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Title: Dual Raster-Scanning Photoacoustic Small-Animal Imager for Vascular Visualization

Authors and Affiliations: Fei Yang[1,2], Zhiyang Wang[1,2], and Sihua Yang[1,2]

[1]MOE Key Laboratory of Laser Life Science & Institute of Laser Life Science, College of Biophotonics, South China Normal University
[2]Guangzhou Provincial Key Laboratory of Laser Life Science, College of Biophotonics, South China Normal University

Corresponding Author:
Sihua Yang
yangsh@scnu.edu.cn

Co-Authors:
yangfeidoudou@163.com
wzy_0617@163.com

Author Questionnaire

1. Microscopy: Does your protocol demonstrate 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: All screen capture files provided, do not film

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

Protocol Length
Number of Shots: **61**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Sihua Yang**: The dual-raster-scanning photoacoustic small-animal imager facilitates wide-field imaging of the vasculature and real-time imaging of the local dynamic tissue, as well as the flexibility to switch between these viewing modes **[1]**.

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

REQUIRED:

1.2. **Fei Yang**: The dual-raster-scanning photoacoustic small-animal imager can be used visualize various tissues in vivo and is easily adapted to many basic biomedical research studies with a need for small-animal imaging **[1]**.

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

Ethics Title Card

1.3. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at the Scientific Research Ethics Committee of South China Normal University.

Protocol

2. System Alignment

2.1. To align the system, cover an entire 10- x 10- x 4.4-centimeter water tank with a 10-micron-thick polyethylene membrane **[1]** and fill the tank with ultrapure water **[2]**.

2.1.1. WIDE: Talent covering tank with membrane

2.1.2. Talent filling tank

2.2. Place the tank on the working stage **[1]** and turn on the laser switch **[2]**.

2.2.1. Talent placing tank onto stage
2.2.2. Talent turning on switch

2.3. Select the laser control program **[1]** and preheat the laser for 5 minutes **[2]**.

2.3.1. Talent selecting program
2.3.2. Talent preheating laser

2.4. Press the **On** button on the pumping switch **[1]** and set the laser parameters **[2-TXT]**.

2.4.1. Talent pressing On button
2.4.2. Talent setting parameters **TEXT: See text for laser parameter setup details**

2.5. Open the laser baffle **[1]** and select the A-line collected program **[2]**.

2.5.1. Talent opening baffle
2.5.2. Talent selecting program, with monitor visible in frame

2.6. Press **Start** to capture the single point signal and to display the amplitude and spectrum of the current A-line signal **[1]**.

2.6.1. SCREEN: screenshot_1: 00:05-00:47 *Video Editor: please speed up*

2.7. Place a blade at the bottom of the water tank **[1]** and immerse the bottom part of ultrasonic transducer in the water tank for acoustic coupling **[2-TXT]**.

2.7.1. Talent placing blade into tank

2.7.2. Talent placing UT in tank **TEXT: Caution: Avoid bubbles**

2.8. Adjust the position of the Galva **[1]** and the XY translator between the ultrasonic transducer and the confocal objective lens to avoid an oscillation signal **[2]**.

2.8.1. Talent adjusting Galva *Videographer: Important step*
2.8.2. Talent adjusting translator *Videographer: Important step*

2.9. Then adjust the height of the working stage to maximize the amplitude of the signal **[1]** and determine the focus position **[2]**.

2.9.1. Talent adjusting stage height *Videographer: Important/difficult step*
2.9.2. Talent determining focus position *Videographer: Important/difficult step*

3. A-line Signal Alignment and Collection

3.1. After confirming a lack of response to pedal reflex **[1-TXT]**, use a trimmer and depilatory cream to remove the hair from the back, ear, scalp, and abdomen of the anesthetized mouse **[2]** and place the mouse onto an 8- x 2.8- x 2-centimeter holder in the prone position **[3]**.

3.1.1. WIDE: Talent pinching toe *Videographer: More Talent than mouse in shot* **TEXT: Anesthesia: urethane 1 g/kg i.p.**
3.1.2. Hair being removed
3.1.3. Talent placing mouse into holder *Videographer: More Talent than mouse in shot*

3.2. Place ultrasound gel onto the area to be imaged **[1-TXT]** and place the holder onto the working stage **[2]**.

3.2.1. Gel being applied **TEXT: Avoid bubbles**
3.2.2. Talent placing holder onto stage *Videographer: More Talent than mouse in shot*

3.3. Press start to collect an A-line signal **[1]** and adjust the position of the Galva, XT (X-T) translator, and working stage to align the region to be imaged with the laser **[2]**.

3.3.1. Talent pressing Start, with monitor visible in frame
3.3.2. Talent adjusting position(s)

3.4. Then press **Stop** to end the collection **[1]**.

3.4.1. SCREEN: screenshot_2: 00:15-00:21

4. Wide-Field Imaging Mode (WIM)

4.1. To collect a wide-field image, select the wide-field imaging mode **[1]** and name the newly created folder **[2]**.

4.1.1. WIDE: Talent selecting WIM, with monitor visible in frame
4.1.2. SCREEN: screenshot_2: 00:22-00:30

4.2. Under the **Scanning Speed** tab, set the scanning speed to 20 millimeters/second, the **Scanning Area** to 20- x 20-millimeters, and the **Step** to 20 **[1]**.

4.2.1. SCREEN: screenshot_2: 00:31-00:38

4.3. When the scanning parameters have been set, click **Collect** to begin scanning **[1]**.

4.3.1. SCREEN: screenshot_2: 00:39-00:52 *Video Editor: can speed up*

4.4. At the end of the acquisition, click **Stop** and **Return to Zero** to bring the motor to zero **[1]**.

4.4.1. SCREEN: screenshot_2: 18:50-19:00

4.5. Close the laser baffle **[1]** and set the **Trigger** setting to internal trigger **[2]**.

4.5.1. Talent closing baffle
4.5.2. SCREEN: screenshot_2: 19:01-19:07

4.6. Press **OFF** to stop the pumping switch **[1]** and select the real-time imaging mode trigger **[1]**.

4.6.1. Talent pressing Off
4.6.2. SCREEN: screenshot_2: 19:08-19:30 *Video Editor: please speed up*

4.7. Connect the real-time imaging mode trigger to the external laser trigger **[1]** and turn on the pumping switch **[2]**.

4.7.1. Talent connecting trigger
4.7.2. Talent pressing On

4.8. Then set the trigger setting to the external trigger and click **Exit** to exit the wide-field imaging mode **[1]**.

4.8.1. SCREEN: screenshot_2: 19:31-19:52 *Video Editor: can speed up*

5. Real-Time Imaging Mode (RIM)

5.1. To collect a real-time image, initiate the A-line signal collection **[1]** and open the laser baffle **[2]**.

5.1.1. WIDE: Talent starting signal collection
5.1.2. Talent opening baffle NOTE: This shot is the same as shot 2.5.1, so, this shot was not filmed

5.2. Align the laser as demonstrated **[1]** and click **Stop** to end the collection after the alignment **[2]**.

5.2.1. Talent aligning laser
5.2.2. SCREEN: screenshot_3: 00:00-00:08

5.3. Select the real-time imaging mode and name the newly created folder **[1]**.

5.3.1. SCREEN: screenshot_3: 00:09-00:13

5.4. Click **Collect** to start scanning. When the acquisition is complete, click **Stop [1]**.

5.4.1. SCREEN: screenshot_3: 00:14-00:23

5.5. Then click **Exit** button to exit the real-time imaging program **[1]**.

5.5.1. SCREEN: screenshot_3: 00:24-00:27

6. WIM Vascular Visualization

6.1. For wide-field imaging of the brain vascular systems, select a focused ultrasonic transducer with a central frequency of 25 megahertz, a bandwidth of more than 90%, and a focal length of 8 millimeters **[1]**.

6.1.1. WIDE: Talent selecting transducer

6.2. Use a scalpel to make a small incision on the lateral side of the cranial temporal top of the mouse **[1]** and use ophthalmic scissors cut the scalp around the outer edge of the skull **[2]**.

6.2.1. Incision being made
6.2.2. Scalp being cut

6.3. Compress the bleeding point to achieve homeostasis **[1]** and wash the wound with normal saline **[2]**.

6.3.1. Bleeding point being compressed
6.3.2. Wound being washed

6.4. Place ultrasound gel onto the exposed skull **[1]** and initiate A-line signal collection **[2-3]**.

6.4.1. Gel being placed
6.4.2. Added shot: Talent placing holder onto stage.
6.4.3. Talent starting signal acquisition, with monitor visible in frame NOTE: This may be slated as 6.4.2.

6.5. Click **Stop** to end the collection after the alignment and select the wide-field imaging mode **[1]**.

6.5.1. SCREEN: screehshot_4: 00:00-00:08

6.6. Name the newly created folder and set the **Scanning speed** to 10 millimeters/second, the **Scanning area** to 10- x 10-millimeters, and the **Step** to 10 **[1]**.

6.6.1. SCREEN: screenshot_4: 00:09-00:30 *Video Editor: please speed up*

6.7. Click **Collect** to start scanning **[1]**. At the end of the acquisition, click **Stop [2]**.

6.7.1. SCREEN: screenshot_4: 00:31-19:10 *Video Editor: please speed up*
6.7.2. SCREEN: screenshot_4: 19:20-19:25

6.8. Then click **Return to Zero** to return the motor to zero and click **Exit** to exit the wide-field imaging program **[1]**.

6.8.1. SCREEN: screenshot_4: 19:25-19:40

7. Dynamic RIM Small Animal Monitoring

7.1. For dynamic real-time imaging, place the mouse in the supine position **[1]** and place ultrasound gel onto the abdomen **[2]**.

7.1.1. WIDE: Talent changing mouse position *Videographer: More Talent than mouse in shot*
7.1.2. Gel being applied

7.2. Collect an A-line signal **[1-added]** and align the laser as demonstrated **[1]**, select real-time imaging mode, and name the newly created folder **[2]**.

- 7.2.1. Added shot: Talent placing holder onto stage.
- 7.2.1. Talent starting collection, with monitor visible in frame NOTE: 7.2.1 added so the other 2 shots may be misslated.
- 7.2.2. SCREEN: screenshot_5: 00:00-00:10

7.3. Then click **Collect** to start scanning. At the end of the acquisition, click **Stop** and **Exit** to exit the real-time imaging program **[1]**.

- 7.3.1. SCREEN: screenshot_5: 00:11-00:25

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
2.8., 2.9.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
2.9.

Results

8. Results: Representative Dual Raster-Scanning Photoacoustic Small-Animal Imaging

8.1. Here a maximum amplitude projection image of the mouse back acquired in wide-field imaging mode can be observed **[1]**.

8.1.1. LAB MEDIA: Figure 4A

8.2. In this B-scan, the mouse back was imaged using the real-time imaging mode **[1]**.

8.2.1. LAB MEDIA: Video 1

8.3. Images of the **[1]** vascular networks of the mouse ear **[2]** and brain can be obtained using a focused ultrasonic transducer **[3]**.

8.3.1. LAB MEDIA: Figure 5
8.3.2. LAB MEDIA: Figure 5 *Video Editor: please emphasize Figure 5A*
8.3.3. LAB MEDIA: Figure 5 *Video Editor: please emphasize Figure 5B*

8.4. In this analysis, B-scan images of the mouse abdomen can be observed **[1]**, revealing the presence of a vessel within the 100-micron imaging range **[2]**.

8.4.1. LAB MEDIA: Figure 6A
8.4.2. LAB MEDIA: Figure 6A *Video Editor: please emphasize red/yellow signal in images*

8.5. In addition, maximum amplitude projection imaging allows comparison of the depth direction of the mouse abdomen versus time **[1]**.

8.5.1. LAB MEDIA: Figure 6B

Conclusion

9. Conclusion Interview Statements

9.1. **Fei Yang**: To obtain an optimal quality image, it is essential to achieve a good confocal adjustment of the optical and acoustic focus **[1]**.

9.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (2.7., 2.8.)

9.2. **Zhiyang Wang**: The special image processing algorithm can be developed for quantitative analysis and can provide valuable information for the early diagnosis and treatment of diseases **[1]**.

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