

Submission ID #: 68420

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Project Page Link: <https://review.jove.com/account/file-uploader?src=20868148>

Title: Live Imaging and Characterization of Microglia Dynamics and Interactions with Synapses in Diseased Murine Retina

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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? **Yes**

If your microscope does not have a camera port, the scope kit will be attached to one of the eyepieces and **you will have to perform the procedure using one eye.**

Nikon Eclipse Ti2-E Inverted Microscope

Videographer: Please record scope shots using a SCOPE KIT, if needed

- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**

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

Within the same building. Dissection takes place on 3rd floor, imaging and analysis take place on 4th 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: 19

Number of Shots: 46

Introduction

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

INTRODUCTION:

AUTHORS: Please note that all statements are restricted to a 20-word limit. Also, please note that the questions will not appear in the video.

~~What is the scope of your research? What questions are you trying to answer?~~

- 1.1. **Camille Fang:** The scope of our research is to study the underlying mechanisms of glaucoma, and microglia in synapse remodeling during development.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. What are the current experimental challenges?

~~What are the current experimental challenges?~~

- 1.2. **Yvonne Ou:** The current experimental challenges involve determining whether microglia actively disassemble retinal ganglion cell synapses or passively clear debris.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What significant findings have you established in your field?~~

- 1.3. **Yvonne Ou:** Our significant findings show increased microglial number, complexity, motility, and synaptic colocalization following transient intraocular pressure elevation in mice.
 - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What advantage does your protocol offer compared to other techniques?~~

- 1.4. **Camille Fang:** Our protocol offers ex vivo imaging, avoiding cataract and corneal opacity issues, enabling easier setup, faster experiments, and higher throughput.
 - 1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How will your findings advance research in your field?~~

1.5. **Yvonne Ou:** Our findings advance research by enabling visualization of microglia–neuron interactions, cellular communication, and dynamics using fluorescent transgenic lines and timelapse imaging.

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**.

How do you think publishing with JoVE will enhance the visibility and impact of your research?

- 1.6. **Camille Fang, Assistant Specialist**: (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.

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

- 1.7. **Camille Fang, Assistant Specialist**: (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 California, San Francisco

Protocol

NOTE: Screen capture timestamps were added at the postshoot stage. If any doubts, please contact Poornima.g@jove.com

2. Time-Lapse Imaging of Mouse Retina for Tracking Analysis of Microglia and Synaptic Puncta

NOTE: The protocol is divided into 3 sections and titles are added at the postshoot stage

Demonstrator: Camille Fang

2.1. Begin with mouse retina positioned on a filter paper and dry it with a laboratory wipe [1]. Invert the retina onto a Matek Petri dish [2]. Weigh down the retina using either metal beads or a ring [3]. Then add three to five drops of artificial cerebrospinal fluid [4].

2.1.1. WIDE: Talent drying a filter paper with the mouse retina using a laboratory wipe.

2.1.2. Talent inverting the retina onto a Matek Petri dish.

2.1.3. Talent placing metal beads or ring to secure the retina.

2.1.4. Talent pipetting ACSF drops onto the retina.

Videographer's NOTE: I included two different takes here. One wider shot and one super tight shot.

2.2. Place the sample under a Spinning disk confocal microscope [1] and adjust the settings to preview the retina [2]. Collect images along the z-axis at 10 to 20 micrometers total depth using a step size of 0.3 micrometer [3]. Maintain the time interval between frames at 20 to 30 seconds to allow for reliable cell tracking [4].

Added shot: 2.2.0. talent loading the microscope. **Videographer's NOTE:** included a couple of different takes of this

Added shot 2.2.0.A: talent setting a preview scan

Videographer's NOTE : The computer used for 2.2 can not screen record, so I recorded the screen with my camera for the step 2.2.

2.2.1. SCREEN: Set the depth to 10-20 μm and step size to 0.3 μm then image the retina.

2.2.2. SCREEN: Set the time-lapse acquisition setup with frame interval to 20–30 seconds.

3. Microglial Surface Rendering and Tracking Analysis

- 3.1. To perform surface rendering of the microglia, convert the timelapse file into IMS format using the file converter software [1-TXT].
 - 3.1.1. SCREEN: Imaris-2.3.mp4. 00:15-00:30
TXT: Set the voxel sizes: X: 0.1272423 μ , Y: 0.1272423 μ , Z: 0.3 μ
- 3.2. In the analysis software Arena, click on the **Observe** folder to open the converted file and ensure the image is in **3D view** [1]. To detect individual microglia, click the **add new surface** icon in the **Object** Toolbar [2].
 - 3.2.1. SCREEN: Imaris-2.4-.mp4. 00:13-00:30
 - 3.2.2. SCREEN: Imaris-2.4-.mp4. 00:44-00:49.
- 3.3. Select **Segment only a region of interest, Track Surfaces (over Time)** (*Track-Surfaces-Over-Time*), **Classify Surfaces**, and **Object-Object Statistics** (*Object-Object-Statistics*) under **Algorithm** settings. Then click the blue **play** button to proceed [1].
 - 3.3.1. SCREEN: Imaris-2.4-2.5-2.6-v2.mp4 00:00-0:13.
- 3.4. Choose the channel for surface rendering. Optionally, enable **Smooth** to smooth the surface creation and enter the surface detail [1]. Under **thresholding**, choose **Machine Learning Segmentation** for detecting cells [2].
 - 3.4.1. SCREEN: Imaris-2.4-2.5-2.6-v2.mp4 00:54-01:05.
 - 3.4.2. SCREEN Imaris-2.6.mp4 00:06-00:12.
- 3.5. Under **Training Data**, use the **Background** and **Foreground** paintbrush tools. Draw paintbrush strokes by selecting the desired paintbrush and using the Shift plus Left click [1]. Ensure **Interpolate Display** is enabled, then select **Train and Predict** [2].
 - 3.5.1. SCREEN: Imaris-2.7-2.8-2.9.mp4 00:00-00:17.
 - 3.5.2. SCREEN: Imaris-2.7-2.8-2.9.mp4 00:17-00:24.
- 3.6. Use the **yellow pointer** in the middle to navigate across the z-stack [1]. Draw multiple strokes across x, y, z planes and across a few time frames. Use three to five short strokes per entry, iteratively adjusting until accurate surface is made [2].
 - 3.6.1. SCREEN: Imaris-2.7-2.8-2.9.mp4 00:25 -00:30.
 - 3.6.2. SCREEN: Imaris-2.7-2.8-2.9.mp4 00:31-00:45.
- 3.7. Use the **yellow rectangle** to toggle between the training display and actual surface created as a final check [1]. Also check across time lapse that the surface matches the cell morphology [2-TXT].

3.7.1. SCREEN: Imaris-2.7-2.8-2.9.mp4 01:50-02:00.

3.7.2. SCREEN: Imaris-2.7-2.8-2.9.mp4 02:01-02:14. **TXT: Once done, deselect split object**

3.8. Adjust the voxel histogram threshold to remove small unconnected objects not part of the primary surface [1]. Then edit the surface manually to remove extraneous objects or cut edges using the scissor tool [2].

3.8.1. SCREEN: Imaris-2.10.mp4 00:30-00:45.

3.8.2. SCREEN: Imaris-2.10.mp4 01:50-02:00.

3.9. If desired, set a threshold of gaps allowed for object and select the tracking algorithm for the moving cell [1]. Filter track objects not associated with the main surface [2]. Using a histogram, define a threshold to remove small tracks lasting less than five frames [3].

3.9.1. SCREEN: Imaris-2.11.mp4 00:00-00:15.

3.9.2. SCREEN: Imaris-2.11.mp4 00:28-00:40.

3.9.3. SCREEN: Imaris-2.11.mp4 00:41-00:51.

4. Spot Classification for Postsynaptic Density Protein-95 (PSD95) to Detect Synaptic Puncta

4.1. To detect whole individual microglia, click on the **blue surface** icon in the **Object Toolbar** [1]. Then select **Track Surfaces (over Time)** (*track-surfaces-over-time*), **Classify Surfaces**, and **Object-Object Statistics** and press play [2].

4.1.1. SCREEN: Imaris-2.12-2.13-2.14.mp4 00:00-00:05.

4.1.2. SCREEN: Imaris-2.12-2.13-2.14.mp4 00:07-00:12.

4.2. Select the **source channel** for the synaptic marker [1]. Turn off slicer and other channels to visualize only PSD95 (*P-S-D-Ninety-Five*) [2]. Use the control button and the **pointer selection tool** to estimate the xy (*X-Y*) diameter of the puncta [3].

4.2.1. SCREEN: Imaris-2.12-2.13-2.14.mp4 00:13-00:19.

4.2.2. SCREEN: Imaris-2.12-2.13-2.14.mp4 00:20-00:23.

4.2.3. SCREEN: Imaris-2.12-2.13-2.14.mp4 00:25-00:30.

4.3. Pick a few bright puncta that persist across frames [1] and Turn off background subtraction [2].

4.3.1. SCREEN: Imaris-2.12-2.13-2.14.mp4 00:35-00:45.

4.3.2. SCREEN: Imaris-2.12-2.13-2.14.mp4 00:48-00:52.

4.4. Now, add a filter based on spot quality. Adjust the histogram to include accurate PSD95

puncta across all time frames [1]. Remove noise spots via edit. Toggle between the cube or circle cursor to delete spots lasting only one or two frames [2].

4.4.1. SCREEN: Imaris-2.15.mp4 00:12-00:30.

4.4.2. SCREEN: Imaris-2.15.mp4 00:45-01:00.

4.5. If desired, set a threshold of gaps allowed and choose the tracking algorithm for synaptic spots [1]. Filter spot tracks not associated with real puncta using a histogram threshold to exclude small object tracks [2].

4.5.1. SCREEN: Imaris-2.16.mp4 00:20-00:30.

4.5.2. SCREEN: Imaris-2.16.mp4 00:35-00:50.

4.6. Define classifications using the **Shortest Distance to Surface** classification [1]. Set **engulfed** as any **puncta < 0 μm** (*puncta-less-than-Zero-micrometer*), **contacted** as between **0 to 0.5 μm from the surface**, or **uncontacted** as **0.5+ (*Zero-point-five-plus*) μm** [2].

4.6.1. SCREEN: Imaris-2.17.mp4 00:00-00:08.

4.6.2. SCREEN: Imaris-2.17.mp4 00:20-00:45.

4.7. Assign labels to events using the classifications defined above [1].

4.7.1. SCREEN: Imaris-2.18.mp4. 0:30-00:40

4.8. Once the surfaces and spots appropriately represent microglia and puncta, export all statistics under the **statistics** tab [1].

4.8.1. SCREEN: Imaris-2.19-v1.mp4. 00:17-00:30

Results

5. Results

- 5.1. Microglia exhibited increased process displacement length after laser-induced intraocular pressure elevation compared to the control [1]. Microglia speed was significantly higher in the laser-treated eyes compared to controls [2].
 - 5.1.1. LAB MEDIA: Figure 3A. *Video editor: Highlight the bar labelled "laser"*
 - 5.1.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the bar labelled "laser"*
- 5.2. The number of contact events between microglia and PSD95 puncta increased following laser treatment [1].
 - 5.2.1. LAB MEDIA: Figure 3C. *Video editor: Highlight the bar labelled "laser"*
- 5.3. Representative images showed increased microglia-synapse interactions in laser-treated retinas compared to controls [1].
 - 5.3.1. LAB MEDIA: Figure 2 *Video Editor: Please highlight the images in the "Laser" column and then "Control"*

1. Cerebrospinal
 Pronunciation link: <https://www.merriam-webster.com/dictionary/cerebrospinal>
 IPA: /səˈriːbroʊˈspainəl/
 Phonetic Spelling: suh·ree·broh·spy·nuhl
2. Confocal
 Pronunciation link: <https://www.merriam-webster.com/dictionary/confocal>
 IPA: /kənˈfoʊkəl/
 Phonetic Spelling: kuhn·foh·kuhl
3. Micrometers
 Pronunciation link: <https://www.merriam-webster.com/dictionary/micrometer>
 IPA: /maɪˈkraːmɪtər/
 Phonetic Spelling: my·krah·muh·ter
4. Microglia
 Pronunciation link: <https://www.merriam-webster.com/dictionary/microglia>

- IPA: /maɪˈkrɒɡliə/
Phonetic Spelling: my·krah·glee·uh
5. Synaptic
Pronunciation link: <https://www.merriam-webster.com/dictionary/synaptic>
IPA: /sɪˈnæptɪk/
Phonetic Spelling: sih·nap·tik
6. Puncta
Pronunciation link: <https://www.merriam-webster.com/dictionary/punctum>
IPA: /ˈpʌŋktə/
Phonetic Spelling: punk·tuh
7. Voxel
Pronunciation link: <https://www.merriam-webster.com/dictionary/voxel>
IPA: /ˈvɑːksəl/
Phonetic Spelling: vok·suhl
8. Interpolate
Pronunciation link: <https://www.merriam-webster.com/dictionary/interpolate>
IPA: /ɪnˈtɜːpəˌleɪt/
Phonetic Spelling: in·tur·puh·layt
9. Histogram
Pronunciation link: <https://www.merriam-webster.com/dictionary/histogram>
IPA: /ˈhɪstəˌɡræm/
Phonetic Spelling: his·tuh·gram
10. Extraneous
Pronunciation link: <https://www.merriam-webster.com/dictionary/extraneous>
IPA: /ɪkˈstreɪniəs/
Phonetic Spelling: ik·stray·nee·uhs
11. Algorithm
Pronunciation link: <https://www.merriam-webster.com/dictionary/algorithm>
IPA: /ˈælgəˌrɪðəm/
Phonetic Spelling: al·guh·ri·thuhm
12. Postsynaptic
Pronunciation link: <https://www.merriam-webster.com/dictionary/postsynaptic>
IPA: /ˌpoʊstsɑːˈnæptɪk/
Phonetic Spelling: pohst·sy·nap·tik
13. Engulfed
Pronunciation link: <https://www.merriam-webster.com/dictionary/engulf>
IPA: /ɪnˈɡʌlf/
Phonetic Spelling: in·gulf
14. Intraocular
Pronunciation link: <https://www.merriam-webster.com/dictionary/intraocular>
IPA: /ˌɪntɹəˈɑːkjələr/
Phonetic Spelling: in·truh·aa·kyuh·ler
15. Morphology
Pronunciation link: <https://www.merriam-webster.com/dictionary/morphology>

IPA: /mɔːrˈfɑːlədʒi/

Phonetic Spelling: mor·faa·luh·jee

16. Segmentation

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

IPA: /ˌseɡməntˈteɪʃən/

Phonetic Spelling: seg·muhn·tay·shuhn

17. Classification

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

IPA: /ˌklæsəfəˈkeɪʃən/

Phonetic Spelling: klas·uh·fuh·kay·shuhn

18. Retinas

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

IPA: /ˈretənə/

Phonetic Spelling: reh·tuh·nuh