



Single-color Miniature Fluorescence Microscopy System

User Manual

Version 3.3.3

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Systems Overview

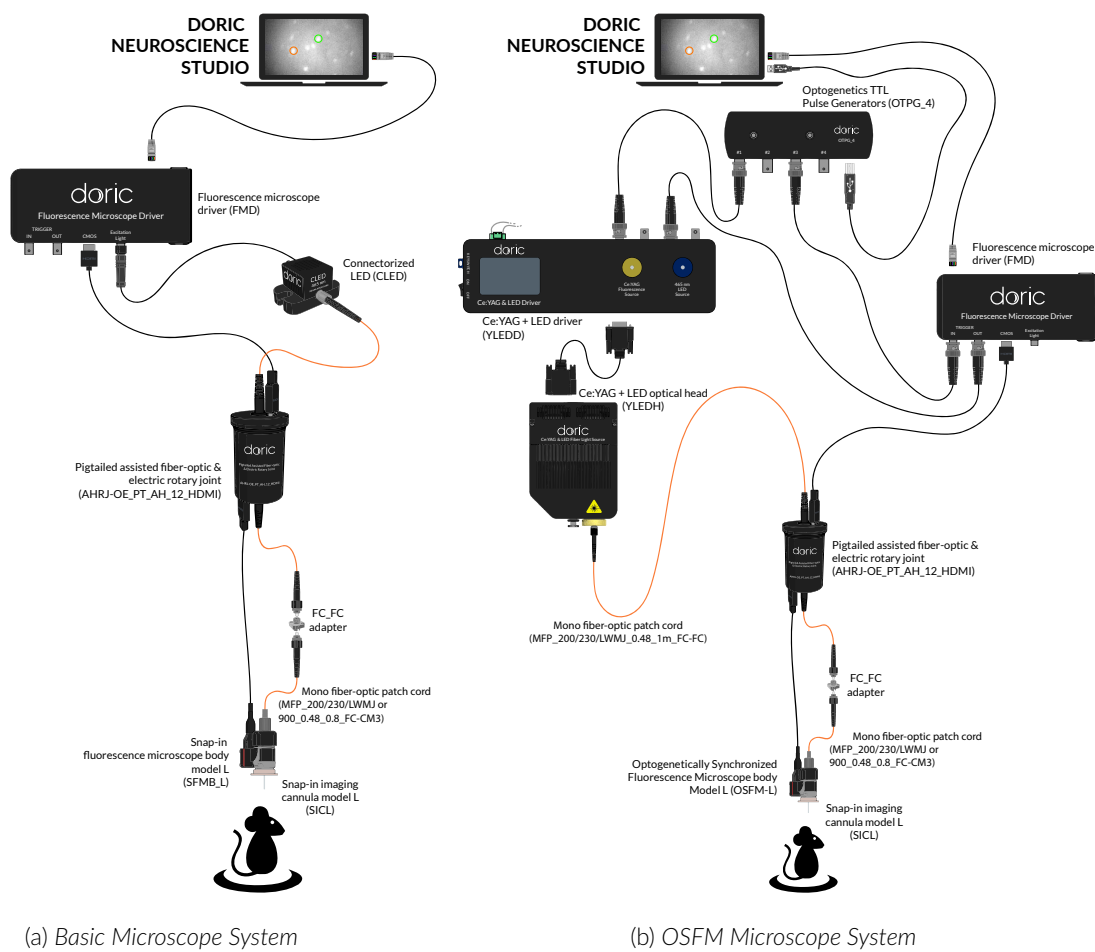


Figure 1.1: Microscope System Configurations

The *Single-color miniature fluorescence microscopy systems* are configured to image a single fluorophore within small animal subject brains. An *Imaging cannula* is used to send excitation light to fluorophores and receive the resulting fluorescence. A *Miniature fluorescence microscope body* (Section 2.1, 2.2) uses a CMOS camera to take fluorescence images transmitted by the cannula and sends them to a computer. Multiple configurations and modalities are available for a multitude of specialized applications.

1.0.1 Basic Fluorescence Microscopy Systems

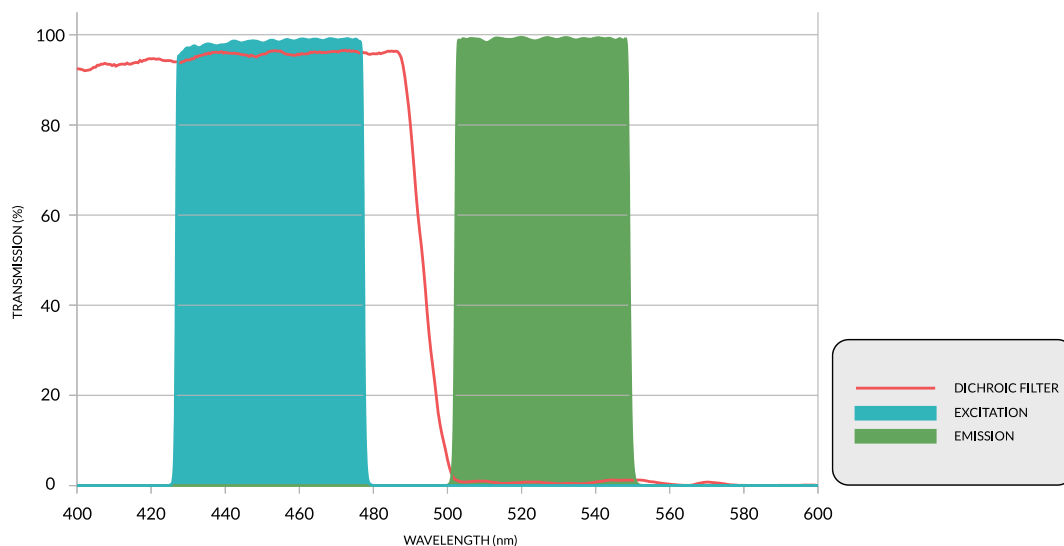


Figure 1.2: GCaMP6 Excitation Filter Set Spectra

The **Basic Fluorescence Microscopy System (BFMS)** (Fig. 1.1a) allows the imaging of a single fluorophore using a single wavelength light source. These systems are available in freely-moving and head-fixed configurations. The standard **GCaMP6** system (Fig. 1.2) uses a *Connectorized LED* as a light source, while the standard **RCaMP2** system (Fig. 1.3) uses a *Ce:YAG optical head*. Each system uses a different *Microscope Body* optimized for each fluorophore excitation/emission wavelengths.

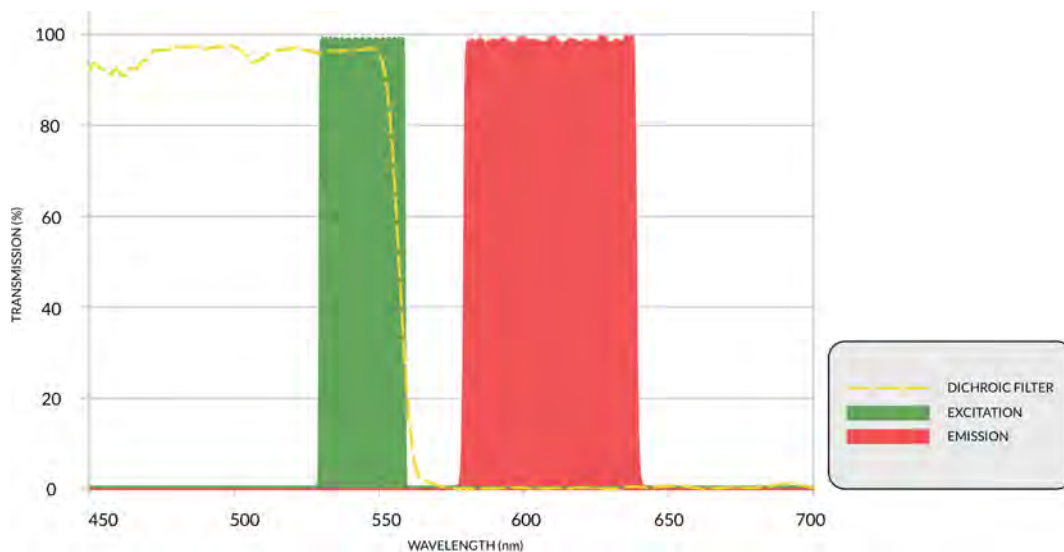


Figure 1.3: RCaMP6 Excitation Filter Set Spectra

1.0.2 Optogenetically Synchronized Fluorescence Microscopy Systems

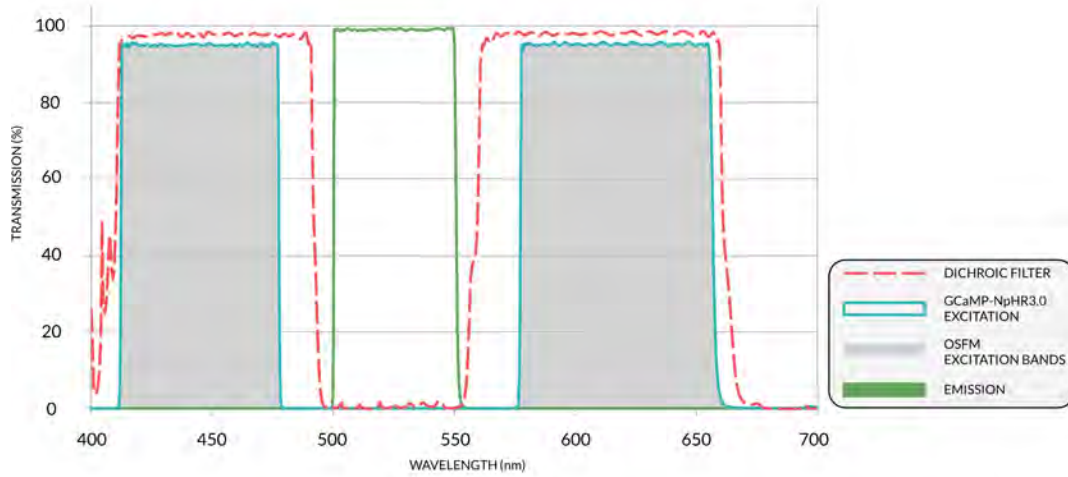


Figure 1.4: GCaMP6+NpHR3.0 Excitation Filter Set Spectra

The **Optogenetically Synchronized Fluorescence Microscopy (OSFM) system** (Fig. 1.1b) allows the imaging of a single fluorophore while performing optogenetic activation/deactivation. These systems are available in freely-moving and head-fixed configurations. When using a **GCaMP6 + NpHR3.0** microscope body a *Ce:YAG + LED optical head* is needed for illumination (Fig. 1.4). When using a **RCaMP2 + Chr2** microscope body a *Ce:YAG + Laser Diode optical head* is needed for illumination (Fig. 1.5).

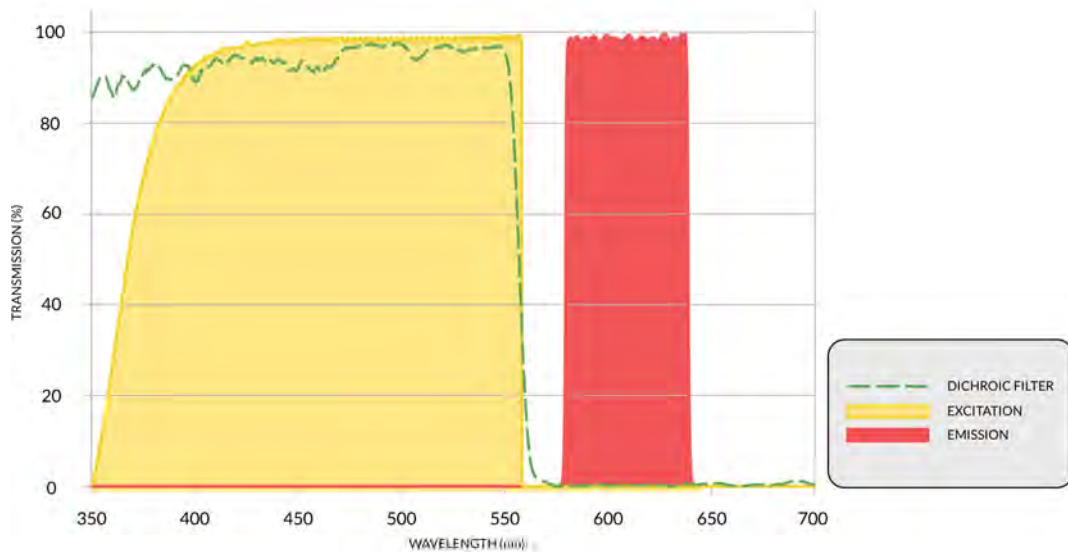


Figure 1.5: RCaMP2+Chr2 Excitation Filter Set Spectra

1.0.3 Freely-moving and Head-fixed Configurations

The **freely-moving** (Fig. 1.1a) configuration allows fluorescence microscopy to be performed while the subject is active thanks to a *Pigtailed assisted fiber-optic & electric rotary joint*. The **head-fixed** configuration is used in combination with stereotaxic instruments. It is also used for high-precision cannula implantation.

Device Overview

2.1 Basic Snap-in Fluorescence Microscope Bodies

The *SFMB Bodies* allow in vivo imaging of fluorescence within small animal subject brains. The microscopes are optimized to image calcium indicators like **GCaMP6** and **RCaMP2**. The Basic Snap-in Fluorescence Microscope Body is offered in two models: Model L for deep brain imaging and Model S for brain surface imaging. The Model L has a 0.5 NA objective lens within its body and is used with an implantable imaging cannula that transfers the image to the microscope CMOS camera. The Model S has a plan-parallel plate instead and relies on the objective lens within the model S imaging cannula to create an image on the CMOS. The *SFMB* are composed of the following elements.

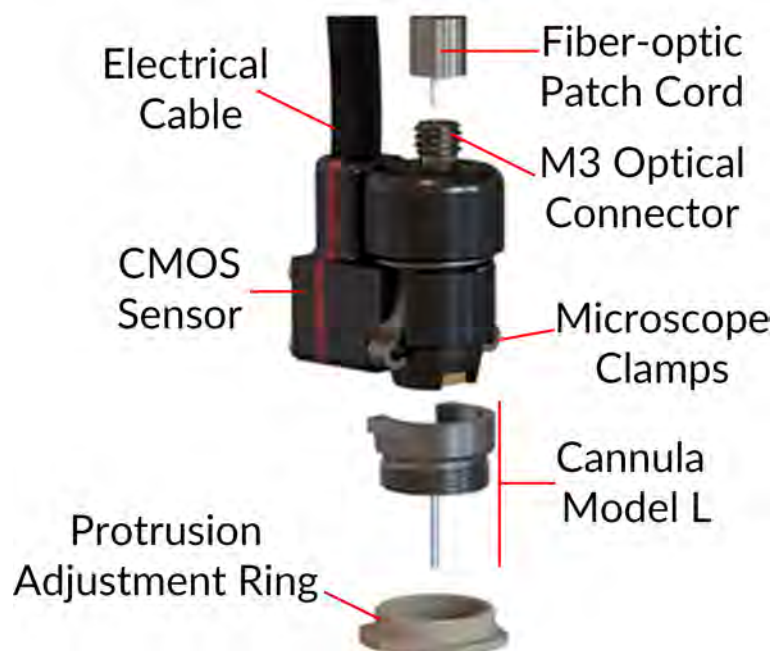


Figure 2.1: Basic Snap-in Fluorescence Microscope Body

- The **Fiber-optic patch cord** connects to the **M3 optical connector**, bringing light to the microscope. The patch cord length is chosen to correspond to the electrical cable length.
- The **Electrical cable** sends and receives electrical signal for the microscope using an HDMI connector, with a standard length of 1 m. The cable is jacketed in either the light and flexible **Ultralight fiberglass jacket** or the robust but heavier **Lightweight metal jacket**.
- The **Microscope clamps** secure the microscope on an imaging cannula. This allows the removal of the microscope body between each imaging session.

- The **CMOS sensor** records fluorescence images. Each **CMOS sensor** has a serial number stored in memory that links each microscope to a specific set of mask correction filters recognizable to our software package.
- The **Imaging cannula** and **Protrusion adjustment ring** are described in Section 2.3.

2.2 Optogenetically Synchronized Fluorescence Microscope Bodies

The *OSFM bodies* combine fluorescence imaging and optogenetic stimulation/inhibition capabilities within the *Single-color miniature fluorescence microscope*. To avoid cross talk between optogenetic stimulation and fluorescence imaging, the *OSFM* hardware provides for at least two distinct spectral bands for optogenetic activation or fluorophore excitation (like blue and yellow). As the field of opsins and calcium indicators is very dynamic, spectral bands can be tailored to specifications. **GCaMP6 + NpHR3.0** and **RCaMP2 + ChR2** microscope versions are currently available. The appearance (Fig. 2.1) and general specifications (Table 5.1) of the *OSFM Body* are identical to the *BSFM Body*.

2.3 Snap-in Imaging Cannulas

Snap-in imaging cannulas transmit images of structures located inside of the brain to the surface of the skull. Cannulas have the following elements.

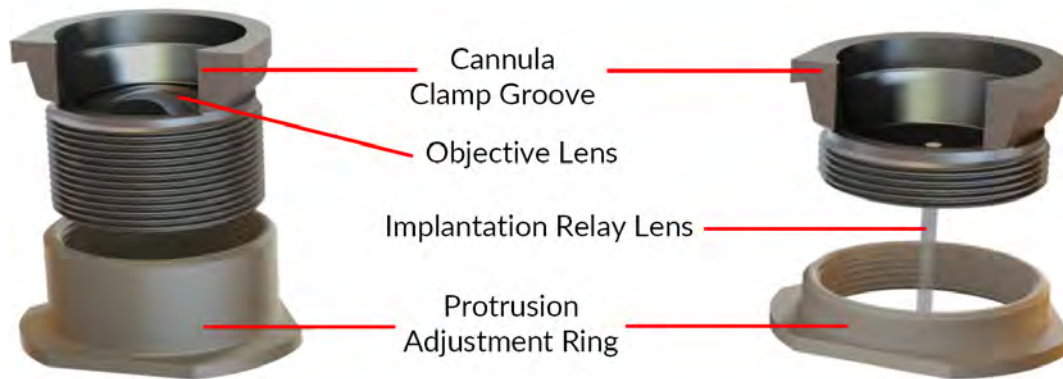


Figure 2.2: Snap-in imaging cannula type S (left) and type L (right)

- The **Cannula clamp groove** is used to secure the microscope body in place using **Cannula clamps**.
- The **Implantation relay lens** is found on the *Model L imaging cannula* (Fig. 2.2 right). It is an image guiding gradient-index rod lens that brings the image from inside the brain to the skull surface.
- The **Objective lens** is found on the *Model S imaging cannula* (Fig. 2.2 left). For areas near the brain surface (less than 150 μm below the skull) it provides higher image quality and a larger FOV than the **Model L imaging cannula**.

A set of rod lenses with different lengths is available to reach different depth ranges of the brain tissue. Fine focusing of the **Model L imaging cannula** is done with the protrusion adjustment ring that comes with each cannula (Fig. 2.3). The **Model S Imaging Cannula** uses a single protrusion adjustment ring (height of 4.5 mm).



Figure 2.3: Model L Imaging Cannula Protrusion Adjustment Rings. Height from left to right: 2.05 mm, 2.77 mm, 3.48 mm, 4.20 mm and 4.92 mm

2.4 Dummy Microscope



Figure 2.4: Dummy Microscope

The **Dummy Microscope** is of similar weight and size as the microscope bodies. It is used to habituate animal subjects to the weight and feel of the microscope.

2.5 Pigtailed Assisted Fiber-optic & Electric Rotary Joint

To use the microscope in experiments using freely-moving animals, the *Pigtailed Assisted Fiber-optic & Electric Rotary Joint* (Fig. 2.5) is provided. This rotary joint allows effectively frictionless rotation of optical fibers and electrical cables connected to the microscope.

- The **Pigtailed Patch Cords** transmit light from the light source to the microscope. They are integrated into the rotary joint for maximal optical signal stability. The fixed and rotary patch cords use an FC connector.
- The **HDMI connectors** are used to provide electrical communication between the driver and the microscope.
- The **5 V mini-USB-B connector** connects to the power supply to allow assisted rotation.



Figure 2.5: Pigtailed Assisted Fiber-optic & Electric Rotary Joint

2.6 Fluorescence Microscope Driver

This driver allows for computer control over the excitation LED light source as well as microscope image capturing and broadcast at video rate to a single computer via high speed Ethernet communication. It can be triggered by or synchronized with external recording devices and it can also trigger other devices using 5V TTL signals (Fig. 2.6). This Fluorescence Microscope Driver is used with the *SFMB*, the *OSFM*, the *eSFMB* and the *eTFMB*.

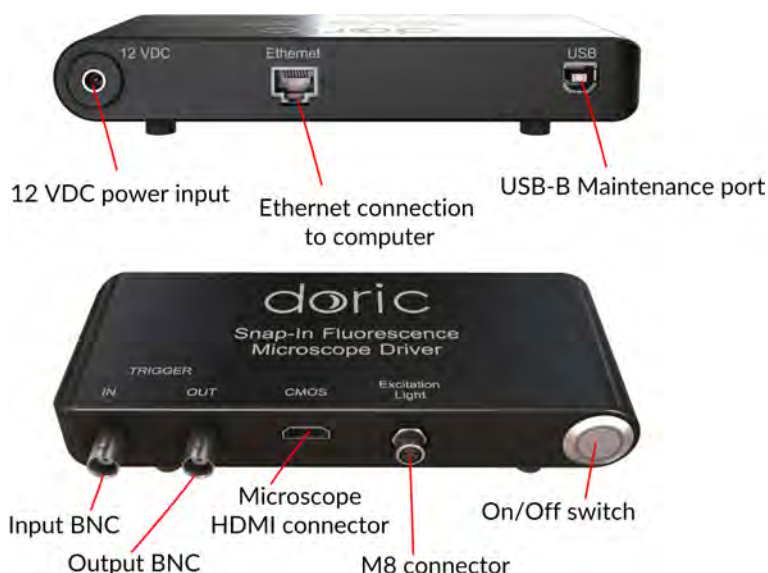


Figure 2.6: Fluorescence Microscope Driver

- The *Fluorescence Microscope Driver* has a **12 VDC** power input (Fig. 2.6) to which the user connects the power supply provided with the system.
- The **Ethernet** port (Fig. 2.6) connects the driver to a computer using an Ethernet cable.
- The **USB-B Maintenance Port** (Fig. 2.6) is used to update driver firmware.
- The **Input BNC** (Fig. 2.6) allows the user to control the microscope using an outside, analog trigger source.
- The **Output BNC** (Fig. 2.6) provides a trigger signal corresponding to the capture of microscope images. This signal is used to trigger other devices.
- The **Microscope HDMI Connector** (Fig. 2.6) is used to connect the microscope to the driver.
- The **M8 connector** (Fig. 2.6) is used to connect the driver to a *CLED* light source.
- The **On/Off Switch** is used to turn the driver On/Off. When linking to the software, a blue circle will flash around the button. Once the driver is properly linked, the blue circle will stop flashing.

2.7 Fluorescence Microscope Snapping Tool



Figure 2.7: Fluorescence Microscope Snapping Tool

The *Fluorescence Microscope Snapping Tool* is used to remove the **Microscope clamps** from the **Cannula clamp groove**.

2.8 Fluorescence Microscope Holder

The *Fluorescence Microscope Holder (FMH)* and the *Clamp for Fluorescence Microscope Holder* are used to secure the microscope in a stereotaxic system.

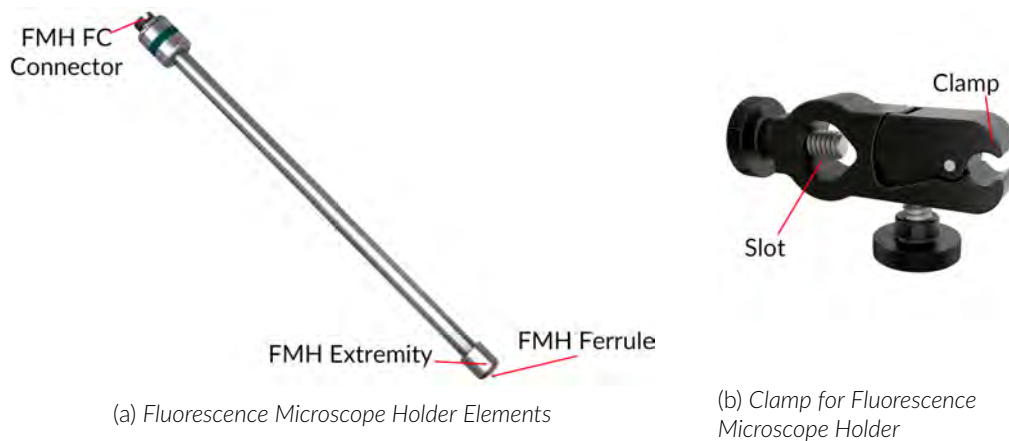


Figure 2.8: Fluorescence Microscope Holder

- The *Clamp for Fluorescence Microscope Holder* (Fig. 2.8b) can be secured to a rod in a stereotaxic system using the **Slot**. The **Clamp** secures the *Fluorescence Microscope Holder* while still allowing easy removal of the holder.
- The **FMH FC Connector** allows the holder to be connected to an FC-connectorized patch cord.
- The **FMH Ferrule** is inserted into the microscope **M3 Optical Connector**. The ferrule is secured by screwing the **FMH Extremity** onto the **M3 Optical Connector**.

Operations Guide

3.1 Connecting the Microscope Driver

- Connect the microscope driver to the power outlet using the 12 V power supply.
- Connect the microscope driver to the computer or to the router using the Ethernet cable.
- Connect the *Electrical Cable* (or the HDMI cable when used with a *Pigtailed Assisted Fiber-optic & Electric Rotary Joint*) to the CMOS port on the driver.
- Push the power button. After ~5 seconds the LED will turn ON and start to blink.

3.2 Setting Up The Communication

In order to communicate with the driver, the IP address of the computer must be static. If the driver is connected to a router, jump to section 3.3.3. If the driver is connected directly to the computer, continue to section 3.2.1.

3.2.1 Configuring Static IP Address

To change the computer's IP address in Windows 7, type *network and sharing* into the Search box in the Start Menu and select Network and Sharing Center. If you are in Windows 8, it will be on the Start Screen itself (Fig. 3.1).

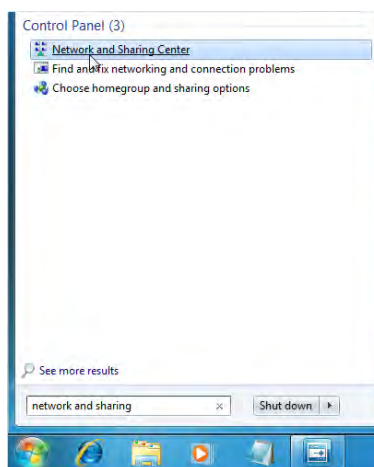


Figure 3.1: Open Network and Sharing Center.

On the side menu, select Change Adapter Settings (Fig. 3.2).

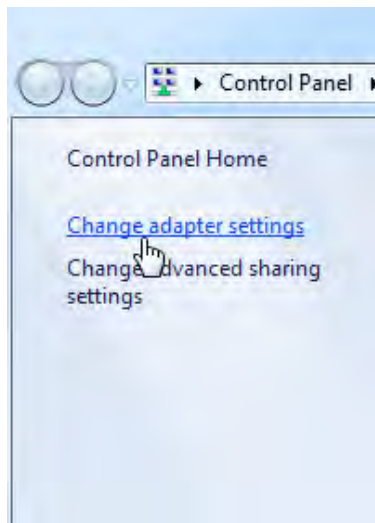


Figure 3.2: Click on *Change Adapter Settings*.

Right-click on the local adapter and select Properties (Fig. 3.3).

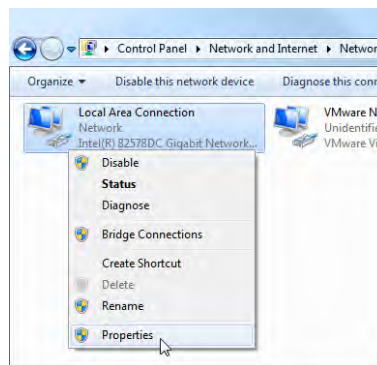


Figure 3.3: Right-click on local adapter Properties.

Select Internet Protocol Version 4 (TCP/IPv4) in the list, and click on Properties (Fig. 3.4).

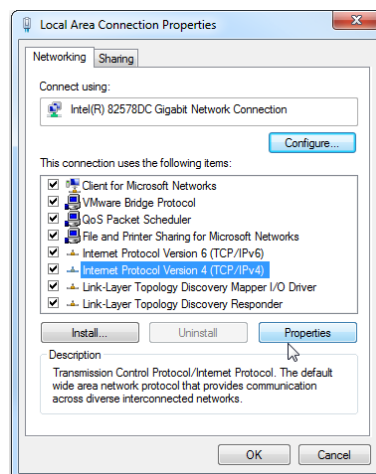


Figure 3.4: Open IPv4 Properties.

Use the following IP address, and set the new IP address to **192.168.1.149**, and the Subnet mask to **255.255.255.0**. Leave the Default gateway and the DNS settings empty. Finally, click OK and close the Network Center (Fig. 3.5).

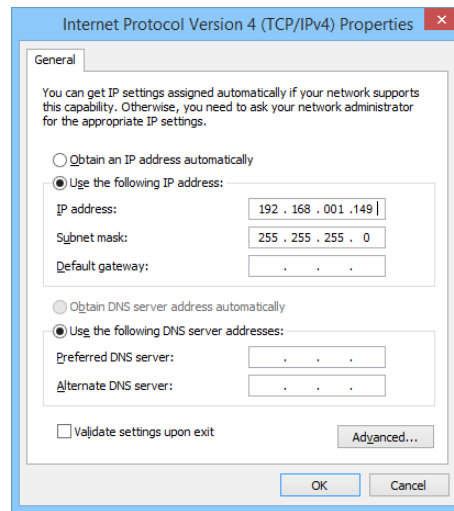


Figure 3.5: Static IP settings.

3.3.2 Activating The JUMBO Ethernet Frames

In order to reduce the load on the computer CPU, the Doric Neuroscience Studio is using JUMBO frames for the image transfer. In order to activate the JUMBO frames, open the Ethernet interface Properties menu as shown in Fig. 3.3 and click *Configure* (Fig. 3.6).

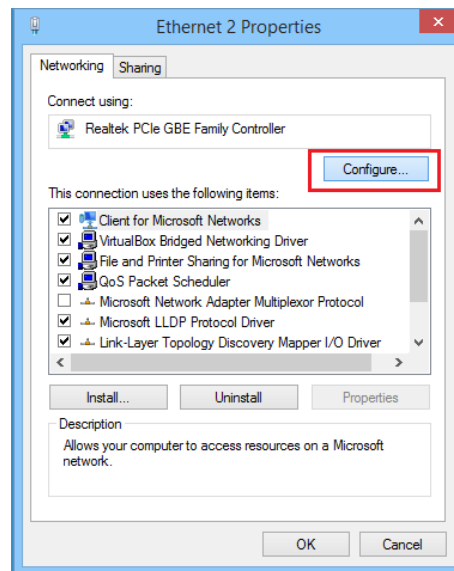


Figure 3.6: Interface *configuration*.

In the Configuration window, click on the *Advanced* tab, and on *jumbo frames* in the list. From the choices, select a value >4 KB MTU, the bigger the better (Fig. 3.7).

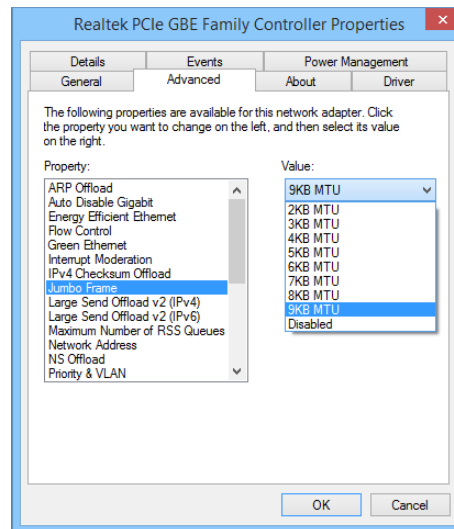


Figure 3.7: Jumbo frame configuration.

3.3.3 Configuring The Driver IP Address

The microscope must be connected to the driver in order to proceed.

When connected for the first time, the microscope driver will broadcast its MAC address. To set the IP address, you can use the built-in ethernet setup interface in the controller software.

- Turn ON the microscope driver.
- Launch the Doric Neuroscience Studio software.
- Allow ~10 seconds for the microscope initialization.
- Choose the proper network interface from the list in the *Ethernet Settings* box, and click on **Pair** and **Remember** to configure the microscope driver.

When the system is ready to use, the power switch LED will stop blinking, and the software status will display *The microscope is ready*.

3.3 General Setup Guidelines

3.3.1 Optical fiber patch cord use

1. Clean the optical fiber connector before insertion. Use isopropanol and a lint-free wipe.
2. With an FC connector, the connector key must be oriented to enter within the receptacle slot to ensure proper connection (Fig. 3.11).

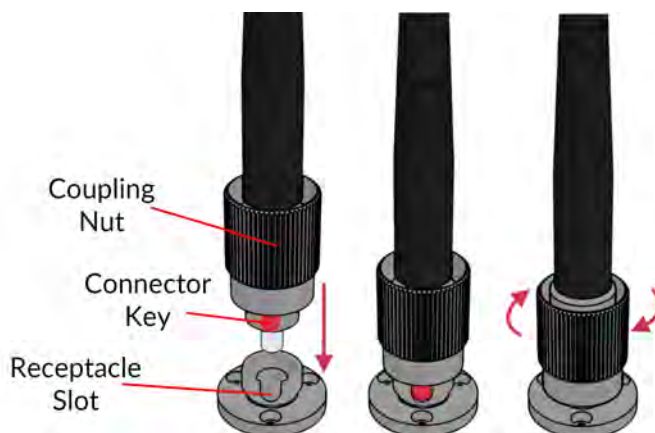


Figure 3.8: FC connector, Fiber Installation

⚠ To reduce the risk of eye injury, it is sound practice to NOT CONNECT/DISCONNECT OPTICAL FIBERS when the light source is turned on.

3.3.2 Microscope Clamping Procedure

For details concerning the imaging cannula handling and implantation, as well as the microscope clamping procedure, it is important to read the [Snap-in Miniature Fluorescence Microscope Surgical Procedure](#), as well as our [Instructional Video](#).

3.4 Connecting the Fluorescence Microscope System

There are multiple different fluorescence microscope system configurations depending on the desired freedom of the animal and the neurons targeted. The animal must be held in one of the two following configurations:

- The **Head-fixed** configuration (Section 3.4.1, Fig. 3.10a) allows the observation of the animal while placed in a stereotaxic apparatus.
- The **Freely-Moving** configuration (Section 3.4.2, Fig. 3.10b) allows the observation of the animal while moving freely in a cage.

Depending on the *Microscope Body* type, several different lighting configurations are used. The most common are presented in this manual.

- The **SFMB GCaMP6** configuration (Section 3.4.3, Fig. 3.11a) allows the imagery of **GCaMP6** fluorescence.
- The **SFMB RCaMP2** configuration (Section 3.4.3, Fig. 3.11b) allows the imagery of **RCaMP2** fluorescence.
- The **OSFM GCaMP6 + NpHR3.0** configuration (Section 3.4.4, Fig. 3.12a) allows the imagery of **GCaMP6** fluorescence with **NpHR3.0** activation.
- The **OSFM RCaMP2 + ChR2** configuration (Section 3.4.4, Fig. 3.12b) allows the imagery of **RCaMP2** fluorescence with **ChR2** activation.

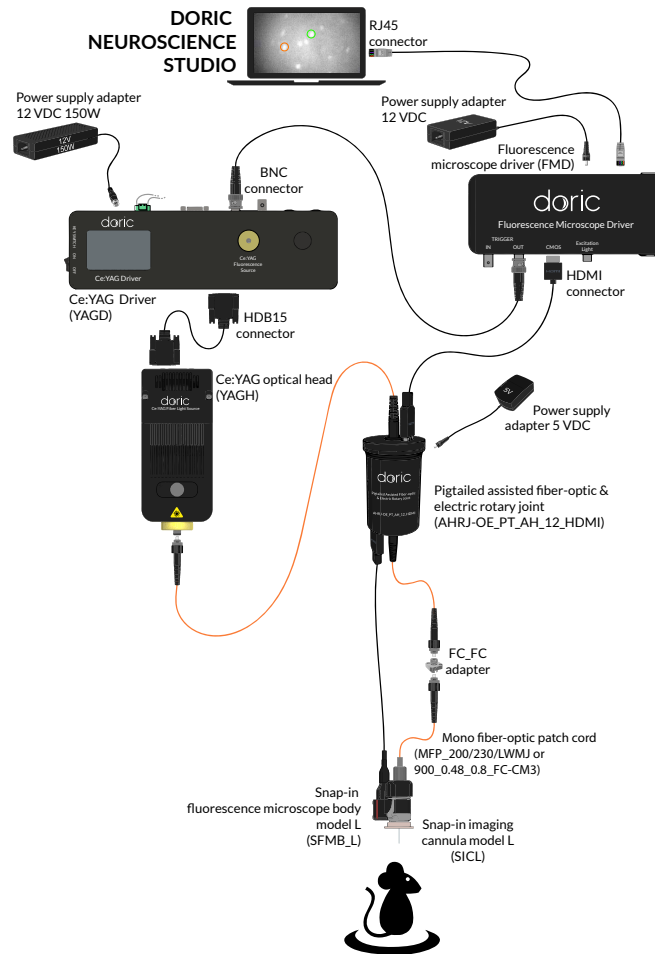


Figure 3.9: Full RCaMP2 Freely-Moving Configuration, combination of (Fig. 3.10b) and (Fig. 3.11b)

Figure 3.10 shows the head-fixed and freely-moving system configurations. Each microscope body and lighting configuration uses the same devices in each of these configurations.

3.4.1 Head-fixed configuration installation

1. Install the *Fluorescence microscope holder* into the *Stereotaxic clamp* (Fig. 3.10a). Ensure the holder is tightly secured inside the clamp.
2. To secure the microscope on the *Fluorescence microscope holder*:
 - a) Remove the **Connector caps** from the microscope **M3 optical connector** and the *Fluorescence microscope holder* ferrule.
 - b) Insert the ferrule into the **M3 optical connector**. Secure them in place by screwing the *Fluorescence Microscope holder* extremity.
3. Install the *Fluorescence microscope holder* in a stereotaxic apparatus.
4. Connect the *Fluorescence microscope holder* and the light source using a fiber-optic patch cord. The patch cord characteristics (MFP 200/230/LWMJ-0.48 1m FC-FC) are set to achieve optimal coupling efficiency with the *Fluorescence microscope holder*.
5. Connect the **Pigtailed microscope cable** to the driver **HDMI connector**.
6. When ready for use, remove the cannula protective cap by unscrewing it. If using a type-L microscope, take great care to remove the cap in a slow, straight motion so as not to break the rod lens inside.

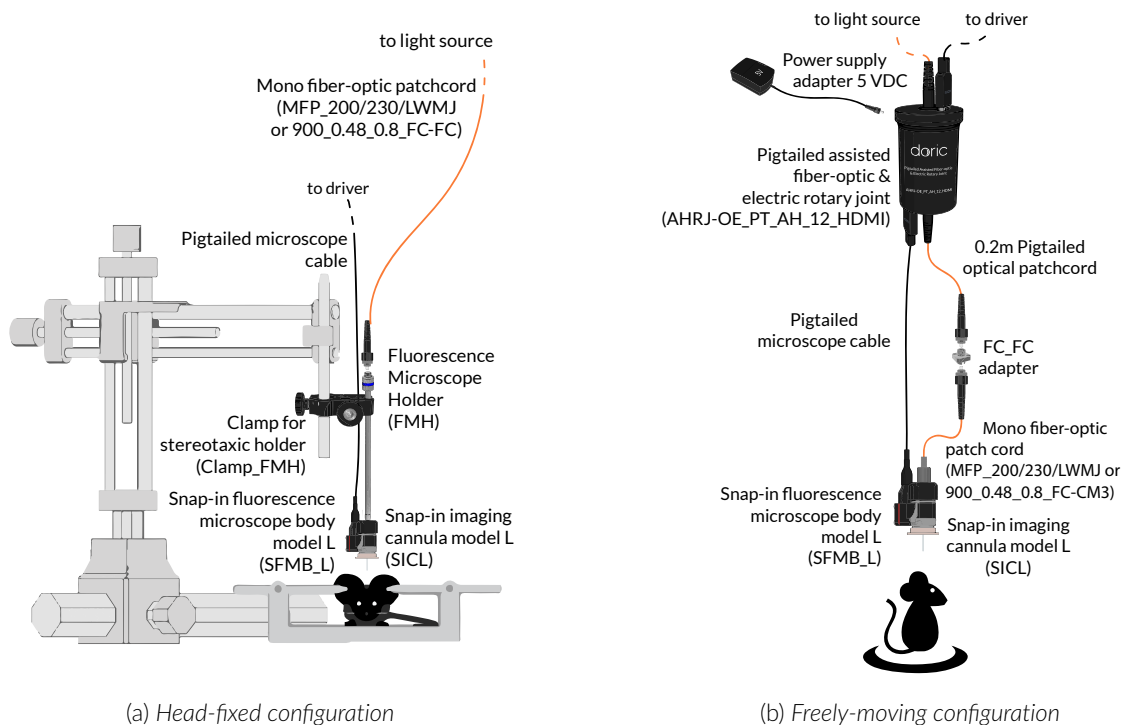


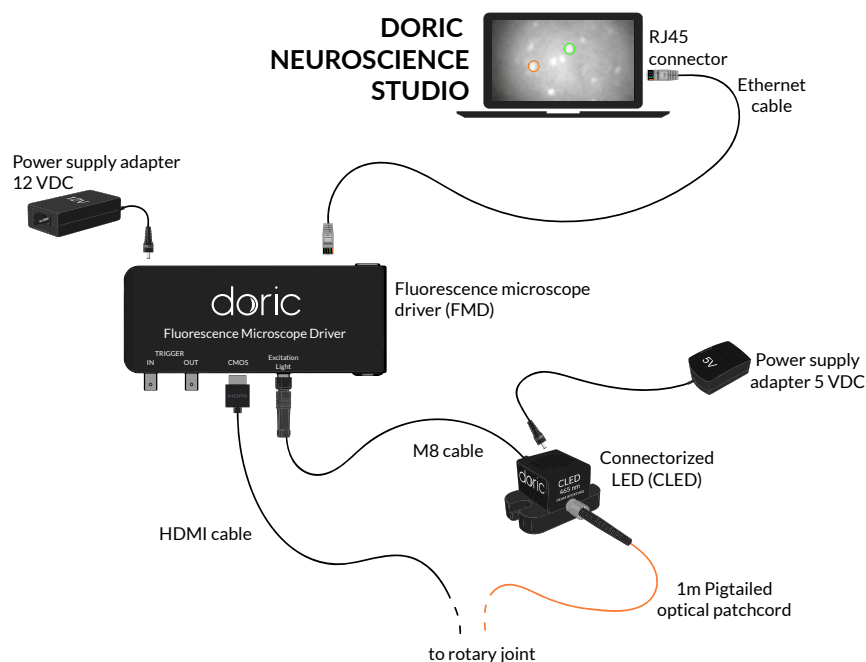
Figure 3.10: BSFM Body System Connections

3.4.2 Freely-moving configuration installation

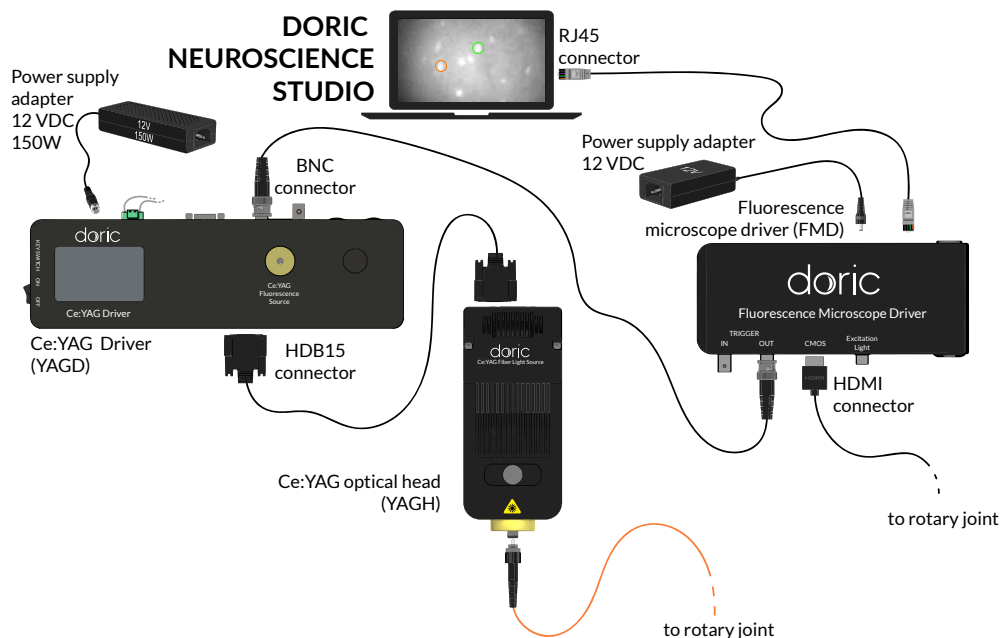
1. Link the driver and the *Pigtailed assisted fiber-optic and electric rotary joint* using the HDMI cable (Fig. 3.10b).
2. Connect the top pigtailed patch cord to the light source **Beam aperture**.
3. Connect the microscope **Pigtailed HDMI Cable** to the rotary joint.
4. Connect the lower pigtailed patch cord of the rotary joint to the *FC mating adapter*, then connect the mono fiber-optic patch cord (FC-CM3) to the microscope. The patch cord (MFP 200/230/LWMJ-0.48 0.8m FC-CM3) linking the Pigtailed Assisted Fiber-optic & Electric Rotary Joint (AHRJ) and the microscope is chosen to ensure proper illumination characteristics.
5. Remove the **Input Protective Cap** from the cannula and insert the microscope body.

3.4.3 Connecting the BSFM

The *BSFM body* comes with two standard models; the first allows **GCaMP6** fluorescence imagery while the second allows **RCaMP2** fluorescence imagery.




(a) GCaMP6 system configuration



(b) RCaMP2 system configuration

Figure 3.11: BFMS Body Illumination System Connections

Microscope Driver and Light Source Installation

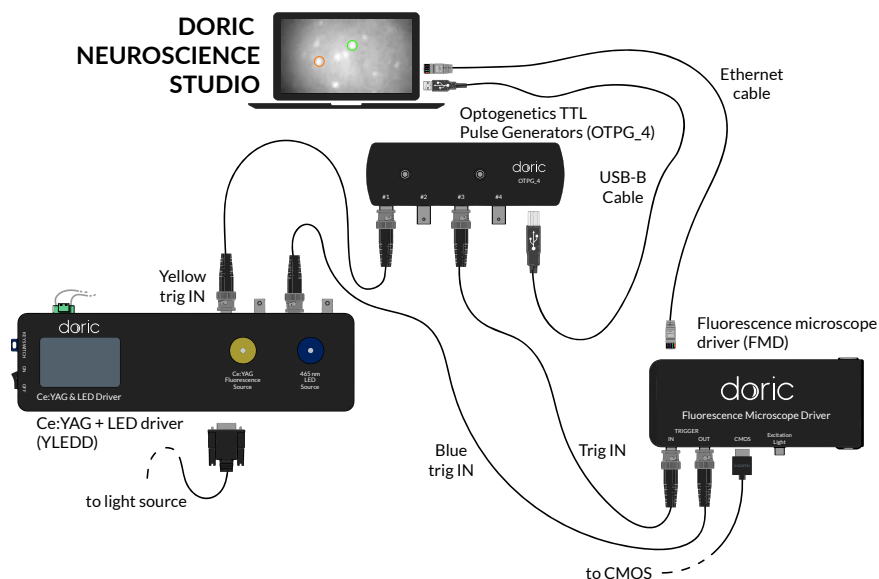
 If using the *Ce:YAG Optical head*, connect the **Interlock connector plug** to a **Laser safety interlock circuit** for safe use. See the application note [Important Laser Safety Information](#) for more information, or contact your institution's laser safety officer.

1. Connect the 12 VDC power supply to the microscope driver **12 VDC power input**.
2. Connect the driver and the light source.
 - If using an LED light source (such as for the **GCaMP6** system (Fig. 3.11a)), connect the driver to the LED using the **M8 connector**.
 - If using a *Ce:YAG Optical Head* (such as for the **RCaMP2** system (Fig. 3.11b)), see the **Ce:YAG manual** for details on driver and light source installation. Once complete, connect the *Fluorescence microscope driver* **BNC Output** to the *Ce:YAG Driver* **BNC Input**. The *Ce:YAG Driver* must be put in **TTL** mode to receive proper signals from the *Microscope driver*.
3. Connect the driver to the computer using the *CAT5E Ethernet Cable*.
4. Turn on the *Fluorescence Microscope Driver*.

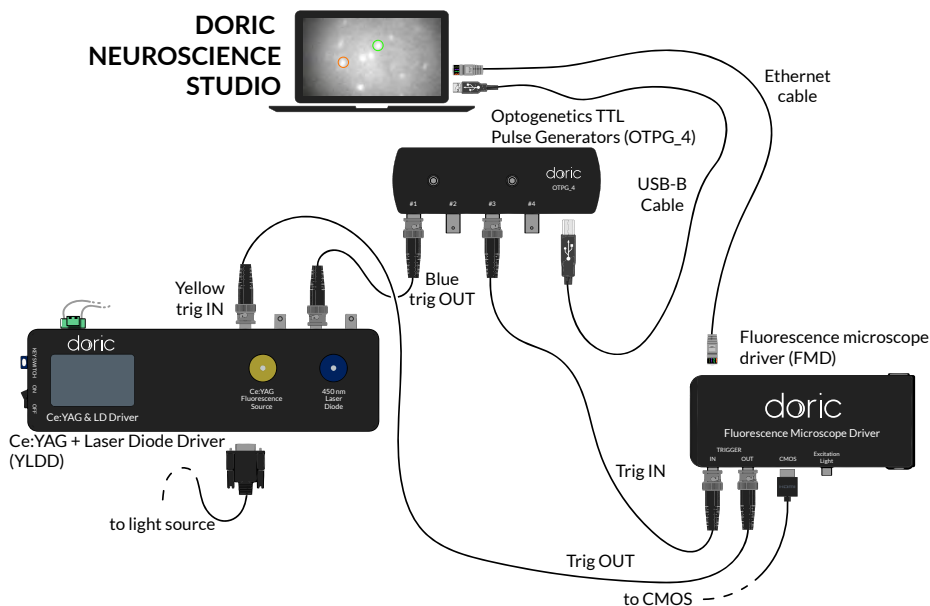
3.4.4 Connecting the OSFM system

The indications in section 3.7, notably Fig. 3.10b and Fig. 3.10a are to be followed for the installation of the **OSFM**. As the **OSFM** uses two light sources, driver and light source installation is more elaborate. Both standard configurations use the *Ce:YAG + LED/LD fiber Light Source* to illuminate the sample¹.

Driver and light source installation



(a) GCaMP6 + NpHR3.0 configuration



(b) RCaMP2 + ChR2 configuration

Figure 3.12: OSFM microscope system connections

¹For details and specifications concerning the Ce:YAG + LED/LD Fiber Light Source, see their manual.

GCaMP6 + NpHR3.0 configuration

For the **GCaMP6 + NpHR3.0 configuration** (Fig. 3.12a), the *Ce:YAG + LED optical head* is used as the light source. The LED light source is used for excitation, while the Ce:YAG light source is used for opsin activation.

1. Connect the *OTPG* to the computer using a **USB-A/USB-B cable**.
2. In this configuration, the LED light source needs to be triggered by the *Fluorescence microscope driver*. Connect the driver **BNC output** to the *Ce:YAG + LED driver* **LED BNC input**.
3. Connect the *Ce:YAG + LED driver* **Ce:YAG BNC input** to an *OTPG* channel.
4. Connect the *Fluorescence microscope driver* **Input BNC** to an *OTPG* channel.
5. When installing in a head-fixed or freely-moving configuration, the rest of the system is connected as described in section 3.4.1 and 3.4.2.

RCaMP2 + ChR2 configuration

For the **RCaMP2 + ChR2 configuration** (Fig. 3.12b), the *Ce:YAG + LD optical head* is used as the light source. The LD light source is used for opsin activation, while the Ce:YAG light source is used for excitation.

1. Connect the *OTPG* to the computer using a **USB-A/USB-B cable**.
2. In this configuration, the Ce:YAG light source needs to be triggered by the *Fluorescence microscope driver*. Connect the driver **BNC output** to the *Ce:YAG + LD driver* **Ce:YAG BNC input**.
3. Connect the *Ce:YAG + LD driver* **LD BNC input** to an *OTPG* channel.
4. Connect the *Fluorescence microscope driver* **Input BNC** to an *OTPG* channel.
5. When installing in a head-fixed or freely-moving configuration, the rest of the system is connected as described in section 3.4.1 and 3.4.2.

Specifications

Table 4.1: *Single-color Fluorescence Microscope Body Specifications*

SPECIFICATION	Microscope Body	
	Model S	Model L
Mass without cables (g)	2.2	
Dimensions without cables in mm (W x L x H)	8.8 x 13.9 x 16.6	
Frame rate (fps)	49	
Objective lens NA	0.5	
FOV at image plane (pixel)	630 x 630	
FOV at object plane (μm)	700 x 700	350 x 350
Lens magnification	3.3x	6x

Table 4.2: *Single-color Fluorescence Microscope Body Excitation and Detection Spectra*

Microscope Bodies	SPECTRUM (nm) ¹		
	Excitation	Detection	Opsin activation
Basic			
GCaMP6	445/62	525/40	—
RCaMP2	549/15	609/57	—
OSFM			
GCaMP6 + NpHR3.0	445/62	525/40	616/76
RCaMP2 + ChR2	549/15	609/57	Compatible with 450, 473, 488

Table 4.3: *Model S Cannula general specifications*

SPECIFICATIONS	VALUE
Magnification	2 x
Working distance	2.4 mm

¹Center wavelength/bandwidth

Table 4.4: Model L Cannula general specifications

SPECIFICATIONS	VALUE
Lens diameter	500 μm
Working distance	80 μm

Table 4.5: Fluorescence Microscope Driver general specifications

SPECIFICATIONS	VALUE	NOTES
Power supply	110 - 240 VAC, 50 - 60 Hz	
DC Power supply	12 VDC	
Dimensions	186 x 90 x 77 mm ³	Including connectors
Data link	Gigabit ethernet	
TTL input voltage	0 to +5 V	
LED Analog input voltage	400 mA/V light source current	Standard 1 A LED Driver
	40 mA/V light source current	Low power mode enabled
LED BNC output voltage	2.5 V/A	
LED Maximum output current range	200, 2000 mA	
LED Maximum forward voltage	7 V	
LED Minimum output current	2.5 mA	Low power mode enabled
LED Rise/Fall time	<10 μs	Typical
LED Connector	M8	See Figure 5.1



Figure 5.1: M8 Female Pinout (Microscope Driver)

Table 4.6: Fluorescence Microscope Driver Software specifications

SPECIFICATIONS	VALUE	NOTES
Current adjustment steps	1 mA	
Modulation minimum frequency	0.01 Hz ²	Internal complex mode : 0.000054 Hz
Modulation maximum frequency	50 kHz	-3 dB attenuation
Minimum ON or OFF time	0.005 ms ²	Internal complex mode : 2 ms
Maximum ON or OFF time	100 s ²	Internal complex mode : 5 h
Maximum number of pulses per sequence	16.68 millions ²	Internal complex mode : 65 535
Maximum number of sequences	4.2 billions ²	Internal complex mode : 65 535
Minimum step increments	39 μsec ²	Internal complex mode only
Number of steps per period	128 ²	Internal complex mode only
Scope Acquisition speed	10 kS/s	Single channel

²For all operation modes, except the internal complex mode

Table 4.7: Connectorized LEDs general specifications

SPECIFICATION	VALUE	NOTE
Input current	Min: 0 mA - Max: 1000 mA	Recommended 700 mA
Forward voltage	3.0 - 4.0 V Typical	
Dimensions	63 x 59 x 21 mm ³	Without cable, with baseplate
Mass	~100 g	
CLED cable connector	M8-4pins - Male	See Figure 5.2
Output NA	0.55	NA of up to 0.63 NA will slightly increase power;
Output optical fiber core diameter	<960 μ m	Power scales up to this core diameter



Figure 5.2: M8 Male Pinout (CLED)

Table 4.8: Doric Neuroscience Studio Hardware Requirements

SPECIFICATIONS	VALUE	NOTES
Operating System	Windows 7, 8 , 10	64-bit
Memory (Minimum/Recommended)	4 GB/16 GB	
Processor Speed (Minimum/Recommended)	2 Ghz Quad-Core i5/ 3.46 Ghz Eight-core i7	
Hard Drive	500 MB	

Annex 1: Cleaning and Handling

5.1 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.** This cable is pigtailed to the CMOS sensor and cannot be easily replaced.
- **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 salted water solution is not recommended. If the microscope comes in contact with these substances, clean the optics (section 6.2) to avoid the apparition of stains.

5.2 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 hard 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.

Annex 2: Troubleshooting

6.0.1 Software

How to make the *Doric Neuroscience Studio* detect the *Microscope Driver*?

1. Ensure that the *Microscope Driver* is plugged into the computer using an Ethernet cable.
2. Ensure that the each *Electrical Cable Connector* is plugged into the appropriate device. The *Microscope Driver* must be linked to the *Microscope*.
3. Ensure that the IP address is static (see section 3.3.1)
4. Ensure the *Jumbo Frames* are activated (see section 3.3.1)
5. *Windows Firewall* can prevent communication. To ensure the communication is not being blocked, open the *Windows Firewall* configuration window, then click on *Allow an app through the firewall*. From there, select the *Change Settings* button, find the *Doric Neuroscience Studio* and check the *Private* and *Public* checkboxes.
6. In the *Network & Sharing Center*, check the Ethernet connection; it should indicated *Unidentified Network*. If *Network Cable Unplugged* is shown despite the Ethernet cable being plugged in and the driver being turned on, disable and re-enable the Ethernet connection.
7. Ensure *Network & sharing* is properly configured at 1 Gbps by double-clicking the Ethernet connection and checking the *Speed*.
8. When the *1-color Microscope Driver* is activated, the *On/Off Switch* should blink blue while initializing. If the light is sustained without any blinking when first turned on, restart the *Microscope Driver*.
9. Certain Intel Ethernet cards must be activated in *Slave Mode* to function. This mode can be found in the same menu as the *Jumbo Frames* (see section 3.3.2).

How can I stop the software lagging and/or dropping frames¹?

1. Deactivate all internet using programs that can conflict with the *Doric Neuroscience Studio* (IE Skype, Firewall, etc.)
2. Use a computer with the recommended specifications:
 - *Operating System*: Windows 10
 - *CPU*: Quad Core I7 3.46 GHz
 - *RAM*: 16 Gb

¹Dropped frames are black frames that occur when an image is lost in communication. They can easily be spotted in the *Average Intensity In ROI* trace if the value descends to 0.

- *Dedicated Graphics Card*: with Open GL version 4.6 recommended
 - *Desktop computer recommended*
3. Windows might limit the Ethernet performances to reduce energy consumption. To ensure that the communication is not limited, open the Power option window:
- Press the Windows + R keys to open the Run dialog box.
 - Type in the following text: "powercfg.cpl", and then press Enter.
 - In the Power Options window, under Select a power plan, choose High Performance.
 - If you do not see the High Performance option, click the down arrow next to Show additional plans.
 - If available, change the System standby and System hibernates settings to Never.
 - Click Save changes or click OK.

How can I visualize recorded frames?

1. All images will appear black in *Window Image Preview*/Traditional Image Viewers as they are special 16 bit .tif files. Use dedicated software such as the *Doric Neuroscience Studio Image Analyzer* or *ImageJ*.
2. Due to library usage conflicts, the *Dell Backup & Recovery* application interferes with the loading of images in the *Doric Neuroscience Studio*. Uninstall the *Dell Backup & Recovery* application from the computer

Can I use a USB to Ethernet adapter to connect the driver?

1. The microscope driver must be connected to a computer ethernet port.
2. Should a USB to Ethernet adapter be used for other function, such as internet access, the adapter must be disabled during the first initialization of the microscope.

6.0.2 Hardware

How do I prevent instability in the *Assisted Opto-electric Rotary Joint*?

1. Ensure the optical fiber *Patch Cord* is of equal or shorter length than the microscope *Electrical Cable* when connector to the *Assisted Opto-Electric Rotary Joint*. Even if the cable is looped, the distance from rotary joint to patch-cord connector should be shorter than the length of the electrical cable.

How do I prevent the *Cannula* turning in the *Protrusion Adjustment Ring*?

1. These two components are meant to be glued together after installation. If they have not been glued during installation, add a drop of quick-drying glue on the border between the *Cannula* and *Protrusion Adjustment Ring*.

How to protect the *Cannula* when the *Input Protective Cap* does not stay inside?

1. Fill the interior of the *Cannula* with KWIK-CAST (WPI) to act as a cap. After removal of the dried sealant, clean the *Rod Lens* outer surface using a cotton swab lightly dipped in isopropyl alcohol.

How do I stabilize the *Cannula-Microscope Body* connection?

1. Ensure that the Microscope Barrel is sufficiently loose (see *Microscope Surgical Procedure Application Note Section 2.1* or the *Instructional Video*).

How to insert the *Microscope Body* into the *Cannula* with minimal force?

1. Ensure that the *Microscope Clamps* are sufficiently loose (screw in the barrel).
2. Ensure that the *Cannula* and *Microscope Body* are properly aligned.
3. Verify the *Cannula* installation instructions in *Section 2.1* of the *Microscope Surgical Procedure Application Note*.

6.0.3 Biology

Why can't I see any individual cells?

1. It generally takes 3 to 8 weeks before the area surrounding the tip of the *Rod Lens* has healed enough for sharp imaging of neurons.

Support

7.1 Warranty

This product is under warranty for a period of 12 months. Contact Doric Lenses for return instructions. This warranty will not be applicable if the unit is damaged or needs to be repaired as a result of improper use or operation outside the conditions stated in this manual.

7.2 Contact us

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

Phone 1-418-877-5600

Email sales@doriclenses.com

The logo for Doric Lenses, featuring the word "doric" in a lowercase, sans-serif font. The letter "o" is stylized with a horizontal line through its center, resembling a lens or a reflection.

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