

Submission ID #: 70396

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Project Page Link: <https://review.jove.com/account/file-uploader?src=21470828>

Title: Mapping Hepatic Stellate Cell Morphology in Mouse Models of Liver Fibrosis

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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**

Videographer: Please capture the screen of the instrument for all shots labeled SCREEN

3. Filming location: Will the filming need to take place in multiple locations? **Yes**

10 m. The locations are different rooms on the same floor.

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: 21

Number of Shots: 41

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. **Tran To:** This protocol presents a comprehensive imaging pipeline to visualize and model hepatic stellate cells' morphology in intact liver tissue.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

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

- 1.2. **Tran To:** Current 2D imaging misses full HSC morphology, limiting structural and functional insights; this protocol enables robust capture of complex 3D architecture.
 - 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. **Tran To:** This pipeline reproducibly maps HSC morphology in the complex liver microenvironment, capturing dendritic processes and spatial relationships with neighboring cells.
 - 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. **Tran To:** Time commitment is an important consideration when performing the protocol. The clearing process takes approximately 3 weeks, which requires advanced planning.

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. **Tran To:** Enabling precise 3D morphometric analysis, this platform links fibroblast structure to function, advancing insights into tissue homeostasis and disease mechanisms.

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**.

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

1.6. **Tran To, PhD Candidate**: (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.

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

1.7. **Tran To, PhD Candidate**: (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 Icahn School of Medicine at Mount Sinai

Protocol

2. In Situ Liver Perfusion and Fixation in the Mouse

Demonstrator: Tran To

- 2.1. To begin, pinch the palms of an anesthetized mouse to ensure proper anesthesia [1-TXT]. Stab the 4 palms with needles to spread the mouse abdomen [2]. Spray the abdomen with 70 percent ethanol to wet the fur [3].
 - 2.1.1. Talent gently pinching the footpad with forceps to check reflex. **TXT: Anesthesia: ketamine/xylazine mixture**
NOTE: 2.1.1- 2.8.2 filmed in one long take due to time sensitivity of the steps in the protocol. Please use take 3 B112_B271_0317US_001
 - 2.1.2. Talent inserting needles into the 4 limbs to secure the mouse.
 - 2.1.3. Talent spraying ethanol over the abdomen.
- 2.2. With a pair of forceps, pick up the skin in the middle of the abdomen aligned with the hind legs [1] and start cutting the skin to reveal the abdomen [2].
 - 2.2.1. Talent lifting abdominal skin with forceps.
 - 2.2.2. Talent cutting open the abdominal skin.
- 2.3. Now use cotton swabs wetted with PBS to move intestines and other organs to the side [1] and lift the left lobe [2].
 - 2.3.1. Talent gently moving organs aside with wet cotton swabs.
 - 2.3.2. Shot of the left liver lobe being lifted.
- 2.4. Carefully cut the membrane attached between the left lobe and the caudate lobe [1]. Then use a suture to tie off at the top base of the left lobe [2].
 - 2.4.1. Talent cutting the membrane between lobes.
 - 2.4.2. Talent tying a suture at the base of the left lobe.
- 2.5. Cut the left lobe below the suture knot [1] and set it aside for tissue sample collection and processing [2].
 - 2.5.1. Talent cutting below the suture knot.
 - 2.5.2. Talent placing the excised lobe into a collection container.
- 2.6. Use PBS-wetted cotton swabs to move the liver and reveal the portal vein [1]. Then, carefully insert the catheter needle into the portal vein at about halfway along its length [2]. Remove the catheter needle [3]. Ensure a backflow of blood through the catheter to indicate successful insertion [4]. **NOTE: The VO is edited for the additional shot**
 - 2.6.1. Talent exposing the portal vein with cotton swabs.

2.6.2. Shot of catheter insertion into the portal vein.

2.6.3. Talent withdrawing the catheter needle.

Added shot: Shot of the blood backflow into the catheter.

2.7. ~~Start the perfusion pump at a flow rate of 2 milliliters per minute [1].~~ Connect the filled tubing from the running perfusion pump to the catheter [1] and perfuse the liver with 20 milliliters of PBS [2]. **NOTE: The VO for the deleted shot is removed**

~~2.7.1. Talent starting the pump.~~ **NOTE: This shot was not filmed – the pump was already turned on and set up.**

2.7.2. Talent connecting tubing to the catheter.

2.7.3. Shot of liver blanching during perfusion.

2.8. Clamp the pulmonary artery above the liver with a hemostat to stop the PBS from going through the heart [1]. Cut the inferior vena cava to release the pressure buildup [2].

2.8.1. Talent applying a hemostat to the pulmonary artery.

2.8.2. Talent cutting the inferior vena cava.

2.9. Now adjust the pump speed to 5 milliliters per minute [1]. After 15 minutes, stop the pump to change the reservoir to 4 percent paraformaldehyde [2]. Then perfuse the liver with 25 milliliters of 4 percent paraformaldehyde [3].

2.9.1. Talent adjusting the pump speed to 5 mL/min.

2.9.2. Talent switching the reservoir to 4 percent paraformaldehyde.

2.9.3. Shot of liver during paraformaldehyde perfusion.

2.10. Once the perfusion is complete, remove the liver from the surrounding organs by cutting the connecting tissues [1].

2.10.1. Talent dissecting and removing the liver.

2.11. Place the liver in a 50-milliliter conical tube filled with 25 milliliters of 4 percent paraformaldehyde [1].

2.11.1. Talent placing liver into conical tube with fixative.

2.12. Place the tube horizontally on a bench rocking platform overnight at 4 degrees Celsius at 1.5 revolutions per minute [1].

2.12.1. Talent placing tube on rocking platform.

2.13. Wash the liver 3 times in PBS on a bench rocking platform for 30 minutes each at room temperature at 1.5 revolutions per minute [1].

2.13.1. Talent placing liver in PBS on rocking platform.

2.14. Transfer the liver to 20 milliliters of PBS with 0.02 percent sodium azide [1] and store at

4 degrees Celsius [2].

2.14.1. Shot of liver being transferred into a labeled tube with 20 mL PBS and 0.02% sodium azide.

2.14.2. Talent placing tube into 4 degrees Celsius storage.

3. Liver Tissue Clearing and Confocal Imaging

3.1. Cut the liver samples into pieces approximately 0.5 to 1 cubic centimeters in size [1]. Transfer the liver samples into glass vials with screw tops [2].

3.1.1. Talent cutting liver into uniform cubes.

3.1.2. Talent transferring samples into glass vials.

3.2. Add the methanol dilutions to the samples [1-TXT]. Dehydrate the samples on a wheel rotator at 8 rotations per minute for 1 hour at room temperature [2].

3.2.1. Talent adds methanol into the tube with the liver sample. **TXT: Dehydrate in methanol from 20% to 80%**

3.2.2. Talent placing vials on a wheel rotator.

ADD TITLE CARD: Complete sample dehydration, bleaching, permeabilization, and immunolabeling

3.3. Then incubate the sample in dibenzyl ether overnight at room temperature before imaging [1]. Store the samples in dibenzyl ether for long-term preservation without affecting the staining signal [2].

3.3.1. LAB MEDIA: IMG_0245.MOV 01:10-01:15, 02:27 – 02:35

3.3.2. LAB MEDIA: IMG_0257.mov 00:00 – 00:23

3.4. For confocal imaging, place the cleared sample in a 35-millimeter glass-bottom dish with a number 1.5 glass bottom [1]. Fill the dish with ethyl cinnamate to completely submerge the sample [2].

3.4.1. LAB MEDIA: IMG_0282.MOV 00:48 – 01:05

3.4.2. LAB MEDIA: IMG_0282.MOV 02:03 – 02:15

3.5. Then place the dish on the stage of the confocal microscope [1-TXT].

3.5.1. LAB MEDIA: IMG_0289.MOV 00:00 – 00:10

TXT: Adjust focus to identify the best plane with brightest signal

3.6. Acquire a tile image on the brightest plane to capture a large area of the sample using 1024 by 1024 format and a speed of 600 [1]. Based on the tile image, select regions of interest to collect a higher resolution z scan [2]. Next, acquire a Z-stack spanning the entire depth of the cleared tissue at the highest resolution supported by the microscope, using a 1024 by 1024 format and a scan speed of 600 [3].

Videographer: Please capture the screen of the instrument for all shots labeled SCREEN

- 3.6.1. SCREEN: A tile image is being captured using 1024x 1024 format and speed of 600
- 3.6.2. SCREEN: Regions of interest are being selected.
- 3.6.3. SCREEN: A z-scan is being captured through the cleared tissue.

Results

4. Results

- 4.1. Successful perfusion and fixation of the mouse liver resulted in a uniform pale, beige-yellow color [1]. Improper perfusion resulted in dark red or brown regions of blood clots, which reduced optical clarity and led to a high background signal in these regions [2].

4.1.1. LAB MEDIA: Figure 1B.

4.1.2. LAB MEDIA: Figure 1C. *Video editor: Highlight the dark red or brown areas indicated by arrows*

- 4.2. Complete optical transparency was achieved after incubation in dibenzyl ether, where the tissue appeared clear compared to before clearing [1].

4.2.1. LAB MEDIA: Alternate-Figure-2_JoVE.pdf. *Video editor: Show the “Before clearing” panel and then the “After clearing” panel*

- 4.3. High-resolution z-stacks revealed fine dendritic processes extending from the cell body of individual hepatic stellate cells [1].

4.3.1. LAB MEDIA: Figure 3. *Video editor: Emphasize the merged image 3C*

- 4.4. Both control and chronic carbon tetrachloride liver samples showed strong and stable fluorescence signals under confocal microscopy [1].

4.4.1. LAB MEDIA: Figure 4. *Video editor: Sequentially show the panels labeled “Control” and “Chronic CCl₄,”*

1. Anesthetized

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

IPA: /əˈnesθəˌtaɪzd/

Phonetic Spelling: uh·nes·thuh·tyzd

2. Ethanol

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

IPA: /ˈeθəˌnɔːl/

Phonetic Spelling: eh·thuh·nawl

3. Forceps

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

- IPA: /'fɔːr,seps/
Phonetic Spelling: for·seps
4. Abdomen
Pronunciation link: <https://www.merriam-webster.com/dictionary/abdomen>
IPA: /'æb.də.mən/
Phonetic Spelling: ab·duh·muhn
5. Intestines
Pronunciation link: <https://www.merriam-webster.com/dictionary/intestine>
IPA: /ɪn'tes.tənz/
Phonetic Spelling: in·tes·tinz
6. Caudate
Pronunciation link: <https://www.merriam-webster.com/dictionary/caudate>
IPA: /'kɔː,deɪt/
Phonetic Spelling: kaw·dayt
7. Suture
Pronunciation link: <https://www.merriam-webster.com/dictionary/suture>
IPA: /'suːtʃər/
Phonetic Spelling: soo·cher
8. Portal vein
Pronunciation link: <https://www.merriam-webster.com/dictionary/portal%20vein>
IPA: /'pɔːr.təl veɪn/
Phonetic Spelling: por·tuhl vayn
9. Catheter
Pronunciation link: <https://www.merriam-webster.com/dictionary/catheter>
IPA: /'kæθ.ə.tər/
Phonetic Spelling: kath·uh·ter
10. Perfusion
Pronunciation link: <https://www.merriam-webster.com/dictionary/perfusion>
IPA: /pər'fjuːʒən/
Phonetic Spelling: per·fyoo·zhuhn
11. Pulmonary
Pronunciation link: <https://www.merriam-webster.com/dictionary/pulmonary>
IPA: /'pʊl.mə,ner.i/
Phonetic Spelling: puul·muh·ner·ee
12. Hemostat
Pronunciation link: <https://www.merriam-webster.com/dictionary/hemostat>
IPA: /'hiː.mə,stæt/
Phonetic Spelling: hee·muh·stat
13. Inferior vena cava
Pronunciation link: <https://www.merriam-webster.com/dictionary/inferior%20vena%20cava>
IPA: /ɪn'fɪr.i.ər 'viː.nə 'keɪ.və/
Phonetic Spelling: in·feer·ee·er vee·nuh kay·vuh
14. Paraformaldehyde
Pronunciation link: <https://www.merriam-webster.com/dictionary/paraformaldehyde>

- IPA: /ˌpɛr.ə.fɔːrˈmæl.dəˌhaɪd/
Phonetic Spelling: peh·ruh·for·mal·duh·hyd
15. Conical
Pronunciation link: <https://www.merriam-webster.com/dictionary/conical>
IPA: /ˈkɑː.nɪ.kəl/
Phonetic Spelling: kah·ni·kuhl
16. Sodium azide
Pronunciation link: <https://www.merriam-webster.com/dictionary/sodium%20azide>
IPA: /ˈsoʊ.di.əm ˈæ.zɑɪd/
Phonetic Spelling: soh·dee·uhm a·zyd
17. Confocal
Pronunciation link: <https://www.merriam-webster.com/dictionary/confocal>
IPA: /kənˈfoʊ.kəl/
Phonetic Spelling: kuhn·foh·kuhl
18. Methanol
Pronunciation link: <https://www.merriam-webster.com/dictionary/methanol>
IPA: /ˈmeθ.əˌnɔːl/
Phonetic Spelling: meh·thuh·nawl
19. Dibenzyl ether
Pronunciation link: <https://www.howtopronounce.com/dibenzyl-ether>
IPA: /dɑːˈben.zɪl ˈiː.θər/
Phonetic Spelling: dye·ben·zuhl ee·ther
20. Permeabilization
Pronunciation link: <https://www.merriam-webster.com/dictionary/permeabilization>
IPA: /ˌpɜː.mi.ə.bə.ləˈzeɪ.fən/
Phonetic Spelling: per·mee·uh·buh·luh·zay·shuhn
21. Immunolabeling
Pronunciation link: <https://www.howtopronounce.com/immunolabeling>
IPA: /ɪˌmjuː.noʊˈleɪ.bəl.ɪŋ/
Phonetic Spelling: ih·myoo·noh·lay·buh·ling
22. Ethyl cinnamate
Pronunciation link: <https://www.howtopronounce.com/ethyl-cinnamate>
IPA: /ˈeθ.əl ˈsɪn.əˌmeɪt/
Phonetic Spelling: eh·thuhl sin·uh·mayt
23. Microscope
Pronunciation link: <https://www.merriam-webster.com/dictionary/microscope>
IPA: /ˈmaɪ.krəˌskoʊp/
Phonetic Spelling: my·kruh·skohp
24. Fluorescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>
IPA: /flʊˈres.əns/
Phonetic Spelling: floor·es·uhns
25. Dendritic
Pronunciation link: <https://www.merriam-webster.com/dictionary/dendritic>

IPA: /denˈdrit.ɪk/

Phonetic Spelling: den·drit·ik

26. Hepatic

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

IPA: /hɪˈpæt.ɪk/

Phonetic Spelling: hi·pat·ik

27. Stellate

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

IPA: /ˈstɛl.ɪt/

Phonetic Spelling: stel·ayt

28. Tetrachloride

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

IPA: /ˌtɛ.trəˈklɔːr.aɪd/

Phonetic Spelling: te·truh·klor·ide