Feb 19, 2025

Multi-Site Optic Fiber Implants

DOI
dx.doi.org/10.17504/protocols.io.6qpvr3dqovmk/v1

Avalon Amaya[1], Kenta M. Hagihara[1], Benjamin Ouellette[1], Conor Grasso[1]
[1]Allen Institute for Neural Dynamics

Allen Institute for Neural...

Avalon Amaya
Allen Institute for Neural Dynamics

DOI: dx.doi.org/10.17504/protocols.io.6qpvr3dqovmk/v1

Protocol Citation: Avalon Amaya, Kenta M. Hagihara, Benjamin Ouellette, Conor Grasso 2025. Multi-Site Optic Fiber Implants. **protocols.io https://dx.doi.org/10.17504/protocols.io.6qpvr3dqovmk/v1**

Protocol status: Working
We use this protocol and it's working

Created: July 14, 2023

Last Modified: February 19, 2025

Protocol Integer ID: 85034

Keywords: Fiber Optic, Fiber Photometry, Optic Fiber, Rodent Neurosurgey, 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

Ethiqa XR 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
Alchohol wipes	Becton, Dickinson and Company	326895
Sterile Surgical Drape, 18×26	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, 3×3" 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.	http://dx.doi.org/10.17504/protocols.io.besjjecn
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 Maufacturing 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/1.25mm

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

Stereotaxic Injection by Nanoject Protocol V.6: 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.

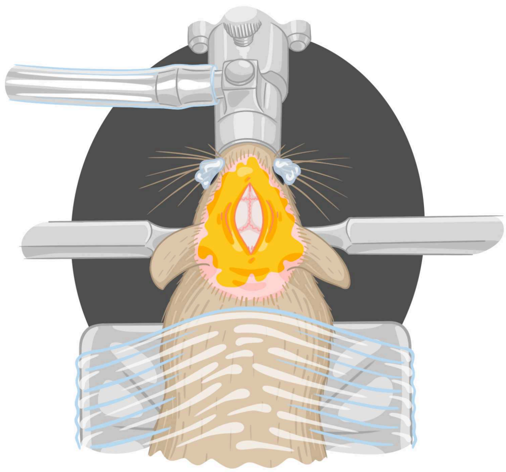


Illustration of initial skin incision on mouse head.
Mouse fixed to stereotax via bite bar and ear bars. Fur from top of head has been removed and skin has been sterilized (see General Setup and Takedown Procedures).

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

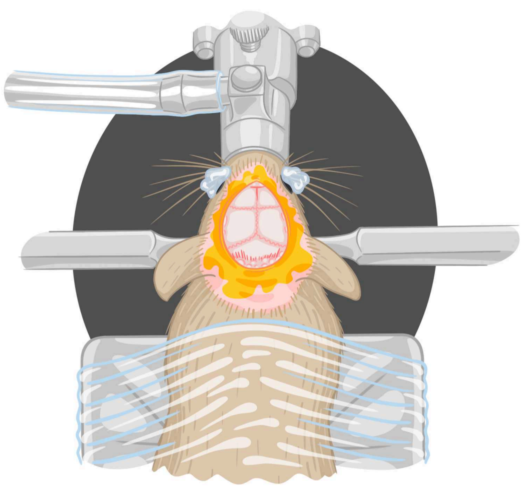


Illustration of skin removal on mouse head.

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.

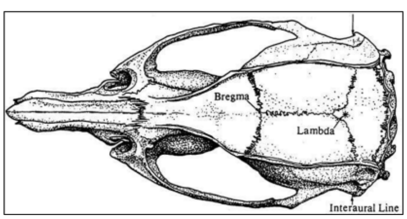


Illustration of Bregma and Lambda landmarks.

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

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 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

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 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.

Fiber Implantation

7m

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 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.

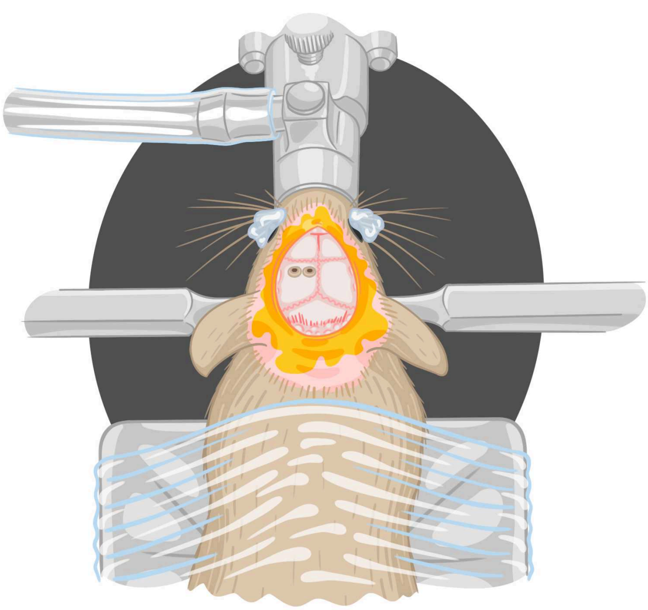


Illustration of two burr holes drilled onto mouse skull.

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.

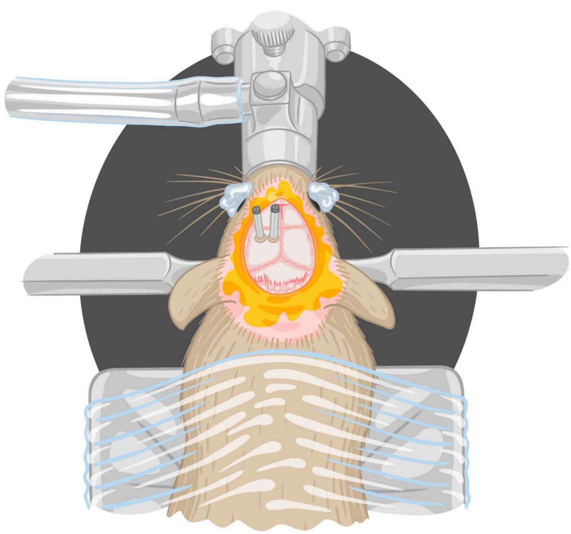


Illustration of optic fiber implants prior to being covered with metabond and black cement.

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 3.5 mm of the ferrule. Let dry completely (around 00:05:00 minutes).

5m

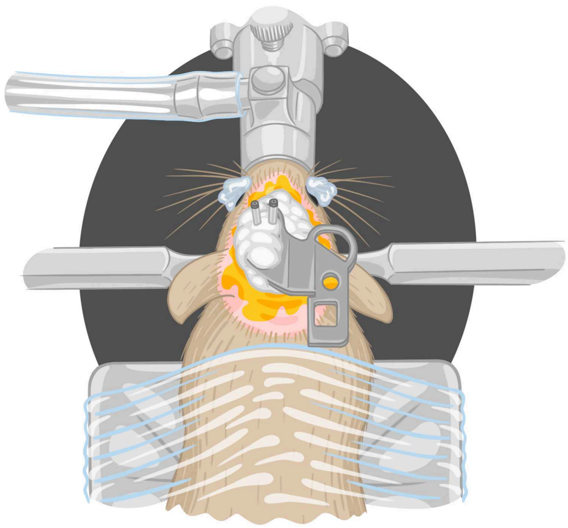


Illustration of metabond placement around the head frame and optic fiber ferrules.
Note: The headframe should be attached to the stereotax during this step. This has been removed for illustration purposes.

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 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).

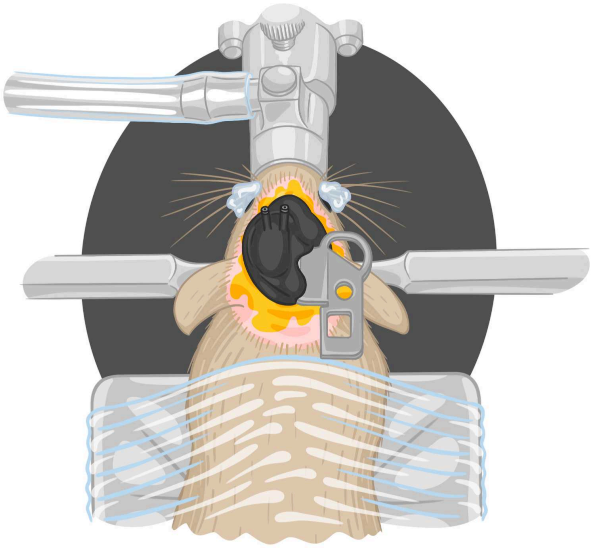


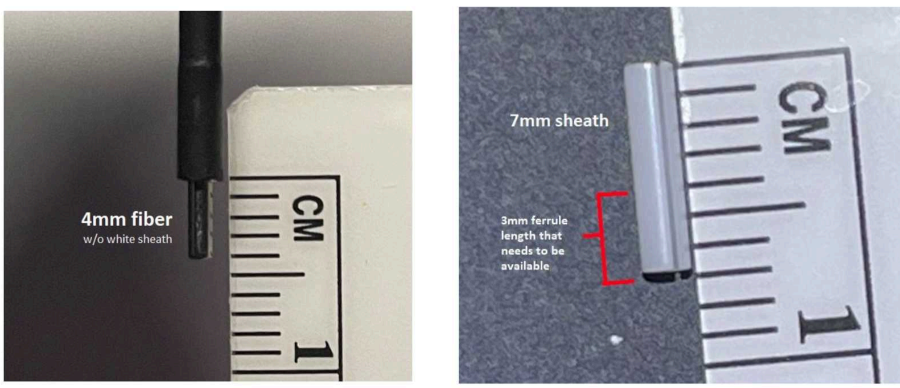
Illustration of black cement placement around head frame and optic fiber ferrules.
Note: The headframe should be attached to the stereotax during this step. This has been removed for illustration purposes.

26 Ensure that the metabond and black cement do not cover the top 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.

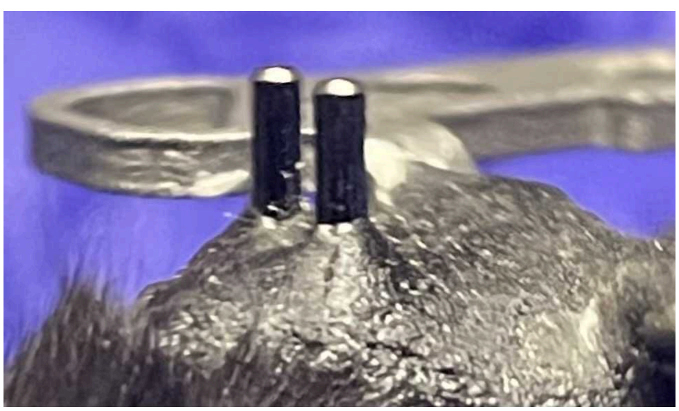
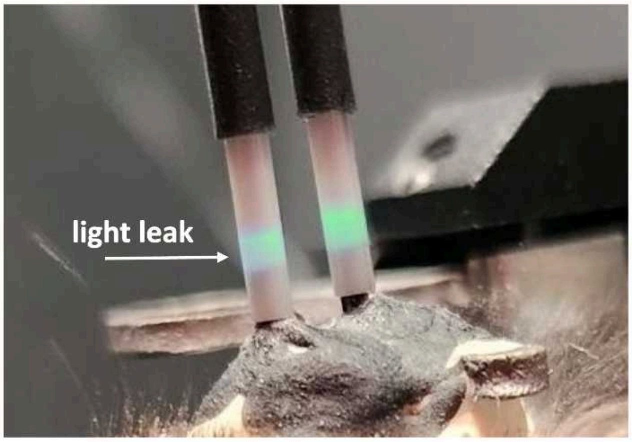


Image of adequate metabond+black cement built up on fiber ferrule.



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.