FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 69236
Scriptwriter Name: Sulakshana Karkala
Project Page Link: https://review.jove.com/account/file-uploader?src=21117028

Title: A Methodological Protocol and Considerations for Transcranial Ultrasonic Stimulation in Exploratory Clinical Human Studies

Authors and Affiliations:
Ziping Huang[1,2,*], Mengyue Chen[3,*], Charalambos C. Charalambous[1], Lei Zhu[1], Ergi Spiro[1,2], Shashank Shekhar[1], Jody A. Feld[1,4], Xiaoning Jiang[1,3], Junjie Yao[1,2], Wuwei Feng[1,2]

[1]Department of Neurology, Duke University School of Medicine
[2]Department of Biomedical Engineering, Duke University
[3]Department of Mechanical and Aerospace Engineering, North Carolina State University
[4]Department of Orthopedic Surgery, Duke University School of Medicine

[*]These authors contributed equally

Corresponding Authors:
Ziping Huang ziping.huang@duke.edu
WuweiFeng wayne.feng@duke.edu
Email Addresses for All Authors:
Ziping Huang ziping.huang@duke.edu
Mengyue Chen mchen23@ncsu.edu
Charalambos C. Charalambous charalambos.charalambous@duke.edu
Lei Zhu l.zhu@duke.edu
Ergi Spiro ergi.spiro@duke.edu
Shashank Shekhar shashank.shekhar@duke.edu
Jody A. Feld jody.feld@duke.edu
Xiaoning Jiang xjiang5@ncsu.edu
Junjie Yao junjie.yao@duke.edu
Wuwei Feng wayne.feng@duke.edu

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.**

Videographer : Please film the screen of the instrument for the following shots:
2.4.1, 2.5.1, 3.1.2, 3.2.1, 3.2.2, 3.3.2, 3.4.1, 3.5.1, 3.5.2

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

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length
Number of Steps: 27
Number of Shots: 56

Introduction

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

INTRODUCTION:

1.1. **Ziping Huang:** Our research encompasses the use of transcranial ultrasonic stimulation (TUS) in neurotypical and neurological adults.

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

1.2. **Ziping Huang:** TUS facilitates basic neuroscience inquiries and is increasingly investigated for neurological and psychiatric diseases like post-stroke complications, depression, and pain.

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

CONCLUSION:

1.3. **Ziping Huang:** We established TUS safety and effective intensity in stroke patients, showing motor-cortex TUS can enhance motor learning and cortical excitation.

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

1.4. **Ziping Huang:** There is no methodological article focusing on the use of TUS in neurologically diseased adults. This protocol addresses this gap.

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

1.5. **Ziping Huang:** We hope this protocol can reduce the methodological variability and improve the reliability and reproducibility of future human ultrasound studies.

1.5.1. 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.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.6. **Ziping Huang, Graduate research assistant**: (authors will present their testimonial statements live)

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

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

1.7. **Ziping Huang, Graduate research assistant**: (authors will present their testimonial statements live)

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

Ethics Title Card

This research has been approved by the Institutional Review Board (IRB) at Duke University

Protocol

2. Hydrophone-Based Characterization of Ultrasound Transducer Acoustic Output in a Water Tank Setup

Demonstrators: Yu Chu, Ziping Huang

2.1. To begin, prepare a water tank with degassed, deionized water to prevent potential damage to the hydrophone **[1]**. Fixate the ultrasound transducer onto the fixture on the water tank **[2]** and mount the hydrophone onto the 3-axis positioning system **[3]**.

2.1.1. WIDE: Talent preparing the water tank with degassed, deionized water.

2.1.2. Talent securing the transducer onto the fixture on the water tank.

2.1.3. Talent mounting the hydrophone onto the 3-axis positioning system.

2.2. Connect the hydrophone to an oscilloscope **[1]** and then connect the needle tip along with the cable **[2]**. Switch on the hydrophone power only after it is fully immersed in water **[3]**. Drive the ultrasound transducer using a function generator **[4-TXT]**.

2.2.1. Talent connecting the hydrophone cable to the oscilloscope.

Added shot: Talent connecting the hydrophone needle tip and the cable to the pre-amp

2.2.2. Talent immersing the hydrophone into the water and then switching on its power.

2.2.3. Talent driving the transducer using the function generator. **TXT: Do this only after the transducer is fully immersed and hydrophone is on**

2.3. Then position the hydrophone tip at the approximate focal region of the ultrasound transducer using the 3-axis positioning system **[1]**. ~~Scan the hydrophone across the water tank at possible locations of primary pressure output **[2]**~~.

2.3.1. Talent adjusting the hydrophone tip to the approximate focal region using the 3-axis positioning system.

~~2.3.2. Talent scanning the hydrophone across the tank.~~

2.4. Record the oscilloscope readings at each location to obtain the acoustic pressure distribution **[1]** and document the hydrophone location corresponding to the focal region **[2]**.

2.4.1. Talent recording readings from the oscilloscope and noting the hydrophone location corresponding to the focal region.

Videographer: Please record the screen of the instrument for this step

2.4.2. Talent noting the hydrophone position corresponding to the focal region. **NOTE**: This was combined with Shot 2.4.1.

2.5. Assess the transmitting sensitivity of the ultrasound transducer at the focal point by applying different input voltages **[1]**.

2.5.1. Talent applying varying input voltages and noting pressure outputs to calculate transmitting sensitivity.
Videographer: Please record the screen of the instrument for this step

3. Configuration and Calibration of Transcranial Ultrasound Stimulation (TUS) System

Demonstrators: Yu Chu, Ziping Huang

3.1. Connect the transcranial ultrasound stimulation hardware and start oscilloscope recording **[1]**. Turn on the oscilloscope, set it to **Run**, and adjust both vertical and horizontal axes to the scale of interest **[2]**.

3.1.1. Talent connecting the TUS hardware.

3.1.2. Talent showing the oscilloscope startup, setting to **Run** mode, and demonstrating adjusting axis scales.
Videographer: Please record the screen of the instrument for this step

3.2. Flip the **MAIN POWER** switch of the amplifier to the **on** position **[1]**. Dial the power output of the amplifier to zero **[2]**. Configure both function generators to the desired waveform, ensuring ramping is added to each pulse duration **[3]**.

3.2.1. Talent flipping the amplifier's **MAIN POWER** switch to on

3.2.2. Shot of the amplifier output being dialed to zero.
Videographer: Please record the screen of the instrument for this step

3.2.3. Talent showing configuration of waveform with settings for ramping on.
Videographer: Please record the screen of the instrument for this step

3.3. Now press **Output** on the function generator **[1]**. Dial the amplifier output to 100 percent using the **ADJUST** wheel and monitor the **Gain** display **[2]**. Press the amplifier **POWER** button to start its output **[3]**.

3.3.1. Talent pressing **Output** on the function generator.

3.3.2. Talent turning the **ADJUST** wheel and checking **Gain** on the amplifier display.
Videographer: Please record the screen of the instrument for this step

3.3.3. Talent pressing the amplifier **POWER** button.

3.4. Confirm the drive signal waveform on the oscilloscope and allow the drive system to run for 15 minutes to stabilize the output **[1]**.

3.4.1. Talent checking the waveform displayed on the oscilloscope.
Videographer: Please record the screen of the instrument for this step

3.5. Check for ramping at the beginning and end of each pulse duration on the drive signal waveform **[1]**. Dial the amplifier output to zero **[2]**.

3.5.1. Talent checking the oscilloscope waveform with clear ramping at start and end of pulse duration.
Videographer: Please record the screen of the instrument for this step

3.5.2. Talent dialing amplifier output to zero.
Videographer: Please record the screen of the instrument for this step

3.6. Fully immerse the transducer in water, positioning the exit plane approximately 1 inch below the surface facing upward **[1]**. Then dial the amplifier output back to 100 percent and observe whether the ramping is successful **[2-TXT]**.

3.6.1. Talent immersing the transducer exit plane 1 inch below water surface, facing up.

3.6.2. Talent dialing amplifier output to 100 percent and observing the water surface.
TXT: Rippling indicates success; Bead ejection indicates failure

3.7. Now mount a neuronavigation tool tracker onto the transducer holder **[1]**. In the software, navigate to **Window** and **Tool Calibrations**, click **New Calibration**, enter a name and select the correct tool tracker **[2]**.

3.7.1. Talent attaching neuronavigation tool tracker to transducer holder.

3.7.2. SCREEN: 69236_3.7.2.mp4 00:00-00:25

3.8. Hold the transducer with the tracker against the calibration block such that the reference indicator pin touches and is perpendicular to the center of the transducer's exit plane **[1]**. Click **Begin Calibration Countdown**, hold steady for 5 seconds, and repeat if necessary until **Success** appears **[2]**. Then close both **Edit Calibration** and **Tool Calibrations** windows **[3]**.

3.8.1. Talent pressing the transducer against the calibration block with correct alignment.

3.8.2. SCREEN: 69236_3.8.2-3.8.3.mp4 00:00-00:12

3.8.3. SCREEN: 69236_3.8.2-3.8.3.mp4 00:15-00:20

3.9. Press the amplifier **POWER** button to turn off its output when the participant is ready for transcranial ultrasound stimulation **[1]**. Disconnect the oscilloscope from the amplifier and connect the transducer to the amplifier output **[2]**.

3.9.1. Talent pressing the amplifier **POWER** button.

3.9.2. Talent disconnecting oscilloscope from amplifier and connecting transducer to amplifier output.

4. Neuronavigation-Guided Transcranial Ultrasound Stimulation (TUS) Administration and Data Export Procedure

Demonstrators: Ziping Huang, Lei Zhu

4.1. If using the simulation-based target, load the target into the neuronavigation software **[1]**. In the **Sessions** tab, open the **New...** *(new)* dropdown and select **Online Session [2]**. Under the **Targets** tab, click on the **Trajectory X-mod** *(X-mod)* target and press **Add =>** *(add)* to add it for the session **[3-TXT]**.

4.1.1. SCREEN: 69236_4.1.1-4.1.3.mp4 00:00-00:10

4.1.2. SCREEN: 69236_4.1.1-4.1.3.mp4 00:11-00:18

4.1.3. SCREEN: 69236_4.1.1-4.1.3.mp4 00:19-00:33
TXT: Follow registration and proceed to Perform

4.2. Bring up the **Bullseye (Tool Centric)** *(Bulls-eye-Tool-centric)* or **Bullseye (Target Centric)** *(Bulls-eye-Target-centric)* view in the **Perform** window **[1]**. Set the **Driver** to the correct TUS transducer and select the correct target in the **Targets to sample** list on the upper left **[2]**.

4.2.1. SCREEN: 69236_4.2.1-4.2.2.mp4 00:00-00:13

4.2.2. SCREEN: 69236_4.2.1-4.2.2.mp4 00:16-00:30

4.3. Comb the participant's hair to expose the scalp at the TUS *(T-U-S)* transducer site and create an anterior-posterior parting line **[1].** Apply degassed ultrasound gel along it **[2-TXT]**.

4.3.1. Talent combing hair to expose scalp at the approximate transducer site and making an anterior posterior parting line,

4.3.2. Talent applying ultrasound gel along the parting line. **TXT: Apply finger pressure from center out to burst air bubbles**

4.4. Create 1 to 3 additional parting lines approximately 1.5 centimeters away on each side and repeat the same process **[1]**. Finally, return to the initial parting line and repeat the process four times **[2]**.

4.4.1. Talent creating additional parting lines and repeating gel application and pressure steps.

4.4.2. Talent returning to initial parting line and repeating actions.

4.5. Now strap the TUS transducer onto the participant's head and align it with the target using the **Bullseye** view **[1]**. Adjust the transducer to minimize distance, tilting, and rotational deviations to within operational limits **[2-TXT]**.

4.5.1. Talent strapping the transducer to the participant's head and checking alignment on **Bullseye** view.

4.5.2. Talent adjusting transducer for minimal deviations. **TXT: Once aligned, tighten strap and check participant comfort**

4.6. If safety monitoring is required, insert a thermocouple wire into the ultrasound gel, close to the scalp **[1-TXT]**. Tape the thermocouple wire on the head using paper tape to prevent movement **[2]**.

4.6.1. Talent inserting thermocouple wire near the edge of the transducer into the ultrasound gel. **TXT: Ensure wire is not centered to avoid interference with ultrasound transmission**

4.6.2. Talent taping the thermocouple wire with paper tape to secure it.

4.7. Press the amplifier's **POWER** button to start output and begin TUS **[1]**. Simultaneously, press **Sample Now** on the neuronavigation software to record initial transducer placement **[2]**.

4.7.1. Talent pressing the amplifier **POWER** button.

4.7.2. SCREEN: 69236_4.7.2-4.8.1.mp4. 00:00-00:13

4.8. Continuously monitor the three alignment metrics in the **Bullseye** view **[1-TXT].**

4.8.1. SCREEN: 69236_4.7.2-4.8.1.mp4. 00:14-00:27 **TXT: Adjust transducer position if metrics deviate from target**

4.9. Monitor the scalp temperature using the thermocouple wire if installed **[1-TXT]**.

4.9.1. Talent monitoring real-time thermocouple temperature. **TXT: Intervene if temperature approaches 43.3 °C**

4.9.2. Talent checking on participant comfort and preparing to intervene if necessary.

4.10. To finish TUS, press the amplifier **POWER** button to stop the output **[1]**. Remove the transducer and all head-mounted equipment from the participant **[2]**.

4.10.1. Talent pressing the amplifier **POWER** button to stop stimulation.

4.10.2. Talent removing the transducer and any attached equipment from the participant's head.

4.11. Inspect the scalp for any signs of redness and ask the participant about any discomfort or adverse effects **[1]**. If no issues are reported or after addressing concerns, gently wipe off the ultrasound gel from the scalp **[2]**.

4.11.1. Talent visually inspecting the scalp and speaking with the participant.

4.11.2. Talent wiping off the ultrasound gel from the participant's scalp.

4.12. Click **Finish** on the neuronavigation window and save the project by selecting **File** and pressing **Save Project [1]**. From the **Sessions** list, select the current session and click **Review [2]**.

4.12.1. SCREEN: 69236_4.12.1.mp4 00:05-00:23

4.12.2. SCREEN: 69236_4.12.2-4.13.2.mp4 00:00-00:10

4.13. In the **Sessions Review** window, choose **Brainsight text file (.txt)** *(Brain-sight-text-file)* from the **Export...** *(Export)* dropdown menu. Ensure **Orientation** is selected and set **Snap to:** *(Snap-to)* to **Nothing [1].** Configure other export settings as needed, then click **Save** to export the stimulation data **[2]**.

4.13.1. SCREEN: 69236_4.12.2-4.13.2.mp4 00:12-00:32

4.13.2. SCREEN: 69236_4.12.2-4.13.2.mp4 00:33-00:42

Results

5. Results

5.1. A satisfactory simulation-based targeting result was achieved where the acoustic focus reached the intended cortical stimulation region **[1]**. A scenario in simulation-based targeting required additional iterations due to misalignment between the acoustic focus and the intended stimulation target **[2]**. Adjusting the Distance Tx *(T-X)* outplane to skin parameter to a more realistic value corrected the visualization scaling issue in the simulation interface **[3]**.

5.1.1. LAB MEDIA: Figure 5A. *Video editor: Highlight the center of the color-mapped acoustic field where the red focal point overlaps with the intended black-cross stimulation region.*

5.1.2. LAB MEDIA: Figure 5B.

5.1.3. LAB MEDIA: Figure 5C. *Video editor: Highlight the field labelled "Distance Tx outplane to skin (mm)"*

5.2. A clean motor evoked potential was recorded, indicating good transcranial magnetic stimulation coil placement and orientation **[1]**. A waveform was observed that might not represent a motor evoked potential **[2]**.

5.2.1. LAB MEDIA: Figure 6A. *Video editor: Highlight the peak bounded by the "0.0373(2)" and the "-0.0307(5)" cursors*

5.2.2. LAB MEDIA: Figure 6C. *Video editor: Highlight the irregular signal fluctuations across the plot without a single sharp peak.*

5.3. A successful MT_{1mV} *(M-T-One-Milli-Volt)* determination was made at 52% maximum stimulator output, with five positive and five negative responses at that level **[1]**. A potentially inconclusive MT_{1mV} determination was recorded at 87% maximum stimulator output due to consecutive rather than alternating positive and negative responses **[2]**.

5.3.1. LAB MEDIA: Figure 6B. *Video editor: Highlight the third column for the last ten 52% MSO rows where the values are five 1.0s and five 0.0s.*

5.3.2. LAB MEDIA: Figure 6D. *Video editor: Highlight the third column for the 87% MSO row where consecutive 1.0s and 0.0s appear instead of alternating responses.*

5.4. A steady, even oscilloscope reading of the transducer driving signal was obtained, indicating proper equipment function **[1]**.

5.4.1. LAB MEDIA: Figure 7C. *Video editor: Highlight the consistent waveform on the* *oscilloscope screen.*

5.5. Good alignment was achieved between the physical transducer placement and the pre-defined target using neuronavigation **[1]**. A misaligned transducer placement was observed, indicating the need for adjustment to match the target **[2]**.

5.5.1. LAB MEDIA: Figure 8A. *Video editor: Highlight the red and green overlay and the three near-zero alignment metrics*

5.5.2. LAB MEDIA: Figure 8B. *Video editor: Highlight the red transducer placement marker that is offset from the central green target area, and the three large alignment metrics.*

1. Degassed
   Pronunciation link: https://www.merriam-webster.com/dictionary/degassed
   IPA: /ˌdiːˈɡæst/
   Phonetic Spelling: dee·gast
2. Deionized
   Pronunciation link: https://www.merriam-webster.com/dictionary/deionized
   IPA: /diˈaɪəˌnaɪzd/
   Phonetic Spelling: dee·eye·uh·nyzd
3. Hydrophone
   Pronunciation link: https://www.merriam-webster.com/dictionary/hydrophone
   IPA: /ˈhaɪdrəˌfoʊn/
   Phonetic Spelling: hy·druh·fohn
4. Ultrasound
   Pronunciation link: https://www.merriam-webster.com/dictionary/ultrasound
   IPA: /ˈʌltrəˌsaʊnd/
   Phonetic Spelling: uhl·truh·sownd
5. Transducer
   Pronunciation link: https://www.merriam-webster.com/dictionary/transducer
   IPA: /trænzˈduːsər/
   Phonetic Spelling: tranz·doo·ser
6. Oscilloscope
   Pronunciation link: https://www.merriam-webster.com/dictionary/oscilloscope
   IPA: /əˈsɪləˌskoʊp/
   Phonetic Spelling: uh·sil·uh·skohp
7. Function Generator
   Pronunciation link: https://www.merriam-webster.com/dictionary/function%20generator
   IPA: /ˈfʌŋkʃən ˈdʒɛnəˌreɪtər/
   Phonetic Spelling: funk·shuhn jen·uh·ray·ter
8. Acoustic
   Pronunciation link: https://www.merriam-webster.com/dictionary/acoustic

IPA: /əˈkuːstɪk/
Phonetic Spelling: uh·koos·tik

9. Hemodynamics
   Pronunciation link: https://www.merriam-webster.com/dictionary/hemodynamics
   IPA: /ˌhiːmoʊdaɪˈnæmɪks/
   Phonetic Spelling: hee·moh·dy·nam·iks
10. Transcranial
   Pronunciation link: https://www.merriam-webster.com/dictionary/transcranial
   IPA: /trænzˈkreɪniəl/
   Phonetic Spelling: tranz·kray·nee·uhl
11. Neuronavigation
   Pronunciation link: https://www.merriam-webster.com/dictionary/neuronavigation
   IPA: /ˌnʊrəˌnævɪˈgeɪʃən/
   Phonetic Spelling: nyur·oh·nav·uh·gay·shuhn
12. Thermocouple
   Pronunciation link: https://www.merriam-webster.com/dictionary/thermocouple
   IPA: /ˈθɝːmoʊˌkʌpəl/
   Phonetic Spelling: thur·moh·kup·uhl
13. Cortical
   Pronunciation link: https://www.merriam-webster.com/dictionary/cortical
   IPA: /ˈkɔːrtɪkəl/
   Phonetic Spelling: kor·tih·kuhl
14. Variability
   Pronunciation link: https://www.merriam-webster.com/dictionary/variability
   IPA: /ˌveriəˈbɪləti/
   Phonetic Spelling: vair·ee·uh·bil·uh·tee
15. Evoked
   Pronunciation link: https://www.merriam-webster.com/dictionary/evoked
   IPA: /ɪˈvoʊkt/
   Phonetic Spelling: ih·vohkt
16. Simulation
   Pronunciation link: https://www.merriam-webster.com/dictionary/simulation
   IPA: /ˌsɪmjəˈleɪʃən/
   Phonetic Spelling: sim·yuh·lay·shuhn
17. Alignment
   Pronunciation link: https://www.merriam-webster.com/dictionary/alignment
   IPA: /əˈlaɪnmənt/
   Phonetic Spelling: uh·lyn·muhnt
18. Calibration
   Pronunciation link: https://www.merriam-webster.com/dictionary/calibration
   IPA: /ˌkæləˈbreɪʃən/
   Phonetic Spelling: kal·uh·bray·shuhn
19. Spectral
   Pronunciation link: https://www.merriam-webster.com/dictionary/spectral

IPA: /ˈspɛktrəl/
Phonetic Spelling: spek·truhl

20. Amplifier
    Pronunciation link: https://www.merriam-webster.com/dictionary/amplifier
    IPA: /ˈæmpləˌfaɪər/
    Phonetic Spelling: am·pluh·fy·er