



efocus Fluorescence Microscope System

Gen3

User Manual

Version 1.5.1

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Important Safety Information

1.1 General Safety Information



WARNING!

For safety precautions, the *Fluorescence Microscope Driver Gen 3* must be powered ONLY by the power supply provided with the product.



The *★LISER™ & LED/LD Light Source* is a new type of optical source which, in addition to the laser-pumped Ce:YAG crystal fluorescence output, can also include standard LED or laser diode outputs. This type of hybrid light source is not specifically considered by international safety committees such as the IEC¹ and the FDA². Consequently, the user should follow all safety procedures related to the worst case scenario, either in working or failure condition. Considering the power level of the fluorescence output of the *★LISER™ & LED/LD Light Source*, this means following Class 3B laser product safety rules even though the output does not necessarily contain laser radiation, depending on the exact model and output filter. The next section on laser safety information should thus be **read and carefully followed**.

1.2 Laser Safety Information

If you are not familiar with laser light sources, ask for advice to qualified personnel **BEFORE FIRST USE** and **READ CAREFULLY** the application note **Important Laser Safety Information** that can be found on the USB key. You can also contact directly Doric Lenses by email (sales@doriclenses.com) to obtain a copy of this application note.



DANGER!

The *LISER & LED/LD Light Source* is a Class 3B laser product. Read the application note *Important Laser Safety Information* BEFORE FIRST USE.



The *★LISER™ & LED/LD Light Source* is a Class 3B laser product emitting visible light at sufficiently high power levels to **PERMANENTLY DAMAGE THE EYES. NEVER LOOK** directly into the optical beam exiting from the output FC connector or from any optical fiber connected to the output FC connector. **NEVER LOOK** directly at specular or diffuse reflections of the output beam. It is important to **WEAR LASER SAFETY GLASSES** (goggles) certified for the wavelength and power level of the light source. Also follow all safety procedures to protect anyone working in the area. Even when wearing laser safety glasses, **NEVER LOOK** directly into the beam or any specular reflection of the optical beam exiting from the *★LISER™ & LED/LD Light Source* or from any optical fiber connected to its output FC connector. The *★LISER™ & LED/LD Light Source* is provided with a safety interlock connector on the rear panel. When the interlock circuit is shorted and the power key is inserted, the driver is enabled (see Section 1.4). For a safe use of the *★LISER™ & LED/LD Light Source*, the safety interlock connector should be connected to the laser safety interlock circuit of the laboratory. You should contact the laser safety officer (LSO) of your institution or company to

¹International Electrotechnical Commission

²Food and Drug Administration

set a proper laser safety interlock circuit for your application and laboratory installation. The ★*LISER*TM & *LED/LD Light Source* emits light spanning over a large bandwidth in the visible light spectrum. Since the output spectrum depends on the exact model and optional output filter, the output power level and the according safety procedures are specific to each application.

1.3 Safety Labels

The laser class labels are provided with the system and the laser aperture is clearly identified by laser warning label and/or the text *LASER APERTURE*.



Figure 1.1: Safety Labels

1.4 Activation Safety Features

The drivers for all Doric Lenses light sources come with a number of safety features. These are built into the driver circuits, as shown in the block diagram (Fig. 1.2).

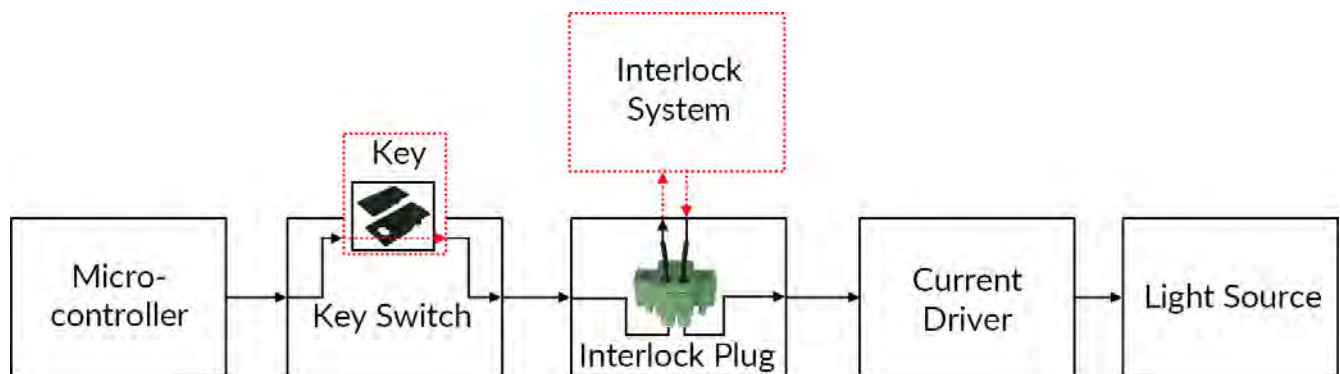


Figure 1.2: Safety feature block diagram

- The **Micro-controller**, **Key Switch**, **Interlock Plug** and **Current Driver** are connected in series. This means that if any single safety feature is not properly in place, the light source cannot be activated.
- The **Micro-controller** is used to control the light source driver.

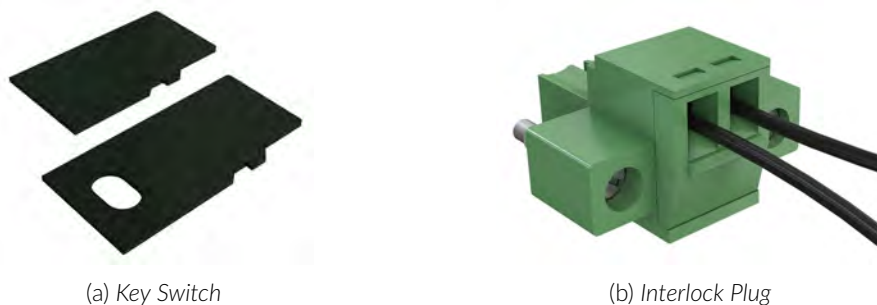


Figure 1.3: Safety Feature Elements

- The **Key Switch** (Safety feature 1) (Fig. 1.3a), located on the left side of the driver, is required to activate any light source. If removed, no data can be sent from the micro-controller to the **Current Driver**.
- The **Interlock Plug** (Safety feature 2) (Fig. 1.3b) is used to integrate the driver into an **Interlock Circuit**.
 - The **Interlock Plug** comes with a small wire short-circuiting it. This wire must be removed before integrating it into an **Interlock Circuit**.
 - Connect the **Interlock Circuit** in series with the **Interlock Plug** so the circuit may function properly.
- The **Current Driver** sends current to any connected light source. If the **Key** is absent or the **Interlock Plug** has an open circuit, it cannot receive signals from the micro-controller, preventing it from sending out current.

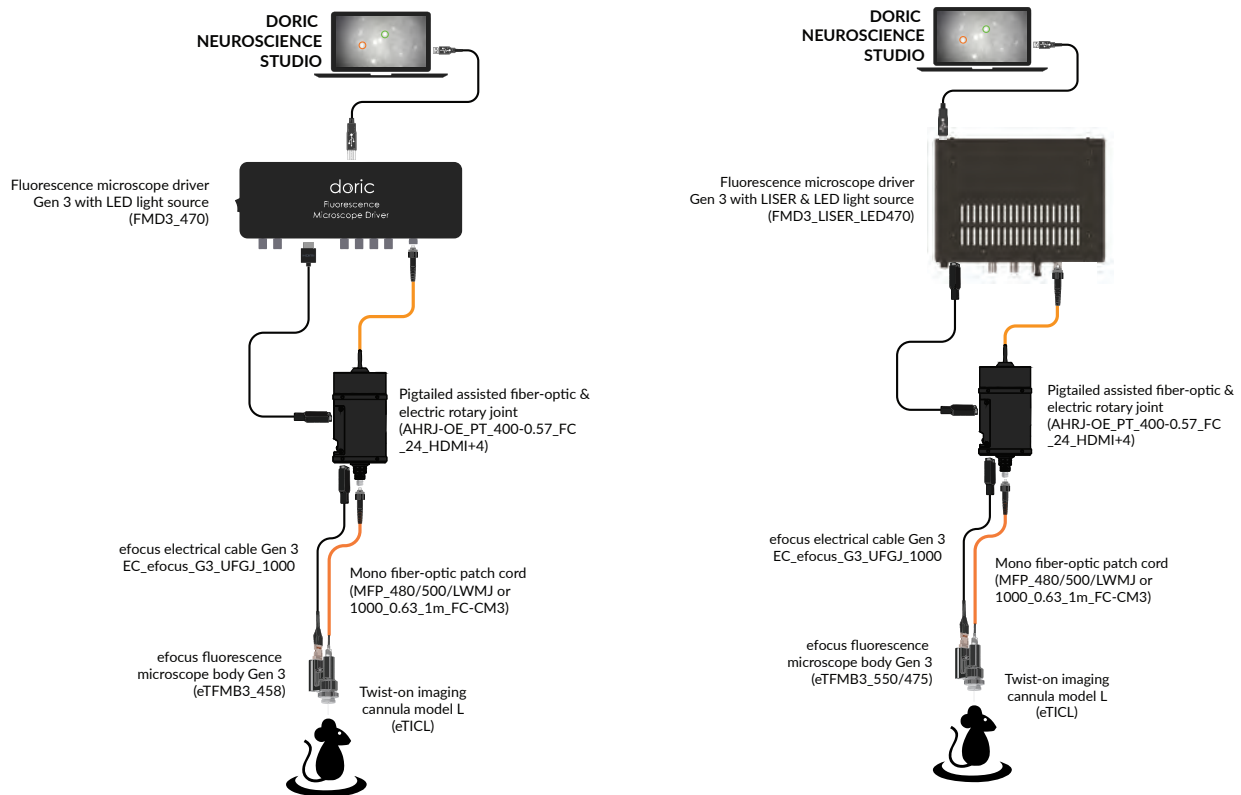
1.5 Emission Indicator

For light sources emitting invisible laser radiation, a dedicated LED indicator is ON when the driver is outputting an electrical current. When the driver is outputting current, the light source will emit light from the aperture.

System Overview

2.1 Introduction

The *efocus Fluorescence Microscopy Systems Gen 3 (eTFMS3)* are state-of-the-art miniature microscopy systems designed to perform calcium imaging *in vivo* on freely moving animals. It images a single fluorophore within the subjects brains via an *Imaging Cannula* that serves as a relay between the microscope and the region of interest within the brain. The *Pigtailed Assisted Rotary Joint* as well as the small size and weight of the *efocus Fluorescence Microscope Body Gen 3* allows the animal to freely move in his habitat. The microscope body also features an electronically adjustable focus adjustment and a simplified easy-to-use twist-on cannula attaching mechanism. The microscope system is available for single-color (Fig. 2.1a) and optogenetically synchronized (Fig. 2.1b) calcium imaging.



(a) GCaMP6 imaging configuration

(b) GCaMP6 imaging and NpHR3 activation configuration

Figure 2.1: *efocus Fluorescence Microscopy System Gen 3 Configurations*

2.2 Fluorescence Microscopy System: GCaMP6 filter set

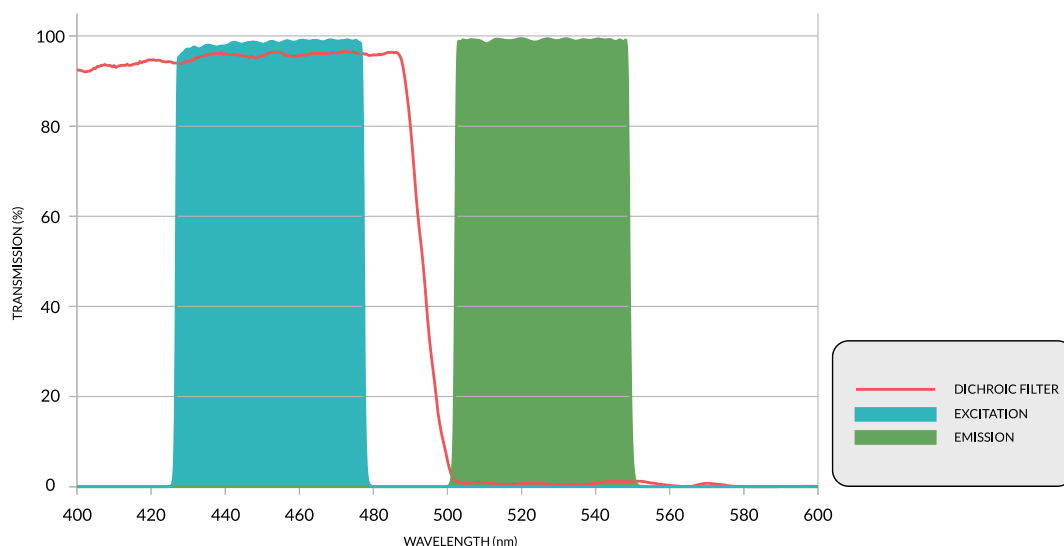


Figure 2.2: GCaMP6 Excitation Filter Set Spectra

The **efocus Fluorescence Microscopy System Gen 3 (eTFMS3)** (Fig. 2.1a) has been designed to image a single fluorophore using a single color light source. This system is available in freely-moving and head-fixed configurations. The standard **GCaMP6** system (Fig. 2.2) uses a LED integrated to the microscope driver as a light source.

2.3 Optogenetically Synchronized Fluorescence Microscopy System: GCaMP6 + NpHR3.0 filter set

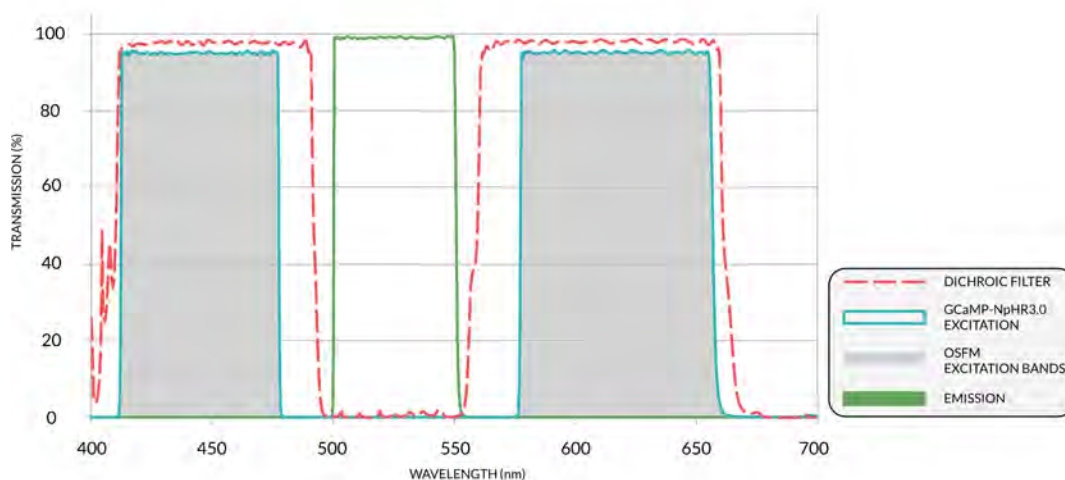


Figure 2.3: GCaMP6+NpHR3.0 Excitation Filter Set Spectra

The **efocus Optogenetically Synchronized Fluorescence Microscopy System Gen 3 (eTOSFS3)** (Fig. 2.1b) images a single fluorophore while performing optogenetic activation/deactivation. This system is available in freely-moving and head-fixed configurations. When using a **GCaMP6 + NpHR3.0** microscopy system, the **★LISER™** with LED is integrated to the microscope driver (Fig. 2.3).

Devices Overview

3.1 efocus Fluorescence Microscope Body Gen 3

The *efocus Fluorescence Microscope Body Gen 3* allows *in vivo* imaging of fluorescence within small animal subjects brains. The microscope is optimized for deep brain imaging of calcium indicators such as **GCaMP6**, it contains a 0.4 NA variable-focus objective lens (in the microscope body), and connects to an implantable imaging cannula that relays the images from the deep structures of the brain to the microscope body. As there are no notable outward differences between the *efocus Fluorescence Microscope Body Gen 3 (eTFMB3)* and the *efocus Optogenetically Synchronized Fluorescence Microscope Body Gen 3 (eTOSFM3)*, these two products are not addressed separately. The different features are shown in Figure 3.1 with their functions described below.



Figure 3.1: *efocus microscope body Gen 3*

- The **M3 Optical Receptacle** is used to receive light from a chosen light source using a fiber-optic patch cord.
- The **Electrical Connector** sends and receives electrical signal for the microscope using a 14-pin electrical connector.
- The **Twist-on Connection System** allows the removal of the microscope body from the imaging cannula between each imaging session without affecting image position. The **Microscope Barrel** secures the microscope on a *Twist-on Imaging Cannula* (see Fig. 3.2, and the *Imaging Cannula Implantation & Microscope Installation for eTFMB3 / eTOSFM3 application note* for more information on the microscope attachment procedure).
- The **CMOS Sensor** captures fluorescence images. Generation 3 of the efocus Fluorescence Microscope Body has an improved CMOS sensor with a higher resolution, dynamic range and sensitivity.
- The **efocus Objective Lens** transmits the images from the Imaging Cannula to the CMOS Sensor.

3.2 Twist-on efocus Imaging Cannula

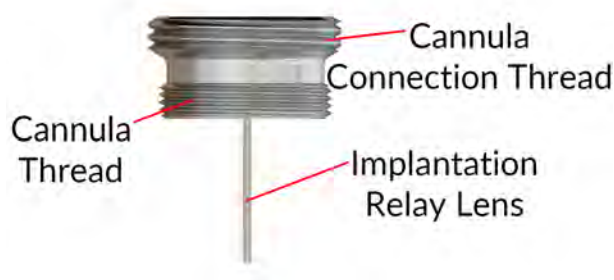


Figure 3.2: Twist-on efocus Type-L Imaging Cannula

The Imaging Cannulas transmit images of structures located inside the brain to the surface of the skull. An imaging cannula (Fig. 3.2) contains the following elements:

- The **Connection System** allows the removal of the microscope body from the cannula between each imaging session without affecting image position. The **Cannula Connection Thread** is used to secure the microscope body in place using the **Microscope Barrel**.
- The **Relay Lens** is an image relaying gradient-index rod lens that brings the image of deep structures located inside the brain to the skull surface.
- The **Cannula Thread** is used to secure the cannula in a *Protrusion Adjustment Ring*. Protrusion adjustment rings are used to support the cannula when there is a gap between the surface of the skull and the base of the cannula. It reduces the amount of dental cement necessary to secure the cannula on the skull.

A set of rod lenses with different lengths is available to reach different depth ranges in brain tissue. Fine adjustment of the protrusion of the rod lens in tissue can be done with the *Protrusion Adjustment Ring Set* that comes with each cannula (Fig. 3.3).



Figure 3.3: 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

3.3 Twist-on Dummy Microscope



Figure 3.4: Twist-on Dummy Microscope

The **Twist-on Dummy Microscope** is of similar weight and size as the efocus microscope body Gen 3. It is used to accustom animal subjects to the weight and feel of the microscope.

3.4 Pigtailed Assisted Fiber-optic & Electric Rotary Joint

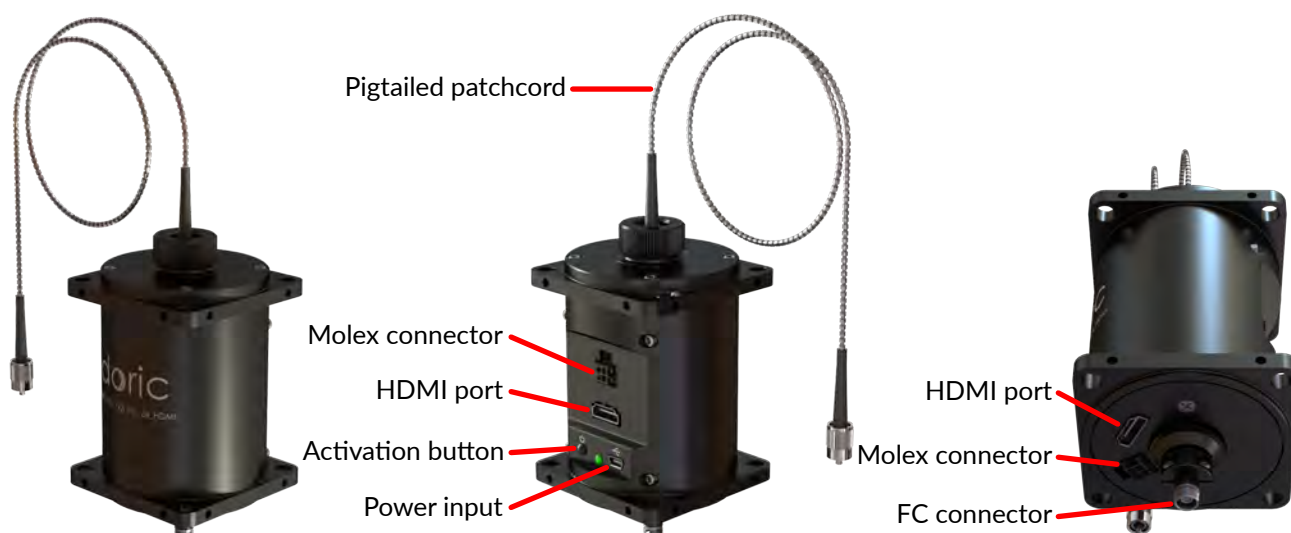


Figure 3.5: 24 contacts *Pigtailed Assisted Fiber-optic & Electric Rotary Joint*

To use the microscope in experiments using freely-moving animals, the 24 contacts *Pigtailed Assisted Fiber-optic & Electric Rotary Joint* (Fig. 3.5) is provided¹. This rotary joint allows effectively frictionless rotation of optical fibers and electrical cables connected to the microscope.

- The rotary joint is connected to the light source with a **Pigtailed Fiber-Optic Patch Cord** (integrated into the rotary joint for optimal performance), and to the microscope via a FC-CM3 Fiber-Optic Patch Cord connected to the **FC connector**².
- The **HDMI Ports** are used to provide electrical communication between the driver and the microscope.
- The **Molex Micro-Fit 3.0 Dual Row 4 Contact Connector** provides additional electrical connection through the rotary joint to connect other devices to the animal.
- The **5 V mini-USB-B Connector** connects to the power supply for the assisted rotation.
- The **Activation Button** is used to activate/deactivate the assistance function. When the rotary assistance is activated, the light next to the button will turn green.

¹To ensure optimal performances, *Twist-on efocus microscope bodies* should not be used with 12 contacts *Pigtailed Assisted Fiber-optic & Electric Rotary Joints*. Contact us for more information.

²MFP_400/430/LWMJ-0.48_1m_FC-CM3 or MFP_480/500/1000-0.63_1m_FC-CM3

3.5 Fluorescence Microscope Driver Gen 3

This driver is used for computer control over the excitation of the LED light source as well as for microscope image capturing and broadcast at video rate to the computer via a USB3 cable (Fig. 3.6). It can be triggered by or synchronized with external recording devices and it can also trigger other devices. The Fluorescence Microscope Driver Gen 3 is used with the eTFMB3 microscope while the Fluorescence Microscope Driver Gen 3 with ★LISER™ (section 3.6) is designed to be used with the eTOSFM3 microscope.



Figure 3.6: Fluorescence Microscope Driver

The Fluorescence Microscope Driver shown in Fig. 3.6 has the following components:

- The **On/Off** switch powers the device.
- The **Microscope Trigger BNCs** are used to receive (**TRIG IN**) or send (**TRIG OUT**) trigger TTL signals tied to the microscope driver as a whole.
- The **HDMI Connector** located on the front of the driver is used to connect the microscope to the Driver.
- The **Digital I/O** BNC ports can send or receive 0-4.75V TTL pulses.
- The **LED FC Connector** is used to connect a Patch Cord to the driver integrated LED.
- The **Molex 4 Contact Connector** can be used to connect an external LED to the microscope driver to be used instead of the integrated LED. For this LED to be controlled by the microscope Driver, the neighbouring switch needs to be switched to EXT.
- The **USB3** port connects the driver to a computer using an USB3-A to USB3-B cable.
- The Service **USB-B port** is to be used for the maintenance of the driver, such as for firmware updates. When such maintenance is necessary, the neighbouring switch needs to be switched.
- The **Power Input** accepts a 12 VDC power supply to power the driver.

3.6 Fluorescence Microscope Driver Gen 3 with ★LISER™

This driver is used for computer control over the excitation of the ★LISER™ and LED light sources as well as for microscope image capturing and broadcast at video rate to the computer via a USB3 cable (Fig. 3.7). It can be triggered by or synchronized with external recording devices and it can also trigger other devices. The Fluorescence Microscope Driver Gen 3 with ★LISER™ is designed to be used with the eTOSFM3 microscope.

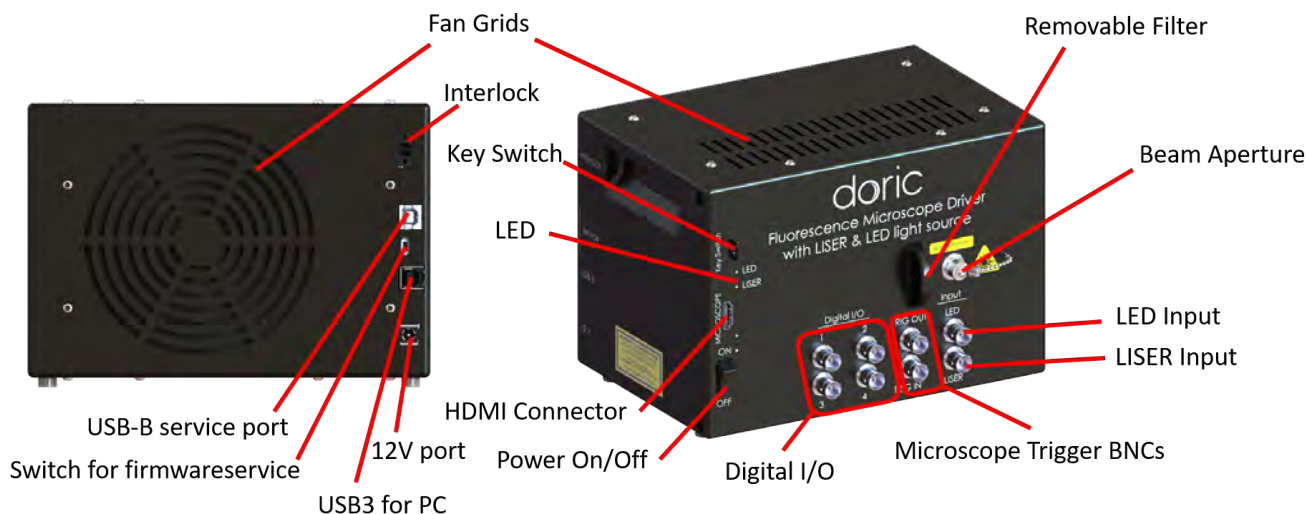


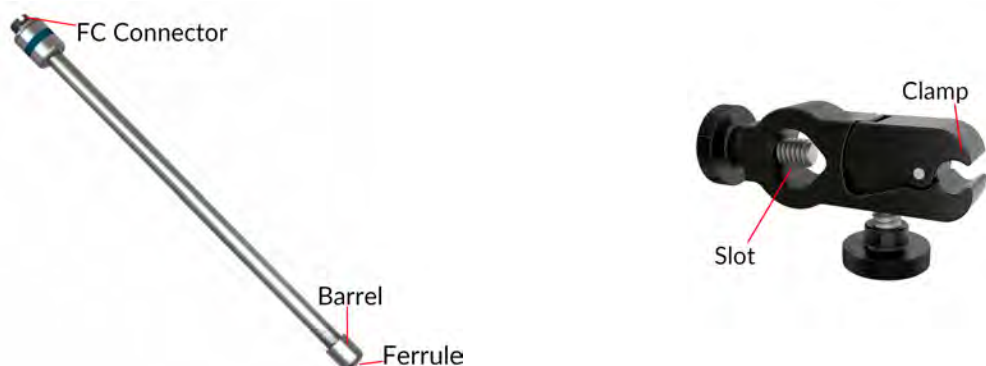
Figure 3.7: Fluorescence Microscope Driver with ★LISER™

The Fluorescence Microscope Driver Gen 3 with ★LISER™ shown in Fig. 3.7 has the following components:

- The **Power On/Off** switch turns on/off the driver.
- The **Microscope Trigger BNCs** are used to receive (**TRIG IN**) or send (**TRIG OUT**) trigger TTL signals tied to the microscope driver as a whole.
- The **HDMI Connector** located on the front of the driver is used to connect the microscope to the Driver.
- The 4 **Digital I/O** BNC ports can send or receive 0-4.75V TTL pulses.
- The BNC **LISER Input** Port is used to command the LISER source.
- The BNC **LED Input** Port is used to command the LED/LD source.
- The **Beam Aperture** is where the light exits the light source. The aperture is composed of a fiber coupling assembly that injects the emitted light into an optical fiber. The standard model uses an FC fiber connector. A safety FC metal cap is attached to the optical head to block the output light beam in absence of optical fiber.
- The **Fan Grids** are found on the top, rear and side of the light source. They must be kept clear at all times to avoid overheating the system.
- The **Removable Filter Holder** is used to insert an optical bandpass filter in the system, allowing the selection of a narrow part of the broad ★ LISER™ spectrum of emission. The filter holder can accept any filter up to a 25.4 mm diameter and a maximum 5 mm thickness.
- The **Key Switch** must be in place to enable light emission. Note that, despite its similar form factor, the power key **is not a standard micro SD card** such as those used in some digital cameras. Do not attach the **Key** to a key fob or similar holder; this may prevent proper insertion of the **Key Switch**.
- The **USB3** port connects the driver to a computer using an USB3-A to USB3-B cable.
- The **USB-B service port** is to be used for the maintenance of the driver, such as for firmware updates. When such maintenance is necessary, the neighbouring switch needs to be switched.
- The **Service switch** is to be used for the maintenance of the driver, such as for firmware updates. When such maintenance is necessary, the switch needs to be switched on **P**. Rest of the time the switch need to be on **O**.
- The **12 V port** connects the Fluorescence Microscope Driver with ★ LISER™ to its 12 VDC power supply.
- The **Interlock** connector plug allows the user to connect the ★ LISER™ to a safety interlock system. It is recommended to connect the interlock plug to a laboratory interlock system (See Chapter 1).

3.7 Fluorescence Microscope Holders

The *Fluorescence Microscope Holder FMH_400* and the *Clamp for Fluorescence Microscope Holder* are used to secure the microscope in a stereotaxic system, for head-fixed experiments.



(a) Fluorescence Microscope Holder FMH_400

(b) Clamp for Fluorescence Microscope Holder

Figure 3.8: Fluorescence Microscope Holder Elements

- The *Clamp for Fluorescence Microscope Holder* (Fig. 3.8b) can be secured to a rod in a stereotaxic system using the **Slot**. The **Clamp** is used to easily secure and release the *Fluorescence Microscope Holder*.
- An FC-connectorized light source can be connected to the **FC Connector** of the *Fluorescence Microscope Holder* (Fig. 3.8a) using a FC-FC optical patch cord. This provides illumination to the microscope while it is connected to the *Fluorescence Microscope Holder*.
- The *Fluorescence Microscope Holder* **Ferrule** is inserted into the microscope **M3 optical connector**. The ferrule is secured by screwing the **Barrel** onto the **M3 Optical Connector**.

3.8 efocus Electrical Cable Gen 3



Figure 3.9: efocus Electrical Cable Gen 3

The *efocus Electrical Cable Gen 3* is used to connect the microscope to the driver. The **HDMI connector** is inserted into the driver, and the **14-pin Omnetics Electrical Connector** is connected to the microscope electrical connector. When used with a *Pigtailed Assisted Fiber-optic & Electric Rotary Joint* (Fig. 3.5), the *efocus Electrical Cable Gen 3* is connected to the HDMI connector located at the base of the rotary joint. To ensure a durable connection between the **14-pin Electrical Connector** and the microscope electrical connector, secure them together using the orange tape provided with the microscope.

Operations Guide

4.1 Connecting the Microscope Driver Gen 3

1. Connect the microscope driver to the power outlet using the 12 V power supply.
2. Connect the microscope driver to the computer using the USB3-A to USB3-B cable.
3. Connect the *efocus Electrical Cable Gen 3* (or the HDMI cable when used with a *Pigtailed Assisted Fiber-optic & Electric Rotary Joint*) to the Microscope port on the driver.
4. Flip the On/Off Switch. Next to the On/Off switch, 2 small white LED light will be in operation. One will be turned on, indicating that the driver is powered on. The other LED will be blinking, indicating that the driver is in standby to be initialized by the Doric Neuroscience Studio Software.

4.2 General Setup Guidelines

4.2.1 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. 4.1).

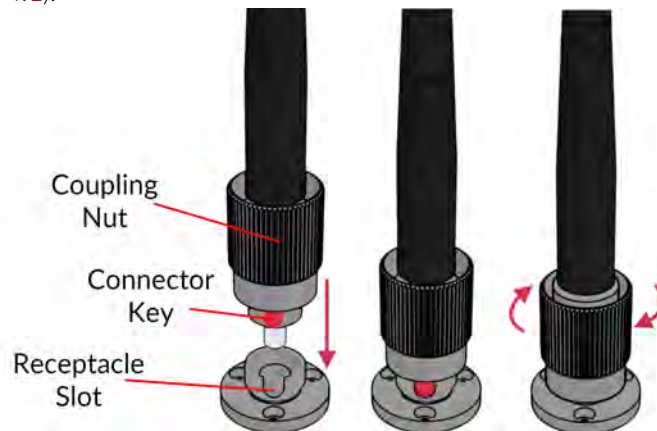


Figure 4.1: FC connector, Fiber Installation



WARNING!

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



4.2.2 Microscope Clamping Procedure

For details concerning the imaging canula handling and implantation, as well as the microscope clamping procedure, it is important to read the Imaging Cannula Implantation and Microscope Installation for eTFMB / eTOSFM.3

4.3 Connecting the efocus Microscopy System Gen 3

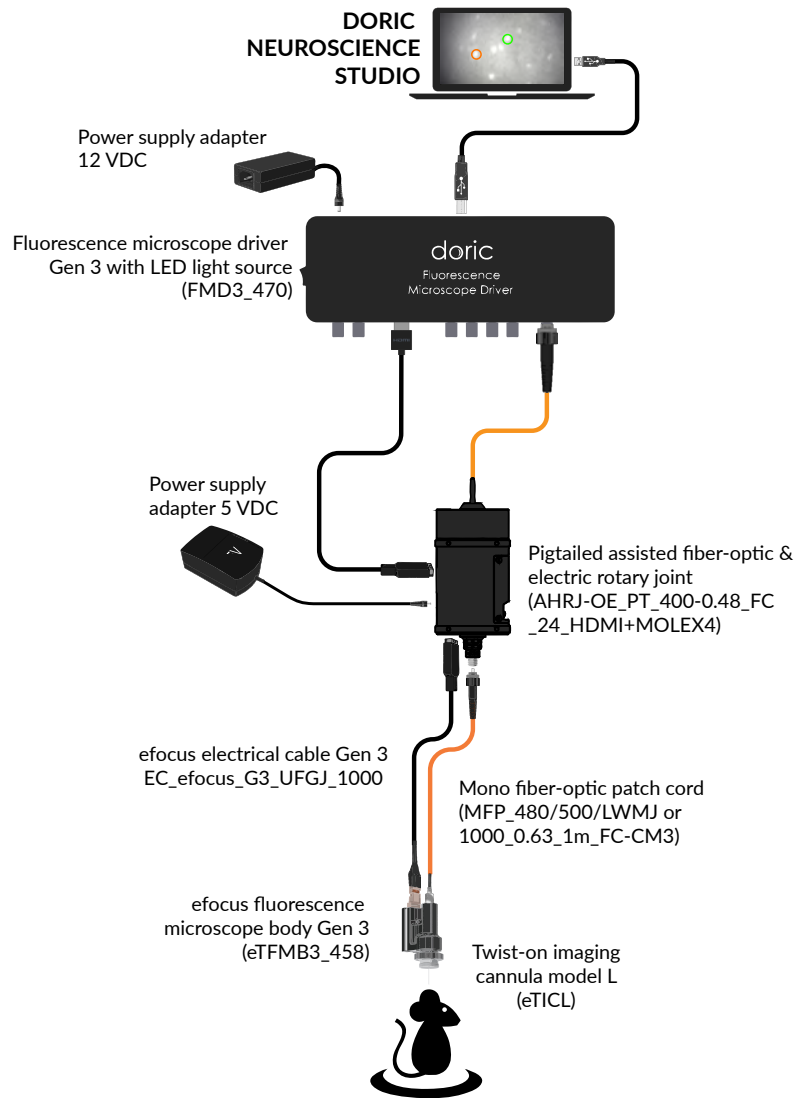


Figure 4.2: Full GCaMP6 Freely-Moving Configuration, combination of Fig. 4.3a and Fig. 4.4b

There are multiple fluorescence microscope system configurations depending on the desired freedom of the animal and the fluorophores/opsin targeted. Depending on the *Microscope Body* type, different lighting configurations are used.

- The *eTFMB3_L_458* body Gen 3 is designed for **GCaMP** fluorescence imaging and uses the Fluorescence Microscope Driver with LED light source.
- The *eTFMB3_L_550* body Gen 3 is designed for **RCaMP** fluorescence imaging and uses the Fluorescence Microscope Driver with LISER and LED light source.
- The *eTOSFM3_L_445_616* body Gen 3 is designed for **GCaMP** fluorescence imaging with red optogenetics and uses the Fluorescence Microscope Driver with LISER and LED light source.
- The *eTOSFM3_L_550_475* body Gen 3 is designed for **RCaMP** fluorescence imaging with blue optogenetics and uses the Fluorescence Microscope Driver with LISER and LED light source.

Section 4.6.1 shows the installation of the Fluorescence Microscope Drivers and light sources. Furthermore, the animal must be held in one of the two following configurations.

- The **Head-fixed** configuration (Section 4.3.2, Fig. 4.4a) allows the observation of the animal while placed in a stereotaxic apparatus.
- The **Freely-Moving** configuration (Section 4.3.3, Fig. 4.4b) allows the observation of the animal while moving freely in a cage.

Figure 4.4 shows the head-fixed and freely-moving system configurations. Each microscope body and lighting configuration uses the same devices in each of these configurations. The indications of section 4.3.2 and 4.3.3 are to be followed for its installation.

4.3.1 Microscope Driver and Light Source Installation

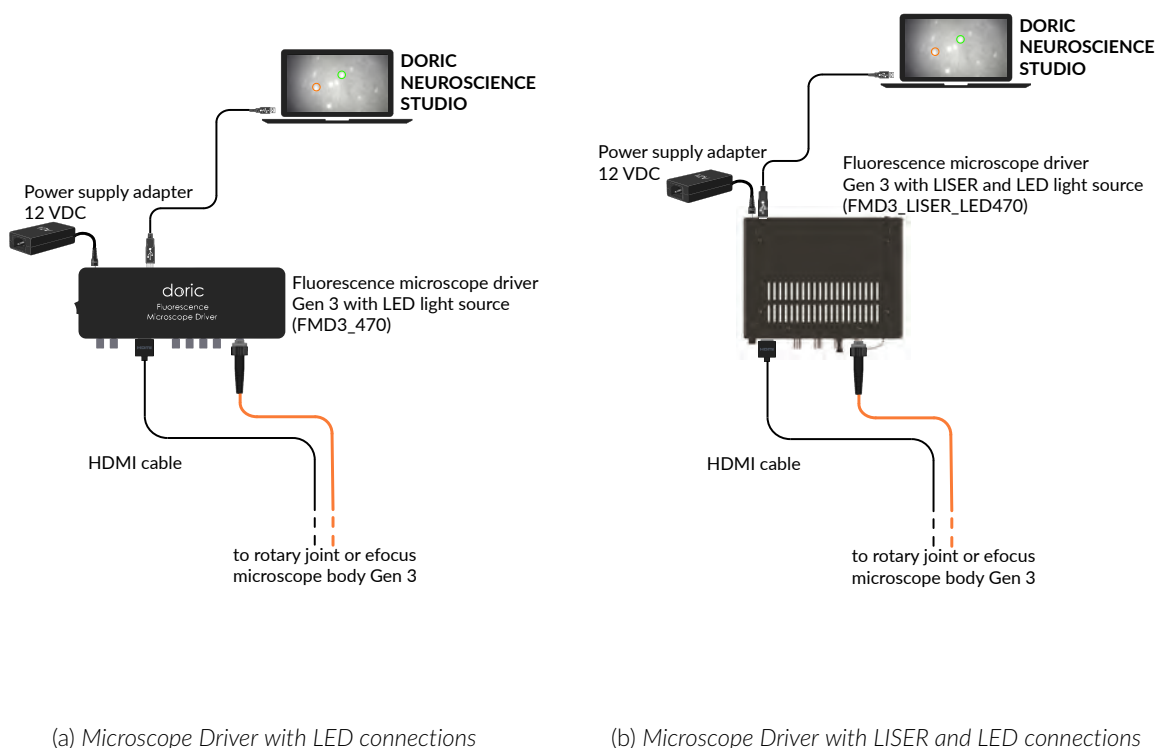


Figure 4.3: Microscope driver connections

1. Connect the 12 VDC *power supply* to the microscope driver **12 VDC power input**.
2. Connect the driver to the computer using the *USB3-A to USB3-B Cable*.
3. Turn on the *Fluorescence Microscope Driver*.



If using the microscope driver Gen 3 with LISER, 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.



4.3.2 Head-fixed Configuration Installation

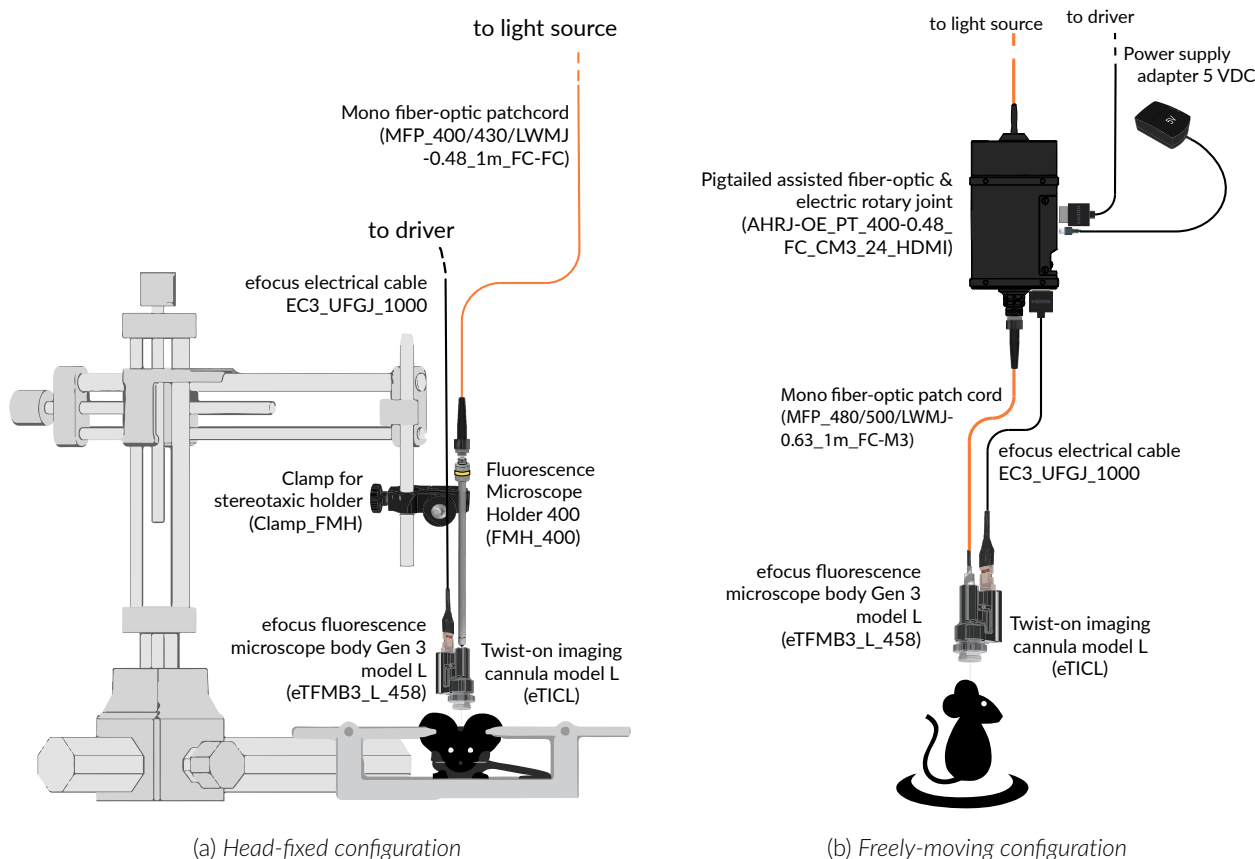
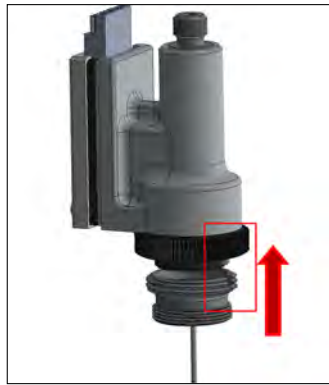


Figure 4.4: efocus Microscopy Systems Connections

1. Install the *Fluorescence Microscope Holder (FMH_400)* into the *Stereotaxic Clamp* (Fig. 4.4a). Ensure the holder is tightly secured inside the clamp.
2. To secure the microscope on the *FMH_400*:
 - a) Remove the **Connector Caps** from the microscope **M3 Optical Connector** and the *FMH_400* ferrule.
 - b) Insert the ferrule into the **M3 Optical Connector**. Secure them in place by screwing the *FMH_400* barrel.
3. Install the *FMH_400* in a stereotaxic apparatus.
4. Connect the *FMH_400* to the light source using a fiber-optic patch cord. The patch cord characteristics¹ are set to achieve optimal coupling efficiency with the *FMH_400*.
5. Connect the **14 pins Electrical Connector** of the *efocus Electrical Cable Gen 3* to the electrical connector of the microscope body. By default, the cable and the body are already connected and an orange tape is wrapped around the connection to secure it. If the cable need to be changed, it's highly recommended to place a new tape to secure the connection. The **HDMI** extremity of the cable need to be connected to the Microscope port of the driver (*FMD3_470* or *FMD3_LISER_LED470*).
6. Unscrew the Input Protective Cap from the *Twist-on imaging cannula* and connect the cannula to the microscope body (Fig. 4.5a). Ensure the microscope key is properly inserted in the cannula slot before screwing the microscope barrel on the cannula (Fig. 4.5b, see *Imaging Cannula Implantation & Microscope Installation for eTFMB / eTOSFM application note* for more information on the microscope clamping procedure). If the key is properly slotted, the microscope will be unable to turn inside the cannula.

¹MFP_400/430/LWMJ-0.48_1m_FC-FC or MFP_400/430/1100-0.48_1m_FC-FC

7. When ready for use, remove the cannula protective cap by unscrewing it. Take great care to remove the cap in a slow, straight motion so as not to break the rod lens inside.



(a) Microscope body insertion in the imaging cannula



(b) Microscope key insertion in the cannula slot

Figure 4.5: Twist-on Microscopy Systems Connections

4.3.3 Freely-moving configuration installation

1. Connect the microscope driver to the side HDMI port of the *Pigtailed Assisted Fiber-optic and Electric Rotary Joint* using the HDMI cable (Fig. 4.4b).
2. Connect the pigtailed patch cord (top end of the rotary joint) to the light source **Beam Aperture**.
3. Connect the **14 pins Electrical Connector** of the *efocus Electrical Cable Gen 3* to the electrical connector of the microscope body. By default, the cable and the body are already connected and an orange tape is wrapped around the connection to secure it. If the cable need to be changed, it's highly recommended to place a new tape to secure the connection. The **HDMI** extremity of the cable need to be connected to the lower end of the *Pigtailed Assisted Fiber-optic & Electric Rotary Joint (AHRJ)*.
4. Connect the lower end of the *Pigtailed Assisted Fiber-optic & Electric Rotary Joint (AHRJ)* to the microscope with the mono fiber-optic patch cord (FC-CM3). This patch cord² has been chosen to ensure proper illumination characteristics.
5. Unscrew the **Input Protective Cap** from the cannula and connect the cannula to the microscope body (Fig. 4.5, and *Imaging Cannula Implantation & Microscope Installation for eTFMB / eTOSFM application note* for more information on the microscope clamping procedure).
6. When ready for use, remove the cannula protective cap by unscrewing it. Take great care to remove the cap in a slow, straight motion so as not to break the rod lens inside.

²MFP_400/430/LWMJ-0.48_1m_FC-CM3 or MFP_480/500/1000-0.63_1m_FC-CM3

Specifications

Table 5.1: *Twist-on eFocus Fluorescence Microscope Specifications*

SPECIFICATION	VALUE
Body Mass	3.0 g
Body dimensions without cables	10 x 15 x 22 mm (W x L x H)
Frame rate	45 fps
Objective lens NA	0.4
FOV at image plane	800 x 800 pixels
Working distance with cannula	0-300 μ m
GRIN lens diameter	500 μ m
FOV at object plane	500 μ m diameter (circular) 1000 μ m 650 μ m x 650 μ m

Table 5.2: *Twist-on eFocus Fluorescence Microscope Body Excitation and Detection Spectra*

Microscope Body	SPECTRUM ¹		
	Excitation ²	Detection	Opsin activation
GCaMP6	458/35 nm	525/40 nm	-
GCaMP6 + NPHR3.0	458/35 nm	525/40 nm	616/76 nm

Table 5.3: *Twist-on Imaging Cannula Model L Specifications*

Brain Zones	Cannula Model	Lens Diameter
0 to 2.6 μ m below the skull surface ³	L type D	1000 μ m
0 to 3.3 mm below the skull surface ³	L type D	500 μ m
2.7 to 5.7 mm below the skull surface ³	L type V	500 μ m
5.1 to 8.1 mm below the skull surface ³	L type E	500 μ m

¹Center wavelength/bandwidth

²With a 470 nm LED light source

³Including the thickness of the skull

Table 5.4: Fluorescence Microscope Driver General Specifications

SPECIFICATIONS	VALUE	NOTES
Power supply	110 - 240 VAC, 50 - 60 Hz	
DC Power supply	12 VDC	
	150 W	for version with ★LISER™ & LED
Dimensions	255 mm x 97 mm x 51 mm	Version with LED
	200 mm x 150 mm x 140 mm	Version with ★ LISER™& LED
Data link	USB-3	
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	1000 mA	
LED Maximum forward voltage	7 V	
LED Minimum output current	2.5 mA	Low power mode enabled
LED Rise/Fall time	<10 μ s	Typical
★LISER™ Analog input voltage	240 mA/V (typical)	See data sheet
★LISER™ Monitor output voltage	4.17 V/A (typical)	See data sheet
★LISER™ Maximum output current range	1200 mA	See data sheet
★LISER™ Maximum forward voltage	6 V typical	See data sheet
★LISER™ Rise/Fall time	<10 μ s	

Table 5.5: Fluorescence Microscope Driver Software Specifications

SPECIFICATIONS	VALUE	NOTES
Current adjustment steps	1 mA	
Modulation minimum frequency	0.01 Hz ⁴	Internal complex mode : 0.000054 Hz
Modulation maximum frequency	50 kHz	-3 dB attenuation
Minimum ON or OFF time	0.005 ms ⁴	Internal complex mode : 2 ms
Maximum ON or OFF time	100 s ⁴	Internal complex mode : 5 h
Maximum number of pulses per sequence	16.68 millions ⁴	Internal complex mode : 65 535
Maximum number of sequences	4.2 billions ⁴	Internal complex mode : 65 535
Minimum step increments	39 μ sec ⁴	Internal complex mode only
Number of steps per period	128 ⁴	Internal complex mode only
Scope Acquisition speed	10 kS/s	Single channel
Operating System	Windows 10, 11	64-bit
Memory (Minimum/Recommended)	4 GB/16 GB	
Processor Speed (Minimum/Recommended)	2 Ghz Quad-Core/ 3.46 Ghz Eight-core i7	
Hard Drive	500 MB	

⁴For all operation modes, except the internal complex mode

Annex 1: Cleaning and Handling

6.1 Important Handling Information



Warning: Handle the microscope and the cannula with care.



Miniature fluorescence microscopes are composed of sensitive electronic and optical components and should always be handled with care. When they are not in use, the microscope body and 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 and plastic, and contact with organic tissues or liquids such as blood or saline solution is not recommended. If the microscope comes in contact with these substances, clean the optics (section 6.2) to avoid the formation of stains.

6.2 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 Guide

7.1 Software

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

1. Ensure that the *Microscope Driver* is plugged into the computer using the provided USB-3 cable.
2. Ensure that the each *Electrical Cable Connector* is plugged into the appropriate device (computer and microscope).
3. When the *1-color Microscope Driver* is activated, the *On/Off Switch* should be on ON and the white LED blink while initializing. If the light is sustained without any blinking when first turned on, restart the *Microscope Driver*.

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
 - *Dedicated Graphics Card*: with Open GL version 4.6 recommended
 - *Desktop computer recommended*
3. Windows might limit the 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.

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.

How can I visualize recorded frames?

1. The images are saved in .doric format, which is a **HDF5** type format. They can be visualized using software supporting HDF5 format such as *Doric Neuroscience Studio* (Image Analyzer plugin), *ImageJ* (Import function and various plugins) or a HDF viewer. Code example are also provided on our [website](#) to read the images in *Python*, *Matlab* and *Octave*.
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

7.2 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.

7.3 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

8.1 Maintenance

The product does not require any maintenance. Do not open the enclosure. Contact Doric Lenses for return instructions if the unit does not work properly and needs to be repaired.

8.2 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. For more information, see our [Website](#).

8.3 Disposition



Figure 10.1: WEEE directive logo

According with the directive 2012/19/EU of the European Parliament and the Council of the European Union regarding Waste Electrical and Electronic Equipment (WEEE), when the product will reach its end-of-life phase, it must not be disposed with regular waste. Make sure to dispose of it with regards of your local regulations. For more information about how and where to dispose of the product, please contact Doric Lenses.

8.4 Contact us

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

Phone 1-418-877-5600

Email sales@doriclenses.com

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