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Obtaining Quality Extended Field-Of-View Ultrasound Images of Skeletal Muscle to Measure Muscle Fascicle Length

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TITLE:

Obtaining Quality Extended Field-Of-View Ultrasound Images of Skeletal Muscle to Measure Muscle Fascicle Length

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SHORT ABSTRACT:

This study describes how to obtain high quality musculoskeletal images using the extended field-of-view ultrasound (EFOV-US) method for the purpose of making muscle fascicle length measures. We apply this method to muscles with fascicles that extend past the field-of-view of common traditional ultrasound (T-US) probes.

LONG ABSTRACT:

Muscle fascicle length, which is commonly measured in vivo using traditional ultrasound, is an important parameter defining a muscle's force generating capacity. However, over 90% of all upper limb muscles and 85% of all lower limb muscles have optimal fascicle lengths longer than the field-of-view of common traditional ultrasound (T-US) probes. A newer, less frequently adopted method called extended field-of-view ultrasound (EFOV-US) can enable direct measurement of fascicles longer than the field-of-view of a single T-US image. This method, which automatically fits together a sequence of T-US images from a dynamic scan, has been demonstrated to be valid and reliable for obtaining muscle fascicle lengths in vivo. Despite the numerous skeletal muscles with long fascicles and the validity of the EFOV-US method for making measurements of such fascicles, few published studies have utilized this method. In this study, we demonstrate both how to implement the EFOV-US method to obtain high quality

musculoskeletal images and how to quantify fascicle lengths from those images. We expect that this demonstration will encourage the use of the EFOV-US method to increase the pool of muscles, both in healthy and impaired populations, for which we have in vivo muscle fascicle length data.

INTRODUCTION:

Fascicle length is an important parameter of skeletal muscle architecture, which overall is indicative of a muscle's ability to produce force[1,2]. Specifically, a muscle's fascicle length provides insight into the absolute range of lengths over which a muscle can generate active force[3,4]. For example, given two muscles with identical values for all isometric force-generating parameters (i.e., average sarcomere length, pennation angle, physiological cross sectional area, contraction state, etc.) except for fascicle length, the muscle with the longer fascicles would produce its peak force at a longer length and would produce force over a wider range of lengths than the muscle with shorter fascicles[3]. Quantification of muscle fascicle length is important for understanding both healthy muscle function and changes in a muscle's force-generating capacity, which can occur as a result of altered muscle use (e.g., immobilization[5,6], exercise intervention[7-9], high heel wearing[10]) or a change in the muscle's environment (e.g., tendon transfer surgery[11], limb distraction[12]). Measurements of muscle fascicle length were originally obtained through ex vivo cadaveric experiments that allow for direct measurement of dissected fascicles[13-16]. The valuable information provided by these ex vivo experiments led to an interest in implementing in vivo methods[17-19] to address questions that could not be answered in cadavers; in vivo methods allow for quantification of muscle parameters in a native state as well as at different joint postures, different muscle contraction states, different loading or unloading states, and across populations with differing conditions (i.e. healthy/injured, young/old, etc.). Most frequently, ultrasound is the method employed to obtain in vivo muscle fascicle lengths[18-20]; it is quicker, less expensive, and easier to implement than other imaging techniques, such as diffusion tensor imaging (DTI)[18,21].

Extended field-of-view ultrasound (EFOV-US) has been demonstrated to be a valid and reliable method for measuring muscle fascicle length in vivo. While commonly implemented, traditional ultrasound (T-US) has a field-of-view which is limited by the ultrasound transducer's array length (typically between 4 and 6 cm, although there are probes that extend to 10 cm [10])[18,20]. To overcome this limitation, Weng et al. developed an EFOV-US technology that automatically acquires a composite, two-dimensional "panoramic" image (up to 60 cm long) from a dynamic, extended distance scan[22]. The image is created by fitting together, in real-time, a sequence of traditional, B-mode ultrasound images as the transducer dynamically scans the object of interest. Because sequential T-US images have large overlapping regions, the small differences from one image to the next can be used to calculate the probe motion without the use of external motion sensors. Once the probe motion between two consecutive images is calculated, the "current" image is merged successively with the preceding images. The EFOV-US method allows direct measurement of long, curved muscle fascicles and has been demonstrated to be reliable across muscles, trials, and sonographers[23-25] and valid for both flat and curved surfaces[23,26].

Implementing ultrasound to measure muscle fascicle length in vivo is not trivial. Unlike other imaging techniques that involve more automated protocols (i.e., MRI, CT), ultrasound is

dependent on sonographer skill and anatomical knowledge[27,28]. There is concern that probe misalignment with the fascicle plane may cause substantial error in fascicle measures. One study demonstrates little difference (on average < 3 mm) in measures of fascicle length taken using ultrasound and DTI MRI but also shows that measurement precision is low (standard deviation of difference ~12 mm)[29]. Still, it has been shown that a novice sonographer, with practice and guidance from an experienced sonographer, can obtain valid meaures using EFOV-US[23]. Thus, efforts should be made to demonstrate appropriate protocols to reduce human error and improve accuracy of measurements obtained using EFOV-US. Ultimately, developing and sharing appropriate protocols may expand the number of experimenters and laboratories that can reproduce fascicle length data from the literature or obtain novel data in muscles which have not yet been studied in vivo.

In this protocol, we demonstrate how to implement the EFOV-US method to obtain high quality musculoskeletal images that can be used to quantify muscle fascicle length. Specifically, we address (a) collecting EFOV-US images of a single upper limb and a single lower limb muscle (b) determining, in real-time, the "quality" of the EFOV-US image, and (c) quantifying muscle architecture parameters offline. We provide this detailed guide to encourage the adoption of the EFOV-US method for obtaining muscle fascicle length data in muscles that have gone unstudied in vivo due to their long fascicles.

PROTOCOL:

Northwestern University's Institutional Review Board (IRB) approved the procedures of this study. All participants enrolled in this work gave informed consent prior to beginning the protocol detailed below.

NOTE: The specific ultrasound system used in this study had EFOV-US capabilities and was adopted because we were able to review details about and validity assessments for the algorithm in the scientific literature[22,26]; multiple other systems with EFOV-US also exist[18,20,30]. A linear array transducer 14L5 (frequency bandwidth 5-14 MHz) was utilized. The muscles imaged in this protocol are just a small subset of muscles for which US images have been captured and fascicle lengths measured (e.g., triceps[25], extensor carpi ulnaris[23], medial gastrocnemius[10], vastus lateralis[24], biceps femoris[8,31]). This protocol is intended to provide pointers and describe the necessary standards so that that it may be applied to muscles beyond the two examples we provide.

1. Collecting EFOV-US images of muscles

Preparation

1.1 Sonographer Preparation

1.1.1 Prior to operating the ultrasound system, read through the system's manual to become familiar with system safety, care for maintaining the system, system setup and controls, etc. In

addition, review the system's instructions for obtaining EFOV-US images and be familiar with the method implemented to obtain the EFOV-US images.

NOTE: Different ultrasound systems name the EFOV-US mode using different terminology. For example, in the system used here, the EFOV mode is referred to as "Panoramic Imaging". While the technical details of the algorithm implemented in various commercial systems are usually intellectual property and therefore not freely available, from a cursory review, many commercial systems with panoramic ultrasound capabilities describe an approach similar to the one described by Weng et al.[22]. Evaluating the general validity of measurements acquired from any system, either by obtaining more detailed information directly from the company who manufactures the system, by using an imaging phantom[26,32], or by other means (e.g., comparison to animal dissection[24]) is recommended as an important step before initiating research involving human participants.

1.1.2 Take time to become familiar with the anatomy of the muscle(s) of interest as well as the surrounding anatomy. It is suggested that the sonographer use an anatomy textbook or preferably an interactive online 3D anatomy model to become familiar with the anatomy of interest.

1.2 Participant Preparation

1.2.1 Explain the protocol of the study to the participant and acquire IRB approved consent prior to beginning the imaging protocol.

1.2.2 Ask the participant to wear appropriate clothing to enable access to the muscle of interest. For example, if the sonographer plans to image a forearm muscle, the participant should be asked to wear a short-sleeved shirt.

1.2.3 Seat the participant in an adjustable chair that can be locked in place. Take time to adjust the chair to make the participant as comfortable as possible while still providing access to the muscle of interest.

NOTE: If an adjustable chair which can lay completely flat is not available, some study designs may require the use of a table to access the muscle of interest (i.e., hamstrings).

1.2.4 Place the joint(s) that the muscle of interest spans in a posture that can be controlled and repeated. Use clinical guidance[33] for locating anatomical landmarks and implementing goniometry and ISB standards for defining the joint coordinate system[34,35]. In general, to measure joint angle, mark anatomical landmarks with skin safe marker (**Table of Materials**) and then align the center of a handheld goniometer up with the axis of rotation of the joint and the arms of the goniometer up with the joint segments.

NOTE: If imaging passive muscle, placing the muscle of interest in a relatively lengthened position is recommended to avoid imaging slack muscle.

1.2.4.1 For imaging the biceps brachii in this study, seat participants with feet supported, back straight, shoulder at 85° of abduction and 10° of horizontal flexion, elbow at 25° flexion, and forearm, wrist, and fingers at neutral.

1.2.4.2 To image the tibialis anterior in this study, seat participants with knee at 60° of flexion and the ankle at 15° of plantar flexion.

1.2.5 Secure the participants limb using cloth straps to minimize movement during the imaging protocol.

Image Acquisition

1.3 Plug in and turn on the ultrasound system. Ensure that the exam is set to Musculoskeletal, the transducer is set to the transducer in use (here we used 14L5), and the transmit frequency is set between 5-17 MHz (here 11MHz was used), a typical frequency range for musculoskeletal imaging. Higher frequencies are generally used for more superficial imaging as they improve resolution but decrease wave penetration.

1.4 Go into the system settings to adjust the footswitch settings. For the purposes of this protocol, we recommend setting the footswitch to start/stop the imaging. If the footswitch in use has multiple pedals, set additional pedals to “Freeze” or “Pause”, and “Print” or “Store” the image.

1.5 Apply a generous amount of ultrasound gel to the head of the transducer.

1.6 Place the transducer on the participant’s skin on the approximate region of interest.

1.7 Move the transducer in the short axis plane of the muscle. Note that the transducer has a small protuberance on one side, called an indicator. The side of the transducer that has the indicator corresponds with the left side of the ultrasound image. When imaging in the short axis, have the sonographer keep the indicator pointed laterally and when the sonographer is in long axis, point the indicator distally.

1.8 Identify the muscle of interest in the short axis plane (perpendicular to muscle fiber direction) and move the transducer distal and proximal to get a full visualization of the muscle path.

1.8.1 Mark important anatomical landmarks (i.e., the lateral and medial edges of the muscle, the muscle tendon junction, and muscle insertion) using skin safe ink markers (**Table of Materials**).

1.9 Once the location of the muscle has been identified and properly marked, have the sonographer move the ultrasound transducer in the long axis plane (parallel to the muscle fiber

direction).

1.10 Beginning at either the distal or proximal end of the muscle, rotate and tilt the transducer to identify the fascicle plane at that point. Make a mark on the skin when the correct transducer position has been established.

1.11 Once the approximate fascicle plane has been established along the entire desired length to be scanned, have the sonographer practice following this path.

1.12 To begin collecting images, put the ultrasound system in EFOV-US mode.

1.13 Starting at one end of the muscle, click the footswitch to start image acquisition and slowly and continuously move the ultrasound transducer in the long axis. Once the end of the muscle has been reached, click the footswitch to end image acquisition.

1.14 Practice and ensure the correct transducer path. This may take several practice images before consistently obtaining "quality" EFOV-US images (See section 2 for explanation of quality images).

1.15 To optimize image visibility and clarity, consider adjustments to the following parameters.

1.15.1 Depth: If image acquisition ends before the desired length of the muscle can be captured, increase the depth of the image (in the system used here, increasing image depth increases the absolute length the scan can be).

1.15.2 Focus: Place the focus arrow in the lower half of the ImageJust below the muscle of interest.

1.15.3 Gain: Ensure the gain is balanced through the depth of the image.

1.15.4 Speed: Image at the optimum speed as guided by the indicator (in most systems a speed indicator displays on the monitor during panoramic imaging).

1.16 Once qualitatively good images have been collected (step 2.1), hit the **Print/Store** footswitch pedal or a synonymous button on the control panel to save the image.

1.17 Repeat steps 1.13-1.16 until 3 quality EFOV-US images of the muscle are obtained.

1.18 Repeat steps 1.6-1.17 until all muscles of interest are obtained.

1.19 Use a towel to gently wipe the gel from the participant's skin. Then have the participant rinse off the area of the skin or use a damp towel to wipe the skin that was exposed to the gel. Dry.

1.20 Wipe gel from the head of the transducer and disinfect.

1.21 Export images as uncompressed DICOM images onto a CD-DVD, flash drive, or through the local network onto a computer.

2. Determining "quality" of the EFOV-US image

2.1 Following step 1.13, have the sonographer identify and evaluate the quality of key anatomical features of the muscle of interest and its surrounding anatomy. This is a qualitative assessment based on the sonographer's knowledge of anatomy and musculoskeletal tissue echogenicity (ability of a tissue to reflect ultrasonic waves). For an EFOV-US images to be considered qualitatively "good" the following should be met:

2.1.1 In any long-axis image of a muscle, check that the sonographer can clearly identify the muscle as a hypoechoic (dark) shape with hyperechoic (bright) boundaries which represent the deep and superficial muscle fascia.

2.1.2 Between the muscle boundaries, check that the sonographer can identify the connective tissue surrounding a muscles fascicle as hyperechoic (bright) lines.

NOTE: When imaging multi-pennated muscles, the image should also contain central tendon(s) that show up in the muscle belly, between the deep and superficial muscle fascia, as a hyperechoic (bright) structure.

2.1.3 Check that the image does not have excessive bending. This is usually indicated by shadows or gaps in the image or a jagged flexible ruler line over the image.

2.2 If the image is missing one or more of the tissue structures described in 2.1, deem the image "qualitatively poor" and return to live 2D-mode.

3. Quanitfying Muscle Fascicle Length

3.1 To quantify muscle fascicle length, use ImageJ, an open source image processing platform. ImageJ can be downloaded at https://imagej.net/Downloads.

NOTE: Though ImageJ is frequently implemented[24,25,31,36-38], quantification of muscle fascicle length may be measured using other image processing software[8,39] or custom codes[40,41].

3.2 Once downloaded, open the ultrasound images as DICOM images in ImageJ by clicking **File | Open** and selecting the image to analyze.

3.3 To ensure that the DICOM image properties have been preserved, click on the **Straight Line** tool in the **Tools** menu and draw a straight line from 0 to 1 cm on the ruler on the side of

the ultrasound image. Then go to **Analyze | Measure** to measure the line made. If the image properties have been preserved, the length of the straight line should be 1 cm.

3.4 To measure fascicle lengths in the image, complete the following.

3.4.1 Right click on the **Straight Line** tool.

3.4.2 Select **Segmented Line**.

3.4.3 Move the cursor onto the image and click at one end of the fascicle that has been chosen to be measured.

NOTE: Only make measurements on fascicles that the entire fascicle path (i.e., from one aponeurosis to the next aponeurosis or aponeurosis to inner tendon) can convincingly be seen.

3.4.4 Click along the path to ensure curvature in the fascicle path is captured.

3.4.5 Once the end of the fascicle path is reached, double click to end the line and go to **Analyze | Measure** to measure the length of the line.

NOTE: A new window, "Results", will pop up the first time a measurement is made. What values are displayed can be managed in the Results window by going to **Results | Set Measurements**.

3.5 Repeat steps 3.4.3-3.4.5 until multiple fascicle measures are made in a single image.

3.6 Save fascicle measurements by clicking **File | Save** on the results tab or the values can be copy and pasted into another document/spreadsheet.

REPRESENTATIVE RESULTS:

Extended field-of-view ultrasound (EFOV-US) was implemented to obtain images from the long head of the biceps brachii and the tibialis anterior in 4 healthy volunteers (**Table 1**). **Figure 1** shows what EFOV-US images of both muscles imaged in this representative imaging session and highlights important aspects of each image such as muscle aponeurosis, central tendon, fascicle path, etc. After the imaging session was over, 3 qualitatively "good" images (**Figure 2**) were analyzed for each muscle in each individual. ImageJ was implemented to measure 4 fascicles per image. In each image, fascicles with paths that could be convincingly visualized from origin to insertion and that were located in different portions of the muscle selected were measured. The average fascicle lengths obtained in this study for the biceps brachii (14.6 ± 1.7 cm) and the tibialis anterior (7.3 ± 0.6 cm) are within the range of fascicle lengths reported previously[25,42] (**Table 1**).

As most challenging and subjective parts of this protocol is determining factors which lead to correctly deeming an image as qualitatively "good" or qualitatively "bad". We provide several examples of "good" and "bad" images (**Figure 2**) and how image landmarks and quality vary across people (**Figure 3**). In addition we have highlighted the portions of the images which are

specifically “bad”.

FIGURE & TABLE LEGENDS:

Table 1: Participant Demographics and Data. Measurements of fascicle length are represented as average ± standard deviation.

Figure 1: Schematic and EFOV images of two example muscles. (left) Illustration of the muscle being studied. (right) Example of “good” images on top and the same image with whole muscle (dark blue), central tendon (light blue), and muscle fascicles (white) outlined. Each image has a corresponding 1 cm scale bar (white) on the bottom right of the image.

Figure 2: Demonstration of Image Quality. Demonstration of three qualitatively “good” and three qualitatively “bad” images obtained from the biceps brachii and the tibialis anterior of participants 1 and 2. (Top **A** & **B**) In all the qualitatively “good” images fascicles which extend from internal tendon to muscle aponeurosis can be visualized. We illustrate images which are qualitatively “bad” and should not be analyzed. Portions of the image which qualify it is as “bad” are emphasized (blue boxes and arrows) and include jagged or broken images, excessive or non-anatomically relevant bending, images which exclude the entire fascicle, and images with blurred central tendons. Each image has a scale bar (white vertical line) which represents 1 cm. This portion of the figure is highlighting the variability among images due mainly to the sonographer’s inconsistency across separate imaging sweeps. (Bottom **A** & **B**) One “good” biceps and one “good” tibialis anterior muscle are shown. The orange box on the original image is then blown up to illustrate more accurately the zoom that is seen when measuring fascicles in ImageJ. The bottom image shows representative outlined fascicles (white dashed lines). These images are deemed “good” because fascicles can be followed from origin to insertion and the zoomed portion of the image doesn't have substantial distortions or artifacts.

Figure 3: Variability in image quality across individuals. Variability in image quality and visibility exists between participants, largely due to anatomical variation (i.e. muscle size, muscle length, subcutaneous fat content) and differences in muscle content (i.e. amounts of intramuscular fat, connective tissue, fibrosis). Specifically, variations in muscle content and layers of tissue above the muscle can affect the echo intensity of the imaged muscle[43]. Natural anatomical differences across individuals will result in muscle architectural features varying in location and/or relative size across US images of different individuals. This demonstration of muscles in different participants stresses the importance of a thorough understanding of anatomy and sufficient practice obtaining images on various individuals for gaining confidence in the quality and accuracy of the images being obtained.

DISCUSSION:

There are a few critical components to obtaining quality EFOV-US images that yield valid and reliable fascicle length measures. First, as indicated in method 1.1.2 it is essential that the sonographer take time to become familiar with the anatomy of the muscle being imaged as well as surrounding muscles, bones, and other soft tissue structures. This will improve the sonographer’s ability to image the correct muscle and determine if multiple images are capturing

the same muscle plane. Second, the sonographer should practice the protocol on phantoms and multiple pilot participants before collecting data for publication. Ultrasound is known to result in measurement error if the sonographer does not properly identify the fascicle plane, a task which is challenging and can improve with practice. Last, it is strongly recommended to ensure that the validity of the measurements made by the EFOV-US algorithm in the ultrasound system being used has been established. If the method's accuracy has not been demonstrated, validation can be done easily using an ultrasound phantom[23,26] or through comparison to another imaging tool[44] or cadaveric dissection[45].

If image visibility is poor or the probe motion is uneven during dynamic scanning, adding ultrasound gel may enhance image quality by improving transducer-to-skin coupling. If image acquisition is cut-off by the algorithm before the entire object of interest is captured, the depth of the image should be increased. Increasing the depth of the image expands the available scanning distance, thus enabling longer objects to be capture within a single EFOV-US image. In general, it is best to refer to the ultrasound system's manual when attempting to improve or troubleshoot image quality or image acquisition.

Here, we demonstrate how to capture EFOV-US images of the entire muscle from the muscle tendon junction of the origin tendon to the insertion tendon. Capturing the entire muscle is necessary for some muscles, such as the biceps brachii, whose fascicles span nearly the entire length of the muscle. However, for other muscles, such as the tibialis anterior or other pennated muscles, shorter scans that do not include the full muscle belly may still capture entire muscle fascicles. For novice sonographers, acquiring images from shorter scans that still capture full fascicle lengths may decrease the chances of probe misalignment with the fascicle plane and improve image quality, decreasing the potential for fascicle measurement error.

Notably, muscle activation can change muscle fascicle length. Due to the nature of the scanning method, the major limitation of EFOV-US is that it cannot be implemented to study muscle fascicle changes due to dynamic muscle contraction (e.g., during walking[46,47]). Additionally, due to the time required to capture an EFOV-US image, imaging a muscle at maximum contraction is likely infeasible due to muscle fatigue. Instead, the EFOV-US method is beneficial for sub-maximal or passive imaging. One way to ensure muscle activity is constant across participants, limbs, or sessions is to simultaneously measure EMG during imaging and analyze only images which are taken when the muscle is at some desired activity level. Though recommended, particularly if studying populations with altered neural drive, measures of EMG were not taken in the population studied here.

Though traditional ultrasound has been shown to be valid and reliable for measuring in vivo muscle fascicle lengths, some fascicle measurement error will occur if the sonographer's alignment of the ultrasound transducer deviates from the fascicle plane[27,29,48]. Due to the nature of the EFOV-US's dynamic scan, there is concern that the EFOV-US method may have more error than T-US[21,24]. While a recent study demonstrated that fascicle measurement error from probe misalignment was not larger in EFOV-US than in the well-established, T-US method[23] in a single wrist muscle, a general limitation of B-mode ultrasound is that you are only able to capture a

relatively small, 2-dimensional view of the muscle. The true path of individual fascicles may be 3D; concerns remain that errors associated with measuring lengths of potentially 3D paths from 2D views may be bigger for longer fascicles.

Static, B-mode ultrasound is a widely accepted method for measuring muscle fascicle lengths in vivo. However, the field-of-view of T-US probes limits the length of fascicles that can be directly measured. Instead, measurement of fascicles longer than the field-of-view of T-US requires trigonometric estimation methods, diffusion tensor imaging (DTI), or EFOV-US[20]. In general, ultrasound imaging is favored over magnetic resonance imaging (MRI) techniques such as DTI because MRI is more expensive and challenging to implement[18]. Fascicle lengths captured with EFOV-US have been shown to be more accurate than trigonometric estimation methods[24,36], which is expected since muscle fascicles regularly follow a curved path, but trigonometric estimation methods assume linearity in their calculation of muscle fascicle length.

It should be noted that though most ultrasound probes are 4-6 cm in length, ultrasound probes up to 10 cm have been used[9,10]. The 10 cm probes enable a wider field-of-view, enabling the capture of longer, straight fascicles. Still, the longer probe length decreases frame rate, would require the imaging surface (the body) to also be straight to avoid uneven compression of the imaged tissue, and may not be able to capture longer curved fascicles (without the use of EFOV)[20].

The guide detailed here for obtaining quality EFOV-US images for measuring muscle fascicle length is intended to encourage the use of the EFOV-US method to expand the pool of muscles for which the field has in vivo muscle architecture data. The expectation is that this method be applied to both healthy and impaired populations (e.g., individuals post-stroke[38,49] or post-orthopedic surgery) to better understand healthy muscle function and muscle adaptation. In addition, these in vivo data are important for development of models that more accurately predict human movement as well as the development of subject specific musculoskeletal models.

Notably, the EFOV-US method is not limited to measurements of muscle fascicle length. The method has been used for measurement of tendon length[50,51] and muscle anatomical cross-sectional area,[52,53] as well as for documentation of various superficial lesions[54,55]. Thus, there is opportunity to develop guides, similar to the one presented here, for obtaining high quality images with the EFOV-US method for various applications.

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DISCLOSURES:
The authors have nothing to disclose.

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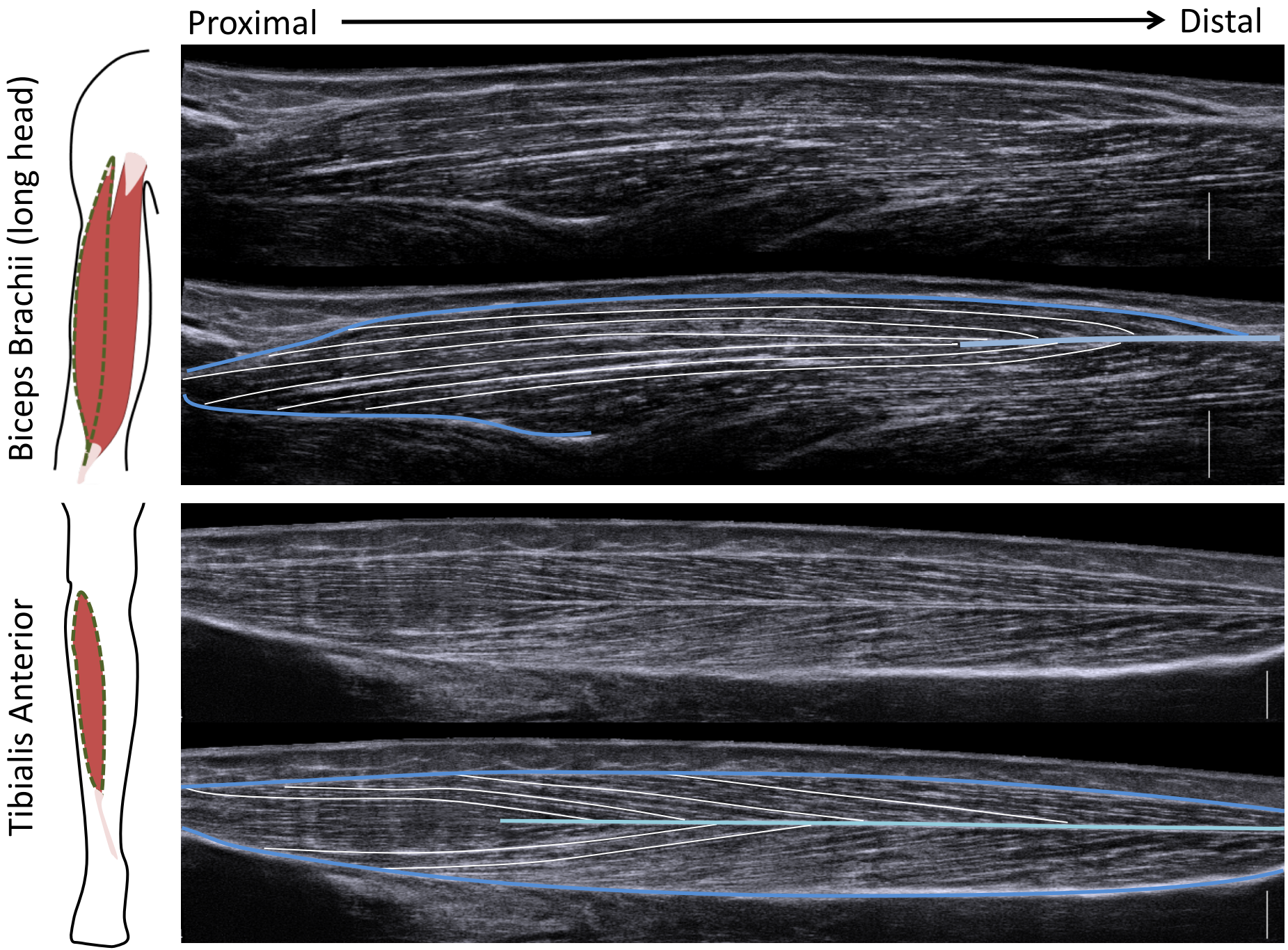

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Figure 2

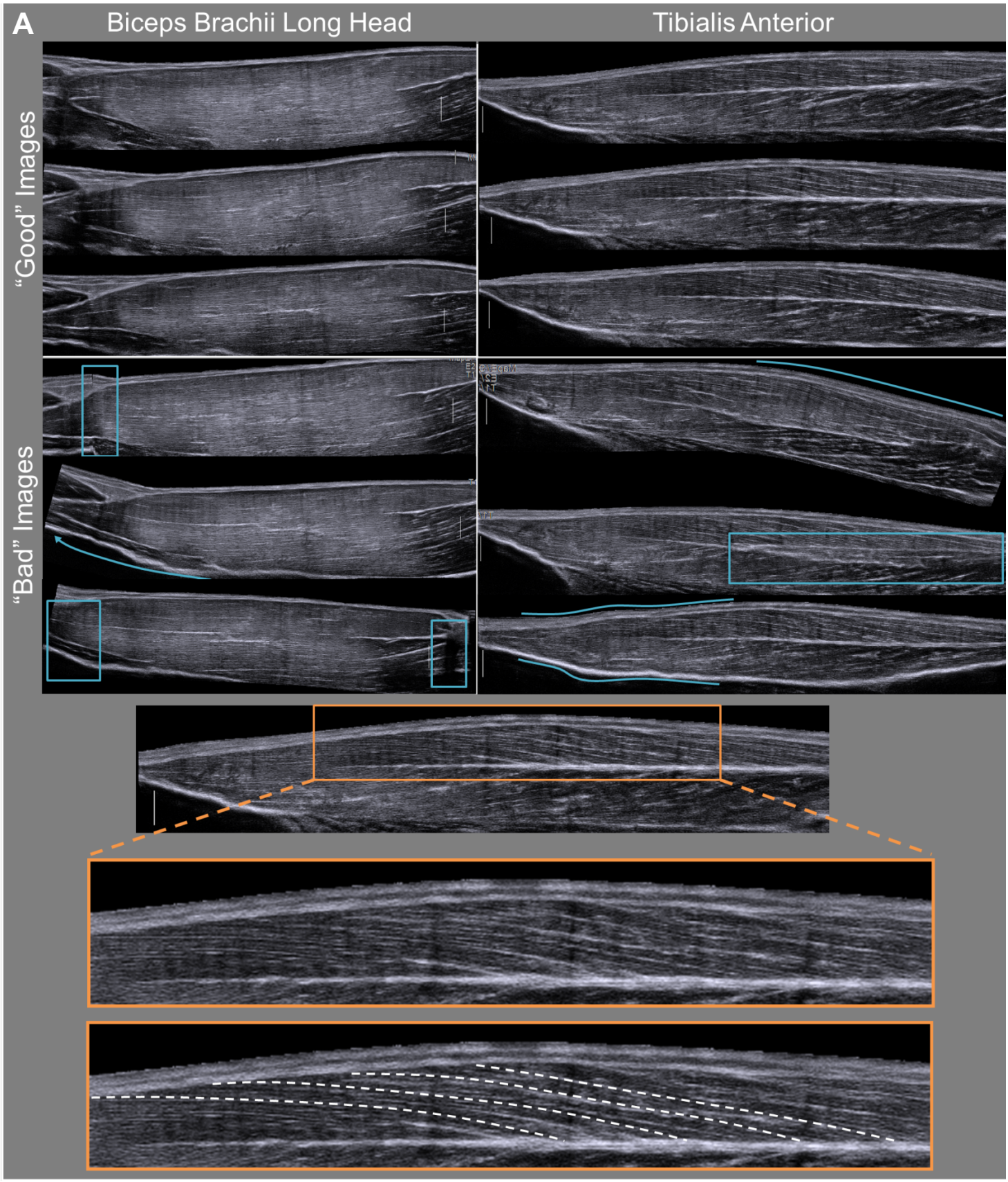
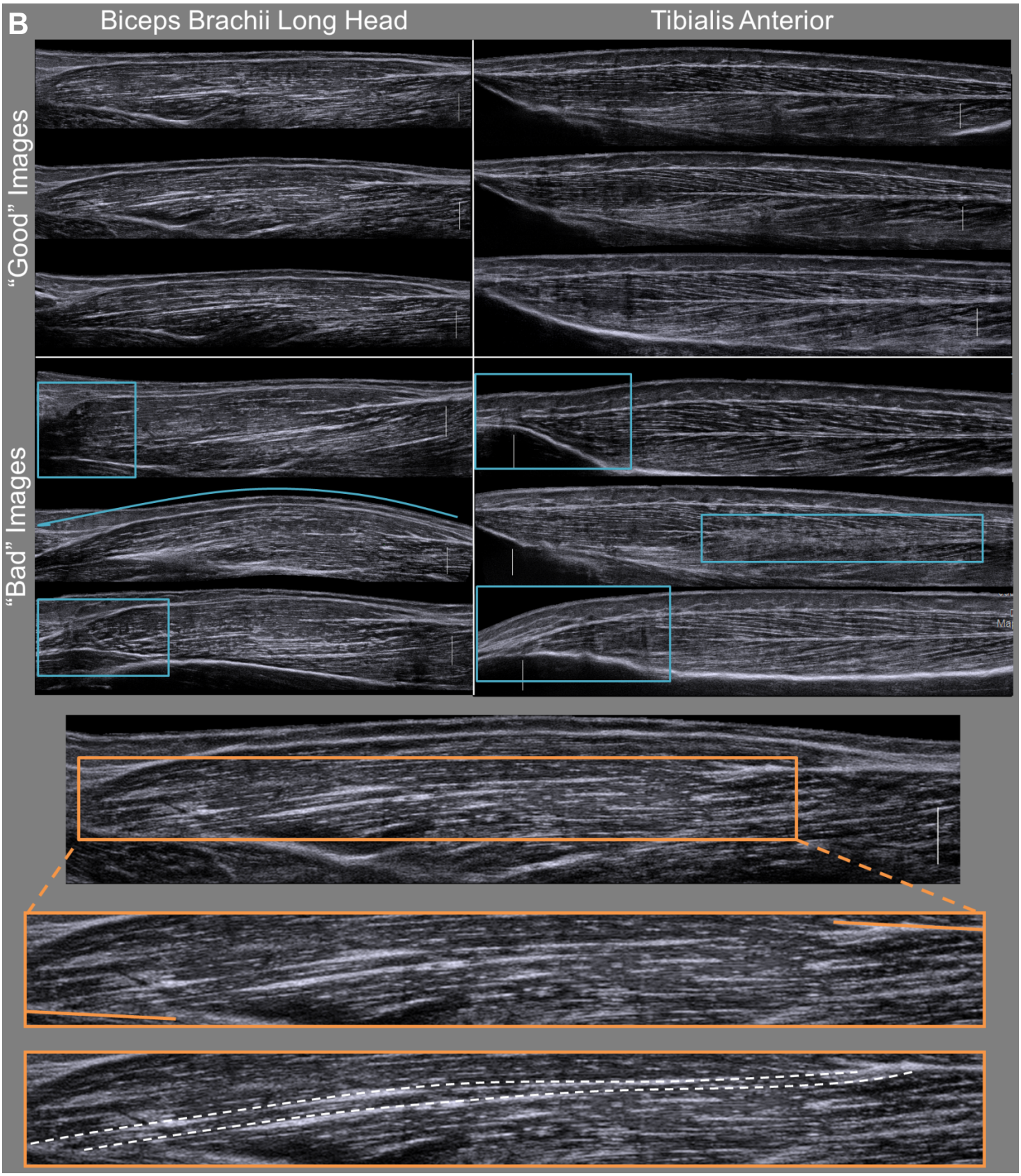


Figure 2


B
Biceps Brachii Long Head
Tibialis Anterior
"Good" Images
"Bad" Images

Figure

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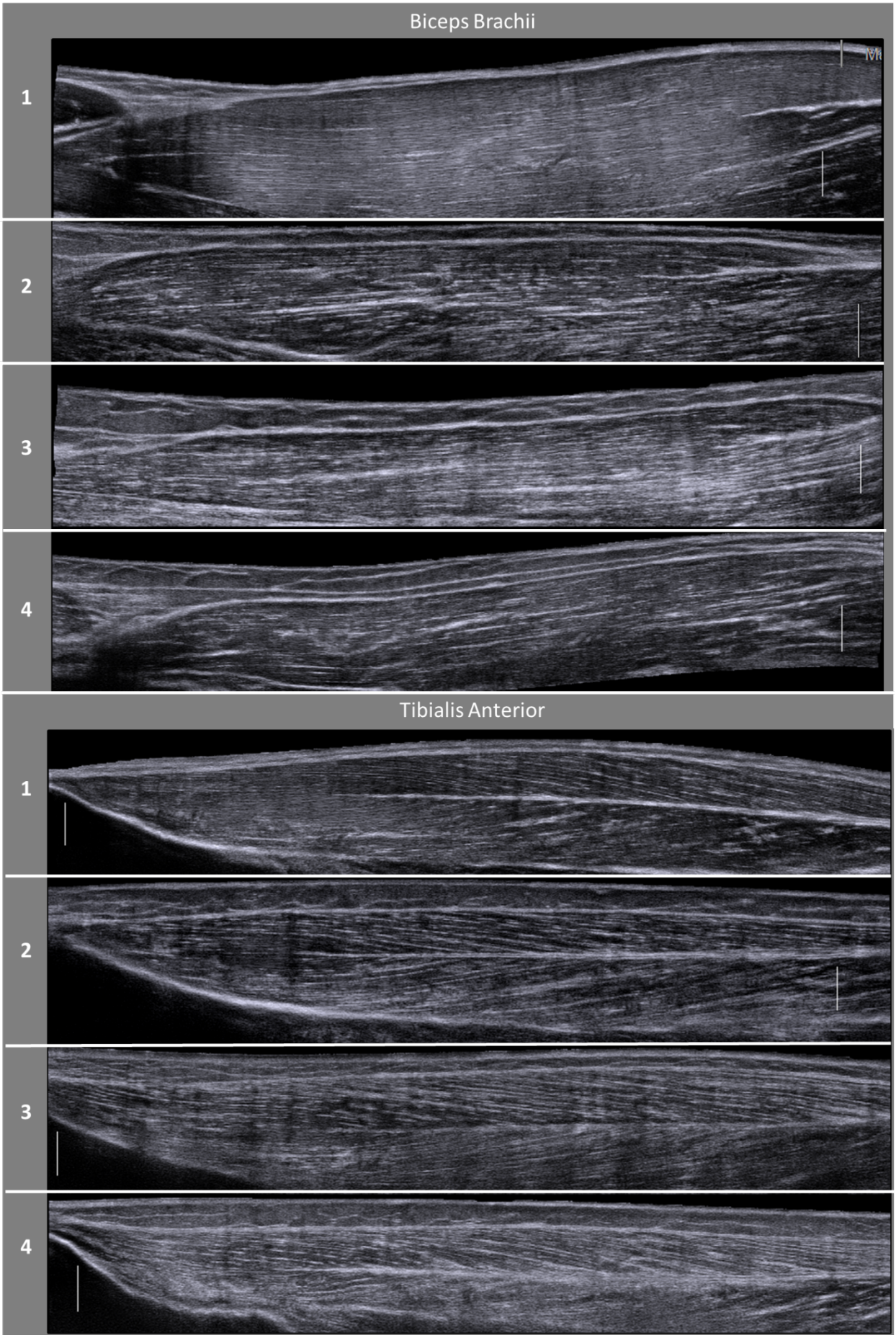

Figure 1


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Figure 2b

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Figure 3

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Table Click here to access/download;Table;Table 1.xlsx

Table 1:

Subject	Gender	Height (m)	Age	Bicep Side	Bicep Fascicle Length (cm)	Tibialis Anterior Side
1	M	1.78	24	L	16.4 ± 0.3	L
2	F	1.8	23	R	12.2 ± 0.2	L
3	M	1.82	24	L	14.9 ± 0.2	R
4	F	1.79	28	R	14.7 ± 0.2	L
				Average	**14.6**	
				SD	**1.7**	

Tibialis Anterior Fascicle length (cm)
7.6 ± 0.1
7.5 ± 0.2
7.7 ± 0.1
6.4 ± 0.3
7.3
0.6

Table of Materials

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Name of Material/ Equipment	Company	Catalog Number	Comments/Description
14L5 linear transducers	Siemens	10789396	
Acuson S2000 Ultrasound System	Siemens	10032746	
Adjustable chair (Biodex System)	Biodex Medical Systems	System Pro 4	
Skin Marker Medium Tip	SportSafe	n/a	Multi-color 4 Pack recommended
Ultrasound Gel - Standard 8 Ounce Non-Sterile Fragrance Free Glacial Tint	MediChoice, Owens &Minor	M500812	

Rebuttal Letter

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This is the first revision of the article submission "Guide to Obtaining Quality Extended Field-Of-View Ultrasound Images of Skeletal Muscle for the Purpose of Measuring Muscle Fascicle Length". The Editor's decision and reviewer comments from the first submission were received on 07/31/2020. We appreciate the comments from the reviewers and the editor. We are resubmitting our article with modifications to the original submission to address the reviewers' comments. We believe that addressing the reviewer critiques has improved the quality of our submission.

We thank the reviewers for their positive feedback regarding our submission. Specifically, Reviewer 1 stated that we "… have provided a clear description of the methods that can be used to obtain EFOV images with the Siemens system." Reviewer 2 stated "It is a good article and definitely of interest…. Being an operator myself and using EFOV a lot, I can only see the benefits that the readers will have from checking this article out!" However, each reviewer pointed out a small number of major concerns. We have responded to each set of reviewer comments and criticisms below. Our response explains how the reviewer comments were addressed and points the reader to the specific area in the paper where changes were made, as applicable.

Editorial Comments:

• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

We have proofread the manuscript for spelling and grammatical errors.

• **Abstracts:**

• Please list a minimum of 6 keywords/phrases.

We had listed 6 keywords/phrases in our original submission.

• **Textual Overlap:** Significant portions show significant overlap with previously published work. Please re-write lines … avoid this overlap.

Through the process of addressing reviewers comments we have re-written significant portions of the text. In addition, we did a comparison of our original submission and there was very minimal overlap with our previous works. We have re-worded where overlap was observed.

• **Introduction:** Please expand your Introduction to include the following:

1.The advantages over alternative techniques with applicable references to previous studies;

2.Description of the context of the technique in the wider body of literature;

3.Information that can help readers to determine if the method is appropriate for their application.

Our introduction included details about point 1 (lines 61-70). In response to this comment and comments from reviewer 2 (see response 1) we have added additional citations to place the context of EFOV US in the wider body of literature. We have added additional information in lines 51-57 about the value and functional importance of measuring

fascicle length which we believe combined with lines 83-85 readers will be able to determine if this method is appropriate for their applications.

• **Protocol Language:** Please ensure that all text in the protocol section is written in the imperative voice/tense as if you are telling someone how to do the technique (i.e. "Do this", "Measure that" etc.) Any text that cannot be written in the imperative tense may be added as a "Note", however, notes should be used sparingly and actions should be described in the imperative tense wherever possible.
1) Some examples NOT in the imperative: entire sections 1.1-1.2, Lines 184-194, etc.
2) Avoid personal pronouns "you", "your" throughout.

We have changed the language in the protocol to be imperative and to exclude the use of pronouns "you" and "your".

• **Protocol Detail:** Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. **Please ensure that all specific details (e.g. button clicks for software actions, numerical values for settings, etc) have been added to your protocol steps.** There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

We believe our protocol has sufficient detail for script generation.

• **Protocol Numbering:**
1) Please adjust the numbering of your protocol section to follow JoVE's instructions for authors, 1. should be followed by 1.1. and then 1.1.1. if necessary.
2) All steps should be lined up at the left margin with no indentations.
3) Please add a one-line space after each protocol step.

We have adjusted the numbering, aligned the text with the left margin with no indentions, and added a one-line space after each step.

• **Protocol Highlight:** After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. There is a 10-page limit for the protocol text, and a 3- page limit for filmable content. If your protocol is longer than 3 pages, please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps.
1) The highlighting must include all relevant details that are required to perform the step. For example, if step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be included in the highlighting.
2) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.
3) Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.
4) Notes cannot be filmed and should be excluded from highlighting.

We have highlighted less than 3 pages of the protocol text for filming.

• **Figures:**

1) Add a common figure title for fig 1.

2) Provide scale references for each ultrasound image.

We have added common figure titles for all figures. Scale references are provided for all images.

• **Commercial Language:** JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Examples of commercial sounding language in your manuscript are Acuson S2000 Siemens,

1) Please use MS Word's find function (Ctrl+F), to locate and replace all commercial sounding language in your manuscript with generic names that are not company-specific. All commercial products should be sufficiently referenced in the table of materials/reagents. You may use the generic term followed by "(see table of materials)" to draw the readers' attention to specific commercial names.

We have removed any commercial sounding language from the manuscript.

• **Table of Materials:**

1) Please sort in alphabetical order.

The materials are in alphabetical order.

• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

Figures and tables have not been previously published.

Comments from Peer-Reviewers:

Reviewer #1:

Manuscript Summary:

1.This paper provides a detailed guide on how to obtain extrapolated field of view ultrasound images from a Siemens system and how to obtain fascicle length from this image. Overall, I think the authors have provided a clear description of the methods that can be used to obtain EFOV images with the Siemens system. However, the suggestions are really specific to the system used in this study. Can the authors also provide some guidelines on how to perform EFOV imaging with other commonly used systems? Without details for other systems the applicability of these

suggestions is limited as the system used in this study is not widely used in the biomechanics community (
see https://journals.physiology.org/doi/full/10.1152/japplphysiol.00835.2019 for an overview of systems and other methods to analyse fascicle length that are commonly used). Therefore, I would suggest the authors to also provide information on how to perform this with other systems to improve the applicability of their paper. If the papers remains very specific to the Siemens system I suggest the authors to adjust the title to reflect that this guide is only applicable to the Siemens system

We agree with the reviewer that some of the language we used in our original manuscript was very specific to the ultrasound machine (Acuson S2000 Siemens) used, narrowing the reach of our paper. We want our paper to be more widely applicable for the biomechanics community, whom, as the reviewer pointed out, use a variety of ultrasound systems. However, based off of an editorial comment (see commercial language above) which states that we "cannot use company brand names before an instrument or reagent", we cannot change the title of the manuscript as the reviewer suggested and are limited in our ability to provide specific information on exactly how to perform EFOV with a variety of systems (as the reviewer has suggested). Still, to address the reviewers concerns we have 1) made edits to the sections of the methods which were too specific to our ultrasound system (see response to specific comment 6 below) and 2) we have pointed readers to Van Hooren et.al, 2020 (mentioned by the reviewer) and other works which list a variety of ultrasound systems which have EFOV-US capabilities (line 124). We thank the reviewer for their comment because in the process of changing the methods to be less system specific and pointing readers to a variety of US systems with EFOV US capabilities, we broadened the applicability of this paper to a wider audience.

Some specific comments:

2. L84-85: how much is little? And please provide some more information on the US technique used. For example was this EFOV? If not, what probe size was used and which muscles were compared?

The difference between ultrasound and DTI measures of muscle fascicle length in this study was on average less than 3mm. The authors did not use EFOV-US. Instead to measure fascicles of the medial gastrocnemius which exceeded the length of a single ultrasound probe, they used a dual probe technique. This technique requires some extrapolation of fascicles across the 18mm gap between the two probes. We thank the reviewer for the comment as we have added detail to the paper to point out what a "little" difference between the methods was and what less precise means to provide the reader more detail about the study (line 99-101).

3. L115-116: it would be helpful to provide an overview in a table of different systems that provide the option to do EFOV. For example, is this available in commonly used ultrasound systems from Telemed, Esaote, and Toshiba? And if so, how are these options called in these systems?

We agree with the reviewer that a table of different systems using EFOV-US would be valuable to the reader. However, as mentioned previously, the editorial reviewer

suggested that company names cannot be used in JoVE publications. To provide reviewers with some information about other US systems we have pointed to the following references (Van Hooren et.al, 2020; Franchi et.al, 2018; Kwah et.al., 2013) which provide a range of studies implementing EFOV-US imaging on systems other than the one used here (line 124). We have also emphasized that readers should take some time to determine what kind of algorithm their system implements, and the validity of measures made using their panoramic setting (line 161-171, also see line 551-555).

4.L128: replace chair by table as for some muscles (e.g. hamstrings) a chair will not work with imaging.

The adjustable chair used in this study is able to lay completely flat for accessing muscles such as the hamstring. However, we have added a sentence addressing the concern of the reviewer for those readers whom may not have a fully adjustable chair (line 185-186).

5. L136-138: what are some suggestions from the authors on determining joint angles in a practical way to improve reliability?

We agree with the reviewer that adding practical details about determining joint angles is valuable for the reader. We have added a couple sentences (lines 188-193) to address this comment. Specifically, we explain the steps to obtaining joint angle using a handheld goniometer and point the reader to a clinical guide for obtaining goniometric measurements and the International Society of Biomechanics (ISB) standards for defining joint coordinate systems.

6. L149-155 & 177-180: these suggestions are really specific to the system used in this study. Can the authors also provide some guidelines for how this works with other commonly used systems?

As described in response 1, we agree with the reviewers that this language is too specific to the ultrasound system we used. Since the reviewer pointed this out, and the editorial reviewer suggested that we cannot use specific company names (Acuson S2000 Siemens) we have made this section a bit more general. It is notable that the protocol (step 1.1.1) begins by telling the reader to become familiar with their own ultrasound system. We have taken out button names which are specific to only our system. See our changes to protocol in lines 216-224 & 246.

7. L186: please replace length by thickness or depth?

We appreciate the reviewers comment however we do mean to say “length” here. The ultrasound image settings are so that the maximum EFOV image length that can be captured is dependent on the depth setting. For example, if your EFOV scan stops before the entire muscle, or desired muscle portion, can be capture, the sonographer should increase the depth (i.e. from 2cm to 3cm) to allow for a longer scan. A small clarifying point has been added to lines 330-331.

8.L238: there are also several other methods to determine muscle fascicle length available. Perhaps the authors can refer to these methods as well for interested readers.

We chose to use ImageJ for this guide as it is, to our knowledge, the most commonly implemented image processing software used for measuring fascicle lengths from ultrasound (I.e. Freitas et al. Ultrasound 2017; Pimenta et al. MSSE 2018; Franchi MSEE 2020; Franchi 2014; Nelson J Biomech 2016 & NNR 2018; Noorkoiv J App Physiol 2010) and it is a freely available, open-source software. Other than ImageJ it seems like some biomechanists often use custom code (i.e. Herbert J. Physiology 2011; Jakubowski Clin Biomech 2017) and few use other software (i.e. Alanso-Fernandez JMSS 2017 – MicroDicom; Seymore EJAP 2017 - OsiriX). We have added a note to the protocol pointing out that ImageJ is not the only available method for determining fascicle length (lines 448-449).

Reviewer #2:

Manuscript Summary:

The manuscript aims to be a sort of a guide for operators to implement the use of EFOV scans. It is a good article and definitely of interest.

Major Concerns:

1. My first major concern is that this guide actually provides data of just two muscles, and actually not the most difficult ones to scan. As this has to be a guide, I would have expected details for scanning more than just the TA for the lower limbs. Previous work on GM, VL, and recently the very heterogeneous Biceps Femoris long head have been published. While this is not preventing the present article for being a good publication nonetheless, the authors should provide (IMHO) references for more muscle groups (I refer the authors to Franchi et al. Ultrasound Med Biol 2018 Review, where EFOV procedure is also throughly explained and in which the concern for b-mode US scan with smaller field of view are discussed - for Biceps Femoris, Franchi et al. MSSE 2020 takes into examination EXACTLY the pitfalls of fascicle length extrapolation, which fully support your points made in the introduction section). Thus, the introduction should be more on-point, as it feels like the some of the literature has been overlooked (Seymore et al. EJAP 2017, Freitas et al. Ulltrasound 2017; Pimenta et al. MSSE 2018 for example of biceps femoris long head)

We agree with the reviewer that we have missed some of the more recent work and appreciate the reviewer pointing us to this literature. We have integrated the works mentioned above throughout both our introduction and discussion sections. In addition, we have further emphasized that the two muscles we chose for this guide are only examples and that there are other muscles which have been previously imaged, as the reviewer points out (line 125-130).

We would like to note that one of our main motivations for creating this guide was to provide readers with information to be able to take quality fascicle measures of a variety of muscle, for the ultimate purpose of increase the pool of muscles for which we have *in vivo* muscle fascicle length data. Thus, we made the choice not to pick the muscles (i.e. GM and VL) which are most commonly measured as it is clear from the literature that FL of those muscles can be captured. The goal of this guide is not to help people take images of the biceps brachii and tibialis anterior, but to show the field that because the EFOV

technique can capture muscles with longer curved fascicles, more muscles than have been studied have the potential to be studied. Further we chose these two muscles as they are different types (fusiform vs bi-pennate) and are in different portions of the body (upper limb vs lower limb).

2. Lastly, the images are very nice! I commend the authors for that. However, we know how difficult is to acquire an image of the full muscle without making any mistakes. Wouldn't be better to state in the discussion (or limitations section) that acquiring scans for smaller field of views (for example 10-12 cm, still larger than the normal FOVs of b-mode scans) will lead to less possibility to obtain "bad" images?

We appreciate the reviewer's compliment about our images! We agree that there is a commonly held perception that the EFOV-US method introduces more error than traditional ultrasound. However, it is unclear the extent to which this is true as only one study (by us) has evaluated this. In this, study we compared fascicle measurements from an EFOV US to 6 traditional ultrasound images which spanned the length of the muscle and found that there was no statistical difference between fascicles obtained from the two different methods (Adkins et. al., J Biomech, 2017). This finding, which contradicts the commonly held perception, suggests EFOV US does not result in additional error due to the longer scan. Still, this has only been tested in a single muscle in a single study. In addition, we have extensive practice with obtaining ultrasound images of muscle and recognize that obtaining quality EFOV-US images requires substantial practice, which our readers may not have. Thus, we have added a paragraph in the discussion (lines 571-579) pointing out this idea of obtaining a full muscle EFOV-US image versus a shorter scan which still captures full fascicles. We point out the finding of our study (Adkins et. al., J Biomech, 2017) but also suggest that shorter scans may be more appropriate for sonographers with little experience.

Minor Concerns:

3. The introduction is sometimes lacking of important concepts. For example, it is not thoroughly explained why is important to measure fascicle length? Maybe Lieber and Friden 2000 can be a starting point (ie., the comparison of two muscle with same PCSA but longer vs. shorter fibres), or any other paper that show a change in the angle-torque relationship in vivo with changes in fascicle length? And in addition: maybe important to detect changes in fascicle length after loading or unloading scenarios? In which training modality fascicle length increase?

We appreciate the reviewers comment and have added a couple sentences to further emphasize the importance of fascicle length measures (line 51-57). We have also added "different loading or unloading" states as an advantage of in vivo methods for quantifying fascicle length (line 66).

4. When talking about transducer's lengths, please acknowledge that 10 cm length has been extensively used in many articles on muscle architecture (work by Narici's group), but maybe that the limitation is that we cannot still see the full length of the fascicles in some cases and that some muscles do show a particularly heterogeneous architecture so already with 10 cm length

there is the risk of not being fully on the same fascicle plane throughout the whole length of the probe (as it's linear and can't have multiple orientation?).

As the reviewer has suggested we have acknowledged the use of 10cm probes citing work from Narici's group (line 75) and have mentioned the advantages and disadvantages of 10cm probes in the discussion (lines 619-624).

5. Nevertheless, I commend the author for the nice guide provided. Being an operator myself and using EFOV a lot, I can only see the benefits that the readers will have from checking this article out!

We appreciate the reviewers support for this work! The reviewer's helpful comments have led us to make changes which we believe improves the quality of the work and will broaden reader interest.