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Short communication

Demonstration of extended field-of-view ultrasound's potential to increase the pool of muscles for which *in vivo* fascicle length is measurable

Amy N. Adkins [a,d,e], Patrick W. Franks [a], Wendy M. Murray [a,b,c,d,e,*]

[a] Department of Biomedical Engineering, Northwestern University, Evanston, IL, USA
[b] Department of Physical Medicine & Rehabilitation, Northwestern University Feinberg School of Medicine, Chicago, IL, USA
[c] Department of Physical Therapy & Human Movement Sciences, Northwestern University Feinberg School of Medicine, Chicago, IL, USA
[d] Arms + Hands Lab, Shirley Ryan AbilityLab (formerly the Rehabilitation Institute of Chicago), Chicago, IL, USA
[e] Edward Hines, Jr. VA Hospital, Hines, IL, USA

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ABSTRACT

Static, B-mode ultrasound is the most common method of measuring fascicle length *in vivo*. However, most forearm muscles have fascicles that are longer than the field-of-view of traditional ultrasound (T-US). As such, little work has been done to quantify *in vivo* forearm muscle architecture. The extended field-of-view ultrasound (EFOV-US) method, which fits together a sequence of B-mode images taken from a continuous ultrasound scan, facilitates direct measurements of longer, curved fascicles. Here, we test the validity and reliability of the EFOV-US method for obtaining fascicle lengths in the extensor carpi ulnaris (ECU). Fascicle lengths from images of the ECU captured *in vivo* with EFOV-US were compared to lengths from a well-established method, T-US. Images were collected in a joint posture that shortens the ECU such that entire fascicle lengths were captured within a single T-US image. Resulting measurements were not significantly different ($p = 0.18$); a Bland-Altman test demonstrated their agreement. A novice sonographer implemented EFOV-US in a phantom and *in vivo* on the ECU. The novice sonographer's measurements from the ultrasound phantom indicate that the combined imaging and analysis method is valid (average error = 2.2 ± 1.3 mm) and the *in vivo* fascicle length measurements demonstrate excellent reliability (ICC = 0.97). To our knowledge, this is the first study to quantify *in vivo* fascicle lengths of the ECU using any method. The ability to define a muscle's architecture *in vivo* using EFOV-US could lead to improvements in diagnosis, model development, surgery guidance, and rehabilitation techniques.

1. Introduction

Muscle architecture is characterized by the number, length, and orientation of its fibers (Gans and Bock, 1965; Gans and de Vree, 1987). Muscle fiber length determines both the absolute range of a muscle's length-tension curve and the absolute maximum shortening velocity of its fibers (Lieber and Friden, 2000). Therefore, measurement of a muscle's fiber length is an important methodological step in the characterization of muscle architecture. Because muscle fibers have a diameter under 100 μm, direct measurements are difficult to obtain (Lieber, 2002). Fascicles, bundles of muscle fibers acting in parallel, are of sufficient size for both *ex vivo* dissection (Cutts et al., 1991; Jacobson et al., 1992; Lieber et al., 1990; Murray et al., 2000) and *in vivo* medical imaging (Kwah et al., 2013; Lieber and Ward, 2011). The lengths of a muscle's fascicles are considered to provide reasonable estimates of the lengths of its fibers (Lieber and Friden, 2000; Noorkoiv et al., 2010; Trotter, 1993).

Ultrasound, the most common method of measuring muscle fascicle lengths *in vivo*, is accepted as reliable and accurate (Kwah et al., 2013; Lieber and Ward, 2011). Traditional static brightness mode (B-mode) ultrasound is used extensively and enables direct measurement of fascicles under 50 mm. For longer fascicles, use of traditional ultrasound (T-US) requires linear extrapolation of the visible portion of the fascicle to estimate the full length from trigonometry (Blazevich et al., 2006; Kellis et al., 2009; Li et al., 2007; Stevens et al., 2014). Extended field-of-view ultrasound (EFOV-US), a less frequently adopted technique, allows

* Corresponding author at: Arms + Hands Lab (21st Floor), Shirley Ryan AbilityLab, 355 E. Erie St., Chicago, IL 60611, USA.
E-mail address: w-murray@northwestern.edu (W.M. Murray).

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direct measurements of longer, curved fascicles. This method fits together a sequence of T-US images taken from a continuous ultrasound scan (Weng et al., 1997). EFOV-US has been demonstrated to be a valid method of measurement of muscle fascicles *in vivo* in a gold standard methods study in the vastus lateralis (Noorkoiv et al., 2010).

Only one published study has reported fascicle lengths of a forearm muscle measured *in vivo*, implementing trigonometric estimation methods on images of the extensor digitorum communis (Brorsson et al., 2008). While cadaveric studies have generated detailed data on forearm muscle architecture (Brand et al., 1981; Cutts et al., 1991; Lieber et al., 1990), anatomical dissection methods pose limitations to studying changes with joint posture and following disease (e.g., (Fukunaga et al., 1997; Gao and Zhang, 2008; Li et al., 2007; Martin et al., 2001; Shortland et al., 2002; Whittaker et al., 2007)). Notably, 24 of 26 muscles and muscle compartments in the forearm have optimal fiber lengths longer than the field-of-view of common ultrasound probes (Holzbaur et al., 2005). Thus, EFOV-US provides a novel opportunity to study forearm muscles *in vivo*. However, misalignment of the ultrasound probe from the plane of the fascicles has been shown to result in fascicle length error using T-US (Bolsterlee et al., 2016; Klimstra et al., 2007). Because EFOV-US requires dynamic scans over extended distances, there is concern such misalignment error could aggregate, reducing accuracy and reliability (Cronin and Lichtwark, 2013; Noorkoiv et al., 2010).

Here, we test the validity and reliability of fascicle measurements obtained from EFOV-US images of the extensor carpi ulnaris, a forearm muscle that has not previously been studied *in vivo*. Additionally, to encourage widespread adoption of the EFOV-US method, we investigated a novice sonographer's ability to obtain valid and reliable fascicle measures.

2. Materials and methods

To test the validity and reliability of fascicle measurements obtained from the EFOV-US method, we completed two studies. In Study 1, images of the extensor carpi ulnaris (ECU) were obtained from eight subjects (Table 1) by an experienced sonographer, implementing and comparing EFOV-US and a well-established method, T-US (Fig. 1A). In Study 2, we evaluated a novice sonographer's ability to obtain valid and reliable fascicle measures by implementing EFOV-US on an ultrasound phantom and *in vivo* on the ECU of six subjects (Fig. 1B, Table 2). All subjects enrolled in both studies (7 female, 7 male, ages 19–29 yrs, height 1.50–1.85 m) gave informed consent and had no history of musculoskeletal disease or injuries of the wrist or elbow. Northwestern University's Institutional Review Board approved the procedures of this study.

2.1. Study 1: EFOV-US vs T-US

The dominant arm of eight healthy subjects was imaged using both EFOV-US and T-US. Subjects were seated with their arm rigidly secured in a joint posture that shortens the ECU to the extent that entire fascicle lengths can be captured within a single T-US image (Fig. 2). To capture the full length of the ECU, the ultrasound machine was set in B-mode and EFOV (Siemens Antares™ Siescape v.5, Siemens Medical Solutions USA, Inc., Mountain View, CA). With the probe at its initial position (Fig. 2), an experienced sonographer initiated data collection and then slowly moved the probe proximally, following the path of the muscle, while imaging data was being continuously collected. When the proximal end of the muscle was reached, the sonographer ended data collection. After identifying a qualitatively acceptable EFOV-US image (Fig. 3), the sonographer implemented the T-US method, capturing an image statically at the initial location. The sonographer then moved the probe until more proximal fascicles could be visualized in the imaging plane, held the probe in place, and captured a second image. This was repeated until 6 T-US images were obtained. Three corresponding "image sets" (1 EFOV-US and 6 T-US images) were collected per subject (Fig. 1A, Fig. 4). Images were exported as DICOM images and fascicle lengths were measured in ImageJ (1842.0.0, Wayne Rasband, National Institutes of Health, Bethesda, MD). On average, eight fascicles were measured per method per image set.

2.2. Study 2: Novice sonographer

To demonstrate the accuracy and reliability of measurements obtained by a novice sonographer, a second sonographer with no previous experience practiced the EFOV-US imaging protocol on the ECU of two individuals over a period of two weeks. Once the sonographer was consistently able to identify the ECU and visualize its muscle fascicles during the imaging session, subjects were enrolled in the study. To evaluate accuracy, an ultrasound phantom was constructed and imaged using EFOV-US. The phantom was constructed from agar, glycerol, and powdered graphite to mimic the sonic properties of muscle tissue (Ortega et al., 2010). Wooden blocks and guitar strings (Custom Gauge Nickle Wound, Ernie Ball, Coachella, CA) with lengths ranging from 19.9 mm to 74.5 mm were measured with a caliper precise to 0.01 mm before placement inside the phantom (Fig. 5). The novice sonographer imaged the phantom objects using EFOV-US and quantified object lengths using ImageJ. During data collection for the ECU, subjects were seated with their elbow at 90° flexion, wrist at neutral, and fingers in a relaxed position. Three qualitatively acceptable images of the ECU (Fig. 3) were collected in the dominant arm of each subject and six fascicles were measured per image. Image quality was evaluated by the sonographer at the time of data collection and

Table 1
Study 1: EFOV-US VS T-US. Subjects' wrist ulnarly deviated 30° and extended 40°.

Subject number	Gender	Age	Dominant hand[a]	Arm length (mm)[b]	Height (m)	EFOV-US (mm)	T-US (mm)	Difference (mm)
1	M	25	R	38	1.84	30.6	30.5	0.1
2	M	27	R	36	1.78	32.0	32.5	−0.5
3	M	26	R	37.5	1.83	31.5	32.9	−1.4
4	F	23	R	35.7	1.75	29.7	30.5	−0.8
5	F	23	R	29	1.50	21.5	21.8	−0.4
6	M	24	R	36.5	1.78	26.7	26.3	0.4
7	F	29	R	36	1.75	25.8	25.6	0.2
8	F	24	R	33.5	1.63	25.2	25.5	−0.2
					Average	**27.9**	**28.2**	**−0.3**

[a] Dominant arm was the arm imaged.
[b] Arm length measured from the medial epicondyle of the humerus to the 5th metacarpophalangeal joint.

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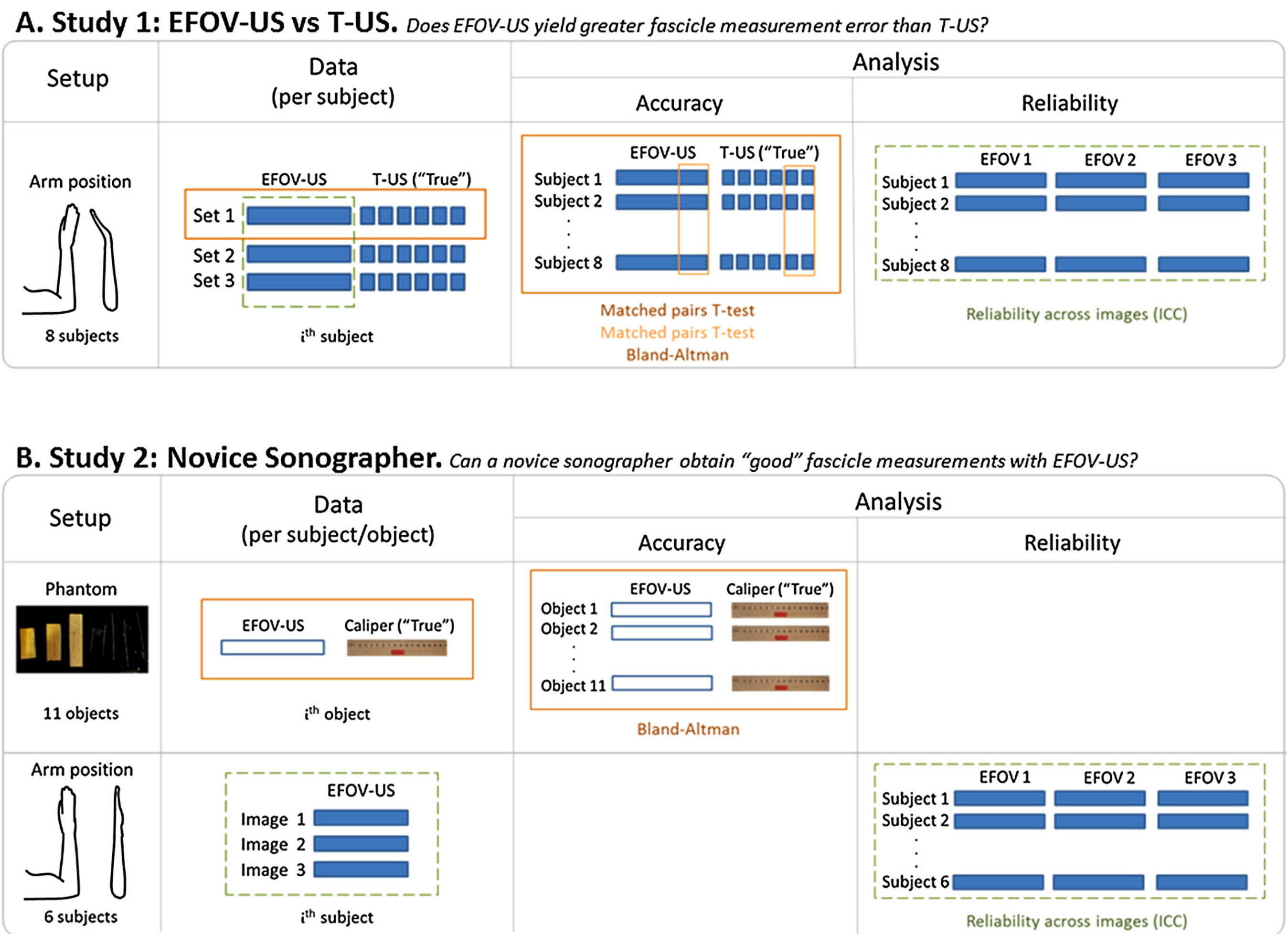

Fig. 1. Methods Diagram. Diagram depicting setup, data, and analysis for (A) Study 1's comparison of EFOV-US and T-US imaging methods, and (B) Study 2's demonstration of the accuracy and reliability of EFOV-US measurements obtained by a novice sonographer. Both A and B show the arm position in which the imaging protocol was performed to obtain *in vivo* ECU fascicle length. Study 1 was completed in a wrist position that shortened ECU fascicles to the extent that they were viewable within the field-of-view of our T-US probe; Study 2 was completed in the neutral wrist position, where ECU fascicles are generally longer than the field-of-view of T-US. The "Data" column uses symbols to illustrate the example data set collected for one subject or object. Each long, filled blue rectangle symbolizes a single image resulting from an EFOV-US scan of the ECU (Study 1 & 2); each filled blue square symbolizes a single T-US image of a portion of the ECU, acquired at different locations along its length (Study 1 only). The long, open rectangle symbolizes the image resulting from an EFOV-US scan of an object embedded in the phantom (Study 2 only); the image of the ruler symbolizes the caliper measurements, taken before each object was embedded in the phantom. Throughout the figure, the orange boxes that outline different data sets identify the portions of each data set that were analyzed to test accuracy; green dashed boxes outline the portions of each data set that were analyzed to evaluate reliability. For Study 1, fascicle lengths measured from the T-US scans were taken to be the "true" value of ECU fascicle lengths for accuracy assessments. For Study 2, caliper measurements of the phantom objects were used to assess the novice sonographers accuracy implementing the EFOV technique. All statistical tests that were performed for accuracy and reliability assessments are listed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Study 2: Novice Sonographer. Subjects' wrist in neutral position.

Subject number	Gender	Age	Dominant hand[a]	Arm length (mm)[b]	Height (m)	Average fascicle length (mm)
9	M	20	R	26.5	1.74	42.9
10	F	20	L	26.0	1.80	44.0
11	M	21	R	27.5	1.83	47.9
12	F	22	R	22.5	1.60	36.5
13	F	23	R	24.8	1.70	37.0
14	M	19	R	29.0	1.85	50.6
					Average	**43.2**

[a] Dominant arm was the arm imaged.
[b] Arm length measured from the medial epicondyle to the ulnar styloid process.

confirmed for acceptable quality by the experienced sonographer after the study was complete.

2.3. Statistical analysis

Bland-Altman tests were implemented to determine agreement between fascicle measurements obtained using EFOV-US and fascicle measurements obtained using T-US (Fig. 1A) as well as between phantom object dimensions measured using the caliper and object dimensions measured using EFOV-US (Fig. 1B). A matched-pairs *t*-test was implemented to compare the average fascicle length obtained from the first EFOV-US image with the average fascicle length of the six corresponding T-US images (Fig. 1A). Based on the image analysis from the ultrasound phantom, a power analysis

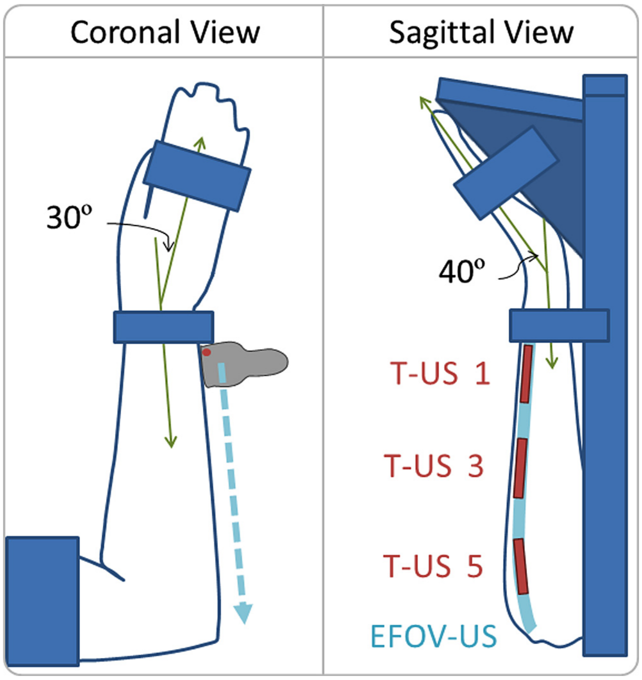

Fig. 2. Arm position for comparing EFOV-US and T-US fascicle lengths. (Left) The coronal view of the right arm showing the elbow at 90° and the wrist ulnarly deviated at 30°. The solid blue rectangles represent straps that rigidly secured the subject in the desired joint posture. The ultrasound probe (grey) was inititally positioned at the distal forearm for both EFOV-US and T-US methods and was moved proximally (dynamically for EFOV-US, in static increments for T-US) until the full muscle was captured. The red circle on the ultrasound probe corresponds to the left side of the ultrasound image, as displayed on the imaging screen (see Fig. 4). (Right) The sagittal view of the arm exhibits the 40° extension of the wrist and neutral position of the forearm. Light blue lines indicate the transducer path for a single EFOV-US scan; superimposed red lines illustrate 3 of the 6 corresponding T-US images collected within a given "image set". (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

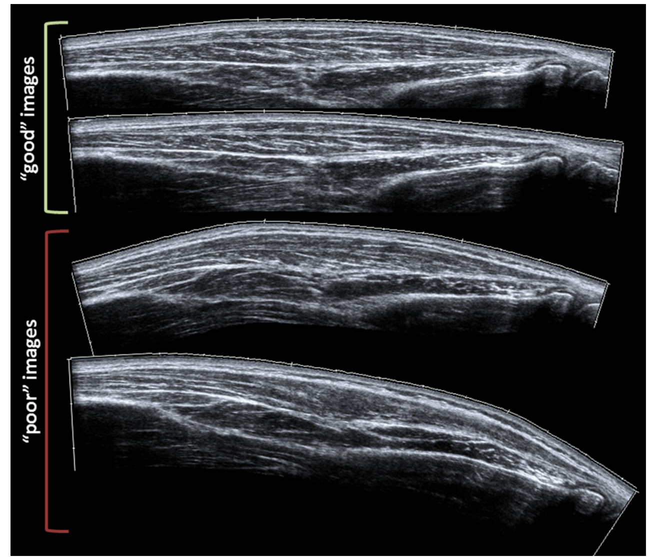

Fig. 3. Qualitatively "good" and "poor" EFOV-US images. EFOV-US images captured from a single subject and qualitatively assessed by an experienced sonographer at the time of data collection as "good" or "poor" (only "good" images were analyzed). The sonographer assesses the presence and clarity of key anatomical features of the image, shown here are differentiations based on echogenicity, the tissue's ability to reflect ultrasound waves (Ihnatsenka and Boezaart, 2010). The "good" ultrasound images (top two images) allow identification of the ECU as the hypoechoic (dark) structure, outlined superficially and deep by muscle fascia, visible as hyperechoic (bright) lines, with connective tissue between muscle fascicles also visible as hyperechoic (bright) striations. Also visible is a hyperechoic central inner tendon that starts distally and spans approximately 75% of the captured muscle belly. "Poor" ultrasound images (bottom two images) lack one or more of these key anatomical features at any point along the length of the image. Here, the "poor" images lack both fascicles and the central tendon across the entirety of the muscle. Deviation from the imaging plane of the object yields blatantly "poor" images, which would not be analyzed. Notably, the EFOV-US algorithm is robust enough that small off plane motions, that occur inadvertently due to manual scanning, do not affect the method's accuracy (Weng et al., 1997).

indicated that a power greater than 0.80 would be achieved with 6 subjects and an effect size of 1.69. To test between image reliability of the EFOV-US method obtained by an individual sonographer, one-way random intraclass correlation coefficients (ICC) were calculated (Fig. 1).

3. Results

3.1. Study 1: EFOV-US vs T-US

Relative to fascicle measurements obtained via T-US images, EFOV-US does not result in additional error due to the method's extended scan distance. EFOV-US fascicle lengths (average 27.9 ± 3.7 mm; male 30.2 ± 2.4 mm; female 25.5 ± 3.4 mm) were not significantly different ($p = 0.18$) than T-US fascicle lengths (average 28.2 ± 4.0 mm; male 30.5 ± 3.0 mm; female 25.8 ± 3.6 mm) (Table 1). Bland-Altman analysis (Fig. 6) indicated a bias of −0.35 mm between the two methods (95% limits-of-agreement = −1.47 mm to 0.77 mm). Fascicle lengths at the end of the EFOV-US image (i.e. where the measurement error is hypothesized to be the largest) were not significantly different ($p = 0.42$) from T-US lengths in the same position. Between image reliability for EFOV-US fascicle measurements was excellent (ICC = 0.99; 95% CI: 0.95–1.00).

3.2. Study 2: Novice sonographer

The novice sonographer's measurements from the phantom indicate that the combined imaging and analysis method is valid. We observed an average error of 2.2 ± 1.3 mm (4.6% of phantom object length). Bland-Altman analysis of agreement between direct caliper and EFOV-US measurements of the phantom objects yielded a bias of −0.67 mm (95% limits-of-agreement = −5.57 mm to 4.23 mm) (Fig. 7).

A novice sonographer was able to implement EFOV-US *in vivo* and obtain reliable ECU fascicle measurements. The average fascicle length observed for the ECU was 43 ± 6 mm with males having longer fascicles than females (males 47 ± 3 mm, females 39 ± 4 mm) (Table 1). Excellent reliability was demonstrated (ICC = 0.97; 95% CI: 0.89–1.00).

4. Discussion

Through comparison with T-US, our results provide evidence that our implementation of EFOV-US in a previously unstudied forearm muscle was valid and reliable. The successful implementation of the method by a novice sonographer encourages investigation of other unstudied muscles with fascicles longer than the field-of-view of T-US. Our work, together with previous studies (Nelson et al., 2016; Noorkoiv et al., 2010) highlight the reliability of the EFOV-US method independent of muscle, imaging session, or sonographer experience.

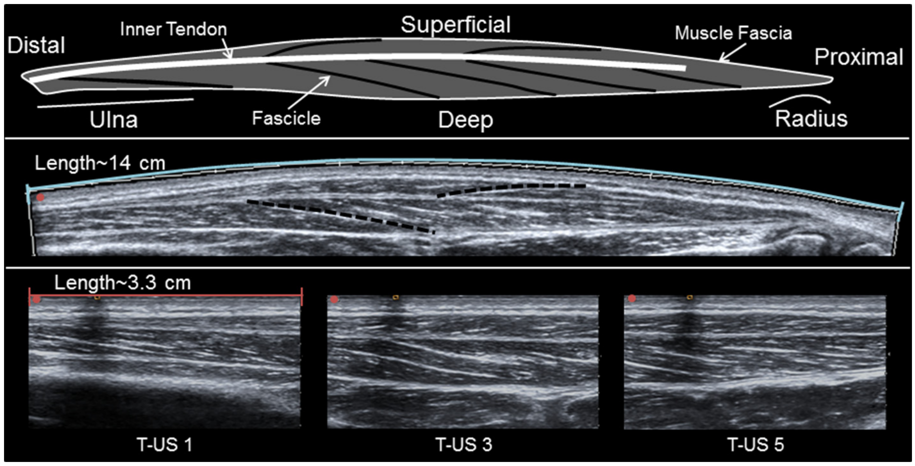

Fig. 4. Extended field-of-view images of the ECU. (Top) Schematic illustration of the ECU muscle captured with EFOV-US. Two bone landmarks are also represented in the schematic, the ulna and the proximal head of the radius seen in the ultrasound image bellow. (Middle) A typical EFOV-US image of the ECU taken by an experienced sonographer. The black dashed lines represent fascicles typically digitized during data analysis. (Bottom) Three example T-US images, taken from the same imaging set as the above EFOV-US image. The images are labeled coresponding to their location in Fig. 2 with the left most T-US image being the most distal. The red circle represents the distal side of each image (see Fig. 2). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 5. Ultrasound phantom. (Left) Image of the wooden blocks and guitar strings placed in the ultrasound phantom. Each wooden block had a distinct width and length, which were imaged separately, yielding two "objects" per block. (Right) An EFOV-US image of the width of a wooden block obtained by the novice sonographer from the ultrasound phantom constructed in this study. The length of the block in the ultrasound image was measured using the segmented line tool in ImageJ. The object measurements obtained from EFOV-US images were compared to direct caliper measures of the object.

Because the EFOV-US method implements extended distance, dynamic scans, there is concern that the method aggrandizes the misalignment error seen in T-US (Cronin and Lichtwark, 2013; Nelson et al., 2016; Noorkoiv et al., 2010). Misalignment of the ultrasound probe with the plane of the fascicles has been shown to result in errors in fascicle length measures (0.4 mm per degree of misalignment) using T-US (Bolsterlee et al., 2016). On average, ultrasound images taken of the medial gastrocnemius muscle were found to be misaligned with the fascicles by 5.5° (Bolsterlee et al., 2016; Bolsterlee et al., 2015) indicating an average error of 2.2 mm; this error is comparable to the absolute error observed in our phantom measurements (2.2 ± 1.3 mm). Our study demonstrates that probe misalignment during EFOV-US does not yield significantly higher error than in T-US.

EFOV-US is more accurate than an approximation method used when fascicles are longer than the field-of-view of T-US. Trigonometric estimation has been shown to yield an absolute error of 7.7 ± 6.2 mm in the vastus lateralis; approximately 9% of total fascicle length (Noorkoiv et al., 2010). Our absolute error, determined by a novice sonographer imaging and analyzing phantom objects, was 4.6% of phantom object length (2.2 ± 1.3 mm). Notably, differences in fascicle length between the paretic and non-paretic arm of individuals post-stroke are larger than the measurement error observed in our phantom study. For example, a study implementing T-US on the brachialis muscle found fascicles of the paretic arm to be 8–21 mm (9–15% of non-paretic fascicle length) shorter than

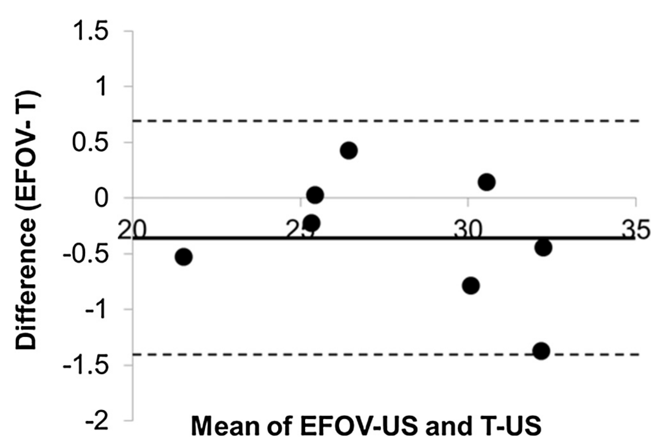

Fig. 6. Bland-Altman test comparing EFOV-US and T-US methods. The Bland-Altman test of agreement is often used to assess agreement of measures taken from two different clinical methods (Bland and Altman, 1986). Here, a Bland-Altman test of agreement was implemented to compare fascicle measurements obtained via T-US with fascicle measurements obtained using EFOV-US. The x-axis is the average of the EFOV-US and T-US fascicle length measurements and the y-axis is the difference between the fascicle measurements. The solid line indicates the bias (−0.35 mm) and the dashed lines represent the lower (−1.47 mm) and upper (0.77 mm) limits-of-agreement (mean difference ± 1.96*standard deviation of the difference). This Bland-Altman graph suggests that fascicle measurements taken via EFOV-US are comparable to the well-established T-US fascicle measurements as there is no observable systematic variance, the measurements lay within the limits-of-agreement, and the bias is small (Bland and Altman, 1986).

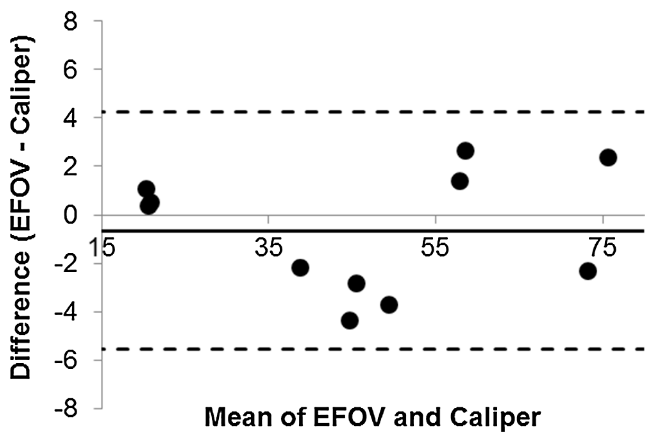

Fig. 7. Bland-Altman test comparing direct caliper and EFOV-US methods. a Bland-Altman test was implemented to compare phantom measurements obtained by a novice sonographer using EFOV-US and direct caliper measurements. The x-axis is the average of the ultrasound and caliper measurements and the y-axis is the difference between the two measurements. The solid line indicates the mean difference and the dashed lines indicate the limits-of-agreement (mean ± 1.96 SD). Measurements of phantom objects from EFOV-US images were compared with caliper measurements yielding a bias of −0.67 mm with 95% confidence intervals of −5.57 and 4.23 mm. No systematic variance was observed over the range of measurements and all measurements lay within the limits-of-agreement. The bias is near zero (−0.67 mm), suggesting agreement between the two measurement methods.

fascicles of the non-paretic arm (Li et al., 2007). Similarly, distal radius fractures, which often result in permanent shortening of the radius, have been shown to shorten the radial wrist flexor and extensor muscles by 4.1–4.4 mm per 10 mm of radius shortening (Tang et al., 1997). Therefore, our relatively small measurement error suggests that EFOV-US may be the best available ultrasound imaging approach to study clinically relevant or intervention-induced changes in forearm fascicle length.

Only one previous study has quantified *in vivo* forearm fascicle lengths, despite the ubiquity of ultrasound studies for other muscle groups (Aggeloussis et al., 2010; Cronin et al., 2008; Duclay et al., 2009; Kawakami et al., 1998; Martin et al., 2001; Stenroth et al., 2012). The EFOV-US method has the capacity to capture the entire length of the fascicle in one image; this is advantageous in the forearm where over 90% of muscles have optimal fiber lengths longer than the field-of-view of T-US. We encourage adoption of this technique for more widespread investigation of *in vivo* muscle architecture in under-studied muscles in both healthy and impaired populations.

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Conflict of interest

None of the authors have any conflict of interest to disclose.

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