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Title: Evaluations of Oxygenation and Vascular Perfusion in Pre-Clinical Models of Cancer and Wound Healing Using OS-DCE OAI

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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**
- 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: 23

Number of Shots: 43

Introduction

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

Videographer's NOTE: Intros are in 4K, steps are in HD. Recorded all intros and testimonials with Euitaek, then re-recorded some of them with David. (in case you wanted variety)

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Euitaek Yang:** I develop optoacoustic imaging to measure oxygenation and perfusion in wounds and tumors for better disease assessment.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.8.1*

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Euitaek Yang:** Current methods cannot simultaneously measure oxygenation and perfusion in real time. This protocol enables noninvasive, dynamic assessment using optoacoustic imaging.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.6.2*

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.3. **Euitaek Yang:** This protocol is applicable to both preclinical and clinical studies of wounds, tumors, and vascular diseases, although it is limited to imaging depths of approximately 10 centimeters.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.4. **Euitaek Yang:** This protocol allows researchers to measure tissue oxygenation and perfusion dynamically and noninvasively.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.5. **Euitaek Yang:** The most important consideration is minimizing motion and maintaining consistent positioning to ensure reliable and reproducible measurements.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.8.3*

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.

What problem in your field of research does your JoVE article address?

- 1.6. **Euitaek Yang,** **Enter author title:** (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.

NOTE: Author Title not provided

What value do you expect this video article to have for other researchers working in your field?

- 1.7. **Euitaek Yang,** **Enter author title:** (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 Animal Care and Use Committee (IACUC) at the University of Wisconsin

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

2. Oxygen Sensitive-Dynamic Contrast Enhanced Optoacoustic Image Acquisition

Demonstrators: Euitaek Yang., David Murphy

- 2.1. To begin, prepare a 2.14 millimolar solution of the Indocyanine Green or ICG (*I-C-G*) contrast agent in sterile water [1]. Filter the solution with a 0.3-micrometer syringe filter [2] and fill a sterile laboratory syringe with 100 microliters of the filtered solution [3].
 - 2.1.1. WIDE: Talent preparing the ICG contrast agent solution in sterile water.
 - 2.1.2. Talent filtering the solution with a 0.3 micrometer syringe filter.
 - 2.1.3. Talent filling a sterile laboratory syringe with 100 microliters of the filtered solution.
- 2.2. Now, fill the imaging tank with deionized distilled water [1] and set the tank temperature to 36 degrees Celsius for a mouse with less than 25 grams body weight [2].
 - 2.2.1. Talent filling the imaging tank with deionized distilled water. NOTE: Started off wide, then moved camera (while still rolling) and went in for closeup of tank filling. Could use one, or the other, or both.
 - 2.2.2. Talent setting the tank temperature to 36 degrees Celsius. NOTE: Take 2 correctly shows it at 36 degrees
- 2.3. If performing DCE OAI (*D-C-E-O-A-I*), flush a 27-gauge tail vein catheter with heparinized saline [1-TXT]. Insert the catheter into a lateral tail vein of an anesthetized mouse [2] and secure it to prevent it from being removed from the tail vein [3]. Confirm that no air bubbles are present [4].
 - 2.3.1. Talent flushing a 27 gauge tail vein catheter with heparinized saline. **TXT: DCE-OAI: Dynamic Contrast Enhanced Optoacoustic Imaging**
 - 2.3.2. Talent inserting the catheter into a lateral tail vein. **TXT: Anesthesia: Induction: 5% Isoflurane; Maintenance: 1.5 to 2% Isoflurane**
 - 2.3.3. Talent securing the catheter to the tail vein.
 - 2.3.4. Talent checking the catheter and syringe line to confirm that no air bubbles are present.
- 2.4. Next, prepare a sterile laboratory syringe with a volume no greater than 500 microliters that can connect to the catheter [1]. Fill the syringe with 100 microliters of 2.14 millimolar ICG [2].

- 2.4.1. Talent shows a sterile laboratory syringe with a volume no greater than 500 microliters for catheter connection.
- 2.4.2. Talent filling the syringe with 100 microliters of 2.14 millimolar ICG.
- 2.5. To cover the mouse and the cradle with ultrasound gel, apply a thin layer of clear ultrasound gel to the tumor or wound area [1]. Apply ultrasound gel to the cradle to make a seal between the cradle and the tumor or wound area [2].
 - 2.5.1. Talent applying a thin layer of clear ultrasound gel to the tumor or wound area.
 - 2.5.2. Talent applying ultrasound gel to the cradle to make a seal between the cradle and the tumor or wound area.
- 2.6. Place the mouse in the cradle according to the manufacturer's instructions [1]. Position the mouse in the holder with the nose firmly inside the nosecone [2]. Use cuffs to restrain the mouse's hands and feet [3]. Use the tooth loop to stabilize the head [4].
 - 2.6.1. WIDE: Talent placing the mouse into the cradle setup.
 - 2.6.2. Talent positioning the mouse with its nose firmly inside the nosecone.
 - 2.6.3. Talent securing the mouse hands and feet with cuffs.
 - 2.6.4. Talent stabilizing the mouse head using the tooth loop. NOTE: Use take 2 for better continuity
- 2.7. When imaging a mouse, use a cradle with a rat nose cone to lift the head from the bottom of the cradle and reduce the risk of accidental drowning if water leaks into the cradle [1].
 - 2.7.1. Talent positioning the mouse in a cradle with a rat nose cone.
- 2.8. Now, place the mouse and cradle into the OAI instrument according to the manufacturer's instructions [1]. Observe the mouse cradle for any leaks [2].
 - 2.8.1. Talent placing the mouse and cradle into the OAI instrument.
 - 2.8.2. Talent inspecting the cradle for leaks inside the instrument.
- 2.9. If performing DCE OAI, connect the catheter to the syringe containing the contrast agent [1].
 - 2.9.1. Talent connecting the catheter to the syringe with contrast agent.
- 2.10. Equilibrate the mouse before imaging by allowing 10 minutes for stabilization of mouse physiology in the warm water tank [1].
 - 2.10.1. Talent placing the mouse in the warm water tank for equilibration.
- 2.11. To position the mouse relative to the OAI transducer, move the mouse or the OAI transducer so that the tumor or wound is located within the multi-slice image set [1]. Optimize the speed of sound for the image set during the equilibration period [2]. Maintain consistent animal orientation and slice position for all mice and at all time

points to ensure rigorous results [4].

2.11.1. Talent moving the mouse or transducer to align the tumor or wound within the imaging region. NOTE: 2.11.1 / 2.11.2 / 2.11.3 – On each of these I shot a wider shot and a closeup shot of the screen so you'd have some options

2.11.2. Show the system interface while adjusting the speed of sound parameter for the image set.

2.11.3. Talent checks mouse orientation and slice positioning in the cradle.

2.12. Now, select the image slice parameters to acquire multiple slices covering the entire tumor or wound area with a 1 millimeter slice thickness [1]. Then, select the absorption wavelengths for OS OAI at 700, 730, 760, 800, 850, and 875 nanometers [2].

2.12.1. SCREEN: Screen2.12.1.MP4: 00:10-00:15

2.12.2. SCREEN: Screen2.12.2.MP4: 00:07-00:09, 00:18-00:30

2.13. Configure signal averaging to acquire 10 averages per wavelength before acquiring the next wavelength and before moving to the next imaging slice [1].

2.13.1. SCREEN: Screen2.13.1.MP4

2.14. Then, set the number of repetitions to maintain a total acquisition time of approximately 2 minutes. Perform a minimum of 3 repetitions to ensure measurement stability [1]

2.14.1. SCREEN: Screen2.13.1.MP4: 00:08-00:16

2.15. Now, acquire the OS OA images using the previously set parameters [1].

2.15.1. SCREEN: Talent initiating image acquisition on the OAI instrument. NOTE: for sections 2.15.1 and 2.20.2, Use scans were acquired by the videographer

2.16. Next, set the DCE OAI acquisition parameters for a single-slice imaging protocol with multiple wavelengths [1]. Position up to 5 imaging slices centered on the tumor or wound area with 1 millimeter slice thickness [2].

2.16.1. SCREEN: Screen2.16.1.MP4: 00:17-00:25. NOTE: If videographer's footage is available for all shots 2.16.1. to 2.19.1, use that

2.16.2. SCREEN: Screen2.16.2.MP4: 00:15-00:30

2.17. Select the optimal absorption wavelength for the contrast agent by setting the wavelength to 800 nanometers for ICG in an in vivo environment [1].

2.17.1. SCREEN: Screen2.17.1.MP4: 00:12-end.

2.18. Set the number of averages and repetitions to maintain a temporal resolution of 5 seconds or less and a total acquisition time of 11.0 to 11.2 minutes for a 10-hertz imaging repetition rate [1-TXT].

2.18.1. SCREEN: Screen2.18.1.MP4: 00:04-00:15.

- 2.19. To acquire the DCE OA images, start the acquisition and collect 12 image sets [1].
 - 2.19.1. SCREEN: Screen2.19.1.MP4.
- 2.20. Then, administer 100 microliters of contrast agent via the catheter while continuing acquisition [1-TXT]. Continue imaging to acquire the remaining 128 image sets for 10.2 minutes post-injection to capture wash-in and washout kinetics [2].
 - 2.20.1. Talent injecting the contrast agent through the catheter during acquisition.
 - 2.20.2. SCREEN: Display ongoing image acquisition showing progression of image sets over time.
- 2.21. Monitor the imaging process to ensure images are acquired [1] and observe the mouse breathing rate to confirm stable physiology during scanning [1].
 - 2.21.1. Talent monitoring the imaging system.
 - 2.21.2. Talent observing the mouse breathing.
- 2.22. Then, remove the mouse and cradle from the instrument [1]. Remove the mouse from the cradle and allow it to recover from anesthesia [2]. Return the mouse to a warmed cage and monitor until ambulatory [3].
 - 2.22.1. Talent removing the mouse and cradle from the instrument.
 - 2.22.2. Talent removing the mouse from the cradle and placing it for recovery.
 - 2.22.3. Talent placing the mouse in a warmed cage and monitoring until ambulatory.
- 2.23. Finally, analyze OA images and compute quantitative parameters to assess tumor or wound physiology and contrast dynamics [1].
 - 2.23.1. Talent at the computer screen with one image opened in the software used for analysis

Results

3. Results

- 3.1. Quantitative maps of oxyhemoglobin [1], deoxyhemoglobin [2], total hemoglobin [3], and blood oxygen saturation were generated from multispectral OAI datasets using spectral unmixing [4].
 - 3.1.1. LAB MEDIA: Figure 4. *Video editor: Show the panel labeled HbO₂ with the colored region inside the yellow circle*
 - 3.1.2. LAB MEDIA: Figure 4. *Video editor: Show the panel labeled Hb with the colored region inside the yellow circle*
 - 3.1.3. LAB MEDIA: Figure 4. *Video editor: Show the panel labeled HbT with the colored region inside the yellow circle*
 - 3.1.4. LAB MEDIA: Figure 4. *Video editor: Show the panel labeled %sO₂ with the colored region inside the yellow circle*
- 3.2. DCE-OAI provided empirical pharmacokinetic parameters, including maximum signal enhancement [1], time-to-peak [2], slope [3], area under the curve [4], signal loss [5], and contrast enhancement at 10 minutes [6].
 - 3.2.1. LAB MEDIA: Figure 5. *Video editor: Highlight the top horizontal line labeled MSE*
 - 3.2.2. LAB MEDIA: Figure 5. *Video editor: Highlight the vertical line labelled TTP*
 - 3.2.3. LAB MEDIA: Figure 5. *Video editor: Highlight the rising slope region from injection to peak*
 - 3.2.4. LAB MEDIA: Figure 5. *Video editor: Highlight the shaded red area labeled AUC*
 - 3.2.5. LAB MEDIA: Figure 5. *Video editor: Highlight the vertical arrow labeled signal loss on the right side*
 - 3.2.6. LAB MEDIA: Figure 5. *Video editor: Highlight the endpoint labeled CE10 at 10 minutes*
- 3.3. Pharmacokinetic modelling enabled estimation of wash-in and washout rates, represented by NK^{trans} (N-K-Trans) and K_{ep} (K-E-P) maps [1].
 - 3.3.1. LAB MEDIA: Figure 6.
- 3.4. Wound models showed slower washout rates immediately after injury that increased during healing, indicating changes in vascular perfusion [1].
 - 3.4.1. LAB MEDIA: Figure 7. *Video editor: Highlight the later part of the curve marked as k*

1. Indocyanine
Pronunciation link: <https://www.merriam-webster.com/dictionary/indocyanine>
IPA: /ˌɪndoʊˈsaɪəniːn/
Phonetic Spelling: in·doh·sy·uh·neen
2. Micrometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/micrometer>
IPA: /maɪˈkrɑːmɪtər/
Phonetic Spelling: my·krom·ih·ter
3. Deionized
Pronunciation link: <https://www.merriam-webster.com/dictionary/deionized>
IPA: /diˈaɪəˌnaɪzd/
Phonetic Spelling: dee·eye·uh·nyzd
4. Catheter
Pronunciation link: <https://www.merriam-webster.com/dictionary/catheter>
IPA: /ˈkæθɪtər/
Phonetic Spelling: kath·ih·ter
5. Heparinized
Pronunciation link: <https://www.merriam-webster.com/dictionary/heparinize>
IPA: /ˈhepərəˌnaɪzd/
Phonetic Spelling: hep·uh·ruh·nyzd
6. Isoflurane
Pronunciation link: <https://www.merriam-webster.com/dictionary/isoflurane>
IPA: /ˌaɪsoʊˈflʊreɪn/
Phonetic Spelling: eye·soh·floor·ayn
7. Ultrasound
Pronunciation link: <https://www.merriam-webster.com/dictionary/ultrasound>
IPA: /ˈʌltrəˌsaʊnd/
Phonetic Spelling: ul·truh·sownd
8. Equilibrate
Pronunciation link: <https://www.merriam-webster.com/dictionary/equilibrate>
IPA: /ˌiːkwəˈlaɪbreɪt/
Phonetic Spelling: ee·kwuh·ly·brayt
9. Transducer
Pronunciation link: <https://www.merriam-webster.com/dictionary/transducer>
IPA: /trænzˈduːsər/
Phonetic Spelling: tranz·doo·ser

10. Multispectral
Pronunciation link: <https://www.howtopronounce.com/multispectral>
IPA: /ˌmʌltiˈspektrəl/
Phonetic Spelling: mul·tee·spek·truhl
11. Nanometers
Pronunciation link: <https://www.merriam-webster.com/dictionary/nanometer>
IPA: /ˈnænəʊˌmi:tər/
Phonetic Spelling: nan·oh·mee·ter
12. Temporal
Pronunciation link: <https://www.merriam-webster.com/dictionary/temporal>
IPA: /ˈtɛmpərəl/
Phonetic Spelling: tem·pruhl
13. Acquisition
Pronunciation link: <https://www.merriam-webster.com/dictionary/acquisition>
IPA: /ˌækwəˈzɪʃən/
Phonetic Spelling: ak·wuh·zish·uhn
14. Kinetics
Pronunciation link: <https://www.merriam-webster.com/dictionary/kinetics>
IPA: /kɪˈnetɪks/
Phonetic Spelling: kuh·net·iks
15. Ambulatory
Pronunciation link: <https://www.merriam-webster.com/dictionary/ambulatory>
IPA: /ˈæmbjələˌtɔːri/
Phonetic Spelling: am·byuh·luh·tor·ee
16. Oxyhemoglobin
Pronunciation link: <https://www.merriam-webster.com/dictionary/oxyhemoglobin>
IPA: /ˌɑːksiˈhi:məˌɡloʊbɪn/
Phonetic Spelling: ok·see·hee·muh·gloh·bin
17. Deoxyhemoglobin
Pronunciation link: <https://www.howtopronounce.com/deoxyhemoglobin>
IPA: /diˌɑːksiˈhi:məˌɡloʊbɪn/
Phonetic Spelling: dee·ok·see·hee·muh·gloh·bin
18. Spectral
Pronunciation link: <https://www.merriam-webster.com/dictionary/spectral>
IPA: /ˈspektrəl/
Phonetic Spelling: spek·truhl
19. Pharmacokinetic
Pronunciation link: <https://www.merriam-webster.com/dictionary/pharmacokinetic>
IPA: /ˌfɑːrməkoʊkɪˈnetɪk/

Phonetic Spelling: far·muh·koh·kih·net·ik

20. Empirical

Pronunciation link: <https://www.merriam-webster.com/dictionary/empirical>

IPA: /ɛmˈpɪrɪkəl/

Phonetic Spelling: em·peer·ih·kuhl

21. Modelling

Pronunciation link: <https://www.merriam-webster.com/dictionary/modeling>

IPA: /ˈmɑːdəlɪŋ/

Phonetic Spelling: mod·uhl·ing

22. Perfusion

Pronunciation link: <https://www.merriam-webster.com/dictionary/perfusion>

IPA: /pərˈfjuːzən/

Phonetic Spelling: per·fyoo·zhuhn