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# Multi-Site Optic Fiber Implants

DOI

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**Protocol status:** Working

**We use this protocol and it's working**

**Created:** July 15, 2023

**Last Modified:** February 19, 2025

**Protocol Integer ID:** 85034

**Keywords:** Fiber Optic, Fiber Photometry, Optic Fiber, Rodent Neurosurgery, Headpost, Optogenetics



## Abstract

This protocol describes the surgical procedure, instrumentation, and reagents necessary for implanting optic fiber probes involving injection(s) and headpost into an adult mouse brain for in-vivo fiber photometry and/or optogenetic manipulation procedures.

## Image Attribution

Gabriel Rodriguez, Allen Institute.

## Guidelines

Only perform this procedure in accordance with IACUC and veterinary requirements.

## Materials

### Anesthesia and other Drugs

⊗ Isoflurane **Patterson Veterinary Catalog #07-890-8115**

⊗ 1 g Dexamethasone **biorbyt Catalog #orb134330**

⊗ Ceftriaxone Injection **MWI Animal Health Catalog # 094311**

⊗ Lactated Ringers Injection, USP, Preservative-Free, Baxter **Henry Schein Animal Health Catalog #059380**

⊗ Ethiq X<sup>®</sup> Buprenorphine Extended-Release Injectable Suspension for Mice and Rats 1.3mg/mL, 3mL **Fidelis Pharmaceuticals Catalog #099114**

⊗ 1 g Atropine **biorbyt Catalog #orb322218**

⊗ 1 g Carprofen **biorbyt Catalog #orb321211**

#### Note

Drugs should only be administered in accordance with IACUC and veterinary requirements. Ensure timing, dosage, and route of administration are accounted for.

### Surgical Tools and Supplies

| A                               | B                           | C        |
|---------------------------------|-----------------------------|----------|
| Black handle scissors, ToughCut | Fine Science Tools          | 14058-11 |
| Scalpel handle                  | Fine Science Tools          | 10003-12 |
| Iris forceps                    | Fine Science Tools          | 11064-07 |
| Dumont #5 45° forceps           | Fine Science Tools          | 11251-35 |
| 45° Vanna scissors, 8cm         | World Precision Instruments | 500260   |
| 45° or 90° Durotomy probe       | Fine Science Tools          | 10066-15 |
| Plastic sterilization container | Fine Science Tools          | 20810-02 |
| Bulldog clamp                   | Fine Science Tools          | 18053-28 |

| A   | B                                   | C   |
|---|-------------------------------------|---|
| PREempt Disinfectant spray                          | McKesson Corporation                | 21101   |
| 70% Ethanol (Diluted in-house)                      | Sigma Aldrich                       | 459836  |
| Alcohol wipes                                       | Becton, Dickinson and Company       | 326895  |
| Sterile Surgical Drape, 18x26                       | Fisher Scientific                   | NC9517505   |
| Sterile Multi-well plate, 24 well                   | Advantor                            | 29443-952   |
| Nair hair removal cream                             | Arm & Hammer                        | 40002957  |
| Betadine Solution 10%                               | McKesson Corporation                | 1073829   |
| Hemostatic Agent Surgifoam                          | McKesson Corporation                | 403360  |
| Sterile Gauze, 3x3" squares, (autoclave sterilized) | Patterson Veterinary                | 07-893-8587   |
| Cotton swabs, double ended, (autoclave sterilized)  | Advantor                            | 89133-810   |
| Sugi pointed sterile swabs                          | Fine Science Tools                  | 18105-01  |
| Insulin syringes, U-100, 0.3 ml, 31G                | Advantor                            | BD328438  |
| Insulin syringes, U-100, 1 ml, 31G                  | Advantor                            | BD328418  |
| Luer-Lock Syringe, 20 ml OR                         | Advantor                            | 53548-025   |
| Luer-Lock Syringe, 10ml                             | Advantor                            | 75846-756   |
| 25G 5/8-inch needle                                 | Advantor                            | 89134-134   |
| 32 mm Syringe Filter 0.2 µm Supor Membrane          | Advantor                            | 75846-756   |
| Press 'n' Seal                                      | Medline                             | CLO70441  |
| Saran Wrap  | GLAD                                | Amazon B015CLAVU  |
| Sterile Drill Bits, 0.5/0.4, FG1/4 AND/OR           | NeoBurr                             | 1734948   |
| Sterile Drill Bits, 1.4/1.1, FG4 AND/OR             | NeoBurr                             | 1734214   |
| Sterile Drill Bits 1.0/4.2, EF4                     | NeoBurr                             | 1730012   |
| Sterile Scalpel blades, #10 OR                      | Advantor                            | 21909-378   |
| Sterile Scalpel blades, #11                         | Advantor                            | 21909-380   |
| Systane Eye Ointment                                | Systane                             | Amazon ALCON293787  |
| Artificial Cerebrospinal Fluid.V                    | Made in-house. Protocol referenced. | <a href="http://dx.doi.org/10.17504/protocols.io.besjjech">http://dx.doi.org/10.17504/protocols.io.besjjech</a> |
| C Universal 4-META Catalyst, 0.7 ml                 | Parkell                             | S371  |
| B Quick Base for MetaBond, 10 ml                    | Parkell                             | S398  |
| Radiopaque L-Powder, white, 5 gm                    | Parkell                             | S396  |
| Radiopaque L-Powder, clear 3 gm                     | Parkell                             | S399  |

| A  | B                                 | C                               |
|--|-----------------------------------|---------------------------------|
| Silicone implant coating, SORTA-Clear 18   | Renolds Advanced Materials        | SORTA-Clear 18                  |
| Loctite 4305   | Henkel                            | 303389                          |
| XLite LED Curing light   | Independent Dental                | Flight Xlite2-CUR               |
| Vetbond Glue   | Patterson Veterinary              | 07-805-5031                     |
| Superglue, Singles   | Krazy Glue                        | Amazon PK4 KG58248SN            |
| 3 ml transfer pipette, plastic   | Avantor                           | 52947-970                       |
| Ortho-Jet BCA Liquid   | Lang Dental Manufacturing Company | Ortho-Jet BCA Liquid            |
| Black cement (1) = 4 parts of Ortho-Jet BCA Powder (mixture) AND                   | Lang Dental Manufacturing Company | Ortho-Jet BCA Liquid            |
| Black cement (2) = 1 part of Powder tempura point, black                           | Jack Richeson & Co                | 1 # Black 62, Amazon B00JGZ8Q1A |
| Kwik-Cast Sealant  | World Precision Instruments       | KWIK-CAST                       |
| Heat-sterilized Glass pipettes AND/OR  | Drummond Scientific               | 3-000-203-G/X                   |
| Heat-sterilized Glass pipettes   | World Precision Instruments       | 1B120F-4                        |
| "Marker" glass pipette, pulled, broken, and Sharpie mark for measuring coordinates | World Precision Instruments       | 1B120F-4                        |
| Microcapillary Pipette tips  | Eppendorf                         | 89009-310                       |
| Parafilm   | Advantor                          | 52858-000                       |
| Lightweight Mineral Oil  | Sigma-Aldrich                     | M8410                           |
| 30 gauge, 2" Backfilling Needle  | Drummond Scientific               | 3-000-027                       |
| Sterile Bone Wax   | Central Infusion Alliance, Lukens | CIA2160287, 901                 |
| 5-0 Monofilament suture with 17mm 1/2C taper needle attached                       | Penn Veterinary Supply            | Monomend MT                     |
| Sterilization pouches  | Advantor                          | 89140-804                       |
| Fiber Optic Cannulae, 200 um fiber core diameter, Black ceramic ferrule            | Neurophotometrics                 | FOC_BF_200um/1.2 5mm            |

***All tools / supplies can be substituted with their equivalent.***

**Key:**

AND = Including the tool/supply in row below.

OR = Can use tool/supply in row below instead.

Autoclaved sterilized = Sterilized in-house.

mixture = Mix with tool/supply in row below.

**Artificial Cerebrospinal Fluid.V**

## Equipment

| Equipment  | Manufacturer / Supplier | Part Number            |
|--|-------------------------|------------------------|
| Small Animal Stereotaxic Instrument                              | Kopf                    | 1900                   |
| Adjustable Stage Platform  | Kopf                    | 901                    |
| Stereo Microscope  | Lecia                   | M80                    |
| Gooseneck Illumination   | AM Scope                | LED-6WA                |
| On-axis Illumination   | Lecia                   | KL2500 LED             |
| Bead sterilizer  | Sigma-Aldrich           | Z378585                |
| Small Animal Temperature Control System                          | CWE Inc.                | TC-1000                |
| Large Heat plate/pad   | Lectro-Kennel           | Outdoor Heated Pet Pad |
| Dental Drill   | NSK                     | Pana-Max2 M4           |
| Oxygen Concentrator  | Nidek Medical Products  | Nuvo Lite Model 525    |
| Isoflurane with oxygen delivery system                           | Patterson Scientific    | Tec 3 EX               |
| Isoflurane induction chamber                                     | Patterson Scientific    | 78933385               |
| Ear bars   | Kopf                    | 1922                   |
| Ultra Fine Point Sharpie   | Sharpie                 | 37001                  |
| Metabond ceramic mixing dish                                     | Parkell                 | S387                   |
| Stylus Pro USB UV Penlight                                       | Streamlight             | 66149                  |
| Electrode Holder   | Kopf                    | 1970                   |
| Stereotaxic Cannula Holder                                       | Inper                   | -                      |
| Galaxy Mini Centrifuge   | Avantor                 | 76269-066              |
| P20 Pipettor   | Gilson                  | F123600                |
| Silver wire  | Stoelting               | 50880                  |
| Midgard Precision Current Source                                 | Stoelting               | 51595                  |
| Nanoject II Variable Volume (2.3 to 69 nL) Automatic Injector OR | Drummond Scientific     | 3-000-204              |
| Nanoject III Programmable Nanoliter Injector                     | Drummond Scientific     | 3-000-207              |

***All equipment can be substituted with their equivalent.***

### Key:

OR = Can use equipment in row below instead.

**Materials/Equipment designed/made in-house (CAD available upon request):**

| Material                           | Part Number   |
|------------------------------------|---------------|
| Titanium 42 Headpost *             | 0160-100-42   |
| Titanium Al Straight Bar *         | 1365-6428-001 |
| Titanium Headpost *                | 0160-100-10   |
| Titanium LC / Brainstem Headpost * | 0160-100-52   |
| Dual Hemisphere Headframe *        | 0160-100-57   |
| Bregma Stylus                      | 0251-900-04   |
| Lambda Stylus                      | 0111-300-01   |
| Dovetail Clamp                     | 0111-200-00   |
| Ear bar Headframe Clamp            | 0155-100-00   |

***All equipment can be substituted with their equivalent.***

\* = Optional

**Personal Protective Equipment (PPE):**

| Suggested PPE                       |
|-------------------------------------|
| Gloves                              |
| Disposable lab coat                 |
| Disposable face mask                |
| Shoe covers / surgery shoes         |
| Scrubs                              |
| Surgical cap                        |
| Biohazard sharps disposal container |
| Biohazard waste disposal container  |
| Blue light blocking glasses         |

Utilize PPE in accordance with IACUC and veterinary requirements. Ensure sterility when necessary.

## Safety warnings

- !
  - Personal Protective Equipment (PPE) should be used at all times while operating this protocol.
  - Isoflurane Warning: Acute over-exposure to waste anesthetic gases (WAG) may cause eye irritation, headache, nausea, drowsiness or dizziness. Repeated exposure may cause damage to cardiovascular system and central nervous system. Refer to MSDS for additional information. Consult the surgical workstation guide to ensure all parts of the dispensation rig are functioning properly.
  - Blue-light filter safety goggles must be worn while using LED curing light.

## Ethics statement

Research focused rodent neurosurgery must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s).

This protocol has been approved by the Allen Institute Animal Care and Use Committee (IACUC).


PHS Assurance : D16-00781

AAALAC : Unit 1854

## Before start

Reference protocol below for all general setup and takedown procedures for rodent neurosurgery:

Protocol



NAME

**General Setup and Takedown Procedures for Rodent Neurosurgery**

CREATED BY

Ali Williford

**PREVIEW**

Reference protocol(s) below if performing injections:

**Stereotaxic Surgery for Delivery of Tracers by Iontophoresis V.6:** [dx.doi.org/10.17504/protocols.io.14egn8ewzg5d/v6](https://dx.doi.org/10.17504/protocols.io.14egn8ewzg5d/v6)

**Stereotaxic Injection by Nanoject Protocol V.6:** [dx.doi.org/10.17504/protocols.io.bp2l6nr7kgqe/v6](https://dx.doi.org/10.17504/protocols.io.bp2l6nr7kgqe/v6)



## Expose and Prepare the Skull Surface for Fiber Implants

5m

- 1 After hair removal and disinfection, create a midline incision with a scalpel blade from approximately behind the eyes to the front of the ears.

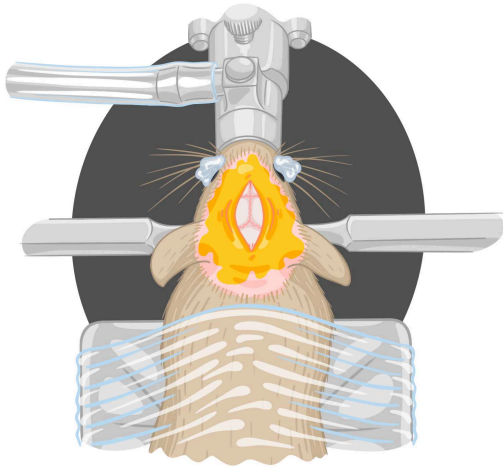


Illustration of initial skin incision on mouse head.  
Mouse fixed to stereotax via bite bar and ear bars. Fur from top of head has been removed and skin has been sterilized (see General Setup and Takedown Procedures).

- 2 Using Vanna scissors, cut a teardrop shape of skin away, ensuring enough skull is exposed for implants.

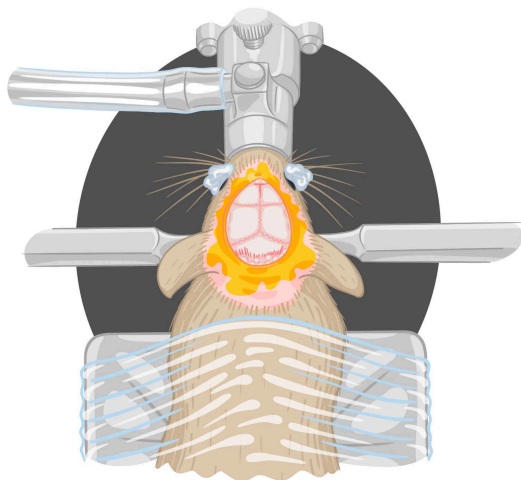


Illustration of skin removal on mouse head.

- 3 Remove exposed periosteum by rubbing it apart with cotton swabs and bunching it near the edges of the skin. Then cut away with Vanna scissors at the skin edge. Use ACSF to rehydrate if necessary (i.e. periosteum dries out).

#### Note

Utilize a 10mL or 20mL syringe, 25G 5/8-inch needle, and a syringe filter to store and dispense ACSF.

- 4 Seal all along incision site with Vetbond. Use a Sugi Absorption Spear to absorb any excess fluid either prior or during. Extend Vetbond seal 1-2mm past incision site along undamaged skin.

#### Note

Extending the Vetbond seal over healthy tissue delays the formation of exudate. Additionally, unsealed soft tissue will weep fluid that can compromise the attachment of the headframe.

## Align the Skull

**5m**

- 5 Locate Bregma and Lambda landmarks with Dovetail Clamp and Bregma Stylus, and use them to level the skull in the anterior-posterior axis within 0.1mm.

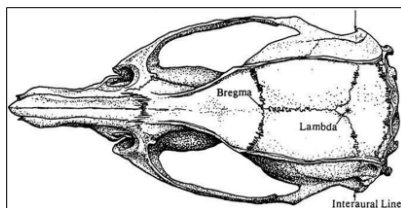


Illustration of Bregma and Lambda landmarks.

- 5.1 If Lambda-Bregma offset is greater than 0.1mm in X, use the yaw adjustment on the stereotaxic alignment system to adjust the yaw within 0.1mm.



- 6 At midline, approximately midway between Bregma and Lambda, measure 2mm laterally on both the left and right hemisphere, ensuring that the skull is level in the medial-lateral axis within 0.15mm.

## Perform Stereotaxic Injections

15m

- 7 Skip to section "Fiber implantation" if not performing virus injections.
- 8 Reference protocol **Stereotaxic Injection by Nanoject Protocol V.6** for performing stereotaxic injections via nanoject:  
[dx.doi.org/10.17504/protocols.io.bp2l6nr7kgqe/v6](https://dx.doi.org/10.17504/protocols.io.bp2l6nr7kgqe/v6)

Begin at step **8.2.6 "Mark the Injection Site"**

Stop at step **8.5 "Suturing"**

### Note

While drilling the burr hole, ensure that the crack/hole in the skull will be wide enough for an optic fiber to pass through. Fibers typically have a  $\pm 0.2$  mm diameter.

### Note

If target coordinates require an angled injection, rotate mouse before proceeding with the injection.

- 9 Reference protocol **Stereotaxic Surgery for Delivery of Tracers by Iontophoresis V.6** for performing stereotaxic injections via Iontophoresis:  
[dx.doi.org/10.17504/protocols.io.14egn8ewzg5d/v6](https://dx.doi.org/10.17504/protocols.io.14egn8ewzg5d/v6)

Begin at step **8.2.6 "Mark the Injection Site"**

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### Note

While drilling the burr hole, ensure that the crack/hole in the skull will be wide enough for an optic fiber to pass through. Fibers typically have a  $\pm 0.2$  mm diameter.

**Note**

If target coordinates require an angled injection, rotate mouse before proceeding with the injection.

## Fiber Implantation

7m

- 10 Use forceps to disinfect optic fiber implant by dipping it in a 70% ethanol well. Rinse twice by dipping it into two wells of ACSF.
- 11 If not using the same burr hole as the one drilled for virus injection, drill a burr hole for optic fiber implant.
  - 11.1 Fibers will have a  $\pm 0.2$  mm diameter. In order for the fiber to pass through the burr hole, ensure that the skull is thin enough to be picked away at this width.

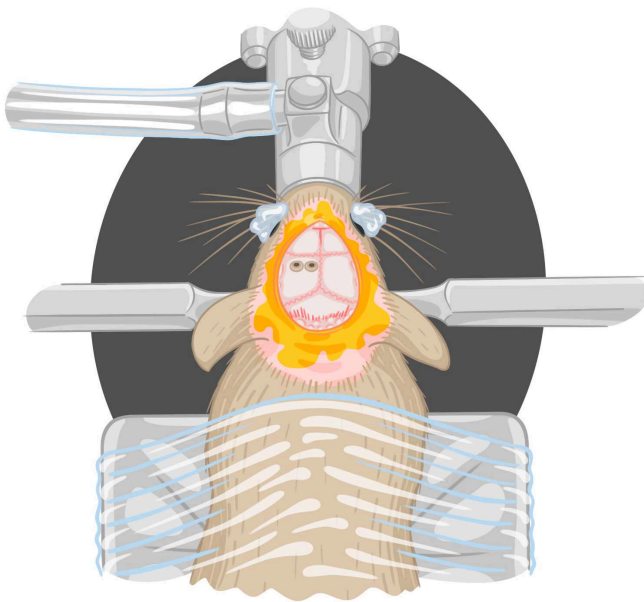


Illustration of two burr holes drilled onto mouse skull.

- 12 Place fiber in the stereotaxic cannula holder.

**Note**


Do not tighten ferrule clamp too tightly, as you will need to avoid using excessive force to untighten.




Illustration of optic fiber in stereotaxic cannula holder.

- 13 If target coordinates require an angled fiber, rotate mouse using stereotaxic controls.
- 14 Position fiber above the pre-drilled burr hole at the desired coordinates and angle for implantation
  - 14.1 If burr hole is not wide enough to allow optic fiber implant to pass through, peel away excess bone using a microprobe, 1 mL insulin syringe, or fine forceps.
- 15 Lower the tip of the optic fiber implant to the brain surface and zero the Z coordinate.
  - 15.1 Ensure that the skull thickness will allow the optic fiber implant to be lowered to desired depth. To do this, measure distance from the dorsal surface of skull to the pia surface, then subtract the measurement from optic fiber length.
- 16 Slowly lower the tip of the optic fiber implant to the designated coordinate.

17 With the optic fiber implant still in the stereotaxic holder, apply several drops of Loctite 4305 light curing glue to the base of the fiber ferrule.

17.1 Glue should not cover more than roughly 1/3 of the ferrule. Most ferrules will have a notch  1 mm from the base. Use this notch as a reference for glue application.



18 Flood area with blue light using the LED curing light until optical glue is hardened. This will take roughly  00:01:00 minutes.

1m

#### Safety information

Use protective blue light blocking eyewear during this step.

19 Carefully release the ferrule from the stereotaxic fiber holder.

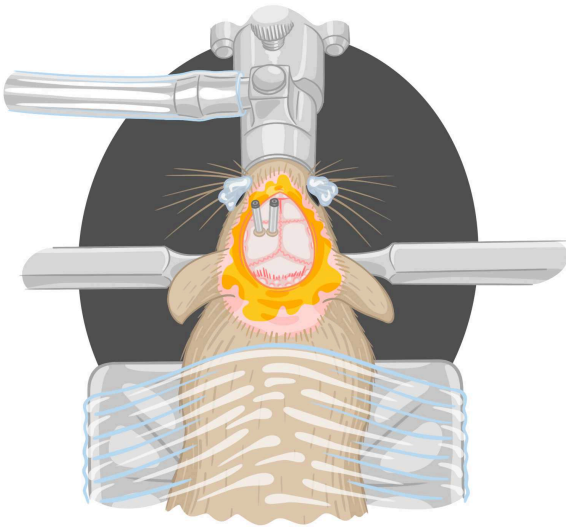




Illustration of optic fiber implants prior to being covered with metabond and black cement.

20 Repeat steps  [go to step #11](#) through  [go to step #19](#) for all desired fiber optic implants.

## Headpost Placement

15m



21 Skip headpost placement if not including headpost.



22 Attach Dovetail Clamp to stereotaxic arm and attach stylus. Center stylus over headframe fiducial point and then raise stylus slightly.


#### Note

Depending on the headpost's fiducial point, use either a Lambda or Bregma stylus.

23 Replace stylus with the appropriate custom headframe and lower the headframe until there is contact with the skull.

#### Note

Depending on the coordinates of the optic fiber implants, headframe and/or headframe placement may vary. Adjust as necessary.

24 Secure the headframe with metabond. Cover all exposed skull with metabond and place enough metabond around the metal of the headframe and base of fiber ferrules to secure them in place. Confirm that metabond does not cover the top  $\pm 3.5$  mm of the ferrule. Let dry completely (around  00:05:00 minutes).

5m

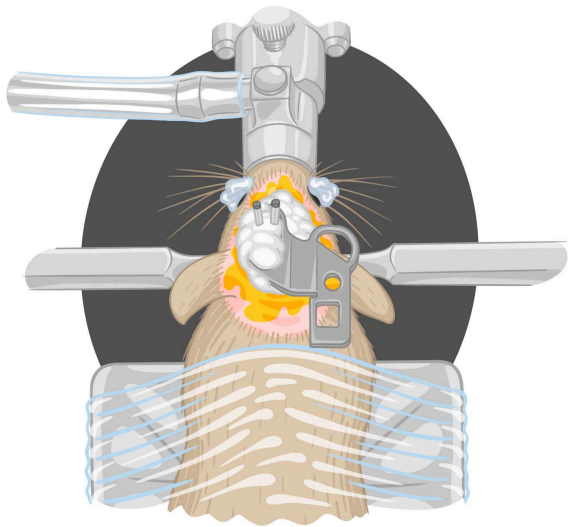



Illustration of metabond placement around the head frame and optic fiber ferrules.

Note: The headframe should be attached to the stereotax during this step. This has been removed for illustration purposes.

25 Cover metabond with black cement.

25.1 Add equal parts Jet Fast Curing Acrylic Resin Liquid and black cement powder to an empty well in the 24 well plate.

25.2 Use the broken end of a swab to layer the black cement. Confirm that black cement does not cover the top  $\rightarrow$  3.5 mm of the ferrule. Let dry completely (around  00:05:00 minutes).

5m

#### Note

Work quickly as black cement hardens rapidly (around  00:02:00 ).



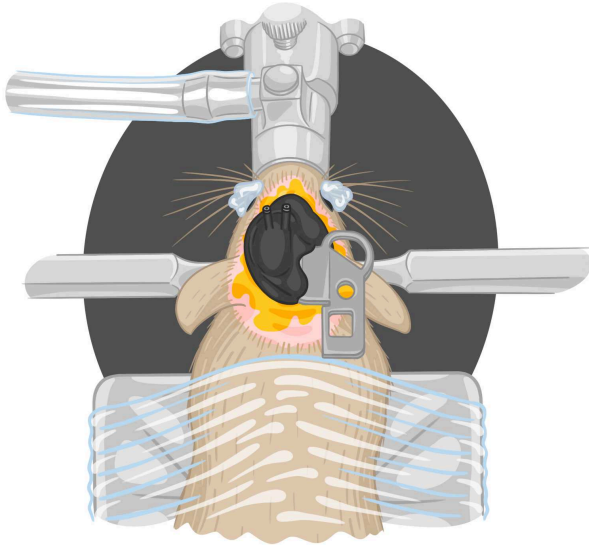


Illustration of black cement placement around head frame and optic fiber ferrules.

Note: The headframe should be attached to the stereotax during this step. This has been removed for illustration purposes.

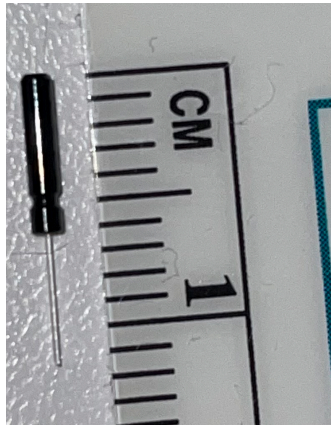
- 26 Ensure that the metabond and black cement do not cover the top  $\pm 3.5$  mm of the ferrule, as it interferes with experimentation. Use the broken end of a dry swab to push down/remove any excess metabond or black cement.



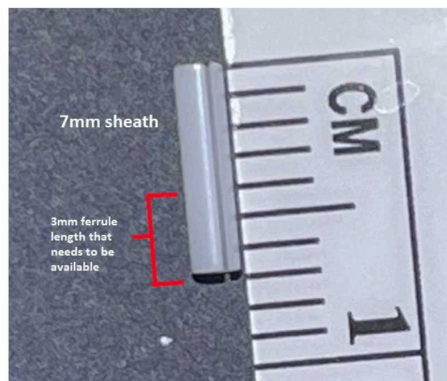
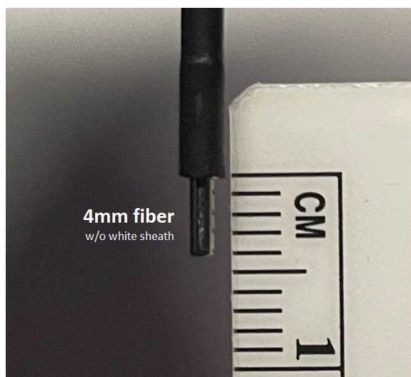
#### Note

Too little metabond can lead to instability and motion artifacts within fiber photometry data.

Too much metabond can cause light leak when LED is not flush with fiber implant (as shown below).



Optic fiber to ferrule length comparison.



Optic fiber cable tip length compared to sheath length.

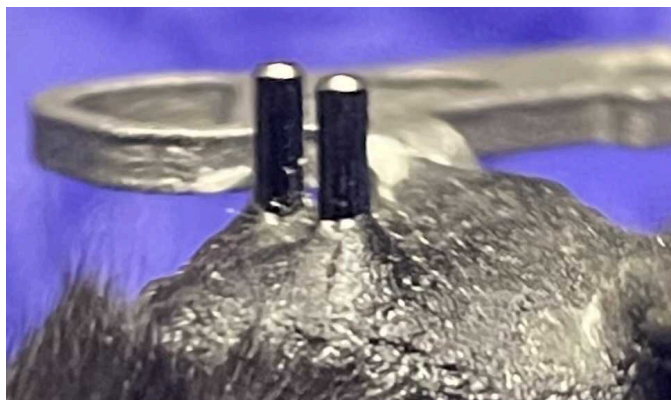



Image of adequate metabond+black cement built up on fiber ferrule.



Example of too much metabond causing LED light to leak.

- 27 Remove mouse from stereotax once metabond and black cement are fully dry.
- 27.1 Detach Dovetail Clamp from headframe carefully.
- 27.2 Remove earbars.
- 27.3 Remove mouse teeth from bitebar by lightly scruffing mouse in a way that raises the teeth out of the bite bar.

## Recover Mouse and Takedown

- 28 Obtain the mouse's postoperative weight.
- 29 Place the mouse back in a recovery cage and put the cage on the  37 °C heat plate.
- 30 Reference **General Setup and Takedown Procedures for Rodent Neurosurgery** protocol for takedown procedures.