize load sharing with other tendons. Velcro was used to secure the position.

A dynamometer (TSD121C, BIOPAC Systems, Inc. Santa Barbara, CA) was placed against medial aspect of the subject’s first metatarsal head. This was similar to our cadaver experiment where we positioned the second load cell, so that we could measure
the inversion force and use the scaling factor to calculate tensile force when necessary. The dynamometer was connected to a data acquisition system (MP100A, BIOPAC Systems, Inc. Santa Barbara, CA) controlled by a PC.

The same 14 MHz linear array ultrasound transducer (L14-5, Zonare Medical System, Mountain View, CA) was positioned posterior to the medial malleolus. The probe was connected to the same portable ultrasound scanner (zOneUltra, Zonare Medical Systems, Mountain View, CA) used in the cadaver experiment. As usual, the ultrasound probe was held by an experienced sonographer with its long axis aligned parallel to the PTT of the subject. An adjustable rotation arm was also used to help the sonographer steady his/her hands.

We first asked the subject to push the dynamometer as hard as he/she could so that we would obtain the maximum inversion force he/she could manage. It was repeated three times, and the averaged value of the maximum inversion force was recorded for future reference: At least 80% of the average maximum value should be reached during
After taking the maximum force measurement, the subject would relax his/her foot but still keep contact with the dynamometer. The operator would count down as “3, 2, 1, go” and the subject would gradually push his/her foot against the dynamometer, from relaxation to at least 80% of maximum in about 2 seconds, hold for about 1 second, then relax gradually in about 2 seconds. The subject would be able to watch the real-time force curve displayed on a computer screen simultaneously so that he/she could be in control of the time and amount of force during the inversion task. However, even with the feedback, the subject often found it difficult to follow the 2s-1s-2s instruction, especially for PTTD subjects.

Figure 4.2: **Left:** Ultrasound B-mode image of PTT from a healthy subject’s left foot, S = skin, M = Malleolus; **Right:** The inversion force vs time curve during a inversion task. The maximum inversion force is much smaller than the maximum tensile load in the cadaver study.

The movement of the PTT over 6 seconds was captured with the ultrasound scanner at a frame rate of 50 Hz. The inversion force applied onto the dynamometer was also recorded. Section 4.2.3 will discuss the synchronization of ultrasound and force recordings. Figure 4.2 shows the ultrasound B-mode image of the PTT and the inversion force curve from a healthy subject.

The subject was then asked to repeat the inversion task several times, until at least 6 clear ultrasound movies were captured. Healthy subject usually took 8 trials to get enough good movies, but it could take more than 10 trials for a PTTD patient to do so with the diseased tendon. There was a 1-2 minute break between each trial for the
subject to recover from the previous trial. On rare occasions, the subject would feel soreness or even pain in the PTT, and we would release the subject from the mounting board and allow a short rest. The maximum force would be re-measured after the rest.

In addition to placing the ankle at maximal plantarflexion, we also instructed the subject to use only the PTT to generate force. We had to keep observing the subjects and prevent them from using their upper body. I found that when told to “relax the knee”, the subject was less likely to use other body parts during the inversion task.

The cross sectional area was measured at three different locations (inframalleolar, retromalleolar, and supramalleolar, as in the cadaver experiments) at the end of the testing for each foot. The averaged value of the cross sectional area of the three location was recorded as the CSA of the testing PTT.

4.2.2 Synchronization of ultrasound and force measurements

One of many important lessons we learned from the cadaver experiment is that we need to synchronize the ultrasound signal and the force recording. Ultrasound recording was controlled by a PC running MATLAB™, while force recording was done by “AcqKnowledge” (version 3.9.1) provided by BIOPAC Systems. After examining the trigger out signal from the L14-5 transducer, we decided to use the negative edge of the trigger out signal as the external trigger for the force recording, by connecting the L14-5 transducer and the dynamometer with a BNC to 3.5mm phone plug connector.

During the in vivo testing, the operator who was in charge of data collection would first freeze the ultrasound signal with a customized MATLAB™ GUI (Appendix D), followed by a countdown of 3 seconds (AKA “pre-scan pause”, which can be changed with the GUI). During the countdown, the operator would start AcqKnowledge, but the dynamometer would wait for the trigger from the L14-5 transducer to start recording. When the 3 second countdown was over, the subject would start the inversion task as instructed, and the transducer would be unfrozen by the GUI automatically and start
transmitting ultrasound pulses. At the same time, a trigger would also signal the dynamometer to start force recording.

In this way, we don’t have to use cross-correlation as in the cadaver project to synchronize displacement and force.

4.3 Speckle tracking results: displacement, strain and elastic modulus

Once the ultrasound data were obtained, the same speckle tracking program was used to calculate the 2D displacement between every other two ultrasound B-mode frames (RF data with phase and amplitude). PCA was then performed with the longitudinal displacement data to separate the PTT from the surrounding tissue and determine the location of the ROI within the tendon.

One difficulty we encountered during the in vivo experiment was that the force-control by a person was often worse than that by the MTS machine. A rapid drop of force after reaching the maximum will cause a rapid drop of the displacement. As a result, the displacement vs time curve was often not symmetrical, and the total displacement of the PTT for the entire inversion task sometimes dropped below zero after the subject decreased the inversion force. This “net negative displacement” would affect the PCA result dramatically. To avoid this kind of problem, I used only the “ramp-up” (i.e. the “loading phase”) of the displacement data to do the PCA. By doing so, the tendon would have the largest total displacement, and therefore the largest change of the displacement.[Figure 4.3]

The same method described in the previous chapter was used to calculate strain. The inversion force was used to calculate the “elastic modulus”, which can be converted to Young’s modulus using the scaling factor we obtained in cadaver experiments.
4.3.1 Displacement and strain

As before, the speckle tracking program calculated the 2D displacement of both longitudinal and transverse directions and 2D strain maps of both directions. The speckle tracking results of one trial from the healthy subject whose data has been used in Figure 4.2 and Figure 4.3 is used here as an example. Figure 4.4 shows the correlation coefficient $R$ of two ultrasound B-mode images (RF data with both amplitude and phase), and averaged $R$ within ROI as a function of time. Skin, which barely moved, has $R$ values near 1. The bone region has poor $R$ values because the ultrasound signal was...
not able to penetrate. The tendon region has very good R values, the low-R-valued region towards the right edge is clearly the result of out-of-plane motion (also due to the fact that speckles were moving out of the right edge). The correlation coefficient stayed above 0.9 for most of the loading phase, and this means the correlation is good and trustworthy. Around $t = 1$ s, the R value fluctuated rapidly, but was still above 0.8. This fluctuation will also be seen in the strain vs time curve.

Figure 4.5: Accumulated displacement of PTT from a healthy subject. **Upper left:** Longitudinal displacement map at $t = 2.4$ s. Skin has near zero displacement. Tendon moves to the right, and the malleolus has very small negative movement to the left. **Upper right:** Average accumulated longitudinal displacement within ROI (green box) vs time. **Lower left:** Transverse displacement map at $t = 2.4$ s. It seems that everything within the imaging area was moving downwards, though slightly (less than 0.5 mm). This could be the result of bulk movement of subject’s foot. **Lower right:** Average accumulated transverse displacement within ROI vs time. It’s much smaller than the longitudinal displacement.

Figure 4.5 shows the displacement map at time $t = 2.4$ s. The longitudinal displacement map once again separates the tendon from the surrounding tissues. Averaged within the ROI determined by PCA, the longitudinal displacement vs time curve was similar to the inversion force vs time curve in Figure 4.4. The transverse displacement indicated that subject’s foot moved downwards during the inversion task. We can barely
separate the tendon from the surrounding tissues in the transverse displacement map. Transverse displacement alone did not provide us much valuable information about the mechanical properties of the PTT.

Figure 4.6 shows the displacement map of at time $t = 2.4$ s. The longitudinal strain pattern was similar to the simulation of phantom B in Chapter 2, with negative (blue) regions mixed in positive (red) regions, but the PCA successfully located the positive region for the ROI. The average strain value within the ROI vs time curve was consistent with the inversion force curve. The transverse strain pattern and curve also provided useful information about the mechanical properties of the PTT, especially the fact that the transverse strain was negative and decreasing (increasing in absolute value) while the PTT was lengthening along the longitudinal direction. Unfortunately, the transverse strain results of the other tendons were not always as good as this example. As a result, like the cadaver study, only the longitudinal strain was used for further analysis.
Figure 4.6: Strain pattern, and averaged strain within ROI of PTT from a healthy subject. **Upper left:** Longitudinal strain \( (S_{xx}) \) at \( t = 2.4 \) s. Once again, the strain pattern at tendon region had non-uniform distribution just like the simulation of phantom B due to out-of-plane motion. The ROI determined by PCA successfully located the positive strain area. Strain at skin region was close to 0 and noisy due to the white noise. Strain values at malleolus was also very noisy, but those values could not be trusted due to low correlation coefficient [Figure 4.4]. The cold (light blue) region to the right of the ROI could be explained as the surrounding tissue being pushed/squeezed by the tendon. **Upper right:** Average longitudinal strain value within the ROI as a function of time. As expected, the strain kept increasing during the loading phase. **Lower left:** Transverse strain \( (S_{yy}) \) at \( t = 2.4 \) s. The tissue under skin was being stretched (color red) by the tissue moving downward. The strain pattern of the PTT was complicated, but the ROI fell onto a negative region. **Lower right:** Average transverse strain value within the ROI. There are two important pieces of information: 1. During the time with “bad” correlation coefficient (see Figure 4.4, around \( t = 1 \) s), the transverse strain had a sudden drop. This means the transverse strain measurement is much more sensitive to movement than the longitudinal strain, and may not be trusted when R value is not large (less than 0.9). 2. After the sudden drop, the transverse strain behaved normally, and the fact that it was negative and decreasing means the PTT was indeed being squeezed more and more along its radial direction while being stretched along longitudinal direction. The fact that transverse strain was not half of the longitudinal strain may indicate that the Poisson’s ratio was not 0.5 for this PTT, which is quite possible considering the longitudinal strain was very large.
4.3.2 Stress strain curve and elastic modulus vs force curve

The stress-strain curve is often used to describe tissue mechanical properties. Figure 4.7 shows the stress-strain curve from one healthy tendon and one PTTD tendon. The color lines in each plots denote different trials. The result from all six “good” trials of the healthy tendon are included but only three of the PTTD tendon are included. This is typical because even if the ultrasound movie seemed to be visually “good”, the tracking result sometimes tells a different story. Therefore, a criteria was set so that “bad” data sets would be excluded; for example, data sets with a strange displacement behavior when compared with force or negative average strain within ROI determined by PCA.

Figure 4.7: Comparing mechanical properties between a healthy and a PTTD tendon, each colored line denotes a different trial. **Upper left:** Stress-strain curve of a healthy tendon. **Upper right:** Stress-strain curve of a PTTD tendon. **Lower left:** Elastic modulus vs inversion force curve of a healthy tendon. **Lower right:** Elastic modulus vs inversion force curve of a PTTD tendon.

Figure 4.7 shows that even for the same tendon, a different trial gives different strain values for the same stress values. For this healthy tendon (the one I used previously as an example), at stress value of 2 MPa, the strain value ranged from 10 % to 40 %; for the PTTD tendon, the strain value varied between 10 % to 30 % at a stress value of
2 MPa. As a result, the elastic modulus also varied a lot between different trials of the same tendon at the same inversion force.

4.3.3 Problem: How to quantify the difference?

Because of the large variance of the strain and the elastic modulus between different trials of the same tendon, it is definitely not ideal to use either of these two values to quantify the differences between healthy and PTTD tendons. [Figure 4.8]

![Figure 4.8: Distribution of elastic modulus at max inversion force. The maximum force from different subjects are very different. The elastic modulus for subjects at their maximum inversion force are also very different. It is hard to separate PTTD tendons from healthy ones.](image)

However, by looking at the stress-strain curves alone (Figure 4.7 Upper row), one can easily identify the difference between the two: the strain of healthy tendon increases as the stress increases (linear behavior), but the strain of the PTTD tendon only increases in the very beginning then almost stops when the stress increases further (nonlinear behavior). Usually, a linear behavior indicates that the tendon is still in its elastic region, while nonlinear behavior indicates the stress (or force) has passed the tendon’s elastic region. This difference causes a flat elastic modulus vs force curve for the healthy tendon, and a steep sloped curve for the PTTD tendon.
4.4 Slope of the elastic modulus vs force curve: a Non-linear Elastic Parameter (NEP)

The slope (or \(\Delta E/\Delta F\)) value of elastic modulus (E) vs force (F) curve was calculated using a best-fit line through the linear region of each trial curve. The rapidly decreasing region in the beginning of the E vs F curve was not included during the fitting because it was due to the near-zero strain value when the inversion force is small. For the healthy tendon in Figure 4.9, \(\Delta E/\Delta F = 0.02 \pm 0.06\) MPa/N. For the PTTD tendon, \(\Delta E/\Delta F = 0.43 \pm 0.12\) MPa/N.

Figure 4.9: Load dependence of elastic modulus of one healthy tendon and one PTTD tendon. A best-fit line was used to determine the slope (\(\Delta E/\Delta F\) value) of each elastic modulus (E) vs inversion force (F) curve. **Left:** A healthy tendon, \(\Delta E/\Delta F = 0.02 \pm 0.06\) MPa/N. **Right:** A PTTD tendon, \(\Delta E/\Delta F = 0.43 \pm 0.12\) MPa/N.

Figure 4.10: Distribution of the NEP of both PTTD and healthy group. The \(p\) value is from the student t test.

Because this value describes the linear or non-linear elastic property of the PTT, it was named the Non-linear Elastic Parameter, or **NEP**.
After obtaining the NEP for all the 34 tendons, the averaged value for healthy tendons is $0.16 \pm 0.20$ MPa/N, and $0.45 \pm 0.43$ MPa/N for PTTD tendons. The distribution of the NEP is shown in the Figure 4.10. The mean value of the PTTD group is much larger than that of the healthy group. A student t test shows a statistically significant differences with a $p < 0.01$ which means those two groups are independent of each other.

### 4.5 Receiver operating characteristic (ROC) curve

By defining the NEP, we now have a feature by which to clarify whether a tendon is healthy or not. If NEP is smaller than a threshold value, the tendon is healthy. If the NEP is larger than the reference value, the tendon is likely to be diseased. The choice of the threshold directly affects the efficiency of the classifier system. A high threshold would make sure the tendons classified as diseased are truly diseased, but would also mark many diseased tendons as healthy only because they have a smaller NEP.

Receiver operating characteristic (ROC) curve, is a graphical plot which illustrates the performance of a binary classifier system as its discrimination threshold is varied. The curve is created by plotting the true positive rate against the false positive rate at various threshold settings. The true positive rate is also known as “sensitivity” in biomedicine, or “recall” in machine learning. The false positive rate is also known as the “fall-out” and can be calculated as 1 - specificity.[55]

With the distribution of the NEP shown in Figure 4.10, a ROC curve was made by a ROC analysis software(ROC-kit, Metz ROC Software, University of Chicago[56, 57, 58]) as in Figure 4.11. The area under the curve (AUC) is $0.83 \pm 0.07$. There is no general guideline for the AUC but an AUC of 0.83 normally indicates that the this classifier system is indeed valid.

For each point on the ROC curve, there is a corresponding threshold, sensitivity
and specificity. This means that when a certain sensitivity/specificity is required to distinguish healthy and PTTD tendon, we can find a threshold of the NEP to fulfill the requirement. However, it is not possible to find the threshold from the fitted ROC curve. Our sample size (34) is also not large enough to determine any desired threshold. A list of thresholds and corresponding sensitivity and specificity can be found in Table 4.1.

<table>
<thead>
<tr>
<th>NEP Threshold [MPa/N]</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>0.5</th>
<th>0.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (True Positive)</td>
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<td>0.83</td>
<td>0.75</td>
<td>0.50</td>
<td>0.33</td>
<td>0.17</td>
</tr>
<tr>
<td>False Positive</td>
<td>0.77</td>
<td>0.32</td>
<td>0.18</td>
<td>0.14</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.23</td>
<td>0.68</td>
<td>0.82</td>
<td>0.86</td>
<td>0.95</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Table 4.1: Table of selected threshold, sensitivity, and specificity from ROC curve. Using NEP of 0.3 MPa/N as the threshold, 75% of the diseased PTTs will be classified correctly as diseased, while 82% healthy PTTs will be marked as healthy. If a sensitivity of 80% is required, the threshold NEP should be set to be around 0.2 MPa/N with specificity of 0.68.

4.6 Explanation of the slope difference between healthy and PTTD subjects

In previous sections, we have claimed that the NEP value describes the non-linearity of the tendon, while near-zero NEP for healthy tendon indicates its linear elastic property.
This does not contradict the well-known fact that tendon exhibits non-linear mechanical behavior.

Figure 4.12: Example stress-strain tensile data of human supraspinatus tendon from previous study [59]. Arrows indicate the zero, transition and linear strain values. The red curve is how PTTD tendon behaves according to our study.

Tendon’s stress-strain curve is usually divided into three parts: zero, transition and linear.[Figure 4.12] The different parts are a result of the organization and behavior of the collagen fiber network. The distribution of collagen fiber alignment can be quantified by measuring the “circular variance”: the larger the variance, the less alignment. In a previous study, the fiber distributions were proven to become more aligned under load.[59]

For a healthy PTT, its stress-strain curve is very similar to the curve in the Figure 4.12, and the NEP is calculated using the linear part. For the PTTD tendon example in Figure 4.7, however, the majority of its stress-strain curve has already passed the linear part, and reached a “post-linear” region. In other words, the linear part of PTTD tendon is much shorter than that of healthy tendon, like the red curve in Figure 4.12. This could be explained by the fact that the collagen fiber of PTTD tendons align much faster under load than those of healthy tendons, due to less interlink caused by the irreversible degradation of its collagen structure.

Now that we know the large NEP value for PTTD tendon may be caused by the exis-
tence of a “post-linear” region, the NEP potentially becomes a fiber alignment indicator and shows the severity of the degradation.

4.7 Discussion, significance and limitations

The results from our initial testing with 8 healthy volunteers and 9 patients demonstrated that we managed to build a inversion task platform for in vivo UEI of human posterior tibial tendon, and we successfully found a detectable and quantifiable difference between healthy and PTTD tendons.

The inversion task platform was relatively easy to construct, and could be quickly modified to fit different subjects. The subjects also had real-time feedback to manage their inversion task performance. We improved the way data were collected by synchronizing the inversion force measurement with the ultrasound signal. Principal component analysis helped place the region of interest at the correct location objectively. The whole analysis demanded minimal subjective handling by the operator. However, to isolate the PTT during the inversion task turned out to be a big challenge. Further detailed instruction is necessary to help the subject develop a better understanding of the task required.

UEI successfully estimated the displacement and strain of the PTT of both healthy volunteers and PTTD patients. Longitudinal displacement was consistent with the inversion force, but transverse displacement mainly came from the bulk displacement of the foot movement. The longitudinal strain pattern was similar to the simulation of phantom B in Chapter 2, and the cadaver experiment; out-of-plane motion had certainly contributed to the mixed negative and positive strain pattern. The transverse strain pattern provided useful information for some tendons but not for the others, due to its high sensitivity to the deformation at the tissue interfaces and pressure applied by the ultrasound probe.
The *in vivo* PTTs exhibited more than 20% longitudinal strain and our cadaver testing measured only 5-10% longitudinal strain. As discussed in Chapter 3, there is evidence that the previously frozen tendons have different mechanical properties than fresh specimens.[52] The previously frozen tendons provide lower stress and strain due to a decrease in collagen fibril density. The dehydration, lack of blood supply, and low temperature during the cadaver study would also cause the tendons to be stiffer than *in vivo* tendons. The cadaver PTTs were also from an old age group (68-92 years old), while most of the *in vivo* subjects were under age of 40. All of these facts caused the large difference in longitudinal strain between *in vivo* and cadaver PTTs.

Inversion force was used to calculate the elastic modulus, instead of Young’s modulus. Unlike the cadaver experiment, we failed to obtain a precise strain value (or elastic modulus value) confined to a narrow range for each tendon. The strain values we obtained had large variations. This caused a large overlap of the results for the healthy and PTTD group. However, instead of using the strain or elastic modulus to distinguish the two groups, we used the slope of elastic modulus (E) vs inversion force (F) curve, a Non-linear Elastic Parameter (NEP), to describe the load dependence, and successfully separated the two groups. The NEP is an indicator of non-linear elasticity, and shows the severity of the degradation of PTT. The NEP of the healthy group was 0.16±0.20 MPa/N, and the NEP for the PTTD tendons was 0.45±0.43 MPa/N. This indicates that healthy tendons exhibit linear elastic behavior while PTTD tendons exhibit nonlinear load-dependent behavior. A ROC curve was made based on the NEP values. The area under curve AUC is 0.83±0.07, which confirms that a valid binary classifier system can be build based on the NEP to separate PTTD tendon from healthy PTTs.

In future studies, the ROC curve may be useful for helping with the prognosis of advanced PTTD and guiding treatment decision making. My hypothesis is that patients with a small NEP may need only bracing and therapy, while those with large NEP values may require surgery. This hypothesis can be tested when more PTTD patients
are evaluated. When PTTD patients, controlled subjects, and healthy volunteers are recruited in the future, it is also important to make sure they are age-matched for a valid comparison. Also, by restricting the age group, it may reduce the variance of the maximum inversion force they can generate, and the elastic modulus may eventually be used as another reliable indicator to help with the monitoring of the PTTD.
Chapter 5

CONCLUSIONS AND FUTURE WORK

Ultrasound elasticity imaging is a powerful tool to characterize the mechanical properties of human tendons. It is noninvasive, real time and affordable. The evaluation of the posterior tibial tendon turns out to be a challenge due to its geometric properties.

Two models with different geometries were used with an acoustic modeling program to verify the speckle tracking algorithm and simulate tendon deformation. The cylinder model demonstrated that the algorithm is capable of precise and accurate measurement of displacement and strain. The curved cylinder model indicated that out-of-plane motion will cause a non-uniform strain pattern within homogeneous isotropic material. The lateral strain pattern was consistent with what we encountered in the cadaver and in vivo experiment. As the result of the overshoot in the lateral displacement map, the negative lateral strain pattern may be reduced by applying spatial filters to the lateral displacement map.

The most important message from the simulation of the deformed curved cylinder is that it gives us the motivation and confidence to use the tracking result within a small region of interest (the optimal window of measurement), regardless of the irregular
negative strain pattern. The fact that the optimal window is around the region where the displacement changes most rapidly (spatially), is the very reason why the weighted centroid of the first principal component can be used to place the region of interest after performing PCA with longitudinal displacement.

The two tendon models were simple, but provided possible explanations for problems encountered during the cadaver and \textit{in vivo} experiment. More simulation should be carried out to answer further questions, such as the curvature-dependence of the optimal window size (Equation 2.10), and how the other sources of error would affect the tracking result. For the curvature-dependence simulation, one should not only change the curvature of the curved cylinder, but also the location of the imaging plane: to move the imaging plane between the outer radius and inner radius. A more sophisticated tendon model other than the simple cylinder would also make the simulation more realistic.

The cadaver experiment was a necessary step before \textit{in vivo} testing. We used the MTS to validate the UEI with 5 human cadaver feet. The displacement, strain and Young’s modulus measured using the MTS and UEI were consistent (coefficient of determination $R^2 = 0.95$, 0.88 and 0.96, respectively). Video tracking was also used to verify UEI ($R^2 = 0.80$). The average Young’s modulus of the 5 cadaver feet was $0.45 \pm 0.16$ GPa calculated by UEI, and $0.52 \pm 0.18$ GPa measured using the MTS. This value is similar to previous studies of human PTT. The MTS, video strain tracking, and results from previous studies all proved that UEI is capable of accurately measuring the mechanical properties of human PTT in the cadaver model.

Another goal accomplished by the cadaver experiment was that the relation between the inversion force (measurable \textit{in vivo}) and the tensile force of PTT (not measurable \textit{in vivo}) was established. The former is approximately one eighth of the latter. With this information, it is possible to use the inversion force to calculate the elastic modulus during an \textit{in vivo} experiment.
The cadaver experiment, as the first step of this project (it actually happened before the simulation), was not developed with enough sophistication. Many improvements could be made if this experiment was to be repeated. The combination of suture and steel cable we used was more than 1 meter in length, while the PTT itself was less than 30 mm. The total length of the system should be minimized, because the less the total length, the less error will be introduced. Steel cable was also not the best choice for our pulling system, because once the cable was bent at the pulley, it introduced extra elasticity to the system. The friction in the pulley system should also be minimized. A constant loading rate would improve the performance. It is also necessary to synchronize the force with the ultrasound signal.

For the ultrasound system, the longitudinal tracking result is capable of describing the movement of the PTT under motion. However, it would be better if the transverse tracking result could be used because of its higher resolution and accuracy. It may require a more stable data collection system including both the subject and the operator. Measuring the Poisson’s ratio in human tendons would be another interesting project, because if the Poisson’s ratio of the PTT is known, the longitudinal strain can be calculated from the transverse strain. It would also be helpful if we could improve the longitudinal tracking result using other signal processing methods.

Our in vivo experiment with 34 tendons is the initial step towards answering the question whether UEI could truly help with treatment decision making. The result we found pointed to a direction different than we expected. Instead of reporting strain or elastic modulus of PTT, we found the load dependence of the elastic modulus to be significantly different between healthy and PTTD tendons. A Non-linear Elastic Parameter (NEP) is used to characterize the difference: NEP = 0.16±0.20 MPa/N for healthy tendons, and 0.45±0.43 MPa/N for PTTD tendons. A ROC curve with AUC of 0.83 indicated that a valid classifier system can be built based on the NEP to separate healthy and PTTD tendons.
Our customized inversion task platform has helped with data collection in the \textit{in vivo} experiment, but it is time to improve it. First, instead of the lab bench on which the platform is currently mounted, a physician’s chair (or a dental chair) with an adjustable leg piece would be more suitable. The inversion task platform could then be fixed to the leg piece. A chair would not only make the subjects more comfortable (a potential subject refused only because the lab bench was too high), but also make sure the subjects would not be able to use their upper body to help with the inversion task. Another suggestion would be to mount the inversion task platform onto a separate and movable block (on wheels) placed on the floor while the subject is sitting in a chair. In that case, whenever the subject uses any body parts above the knee, the block would move. A better dynamometer might be a critical improvement too. The current dynamometer is originally designed to measure gripping or pulling force, as a result, several modifications were applied to make it suitable for the measurement of inversion force.

As for the use of the ultrasound system \textit{in vivo}, previous comments regarding the cadaver experiment still applies. The longitudinal tracking was capable of estimating strain, but the large variance of the longitudinal strain measurement suggested the necessity of using the transverse tracking as an alternative way to precisely measure the longitudinal deformation. Other imaging techniques may also provide help. Take the Shear Wave Elasticity Imaging as an example, the fact that SWEI does not require external driving force will minimize the unnecessary movement from both the operator and subjects. Preliminary \textit{in vivo} SWEI results of human PTT have been collected recently.[Appendix F]

The next step of the project “Ultrasound Elasticity Imaging of Human Posterior Tibial Tendon” would be to focus on patients with advanced PTTD, before and after different treatments, to determine if there is any differences between patients who recover from therapy and those who need surgery. A group of close-aged subjects should
be recruited to reduce the variability of the maximum inversion force. It is also necessary to build an improved inversion task platform. As an indicator of non-linearity and the severity of degradation of PTT, the Non-linear Elastic Parameter (NEP), measured by UEI, could become a newly-found parameter to evaluate the PTT, help classify advanced-stage PTTD, determine its prognosis, and inform treatment decisions.
Appendix A

MATLAB® Code for Simulation

MATLAB® code for generate a curved cylinder

```matlab
[phantom_positions, phantom_amplitudes, inside] = curved_cylinder(25000);
save Pht_B_data.mat phantom_positions phantom_amplitudes inside

function [positions, amp, inside] = curved_cylinder(N)
% N is the total number of the scatterers in the whole block
x_size = 30/1000; % Width of phantom [mm]
y_size = 20/1000; % Transverse width of phantom [mm]
z_size = 20/1000; % Height of phantom [mm]
z_start = 30/1000; % Start of phantom surface [mm];

% generate x, y and z coordinates for random scatterers
x = (rand(N,1) - 0.5)*x_size; % -15<x<15
y = (rand(N,1) - 0.5)*y_size; % -10<y<10
z = rand(N,1)*z_size + z_start; % 30<z<50

% find out the scatterers inside the curved cylinder
z_center = z_start + z_size/2;
x_center = 0/1000;
y_center = -12/1000;
x = x - x_center;
y = y - y_center;

R = 13/1000; % curvature of the cylinder
r = 3/1000; % radius of the cylinder
theta = atan(y./x);
index1 = (theta > 0) & (x > 0); % -15<x<15
index2 = (theta < 0) & (x < 0); % -10<y<10
index3 = (theta > 0) & (x < 0);
index4 = (theta < 0) & (x > 0);
xc = zeros(size(x));
yc = xc;
zc = ones(size(z)) + z_center;
x(index1) = R*cos(theta(index1));
yc(index1) = R*sin(theta(index1));
x(index2) = -R*cos(theta(index2));
yc(index2) = -R*sin(theta(index2));
x(index3) = -R*cos(theta(index3));
yc(index3) = -R*sin(theta(index3));
x(index4) = R*cos(theta(index4));
yc(index4) = R*sin(theta(index4));

theta0 = linspace(0,1,N)*2*pi;
xc0 = (R-r)*sin(theta0);
yc0 = (R-r)*cos(theta0);
x1 = (R+r)*sin(theta0);
```

80
yc1 = (R+r) * cos(theta0);

rc = sqrt((x - xc).^2 + (y - yc).^2 + (z - zc).^2);
inside = (rc < r);

x = x + x_center;
y = y + y_center;
xc = xc + x_center;
xc0 = xc0 + x_center;
xc1 = xc1 + x_center;
yc = yc + y_center;
yc0 = yc0 + y_center;
yc1 = yc1 + y_center;

figure; clf; hold on
plot3(xc,yc,zc,'.');
plot3(xc,yc,zc+r,'.');
plot3(xc,yc,zc-r,'.');
plot3(xc0,yc0,zc,'.');
plot3(xc1,yc1,zc,'.');
grid on
axis equal
xlim([-x_size x_size]/2);
ylim([-y_size y_size]/2);
zlim([0 z_size]+z_start);
xlabel('x');
ylabel('y');
plot3(x(inside),y(inside),z(inside),'r.');
view(3);

amp = randn(N,1);  % (N,1) is 1-D or column vector
amp = amp.*(1-inside) + 10*amp.*inside;
positions = [x y z];

MATLAB™ code for Coordinate Transform of the Curved Cylinder

load Pht_B_data
x = phantom_positions(:,1);
y = phantom_positions(:,2);
z = phantom_positions(:,3);
N = length(x);
phantom_amplitudes = (1-inside).*randn(N,1) + inside.*phantom_amplitudes;

figure;
plot3(x(inside),y(inside),z(inside),'r.');
xlim([min(x) max(x)]); ylim([min(y) max(y)]);
title('before'); grid on;

x_center = 0/1000;
y_center = -12/1000;
x = x - x_center;
y = y - y_center;

r = sqrt(x.^2 + y.^2);
theta = atan(y ./ x);
theta(theta<0) = theta(theta<0) + pi;
disp(num2str(min(theta)));
disp(num2str(max(theta)));
zM = mean([min(z) max(z)]);
z = z - zM;
s = 2/100;
z1 = z/sqrt(1+s);
r1 = r - min(r);
r1 = r1/sqrt(1+s);
r1 = r1 + min(r);
theta1 = (1+s)*theta;
x1 = r1.*cos(theta1);
y1 = r1.*sin(theta1);
z1 = z1 + zM;
x1 = x1 + x_center;
y1 = y1 + y_center;
x = x + x_center;
y = y + y_center;

figure;
plot3(x1(inside),y1(inside),z1(inside),'.');
xlim([min(x) max(x)]); ylim([min(y) max(y)]);
title('after'); grid on

clear phantom_positions
phantom_positions(:,1) = x1;
phantom_positions(:,2) = y1;
phantom_positions(:,3) = z1;
save Pht_B_NEW.mat phantom_positions phantom_amplitudes inside

sim_img   % command for generating ultrasound data
make_image % command for display simulated ultrasound B-mode image

MATLAB™ code for function “sim_img”:

% Example of use of the new Field II program running under
% Matlab.
% This example shows how a linear array B-mode system scans an image
% This script assumes that the field_init procedure has been called
% Here the field simulation is performed and the data is stored
% in rf-files; one for each rf-line done. The data must then
% subsequently be processed to yield the image. The data for the
% scatterers are read from the file pht_data.mat, so that the procedure
% can be started again or run for a number of workstations.
% Example by Joergen Arendt Jensen and Peter Munk,
% Version 1.2, August 14, 1998, JAJ.
% Ver. 1.1: 1/4-98: Procedure xdc_focus_center inserted to use the new
% focusing scheme for the Field II program
% Ver. 2.0: 13/8 2007: Parallel version that checks whether the simulation
% of a line has been made before, which makes it possible
% to run the code in parallel on multiple workstations.

% Generate the transducer apertures for send and receive

f0=3.5e6; % Transducer center frequency [Hz]
fs=100e6; % Sampling frequency [Hz]
c=1540; % Speed of sound [m/s]
lambda=c/f0; % Wavelength [m]
width=lambda;
% Width of element
element_height=5/1000; % Height of element [m]
kerf=0.05/1000; % Kerf [m]
focus=[0 0 70]/1000; % Fixed focal point [m]
N_elements=192; % Number of physical elements
N_active=64; % Number of active elements

% Set the sampling frequency
set_sampling(fs);

% Generate aperture for emission
xmit_aperture = ...
xdc_linear_array (N_elements, width, element_height, kerf, 1, 10, focus);

% Set the impulse response and excitation of the xmit aperture
impulse_response=sin(2*pi*f0*(0:1/fs:2/f0));
impulse_response=impulse_response.*hanning(max(size(impulse_response)))';
xdc_impulse (xmit_aperture, impulse_response);

excitation=sin(2*pi*f0*(0:1/fs:2/f0));
xdc_excitation (xmit_aperture, excitation);

% Generate aperture for reception
receive_aperture = ...
xdc_linear_array (N_elements, width, element_height, kerf, 1, 10, focus);

% Set the impulse response for the receive aperture
xdc_impulse (receive_aperture, impulse_response);

% Load the computer phantom
if ~exist('pht_data.mat')
    disp('Scatterer positions should be made by the script mk_pht')
    disp('before this script can be run')
    return
else
    load pht_data
end

% Set the different focal zones for reception
focal_zones=[30:20:200]/1000;
Nf=max(size(focal_zones));
focus_times=(focal_zones-10/1000)/1540;
z_focus=60/1000; % Transmit focus

% Set the apodization
apo=hanning(N_active)';

% Do linear array imaging
no_lines=150; % Number of lines in image
image_width=40/1000; % Size of image sector
d_x=image_width/no_lines; % Increment for image

% Do imaging line by line
for i=[1:no_lines]

% Test if the file for the line exist.
% Skip the simulation, if the line exits and
% go the next line. Else make the simulation
file_name=['rf_data/rf_ln',num2str(i),'.mat'];
if ~exist(file_name)

% Save a file to reserve the calculation

cmd=['save rf_data/rf_ln',num2str(i),'.mat '];
eval(cmd);

disp(['Now making line ',num2str(i)])

% The the imaging direction
x= -image_width/2 +(i-1)*d_x;
% Set the focus for this direction with the proper reference point
% Calculate the apodization
N_pre = round(x/(width+kerf) + N_elements/2 - N_active/2);
N_post = N_elements - N_pre - N_active;
apo_vector=[zeros(1,N_pre) apo zeros(1,N_post)];
xdc_apodization (xmit_aperture, 0, apo_vector);
xdc_apodization (receive_aperture, 0, apo_vector);

% Calculate the received response
[rf_data, tstart]= ...
calc_scat(xmit_aperture, receive_aperture, phantom_positions, phantom_amplitudes);

% Store the result
cmd=['save rf_data rf_ln',num2str(i),'.mat rf_data tstart'];
disp(cmd);
eval(cmd);
else
disp(['Line ',num2str(i),' is being made by another machine.'])
end
end
%
% Free space for apertures
xdc_free (xmit_aperture)
xdc_free (receive_aperture)
disp('You should now run make_image to display the image')

MATLAB™ code for function “make_image”:

% Compress the data to show 60 dB of
% dynamic range for the cyst phantom image
% % version 1.3 by Joergen Arendt Jensen, April 1, 1998.
% % version 1.4 by Joergen Arendt Jensen, August 13, 2007.
% % Clibrated 60 dB display made
f0=3.5e6; % Transducer center frequency [Hz]
fs=100e6; % Sampling frequency [Hz]
c=1540; % Speed of sound [m/s]
no_lines=150; % Number of lines in image
image_width=40/1000; % Size of image sector
d_x=image_width/no_lines; % Increment for image

% Read the data and adjust it in time
min_sample=0;
for i=1:no_lines
% Load the result
cmd=['load rf_data_00_new rf_ln',num2str(i),'.mat'];
disp(cmd);
eval(cmd);

% Find the envelope
% new cmd
% rf_env=abs(hilbert([zeros(round(tstart*fs-min_sample),1); rf_data]));
% % added by Liang
rf = hilbert([zeros(round(tstart*fs-min_sample),1); rf_data]);
% rf = ([zeros(round(tstart*fs-min_sample),1); rf_data]);
% no hilbert, liang 110713
rf_all(1:max(size(rf)),i)=rf;
rf_env = abs(rf);
%% old cmd
eenv(l:max(size(rf_env)),i)=rf_env;
end

%% Do logarithmic compression
D=10; % Sampling frequency decimation factor

%% added by Liang 1/31/14
% rf_D = rf_all(1:D:max(size(rf_all)),:);
rf_D = baseband_russ3(rf_all,fs,f0);
rf_D = rf_D(1:D:max(size(rf_D)),:);

% Finding the envelope
% log_env=env(1:D:max(size(env)),:)/max(max(env));
log_env=abs(rf_D)/max((max(abs(rf_D))));
log_env=20*log10(log_env);
log_env=127/60*(log_env+60);

% Make an interpolated image
disp('Doing interpolation')
ID=1; % 20
[n,m]=size(log_env);
new_env=zeros(n,m*ID);
for i=1:n
    new_env(i,:)=abs(interp(log_env(i,:),ID));
end
[n,m]=size(new_env);

fn=fs/D;
figure;
% clf
image(((1:(ID*no_lines-1))*d_x/ID_no_lines*d_x/2)*1000, ...
((1:n)/fn+min_sample/fs)*1540/2*1000,new_env)
xlabel('Lateral distance [mm]')
ylabel('Axial distance [mm]')
colormap(gray(127))
axis('image')
% axis([-20 20 35 90])
Appendix B

Principal Component Analysis (PCA)

Principal component analysis (PCA) is a statistical procedure that uses an orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables called principal components.[60] In many applications, the dimension of a data set was reduced to only the first few principal components, or even the first principal component alone, because the first principal component has the largest possible variance.

One thing needs to be noticed is that PCA is sensitive to the scaling of the original variables, and there is no consensus as to how to best scale the data to obtain optimal results.

To understand the process of PCA, some elementary background in mathematics and statistics is need. For a set of numbers $X = [X_1, X_2, X_3, ... X_n]$, the mean ($\bar{X}$) can be calculated using the formula:

$$\bar{X} = \frac{\sum_{i=1}^{n} X_i}{n} \quad (B.1)$$
The Standard Deviation (SD) is defined as

$$s = \sqrt{\frac{\sum_{i=1}^{n}(X_i - \bar{X})^2}{n-1}}$$  \hspace{1cm} (B.2)

where \(s\) is the usual symbol for standard deviation. Variance is almost identical to the standard deviation:

$$s^2 = \frac{\sum_{i=1}^{n}(X_i - \bar{X})^2}{n-1}.$$  \hspace{1cm} (B.3)

Notice that it is simply the SD squared, and the symbol \(s^2\) is the usual symbol for variance. Sometimes the symbol “\(var(X)\)” is also used to indicate the variance of data set \(X\).

Both standard deviation and variance only operate on 1D. Covariance is the measurement to find out how data from one dimension vary from the mean with respect to another dimension. For a 2D data set \(X = [X_1, X_2, ..., X_n]\) and \(Y = [Y_1, Y_2, ..., Y_n]\), the covariance is defined as

$$cov(X, Y) = \frac{\sum_{i=1}^{n}(X_i - \bar{X})(Y_i - \bar{Y})}{n-1}.$$  \hspace{1cm} (B.4)

The covariance matrix of the 2D data set is a \(2 \times 2\) matrix:

$$C' = \begin{pmatrix} cov(X, X) & cov(X, Y) \\ cov(Y, X) & cov(Y, Y) \end{pmatrix}. \hspace{1cm} (B.5)$$

From the covariance matrix, one should be able to calculate the eigenvectors and corresponding eigenvalues.

With the background above, I will briefly describe how to perform PCA. the first step of PCA is to subtract the mean from each of the data dimensions. For a 2D data set, it means all the \(X\) values have \(\bar{X}\) subtracted, and all the \(Y\) values have \(\bar{Y}\) subtracted.
The data set will have zero-mean after the subtraction.

The second step of PCA is to calculate the covariance matrix, and then the eigenvectors and eigenvalues of the covariance matrix.

The third step is to order the eigenvectors by eigenvalues, highest to lowest. The eigenvector with the largest eigenvalue is called the first principal component (someone also call it the principle component), because it represents the direction which the data have the maximum variance.

Figure B.1 shows an example of PCA with a 2D data set

\[ X = [5.00, 1.00, 4.40, 3.80, 6.20, 4.60, 4.00, 2.00, 3.00, 2.20] \]
\[ Y = [1.20, 0.35, 1.45, 1.10, 1.50, 1.35, 0.80, 0.55, 0.80, 0.45] \]

The two principal components are (0.97, 0.24) and (-0.24, 0.97) with eigenvalues 2.62 and 0.02, respectively.

Figure B.1: Example: PCA of a 2D data set. The data points are label as ×, the red line denotes the first principal component, the blue line denotes the second principal component. The length for the red line represents the eigenvalue for the first principal component. The eigenvalue of the second principal component is so small that the blue line is 10 times of the actual value.

In this example, the PCA can be thought of as fitting a ellipse to the data. The two principal components are the two axis of the ellipse. The first principal component is the major axis, and the direction with the largest variance. For the first principal component (0.97, 0.24), its direction is the Tangent of its two values. The fact 0.97>0.24 means maximum variance direction is closer to the x-axis than to the y-axis. It may sound
trivial here, but it is the most important concept in our application.

For the next step of PCA, we can decide to ignore the components with lower eigenvalues because they are of less significance. Of course, some information will be lost when components are ignored, but if the corresponding eigenvalues are small, we don’t lose much. With the chosen component or components, a feature vector can be made

$$\text{FeatureVector} = (\text{eigenvector}_1, \text{eigenvector}_2, \ldots, \text{eigenvector}_m)$$ (B.6)

and a new data set can be derived. This is the whole process of PCA. However, we don’t need to reconstruct the data set from the feature vector so I will just skip the detail of these steps. If interested, a tutorial on PCA can be found online[61].

In our case, instead of a bunch of 2D data points, we have 300 frames of 2D displacement map, and for each map there are approximately $200 \times 500 = 100,000$ pixels (lateral $\times$ axial $= 25 \times 20$ mm). The value at each pixel denotes the accumulated displacement of the pixel. In order to segment out the tendon from its surrounding tissue, we want to find out the region in which the pixels are “related” to each other, because the speckles inside the tendon would have the similar movement, and they should be very different than the speckles outside the tendon. Furthermore, the speckles inside the tendon should also have moved more and faster during the inversion task than the speckles outside.

To perform PCA with the displacement map, I treated the data set (300 frames of 2D maps) as 300 measurements (or observations), and each measurement is 100,000-Dimensional. This means each pixel in a 2D map represents a dimension (a variable), and each frame is a measurement in this 100,000-D coordinate system. In this case the size of the covariance matrix will be $100,000 \times 100,000$. Each element of the covariance matrix represents the covariance between one pixel location and another pixel location. This way of performing PCA is essentially the same way used in computer vision.
For each 2D displacement map

\[
\begin{pmatrix}
\begin{array}{c c c}
D_{1,1} & D_{1,2} & \cdots & D_{1,200} \\
D_{2,1} & D_{2,2} & \cdots & D_{2,200} \\
\vdots & \vdots & \ddots & \vdots \\
D_{500,1} & D_{500,2} & \cdots & D_{500,200}
\end{array}
\end{pmatrix},
\]

(B.7)

the rows of pixels in the 2D map were placed one after another to form a 1D vector

\[
\begin{pmatrix}
D_{1,1} & D_{1,2} & \cdots & D_{1,200} & D_{2,1} & D_{2,2} & \cdots & D_{2,200} & \cdots & D_{500,1} & \cdots & D_{500,200}
\end{pmatrix}.
\]

(B.8)

The values in the vector were the displacement.

Then all the 300 vectors were combined together to form a big matrix

\[
M = \begin{pmatrix}
D_{1,1} & D_{1,2} & \cdots & D_{1,500,200} \\
D_{2,1} & D_{2,2} & \cdots & D_{2,500,200} \\
\vdots & \vdots & \ddots & \vdots \\
D_{300,1} & D_{300,2} & \cdots & D_{300,500,200}
\end{pmatrix}.
\]

(B.9)

Each column (100,000 in total) is a variable like the \(X\) or \(Y\) in the previous 2D example, and each row corresponds to a measurement.

From here, the PCA analysis can be performed by either calculating the covariance matrix or using a MATLAB\textsuperscript{TM} function

\[
[\text{COEFF}, \text{SCORE}, \text{latent}] = \text{princomp}(M)
\]

where \text{COEFF} contains all the eigenvectors, and \text{latent} returns all the corresponding eigenvalues.
I included the MATLAB™ code I used to perform PCA with the displacement data in the Appendix C. The first principal component of a set of displacement maps from one of *in vivo* experiment is shown in the Figure B.2.

![Figure B.2: The first principal component of PCA with displacement maps from a *in vivo* data set](image)

The first principal component easily segmented out the tendon from its surrounding tissue. But we also need to find a region of interest to get an average value, objectively. (Actually I can just use the whole positive area in the first principal component map but it is usually a good idea to stay away from the edge, so to find a region of interest inside the positive area seems to be a wiser choice.) Like what I said about the previous 2D example, the value of the first principal component for each dimension indicates how close each dimension is to the maximum variance direction. A dimension with larger value is closer to the maximum variance direction. Like the Tangent we used in the previous 2D example to denote the maximum variance direction, the weighted centroid of the first principal component indicates the maximum variance direction. The region of interest is then placed around the weighted centroid for further analysis. In this case, the calculation can be done by a program objectively, with minimum influence from the operator.

In the situation that the major displacement is negative (to the left), the major value for the first principal would be negative too. This means the maximum variance is along the negative direction, and we can still use the weighted centroid by reverse the sign of the first principal component.
Appendix C

MATLAB™ Code for PCA

The following is from my MATLAB™ GUI for data analysis [Appendix E]. It’s the Callback for a pushbutton which will perform PCA with displacement data.

```matlab
function pushbutton_PCA_Callback(hObject, eventdata, handles)
% hObject handle to pushbutton_PCA (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)
disp('====== performing PCA ======');
PCA_L = handles.analysis.PCA_L; % ROI used for PCA, default is the full image
PCA_R = handles.analysis.PCA_R;
PCA_U = handles.analysis.PCA_U;
PCA_D = handles.analysis.PCA_D;
disp('the current ROI is:');
disp(['x: ' num2str(PCA_L) 'mm to ' num2str(PCA_R) 'mm.'])
disp(['z: ' num2str(PCA_U) 'mm to ' num2str(PCA_D) 'mm.'])
Xacc = handles.analysis.Xacc;
DirectionLeft = handles.analysis.radiobutton_DirectionLeft;
DirectionRight = handles.analysis.radiobutton_DirectionRight;
Direction = DirectionRight - DirectionLeft;

Xacc01 = zeros(size(Xacc,1)*size(Xacc,2),size(Xacc,3));
for n = 1:size(Xacc01,2)
    tmpXacc = squeeze(Xacc(:,:,n));
    Xacc01(:,n) = tmpXacc(:);
end
Xacc01Mean = mean(Xacc01,1);
Xacc01Mean = repmat(Xacc01Mean,[size(Xacc01,1) 1]);
[coeff, score, latent] = princomp(Xacc01);
V00 = coeff(:,1:2)';
figure(1);clf;
plot(V00(1,:),V00(2,:),o);
XaccPCA = score(:,1:2)';
dx = handles.analysis.dx; %0.1330;
dz = handles.analysis.dz; %0.0409;
depth = handles.analysis.depth;
x = handles.analysis.xMM;
z = handles.analysis.zMM;
```
f1 = reshape(XaccPCA(1,:),size(Xacc,1),size(Xacc,2));
figure(2)
imagesc(x,z,f1');colormap(jet);colorbar;axis([-300 300]);
axis equal tight
f2 = reshape(XaccPCA(2,:),size(Xacc,1),size(Xacc,2));
figure(3)
imagesc(x,z,f2');colormap(jet);colorbar;axis([-300 300]);
axis equal tight
f_selection = ((Direction*f1) > 0);
f0 = f1.*f_selection;
figure(4);clf
imagesc(x,z,f0');colormap(jet);colorbar;axis([-300 300]);
axis equal tight
L = handles.analysis.PCA_L;
R = handles.analysis.PCA_R;
U = handles.analysis.PCA_U;
D = handles.analysis.PCA_D; U = U - depth; D = D - depth;
L = round(L/dx)+1; R = round(R/dx)+1;
U = round(U/dz)+1; D = round(D/dz)+1;
line([L,R,R,L]*dx,[U,U,D,D,U]*dz,'color','r','linewidth',2);
f_selection_roi = zeros(size(f1));
f_selection_roi(L:R,U:D) = 1;
f_selection = f_selection.*f_selection_roi;
f0 = f1.*f_selection;
figure(5);clf
imagesc(x,z,f0');colormap(jet);colorbar;axis([-300 300]);
axis equal tight
handles.analysis.f_selection = f_selection;
disp('done with PCA');

% Two ways to calculate the centroid, but I am not going to use it
X = repmat(x',[1,length(z)]);
Z = repmat(z,[length(x),1]);
Z1 = Z.*f_selection; X1 = X.*f_selection;
roi_x0o = sum(X1(:))/sum(f_selection(:));
roi_z0o = sum(Z1(:))/sum(f_selection(:));

bw = f_selection';
s = regionprops(bw,'centroid');
roi_x0c = s.Centroid(1)*dx;
roi_z0c = s.Centroid(2)*dz + depth;

% calculate weighted centroid:
bw = f0';
s = regionprops(f_selection',bw,'WeightedCentroid');
roi_x0w = s.WeightedCentroid(1)*dx;
roi_z0w = s.WeightedCentroid(2)*dz + depth;
disp('weighted centroid.');

% use the weighted centroid for deciding the ROI
roi_x0 = roi_x0w;
roi_z0 = roi_z0w;

hold on
plot(roi_x0o,roi_z0o,'o');
plot(roi_x0c,roi_z0c,'go');
plot(roi_x0w,roi_z0w,'ko');
wdwtmp = handles.analysis.roi_size;
ll = roi_x0 - wdwtmp*dx; rr = roi_x0 + wdwtmp*dx;
tt = roi_z0 - wdwtmp*dz; bb = roi_z0 + wdwtmp*dz;
line([ll,rr,rr,ll],[tt,tt,bb,bb,tt],'color','b','linewidth',3);
handles.analysis.roi_x0 = roi_x0;
handles.analysis.roi_z0 = roi_z0;
disp('the rectangular will be used as the RoI');
disp(['the centre coordinate: (' num2str(roi_x0) ',' num2str(roi_z0) ')']);

handles.analysis.roiXL = ll;
set(handles.edit_roixl,'string',num2str(handles.analysis.roiXL));
handles.analysis.roiXR = rr;
set(handles.edit_roixr,'string',num2str(handles.analysis.roiXR));
handles.analysis.roiZT = tt;
set(handles.edit_roizt,'string',num2str(handles.analysis.roiZT));
handles.analysis.roiZB = bb;
set(handles.edit_roizb,'string',num2str(handles.analysis.roiZB));

disp([ll rr]);
disp([tt bb]);
disp('------- PCA finished -------');
guidata(hObject,handles)
Appendix D

Customized MATLAB™ GUI for Data Collection

Figure D.1: MATLAB™ GUI for data collection

1. Connect the USB-Serial-USB cable and an USB drive to an USB hub; (USB drive is for data collection. I also recommend always using the USB hub.) Connect the USB hub to Zonare scanner. Connect the USB-Serial-USB cable to PC.

2. Turn on Zonare scanner and run the GUI.

3. Use the GUI: Select the correct PC COM Port connected with the Zonare, then press Initialize Zonare.
4. Set scan parameters. Scan structure file will be stored in the Output Folder. (Ultrasound data will be dumped into the USB drive, no need to set.) For frame rate 50 Hz and frame last 300, the collection time is \( \frac{300}{50} = 6 \) s. I usually add a few extra frames to make sure enough frame data will be collected.

5. Usually Harmonics and Compounding are both off.

6. Press Set Zonare after all the parameters are set.

7. Press Start to start data collection. The scanner will freeze for 3 seconds (Pre-Scan Pause), and then unfreeze to fire ultrasound pulse. After 6 seconds, the scanner will freeze again for 10 s (hard coded) and then back to normal. Ready for next collection.

8. In order to make and view ultrasound B-mode movies, the ultrasound data needs to be copied from USB drive to the Data Folder, together with the structure files in Output Folder. Press “Get .m file”, then “Import Data”, then “Make Movie”.

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Appendix E

Customized MATLAB™ GUI for Data Analysis

E.1 GUI For speckle tracking

Figure E.1: MATLAB™ GUI for data analysis, Part 1 Correlation

This GUI can automatically perform speckle tracking and accumulation with up to 12 data sets, which are usually the total data sets obtained from one subject. In case of
less than 12 data sets, just uncheck the checkbox.

All I need to do is to set the Raw Data Folder, fill the File Name(s), press “Extract” (if the data set hasn’t been extracted), and press “Load Data and Do Everything”. The GUI will start with the first data set with checked checkbox. I can also manually perform the speckle tracking by press “Load Data Only”, then go through panel “Correlation Settings”.

Usually I do it manually first to find the best settings for correlation.

### E.2 GUI for PCA and plotting

Figure E.2: MATLAB™ GUI for data analysis, Part 2 PCA and Plotting

This GUI is for PCA and determine the correct ROI for displacement and strain estimation. Once loaded the frame data, calculated the strain, the PCA can be performed. The ROI setting in the PCA panel is for the situation that the first principal component has separate peaks. In such a case, I need to hand pick one region and then calculate the weighted centroid.

After obtain the displacement and strain, elastic modulus can be calculated.
Appendix F

A Figure from Current Shear Wave Elasticity Imaging Study

In the Figure F.1 above, the figures in the lower row are the ultrasound B-mode image of human PTT under different load. The figures in the upper row shows the distribution of the shear wave speed. The shear wave travels faster in stiffer tissue. It is clear that the PTT became stiffer under load.

Figure F.1: A figure from current Shear Wave Elasticity Imaging study
Appendix G

Published Works

G.1 Journal papers (1st Author)


G.2 Conference proceedings (1st Author)


G.3 Other conference presentations


2. Gao L, Yuan JS, Heden G, Szivek J, Taljanovic M, Latt LD, Witte RS. Ultrasound elastography can be used to measure the stiffness of the posterior tibial tendon in vivo. Duke Foot and Ankle Society Meeting, May 25-16, 2013, Durham, NC.


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[51] https://www.cabrillo.edu/~dbrown/tracker/


[60] https://en.wikipedia.org/wiki/Principal_component_analysis