



Optofluid Cannulas Implantation and fluid injection

Application Note

Version 1.1.0

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Opto-Fluid Cannulas (OFC)

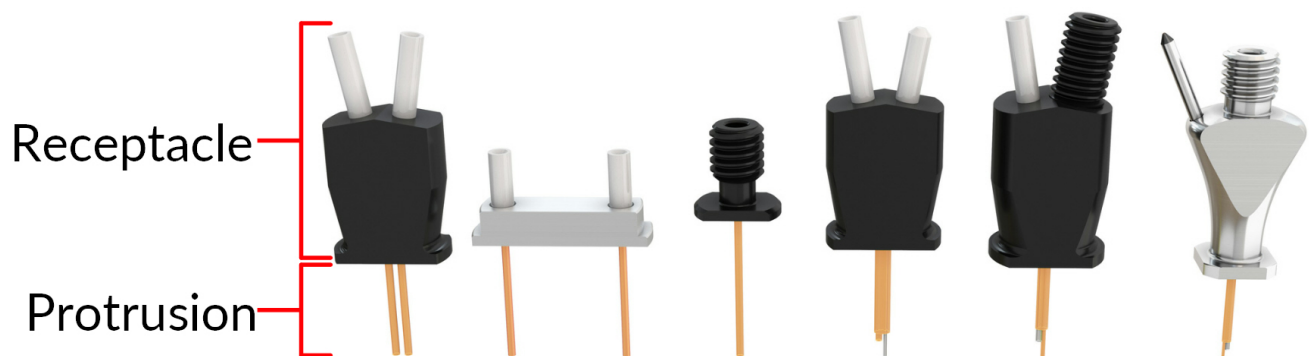


Figure 1.1: (Left to right) DiOFC_S, DiOFC_L, iOFC, OmFC-ZF, OMFC-M3, and OsFC

Opto-fluid cannulas (OFC) allows easy access to the brain for liquids and light by hybridization of fiber-optic and fluid delivery cannulas. Designed for chronic implantation, they remove the need for frequent cannula replacement. This greatly decreases the risk of infection and ensures the spatial accuracy of fluid delivery and optical illumination to the brain region of interest. These factors allow consistent results in behavioral and longitudinal studies, even over long periods of time.

OFC are most often used in experiments that require the delivery of a fluid solution (such as a solution containing viruses or pharmacological agents) alongside illumination of the region of interest. When an **Opsin** (such as Channelrhodopsin) is expressed in the region of interest, the light-sensitive ion channels can be activated by outside illumination. OFC allow illumination weeks later when the opsins are properly expressed. OFC can also be used for fiber photometry recording, measuring activity induced fluorescence of all neurons expressing a calcium indicator in the region of interest. This can be done with various pharmacological agents, allowing real-time measurement of the effect of these agents on nervous tissue.

Several models of opto-fluid cannulas are offered for single and multiple injections or with interchangeable injections. The lengths of the optical fiber, the injector and the injector guiding tube are customized to reach targeted regions of interest. These cannulas are separated into two primary sections:

- The implantable **Protrusion** guides light and/or fluids deep into the brain.
- The **Receptacle** is secured on the head of the subject, connecting the cannula to sources of light and/or fluid.

1.1 OsFC - Optical Single-shot Fluid Cannula

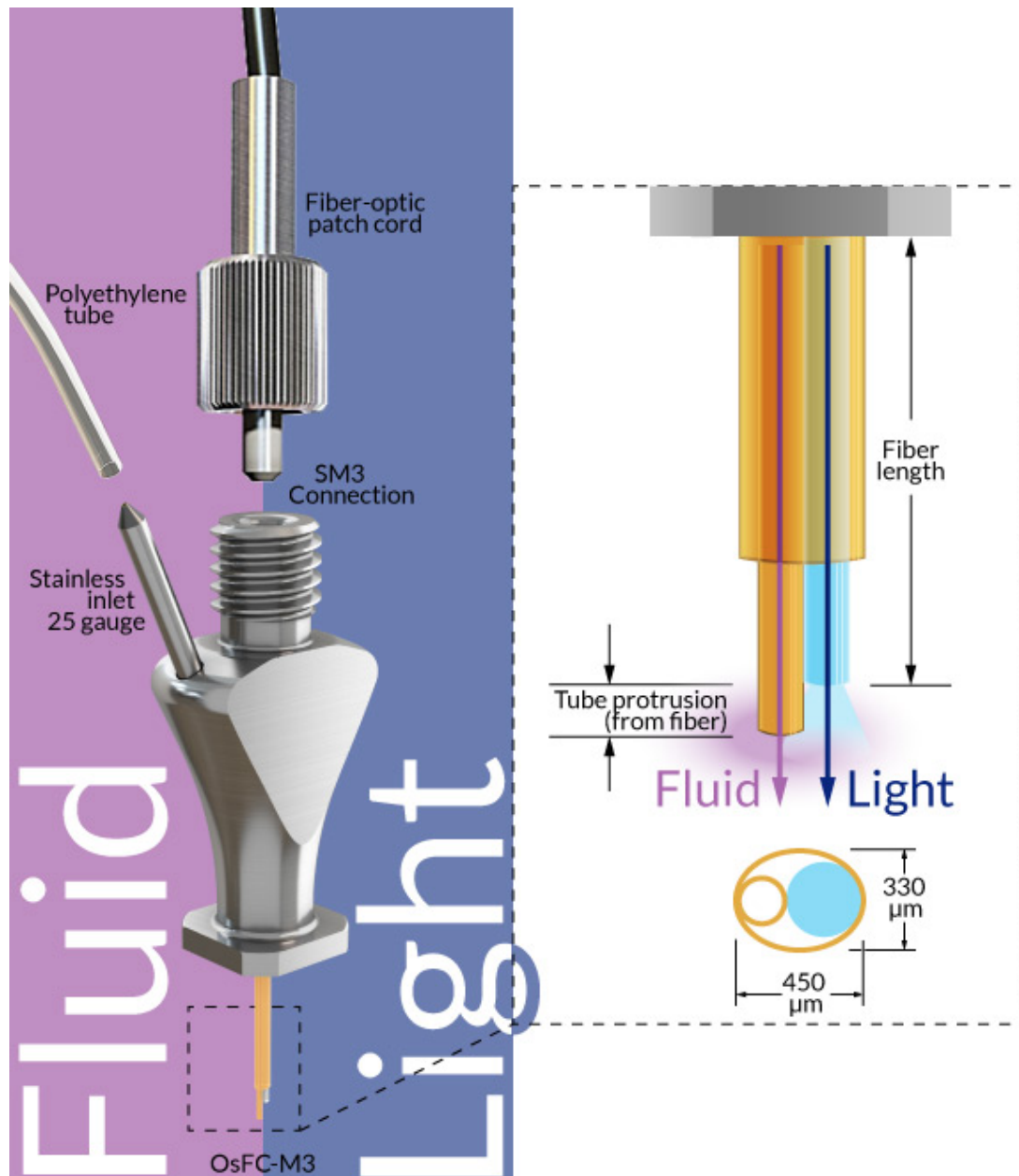


Figure 1.2: OsFC - Optical Single-shot Fluid Cannula

The *Optical Single-shot Fluid Cannula (OsFC)* is designed for optical delivery/detection with a single fluid delivery. An optical **SM3 connector** (Fig. 1.2) connects to the optical fiber patch-cord and allows optical input. A 25-gauge **Stainless inlet** on the side (Fig. 1.2) is used for fluid input when connected to a polyethylene tube. The outputs are brought together in the **Protrusion** with a polyimide tube.

- The OsFC is designed to be **Loaded with liquid before implantation**. Fluid delivery is performed once the cannula has been implanted.
- It is not recommended to perform a second injection as the passage can become clogged after first use.
- When delivering a virus-containing solution, it is important to remember that the solution stays in the tubing during the implantation process. This requires quick implantation of the cannula to avoid reducing infectivity due to exposure of the virus to room temperature.

1.2 OmFC - Optical Multiple Fluid Cannula

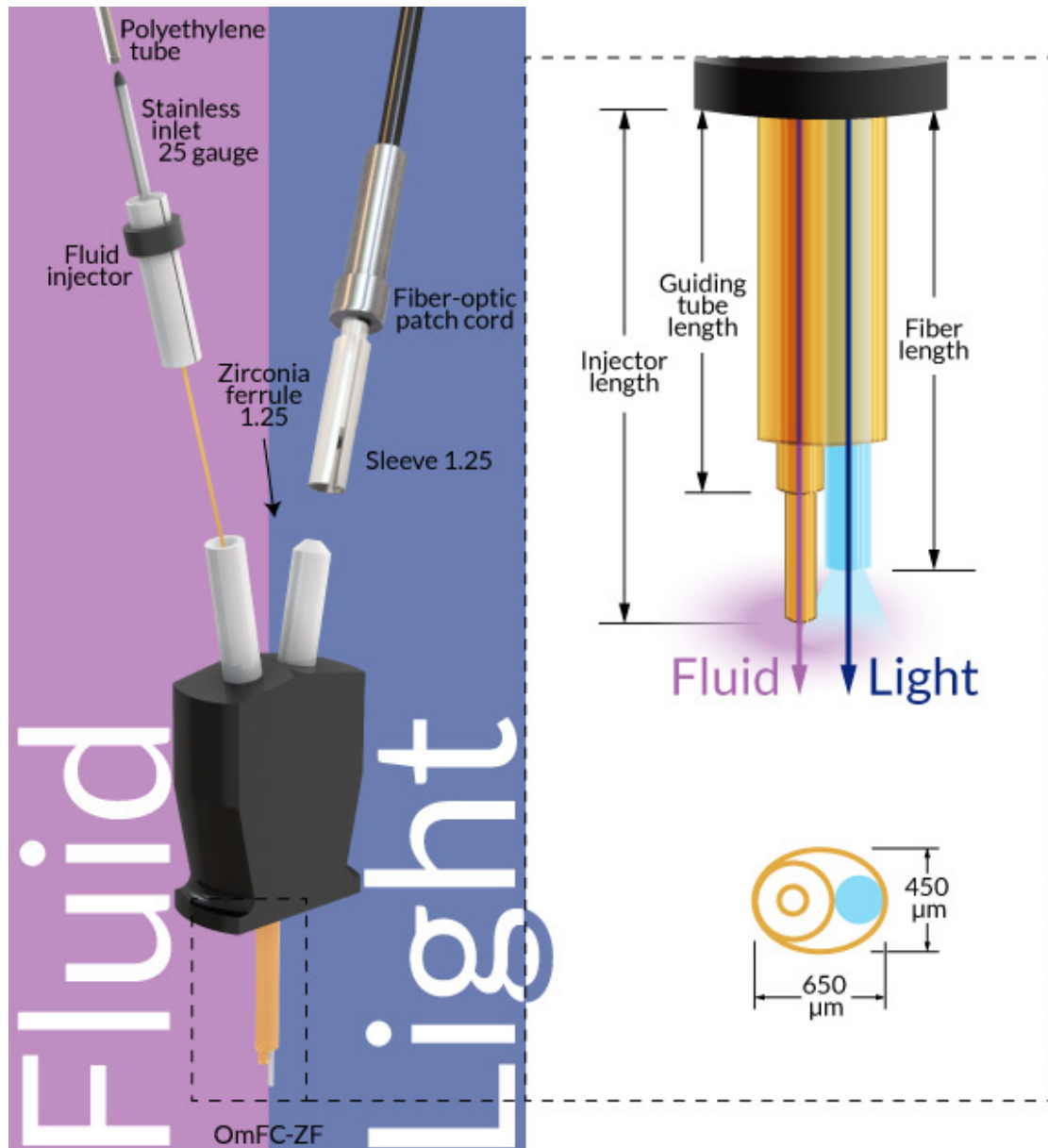


Figure 1.3: OmFC - Optical Multiple Fluid Cannula

The *Optical multiple Fluid Cannula (OmFC)* is designed for optical delivery/detection with the possibility of multiple fluid deliveries. An optical **SM3 or ZF connector** (Fig.1.3) connects to the optical fiber patch-cord and allows optical input. A **Guiding tube** (Fig.1.3) allows the insertion of a *Fluid Micro-injector (FI-OmFC)* (Fig.1.3) that can be loaded with a single fluid.

- The **Guiding tube** is kept free of debris using an **Injector plug** which comes pre-installed on the cannula. Insert this plug during implantation and whenever the *FI-OmFC* is not in use to avoid clogging the guiding tube. When in place, the plug is 100 μm longer than the guiding tube protrusion.
- The *FI-OmFC* can be reused multiple times on the same animal if properly cleaned and sterilized. Use isopropanol or a similar cleanser.
- The *FI-OmFC* can be loaded separately from the cannula, and does not require loading during implantation. The stainless inlet on the fluid micro-injector is used to connect a syringe or a liquid delivery system using polyethylene tubing.

1.3 iOFC - interchangeable Optical Fluid Cannula

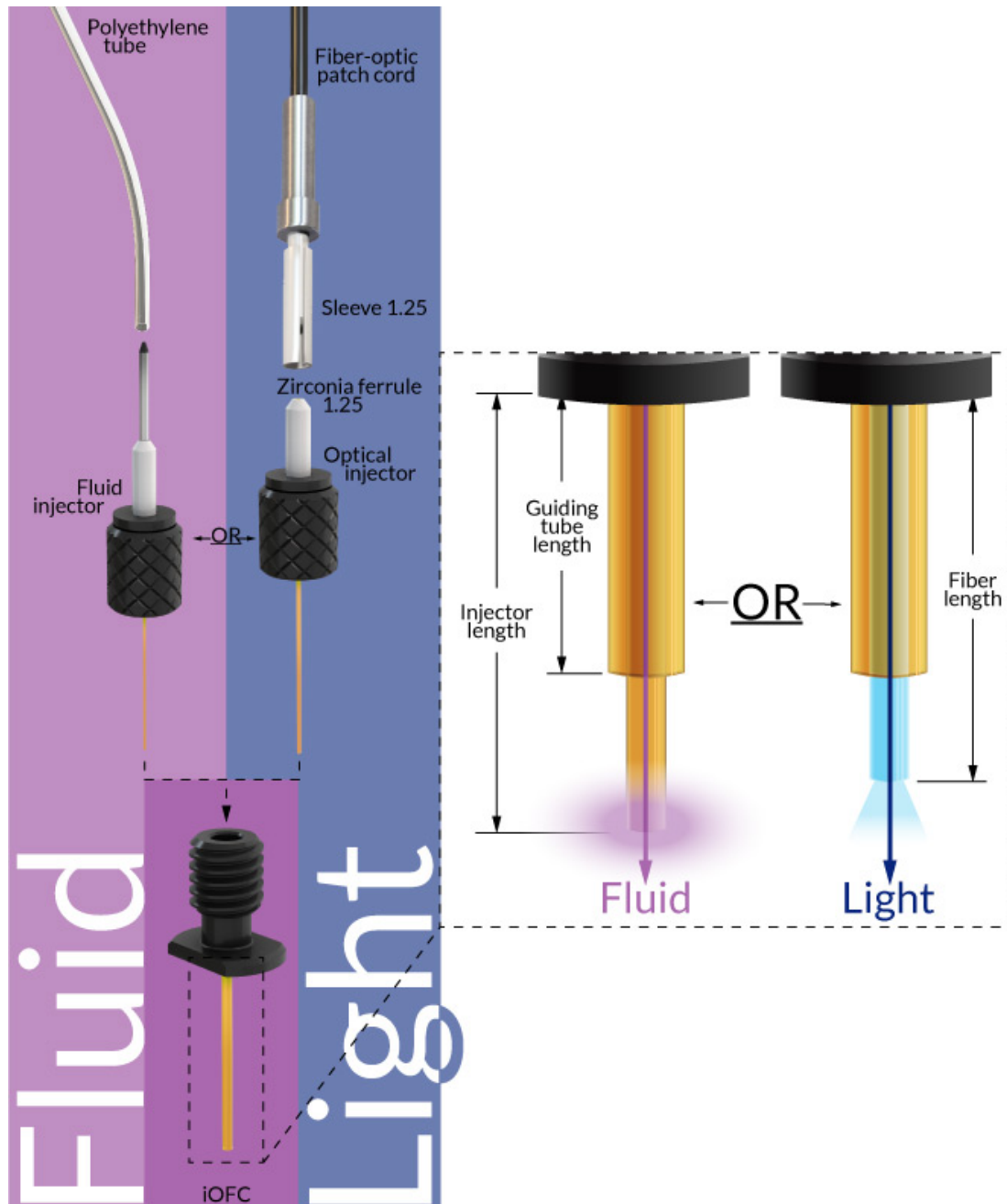


Figure 1.4: interchangeable Optical Fluid Cannula

The interchangeable Optical Fluid Cannula (iOFC) is the smallest and lightest OFC due to its single **Guiding tube**. The **Guiding tube** (Fig.1.4) is used with interchangeable *Fluid micro-injectors (FI-iOFC)* and *Optical fiber injectors (OI-iOFC)*. These injectors are secured to the cannula using an SM3 receptacle.

- The **Guiding tube** is kept free of debris using an **Injector plug** which comes pre-installed on the cannula. Insert this plug during implantation and whenever a **Micro-injector** is not in use to avoid clogging the guiding tube.
- The FI-iOFC can be reused multiple times on the same animal if properly cleaned and sterilized. Use isopropanol or a similar cleanser.

- The *FI-iOFC* can be loaded separately from the cannula, and does not require loading during implantation. The stainless inlet on the fluid micro-injector is used to connect a syringe or a liquid delivery system using polyethylene tubing.
- The *OI-iOFC* connects to a patch-cord using a **ZF** connection.

1.4 DiOFC - Dual interchangeable Optical Fluid Cannula

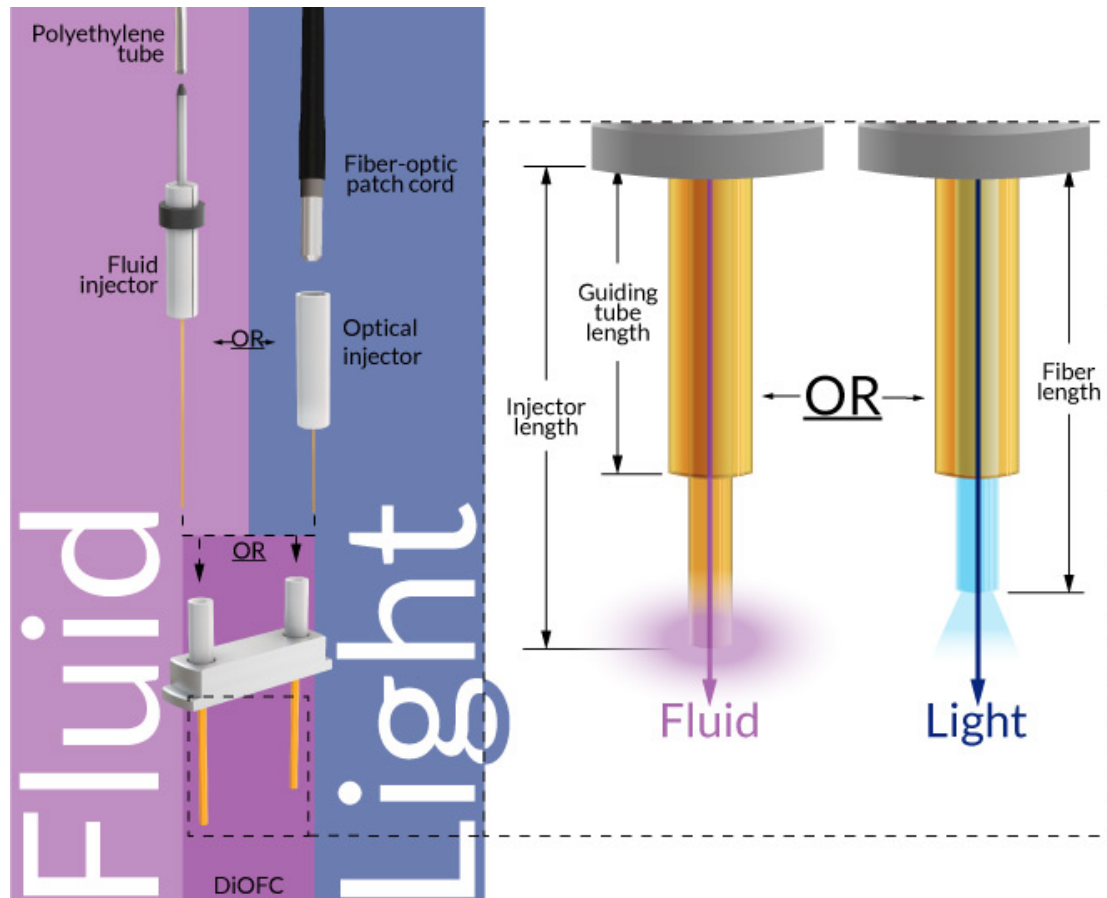


Figure 1.5: Dual interchangeable Optical Fluid Cannula

The precise pitch of the DiOFC guarantees an optimal bilateral implantation where both light and fluid injection can be used. The interchangeable configuration saves space and weight and can be used with multiple lengths of optical and fluid injector. The guiding tube (Fig.1.4) is used with interchangeable *Fluid micro-injectors (FI-DiOFC)* and *Optical fiber injectors (OI-DiOFC)*. These injectors are secured to the cannula using an ZF receptacle.

- The **Guiding tubes** are kept free of debris using an **Injector plug** which comes pre-installed on the cannula. Insert this plug during implantation and whenever a **Micro-injector** is not in use to avoid clogging the guiding tube.
- The **Guiding tubes** have a minimum pitch (center to center distance) equal to the tube outer diameter.
- The *FI-DiOFC* can be reused multiple times on the same animal if properly cleaned and sterilized. Use isopropanol or a similar cleanser.
- The *FI-DiOFC* can be loaded separately from the cannula, and does not require loading during implantation. The stainless inlet on the fluid micro-injector is used to connect a syringe or a liquid delivery system using polyethylene tubing.
- The *OI-DiOFC* connects to a patch-cord using a ZF connection.

OFC implantation and usage

As all OFC share basic design elements, implantation is very similar for each type of cannula. The following section describes the implantation process, followed by general usage guidelines.

2.1 OFC implantation

All OFC are delivered with *Protective caps* and *Injector plugs* to protect the **Protrusion**. Handle the cannula with care, as both the cannula and injectors are fragile.

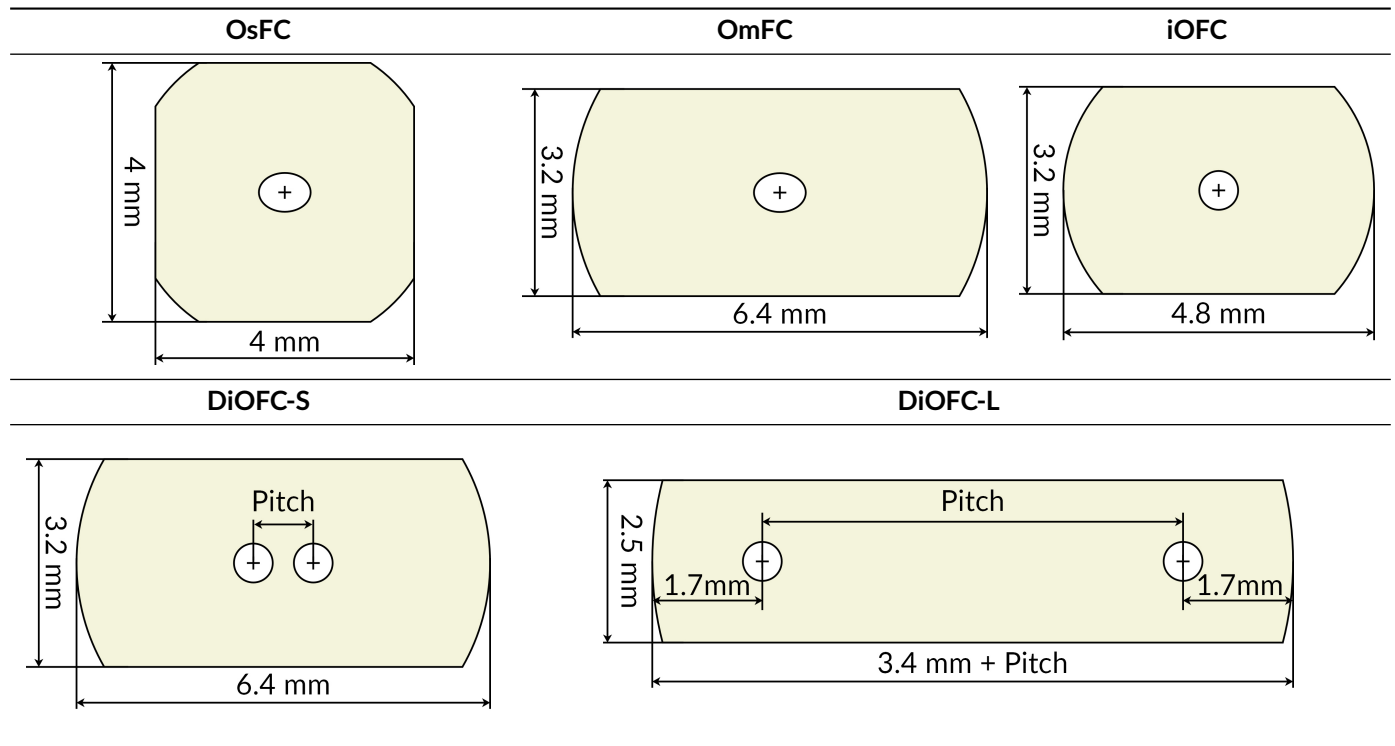
2.1.1 OFC preparation

1. Install the cannula in the appropriate holder.
 - Screw onto the OsFC an *SM3 Receptacle Adapter (FCA-SM3)* that is compatible with your *Stereotaxic Cannula Holder (SCH)*. Secure the assembly in the SCH, then integrate it into the stereotaxic apparatus.
 - The OmFC is installed into the *OmFC Cannula Holder*. The **Injector plug** must be left in place during installation and implantation. The plug must not be removed, as otherwise the **Guiding tube** could become clogged during implantation.
 - The iOFC is installed into the **SCH.1.25**, where it is held by the connection of the *Injector plug*. The plug must not be removed, as otherwise the **Guiding tube** could become clogged during implantation.
2. Install the appropriate holder in a stereotaxic apparatus. In most cases, this involves a *Stereotaxic Cannula Holder*. The holder is required to accurately position the cannula for implantation.
 - The *OmFC stereotaxic cannula holder (SCH.OmFC)* must be installed into a *SIA* to be integrated in a stereotaxic apparatus.
3. Once the cannula is in place, the setup can be prepared for implantation.
 - **If using an OsFC, the injection tube must be filled PRIOR to implantation. See section 2.2.1 for details on filling the injection tubing.**

2.1.2 Implantation site preparation

1. Determine the stereotaxic coordinates for implantation.
2. Drill holes to allow the placement of skull screws around the craniotomy site. These help anchor the dental cement that holds the cannula in place. Use table 2.1 to identify the cannula footprint.
3. Perform the craniotomy. The craniotomy must be about **1.5 x** larger than the guiding tube diameter.
 - When using a DiOFC, the craniotomy must accommodate both guiding tubes.
4. Place the supporting screws in the prepared holes around the craniotomy site.

Table 2.1: Cannula Footprint; The white space represents the guiding tube, the cross the guiding tube center, and the beige shape the cannula receptacle



2.1.3 Cannula Implantation

1. Bring the cannula installed in the stereotaxic apparatus above the animal.
2. Slowly lower the cannula into the craniotomy until you reach the desired region of interest. A slow speed is necessary to allow good tissue penetration.
3. Spread fast-drying bio-compatible glue between the receptacle and the skull.
 - If the receptacle is very close to bone, capillary action will ensure a liquid strong-adherence glue spreads underneath the receptacle.
 - If the receptacle is not very close to bone, use a gel glue to fill the gap.
 - Placing glue around the skull screws can increase their stability.
4. When the glue has dried, secure the cannula to the skull using dental cement on the screws and the base of the receptacle.
 - If gel glue was used, it is important to place cement on it, from the cannula to the skull, to stabilize the system.
 - The dental cement should not come into contact with tissues, muscles, skin or fur, as this could drastically reduce the adhesion of the cannula.
5. Once the cement is dry, remove the cannula from the stereotaxic apparatus.

2.2 OFC usage

The following section details the usage of the various OFC and their injector. As a reminder, **Handle the cannulas and injectors with care; optical fibers and tubing are fragile and can easily be broken.**

Table 2.2: Fluid Injector Dead Volume

INJECTOR	DEAD VOLUME
OsFC	110 nL+8 nL per mm of protrusion
FI_OmFC	175 nL+8 nL per mm of protrusion
FI_iOFC	115 nL+8 nL per mm of protrusion
FI_DiOFC-S	175 nL+8 nL per mm of protrusion
FI_DiOFC-L	110 nL+8 nL per mm of protrusion

2.2.1 Injection tube/micro-injector loading

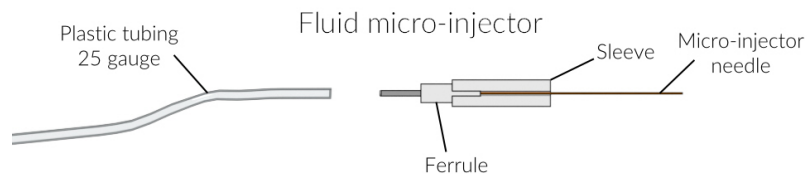


Figure 2.1: OmFC Fluid Injector

1. Connect the fluid injector stainless inlet (fluid input) to the polyethylene tubing.
2. Connect the opposite end of the tubing to a gastight syringe or other fluid delivery system.
3. Fill the injection tubing with a volume equal to the volume required and the dead volume. The dead volume of each fluid injector can be found in table 2.2.
 - The injector should be filled until a small droplet appears at the tip.
4. Using a saline solution and a light-duty tissue paper clean the injector tip before placing it in the guiding tube.
 - It is important to collect any drops that leave the injector, to ensure no fluid clogs the guiding tube or soils the optical fiber.
 - The OsFC does not use a guiding tube. The protrusion itself should be cleaned before implantation.

2.2.2 Fluid injection

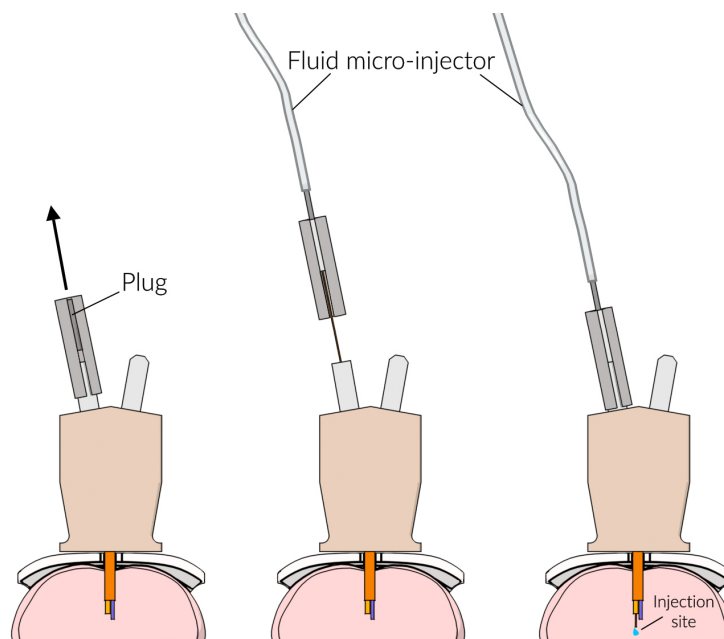


Figure 2.2: OmFC Fluid Injection

1. Verify that the dental cement is completely dry before starting the injection.
2. Remove the plug from the guiding tube and install the pre-loaded fluid injector.
 - For the OsFC, it is the cannula itself that is pre-loaded. Thus, there is no plug to remove or injector to place.
 - For the iOFC, the injector is screwed onto the **SM3 connector**.
3. Slowly inject the fluid at a controlled speed. Leave the injector in place while the fluid is diffusing within the tissue.
4. Once the injection is complete, remove the injector and replace the plug.

2.2.3 Optical probing

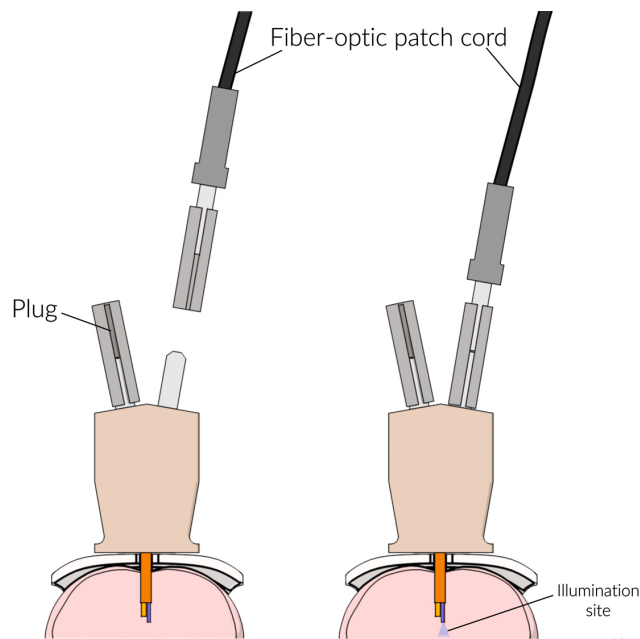


Figure 2.3: *OmFC Optical Injector*

1. Prepare the optical injector.
 - For the *OsFC* and *OmFC*, the optical injector is integrated into the cannula, and no additional action is required
 - For the *iOFC* and *DiOFC*, the optical injector is separate from the cannula. The optical injector is inserted into the guiding tube. If an **SM3 receptacle** is used, the injector must be screwed tight.
2. Connect the fiber-optic patch-cord to the injector.
 - For the *OsFC* and the *OmFC-M3*, an *M3 connector* patch-cord is used. The connector must be screwed in tightly to ensure a stable connection.
 - For the *iOFC* and *OmFC-ZF*, a **Zirconia Ferrule connector** patch-cord is used. The two ferrules are linked using a *Zirconia Mating Sleeve*.
 - For the *DiOFC*, a **Zirconia Ferrule connector** patch-cord is used. The *OI-DiOFC* has an integrated sleeve, into which the patch-cord ferrule is inserted.
3. Activate the illumination system and perform the experiment.
4. Once the experiment is complete, detach the patch-cord. The subject can move freely with the cannula on its head between experiments.

3.1 Contact us

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

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