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Title: Blood Flow Imaging with Ultrafast Doppler

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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 similar? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **Y**

Videographer: screen capture files provided, do no film

3. Interview statements: Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

☒ Interviewees self-record interview statements outside of the filming date. JoVE can provide support for this option.

4. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **10**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Olivier Villemain**: The quantitative evaluation of blood flow by ultrasound is an extremely useful parameter in medicine for the evaluation of many organs **[1]**.

1.1.1. LAB MEDIA: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Jerome Baranger**: Ultrafast Doppler ultrasound provides spatio-temporal coherence and facilitates an increased sensitivity to measuring small blood flow velocities **[1]**.

1.2.1. LAB MEDIA: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL:

1.3. **Olivier Villemain**: Having access to microperfusion opens the door to a more precise understanding of the perfusion of tumors or of organs such as the heart and the brain **[1]**.

1.3.1. LAB MEDIA: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Protocol

2. Doppler Phantom Preparation

2.1. To set up the Doppler phantom, first use plastic tubes to connect the peristaltic pump, the blood mimicking fluid reservoir, the pulse dampener, and the Doppler flow phantom **[1]**.

2.1.1. LAB MEDIA: Talent connecting instruments

2.2. Select the canal with a 4-millimeter diameter **[1]** and program the pump to eject 720 milliliters/minute of fluid for 0.3 seconds followed by 0.7 seconds of ejection at 50 milliliters/minute to mimic the systole and diastole cardiac phases respectively **[2]**.

2.2.1. SCREEN: screenshot_1: 00:02-00:03

2.2.2. SCREEN: screenshot_1: 00:03-00:19 *Video Editor: please speed up*

2.3. Then run the pump **[1]** and gently shake the pipes to expel any potential air bubbles **[2]**.

2.3.1. SCREEN: screenshot_1: 00:19-00:33 *Video Editor: please speed up*

2.3.2. LAB MEDIA: Talent shaking pipe(s) *Videographer: Important step*

3. Ultrafast Ultrasound Scanner Setup and Sequence Programming

3.1. To set up the ultrafast ultrasound scanner, use the PCI (P-C-I) express link to connect the ultrafast-enabled research scanner to the host computer **[1]** and change the transducer adapter on the scanner to match the probe connector **[2]**.

3.1.1. LAB MEDIA: Talent connecting scanner to computer

3.1.2. LAB MEDIA: Talent changing adapter to match connector

3.2. Connect the probe **[1]** and run Matlab to activate the ultrasound scanner license **[2]**.

3.2.1. LAB MEDIA: Talent connecting probe *Videographer: Important step*

3.2.2. LAB MEDIA: Talent running Matlab

3.3. To set up the ultrasound sequence program, set the imaging depth to 50 millimeters and the focal depth to 35 millimeters **[1-TXT]**.

3.3.1. SCREEN: screenshot_2: 00:03-00:10 **TEXT: Here Verasonics Vantage system shown**

3.4. To design an ultrafast ultrasound sequence, set the imaging depth to 50 millimeters, program three tilted plane waves at minus 3, 0, and 3 degrees, and set the pulse repetition frequency to 12 kilohertz **[1]**.

3.4.1. SCREEN: screenshot_2: 00:11-00:25 *Video Editor: can speed up*

3.5. Then select 4 half-cycles for the ultrasound waveform with the center frequency set according to the probe used and set the total duration to 1 second **[1]**.

3.5.1. SCREEN: screenshot_2: 00:25-00:30

4. Probe Positioning and Data Acquisition

4.1. When the sequence parameters have been set, apply ultrasound gel to the probe lens **[1]** and place the probe onto the phantom **[2]**.

4.1.1. LAB MEDIA: Talent applying gel

4.1.2. LAB MEDIA: Talent placing probe onto phantom

4.2. Launch the B Mode ultrasound sequence **[1]** and locate the canal of interest. The fluid will appear darker than the surrounding tissue **[2]**.

4.2.1. SCREEN: screenshot_2: 00:30-00:40

4.2.2. SCREEN: screenshot_3: 00:03-00:09 *Video Editor: please emphasize fluid when mentioned*

4.3. Switch to the longitudinal view **[1]**. Without changing the position of the probe, end the B mod sequence and launch the ultrafast sequence acquisition script **[1]**.

4.3.1. SCREEN: screenshot_3: 00:09-00:20

4.3.2. SCREEN: screenshot_3: 00:20-00:50 *Video Editor: please speed up*

5. Image Reconstruction and Clutter Filtering

5.1. When the sequence is over, save the raw data **[1]** and use the ultrasound system default software to launch the image reconstruction script **[2]**.

5.1.1. LAB MEDIA: Talent saving data, with monitor visible in frame *Videographer: Important/difficult step*

5.1.2. SCREEN: screenshot_4: 01:48-01:58

5.2. For clutter filtering, use the Matlab script to reshape the 3D space x space x time IQ matrix into a 2D space x time Casorati matrix **[1-TXT]**.

5.2.1. SCREEN: screenshot_5: 00:01-01:29 *Video Editor: please speed up* **TEXT: Script provided in Supplementary Materials**

5.3. Then use the formula to compute the singular value decomposition of the matrix and use the spatial singular vectors to compute the Spatial Similarity Matrix C and to identify the blood subspace boundaries. Use the blood space boundary cutoff to filter the IQ data **[1]**.

5.3.1. SCREEN: screenshot_5: 01:30-2:01 *Video Editor: please speed up*

6. Flow Visualization and Velocity Measurements

6.1. To compute the power Doppler map **[1]**, use the formula to integrate the envelope of the filtered data along the temporal dimension and display the power Doppler map in a logarithmic scale **[2]**.

6.1.1. LAB MEDIA: Talent at computer, computer power Doppler map, with monitor visible in frame *Videographer: Important/difficult step*

6.1.2. SCREEN: screenshot_5: 02:23-02:28

6.2. Define a circular region of interest containing 1 to 30 pixels in the image **[1]** and average the filtered data signal over the pixels within the region of interest to obtain a vector for the filtered data of the relevant number of experimental time points **[2]**.

6.2.1. SCREEN: screenshot_5: 02:28-02:44

6.2.2. SCREEN: screenshot_5: 02:44-02:50

6.3. To compute and display the Doppler spectrogram of these data, set the Short-Time Fourier Transform window to a 60-samples Hann window and set the Short-Time Fourier Transform overlap to 90% of the window length **[1]**.

6.3.1. SCREEN: screenshot_5: 02:50-03:05

6.4. Then overlay the center frequency at each time point of the spectrogram **[1]** and use the Doppler formula to convert the frequency values into blood axial velocities **[2]**.

6.4.1. SCREEN: screenshot_5: 03:05-03:11

6.4.2. SCREEN: screenshot_5: 03:11-03:29

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see? Please list 4 to 6 individual steps.
2.3.2., 3.2.1., 5.1.1. 6.1.1.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success? Please list 1 or 2 individual steps from the script above.
5.1.1., 6.1.1.

Results

7. Results: Representative Ultrafast and Power Doppler Imaging

7.1. If a good quality spectrogram has been acquired **[1]**, the blood velocities can be extracted from any region of interest within the image **[2]**.

7.1.1. LAB MEDIA: Figure 2C bottom left image
7.1.2. LAB MEDIA: Figure 2C bottom left image *Video Editor: please emphasize vertical blue arrow and/or Mean velocity in ROI text*

7.2. In this image of a neonate brain, acquisition vessels with very different flow characteristics **[1]**, from small cortical venules and arterioles **[2]** to the major pericallosal artery, can be observed **[3]**.

7.2.1. LAB MEDIA: Figure 3B
7.2.2. LAB MEDIA: Figure 3B *Video Editor: please emphasize small vessels*
7.2.3. LAB MEDIA: Figure 3B *Video Editor: please emphasize major artery*

7.3. Here, the ability of ultrafast Doppler to extract a blood flow signal in a strongly moving organ such as the myocardium is shown **[1]**.

7.3.1. LAB MEDIA: *Video Editor: please emphasize red signal*

Conclusion

8. Conclusion Interview Statements

8.1. **Jerome Baranger**: Although we demonstrated the use of singular value decomposition, other types of filters can also be used **[1]**.

8.1.1. LAB MEDIA: Named talent says the statement above in an interview-style shot, looking slightly off-camera (5.3.)

8.2. **Olivier Villemain**: The use of ultrafast Doppler allows us to better understand the perfusion of the myocardium or the brain. It is a real revolution in medical imaging **[1]**.

8.2.1. LAB MEDIA: Named talent says the statement above in an interview-style shot, looking slightly off-camera Note: Statement has been changed slightly in video (did not open movie file and text not provided)