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Scriptwriter Name: Deepthi Das

Project Page Link: https://review.jove.com/files_upload.php?src=21660233

Title: An Integrated Method for Photothrombotic Stroke Modeling and In Vivo Optrode Recording of Neuronal and Astrocytic Activity in Behaving Mice

Authors and Affiliations: Siyun Chen¹, Wenwen Zhang¹, Zhuorui Huang¹, Jiahui Zhang¹, Wenkun Huang¹, Yunyao Zheng¹, Kangwen Ming², Lin Yu², Wei Yi¹, Xiaorong Tang¹

¹South China Research Center for Acupuncture and Moxibustion, Medical College of Acu-Moxi and Rehabilitation, Guangzhou University of Chinese Medicine

²Rehabilitation Department, The Affiliated Traditional Chinese Medicine Hospital, Guangzhou Medical University

Corresponding Authors:

Xiaorong Tang tangxrkk@163.com, tangxr@gzucm.edu.cn

Email Addresses for All Authors:

csyday@hotmail.com
zww18831413406@163.com
2023051116@stu.gzucm.edu.cn
2023051173@stu.gzucm.edu.cn
2023051113@stu.gzucm.edu.cn
2081795053@qq.com
mkw1976828@sina.com
yul@gzhmu.edu.cn
acupaper@163.com
tangxr@gzucm.edu.cn

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? **No**

If a dissection or stereo microscope is required for your protocol, please list all shots from the script that will be visualized using the microscope (shots are indicated with the 3-digit numbers, like 2.1.1, 2.1.2, etc.).

WF 10*20(SWG-WF10X/20)

SCOPE Shots: 2.4.1, 2.4.2, 2.4.3, 2.5.1, 2.5.2, 3.2.1, 3.2.2, 3.3.1, 3.3.2, 3.4.1, 3.4.2, 3.4.3, 3.4.4, 3.5.1, 3.5.2, 3.6.1, 3.6.2, 3.7.1, 3.7.2

NOTE: Videographer filmed these

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>

Please upload all screen-captured video files to your project page as soon as possible.

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: 21

Number of Shots: 58-3=55

Interviews

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. **Siyun Chen:** Our research develops an integrated method to simultaneously record astrocyte and neuron activity during ischemic stroke in behaving mice.

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

- 1.2. **Siyun Chen:** Previous methods record neurons or astrocytes separately; our protocol captures astrocyte and neuron signals simultaneously from the very moment of stroke onset.

CONCLUSION:

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

- 1.3. **Xiaorong Tang:** This protocol allows uninterrupted recording of astrocyte and neuron activity across the full stroke timeline, from acute onset to chronic stages.

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

- 1.4. **Xiaorong Tang:** Success in this protocol relies on precise coordinate alignment, meticulous optrode implantation, and sufficient viral expression time across surgeries.

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

- 1.5. **Xiaorong Tang:** Future studies could use multiple implants to monitor broader brain regions or target different cell types with diverse behavioral assays.

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 at Guangzhou University of Chinese Medicine

Protocol

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

2. Stereotaxic Viral Vector Delivery to the Mouse Motor Cortex

Demonstrator: Siyun Chen

- 2.1. To begin, obtain and transfer a deeply anesthetized C57BL-6J mouse to the stereotaxic frame equipped with an anesthesia mask **[1-TXT]**. Monitor the depth of anesthesia every 10 minutes while maintaining the mouse body temperature at 36 to 37.5 degrees Celsius with a feedback-controlled heated pad **[2]**.
 - 2.1.1. WIDE: Shot of the mouse in deep anesthesia. **TXT: Anesthesia: 1-2% isoflurane**
Videographer: Please obtain CU shot as well for this shot.
 - 2.1.2. Talent checking anesthesia depth of the mouse lying on the feedback-controlled heated pad.
- 2.2. Secure the mouse to the stereotaxic frame with ear bars and a mouth bar after applying ophthalmic ointment to the eyes **[1]**. Confirm head immobilization by gentle probing once the head is symmetrically fixed **[2]**.
 - 2.2.1. Talent securing the mouse with ear bars and a mouth bar. **NOTE: 2.2.1 & 2.2.2 were shot together**
 - 2.2.2. Talent gently probing the head to confirm immobilization.
- 2.3. Use surgical scissors to make a midline incision after removing the hair using a depilatory cream **[1]**. Clean the periosteum with saline and cotton swabs to expose Bregma and Lambda **[2]**. Lower the glass micropipette to touch Bregma and Lambda successively **[3]**. After noting the Z-coordinate on the digital vernier, adjust the mouth bar until the difference is less than 0.04 millimeters **[4]**.
 - 2.3.1. Talent making a midline incision with surgical scissors.
 - 2.3.2. Talent cleaning the periosteum with saline and cotton swabs.
 - 2.3.3. Talent lowering the glass micropipette to Bregma and Lambda.
 - 2.3.4. The Z-coordinate is being displayed on the digital vernier, and the mouth bar is being adjusted.
- 2.4. Lower the glass micropipette to touch Bregma and reset the digital vernier to zero **[1]**. Move the micropipette 2.3 millimeters to the left and right of Bregma successively **[2]**.

After noting the Z-coordinate, adjust the ear bars until the bilateral differences are less than 0.04 millimeters [3].

- 2.4.1. SCOPE: Talent lowering the glass micropipette to Bregma and resetting the digital vernier. **NOTE: 2.4.1 & 2.4.2 were shot together**
- 2.4.2. SCOPE: Talent moving the micropipette to the left and right of Bregma.
- 2.4.3. SCOPE: Talent adjusting the ear bars until the bilateral differences are less than 0.04 millimeters

2.5. After resetting the digital vernier to zero at Bregma, mark the motor cortex forelimb area at anterior-posterior plus 1.5 millimeters and medial-lateral plus 0.74 millimeters [1]. Use a microdrill and 0.5-millimeter round-tip drill bits to drill a small hole approximately 0.5 millimeters in diameter at the marked location [2].

- 2.5.1. SCOPE: Talent marking the motor cortex forelimb area.
- 2.5.2. SCOPE: Talent drilling the marked location with the microdrill.

2.6. Before loading, mix the two adeno-associated viral vectors in equal volumes and briefly centrifuge the sample [1-TXT]. Lower the glass micropipette connected to the micro-infusion pump to the target site at a dorsal-ventral depth of minus 0.94 millimeters from the brain surface [2]. Infuse 400 nanoliters of the viral mix, containing 200 nanoliters of each vector, at 50 nanoliters per minute [3].

2.6.1. Talent mixing the two adeno-associated virus vectors. **TXT: Viral mix: AAV9-GfaABC1D-GCaMP6s + AAV9-GfaABC1D-opto-vTRAP**

NOTE: 2.6.1 had been split into 2 shots- 2.6.1(1) and 2.6.1(2):

1.Mixing the two adeno-associated virus vectors.

2.Vibrate and centrifuge the virus mixture

- 2.6.2. Talent lowering the glass micropipette to the target site.
- 2.6.3. Talent starting viral infusion with the micro-infusion pump.

2.7. Slowly withdraw the pipette 10 minutes after the infusion is completed [1]. Suture the scalp with 4-0 (*four-oh*), 20-millimeter, 3/8 (*three-eighths*) circle suture [2] and place the mouse on a heating pad until full recovery [3].

- 2.7.1. Talent slowly withdrawing the pipette.
- 2.7.2. Talent suturing the scalp.
- 2.7.3. Talent placing the mouse on a heating pad.

3. Craniotomy and Chronic Optrode Implantation

Authors: During filming, please point out the organs or veins before dissecting them.

- 3.1. Follow the previously demonstrated procedure up to scalp incision [1]. During the scalp incision, remove 1.5 centimeters by 1.5 centimeters of scalp while keeping sufficient skin around the eyes [2]. Clean the skull with hydrogen peroxide and 0.9% saline [3]. After identifying Bregma, reset the digital vernier to zero [4].
 - 3.1.1. Representative shot of the talent immobilizing the head.
 - 3.1.2. Talent removing the specified area of scalp
 - 3.1.3. Talent cleaning the skull with hydrogen peroxide and saline.
 - 3.1.4. Talent resetting the digital vernier after identifying Bregma.
- 3.2. Use a 0.8-millimeter round-tip drill to lightly roughen the skull surface [1]. Mark the motor cortex forelimb area at anterior-posterior 1.5 millimeters and medial-lateral 0.74 millimeters and use the mark as the center point to draw a 5-millimeter by 5-millimeter square [2].
 - 3.2.1. SCOPE: Talent roughening the skull surface with the drill.
 - 3.2.2. SCOPE: Talent marking the motor cortex forelimb area and drawing a square centered on the marked location.
- 3.3. Use a microdrill at approximately 10,000 revolutions per minute to trace the square [1]. Continuously apply ice-cold saline to the drill path and stop immediately if the bone flap loosens [2].
 - 3.3.1. SCOPE: Talent tracing the square with the microdrill. **NOTE: 3.3.1 & 3.3.2 shot together**
 - 3.3.2. SCOPE: Talent applying ice-cold saline to the drill path and stopping drilling if the bone flap loosens.
- 3.4. Drill three small 0.5-millimeter-diameter holes over the contralateral hemisphere and cerebellum [1]. Implant cranioplasty screws into the holes so that each screw reaches the dura mater without further penetration [2]. Use forceps to remove the loosened bone flap [3]. Use ultra-fine forceps with an ultra-fine tip less than 0.03 millimeters by 0.01 millimeters to remove the dura at the cranial window, while avoiding direct contact with brain tissue [4].
 - 3.4.1. SCOPE: Talent drilling three small holes.
 - 3.4.2. SCOPE: Talent implanting cranioplasty screws.
 - 3.4.3. SCOPE: Talent removing the loosened bone flap with forceps.
 - 3.4.4. SCOPE: Talent removing the dura mater with ultra-fine forceps.

- 3.5. Fix the optrode to the micromanipulator arm of the stereotaxic frame and move it above the cranial window [1]. Securely intertwine the silver ground and reference wires of the optrode to the three cranioplasty screws [2].
 - 3.5.1. SCOPE: Talent positioning the optrode above the cranial window.
 - 3.5.2. SCOPE: Talent intertwining the silver ground and reference wires to the screws.
- 3.6. Clean the cranial window surface with sterile saline [1]. Slowly lower the optrode to the desired dorsal-ventral depth of minus 0.94 millimeters at 1 millimeter per 10 seconds [2].
 - 3.6.1. SCOPE: Talent cleaning the cranial window surface with sterile saline.
 - 3.6.2. SCOPE: Talent lowering the optrode into the cranial window.
- 3.7. Apply a layer of agarose over the cranial window after implantation [1]. Apply 3 to 4 layers of dental cement [2]. Once the dental cement solidifies, provide analgesia for 3 to 5 days and allow the mouse to recover for 1 to 2 weeks [4].
 - 3.7.1. SCOPE: Talent applying agarose over the cranial window.
 - 3.7.2. SCOPE: Talent applying dental cement over the exposed skull and optrode.
 - ~~3.7.3. Talent removing the mouse from the stereotaxic frame.~~
 - 3.7.4. Talent providing analgesia to the mouse and leaving it undisturbed.

4. Fiber Photometry Recording and Stroke Induction

Demonstrators: Siyun Chen and Wenwen Zhang

- 4.1. Use a dual- or triple-wavelength fiber photometry system to excite GCaMP6s (*G-camp six S*) [1]. Set the signal channel to 470 nanometers and the control channel to 410 nanometers. Adjust the sampling rate to 60 frames per second [2].
 - 4.1.1. SCREEN: 411&412_screenshot_1_new.mp4 00:13-00:23.
 - 4.1.2. SCREEN: 411&412_screenshot_1_new.mp4 00:23-00:32.
- 4.2. Now, ground all recording system components properly [1]. Use a common median reference during recording. Verify that wide-band and local field potential signals are selected in the recording software while monitoring for noise throughout the recording session [2].
 - 4.2.1. Talent grounding the recording system components.
 - 4.2.2. SCREEN: 422&423_screenshot_2_new 00:04-00:11 and 00:13-00:20.

- 4.3. After anesthetizing the mouse briefly, connect the optrode to the recording line through an adaptor [1-TXT]. Place the mouse in a home-cage-like environment for recording [2]. Allow 10 minutes of acclimatization after awakening [3].
- 4.3.1. Talent connecting the optrode to the recording line through an adaptor. **TXT: Anesthesia: Isoflurane for up to 60 seconds** **NOTE: All shots in 4.3 was shot together**
- 4.3.2. Talent placing the mouse in a home-cage-like recording environment.
- 4.3.3. Shot of the mouse acclimatizing after awakening.
- 4.4. Record the mouse performing the grip strength test, requiring one pull per minute for 5 minutes [1]. Timestamp behavioral tests precisely in both multichannel and fiber photometry systems [2].
- 4.4.1. Talent recording while the mouse performs the grip strength test.
Videographer: Please record the action of the mouse and then the monitor.
- 4.4.2. ~~SCREEN:~~ Show synchronized timestamps in the multichannel and fiber photometry systems **Author's NOTE: 4.4.2 was shot by the videographer using camera**
Videographer's NOTE: 4.4.1–4.4.2(1–3) show different angles of the same step
- 4.5. Administer 1.5% Rose Bengal dye solution intraperitoneally at 10 to 20 milligrams per kilogram and wait for 5 minutes [1]. Connect the optrode probe to the laser source once deep anesthesia is achieved and maintained [2]. Deliver a 530-nanometer laser beam at 15 milliwatts through the implanted optrode for 5 to 8 minutes to induce focal ischemia [3].
- 4.5.1. Talent administering Rose Bengal dye solution intraperitoneally.
- 4.5.2. Talent connecting the optical probe to the laser source.
- 4.5.3. ~~SCREEN:~~ Talent delivering the laser beam through the implanted optrode.
NOTE: 4.5.3 was not on computer screen
4.5.2 & 4.5.3 were shot together
- 4.6. To perform optogenetic stimulation, connect the laser stimulator to the optrode. Turn on the 488-nanometer optogenetic laser stimulator and set it to 10 milliwatts for 5 minutes for opto vTRAP (*op-toe vee-trap*) [1]. Finally, place the mouse onto a heated pad for recovery [2].
- ~~4.6.2. SCREEN: Talent initiating the first post-stroke acute recording.~~
- 4.6.3. Talent turning on the stimulator for optogenetic manipulation. **NOTE: 4.6.3 was shot with the camera by the videographer, not recorded on screen**
- 4.6.1. Talent placing the mouse onto a heated pad. **NOTE: 4.6.1 should come after 4.6.3, VO adjusted**

Results

5. Results

- 5.1. Compared with baseline [1], cerebral blood flow declined after photothrombosis [2] and remained suppressed at 1 day [3] and 7 days post-stroke [4].

- 5.1.1. LAB MEDIA: Figure 2A, 2B. *Video editor: Highlight the “Baseline” image and bar.*
- 5.1.2. LAB MEDIA: Figure 2A, 2B. *Video editor: Highlight the “PST” image and “Post-stroke” bar.*
- 5.1.3. LAB MEDIA: Figure 2A, 2B. *Video editor: Highlight the “D1” image and bar.*
- 5.1.4. LAB MEDIA: Figure 2A, 2B. *Video editor: Highlight the “D7” image and bar.*

- 5.2. Forelimb grip strength showed a highly significant decline at 1 day [1] and remained suppressed at 7 days post-stroke [2].

- 5.2.1. LAB MEDIA: Figure 2D. *Video editor: Highlight the “D1” bar.*
- 5.2.2. LAB MEDIA: Figure 2D. *Video editor: Highlight the “D7” bar.*

- 5.3. The astrocytic calcium transients recorded by fiber photometry and neuronal spikes from electrophysiology were aligned based on timestamps [1].

- 5.3.1. LAB MEDIA: Figure 3.

- 5.4. Following photothrombotic stroke, firing rates were significantly suppressed in both pyramidal neurons [1] and interneurons [2] as compared to the baseline firing during the forelimb grip task [3].

- 5.4.1. LAB MEDIA: Figure 3CD. Video editor: Highlight the lower “Post” bar in C
- 5.4.2. LAB MEDIA: Figure 3CD. Video editor: Highlight the lower “Post” bar in D .
- 5.4.3. LAB MEDIA: Figure 3C, D. Video editor: Highlight the “Pre.” Bars in C and D

- 5.5. At baseline, the astrocytic calcium transients peaked within 4 to 6 seconds of task onset [1], but the amplitude was significantly reduced after photothrombotic stroke, reflecting weakened calcium activity [2].

- 5.5.1. LAB MEDIA: Figure 3E,F. Video editor: Highlight the “Pre” calcium trace peak around 4 to 6 seconds.

- 5.5.2. LAB MEDIA: Figure 3E,F. Video editor: Highlight the lower “Post” calcium curve trace in E and the lower “Post” bar in F.
- 5.6. Raster plots [1] and peri-stimulus time histograms showed changes in neuronal activity during optical stimulation [2].
- 5.6.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the vertical shaded area labeled “Light ON”, then show the dense short vertical lines before this region and compare them to the lines inside the shaded area for both the top blue rows and bottom red rows.*
- 5.6.2. LAB MEDIA: Figure 4B. *Video editor: Highlight the vertical shaded area labeled “Light ON,” then show the height of the bars before this region and compare them to the bars inside the shaded area for both the top blue graph and bottom red graph.*
- 5.7. Pyramidal neuron firing rate showed a significant decrease after optical stimulation compared with pre-stimulus baseline [1].
- 5.7.1. LAB MEDIA: Figure 4C. *Video editor: Highlight the shorter bar labeled “Post,” along with the dots above each bar showing individual points.*
- 5.8. Interneuron firing rate showed a significant increase after optical stimulation compared with pre-stimulus baseline [1].
- 5.8.1. LAB MEDIA: Figure 4D. *Video editor: Highlight the taller bar labeled “Post,” along with the dots above each bar showing individual points.*

1. Stereotaxic

Pronunciation link: https://www.merriam-webster.com/dictionary/stereotaxic
IPA: /ˌster.i.əʊˈtæks.ɪk/
Phonetic Spelling: steh·ree-oh-tak-sik

2. Anesthetized

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

IPA: /əˈnes.θəˌtaɪzd/

Phonetic Spelling: uh·ness·thuh·tyzd

3. Isoflurane

Pronunciation link: https://www.merriam-webster.com/medical/isoflurane

IPA: /ˌaɪ.sooˈflʊr.eɪn/

Phonetic Spelling: eye·soh·floor·ayn

4. Periosteum

Pronunciation link: https://www.merriam-webster.com/medical/periosteum

IPA: /ˌper.iˈɑːs.ti.əm/

Phonetic Spelling: peh·ree·oss·tee·uhm

5. Bregma

Pronunciation link: https://www.howtopronounce.com/bregma

IPA: /ˈbreg.mə/

Phonetic Spelling: breg·muh

6. Lambda

Pronunciation link: https://www.merriam-webster.com/dictionary/lambda

IPA: /ˈlæm.də/

Phonetic Spelling: lam·duh

7. Micropipette

Pronunciation link: https://www.merriam-webster.com/dictionary/micropipette

IPA: /ˌmaɪ.kroʊ.paiˈpet/

Phonetic Spelling: my·kroh·pie·pet

8. Vernier

Pronunciation link: https://www.merriam-webster.com/dictionary/vernier

IPA: /ˈvɜːni.ə/

Phonetic Spelling: vur·nee·ur

9. Adeno-associated

Pronunciation link: https://www.howtopronounce.com/adeno-associated

IPA: /əˌdiː.nəʊ əˈsoʊ.si.eɪ.tɪd/

Phonetic Spelling: uh·dee·noh uh·soh·see·ay·tuhd

10. Centrifuge

Pronunciation link: https://www.merriam-webster.com/dictionary/centrifuge

IPA: /ˈsen.trəˌfjuːdʒ/

Phonetic Spelling: sen·truh·fyooj

11. Craniotomy

Pronunciation link: https://www.merriam-webster.com/medical/craniotomy

IPA: /ˌkreɪ.niˈɑː.tə.mi/

Phonetic Spelling: kray·nee·aa·tuh·mee

12. Cerebellum

Pronunciation link: https://www.merriam-webster.com/dictionary/cerebellum

IPA: /ˌser.əˈbel.əm/

Phonetic Spelling: seh·ruh·bell·uhm

13. Agarose

Pronunciation link: https://www.merriam-webster.com/medical/agarose

IPA: /ˈæg.əˌroʊs/

Phonetic Spelling: ag·uh·rohs

14. Photometry

Pronunciation link: https://www.merriam-webster.com/dictionary/photometry

IPA: /foʊˈtɑː.mə.tri/

Phonetic Spelling: foh·taa·muh·tree

15. Acclimatization

Pronunciation link: https://www.merriam-webster.com/dictionary/acclimatization

IPA: /əˌklaɪ.mə.təˈzeɪ.ʃən/

Phonetic Spelling: uh·kly·muh·tuh·zay·shuhn

16. Intraperitoneally

Pronunciation link: https://www.howtopronounce.com/intraperitoneally

IPA: /ɪn.trəˌper.ə.təˈniː.ə.li/

Phonetic Spelling: in·truh·peh·ruh·tuh·nee·uh·lee

17. Ischemia

Pronunciation link: https://www.merriam-webster.com/medical/ischemia

IPA: /ɪˈskiː.mi.ə/

Phonetic Spelling: iss·kee·mee·uh

18. Photothrombosis

Pronunciation link: https://www.howtopronounce.com/photothrombosis

IPA: /ˌfoʊ.təʊ.θrɑːmˈboʊ.sɪs/

Phonetic Spelling: foh·toh·throm·boh·suhs

19. Astrocytic

Pronunciation link: https://www.howtopronounce.com/astrocytic

IPA: /ˌæs.troʊˈsaɪ.tɪk/

Phonetic Spelling: ass·troh·syeh·tik

20. Electrophysiology

Pronunciation link: https://www.merriam-webster.com/medical/electrophysiology

IPA: /ɪˌlek.troʊˌfɪz.iˈɑː.lə.dʒi/

Phonetic Spelling: ee·lek·troh·fiz·ee·aa·luh·jee

21. Interneurons

Pronunciation link: https://www.merriam-webster.com/medical/interneuron

IPA: /ˌɪn.təˈnɜr.ɑːn/

Phonetic Spelling: in·tur·nyur·on

22. Peri-stimulus

Pronunciation link: https://www.howtopronounce.com/peri-stimulus

IPA: /ˌper.iˈstɪm.jə.ləs/

Phonetic Spelling: peh·ree·stim·yuh·luhs

23. Optogenetic

Pronunciation link: https://www.howtopronounce.com/optogenetic

IPA: /ˌɑːp.təʊ.dʒəˈneɪ.ɪk/

Phonetic Spelling: op·toh·juh·net·ik

24. Optrode

Pronunciation link: https://www.howtopronounce.com/optrode

IPA: /ˈɑːp.troʊd/

Phonetic Spelling: op·trohd

25. Nanoliters

Pronunciation link: https://www.merriam-webster.com/dictionary/nanoliter

IPA: /ˈnæn.əʊ.liː.tər/

Phonetic Spelling: nan·oh·lee·tur