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Imaging Cannula Implantation & Microscope Installation for 2CFM

Application Note

Version 1.2.0

Contents

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Microscope Model Specifications

There are several types of microscopes sharing identical implantation methods, such as the *2CFM-S* and *2CFM-L*. This document describes how to install and implant these miniature fluorescence microscopes and their accessories on an animal subject.

1.1 Fluorescence Microscope Models

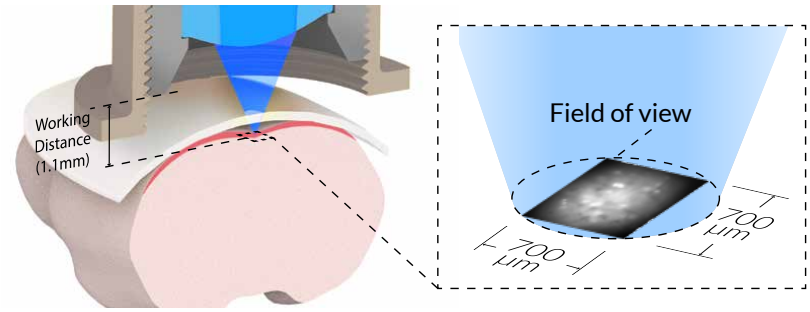
The depth of the region-of-interest determines the choice of microscope body and imaging cannula. For brain regions up to 8.3 mm in depth, the *Model-L Cannula* is implanted within the brain, while the *Model-L Microscope* allows imagery of brain tissue at these locations. The Model-S Microscope is preferred for surface observation as it is optimized for a field of view between 0 and 150 µm below the brain surface (Fig. 1.1b).

(a) *2-color Model-L* (b) *2-color Model-S*

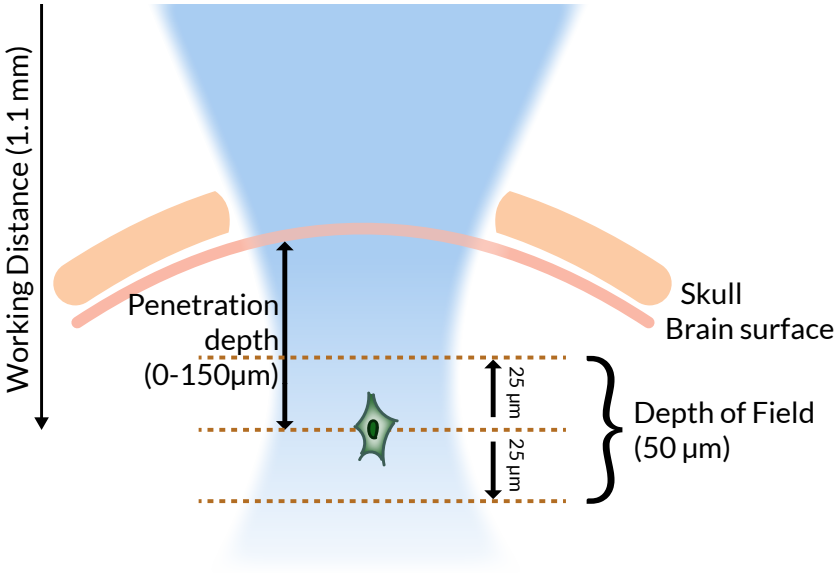
Figure 1.1: *Miniature Fluorescence Microscopes In Exploded Format*

1.1.1 Model-S Cannula

For the *Model-S Cannula* used with the *S-Type Two-color Fluorescence Microscope*, the field of view obtained is 700 µm X 700 µm (Fig. 1.2a) and the depth of field (focus range) is 50 ± 25 µm (Fig. 1.2b). The working distance is 1.1 mm and defined as the distance from the bottom of the metal part of the cannula to the focal plane. Due to light scattering and absorption in the brain, the penetration depth for imagery is typically of 0-150 µm. For the *Model-S Cannula*, a single **Protrusion Adjustment Ring** (height of 4.5 mm) is required to observe the full range.



(a) *Field of View*



(b) *Depth Range*

Figure 1.2: *Model-S Characteristics*

1.1.2 Model-L Cannula

For deeper brain regions, a gradient-index rod lens is needed to guide the image from inside of the brain to the microscope body objective lens. The information in this section applies specifically to the *Snap-in Imaging Cannulas Model L*. Depending on the depth of the region of interest, two types of imaging cannulas are available (LD or LV) with different rod lens lengths. Table 1.1 gives the range of penetration depths obtained with each cannula type. The penetration depth for this model is measured from the surface of the skull or the bottom of the protrusion adjustment ring to the region of interest.

To ensure that the *Twist-on efocus Imaging Cannula Model L* is well secured on the skull, it is recommended to combine the imaging cannula with a *Protrusion Adjustment Ring*. To select the right protrusion ring, follow these steps.

[1] including a working distance of 80 µm

Table 1.1: *Range of Penetration Depth For Rod Lens*

Cannula type	Range of penetration depths *d* (mm) [1]
LD	0 - 3.4
LV	2.8 - 5.9
LE	5.3 - 8.3

1. Measure the protrusion length (L) (Fig. 1.3) of the lens from the bottom of the metal part of the cannula to the extremity of the lens.
2. Determine at which penetration depth you want to position the extremity of the lens in the subject's brain. Add to this measure the thickness of the skull and the working distance of the GRIN rod lens (80 µm). To ensure that the Protrusion Adjustment Ring will not be too low, it is recommended to add 100 µm to this measure. You will obtain the protrusion distance (d) (Fig. 1.3) representing the distance from the tip of the lens to the bottom of the Protrusion Adjustment Ring. (d = penetration depth + skull thickness + working distance$(80\ \mu m) + 100\ \mu m$).
3. The protrusion of the ring (p) (Fig. 1.3) is given by : $p = L - d$ and can be related to the Protrusion Adjustment Ring with closest matching protrusion length (Table 1.2). Each ring is identified by its height (h) (Fig. 1.3).
4. Place the selected Protrusion Adjustment Ring on the cannula and adjust the position of the ring to obtain the required protrusion distance (d).

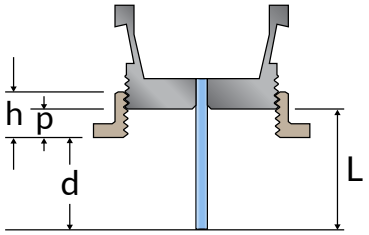


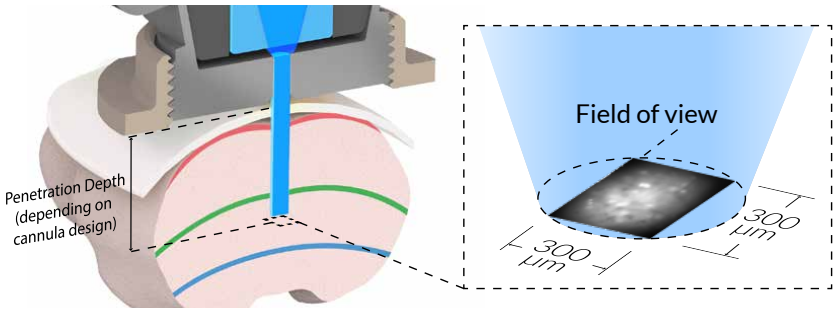
Figure 1.3: *Protrusion Adjustment Ring Parameters*

Table 1.2: *Protrusion Adjustment Ring Model L Selection*

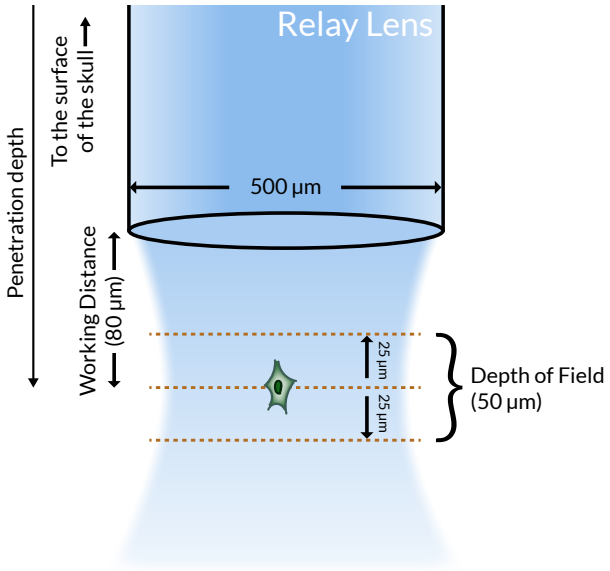
Ring #	Height (h) (mm)	Protrusion Length (p) (mm)
1	2.0	0 - 1.5
2	2.7	0.7 - 2.2
3	3.4	1.4 - 3.0
4	4.2	2.2 - 3.7
5	4.9	2.9 - 4.4

The working distance of our standard model-L imaging cannulas is 80 µm and represents the distance from the extremity of the rod lens to the focal plane[2]. It means that the microscope does not image immediately underneath the lens, and this working distance must be considered when calculating the required depth. The field of view of the two-color model-L microscope is 300 µm X 300 µm (Fig. 1.4a), and the depth of field is 50 µm (Fig. 1.4b).

[2]Cannulas with different working distances are offered on request



(a) *Field of View*



(b) *Depth Range*

Figure 1.4: *Model-L Characteristics*

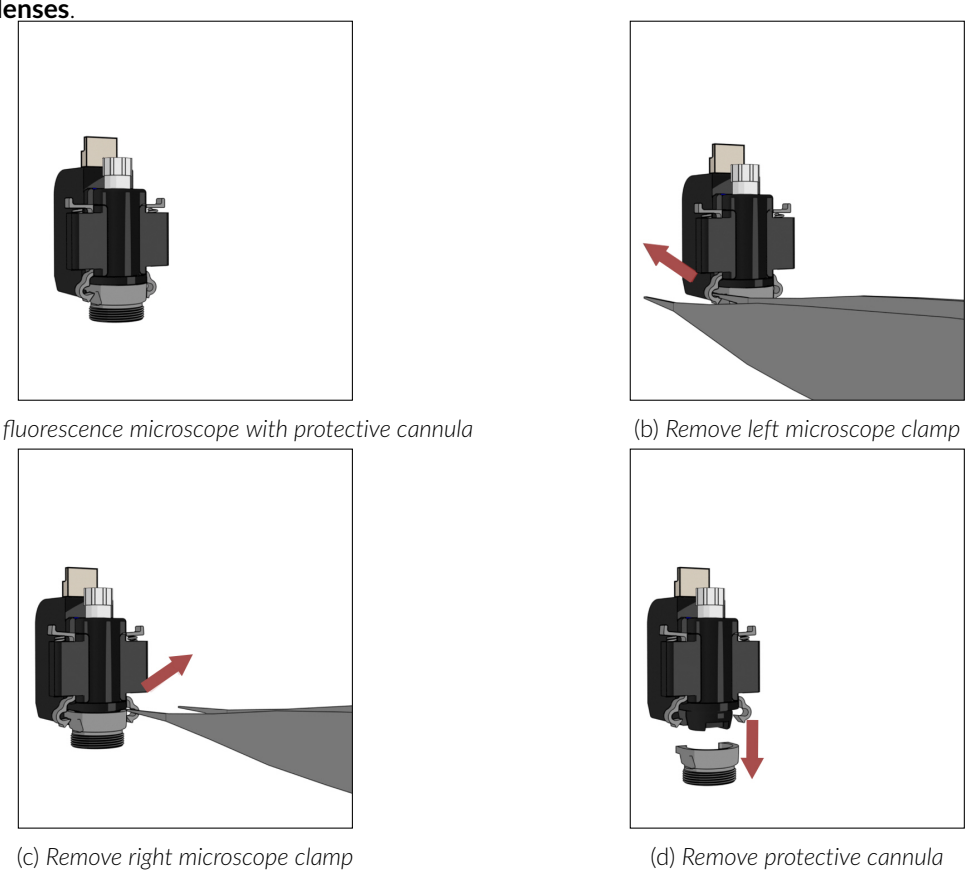
2

Implantation of the Imaging Cannula

The following section describes the use and implantation of the 2CFM-L and 2CFM-S microscope bodies and related cannulas. The 2CFM-L is used as an example in the figures of the following section.

2.1 Snap-in Cannula Removal and Installation

The microscope body is delivered with a protective cannula that should be removed for the first use and re-installed after each imaging session to protect the microscope. To remove the protective cannula and secure the imaging cannula on the microscope body, follow the procedure described in this section. Handle the microscope and cannula with care. The relay lens and objective are fragile and any stain or scratch can affect image quality. **Do not touch the surface of the lenses**.



(a) *2-color fluorescence microscope with protective cannula*

(b) *Remove left microscope clamp*

(c) *Remove right microscope clamp*

(d) *Remove protective cannula*

Figure 2.1: *Cannula Removal Procedure*

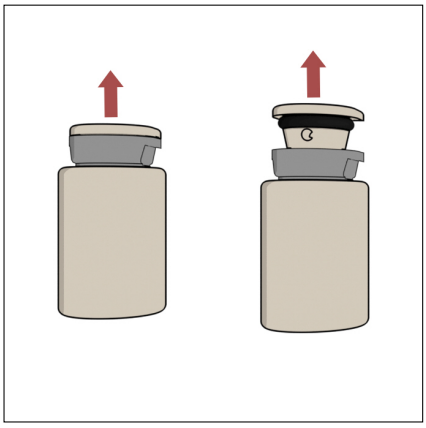
1. Remove the **Protective Cannula** from the *Microscope Body* (Fig. 2.1). Any other 2-color microscope cannula can be removed in the same way.

a) The microscope is provided with a protective cannula (Fig. 2.1a)

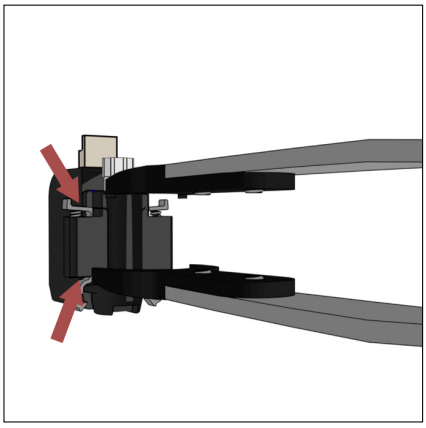
b) Using the *Microscope Snap-off Tool*, remove the left **Microscope Clamp** from the **Cannula Clamp Groove** (Fig. 2.1b).

c) Remove the right **Microscope Clamp** from the **Cannula Clamp Groove** (Fig. 2.1c).

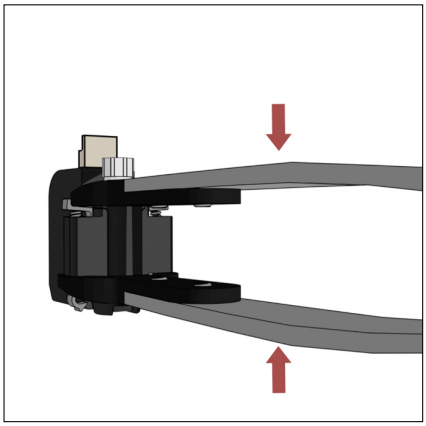
d) Remove the **Protective Cannula** (Fig. 2.1d).



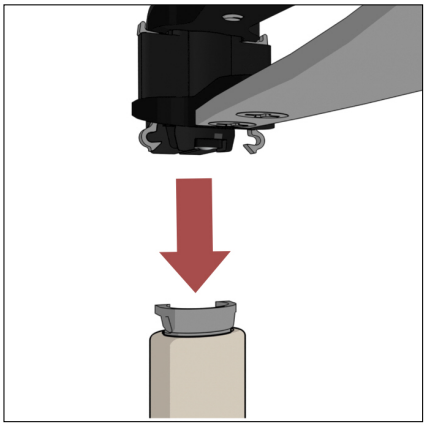
(a) *Remove the input protective cap from the Imaging Cannula*



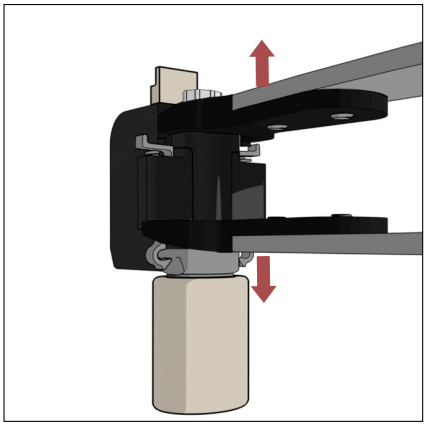
(b) *Place the snap-on tool in the appropriate position*



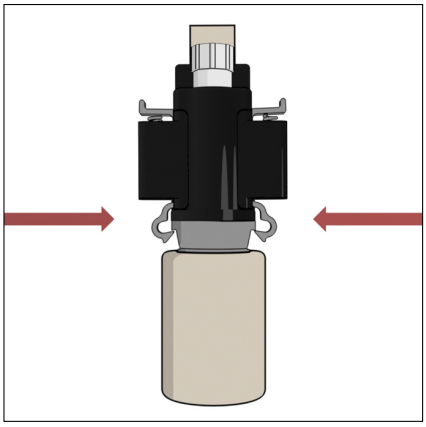
(c) *Close the snap-on tool*



(d) *Place the microscope on the imaging cannula*



(e) *Open the snap-on tool to close the microscope clamps*



(f) *Verify microscope clamp closure*

Figure 2.2: *Cannula Installation Procedure*

2. Secure the imaging cannula on the microscope body (Fig. 2.2). Any other 2-color microscope cannula can be installed in the same way.

   a) Remove the **Input Protective Cap** from the *Imaging Cannula* (Fig. 2.2a).

b) The **Snap-on Tool** has two unique tines, each of a different size. Take the smaller tine and place it on the clamps. The larger tines are placed under the barrel (Fig. 2.2b). Each tine has a small tooth that is used to prevent the microscope from moving; ensure they are properly in place.

c) Press the **Snap-on Tool** to close the tines and open the clamps (Fig. 2.2c).

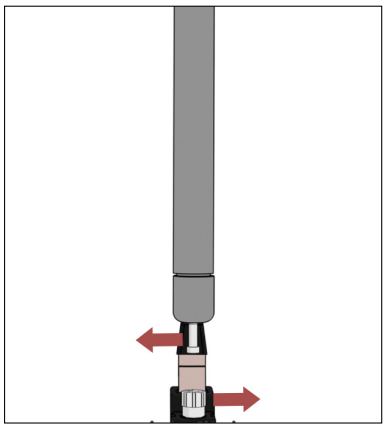
d) Deposit the microscope onto the **Cannula** (Fig. 2.2d).

e) Open the **Snap-on Tool**, and the **Microscope Clamps** will close onto the **Cannula Clamp Groove** (Fig. 2.2e).

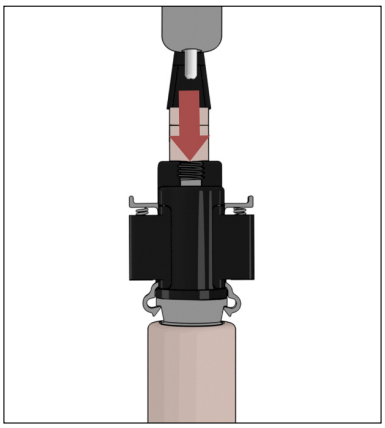
f) Inspect the **Microscope Clamps**; if they are not completely inside the **Cannula Clamp Groove**, gently press them into place (Fig. 2.2f).

2.2 Fluorescence Microscope Holder Installation

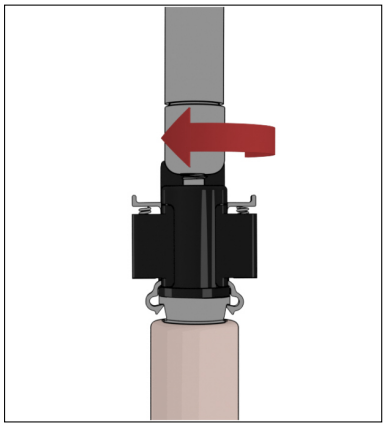
For cannula implantation, the microscope can be secured on its *Fluorescence Microscope Holder*. The holder allows imagery during the descent of the relay lens into the brain. Experiments not requiring a freely-moving animal can be done with this head-fixed configuration. The 2CFM-L is used as an example in the following figures as the process is identical for all microscope types.



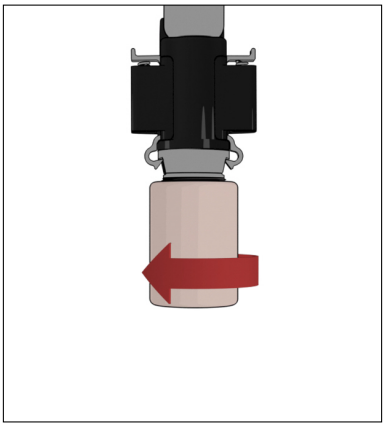
(a) *Remove the protective caps from the microscope and the fluorescence microscope holder-2*



(b) *Place the holder ferrule inside the microscope M3 connector*



(c) *Screw the holder extremity onto the M3 connector*



(d) *Unscrew and remove the cannula output cap*

Figure 2.3: *Cannula Installation Procedure*

1. Install the microscope onto the *Fluorescence Microscope Holder 2 (FMH_2)*.

   a) Remove the **Connector Caps** from the microscope **M3 Optical Connector** and the **Fluorescence Microscope Holder 2 ferrule** (Fig. 2.3a). Clean the **Fluorescence Microscope Holder 2 ferrule** tip using a lint-free wipe and isopropyl alcohol before the next step.

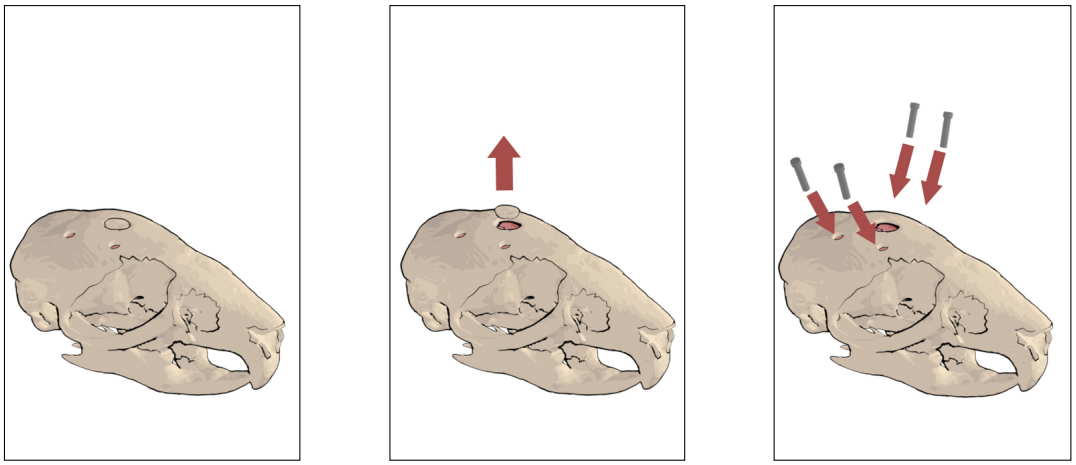
   b) Insert the ferrule into the **M3 Optical Connector** (Fig. 2.3b). Secure them in place by screwing the **Fluorescence Microscope Holder 2 barrel** (Fig. 2.3c).

c) Install the *Fluorescence Microscope Holder 2* into a stereotaxic apparatus.

d) When ready for use, remove the **Output Protective Cap** from the cannula by unscrewing it (Fig. 2.3d). If using a type-L cannula, take great care to remove it in a straight motion so as not to touch or break the rod lens.

2. Before implantation, remove the protective cap and start the acquisition system to check the image quality obtained. Make sure **not to touch the surface of the lens.** If the image shows spots, there may be dust on the lens. Use a cotton swab to clean the tip of the lens with acetone or isopropyl alcohol. **Never dip the lens in acetone.** After this test, close the acquisition system.

2.3 Animal Surgical Preparation



(a) *Create holes for the skull screws* (b) *Perform the craniotomy* (c) *Secure the skull screws*

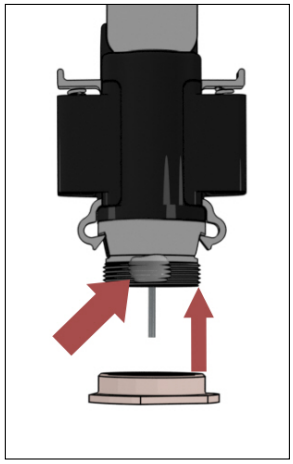
Figure 2.4: *Surgical Preparation*

Before implantation, the animal subject must be prepared. This includes the placement of **Skull Screws** to secure the cannula, and a craniotomy to allow observation of the brain[1].

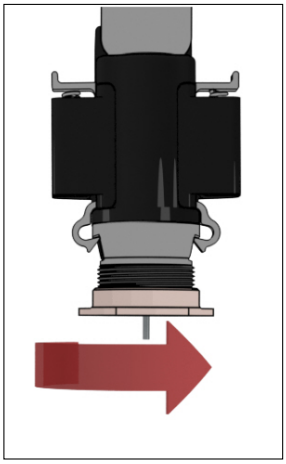
1. Determine the stereotaxic coordinates for cannula implantation.
2. Drill holes to allow the placement of skull screws (Fig. 2.4a). This requires a distance of at least 5 mm from craniotomy center to allow rotation of the *Protrusion Adjustment Ring*.
3. Perform the craniotomy (Fig. 2.4b). When using a type-L cannula, the hole must have a diameter larger than the rod lens diameter. When using a type-S cannula, the hole must have a diameter of at least 2 mm.
4. Place 4 supporting screws in the prepared holes around the craniotomy site (Fig. 2.4c).

[1] Skull screws not provided with microscope

2.4 Protrusion adjustment ring installation



(a) *Place glue drops into cannula thread*



(b) *Thread the protrusion adjustment ring onto the cannula.*

Figure 2.5: *Protrusion Adjustment Ring Installation*

To prepare for implantation, the *Protrusion Adjustment Ring* must first be installed. The *Protrusion Adjustment Ring* is used to stabilize the system on the skull when the implanted cannula is at the appropriate depth. Its position is determined by the depth of the structure of interest relative to the top of the skull (see section 1.1.2).

1. Place a couple of drops of a slow drying glue (e.g. epoxy adhesive) to secure the ring to the metal thread of the imaging cannula[2].
    - **Be careful not to apply glue on the microscope body or the imaging lens.**
    - A slow drying will allow small adjustments during implantation.
    - Each full rotation of the protrusion adjustment ring represents a vertical distance of 300 µm.
2. Attach the proper protrusion adjustment ring to the cannula at its approximate position. For type-L cannulas, place the *Protrusion Adjustment Ring* in a slow, vertical motion to avoid making contact with the rod lens.
3. Once the *Protrusion Adjustment Ring* is in place, the microscope can be moved above the animal subject.

2.5 Type-S Cannula Implantation

When using a type-S cannula, no penetration is necessary as the lens does not enter the brain. Only *2CFM-S Microscope Bodies* are capable of using *Type-S Cannulas*. The region must however be prepared for observation. The following section details a few recommended protocols for surface imagery.

1. Prepare the animal for observation. There are several methods possible to obtain optimal image quality. It should be noted that the type-S *Imaging Cannula* leaves a small air pocket between the brain and the objective lens. Dura exposed to air becomes opaque (white) over time, while brain matter can be damaged by air exposure.
    - The use of a **Cranial Window** is common. These thin, small-diameter glass windows are placed above the craniotomy opening, reducing air exposure of the brain. The space between the window and the brain can also be filled with a biocompatible transparent medium, such as agarose.
    - The **Thin-skull Window Technique** involves progressive thinning of the animal subject's skull. This thinning allows light to be transmitted through the skull, thus allowing imagery without penetrating the skull itself.
    - The **Side-prism Method** can be used in conjunction with a **Cranial window**. A microprism is inserted into the surface areas of the brain (Fig. 2.6), which allows the viewing of regions perpendicular to the normal focal plane.

[2] Glue not provided with microscope

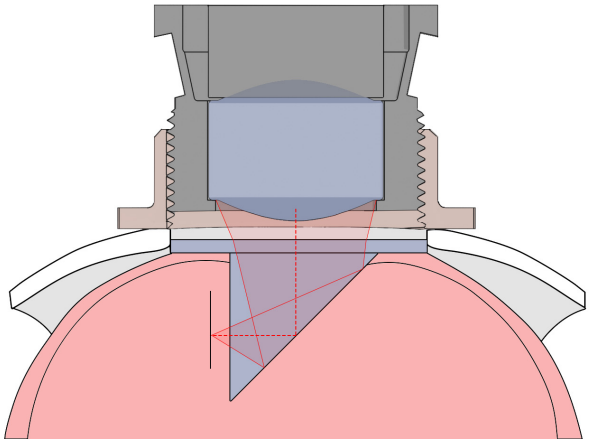


Figure 2.6: *Side-prism Method*

2. Connect the *Fluorescence Microscope Holder 2* to the illumination system.
3. Lower the microscope above the craniotomy hole.
    - The *Fluorescence Microscope Holder 2* allows illumination during implantation.
    - If the cannula is installed when sufficient viral expression has been reached, a diffuse fluorescence signal will confirm the positioning of the focal plane in the targeted area (the injection site).
    - If the cannula is installed immediately after viral injection, the fluorescence signal cannot be used to confirm the position of the focal plane at the targeted site.
4. If there is a significant gap between the **Protrusion Adjustment Ring** and the skull, the position can be adjusted.
    - Verify that the *Fluorescence Microscope Holder 2* is secure and that the microscope will not move if touched.
    - Slowly unscrew the **Protrusion Adjustment Ring** to bring it closer to the skull.

2.6 Type-L Cannula Implantation

When using a type-L cannula, the rod lens must penetrate the brain. Only type-L microscope bodies are capable of using a type-L cannula. The following section details the standard method of cannula implantation.

2.6.1 Setting the Depth reference

When using a type-L cannula, a depth reference must be taken on the brain surface with the tip of the relay lens.

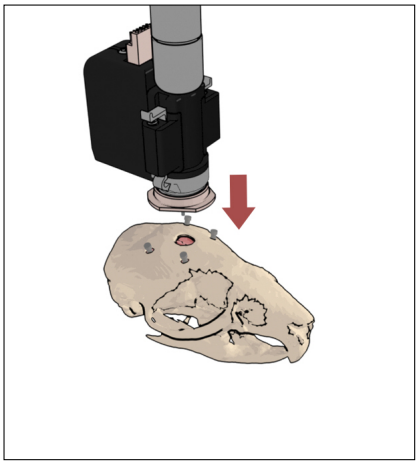
1. Place the microscope above the prepared animal subject. Lower the system into the craniotomy hole, so that the tip of the rod lens is touching the brain surface without penetrating it.
    - Given the size of the cannula, the tip can be difficult to see. A mirror or a camera can be helpful to see exactly when the extremity of the lens touches the brain.
2. When the reference point is found, note it and elevate the microscope.
3. Carefully remove the dura from the reference point. A clean brain surface without any bleeding will allow optimal image quality while lowering the rod lens.

2.6.2 Implantation of the Cannula

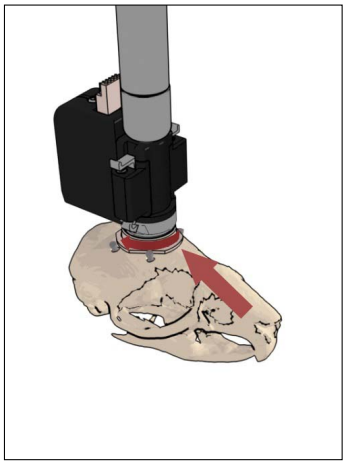
From the beginning to the end of implantation, handle the imaging cannula with care. The relay lens is very fragile and any stain or scratch can affect image quality.

1. Connect the *Fluorescence Microscope Holder 2* to the illumination system.

2. Slowly lower the rod lens (around 1 µm/s) to allow proper tissue penetration (Fig. 2.7a).
    - The *Fluorescence Microscope Holder 2* allows illumination during the implantation of the lens.
    - If the cannula is implanted when a sufficient viral expression has been reached, a diffuse fluorescence signal will confirm the positioning of the lens in the targeted area (the injection site).
    - If the cannula is implanted immediately after viral injection, the fluorescence signal cannot be used to confirm the position of the lens in the targeted site.
3. If the imaging cannula is not in the desired area, it is possible to make another descent to place the lens.
4. If there is a significant gap between the *Protrusion adjustment ring* and the skull:
    - Verify that the *Fluorescence Microscope Holder 2* is secure and that the microscope will not move if touched.
    - Slowly unscrew the protrusion adjustment ring to bring it closer to the skull. Stop unscrewing if the **Rod Lens** starts moving inside the brain (Fig. 2.7b).



(a) *Position the microscope on the skull, lower the lens slowly*

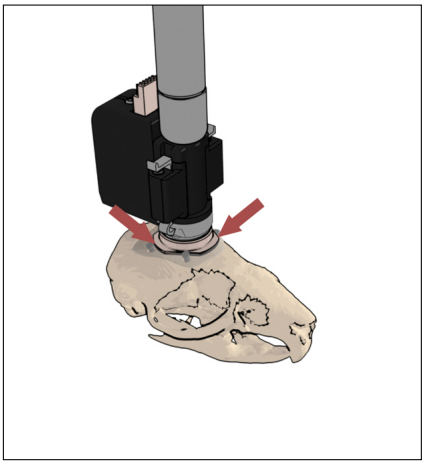


(b) *Unscrew the protrusion adjustment ring closer to skull*

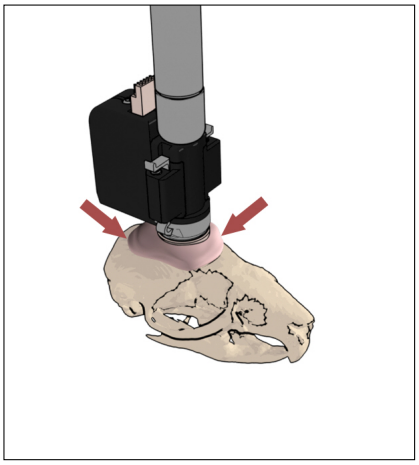
Figure 2.7: *Implantation Preparation*

2.7 Securing the Cannula on the Skull

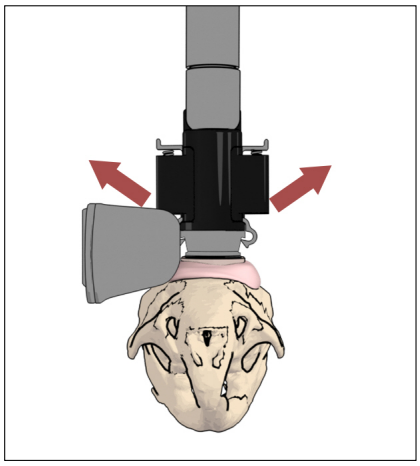
Once the cannula is implanted in the region-of-interest, it is necessary to secure it and the **Protrusion Adjustment Ring** onto the skull.



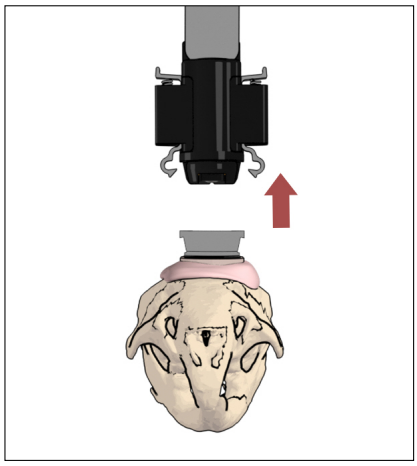
(a) *Use a fast drying glue to secure the protrusion adjustment ring on the skull*



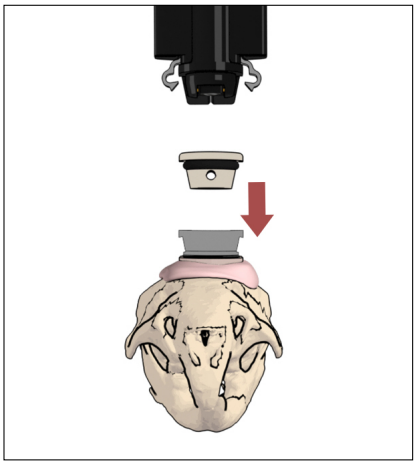
(b) *Use dental cement to secure the protrusion adjustment ring on the skull*



(c) *Disconnect the microscope connection system*



(d) *Remove the microscope body from the cannula*



(e) *Place the input protective cap on the cannula*

Figure 2.8: *Securing the Imaging Cannula on the Skull*

1. Put a fast drying glue between the **Protrusion Adjustment Ring** and the skull (Fig. 2.8a)[3]. If the ring is near the bones, the capillary action of a strong adherence liquid glue will ensure that the glue will pass below the ring. Otherwise, a gel glue should be used to fill the gap.
2. To improve support for the screws, it is recommended to put glue on them.
3. When the glue on the surface of the skull is completely dried, secure the microscope to the skull by applying dental cement on the **Protrusion Adjustment Ring** and on the screws (Fig. 2.8b)[4].
    - If the **Protrusion Adjustment Ring** is not touching the skull, it is important to put some cement on the glue between the ring and the bones to stabilize the system.
    - The **Connection System** must remain free of cement to be able to separate the microscope and the cannula.
    - Tissue, muscles, skin or fur should not come in contact with the dental cement. This is necessary to ensure proper adhesion of the cannula to the skull.
4. Once the dental cement has completely dried, the microscope can be removed. Detach the **Connection System** (Fig. 2.8c).
5. Remove the microscope from the cannula (Fig. 2.8d).
6. Place the **Input Protective Cap** onto the imaging cannula to protect the lens (Fig. 2.8e).
7. Place the protective cannula onto the microscope.
    - If the imaging sessions are done in a head-fixed configuration, the microscope body can be left attached to the *Fluorescence Microscope Holder* until the first imaging session.
    - For freely-moving experiments, unscrew the *Fluorescence Microscope Holder* and put its protective M3 cap to protect the microscope body's optical pathway.

2.8 Tissue Healing and Training of the Animal (3 weeks or more)

Usually, the system can distinguish cells 2 or 3 weeks after the implantation of the cannula. Nonetheless, better image quality is obtained 2-8 weeks after the implantation. The waiting time for tissue repair can be a good period to use the *Dummy Microscope* (Fig 2.9). It is important to train the animal, using the dummy, to tolerate the encumbrance of a microscope on its head and to get used to moving easily in its cage with it.

Figure 2.9: *Dummy Microscope*

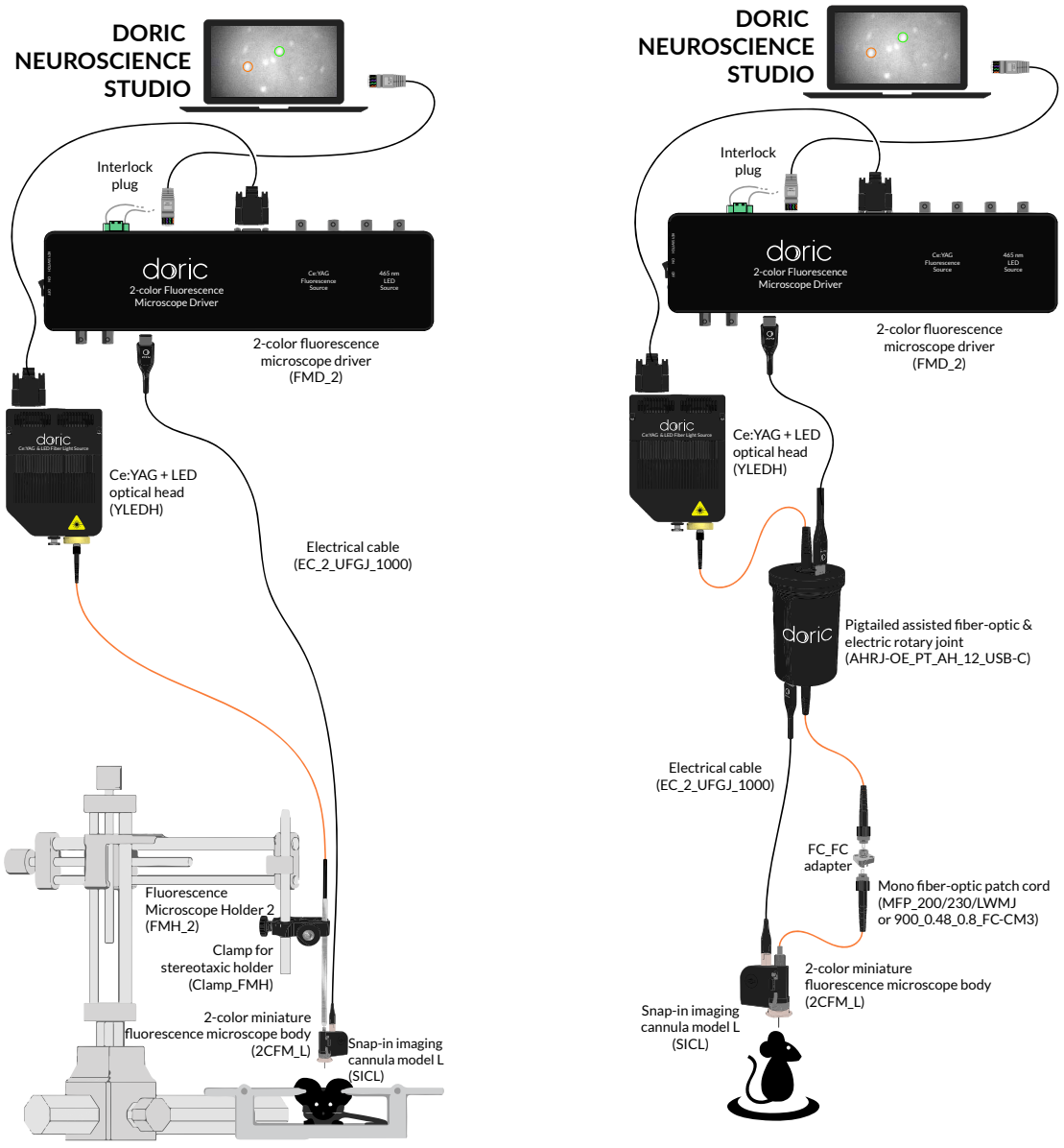
[3] Glue not provided with microscope
[4] Dental cement not provided with microscope

3

Imaging Sessions

The following section describes the basics of an imaging session using the *2-color Miniature Fluorescence Microscope*.

1. Prepare the microscope for a head-fixed configuration session, as described in section 2.2.
2. Before installing the microscope, place the anesthetized subject in a stereotaxic head restraint.
3. Remove the **Input Protective Cap** from the cannula (Fig. 2.8e).
4. Install the microscope in the cannula. The stereotaxic head restraint decreases the pressure applied on the head of the animal when the microscope body is secured in the implanted cannula.
5. If performing a head-fixed experiment, complete the connections of the system and open the acquisition system. This allows imaging of the region of interest.
6. If performing a freely-moving experiment, remove the animal from the stereotaxic apparatus and let the animal recover from the anesthesia.
    - For more details about the connections of the system and the use of the Doric Neuroscience Studio Software, see our website.
    - During behavior imaging sessions, it is recommended to put the metal shield at the base of the ultralight cable to protect it from animal interference, such as chewing or scratching.



(a) *Head-fixed Configuration for Deep-brain Imaging of two colors*

(b) *Freely-moving Configuration for Deep-brain Imaging of two colors*

Figure 3.1: *2-color Fluorescence Microscope System Configurations*

4

Handling & Cleaning

4.1 Important handling information

WARNING!
Handle the microscope and the cannula with care.

Miniature fluorescence microscopes are composed of sensitive electronic components and should always be handled with care. When they are not used, the microscope body with its cannula should be stored in a closed, dust-free environment. Some microscope components must be handled with extra care:

- **Electrical cable: Do not twist or pull on the cable.**
- **Relay lens:** The cannula lens is made of glass and is unprotected. **Abrasive materials can scratch the surface** and reduce the image quality.

The microscope bodies and the cannula lenses are made of glass, metal, plastic and the contact with organic tissues or liquids, like blood or saline solution is not recommended. While the cannula is designed to be in contact with such substances, the microscope body is not. If the body comes in contact with these substances, clean the optics (section 4.2) to avoid the appearance of stains.

4.2 Cleaning optics

The microscope objective lens should be cleaned before each use. The procedure explained here can also be used to clean the cannula relay lenses.

- Turn the driver OFF.
- **Wear gloves to manipulate the microscope.** Finger oil can stain the glass and is often difficult to remove properly.
- Use isopropyl alcohol on a cotton swab to gently clean the lens.
- **Do not blow on the optics.** Saliva particles will often stain the surface. Larger dust particles can be removed using a dust-free blower before cleaning with a cotton swab.

4.3 Imaging cannula reuse

The implanted cannulas are sold as disposable but can be re-used if removed carefully. To do so, simply remove the glued protrusion adjustment ring from the metal part. In this case, plan spare sets of protrusion adjustment rings. Acetone can be used to clean the cannula lens with a cotton swab (never dip the cannula in acetone), but care should be taken not to expose the binding site between the lens and the metal part of the cannula.

5

Support

5.1 Contact us

For any questions or comments, do not hesitate to contact us by:

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