Single-cell Optical and Electrical Recordings

Getting Started Guide

Version 1.0.0

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1

Single-cell Monitoring and Recording

Being interested in the activity of individual cells can be a real challenge in regions of the brain where all cells have the same electrophysiological signatures. A way to distinguish cells is to label them with fluorophores depending on their protein expressions. The combination of two techniques, fiber photometry and electrophysiology, gives the possibility to link patterns of neuronal activity to specific cells. Another way is to use an optogenetic tagging strategy in order to link the electrophysiological fluctuations with a specific type of cells.

Single-cell recording opto-electric probes allow **in vivo** optical excitation and monitoring, combined with electrophysiological recordings at a single-cell resolution. The probe consists of an adapter, a holder, an interconnect wire and a glass probe tip (Fig. 1.1). The tip of these tapered glass probes (also called micro-optrodes) are smaller than the cell itself to ensure a sufficient spatial resolution. The probe is designed for head-fixed experiments and its opto-electrical interface enables optogenetic activation, photometry applications and single-cell extracellular electrophysiological recording.

Figure 1.1: *Single-cell Recordings Opto-electric Probe*

For photometry measurements, the optical excitation is done with a *Connectorized laser diode module*. The excitation light passes through a fluorescence module and is injected within the optical core of the probe. The emitted fluorescence follows the same path in the optical core but in the opposite direction. Afterwards, the light goes in the fluorescence cube via an optical patch cord. The photosensor modules (PMT) attached to the detection ports amplify the signal which is read by the fiber photometry console (Fig. 1.2).

For electrophysiological recordings, the hollow core of the probe tip is filled with an electrolyte solution and is used as an electrode. The interconnect wire is inserted in the electrolyte solution, linking it and the probe adapter (SCRA). With the SCRA connected to the headstage of a conventional electrophysiological recording system, the probe allows the detection of electric field signal at single-cell resolution (Fig. 1.2 and 1.3)[1].

[1]The following video demonstrates the recording process (animation by Stuart Jantzen from University of Toronto).

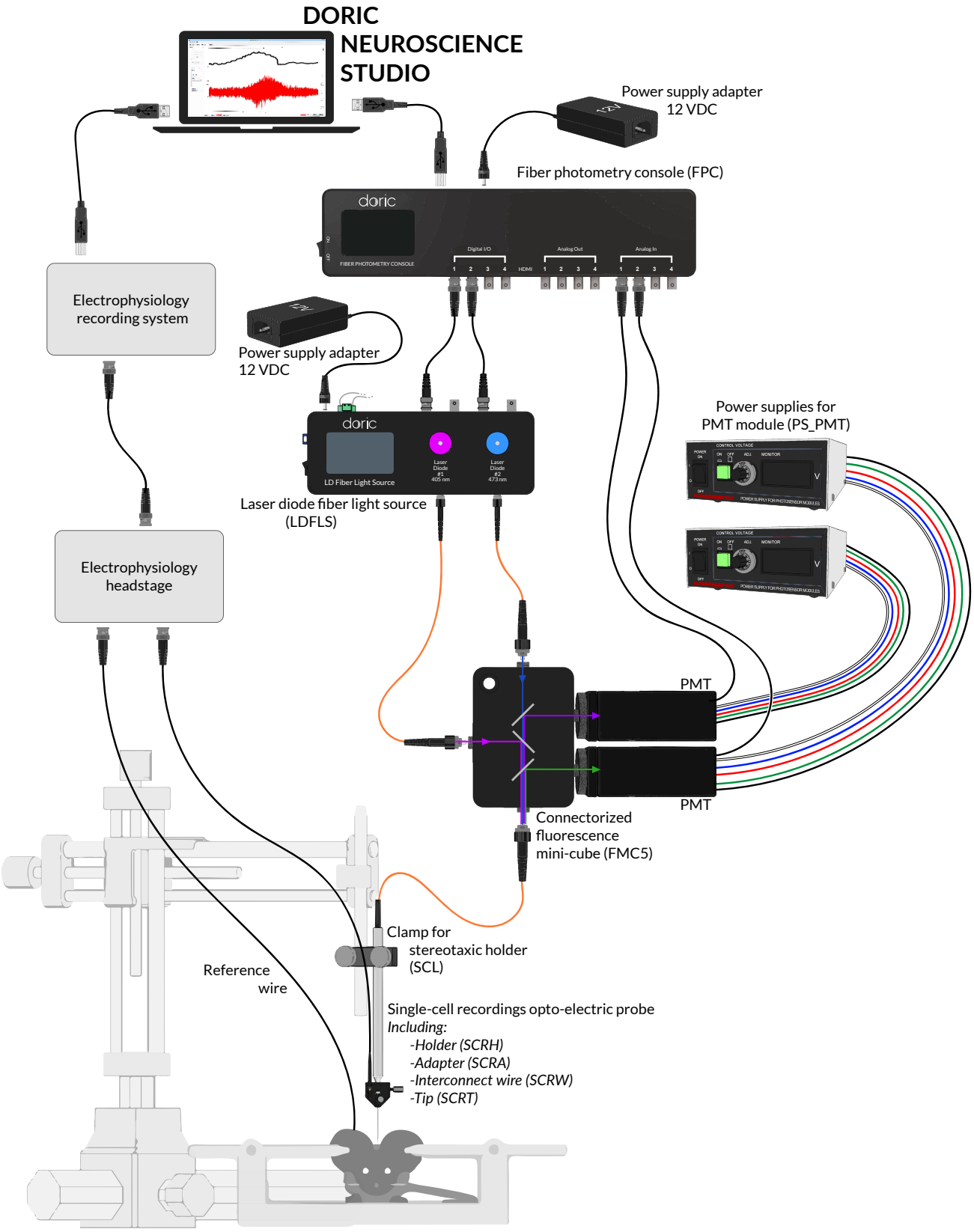


Figure 1.2: *Single-cell Fiber Photometry and Electrophysiology System*

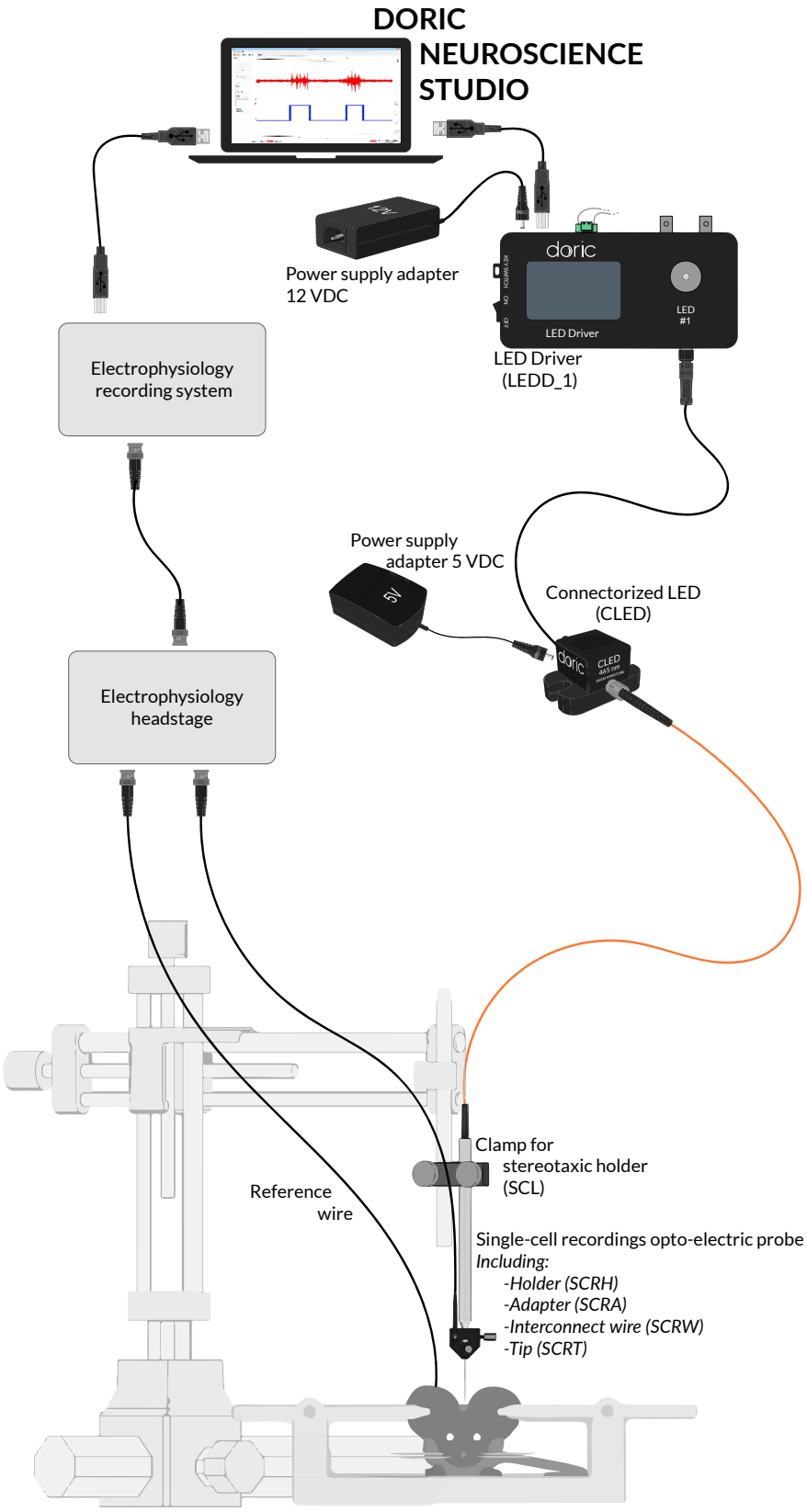


Figure 1.3: *Single-cell Optogenetic and Electrophysiology System*

2

Single-cell Recordings Opto-electric Probe

2.1 Single-cell Opto-electric Probe Holder

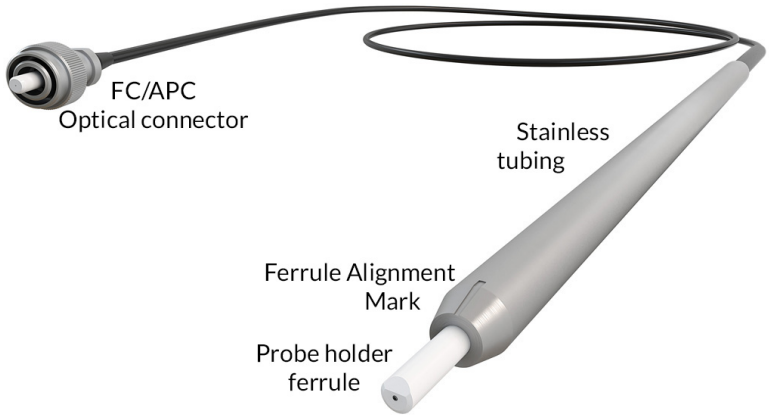


Figure 2.1: *Single-cell Opto-electric Probe Holder*

The *Single-cell Opto-electric Probe Holder* is used to transmit light to the probe and secure it in place.

- The **FC/APC optical connector** is used to connect the holder to an optical input/output.
- The **Stainless Tubing** is used to secure the holder in a stereotaxic apparatus using a *Clamp for single-cell opto-electric probe holder*.
- The **Probe holder ferrule** is a 2.5 mm diameter ferrule that is inserted into the *Single-cell Opto-electric Probe Adapter*.
- The **Ferrule alignment mark** is used to orient the probe holder when placed in the probe adapter.

2.2 Single-cell Opto-electric Probe Adapter

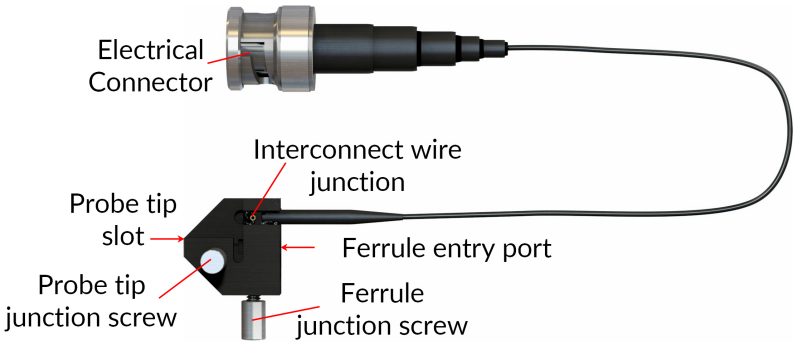


Figure 2.2: *Single-cell Opto-electric Probe Adapter*

The *Single-cell opto-electric probe adapter* is the link between the *Single-cell opto-electric probe holder* and the *Single-cell opto-electric probe tips*.

- The **Electrical connector** connect the adapter to an electrophysiology system. This connector can be either a *BNC* or a *Gold Pin*.
- The **Interconnect wire junction** is used to secure the connector of the *Single-cell opto-electric interconnect wire* and link it to the **Electrical connector**.
- The **Ferrule entry port** receives the **Probe holder ferrule**.
- The **Ferrule junction screw** is used to secure the **Probe holder ferrule**.
- The **Probe tip slot** accepts and aligns the *Single-cell Opto-electric Probe Tips*.
- The **Probe tip junction screw** secures the probe to the adapter.

2.3 Single-cell Opto-electric Probe Tips

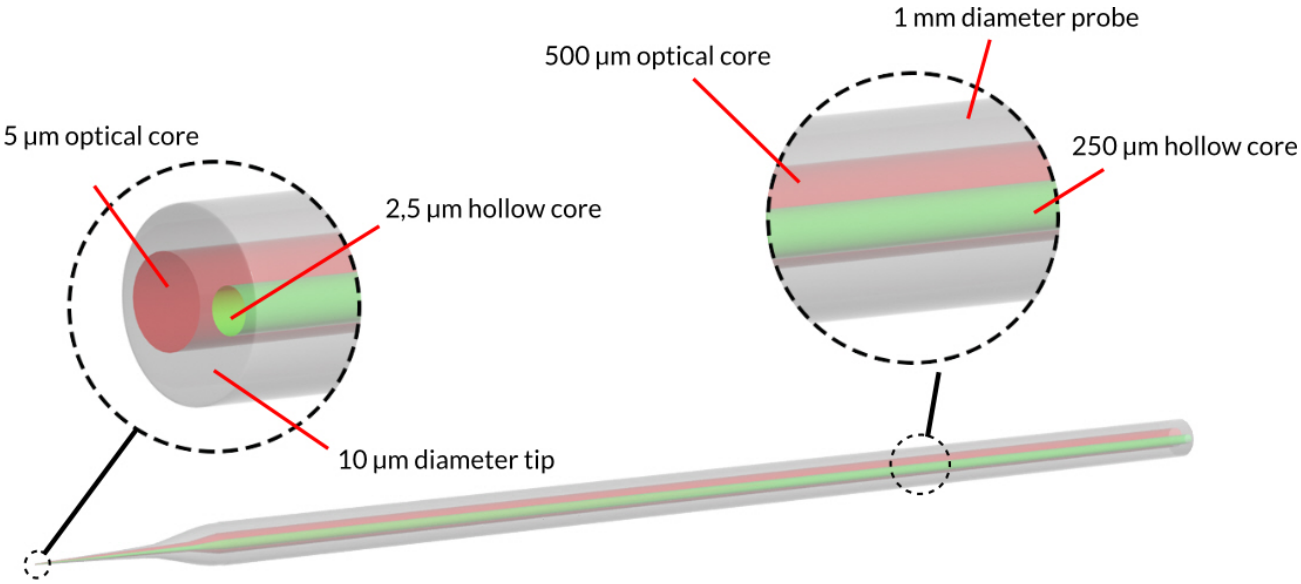


Figure 2.3: *Single-cell Opto-electric Probe Tips; diameters indicated are typical values*

The *Single-cell opto-electric probe tip* is a 1 mm diameter piece of dual-core optical fiber. One is end pulled and cut to a 10-20 μm diameter tip (Fig. 2.3). The probe is inserted into the *Single-cell opto-electric probe adapter* to align the optical core with the *Single-cell opto-electric probe holder*. All values indicated are typical.

- The **500 μm diameter optical core** is used to transmit light.
- The **250 μm diameter hollow core** is used to contain an electrolyte solution.
- The **10 μm diameter tip** allows the delivery of light into a single cell. At the tip, the optical core has a diameter of 5.0 μm, and the hollow core a diameter of 2.5 μm.

2.4 Single-cell Opto-electric Interconnect Wire

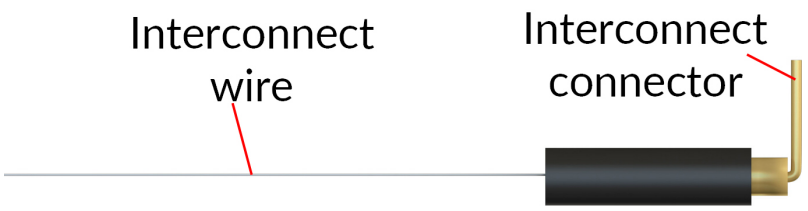


Figure 2.4: *Single-cell Opto-electric Interconnect Wire*

- The **Interconnect wire** is a 100 μm diameter silver wire that is inserted into the *Single-cell opto-electric probe tip* hollow core to provide electrical transmission.
  - It is recommended to **Chloride** the silver wire before every use.
- The **Interconnect connector** is an electrical pin made of gold-plated copper. It is inserted into the **Interconnect junction** to ensure electrical transmission.

2.5 Single-cell Recording Opto-electric Probe - Raw Fiber

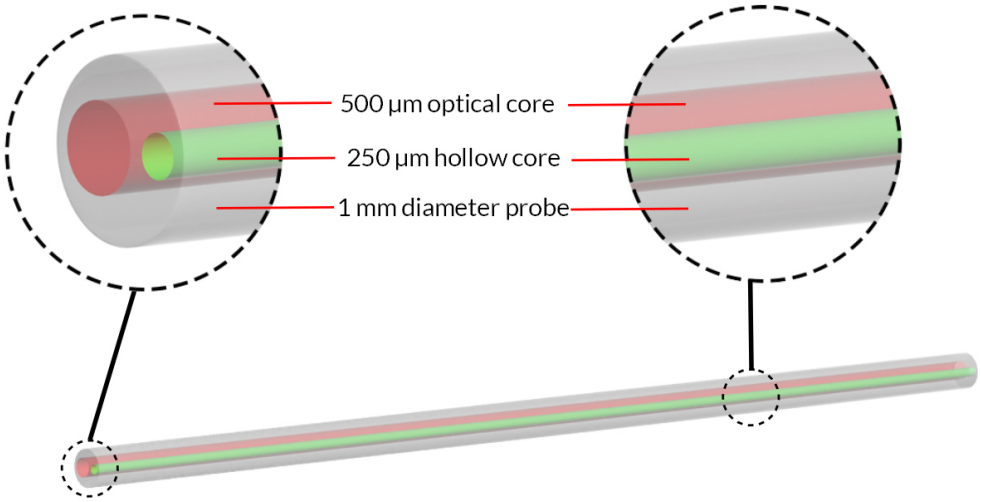


Figure 2.5: *Single-cell Opto-electric Probe-Raw Fiber*

Raw silica dual core fiber in lengths of 55 mm are available for those who prefer to prepare their own probe tips.

2.6 Stereotaxic Clamp for Single-cell Opto-electric Probe Holder

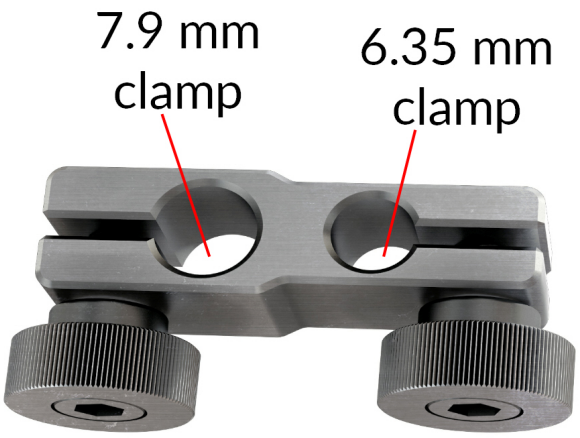


Figure 2.6: *Stereotaxic Clamp for Single-cell Opto-electric Probe Holder*

The *Single-cell recording systems* are provided with a *Stereotaxic clamp* to secure the *Single-cell opto-electric probe holder* in place. There are two clamp holes.

- The **7.9 mm diameter clamp** is secured on a stereotaxic apparatus.
- The **6.35 mm diameter clamp** secures the *Single-cell opto-electric probe holder*.

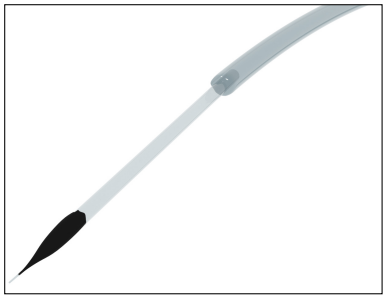
3.1 Electrophysiological recordings preparation

3.1.1 Filling the probe tip

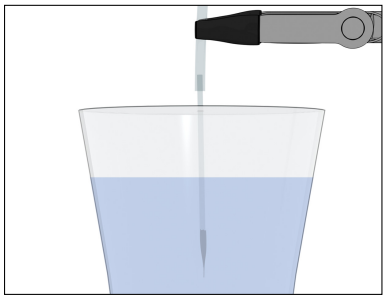
For electrophysiological recordings, the hollow core must be filled with a conductive electrolyte solution, **typically 1 to 3 M NaCl**. The filling requires negative pressure that can be obtained with a syringe or a vacuum.

When using a vacuum pump setup

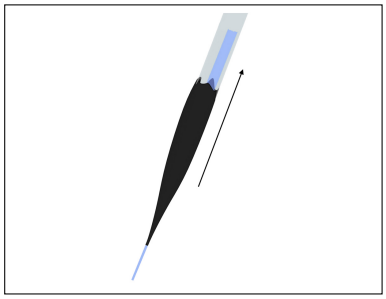
Connect one side of the silicone tubing to the vacuum setup and the other side to the large end of the hollow-core fiber (Fig. 3.1a). Seal the connections with a sheet of Parafilm M (Pechiney Plastic Packaging Company). Place the tapered tip of the probe in an electrolyte solution (Fig. 3.1b). At this step, make sure that the setup is stable to avoid any movement that could break the tip. Activate the pump to create a negative pressure in the hollow core fiber (Fig. 3.1c). Leave the probe tip in the solution long enough to fill the hollow-core up to 5 to 10 mm from the large side (Fig. 3.1d). The filling could take 1 to 15 minutes depending on the diameter of the tip and the negative pressure level. The tip must always be in the solution when vacuum is applied. **To prevent bubble insertion, always stop the vacuum before removing the tip from the solution**. Verify the filling level of the hollow-core.



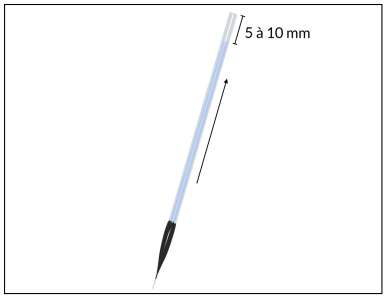
(a) *Install the silicone tubing on the large end of the hollow-core fiber*



(b) *Place the tapered tip of the probe in an electrolyte solution*



(c) *Activate the pump to create negative pressure in the hollow-core fiber*



(d) *Fill the hollow-core up to 5 to 10 mm from the large side*

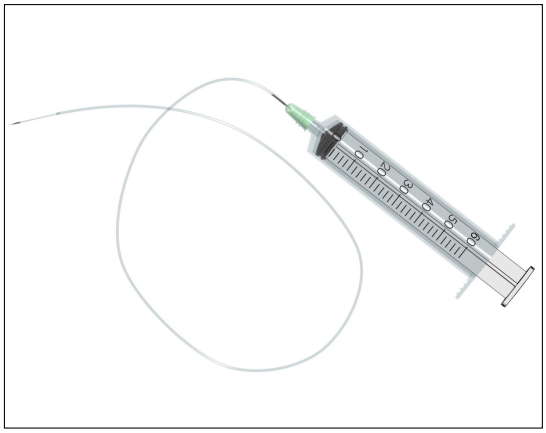
Figure 3.1: *Filling the probe tip with a vacuum pump setup*

It is critical to fill the tip with enough liquid to enable a contact between the electrolyte solution meniscus and the interconnect wire. If the filling is not sufficient, the electrolyte solution could flow along the wire due to capillary action. In

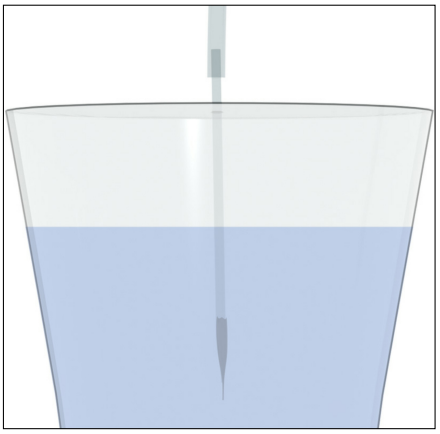
this case, the meniscus could drop and lose its contact with the tip of the wire, making the probe tip unusable for electrophysiological recordings. If the probe is not filled enough, submerge it into the electrolyte solution and recreate a vacuum. When the filling is sufficient, the probe can be connected to the holder. If the tapper is well filled, but lacking a small amount of fluid, it is possible to back-fill the hollow core with a micro-capillary and a syringe.

When using a syringe

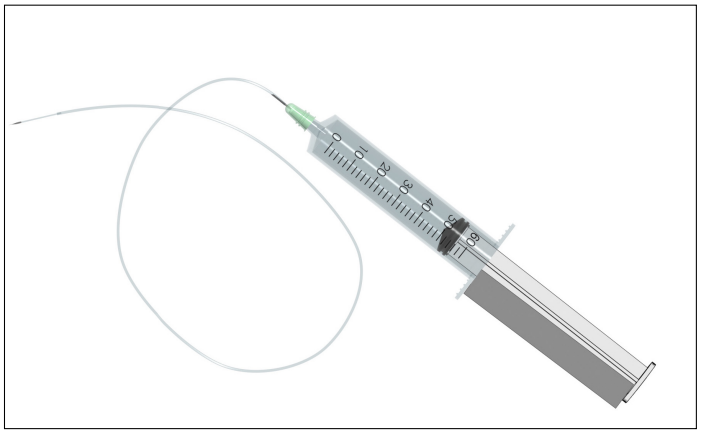
Put a plastic tube at the tip of a large volume syringe (60 ml is sufficient). Install the probe tip at the end of the tube (Fig. 3.2a) and insert the tapered tip in the electrolyte solution. Pull the plunger of the syringe to create a vacuum inside the syringe (Fig. 3.2b). Block the plunger with a syringe stopper to keep the hollow-core under negative pressure (Fig. 3.2c). Let the probe tip in the solution until it is sufficiently filled (the meniscus should be at 5 to 10 mm from the large end of the probe; Fig. 3.1d). The tip of the probe should be in the liquid when the tube is removed to avoid the insertion of bubbles. Seal with a sheet of Parafilm M (Pechiney Plastic Packaging Company). Finally, verify the filling level of the hollow-core fiber and if it is not filled enough, submerge it into the solution and recreate a vacuum.



(a) *Put a plastic tube at the tip of a large volume syringe and install the probe tip at the end*



(b) *Fill the syringe by pulling back the plunger*



(c) *Block the plunger with a syringe stopper to keep the hollow-core under negative pressure*

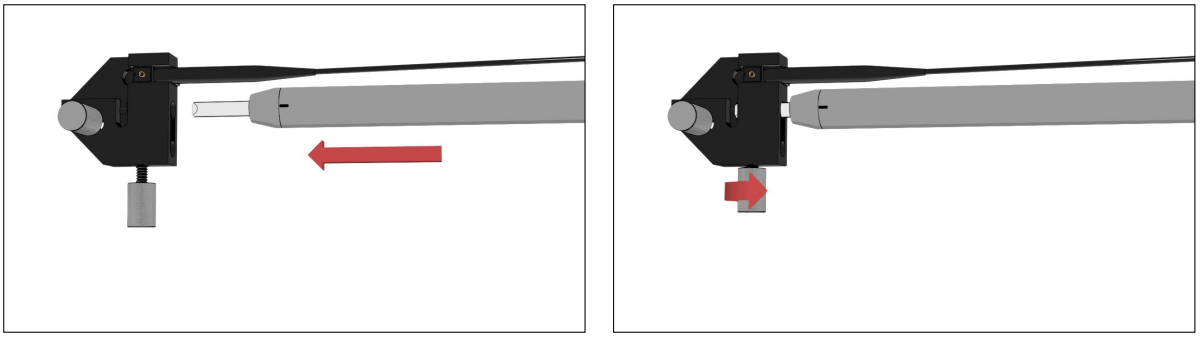
Figure 3.2: *Filling the probe tip with a large volume syringe*

3.2 Assembling the Single-cell Recordings Opto-electric Probe

Read the following instructions and watch our Instructional Video on the assembling of the Single-cell Recordings Opto-electric Probe.

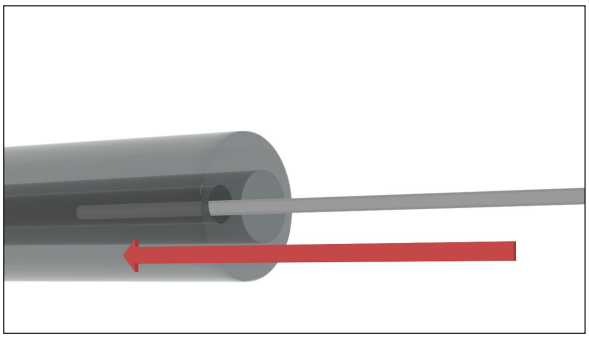
1. Insert the **Probe holder ferrule** into the **Ferrule entry port**, in such way that the **Ferrule alignment mark** faces the same direction as the **Interconnect wire junction** and is aligned with the **Probe tip slot** (Fig. 3.3a). **It is recommended to measure the impedance of the probe before insertion.**
2. Secure the holder with the **Ferrule junction screw** (Fig. 3.3b).
3. Localize the **Hollow-core** at the surface of the fiber (using a magnifying glass if necessary) and insert the **Interconnect wire** inside the hollow core (Fig. 3.3c).
4. The wire should be as straight as possible because a curved point could prevent insertion. This step is difficult at first, but becomes easier with practice. Once the wire is inserted further than the shoulder of the taper, bend the wire so that it forms an angle of 90 degrees (Fig. 3.3d).
5. Insert the probe tip in the **Probe tip slot** and align the interconnect wire with the slot (Fig. 3.3e).
6. Push the probe to create a contact with the **Probe holder ferrule** (Fig. 3.3f).
7. Rotate the probe tip and the interconnect wire together in the receptacle to align the **Optical core** of the probe tip and the optical core of the **Probe holder ferrule** in the holder (Fig. 3.3g). **The wire must point upward and be aligned with the *Ferrule alignment mark* on the probe holder** (Fig. 3.3j).
8. Secure the probe tip position using the **Probe tip junction screw** (Fig. 3.3h).
9. Insert the **Interconnect connector** in the **Interconnect wire junction** (Fig. 3.3i and 3.3j).
10. When all these steps are done, the single-cell opto-electric probe is ready to illuminate and collect fluorescence and electrical signal via the probe tip.

Warning: The probe tips are very brittle and can break easily. With the exception of brain tissue, the probe tip should not come into contact with other objects.



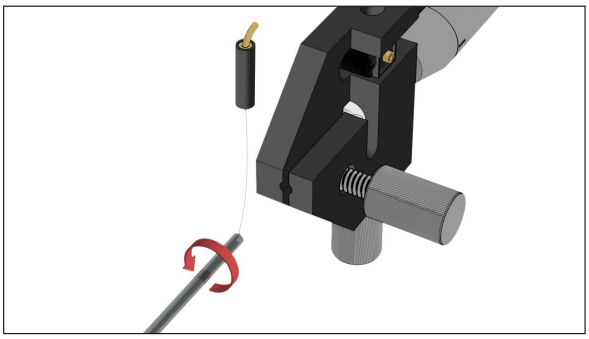
(a) *Insert the opto-electric probe holder in the adapter* (b) *Secure the holder with the patch cord junction screw*

Figure 3.3: *Assembling the Single-cell Recordings Opto-electric Probe*

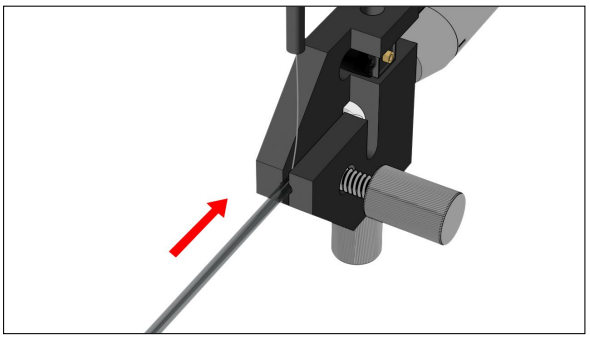


(c) *Insert the wire inside the hollow core*

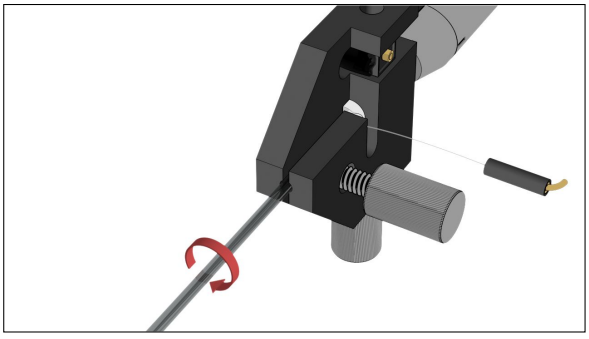
(d) *Bend the wire so that it forms an angle of 90°*



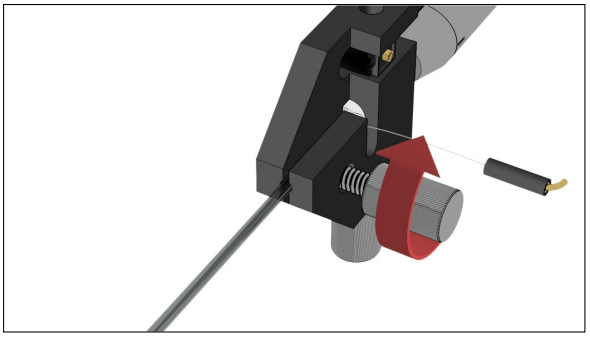
(e) *Align the interconnect wire with the slot*



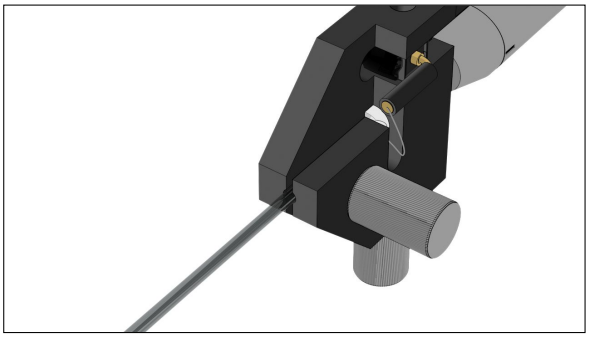
(f) *Push the probe to create a contact with the zirconia ferrule*



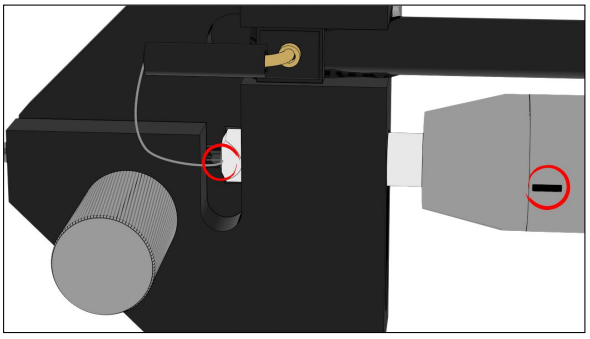
(g) *Rotate the probe in the receptacle to align the two optical cores*



(h) *Secure the probe tip position using the probe tip junction screw*



(i) *Insert the gold metal end of the interconnect wire in the hole on the adapter*



(j) *Ensure the wire and hollow core are aligned with the alignment mark*

Figure 3.3: *Assembling the Single-cell Recordings Opto-electric Probe (continued)*

3.3 Connecting the Single-cell Recording Opto-electric Probe

3.3.1 Electrical connection

The link between the electrolyte solution and the electrophysiological recording system is relayed by a metallic junction wire. The thin wire is combined with an electrical connector to pick up the signal. The **BNC or Gold pin electrical connector** of the *Single-cell Recordings Opto-electric Probe* should be connected directly to the electrophysiological recording headstage (Fig. 1.2 and 3.4). This probe system can be used as any standard electrophysiological glass probe.

> **Caution**: Standard coaxial BNC wire uses a grounded metallic shield. In this setup the wire is not coaxial and cannot be grounded/shielded. There is no metallic shield to prevent environmental noise amplification. Consequently, if a wire extension is used, ensure you do not use a wire with a metallic overlay.

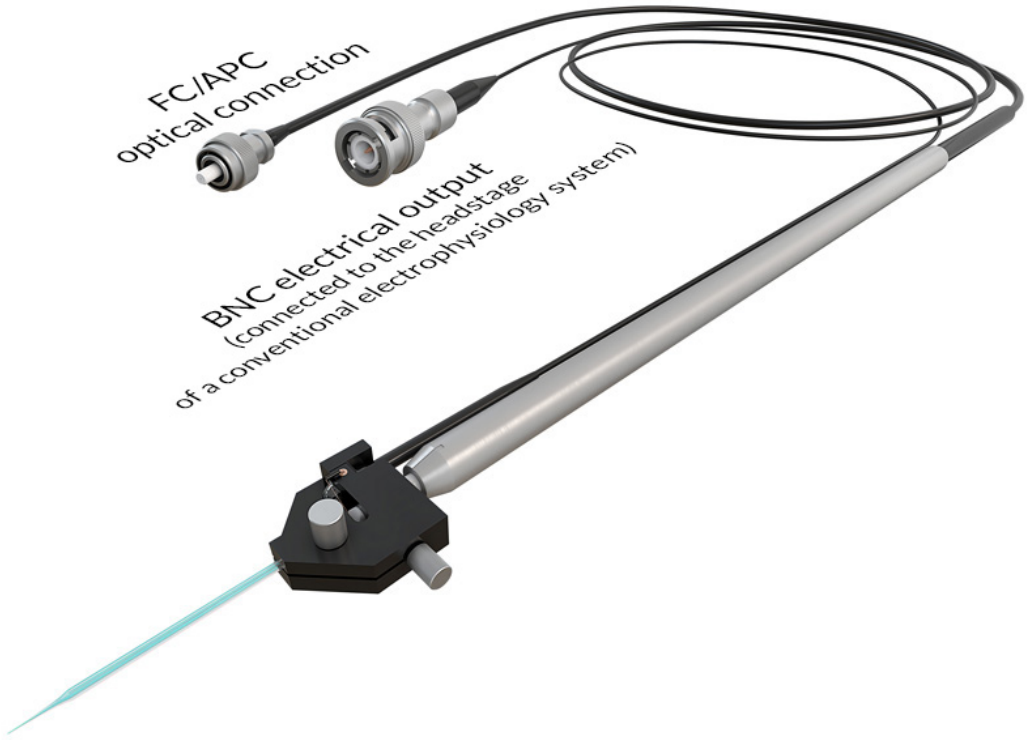


Figure 3.4: *Single-cell Recordings Opto-electric Probe connectors*

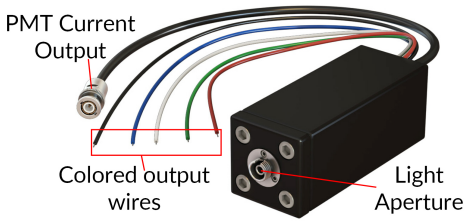
3.3.2 Optical connection

For fluorescence measurements, the FC/APC connector is connected to the sample port of a *Connectorized fluorescence mini cube* (Fig. 1.2 and 3.4). For optogenetic activation/deactivation, the fiber can be connected directly to a light source.

3.4 Fiber Photometry and Electrophysiology

3.4.1 Connection and activation of the photosensor modules

These steps should be done before the beginning of the experiment to set the gain and the sensitivity of the photosensors.



(a) *Hamamatsu H10722-20 Photosensor Module*

(b) *Hamamatsu H10722-20 Photosensor Module Power Supply*

Figure 3.5: *Hamamatsu H10722-20 Photosensor Module*

Warning: The *Hamamatsu H10722-20 Photosensor Module* is highly sensitive to light and can be easily damaged if too much light intensity is directed towards it when the power supply is activated.

1. Connect the *Hamamatsu H10722-20 photosensor modules (PMTs)* to the power supply (Fig. 3.5). Each **Colored output wire** (white, black, green, blue and red) corresponds to the same color connector at rear of the power supply. If two photosensor modules are connected to the same power supply, the latter will apply the same voltage simultaneously on both modules.
2. Connect the PMT **Current output** to the **Analog Input** of the *Fiber Photometry Console*. This output signal corresponds to the optical signal.
3. Turn down the **Voltage adjustment** to 0 V. It is very important to do this step before turning on the control voltage.
4. Verify that the **Control voltage** is in the OFF position and **Switch on** the power supply.
5. Turn on the **Control voltage**. The **Monitor** should light up and indicate 0.000.
6. At this point, you can control the voltage applied on the photosensor modules with the **Voltage adjustment**. This voltage will control the gain and the sensitivity of the photosensors. The power supply allows a voltage between .000 and 1.000 V. Note that the detector gain is not linear with the applied voltage. Refer to the photosensor module data sheet to know the exact value.

3.4.2 Preparation for the fluorescence detection

To perform fluorescence detection, follow these steps.

Warning: Before connecting or disconnecting patch cords to the excitation ports of the *connectorized fluorescence mini cube*, make sure that the power supply for *Hamamatsu H10722-20 photosensor modules* is off.

1. Connect the optical fiber patch cords (200 μm, NA 0.22) to the *Connectorized laser diode module* (FC connector, black strain relief) and to the *Fluorescence mini cube* excitation ports (E1, E2, E3 or AE) (FC/APC connector, green strain relief). Patch cords bringing light to the *Fluorescence mini cube* must be connected to the excitation ports (E1, E2, E3 or AE). Never connect these patch cords directly in the sample port as too much light damages the photosensor modules.

2. **Switch on** the photosensor power supply and set the **voltage adjustment** to 0.000 V.
3. Connect the FC/APC connector of the *Single-cell recording opto-electric probe* to the fluorescence mini cube sample port.
4. Turn on the light source driver. Light should be emitted from the tip.

3.4.3 Fluorescence detection validation

1. Remove the *Single-cell opto-electric probe adapter* from the *Single-cell recording opto-electric probe*.
2. Activate the *connectorized laser diode module*.
3. Turn up the **Voltage adjustment** of the detector to 0.300 — 0.400 V.
4. Bring the probe tip near a fluorescent object, such as a post-it note. If the system is properly connected, a signal should appear on the detector. This signal should fluctuate as the probe is brought closer/further from the fluorescent object.
5. Once these tests have confirmed proper fluorescence detection, it is necessary to repeat the steps taken in section 3.4.2 before starting any measurements.

4

Support

4.1 Contact us

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

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