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Title: Microvascular Perfusion Monitored by Laser Speckle Contrast Imaging during Renal Ischemia-Reperfusion Injury in Mice

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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**

If **Yes**, we will need you to record using screen recording software.

We recommend using the screen capture program [OBS](https://review.jove.com/v/5848/screen-capture-instructions-for-authors?status=a7854k). JoVE's tutorial for using OBS Studio is provided at this link: <https://review.jove.com/v/5848/screen-capture-instructions-for-authors?status=a7854k>

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. **No**

Current Protocol Length

Number of Steps: 23

Number of Shots: 47

Introduction

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

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**.

INTRODUCTION:

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

- 1.1. **Weibin Ye:** In this study, we use the Laser Speckle Contrast Imaging system to monitor the microperfusion in the kidney during ischemia-reperfusion.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

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

- 1.2. **Weibin Ye:** This technology can be applied to research related to changes in blood flow like solid organ transplantation, drug efficacy, and ischemia studies.
 - 1.2.1. 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?~~

- 1.3. **Weibin Ye:** We can obtain the renal micro-perfusion blood flow through the LSCI system, thereby verifying the process of ischemia and reperfusion.
 - 1.3.1. 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.~~

- 1.4. **Weibin Ye:** To obtain a clear kidney view with good contrast, we need to be patient and spend time separating the kidney from the surrounding vessels and tissues.
 - 1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

1.5. **Weibin Ye:** In the future, additional indicators of pathophysiology can be incorporated, and this technology can be used to monitor the effect of drugs on renal ischemia.

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.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at Shantou University Medical College

Protocol

Author's NOTE: During the procedures of renal vessels ligation and renal resection, to maintain the continuity of the surgery, several shots were captured in one segment rather than being shot separately.

2. Right Nephrectomy Procedure for Renal Ischemia Model Preparation in Mice

Demonstrators: Weibin Ye, Yukuan Chen

- 2.1. To begin, sterilize the surgical table before each procedure [1]. Arrange sterilized instruments, cotton swabs, gauze, pre-diluted anesthetics, and all other required items neatly on the table [2].
 - 2.1.1. WIDE: Talent disinfecting the surgical table surface.
 - 2.1.2. Talent organizing all required sterile materials on the surgical table.
- 2.2. Administer meloxicam at 0.2 milligram per kilogram body weight by subcutaneous injection 30 minutes before surgery to alleviate pain [1].
 - 2.2.1. Talent performing a subcutaneous injection of meloxicam in the mouse.
- 2.3. Pour sterilized saline into a clean container [1]. Using scissors, cut a suitable opening in the center of a piece of gauze [2].
 - 2.3.1. Talent pouring sterile saline into a sterile container.
 - 2.3.2. Talent cutting an opening in the middle of a gauze pad.
- 2.4. Place an anesthetized mouse on the heating pad [1] and secure it with tape [2].
 - 2.4.1. Talent positioning the mouse on the heating pad.
 - 2.4.2. Talent taping the mouse in place.
- 2.5. Apply depilatory cream to remove abdominal hair [1]. Using the heating pad, maintain rectal temperature at 37 degrees Celsius throughout surgery [2].
 - 2.5.1. Talent applying depilatory cream to the abdomen.
 - 2.5.2. Shot of temperature display on the heat pad showing 37 degrees Celsius.
- 2.6. With a cotton swab, disinfect the surgical area first with 75 percent alcohol and then with iodophor [1].
 - 2.6.1. Talent disinfecting the abdominal skin sequentially with alcohol and iodophor.
- 2.7. Then use forceps to pinch the mouse toe to confirm absence of pain response [1]. With a pair of scissors, make a vertical midline abdominal incision [2]. Gently separate the skin from the peritoneum and then incise the peritoneum to enter the abdominal cavity

- [3].
 - 2.7.1. Talent pinching the toe to check anesthesia depth.
 - 2.7.2. Talent making a vertical abdominal incision with scissors.
 - 2.7.3. Talent opening the peritoneum to expose the abdominal cavity.
- 2.8. Apply a retractor to improve visibility [1]. Cover the operative field with gauze, aligning the opening with the abdominal cavity [2]. Moisten the gauze with saline [3].
 - 2.8.1. Talent placing the retractor to hold the incision open.
 - 2.8.2. Talent positioning the gauze over the surgical field.
 - 2.8.3. Shot of saline being poured over the gauze.
- 2.9. With a cotton swab, gently move the intestine to one side to expose the right kidney and surrounding tissues [1]. Fold the gauze to cover the intestine [2], then moisten it with saline to prevent drying [3].
 - 2.9.1. Talent gently repositioning the intestine with a cotton swab.
 - 2.9.2. Talent covering the intestine with gauze.
 - 2.9.3. Shot of saline being poured over the gauze.
- 2.10. Now, use a pair of angled forceps to lift the right ureter [1]. Pass two 6-0 (six-Oh) suture threads through the ureter and ligate at two positions [2].
 - 2.10.1. Talent lifting the right ureter with angled forceps.
 - 2.10.2. Talent placing and tying two 6-0 sutures around the ureter.
- 2.11. Using microscissors, transect the ureter between the two ligatures [1].
 - 2.11.1. Talent cutting the ureter between ligatures with microscissors.
- 2.12. Then use saline-moistened cotton balls held with fine forceps to gently push the liver upward [1]. Fix the liver with the cotton balls to expose the kidneys and right-sided vessels [2].
 - 2.12.1. Talent elevating the liver with moistened cotton balls.
 - 2.12.2. Shot of liver being fixed in position with the cotton balls.
- 2.13. Next, use angled forceps to bluntly separate the perinephric fat pads until the renal artery and vein are fully exposed [1].
 - 2.13.1. Talent dissecting perinephric fat to expose renal vessels.
- 2.14. Carefully slide the forceps underneath the renal artery and vein to create a channel [1]. Pass a 6-0 silk braided suture around both vessels and ligate them together [2].
 - 2.14.1. Talent sliding angled forceps under the renal vessels.
 - 2.14.2. Talent tying a 6-0 silk suture around the artery and vein.

2.15. Cut the occluded renal artery and vein near the kidney [1]. Remove the kidney with adherent tissue [2]. Then remove the cotton ball used to hold the liver [3].

2.15.1. Talent cutting the occluded vessels near the kidney.

2.15.2. Talent removing the kidney from the abdominal cavity.

2.15.3. Talent removing the supporting cotton ball from beneath the liver.

3. Laser Speckle Contrast Imaging of Renal Ischemia–Reperfusion Injury in Mice

Demonstrator: Ye Weibin

3.1. Turn on the laser speckle contrast imaging system and launch the dedicated software [1]. Click **Original Image** and then **Preview** to display the raw surgical field image [2].

3.1.1. LAB MEDIA: 3.1.1.mp4: 00:10-00:13

3.1.2. LAB MEDIA: 3.1.2.mp4: 00:02-00:12

3.2. Reposition the mouse [1-TXT]. Adjust camera magnification while viewing the original image to obtain a clear view of the target kidney [2].

3.2.1. Talent adjusting the mouse position and nose cone. **TXT: Maintain 1% isoflurane inhalation**

3.2.2. LAB MEDIA: 3.2.2.mp4: 00:05-00:20

3.3. Now click **Simultaneous Display** to view real-time blood flow imaging [1]. Set magnification to 2.5X and select **Automatic Blood Flow Adjustment**, then lock the blood flow parameters [2]. Set recording to capture 1 frame every 5000 milliseconds [3].

3.3.1. LAB MEDIA: 3.3.1.mp4: 00:02-00:10

3.3.2. LAB MEDIA: 3.3.2.mp4: 00:02-00:13

3.3.3. LAB MEDIA: 3.3.3.mp4: 00:02-00:10

3.4. Next, set the square area size parameter to 112 and uniformly position 5 square regions on the kidney image [1]. Click **Real-time Image Registration** and **Record** to begin blood flow recording [2].

3.4.1. LAB MEDIA: 3.4.1.mp4: 00:08-00:20.

3.4.2. LAB MEDIA: 3.4.2.mp4: 00:03-00:13

3.5. After recording a 30-minute baseline, bluntly separate adipose tissue around the left kidney vessels [1]. Using hemostatic forceps, place a micro clamp across the left renal artery and vein near the hilum [2]. Continue real-time monitoring for another 30 minutes [3].

3.5.1. Talent dissecting adipose tissue around left renal vessels.

- 3.5.2. Talent applying a micro clamp across the left renal artery and vein.
- 3.5.3. LAB MEDIA: 3.5.3.mp4: 00:02-00:12
- 3.6. Release the micro clamp to initiate reperfusion [1]. Monitor and record real-time recovery of renal microvascular blood flow [2].
 - 3.6.1. Talent removing the micro clamp from the vessels.
 - 3.6.2. LAB MEDIA: 3.6.2.mp4: 00:06-00:15
- 3.7. After monitoring reperfusion for at least 60 minutes, stop recording and suture the abdominal incision using a 7-0 silk braided suture [1]. Administer 1 milliliter saline by intraperitoneal injection to prevent dehydration [2].
 - 3.7.1. Talent suturing the abdominal incision with 7-0 silk suture.
 - 3.7.2. Talent performing intraperitoneal saline injection.
- 3.8. Place the mouse on the heating pad and monitor until it regains consciousness and can right itself [1].
 - 3.8.1. Talent observing the recovering mouse on the heating pad until alert and mobile.

Results

4. Results

- 4.1. During the initial 30 minutes, renal microvascular blood flow gradually became stable at baseline [1]. Heterogeneity in perfusion levels among different regions of interest was observed before ischemia [2].
 - 4.1.1. LAB MEDIA: Figure 2A. *Video editor: Show the normal perfusion image*
 - 4.1.2. LAB MEDIA: Figure 2B. *Video editor: Highlight the multiple colored curves labeled as different regions of interest between 0 to 30 min in B*
- 4.2. Following clamping of the renal artery and vein, blood flow dropped sharply, and the renal microcirculation entered a no-perfusion state [1].
 - 4.2.1. LAB MEDIA: Figure 2A, B and D . *Video editor: Highlight the ischemia image in A then Highlight the point on the curves where the lines abruptly fall to near zero in B and Highlight the bar corresponding to ischemia in D*
- 4.3. Upon release of the clamp, blood flow surged instantly to a peak and then decreased before gradually recovering to a comparatively stable level [1].
 - 4.3.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the sharp upward spike in the curves immediately after 60 min*
 - 4.3.2. LAB MEDIA: Figure 2C. *Video editor: Highlight the peak followed by a decline and gradual stabilization in the single-region curve.*
- 4.4. Microvascular perfusion during 60 minutes of reperfusion was much lower than that before ischemia [1].
 - 4.4.1. LAB MEDIA: Figure 2D. *Video editor: Highlight the bar labeled "Reperfusion"*

1. meloxicam

Pronunciation link: <https://www.merriam-webster.com/medical/meloxicam>

IPA: /məˈlɔːk.sɪˌkæm/

Phonetic Spelling: muh·laak·see·kam

2. anesthetized
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetized>
IPA: /əˈnɛs.θəˈtaɪzd/
Phonetic Spelling: uh·नेसθ·thuh·tyzd
3. depilatory
Pronunciation link: <https://www.merriam-webster.com/dictionary/depilatory>
IPA: /dɪˈpɪl.əˈtɔːr.i/
Phonetic Spelling: dih·pil·uh·tor·ee
4. iodophor
Pronunciation link: <https://www.merriam-webster.com/dictionary/iodophor>
IPA: /aɪˈoʊd.əˈfɔːr/, /aɪˈɑːd.əˈfɔːr/
Phonetic Spelling: eye·ohd·uh·for, eye·aad·uh·for
5. peritoneum
Pronunciation link: <https://www.merriam-webster.com/dictionary/peritoneum>
IPA: /ˌpɛr.ə.təˈniː.əm/
Phonetic Spelling: peh·ruh·tuh·nee·uhm
6. subcutaneous
Pronunciation link: <https://www.merriam-webster.com/dictionary/subcutaneous>
IPA: /ˌsʌb.kjuːˈteɪ.ni.əs/
Phonetic Spelling: sub·kyoo·tay·nee·uhs
7. ureter
Pronunciation link: <https://www.merriam-webster.com/dictionary/ureter>
IPA: /ˈjʊr.ə.tər/, /jʊˈriː.tər/
Phonetic Spelling: yoor·uh·ter, yuh·ree·ter
8. ligatures
Pronunciation link: <https://www.merriam-webster.com/dictionary/ligatures>
IPA: /ˈlɪɡ.ə.tʃəz/
Phonetic Spelling: lig·uh·churz
9. microscissors
Pronunciation link: <https://www.merriam-webster.com/medical/microscissors>
IPA: /ˈmaɪ.kroʊˌsɪz.əz/
Phonetic Spelling: my·kroh·siz·erz
10. intraperitoneal
Pronunciation link: <https://www.merriam-webster.com/dictionary/intraperitoneal>
IPA: /ˌɪn.trəˌpɛr.ə.təˈniː.əl/
Phonetic Spelling: in·truh·peh·ruh·tuh·nee·uhl
11. hemostatic
Pronunciation link: <https://www.merriam-webster.com/dictionary/hemostatic>
IPA: /ˈhiː.məˈstæt.ɪk/
Phonetic Spelling: hee·muh·stat·ik
12. adipose
Pronunciation link: <https://www.merriam-webster.com/dictionary/adipose>
IPA: /ˈæd.əˈpoʊs/
Phonetic Spelling: ad·uh·pohs

13. ischemia
Pronunciation link: <https://www.merriam-webster.com/dictionary/ischemia>
IPA: /ɪsˈkiː.mi.ə/
Phonetic Spelling: iss·kee·mee·uh
14. isoflurane
Pronunciation link: <https://www.howtopronounce.com/isoflurane>
IPA: /ˈaɪ.sə.flʊˌreɪn/
Phonetic Spelling: eye·suh·flur·ayn
15. reperfusion
Pronunciation link: <https://www.merriam-webster.com/dictionary/reperfusion>
IPA: /ˌriː.pəˈfjuː.ʒən/
Phonetic Spelling: ree·pur·fyoo·zhuhn
16. microvascular
Pronunciation link: <https://www.merriam-webster.com/dictionary/microvascular>
IPA: /ˌmaɪ.kroʊˈvæs.kjə.lə/
Phonetic Spelling: my·kroh·vas·kyuh·ler