

Submission ID #: 70582

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Project Page Link: <https://review.jove.com/account/file-uploader?src=21527558>

Title: A Dual-Color Fiber Photometry Method for Recording Astrocyte–Neuron Activity Across Multiple Brain Regions During Learning and Memory Behaviors

Authors and Affiliations:

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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 **Yes**, can you record movies/images using your own microscope camera?

Yes

2.5.1, 2.7.1, 2.9.2, 2.12.1, 2.14.3, 2.15.1

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? **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: 27

Number of Shots: 50

Introduction

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

INTRODUCTION:

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

- 1.1. **Jihua Fan:** This protocol enables recording of calcium signals from neurons and astrocytes across multiple brain regions during learning and memory behaviors.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 3.9.1*

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

- 1.2. **Jihua Fan:** This protocol can be applied to study astrocyte–neuron network dynamics during learning, memory, and other behaviors.
 - 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. **Jihua Fan:** This protocol enables simultaneous measurement of astrocyte–neuron activity and functional coupling across multiple brain regions.
 - 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. **Jihua Fan:** The key challenge is maintaining precise alignment across surgeries while minimizing tissue damage during injection and fiber implantation.
 - 1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.7.1, 2.7.3*

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

- 1.5. **Jihua Fan:** Future studies can apply this method to disease models to examine how astrocyte–neuron coordination is altered.
- 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 Animal Care and Use Committee of the Third
Military Medical University

Protocol

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

2. Stereotaxic Viral Injection and Multi-site Fiber Photometry Probe Implantation

Demonstrator: Jihua Fan

- 2.1. To perform the first surgery for astrocyte injection, take an anesthetized mouse [1-TXT] and maintain body temperature at 37 to 38 degrees Celsius with a heating pad [2]. Apply ophthalmic ointment to prevent corneal desiccation [3].
 - 2.1.1. WIDE: Talent at the working area with the anesthetized placed on the surgical platform **TXT: Anesthesia: 3% isoflurane; Plasmid injected: AAV5-GfaABC1D-GCaMP6f**
 - 2.1.2. Talent placing the animal on the heating pad.
 - 2.1.3. Talent applying ophthalmic ointment to the eyes.
- 2.2. Then, remove the scalp hair [1] and disinfect the skin thoroughly [2]. Make a midline incision to reveal the skull surface [3].
 - 2.2.1. Talent removing the scalp hair.
 - 2.2.2. Talent disinfecting the skin.
 - 2.2.3. Talent making a midline incision to reveal the skull surface.
- 2.3. Place the head in the stereotaxic instrument [1]. Adjust the bregma and lambda sutures to the same horizontal plane [2] and adjust the frame further to ensure that points 2 millimeters lateral to the bregma on both sides are level [3].
 - 2.3.1. Talent positioning the head in the stereotaxic instrument.
 - 2.3.2. Talent adjusting the bregma and lambda sutures to the same horizontal plane.
 - 2.3.3. Talent adjusting the frame to level the points 2 millimeters lateral to the bregma on both sides.
- 2.4. Identify the stereotaxic coordinates with a mouse brain atlas using the bregma as the reference point [1].
 - 2.4.1. TEXT ON A PLAIN BACKGROUND
mPFC: AP +1.8 mm, ML +0.3 mm, DV -3.0 mm
CA1: AP -2.1 mm, ML +1.6 mm, DV -1.2 mm
MEC: AP -4.5 mm, ML -2.9 mm, DV -2.0 mm
- 2.5. Drill a 0.6-millimeter diameter circular craniotomy above each site [1-TXT]. Keep the

- dura mater moist and intact after opening the skull [2].
- 2.5.1. SCOPE: 70582_SCOPE_2.5.1.MP4: 00:05-00:16
- 2.5.2. Talent applies saline to keep the duramater moist.
- 2.6. Load the GCaMP6f (*G-Camp-Six-F*) virus into the syringe at 5 nanoliters per second [1] and install the syringe in the stereotaxic arm [2].
- 2.6.1. Talent loading the GCaMP6f virus into the syringe.
- 2.6.2. Talent installing the syringe in the stereotaxic arm.
- 2.7. Lower the pipette slowly into the target region and inject 150 nanoliters at 0.6 nanoliters per second, hold for 8 minutes [1], and withdraw at 0.1 millimeters per second [2]. After repeating this procedure for all the regions, seal the incision with tissue adhesive and allow 2 weeks for viral expression [3]. NOTE: The numbering is modified for the modified shot
- 2.7.1. SCOPE: 70582_SCOPE_2.5.1.MP4: 00:03-00:18
- 2.7.2. Slowly withdraw the pipette after injection. NOTE: This shot is modified
- 2.7.3. Talent applying tissue adhesive to seal the incision.
- 2.8. To perform the secondary surgery for neuronal injection with AAV9-hSyn-jRGECO1a (*A-A-V-H-Syn-J-R-Geko-One-A*), prepare the mouse as demonstrated earlier [1].
- 2.8.1. Shot of the animal prepared for injection.
- 2.9. Reopen the scalp along the midline and level the skull [1]. Clear tissue debris at the same locations as the craniotomy windows from the first surgery to expose the dura mater intact [2].
- 2.9.1. Talent reopening the scalp along the midline and adjusting the skull to a level position.
- 2.9.2. SCOPE: 70582_SCOPE_2.9.2.MP4: 00:00-00:10
- 2.10. Adjust coordinates using the bregma as the reference point. Then, perform injections at the same coordinates and depths as described previously [1].
- 2.10.1. Talent adjusting stereotaxic coordinates using the bregma as the reference point.
- 2.11. Next, load the jRGECO1a (*J-R-Geko-One-A*) virus into the syringe at 5 nanoliters per second [1] and install the syringe in the stereotaxic arm [2].
- 2.11.1. Talent loading the jRGECO1a virus into the syringe.
- 2.11.2. Talent installing the syringe in the stereotaxic arm.
- 2.12. Inject 150 nanoliters at 0.6 nanoliters per second, hold for 8 minutes, and withdraw slowly. Repeat this procedure for all regions and allow 3 weeks for viral expression [1].

2.12.1. SCOPE: 70582_SCOPE_2.12.1.MP4: 00:03-00:13, 00:35-00:37, 00:48-00:53

2.13. Next, install the optical fiber ferrule in the stereotaxic holder [1].

2.13.1. WIDE: Talent installing the optical fiber ferrule in the stereotaxic holder.

2.14. Using the presented coordinates [1], position the fiber at 100 to 150 micrometers above the viral injection depth [2-TXT]. Lower the fiber slowly to minimize tissue damage [1].

2.14.1. TEXT ON A PLAIN BACKGROUND

mPFC: AP +1.8 mm, ML +0.3 mm, DV -2.9 mm

CA1: AP -2.1 mm, ML +1.6 mm, DV -1.1 mm

MEC: AP -4.5 mm, ML -2.9 mm, DV -1.9 mm

2.14.2. Talent positioning the optical fiber above the viral injection depth

2.14.3. SCOPE: 70582_SCOPE_2.14.3.MP4: 00:12-00:30.

2.15. Seal the craniotomy with tissue adhesive [1] and secure the ferrule with dental cement [2].

2.15.1. SCOPE: 70582_SCOPE_2.5.1.MP4: 00:04-end

2.15.2. Talent securing the ferrule with dental cement.

3. Multi-channel Fiber Photometry Recording

3.1. Perform multi-channel fiber photometry recording three weeks after the second virus injection to ensure stable and full expression of both calcium indicators [1].

3.1.1. WIDE: Talent preparing the setup for multi-channel fiber photometry recording.

3.2. To habituate the mice to the fiber system and arena, first connect one end of the bundled optical fiber to the fiber photometry acquisition system [1].

3.2.1. Talent connecting the bundled optical fiber to the fiber photometry acquisition system.

3.3. Connect the three branches at the other end of the bundled fiber to the three brain regions, connect Channel 1 to the medial prefrontal cortex [1], Channel 2 to CA1 [2], and Channel 3 to the medial entorhinal cortex [3].

3.3.1. Talent connecting Channel 1 to the medial prefrontal cortex.

3.3.2. Talent connecting Channel 2 to CA1.

3.3.3. Talent connecting Channel 3 to the medial entorhinal cortex.

3.4. After briefly anesthetizing the mouse, place it in an open area with a side length of 50 centimeters and allow free exploration [1].

3.4.1. Talent placing the mouse in the open arena.

- 3.5. After 10 minutes, disconnect the fiber [1] and return the mouse to the home cage. Repeat once per day for three days [2].
 - 3.5.1. Talent disconnecting the fiber from the mouse.
 - 3.5.2. Talent placing the mouse back into the home cage.
- 3.6. For Fiber photometry recording during the Novel Object Recognition Task, place two identical objects in diagonally opposite quadrants [1]. Connect the fiber bundle in a lightly anesthetized state [2].
 - 3.6.1. Talent positioning two identical objects in opposite quadrants of the arena.
 - 3.6.2. Talent connecting the fiber bundle while the mouse is lightly anesthetized.
- 3.7. Allow the mouse to explore the objects for 10 minutes [1]. After that, return the mouse to the home cage without removing the fiber [2].
 - 3.7.1. Shot of the mouse exploring the objects in the arena.
 - 3.7.2. Talent placing the mouse back into the home cage with the fiber attached.
- 3.8. Now, adjust the stimulation parameters of the lasers. Set 470 nanometers for GCaMP6f and 560 nanometers for jRGECO1a [1].
 - 3.8.1. SCREEN: 70582_screenshot_1.mp4: 00:06-00:12, 00:20-00:28
- 3.9. Record calcium signals at 30 hertz synchronized with video tracking at 30 hertz [1]. Then, position a laser diode within the video frame outside the mouse's visual field at the start of fiber photometry acquisition to serve as a temporal marker [2].
 - 3.9.1. SCREEN: 70582_screenshot_2.mp4 00:06-00:10, 00:14-00:16, 00:27-00:34
 - 3.9.2. Talent positioning a laser diode within the video frame outside the mouse visual field.
- 3.10. After 10 minutes from the learning phase, replace one object with a novel object [1]. Place the mouse back into the arena for 10 minutes of exploration while recording [2].
 - 3.10.1. Talent replacing one object with a novel object in the arena.
 - 3.10.2. Talent placing the mouse back into the arena for exploration during recording.
- 3.11. Disconnect the fiber from the ceramic ferrule [1] and return the mouse to the home cage [2].
 - 3.11.1. Talent disconnecting the fiber with the ceramic ferrule.
 - 3.11.2. Talent placing the mouse back into the home cage.
- 3.12. After imaging, quantify the calcium responses during novel versus familiar object exploration using the appropriate analysis software [1].
 - 3.12.1. Shot of the computer screen with the images opened in the software.

Results

4. Results

- 4.1. Confocal imaging revealed strong overlap between GCaMP6f fluorescence and the astrocyte marker S100 β (*S-Hundred-Beta*), and [1] robust co-localization of jRGECO1a with the neuronal marker NeuN (*Neu-N*) in the mPFC (*M-P-F-C*), CA1 (*C-A-One*), and MEC (*M-E-C*) [2].
 - 4.1.1. LAB MEDIA: Figure 2A–C. *Video editor: In each region panel, highlight the green and orange signals in the lower images.*
 - 4.1.2. LAB MEDIA: Figure 2A–C. *Video editor: In each region panel, highlight the red and yellow signals in the upper images.*
- 4.2. These results confirmed stable and specific expression of GCaMP6f in astrocytes and [1] jRGECO1a in neurons within the targeted memory-related brain regions [2].
 - 4.2.1. LAB MEDIA: Figure 2D–E. *Video editor: Highlight the bar graphs for GCaMP6f in the mPFC, CA1, and MEC.*
 - 4.2.2. LAB MEDIA: Figure 2F–G. *Video editor: Highlight the bar graphs for jRGECO1a in the mPFC, CA1, and MEC.*
- 4.3. Statistical analyses of event frequency [1], duration [2], and cross-regional distribution demonstrated that this method reliably and stably measured dual cell-type-specific calcium signals across multiple brain regions [3].
 - 4.3.1. LAB MEDIA: Figure 4C.
 - 4.3.2. LAB MEDIA: Figure 4D.
 - 4.3.3. LAB MEDIA: Figure 4E
- 4.4. The cross-correlation coefficients between astrocytes and neurons across multiple brain regions during the stationary state [1] were significantly lower than during the freely moving state [2].
 - 4.4.1. LAB MEDIA: Figure 4F,G. *Video editor: Highlight the stationary condition in the comparison panels for the mPFC, CA1, and MEC.*
 - 4.4.2. LAB MEDIA: Figure 4F,G. *Video editor: Highlight the movement condition in the comparison panels for the mPFC, CA1, and MEC.*
- 4.5. Astrocytic calcium signals exhibited lower peak amplitudes during exploration of the novel object compared to the familiar object [1].

- 4.5.1. LAB MEDIA: Figure 5B. *Video editor: Highlight the blue and orange traces after time 0 in the mPFC, CA1, and MEC, and emphasize that the blue peaks stay lower than the orange peaks.*
- 4.6. Neuronal calcium signals also showed a similar decreasing trend during exploration of the novel object compared to the familiar object [1].
 - 4.6.1. LAB MEDIA: Figure 5C. *Video editor: Highlight the blue and orange traces after time 0 in the mPFC, CA1, and MEC, and emphasize that the blue traces remain lower than the orange traces.*
- 4.7. Quantitative analysis of the area under the curve further confirmed that calcium responses in astrocytes and neurons were significantly attenuated during novel object exploration [1].
 - 4.7.1. LAB MEDIA: Figure 5D and E .

- 1. astrocyte
Pronunciation link: <https://www.merriam-webster.com/dictionary/astrocyte>
IPA: /'æs.trəˌsaɪt/
Phonetic Spelling: ass·truh·syte
- 2. anesthetized
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetize>
IPA: /əˈnɛs.θəˌtaɪzd/
Phonetic Spelling: uh·ness·thuh·tyzed
- 3. ophthalmic
Pronunciation link: <https://www.merriam-webster.com/dictionary/ophthalmic>
IPA: /ɑːfˈθæl.mɪk/
Phonetic Spelling: off·thal·mik
- 4. corneal
Pronunciation link: <https://www.merriam-webster.com/dictionary/corneal>
IPA: /ˈkɔːr.ni.əl/
Phonetic Spelling: kor·nee·uhl
- 5. desiccation
Pronunciation link: <https://www.merriam-webster.com/dictionary/desiccation>
IPA: /ˌdɛs.əˈkeɪ.ʃən/
Phonetic Spelling: dess·uh·kay·shuhn

6. isoflurane
Pronunciation link: <https://www.merriam-webster.com/dictionary/isoflurane>
IPA: /,aɪ.souˈflʊr.əm/
Phonetic Spelling: eye·soh·floor·ayn
7. plasmid
Pronunciation link: <https://www.merriam-webster.com/dictionary/plasmid>
IPA: /ˈplæz.mɪd/
Phonetic Spelling: 3laz·mid
8. stereotaxic
Pronunciation link: <https://www.merriam-webster.com/dictionary/stereotaxic>
IPA: /,stɛr.i.ouˈtæks.ɪk/
Phonetic Spelling: steh·ree·oh·tak·sɪk
9. bregma
Pronunciation link: <https://www.merriam-webster.com/dictionary/bregma>
IPA: /ˈbrɛɡ.mə/
Phonetic Spelling: breg·muh
10. lambda
Pronunciation link: <https://www.merriam-webster.com/dictionary/lambda>
IPA: /ˈlæm.də/
Phonetic Spelling: lam·duh
11. craniotomy
Pronunciation link: <https://www.merriam-webster.com/dictionary/craniotomy>
IPA: /ˌkreɪ.niˈɑː.tə.mi/
Phonetic Spelling: kray·nee·ah·tuh·mee
12. dura mater
Pronunciation link: <https://www.merriam-webster.com/dictionary/dura%20mater>
IPA: /ˈdʊr.əˈmeɪ.tər/, /ˈdjʊr.əˈmeɪ.tər/
Phonetic Spelling: dur·uh may·ter
13. nanoliters
Pronunciation link: <https://www.merriam-webster.com/dictionary/nanoliter>
IPA: /ˈnæn.ou.liː.təz/
Phonetic Spelling: nan·oh·lee·terz
14. pipette
Pronunciation link: <https://www.merriam-webster.com/dictionary/pipette>
IPA: /paɪˈpet/
Phonetic Spelling: pie·pet
15. neuronal
Pronunciation link: <https://www.merriam-webster.com/dictionary/neuronal>
IPA: /nuˈrɑː.nəl/, /njʊˈrɑː.nəl/
Phonetic Spelling: noor·ah·nuhl
16. ferrule
Pronunciation link: <https://www.merriam-webster.com/dictionary/ferrule>
IPA: /ˈfɛr.uːl/
Phonetic Spelling: feh·rool

17. micrometers
 Pronunciation link: <https://www.merriam-webster.com/dictionary/micrometer>
 IPA: /maɪˈkraɪ. mə. tər/ ~~z~~
 Phonetic Spelling: my·krah·muh·terz
18. photometry
 Pronunciation link: <https://www.merriam-webster.com/dictionary/photometry>
 IPA: /foʊˈtɑː.mə.tri/
 Phonetic Spelling: foh·tah·muh·tree
19. habituate
 Pronunciation link: <https://www.merriam-webster.com/dictionary/habituate>
 IPA: /həˈbɪtʃ.u.ert/
 Phonetic Spelling: huh·bich·oo·ayt
20. entorhinal
 Pronunciation link: <https://www.merriam-webster.com/dictionary/entorhinal>
 IPA: /ˌɛn.təˈrˈhaɪ.nəl/
 Phonetic Spelling: en·tor·hy·nuhl
21. anesthetizing
 Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetize>
 IPA: /əˈnɛs.θəˌtaɪ.zɪŋ/
 Phonetic Spelling: uh·ness·thuh·ty·zing
22. synchronized
 Pronunciation link: <https://www.merriam-webster.com/dictionary/synchronize>
 IPA: /ˈsɪŋ.krəˌnaɪzd/
 Phonetic Spelling: sing·kruh·nyzed
23. confocal
 Pronunciation link: <https://www.merriam-webster.com/dictionary/confocal>
 IPA: /kɑːnˈfoʊ.kəl/
 Phonetic Spelling: kon·foh·kuhl
24. fluorescence
 Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>
 IPA: /flʊˈrɛs.əns/, /flɔːˈrɛs.əns/
 Phonetic Spelling: floor·ess·uhns
25. co-localization
 Pronunciation link: <https://www.merriam-webster.com/dictionary/colocalization>
 IPA: /ˌkoʊ.loʊ.kə.ləˈzeɪ.ʃən/
 Phonetic Spelling: koh·loh·kuh·luh·zay·shuhn
26. neuronal
 Pronunciation link: <https://www.merriam-webster.com/dictionary/neuronal>
 IPA: /nɜːˈrɑː.nəl/, /njʊˈrɑː.nəl/
 Phonetic Spelling: noor·ah·nuhl
27. statistical
 Pronunciation link: <https://www.merriam-webster.com/dictionary/statistical>
 IPA: /stəˈtɪs.tɪ.kəl/
 Phonetic Spelling: ~~st~~uh·tis·ti·kuhl

28. correlation

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

IPA: /ˌkɒr.əˈleɪ.ʃən/

Phonetic Spelling: kor·uh·lay·shuhn

29. astrocytic

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

IPA: /ˌæs.trəˈsaɪ.tɪk/

Phonetic Spelling: ass·truh·sy·tik

30. attenuated

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

IPA: /əˈtɛn.ju.eɪ.tɪd/

Phonetic Spelling: uh·ten·yoo·ay·tuhd