## An Automated Tracking Algorithm Characterizing Deformation of Fatigue-Induced Achilles Tendons

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INTRODUCTION: Ultrasound is widely used in clinical research as a non-invasive and effective modality for imaging the Achilles tendon. Our recent work developed an ultrasound stress imaging approach to predict Achilles tendon failure under fatigue loading in vitro [1]. However, these published findings did not characterize tendon mechanics to assess fatigue damage of the Achilles tendon mid-substance. Therefore, the purpose of this study was to develop an automated ultrasound tracking tool to characterize mid-substance strain mechanics across the fatigue life of human Achilles tendon. We then tested its efficacy by correlating the bulk strains measured using the tensile testing system and our automated ultrasound tracking tool. We hypothesized that the Achilles mid-substance bulk strain measurements would correlate with the tensile testing system across the fatigue life. Establishing this tool is a critical step towards longitudinally monitoring Achilles tendon fatigue damage *in vivo* to guide personalized rehabilitative care.

METHODS: Ten cadaveric Achilles tendons (4M, 3F; Age: 60 ± 15) were dissected, cut into a dog-boned shape, and mounted onto a custom-built tank and pulley system as previously described (Fig 1A) [1]. The tendons were loaded up for 150,000 cycles or until rupture using a sine waveform that generated loading cycles between 10 and 20 MPa at 1 Hz. After every 500 cycles, continuous B-mode ultrasound images of the mid-substance of each specimen were acquired at 41 Hz during 2 loading cycles at 0.25 Hz to remove motion artifact [1]. After the experiment, a custom script (MATLAB 2022b.) was implemented to track the deformation of the tendon mid-substances through the acquired ultrasound images. A rectangular region of interest (ROI) was drawn over the mid-substance (yellow rectangle), in which a 11 x 3 grid of kernels (red rectangles) was formed inside to define the subregions of the midsubstance (Fig 1B). Each kernel was seeded with point trackers (white dots) generated by Kanade-Lucas-Tomasi point tracking algorithm that detects corner point eigenvalues, as we previously used in our recent work, and a speckle inside was defined as the mid-point of the kernel [2]. The point trackers returned their x- and y-coordinates after tracking each frame of the ultrasound images. The x-displacement of the point tracker at a frame was calculated from subtracting its x-coordinate by its x-coordinate at the reference frame. Further, the x-displacement of the speckle in each kernel was calculated by averaging all point trackers' x-displacements in that kernel. The displacements of the three speckles in the furthest right/left three kernels were averaged and divided by the original gauge length determined from the distance between three speckles in the furthest right kernels and three speckles in the furthest left kernels to calculate the bulk strain of the mid-substance. We calculated the crosshead bulk strain of the whole specimen by dividing crosshead displacement by the measured gauge length. We correlated the bulk strains of both mid-substance and crosshead from the references to the first peaks every 10% of the fatigue life in the 6 specimens that ruptured and the 4 specimens that survived 150,000 cycles to determine whether mid-substance ultrasound tracking is robust in human tendon with differing magnitudes of mechanical fatigue damage (Fig 1C).

RESULTS SECTION: Our automated ultrasound tracking tool accurately quantified bulk tendon strain across (Fig 1C). The average correlation between mid-substance bulk strain and crosshead bulk strain was  $0.991 \pm 0.004$  in the ruptured group and  $0.995 \pm 0.014$  in the non-ruptured group. This very strong tracking fidelity was similarly strong at the 100% cycles  $(0.995 \pm 0.002)$  for the ruptured group. The average mid-substance bulk strain in the ruptured group  $(2.193\% \pm 0.316\%)$  was 45% greater (p < 0.001) than the average mid-substance bulk strain in the survived group  $(1.508\% \pm 0.459\%)$ .

DISCUSSION: In this study, we implemented our tracking algorithm over ultrasound continuous images to measure mid-substance bulk strains as well as validate its performance throughout the fatigue life. Regardless of ruptured and survived conditions or the numbers of applied cycles, the average correlations between both bulk strains remained very strong, beyond 0.990. This indicates that our automated tracking tool works effectively for all mechanically induced tendon fatigue damage. It is important to note that the mid-substance bulk strain values we report do not represent the failure strains because these stress-images were acquired at relatively low tendon stresses (10-20 MPa) compared to experimentally derived values of tendon rupture [3]. These low tendon stresses are clinically important because they are safe to apply in patients with symptomatic Achilles tendinopathy. Our tool uses a 11 x 3 tracking grid (and user customizable) that allows researchers to quantify regional strain mechanics. This is an important feature to evaluate local strains in the mid-substance and we are currently linking these mid-substance strain mechanics with altered neuromechanics profiles in patients with tendinopathy.

SIGNIFICANCE/CLINICAL RELEVANCE: Our automated ultrasound tracking tool reliably quantifies mid-substance Achilles tendon strain across the fatigue life. Our future work will use this tool on different Achilles tendon injuries like tendinopathic tendons and ruptured tendons to assess tendon mechanical status throughout treatment and guide precision rehabilitation.

REFERENCES: [1] Schmidt et al (2020). J. Biomech. [2] Drazan et al (2019). Peer J. [3] Maganaris et al (2008). Disabil Rehabil.

 $ACKNOWLEDGEMENTS: This study was funded by NIH/NIAMS\ R01AR078898\ and\ P50AR080581.$ 

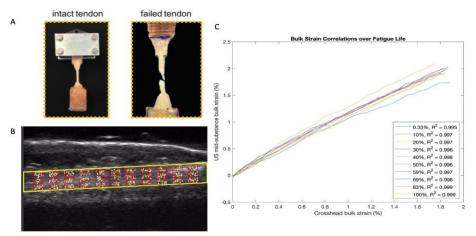


Fig 1. (A) Schematic of specimen preparation of a cadaveric Achilles tendon mounted on a moveable crosshead and fixed end excerpted from Schmidt et al.,2020. (B) A representative ultrasound image with an ROI (yellow rectangle), a 11 x 3 set of kernels (red rectangles), point trackers (white dots), and speckles (green dots) centered inside the kernels. (C) A representative correlation between mid-substance bulk strain and crosshead bulk strain over the 10 points of fatigue life, from the unstretched position to fully stretched position.