FINAL SCRIPT: APPROVED FOR FILMING

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Title: Ultrasound Tissue Characterization of Human Achilles Tendon by Stability Quantification of Echo Patterns

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **NO**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **YES, all done**

3. **Filming location:** Will the filming need to take place in multiple locations? **NO**

Current Protocol Length
Number of Steps: 25
Number of Shots: 46

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Chiara Busso:** We aimed to describe a standardized protocol using Ultrasound Tissue Characterization to assess Achilles tendon structure, and to support its application in both research and clinical practice for diagnosing injuries and monitoring treatment response.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:5.1*
Videographer's Note: Use A7sIII_0160

What are the most recent developments in your field of research?

1.2. **Marco A. Minetto:** Many approaches for the assessment of tendon disorders have been proposed. The most recent advances are in the area of ultrasound imaging, which provides an accessible approach to evaluating tendon size and structure.
Videographer's Note: Use A7sIII_0158, A7sIII_0159, A7sIII_0160

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.9*

What research gap are you addressing with your protocol?

1.3. **Chiara Busso:** We addressed the need for a standardized, operator-independent method to quantitatively assess tendon structure, overcoming key limitations of traditional ultrasound imaging.
Videographer's Note: Use A7sIII_0164

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What research questions will your laboratory focus on in the future?

1.4. **Marco A. Minetto:** Among the many new questions, the method we described will put forth the question of what echo-types are associated with various disease states. Do echo-type changes precede and predict the occurrence of clinical symptoms?
Videographer's Note: Use A7sIII_0162

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the local ethics committees (Comitato Etico Interaziendale) at the Politecnico di Torino

Protocol

2. Transducer Assembly and System Setup for Ultrasound Imaging

Demonstrator: Chiara Busso

2.1. To begin, unscrew the two metal screws underneath the tracker to release the stand-off **[1]**. Cover the superior surface of the stand-off with a thick layer of acoustic gel **[2]**. Then reattach the stand-off to the tracker **[3]**.

2.1.1. WIDE: Talent unscrewing the metal screws below the tracker to release the stand-off.
Videographer's Note: Use A7sIII_0166

2.1.2. Talent applying a thick layer of acoustic gel on top of the stand-off.
Videographer's Note: Use A7sIII_0165

2.1.3. Talent reassembling the tracker and stand-off.
Videographer's Note: Use A7sIII_0165

2.2. Unscrew the two black screws of the transducer strap and the locking screw of the transducer holder base **[1]**.
Videographer's Note: Use A7sIII_0168, A7sIII_0169 for 2.2.1-2.4.2

2.2.1. Talent unscrewing the black screws of the strap and the locking screw of the holder base.

2.3. Insert the transducer into the transducer holder base and fasten the locking screw and the two strap's screws **[1].**

2.3.1. Talent inserting the transducer into the transducer holder base and tightening the locking screw at the base and on the strap.

2.4. Next, unscrew the two black screws securing the transducer holder **[1]**. Adjust the holder height so the transducer runs half a millimeter to one millimeter above the stand-off, remaining in contact with the acoustic gel **[2]**.

2.4.1. Talent unscrewing the two screws fixing the transducer holder.

2.4.2. Talent adjusting the holder height so that it is nearly flush with the stand-off and in contact with gel.

2.5. Connect the tracker using the USB cable and attach the transducer to the Smart System **[1]**. Plug in the power-brick to supply power **[2]**.

2.5.1. Talent connecting the tracker and transducer cables to the Smart System ports.
Videographer's Note: Use A7sIII_0170, A7sIII_0172, A7sIII_0173

2.5.2. Talent plugging in the power-brick to power up the system.

Videographer's Note: Use A7sIII_0171

3. System Initialization, Subject Positioning, and Ultrasound Data Acquisition

3.1. Press the power button on the top-left edge of the Smart System until the blue indicator lights up. Few beeps will be heard **[1]**.

3.1.1. Talent pressing the top-left power button and blue light appearing on the Smart System.
Videographer's Note: Use A7sIII_0175

3.2. Double-click on the ultrasound software icon on the desktop to launch it **[1]**. Then minimize the ultrasound screen from the Smart System toolbar **[2]**. Press the **ON/OFF** *(on-off)* button to power on the tracker **[3]**.

3.2.1. SCREEN Screen-3.2.1---3.2.2.mp4 00:04-00:19.
3.2.2. SCREEN: Screen-3.2.1---3.2.2.mp4 00:20-00:25 .
3.2.3. Talent pressing the ON/OFF button to turn on the tracker.
Videographer's Note: Use A7sIII_0176

3.3. Position the subject in a comfortable prone position with the bare foot relaxed and dangling off the end of the table **[1]**. Apply a thick layer of acoustic gel to the skin on the distal posterior part of the leg to ensure good contact with the transducer **[2]**.

3.3.1. Shot of Subject positioning themselves prone on the table with foot dangling off the edge.
Videographer's Note: Use A7sIII_0177
3.3.2. Talent applying a thick layer of gel on the distal posterior leg of the subject.
Videographer's Note: Use A7sIII_0178 for 3.3.2 to 3.4.1

3.4. Then push the knee against the subject's metatarsal heads to passively dorsiflex the ankle until it reaches maximum dorsiflexion **[1]**.

3.4.1. Talent pushing the subject's knee forward to dorsiflex the ankle.

3.5. Next, double-click on the analysis software icon on the Smart System **[1]**. Then click the red **Acquisition** button on the upper toolbar **[2]**. When the message **Tracker initialization OK** appears, click **OK [3]**.

3.5.1. SCREEN: Screen-3.5.1---3.7.2.mp4 00:03-00:09.
3.5.2. SCREEN: Screen-3.5.1---3.7.2.mp4 00:10-00:15 .
3.5.3. SCREEN: Screen-3.5.1---3.7.2.mp4 00:16-00:20 .

3.6. When the acquisition control window opens, click **START US** *(Start-U-S)* to display the ultrasound image from the ultrasound software **[1]**.

3.6.1. SCREEN: Screen-3.5.1---3.7.2.mp4 00:21- 00:29 .

3.7. In the acquisition control window, click **ACQUISITION [1]**. When prompted with "Press

the Start-button on the tracker to start the acquisition", click **OK [2]**.

3.7.1. SCREEN: Screen-3.5.1---3.7.2.mp4 00:30-00:38 .
3.7.2. SCREEN: Screen-3.5.1---3.7.2.mp4 00:39-00:45 .

3.8. Place the tracker on the calcaneal region so that it is perpendicular to the long axis of the Achilles tendons **[1]**.

3.8.1. Talent positioning the tracker perpendicularly on the calcaneal region.
Videographer's Note: Use A7sIII_0180

3.9. Then press the **START** button on the tracker to begin the acquisition **[1]**. The transducer begins to move automatically along the tracker over the length of the tendon **[2].** Monitor the full scanning procedure on the ultrasound scanner screen of the Smart System **[3]**.

3.9.1. Talent pressing the **START** button on the tracker.
Videographer's Note: Use A7sIII_0182, A7sIII_0181
3.9.2. Shot of the transducer moving automatically along the tracker over the length of the tendon.
Videographer's Note: Use A7sIII_0180
3.9.3. SCREEN: Screen-3.8.4.mp4 00:00-00:14

3.10. At the end of data collection, click **OK** on the acquisition control window. Then click **EXIT** to close the window **[1]**.

3.10.1. SCREEN: Screen-3.9.1---3.9.2.mp4. 00:00-00:06

3.11. Click on the transverse cross-section in the top-left of the analysis software screen to refresh and display acquired images **[1]**. From the **FILE** dropdown menu, select **SAVE SCAN** and save images into the designated folder **[2]**.

3.11.1. SCREEN: Screen-3.10.1.mp4. 00:00-00:07
3.11.2. SCREEN: Screen-3.10.2.mp4. 00:00-00:12

4. Image Analysis

4.1. Click the appropriate button on the upper toolbar of the analysis software to switch from qualitative to quantitative parameter set **[1]**. Then press the **UTC Quantitative Analysis Properties** setting button on the toolbar to open the window **[2]**.

4.1.1. SCREEN: Screen-4.1.1---4.2.2.mp4. 00:00-00:03
4.1.2. SCREEN: Screen-4.1.1---4.2.2.mp4. 00:04-00:07

4.2. Now, flag **CUSTOMIZED SET 1** from the settings window **[1]**. Set the sample window to 25, click **SAVE ALL SETTINGS** to save changes and then click **OK** to close the window **[2]**.

4.2.1. SCREEN: Screen-4.1.1---4.2.2.mp4. 00:08-00:11
4.2.2. SCREEN: Screen-4.1.1---4.2.2.mp4. 00:12-00:21

4.3. Next, press the Show/edit *(Show-or-edit)* contour button on the upper toolbar to open contour mode window and flag **CONTOUR [1]**. Click the analysis 1 button on the toolbar to start contouring **[2].** Slide through sagittal scans and click at intervals to draw a contour around the tendon in the transverse cross-section, starting from the edge of the calcaneus bone, switching from analysis to ultrasound mode to properly identify tendon margins **[3]**.

4.3.1. SCREEN: Screen-4.4.1---4.5.1.mp4. 00:00-00:07
4.3.2. SCREEN: Screen-4.4.1---4.5.1.mp4. 00:08-00:17
4.3.3. SCREEN: Screen-4.4.1---4.5.1.mp4. 00:18-01:00

4.4. Continue drawing a contour every 25 images while moving through the sagittal section **[1]**.

4.4.1. SCREEN: Screen-4.4.1---4.5.1.mp4. 01:07-01:30

4.5. Once all contours are completed, select **SAVE UTC CONTOUR** from the **FILE** dropdown menu,to save all drawn contours **[1]**.

4.5.1. SCREEN: Screen-4.6.1.mp4. 00:00-00:12

4.6. Click the specific analysis launch button, which corresponds to the sample window ideal for general analysis for statistics, on the toolbar to start a new analysis **[1]**.

4.6.1. SCREEN: Screen-4.7.1---4.8.2.mp4. 00:00-00:11

4.7. Then press the statistics button on the toolbar to open the statistical graph window **[1]**. Click **CONTOUR** to display graphs for all drawn and interpolated contours **[2]**.

4.7.1. SCREEN: Screen-4.7.1---4.8.2.mp4. 00:12-00:16
4.7.2. SCREEN: Screen-4.7.1---4.8.2.mp4. 00:17-00:21

4.8. For reporting, navigate to the sagittal cross-section of the tendon and click the **STAR** button on the toolbar to select reference image at significant positions **[1]**.

4.8.1. SCREEN: Screen-4.9.1---4.10.1.mp4. 00:00-00:28

4.9. Press the *export pdf* button on the toolbar to import images and statistics into the PDF editor **[1]**.

4.9.1. SCREEN: Screen-4.9.1---4.10.1.mp4. 00:29-00:37

Results

5. Results

5.1. The normal Achilles tendon showed homogeneous echogenicity in both longitudinal and transverse scans, with the thickest region measured at 4 millimeters at position #2 **[1]**. UTC analysis of the normal tendon revealed a high percentage of echo-type I, indicating intact and aligned tendon bundles, across all three positions **[2]**.

5.1.1. LAB MEDIA: Figure 1. *Video editor: Please position #2 in top image*

5.1.2. LAB MEDIA: Figure 1. *Video editor: Highlight the green pixel clusters in the bottom panels of positions #1, #2, and #3.*

5.2. The pathological Achilles tendon showed moderate hypoechogenic regions proximally and extensive hypoechogenic areas in both the midportion and distal positions **[1]**.

5.2.1. LAB MEDIA: Figure 2. *Video editor: Highlight the positions #2 and #3 in top image*

5.3. UTC analysis of the pathological tendon showed a reduced percentage of echo-type I and a high presence of echo-types III and IV, especially in the central and distal portions **[1]**.

5.3.1. LAB MEDIA: Figure 2. *Video editor: Highlight the bottom panels with a focus on the red and grey pixels in positions #2 and #3.*

5.4. The echo-type I percentage in the normal tendon was consistently high, ranging from 86.9% to 90.3% at all positions **[1]**, and remained elevated in the total tendon volume at 83.8% **[2]**.

5.4.1. LAB MEDIA: Figure 3. *Video editor: Highlight the green lines in the left graph*

5.4.2. LAB MEDIA: Figure 3. *Video editor: Highlight the total volume value (83.80) in Echo Type 1 in the left table*

5.5. In contrast, the pathological tendon exhibited a much lower echo-type I percentage at all positions **[1]**, while echo-types III and IV were significantly elevated, comprising 50.7% of the total volume **[2]**.

5.5.1. LAB MEDIA: Figure 3. *Video editor: Highlight the green lines in the right graph*

5.5.2. LAB MEDIA: Figure 3. *Video editor: Highlight the total volume value (9.41 and 41.33) in Echo Type III and Echo Type IV in the right table*

Pronunciation Guide:

1. **Ultrasound Tissue Characterization (UTC)**
    - **Pronunciation link: No single authoritative link, but "ultrasound" and "characterization" can be found in dictionaries.**
    - **IPA: /ˈʌltrəˌsaʊnd ˈtɪʃu ˌkɛrəktəraɪˈzeɪʃən/**
    - **Phonetic: UL-truh-sound TISH-oo kah-rak-tuh-rye-ZAY-shun**
2. **Achilles tendon**
    - **Pronunciation link: https://www.merriam-webster.com/dictionary/Achilles**
    - **IPA: /əˈkɪliːz ˈtɛndən/**
    - **Phonetic: uh-KIL-eez TEN-dun**
3. **Echogenicity / Echogenic**
    - **Pronunciation link: https://www.merriam-webster.com/dictionary/echogenicity**
    - **IPA: /ˌɛkoʊdʒəˈnɪsəti/**
    - **Phonetic: eh-ko-juh-NIS-i-tee**
4. **Hypoechogenic / Hypoechogenicity**
    - **(Hypo- prefix + echogenic)**
    - **IPA: /ˌhaɪ.poʊˌɛkoʊdʒəˈnɪsɪti/**
    - **Phonetic: hy-poh-eh-ko-juh-NIS-i-tee**
5. **Intraoperator / Intraoperative**
    - **Pronunciation link: https://www.merriam-webster.com/dictionary/intraoperative**
    - **IPA: /ˌɪntrəˈɑpərətɪv/**
    - **Phonetic: in-truh-OP-er-uh-tiv**
6. **Anteroposterior**
    - **Pronunciation link: https://www.merriam-webster.com/dictionary/anteroposterior**
    - **IPA: /ˌæn.təroʊˈpɒstəriər/**
    - **Phonetic: an-ter-oh-PAH-steer-ee-er**
7. **Sagittal / Coronal / Transverse**
    - **Sagittal: /ˈsædʒɪtəl/ — SAJ-ih-tul**
    - **Coronal: /ˈkɔːrənəl/ — KOR-uh-nul**
    - **Transverse: /trænsˈvɜrs/ — tranz-VERS**
8. **Quantification / Quantitative**
    - **Pronunciation link: https://www.merriam-webster.com/dictionary/quantification**
    - **IPA (quantification): /ˌkwɑntɪfɪˈkeɪʃən/**
    - **Phonetic: kwan-tih-fuh-KAY-shun**
    - **Quantitative: /ˈkwɑntɪtətɪv/ — KWAN-tih-tuh-tiv**
9. **Echo-type**
    - **(Echo + type)**
    - **IPA: /ˈɛkoʊˌtaɪp/**
    - **Phonetic: EH-ko-type**

10. **Fibrillar / Fibrils**
    - **Pronunciation link: https://www.merriam-webster.com/dictionary/fibrillar**
    - **IPA: /ˈfaɪbrələr/**
    - **Phonetic: FYE-bruh-lur**
11. **Amorphous**
    - **Pronunciation link: https://www.merriam-webster.com/dictionary/amorphous**
    - **IPA: /əˈmɔrfəs/**
    - **Phonetic: uh-MOR-fuss**
12. **Reproducibility / Reliability**
    - **Reproducibility: /ˌriːprəˌdʌsəˈbɪlɪti/ — re-pro-du-si-BIL-i-tee**
    - **Reliability: /ˌriːlaɪˈæbɪlɪti/ — ree-lye-AB-i-lity**
13. **Operator-independent**
    - **IPA: /ˌɒpəˌreɪtər ˌɪndɪˈpɛndənt/**
    - **Phonetic: OP-uh-rayter in-di-PEN-dunt**
14. **Disorganized / Disorganisation**
    - **Disorganized: /dɪsˈɔrgənaɪzd/ — dis-OR-guh-nyzd**
    - **Disorganization: /ˌdɪsˌɔrgənaɪˈzeɪʃən/ — dis-or-guh-nuh-ZAY-shun**