doric

2-color Fluorescence Microscope System

User Manual

Version 1.2.1

Contents

1

Important Safety Information

1.1 General Safety Information

As the 2-color Fluorescence Microscope Driver includes a **Ce:YAG light source driver**, additional safety information is required. The *Ce:YAG + LED Fiber 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 IEC[1] and the FDA[2]. 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 *Ce:YAG + LED Fiber 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** even for the base model of the *Ce:YAG + LED Fiber Light Source* which does not include a laser output in working condition.

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 provided USB key. You can also contact directly Doric Lenses by email (sales@doriclenses.com) to obtain a copy of this application note.

DANGER!

The *Ce:YAG + LED Fiber Light Source* is a Class 3B laser product.
Read the application note *Important Laser Safety Information* BEFORE FIRST USE.

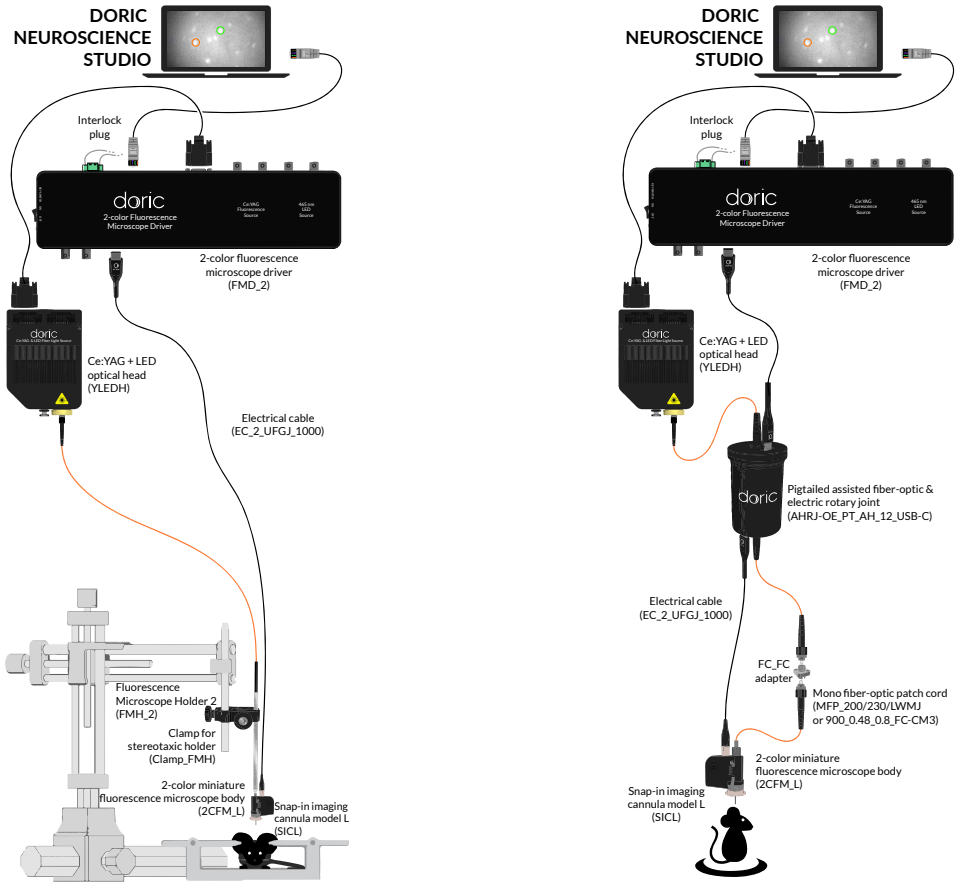
The *Ce:YAG + LED Fiber Light Source* is a Class 3B laser product emitting visible light at sufficiently high power levels to **PERMANENTLY DAMAGE THE EYES. NEVER LOOK** directly into the optical beam exiting from the output FC connector or from any optical fiber connected to the output FC connector. **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 *Ce:YAG + LED Fiber Light Source* or from any optical fiber connected to its output FC connector. The *Ce:YAG + LED Fiber Light Source* is provided with a safety interlock connector on the rear panel of its driver. When the interlock circuit is shorted and the power key is inserted, the driver is enabled. For a safe use of the *Ce:YAG + LED Fiber 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 set a proper laser safety interlock circuit for your application and laboratory installation. The *Ce:YAG + LED Fiber Light Source* emits light spanning over a large bandwidth in the visible light spectrum. Since the output spectrum depends on the exact model, a typical output spectrum is provided in the Data Sheet of your specific model.

[1] International Electrotechnical Commission
[2] Food and Drug Administration

2

Overview

2.1 2-color Fluorescence Microscope Systems



(a) *Head-fixed system configuration*

(b) *Freely-moving system configuration*

Figure 2.1: *2-color Fluorescence Microscope System Layouts*

The **2-color fluorescence microscope system** is configured to image the fluorescence of two different fluorophores inside the brain. The **Head-fixed** configuration (Fig. 2.1a) is modular and consists of a connectorized *Ce:YAG + LED Optical Head*, a *2-color Fluorescence Microscope Driver*, a *2-color Miniature Fluorescence Microscope Body*, an *Imaging cannula*, a *Fluorescence Microscope Holder*, and a *Stereotaxic adapter*. This configuration is used when the animal is placed in a stereotaxic apparatus such as during the cannula implantation. The **Freely-moving** configuration (Fig. 2.1b) is modular and consists of a connectorized *Ce:YAG + LED Optical Head*, a *2-color Fluorescence Microscope Driver*, a *Pigtailed Assisted Fiber-optic & Electric Rotary Joint*, a *2-color Miniature Fluorescence Microscope Body* and an *Imaging cannula*. This configuration is used when the animal is moving freely in a cage.

2.2 Snap-in Fluorescence Microscope Bodies

Depending on the cannula type used, three models of microscope bodies (Fig. 2.2) are offered: *Model LD* for deep brain imaging, *Model LV* for very deep brain imaging and *Model S* for surface brain areas. Both models have a 0.5 NA objective lens, an M3 connector to connect a Fiber-optic Patch Cord, a 12 pins connector to connect the microscope to the Fluorescence Microscope Driver, and 2 imaging sensors to detect each fluorophore.The first imaging sensor is configured to detect GFP-like fluorophores and the second is configured to detect RFP-like fluorophores. Each microscope is provided with a protective cannula to prevent damage to the objective lens.

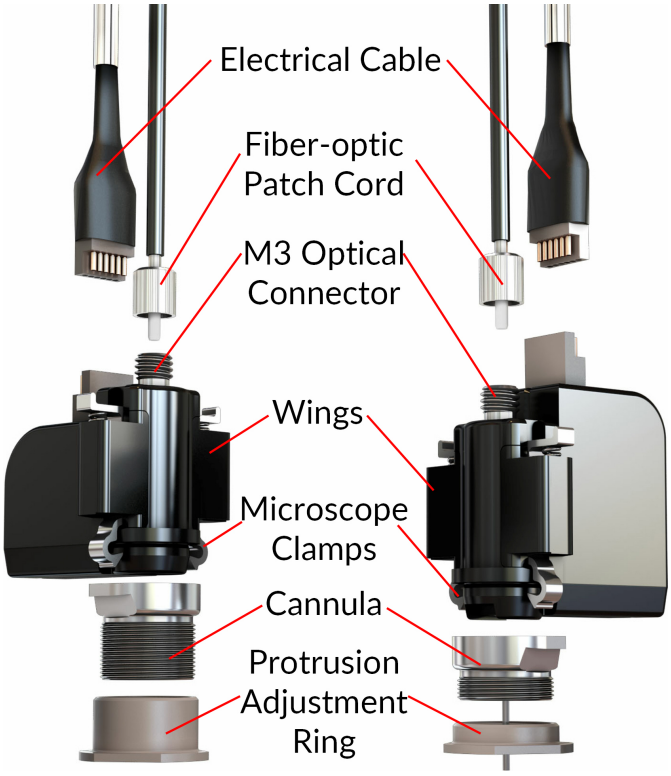


Figure 2.2: *2-color Fluorescence Microscope Bodies, Model S (left) and Model L (right)*

- The **Electrical cable** sends and receives electrical signal for the microscope. This is done using a 12-pin connector to USB-C.
- The **Fiber-optic patch cord** sends optical signal to the microscope from the light source. It connects to the **M3 optical connector**.
- The **Wings** are used to stabilize the microscope while clamping/unclamping.
- The **Microscope clamps** are used to secure the microscope on a cannula base and are part of the **Connection System**.
- The **Cannula** and **Protrusion adjustment ring** are described in section 2.4.

2.3 2-color Fluorescence Microscopy System: Filter Set

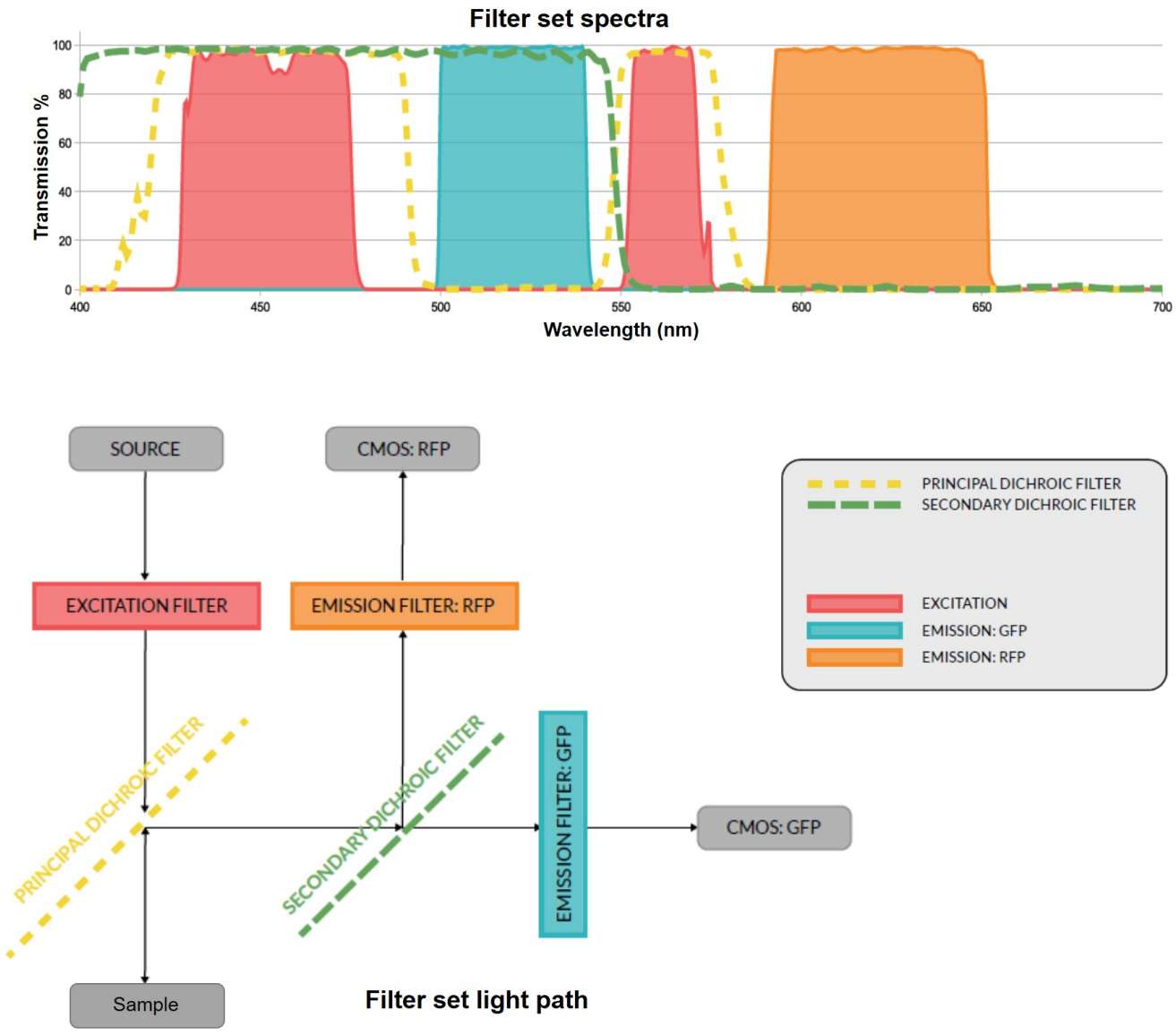


Figure 2.3: *2-Color Fluorescence Microscope: Filter Set spectra and Light path*

The **2-color Fluorescence Microscope system** has been designed for imaging of two fluorophores using two sensors and and uses the Ce:YAG + LED optical head as the excitation light source. The standard system (Fig. 2.3) uses the blue LED as the light source to excite the GFP-like fluorophore and the Ce:YAG light source to excite the RFP-like fluorophore.

2.4 Snap-in Imaging Cannulas

Snap-in imaging cannulas transmit images of structures located inside the brain to the surface of the skull. Each of the three microscope types (LD, LV and S) have their own optimized cannulas. The *Model LD and LV Imaging Cannulas* use an image guiding gradient-index rod lens that relays the image from inside the brain to the skull surface (Fig. 2.4 right). For areas near the brain surface (less than 150 µm below the skull), the *Model S Imaging Cannula* (Fig. 2.4 left) provides higher image quality and a larger FOV than the *Model L imaging cannula*.

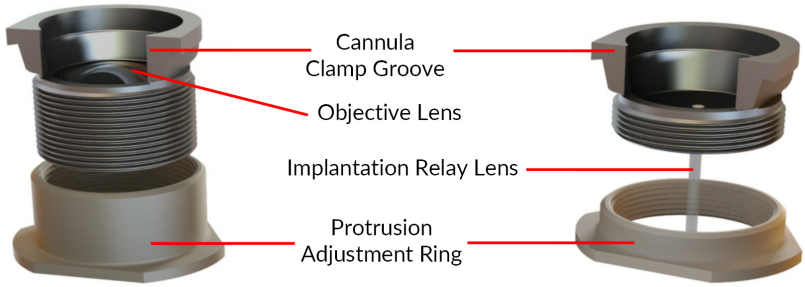


Figure 2.4: *Snap-in Imaging Cannula Type S (left) and Type L (right)*

Field of view position adjustment of the *Model LD and LV imaging cannulas* are done with a protrusion adjustment ring set that comes with each cannula (Fig. 2.5). The *Model S imaging cannula* uses a single protrusion adjustment ring with a height of 4.5 mm.

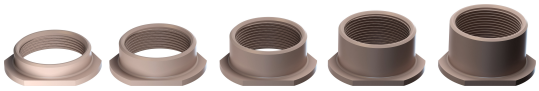


Figure 2.5: *Model L Imaging Cannula Protrusion Adjustment Rings. Height (in mm) from left to right: 2.05, 2.77, 3.48, 4.2 and 4.9*

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. 2.6) is provided with the microscope system. 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 optimal performance. The fixed and rotary patch cords use an FC connector.
- The **USB-C connectors** are used to provide electrical communication between the driver and the microscope body.
- The **5 V mini-USB-B connector** connects to the power supply to allow assisted rotation. For a proper transmission of the microscope images to the microscope driver, connecting the power supply to the rotary joint is required, even if an assisted rotation is not needed.

Figure 2.6: *Pigtailed Assisted Fiber-optic & Electric Rotary Joint*

2.6 2-color Dummy Microscope

The **2-color Dummy Microscope** is of similar weight and size as the **2-color Miniature Fluorescence Microscope Bodies**. It is used to accustom animal subjects to the weight and feel of the microscope.

2.7 2-color Fluorescence Microscope Driver

This driver (Fig. 2.7) allows for computer control over the excitation light sources, image capturing and broadcast at video rate to a computer via high speed Ethernet communication. It can be triggered by or synchronized with external recording devices, and it can also trigger other devices. This *2-color Fluorescence Microscope Driver* is only used with the *2-color Fluorescence Microscope System*. The standard model has an integrated **Ce:YAG** + **LED** optical head driver.

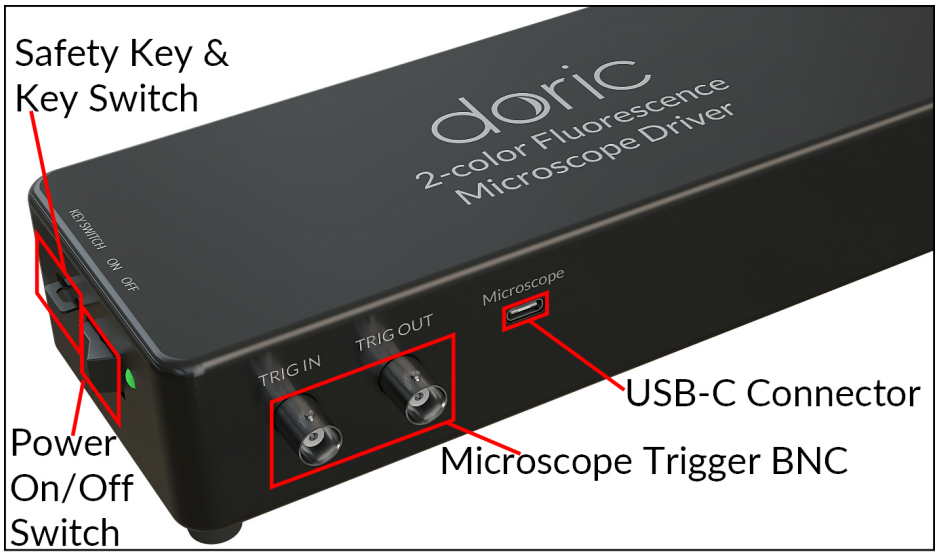


Figure 2.7: *2-color Fluorescence Microscope Driver, Front and Side*

- The **USB-C Connector** is located on the front of the driver. The connector links the driver and the microscope.
- The **Microscope Trigger BNCs** are used to receive (**TRIG IN**) or send (**TRIG OUT**) trigger signals tied to the microscope driver as a whole.
- The **On/Off** switch powers the device.
- The **Safety Key** and **Key Switch** are located beside the **On/Off** switch. The **Safety Key** must be inserted in the **Key Switch** to be able to activate the light sources.
- The **Driver Indicator** blinks red and green when the driver is initializing, and stops blinking when the driver is ready.

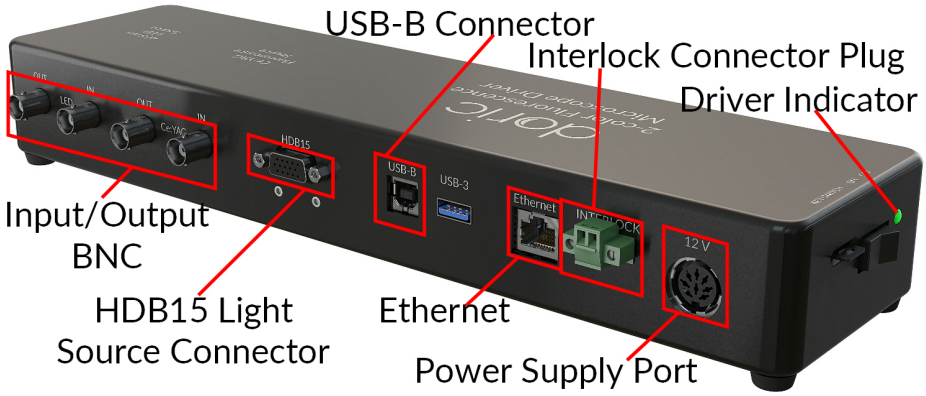


Figure 2.8: *2-color Fluorescence Microscope Driver, Back*

- The LED and Ce:YAG **Input BNC** connectors are used to receive analog signals for a given light source.
- The LED and Ce:YAG **Output BNC** connectors are used to send analog signals from a given light source.

- The **Interlock Connector Plug** is used to connect the driver to an interlock system. See chapter 1 - "Important Safety Information" for more information.
- The **Ethernet** port connects the driver to a computer using an ethernet cable.
- The **Power Input** accepts a 12 VDC power supply.
- The **HDB15 connector** links the driver with the **Ce:YAG + LED Optical Head**.

2.8 Ce:YAG + LED Optical Head

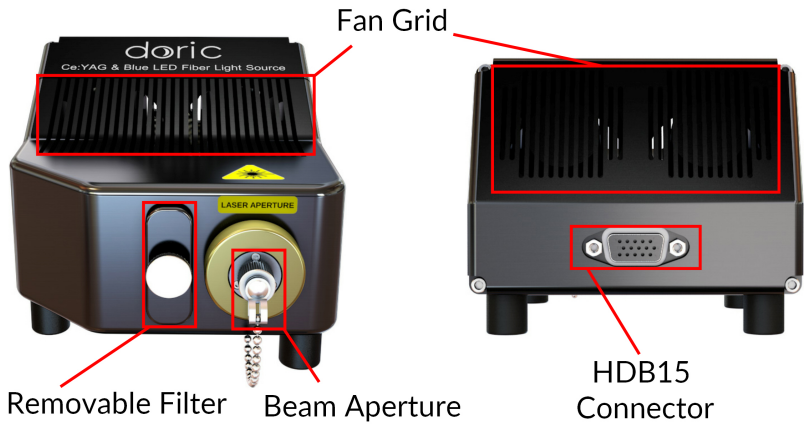


Figure 2.9: *Ce:YAG + LED Optical Head*

The *Ce:YAG + LED Optical Head* (Fig. 2.9) is the standard light source provided with the **2-color Fluorescence Microscope System**. It includes a light source combined with the Ce:YAG Fluorescence output. For further information, see the ***Ce:YAG + LED Fiber Light Source*** user manual.

DANGER!

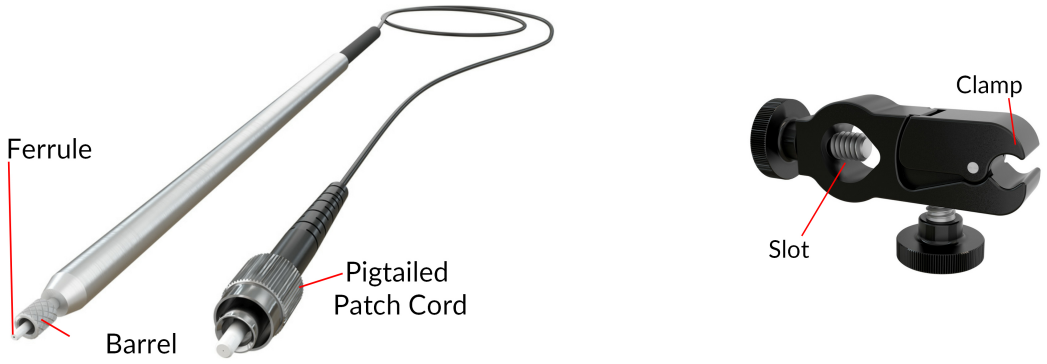
The *Ce:YAG + LED Optical Head* is a Class 3B laser product.
Read the application note *Important Laser Safety Information* BEFORE FIRST USE.

The *Ce:YAG + LED Optical Head* is considered a Class 3B laser product, and it is critical to follow the safety instructions in this manual. 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 provided USB key. You can also contact directly Doric Lenses by email (sales@doriclenses.com) to obtain a copy of this application note.

2.9 Fluorescence Microscope Holder 2

The *Fluorescence Microscope Holder 2* and the *Clamp for Fluorescence Microscope Holder* are used to secure the microscope in a stereotaxic system.

- The *Clamp for Fluorescence Microscope Holder* (Fig. 2.10b) 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 **Pigtailed Patch Cord** allows the holder to be connected to an FC-connectorized light source.
- The **Ferrule** is inserted into the microscope **M3 optical connector**. The ferrule is secured by screwing the **Barrel** onto the **M3 Optical Connector**.



(a) *Fluorescence Microscope Holder 2* (b) *Clamp for Fluorescence Microscope Holder*

Figure 2.10: *Fluorescence Microscope Holder Elements*

(a) *2-color Fluorescence Microscope Snap-on Tool* (b) *2-color Fluorescence Microscope Snap-off Tool*

Figure 2.11: *Snapping tools*

2.10 Microscope Snap-on and Snap-off tools

The microscope **Snap-on Tool** (Fig. 2.11a) and **Snap-off Tool** (Fig. 2.11b) are used to attach and separate the *Microscope Body* and the *Imaging cannula*.

2.11 Electrical cable for 2-color Fluorescence Microscope Bodies

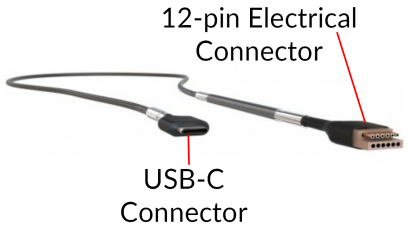


Figure 2.12: *Electrical Cable for 2-color Fluorescence Microscope Bodies*

The *Electrical cable for 2-color Fluorescence Microscope Bodies* is used to connect the *2-color Fluorescence Microscope Body* to the *Rotary Joint*.

- The **12-pin Electrical Connector** is linked to the microscope body.
- The **USB-C Connector** is linked to the rotary joint. When connecting the USB-C connector, ensure that the logo on the connector is facing outward.

3

Operations Guide

3.1 Connecting the driver

The system is provided with a safety interlock connector on the rear panel of its driver. When the interlock circuit is shorted and the power key is inserted, the light sources are enabled. For a safe use of the driver, the safety interlock connector should be connected to the laser safety interlock circuit of the laboratory.

1. Connect the driver's safety interlock circuit.
2. Ensure the **Safety Key** is properly inserted into the **Key switch**.
3. Connect the microscope driver to the wall using the 12 VDC power supply.
4. Connect the microscope driver to the computer or to the router using the Ethernet cable.
5. Connect the microscope USB-C electrical cable to the USB-C port on the driver. Ensure that the logo on the USB-C connector is facing upward.
6. Turn on the power switch. The **Driver Indicator Light** will blink red and green. After opening the Doric Neuroscience Studio, when the driver is properly initialized, the light will stop blinking.

3.2 Installing the software

1. **Run** the Doric Neuroscience Studio Installer from the supplied USB key or download the latest version of the software from our website.
2. **Select** the language to use during the installation.
3. In the license agreement window (fig. 3.1), you need to accept the agreement and **click Next** to continue the process.

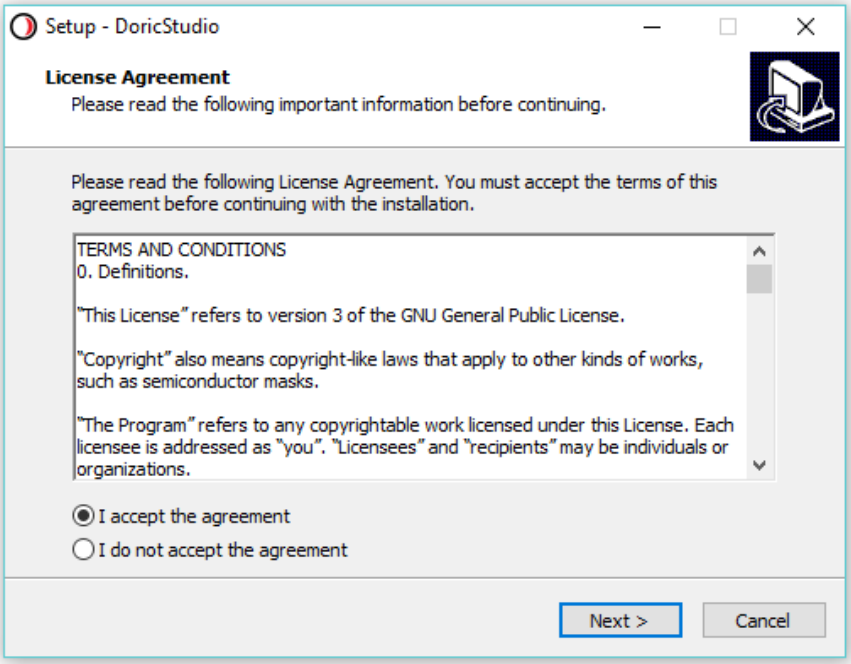


Figure 3.1: *Doric Neuroscience Studio License Agreement*

4. **Click Next** in the Information window.
5. **Choose** where to install the software (Fig. 3.2) and **Click Next**.

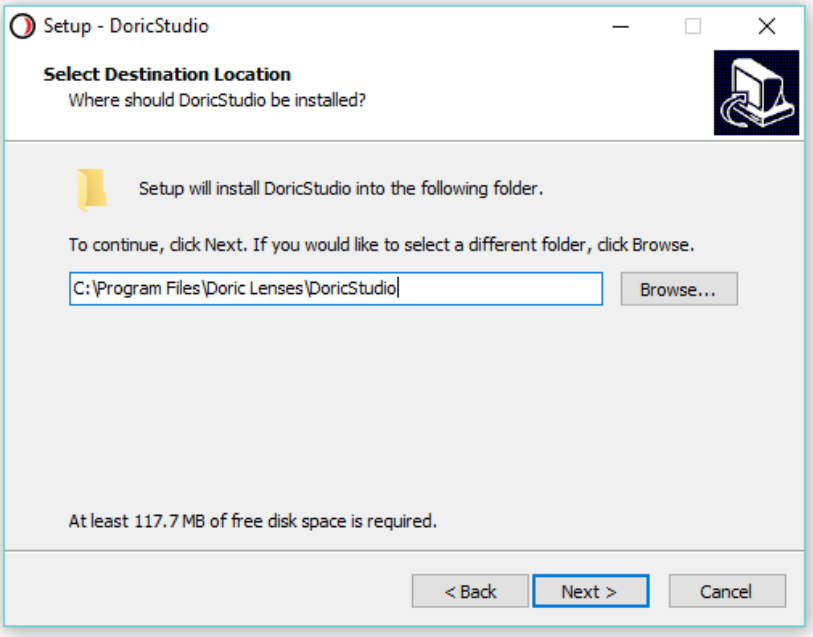


Figure 3.2: *Select Destination Location*

6. **Choose** if you want to create a shortcut in the Start Menu folder and **click Next**.

7. **Choose** if you want to create a desktop icon and **click Next**.
8. When you are ready, **click Install** to begin the process. This should take a few moments. When the installation is done, the message in Fig. 3.3 will show up.

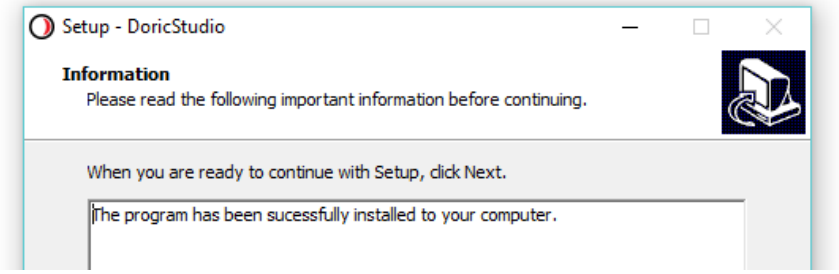


Figure 3.3: *Successful installation of the Doric Neuroscience Studio*

9. **Click Next** and **Finish** to exit the setup.
10. You can now run the software!

3.3 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 3.3.1.

3.3.1 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 or 10, type *network connections* on the Start Screen itself (Fig. 3.4).

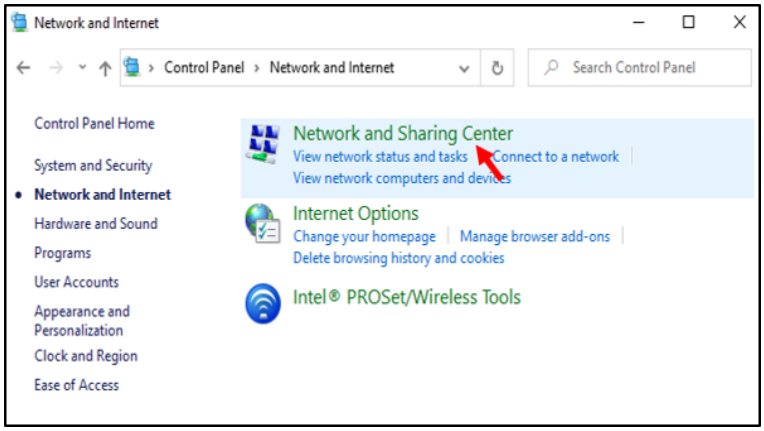


Figure 3.4: *Open Network and Sharing Center.*

For windows 7 users - on the side menu, select Change Adapter Settings (Fig. 3.5).

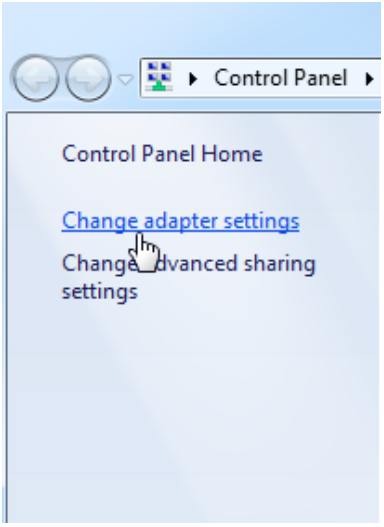


Figure 3.5: *Click on Change Adapter Settings.*

Right-click on the local adapter and select Properties (Fig. 3.6).

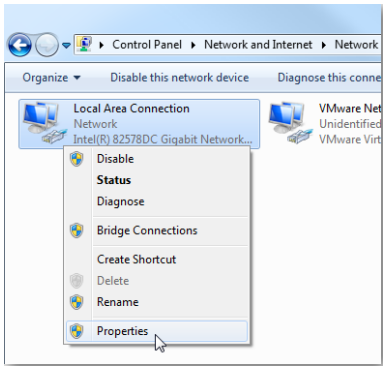


Figure 3.6: *Right-click on Local Adapter Properties.*

Select Internet Protocol Version 4 (TCP/IPv4) in the list, and click on Properties (Fig. 3.7).

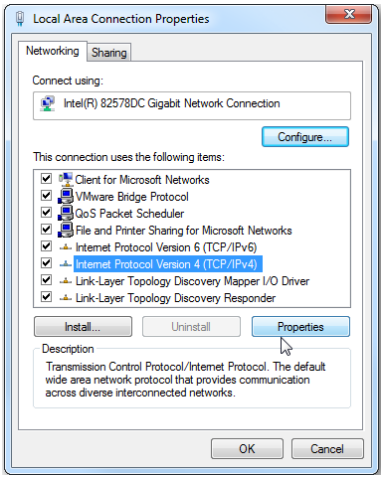


Figure 3.7: *Open IPv4 Properties.*

Select 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. 3.8).

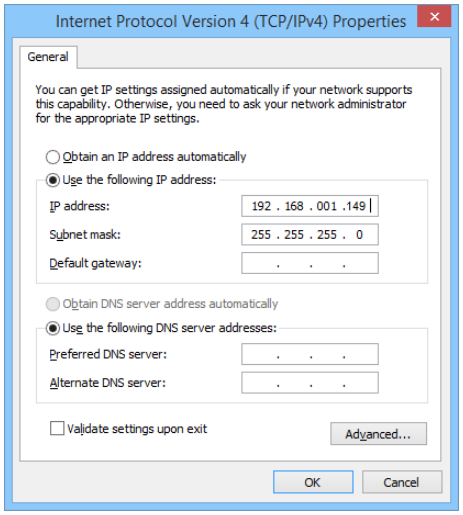


Figure 3.8: *Static IP Settings.*

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. 3.6 and click *Configure* (Fig. 3.9).

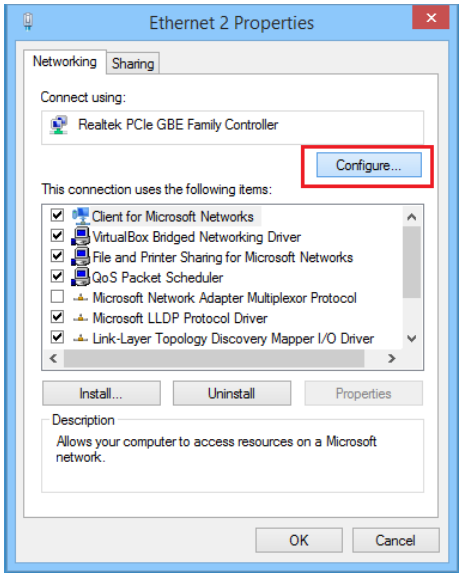


Figure 3.9: *Interface Configuration.*

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. 3.10).

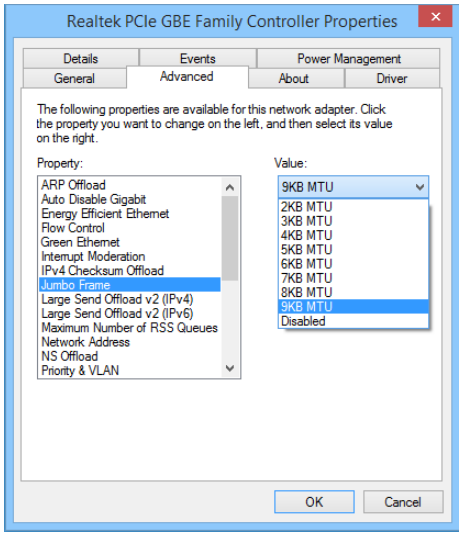


Figure 3.10: *Jumbo Frame Configuration.*

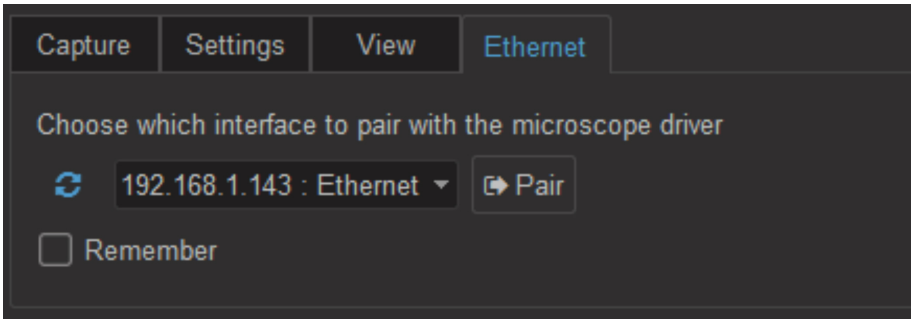


Figure 3.11: *IP Address Configuration*

3.3.3 Initial Microscope Configuration

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

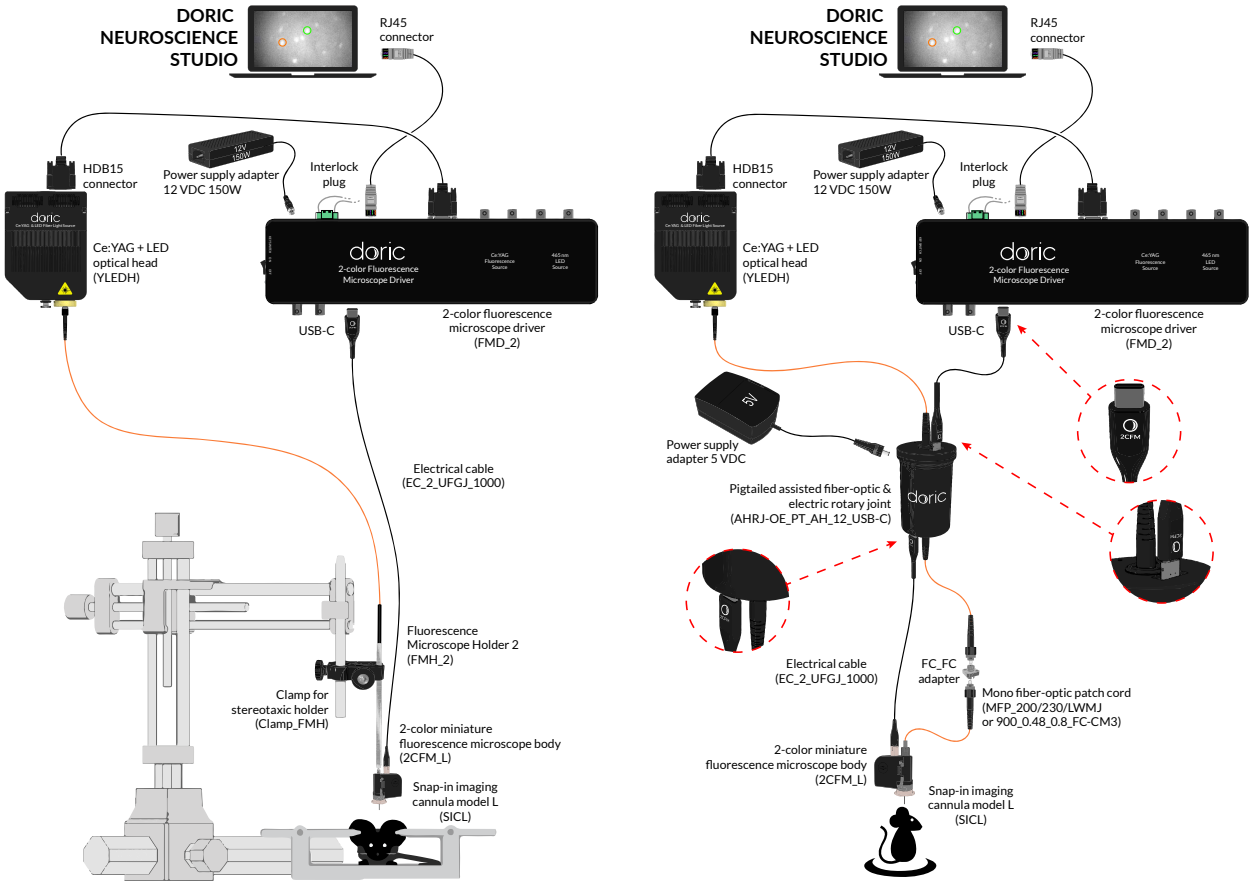
When connected for the first time, the microscope driver will broadcast its MAC address. To set the IP address, you can use the built-in ethernet setup interface in the Doric Neuroscience Studio software.

1. Turn ON the microscope driver.
2. Launch the Doric Neuroscience Studio software.
3. Allow ∼10 seconds for the microscope initialization.
4. Choose the proper network interface from the list in the *Ethernet Settings* box (Fig. 3.11), and click *Pair and Remember* to configure the microscope driver.
5. For proper microscope function, the microscope **Masks** must be installed; see section 3.5.1.

When the system is ready to use, the software status will display *The microscope is ready*.

3.4 Connecting the system

Figure 3.12 shows the head-fixed and freely-moving configurations connections.



(a) *Head-fixed Configuration* (b) *Freely-moving Configuration*

Figure 3.12: *2-color Microscope System Connections*

3.4.1 Microscope driver and optical head installation

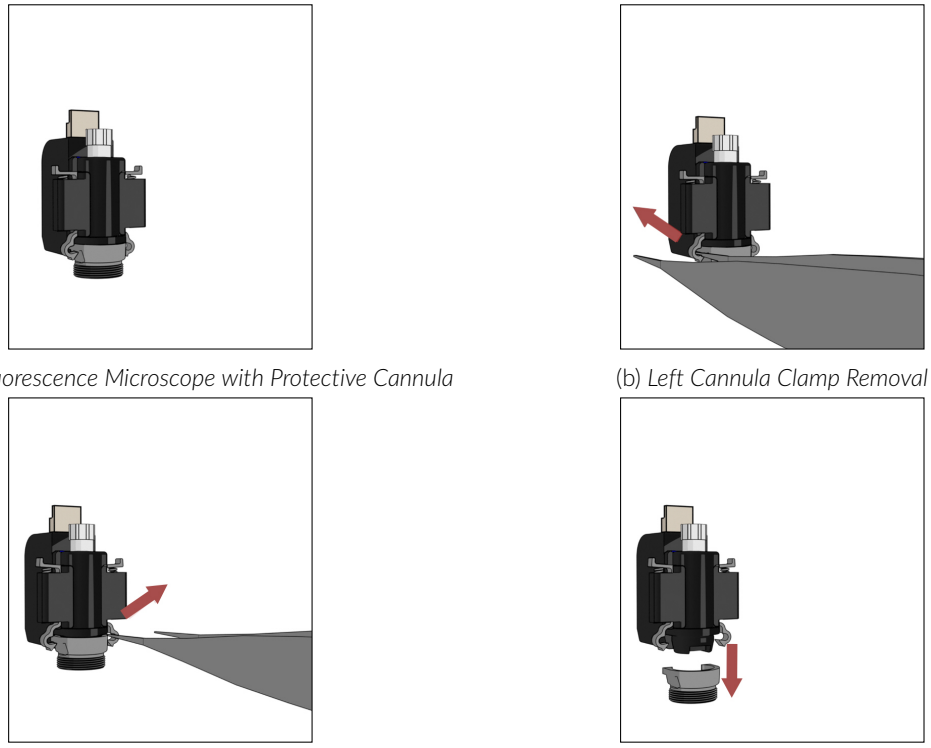
⚠ For safe use of the *Ce:YAG + LED Optical head*, connect the driver **Interlock connector plug** to a **Laser safety interlock circuit**. See the application note ***Important Laser Safety Information*** for more information, or contact your institution's laser safety officer.

1. Connect the *12 VDC power supply* to the driver **12 VDC power input**. Turn on the *2-color fluorescence microscope driver*.
2. Connect the driver to the computer using the *CAT5E Ethernet Cable*.
3. Ensure the **Key** is inside the driver **Key Switch**. If the **Key** is removed or improperly inserted, the *Ce:YAG + LED Optical head* cannot be activated.
4. Connect the driver to the **Ce:YAG + LED optical head** using the custom *HDB15 cable*.

5. The driver has multiple usage modes.

    - The **2-color Fluorescence Microscope Driver** integrates control electronics for the **2-color Fluorescence Microscope Body** and a *Ce:YAG + LED Optical Head*.
    - When the microscope is in use, each microscope sensor is linked to a single **Illumination source**. Once an **Illumination Source** has been linked to a microscope sensor, it can no longer be controlled independently.
    - If the **Illumination Sources** are not linked to the microscope, they can be controlled independently. The **Input/Output BNC** on the back of the driver are used to receive/send analog signals for each individual light source driver.

3.4.2 Imaging cannula removal and installation



(a) *2-color Fluorescence Microscope with Protective Cannula*

(b) *Left Cannula Clamp Removal*

(c) *Right Cannula Clamp Removal*

(d) *Remove Protective Cannula*

Figure 3.13: *Cannula Removal Procedure*

Handle the microscope and cannula with care. The relay lens and objective are fragile and any stain or scratch can affect image quality. **Do not touch the surface of the lenses**.

1. Remove the **Protective Cannula** from the *Microscope Body* (Fig. 3.13). Any other microscope cannula can be removed in the same way.

    a) Using the *Microscope Snap-off Tool*, remove the left **Cannula clamp** from the **Microscope Clamp Groove** (Fig. 3.13b).

    b) Remove the right **Cannula Clamp** from the **Microscope Clamp Groove**(Fig. 3.13c).

    c) Remove the **Protective Cannula** (Fig. 3.13d).

2. Secure the imaging cannula on the microscope body (Fig. 3.14). Any other microscope cannula can be installed in the same way.

    a) Remove the **Input Protective Cap** from the *Imaging Cannula* (Fig. 3.14a).

    b) The **Snap-on Tool** has two unique tines, each of a different size. Take the smaller tine and place it on the clamps. The larger tines are placed under the **Wings** (Fig. 3.14b).

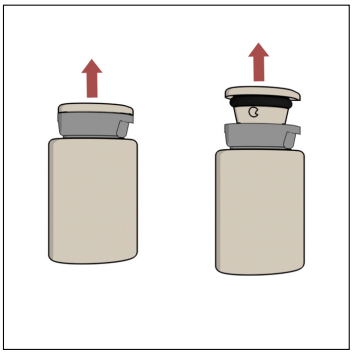
    c) Each tine has a small tooth that is used to prevent the microscope from moving; ensure they are properly in place.

d) Press the **Snap-on Tool** to close the tines and open the clamps (Fig. 3.14c).

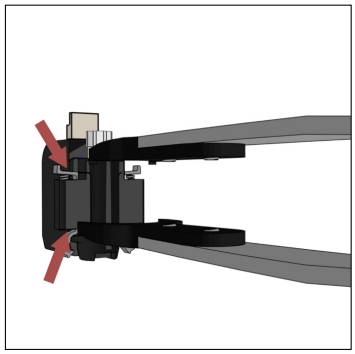
e) Deposit the microscope onto the **Cannula** (Fig. 3.14d).

f) Open the **Snap-on Tool**, and the **Cannula clamps** will close onto the **Microscope Clamp Groove** (Fig. 3.14e).

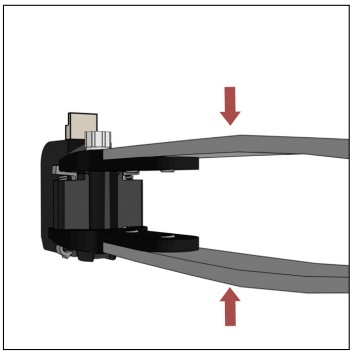
g) Inspect the **Cannula Clamps**; if they are not completely inside the **Microscope Clamp Groove**, gently press them into place using your fingers (Fig. 3.14f).



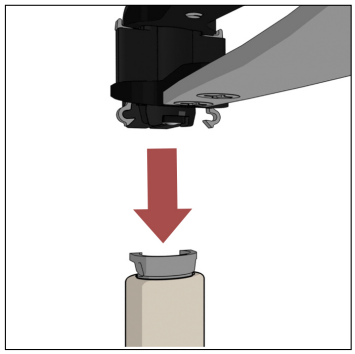
(a) *Remove the Input Protective Cap from the Imaging Cannula*



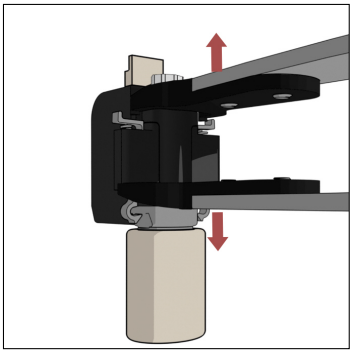
(b) *Place the Snap-on Tool in the Appropriate Position*



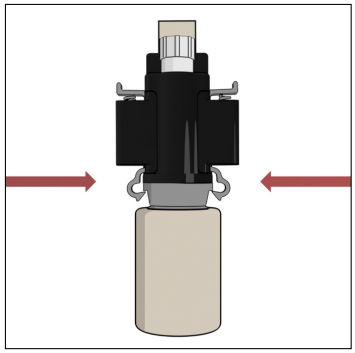
(c) *Close the Snap-on Tool*



(d) *Place the Microscope on the Imaging Cannula*



(e) *Open the Snap-on Tool to Close Cannula Clamps*



(f) *Verify Cannula Clamp Closure*

Figure 3.14: *Cannula Installation Procedure*

3.4.3 FC Connector 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. 3.15).

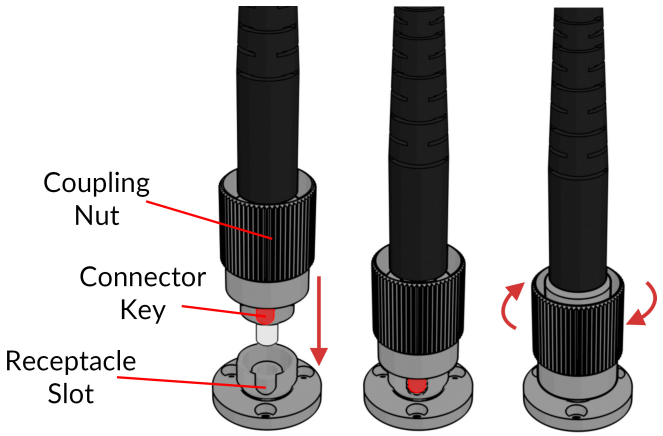
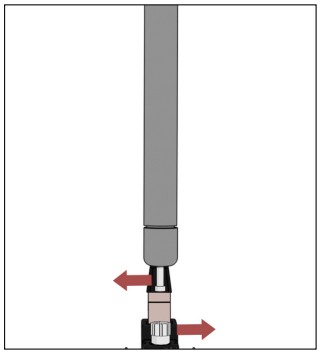


Figure 3.15: *FC connector, Fiber Installation*

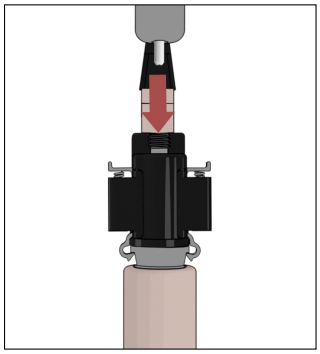
> ⚠ To reduce the risk of eye injury, **it is sound practice to NOT CONNECT/DISCONNECT OPTICAL FIBERS when the light source is turned on**.

3.4.4 Head-fixed configuration installation

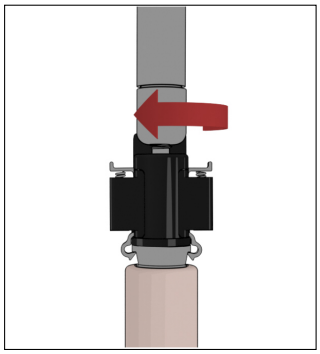
1. Connect the **USB-C** connector of the *Electrical Cable* to the driver.
    - The **USB-C connectors** must be inserted in the correct orientation. The logo on the USB-C connector must be oriented upward (Fig. 3.12b).
2. Connect the **Electrical Cable** to the microscope using the **12-pin Electrical Connector**.
3. Install the *Fluorescence Microscope Holder 2* in the **Stereotaxic Clamp**. Ensure that the holder is tightly secured in the clamp.



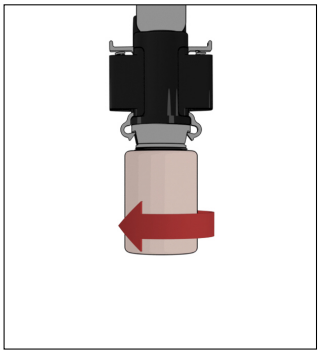
(a) *Remove the Protective Caps from the Microscope and Fluorescence Microscope Holder*



(b) *Place the FMH 2 Ferrule Inside the Microscope M3 connector*



(c) *Screw the Microscope Holder Barrel Onto the M3 Connector*



(d) *Unscrew and Remove the Cannula Output Cap*

Figure 3.16: *Cannula Installation Procedure*

4. Secure the microscope onto the *Fluorescence Microscope Holder 2*.
    a) Remove the **Connector Caps** from the microscope **M3 optical connector** and the *Fluorescence Microscope Holder 2* ferrule (Fig. 3.16a).
    b) Clean the ferrule of the Fluorescence Microscope Holder 2 using isopropanol and a lint-free wipe.
    c) Insert the ferrule into the **M3 Optical Connector** (Fig. 3.16b). Secure them in place by screwing the *Fluorescence Microscope Holder 2* barrel (Fig. 3.16c).
5. Connect the *Ce:YAG + LED optical head* and the *Fluorescence Microscope Holder 2* using the holder's pigtailed optical fiber.
6. When ready for use, remove the **Output Protective Cap** from the cannula by unscrewing it (Fig. 3.16d). If using a type-L cannula, take great care to remove it in a straight motion so as not to break the rod lens.

3.4.5 Freely-moving configuration installation

1. Link the driver and the *Pigtailed Assisted Fiber-optic and Electric Rotary Joint* using the USB-C/USB-C cable (Fig. 3.12b).
    - The **USB-C Connectors** must be inserted in the correct orientation. The direction is indicated by the small **Logo** on the **USB-C Connector**.
    - When inserting the connector into the *2-color fluorescence microscope driver*, the **Logo** (Fig. 3.12b) should be on the same side as the top of the driver.
    - The **Logo** should face outside when inserting the connector into the rotary joint.
2. Connect the top **Pigtailed Patch Cord** of the rotary joint to the *Ce:YAG +LED Optical Head* using the FC connector.
3. Link the rotary joint **USB-C Connector** and the microscope **Electrical Connector** using the *Electrical Cable*.
4. Connect the lower pigtailed patch cord of the rotary joint to the *FC Mating Adapter*, then connect the mono fiber optical patch cord (FC-CM3) to the microscope M3 connector. Before inserting the patch cord into the M3 connector, clean the ferrule of the patch cord using isopropanol and a lint-free wipe.

3.5 Microscope

The Microscope module of the Doric Neuroscience Studio provides an interface to control our Fluorescence Microscope Driver. The module enables image acquisition and its export in 16 bit .tif or in .doric (hdf5-based) files. The TIF format can easily be read with any standard imaging software. Doric files can be read by the Doric Neuroscience Studio **Image Analysis Module** or using an HDF5 library . Despite the fact that the images are saved with a 16 bit pixel depth, the true image pixel depth is 10 bit, so pixel gray values are contained between 0 and 1020 counts.

Below is the user interface (Fig. 3.17) and a complete description of all the functions.

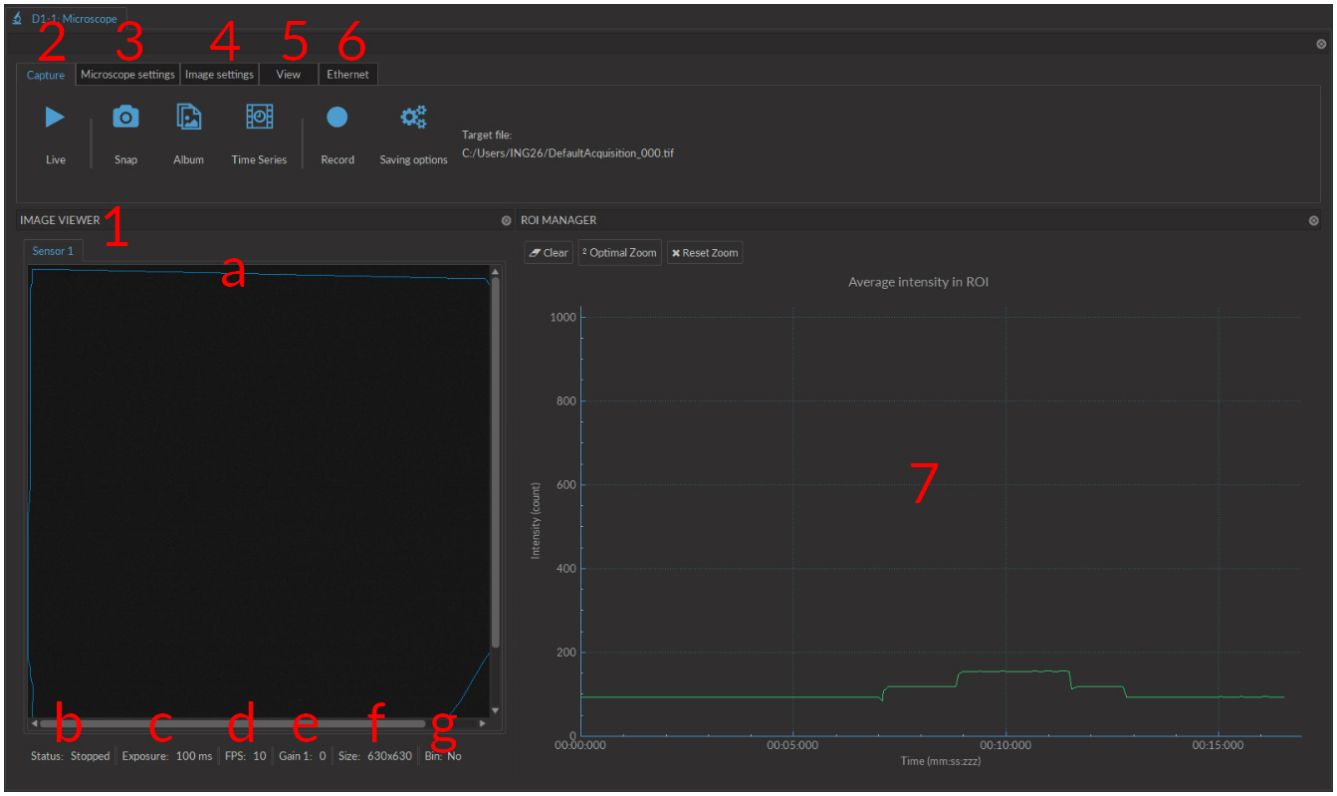


Figure 3.17: *Microscope Module Interface*

1. The **Image Box** (Fig. 3.17) displays images from the microscope and allows region of interest (ROI) drawing by clicking and dragging the mouse over the image.
    a) The **Sensor Tabs** (Fig. 3.17) display the sensors available to view. For multi-sensor microscopes, changing tabs allows you to see the image available to each.
    b) The microscope **Status** (Fig. 3.17) will indicate the current microscope state (Live/Stopped).
    c) The **Exposure** (in ms) (Fig. 3.17) indicates the exposure time of the microscope sensor.
    d) The **FPS** (Frames Per Second) (Fig. 3.17) indicates the number of frames per second taken by the sensor.
    e) The **Gain** (Fig. 3.17) indicates the electrical gain of the sensor.
    f) The **Size** (Fig. 3.17) indicates the resolution of the sensor images (in Pixels x Pixels).
    g) The **Bin** (Fig. 3.17) status indicates whether or not the sensor image is being binned (yes/no).
2. The **Capture** tab (Fig. 3.17) contains different image-capturing functions of the microscope.

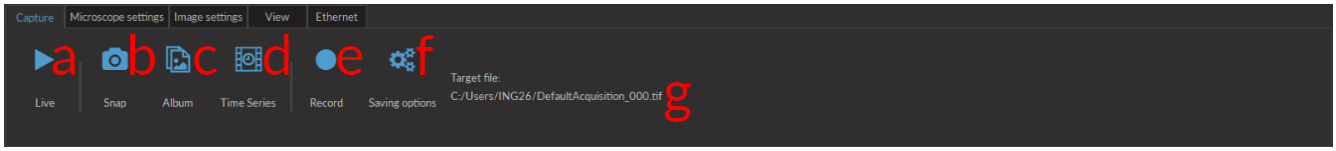


Figure 3.18: *Capture Tab*

a) The **Live** button (Fig. 3.18), when pressed, displays images from the microscope. These images are not saved.

b) The **Snap** button (Fig. 3.18), when pressed, takes a snapshot of the current image and saves it in the requested directory with the desired name (**Saving Options**) as a single image.

c) The **Album** button (Fig. 3.18), when pressed, acquires a snapshot and adds it to an album stack. The whole stack can be saved as one image stack.

d) The **Record** button (Fig. 3.18), when pressed, acquires a continuous image stream, until **Stop** is pressed, and saves it in the requested directory with the desired name (**Saving Options**) as one image stack.

Figure 3.19: *Time Series Window*

e) The **Time Series** button (Fig. 3.18), when pressed, opens the time series interface (Fig. 3.19).

   i. The **Number of time points** (Fig. 3.19) defines the number of moments when a set of images will be recorded.

   ii. The **Images per time point** (Fig. 3.19) defines the number of images taken in each set.

   iii. The **Time interval between points** (Fig. 3.19), defined in ms, s and min, defines the duration between each image set. This duration always has a minimum value of **Exposure time**x**Images per time point**.

   iv. The **Summary** box (Fig. 3.19) shows many values related to the time series, including the **Total images** recorded, the **Total memory** occupied by the full series, the **Time point duration** and the **Total duration** of the full series.

   v. The **Progression bar** (Fig. 3.19) displays the progress (in %) of the time series.

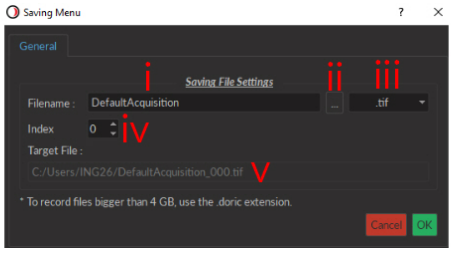


Figure 3.20: *Saving Options Window*

f) The **Saving options** button opens the **Saving options window**.

   i. The **Filename** box (Fig. 3.20) is used to define the recorded file name.

   ii. The **...** button (Fig. 3.20) opens a window used to choose the save file location.

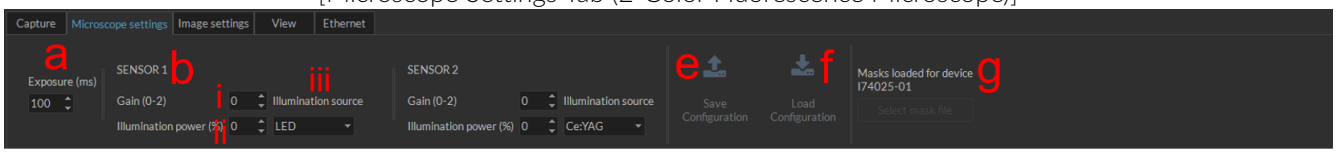
   iii. The **File type** drop-down menu (Fig. 3.20) is used to decide which file type is used to save images. For files larger than 4 GB, the .doric extension is recommended.

   iv. The **Index** box (Fig. 3.20) displays the current index that will be added to the filename.

v. The **Target File** box (Fig. 3.20) shows the full location and name of the file being saved when an image sequence is recorded.

3. **Microscope settings** tab (Fig. 3.17) is used to set parameters related to the microscope recording images.

[Microscope Settings Tab (2-Color Fluorescence Microscope)]



[Microscope Settings Tab (eFocus Miniature Fluorescence Microscope)]

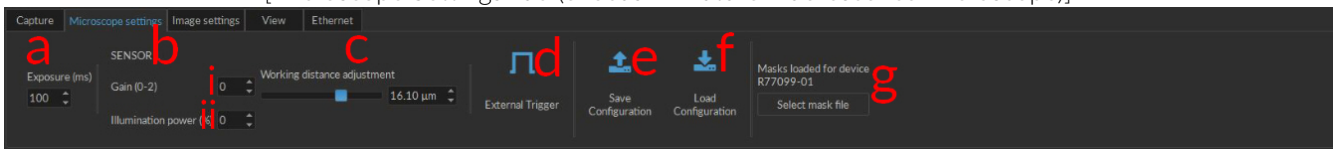


Figure 3.21: *Microscope Settings Tab*

a) The **Exposure** box (Fig. 3.21) sets the exposure time of the sensor. The time can be set between 22 and 1000 ms.

b) The **SENSOR** section (Fig. 3.21) defines characteristics for a single sensor and the associated excitation source. When a microscope used has multiple sensors, multiple **SENSOR** sections will be displayed, one for each sensor.

   i. The **Gain** box defines the sensor gain.

   ii. The **Illuminator power (%)** box defines the power emitted by the excitation light source. The light sources will be activated when the image acquisition is started. The maximum optical power (in mW) depends on the light source model.

   iii. The **Illumination source** box defines if a light source is linked to a given sensor. If No source is selected, this light source can be controlled independently using the light source Tab. For more information on how to control the light sources using the Light Source Tab, please refer to the ***Doric Neuroscience Studio*** User Manual.

c) The **Working Distance Adjustment Slider** appears when an eFocus Miniature Fluorescence Microscope is connected to the driver. This slider will adjust the working distance from -45 to 45 um for snap-in fluorescence microscope bodies and from 0 to 350 um for twist-on fluorescence microscope bodies.

d) The **External Trigger** button (Fig. 3.21) opens the **external trigger** window.

   i. The **Number of images per trig** box (Fig. 3.22a) defines the number of images acquired at each trigger pulse.

   ii. The **File name/location** (Fig. 3.22a) box displays the location where the images are saved as well as their file name.

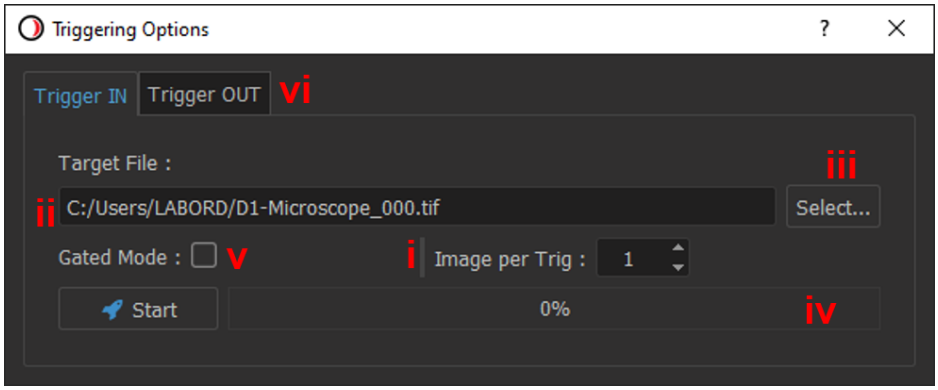
   iii. The **Select...** (Fig. 3.22a) button allows the selection of the **File name/location**.

   iv. The **Progression bar** (Fig. 3.22a) displays the advancement of the triggered sequence (in %).

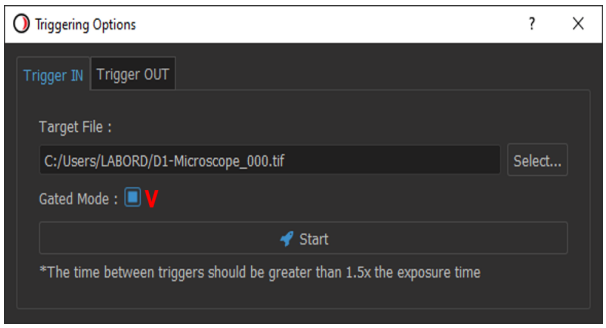
   v. The **Gated mode** checkbox (Fig. 3.22a) will change the external trigger to gated mode (Fig. 3.22b). In this mode, the microscope will only aquire images when a high TTL signal is received on the TRIG IN input.

   vi. Selecting the **Trigger Out** Tab will change the external trigger to *Trigger Out* mode. This mode is used to select the type of TTL signal generated at the output of the Microscope Trigger Out BNC. In Normal Mode, the TTL signal is high whenever the microscope is live and in Triggered with each frame Mode, a TTL pulse is generated at each frame.

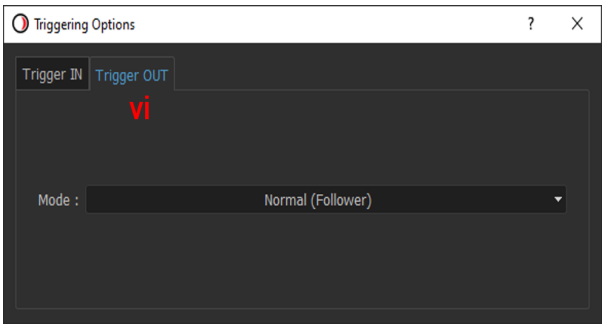
e) The **Save configuration** button (Fig. 3.21) will save all **Microscope settings** and **Image settings** in a **.doric** format file.



(a) *External Trigger Settings Window*



(b) *Gated Mode*



(c) *Trigger Out Mode*

Figure 3.22: *External Trigger Settings Windows*

f) The **Load configuration** button (Fig. 3.21) will load a selected configuration file.

g) The **Select mask file** button opens a window to select a mask file for the microscope used. This section only appears when a *2-color Fluorescence Microscope* or an *efocus Microscope* is connected. The mask file currently loaded will be shown just above it. For more information on masks, see section 3.5.1.

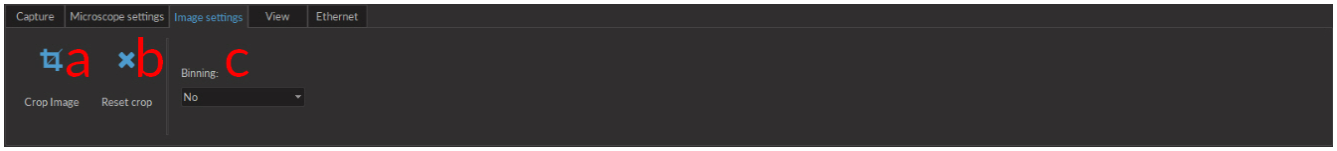


Figure 3.23: *Image Settings Tab*

4. The **Image settings** tab (Fig. 3.23) is used to define certain settings related to the displayed and recorded images.

   a) The **Crop Image** button (Fig. 3.23) allows a square to be drawn onto the image. When a new **Capture** sequence is activated, only the cropped region will be captured.

   b) The **Reset crop** button (Fig. 3.23) resets the cropped image to its original state. The change will only appear when a new **Capture** sequence is activated.

   c) The **Binning** drop-down list (Fig. 3.23) allows the binning of pixels. This reduces the number of pixels for smaller save file sizes.

5. The **View** tab (Fig. 3.17) is used to change viewing parameters of the sensor image. These changes will only appear on the sensor image when a new **Capture** sequence is started. Any adjustments made affect only the displayed image and not the recorded images.

Figure 3.24: *View Tab*

a) The **Zoom In/Zoom Out** buttons (Fig. 3.24) will increase/decrease the zoom of the sensor image.

b) The **Reset Zoom** button (Fig. 3.24) will reset the **Zoom factor** to 100%.

c) The **Zoom Factor** drop-down list (Fig. 3.24) allows the selection of a zoom factor from a pre-set list. The box will also display the current zoom if it was changed using different buttons.

d) The **Fit In View** button (Fig. 3.24) resize the image to fit the size of the Image Viewer box.

e) The **Roi shape** drop-down list (Fig. 3.24) allows the selection of the shape used when drawing a **Region Of Interest** onto a sensor image. These shapes include **Freehand**, **Circle**, **Rectangle** and **Square**.

f) The **SENSOR** section (Fig. 3.24) is used to adjust contrast on a given sensor. When a microscope used has multiple sensors, multiple **SENSOR** sections will be displayed, one for each sensor.

   i. The **Contrast** slider (Fig. 3.24) allows the adjustment of contrast from 0.1 to 5.

   ii. The **Min/Max** sliders (Fig. 3.24) indicate the minimum/maximum number of counts displayed. Should the **Min** be above 0, all pixels with lower count will display a minimal value. Should the **Max** be below 1020, all pixels with a higher count will appear saturated.

   iii. The **Auto contrast** slider button (Fig. 3.24) will active an automatic contrast adjustment algorithm.

   iv. The **Reset** button resets contrast functions to their default settings.

g) The **Pseudocolor** drop-down list (Fig. 3.24) allow the sensor image color palette to be changed.

h) The **Show saturation** checkbox (Fig. 3.24) allows all saturation on the sensor image to be displayed in red. This function is only available if no pseudocolor is selected.

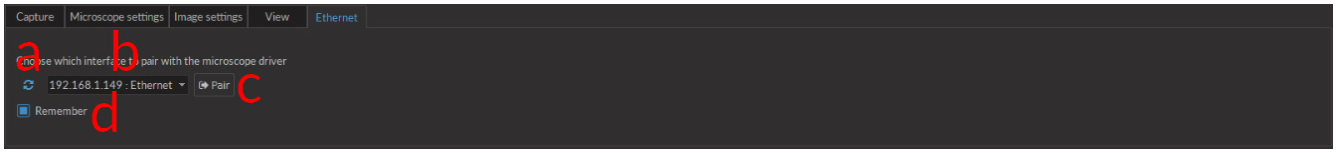


Figure 3.25: *Microscope Ethernet Tab*

6. The **Ethernet** tab (Fig. 3.17) is used to define the ethernet connection used to connect the computer to the microscope driver.
    a) The **Refresh** button (Fig. 3.25) will identify any accessible IP addresses and add them to the drop-down list.
    b) The **Ethernet** drop-down list (Fig. 3.25) includes all IP addresses connected to an ethernet adapter. The proper one must be selected to properly connect the microscope.
    c) The **Pair** button (Fig. 3.25) connects the software to the driver.
    d) The **Remember** checkbox (Fig. 3.25) will keep the chosen IP address so that the chosen microscope driver will be connected automatically next time the software is opened.

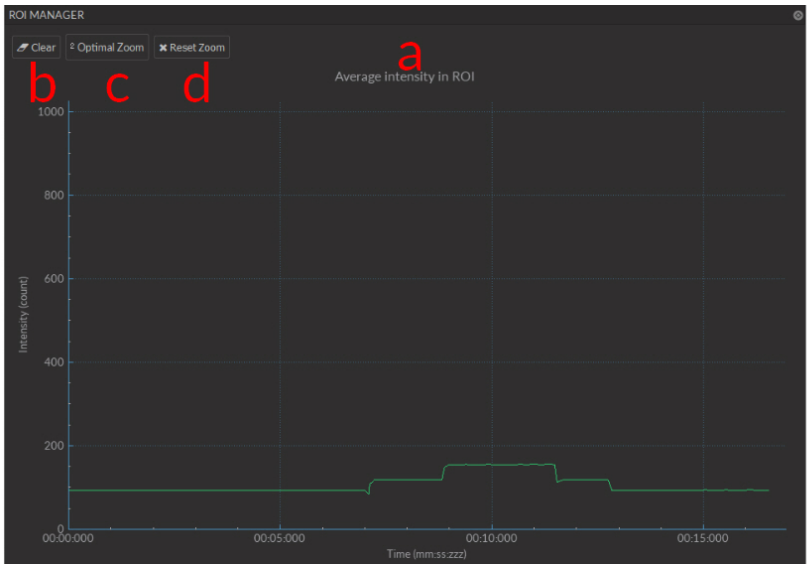


Figure 3.26: *ROI Manager*

7. The **ROI Manager** (Fig. 3.17) displays the live mean pixel intensity from a drawn ROI.
    a) The **Average Intensity in ROI** plot (Fig. 3.26) displays the average intensity over time inside a drawn ROI. *CTRL + mouse wheel* will adjust the x-axis zoom, while *SHIFT + mouse wheel* will adjust the y-axis zoom.
    b) The **Clear** button (Fig. 3.26) will clear any data displayed in the ROI manager and the ROI on the **Image Viewer**.
    c) The **Optimal Zoom** button (Fig. 3.26) sets the zoom factor on the plot to best display all data.
    d) The **Reset Zoom** button (Fig. 3.26) resets the zoom to its default setting.

3.5.1 Mask Installation

For the *2-color fluorescence microscope* and the *eFocus fluorescence microscope* to function properly, a series of **Masks** must be loaded onto the *Doric Neuroscience Studio* at the first use of each microscope body on a given computer. The following section explains how to install said **Masks**.

1. With each microscope is provided a single USB key. The mask file has the name **DoricMaskFile_X00000-00.zip**, where **X00000-00** is replaced by the microscope serial number. Save this file in a secure location, as it is required should the *Neuroscience Studio* be installed on a different computer.
2. Once the system is connected and the microscope in place, go to the **Microscope settings** tab and click **Select Mask File**. This opens a file selection window. Travel to the location of the mask file, select it, and click **OK**.
3. After the file has been selected, the studio will show **Loading Masks** above the **Select Mask File** button. This is replaced by **Masks Loaded** once loading is complete.
4. With the masks installed, the microscope is ready for use.

3.6 Image Analyser

This module provides an easy way to extract relevant data from the images acquired by the Doric miniature fluorescence microscopes. The software loads images in .TIF and .doric formats, implements image processing functions and an export tool to save the fluorescence data in .CSV or .doric format. This software does not replace standard analysis tools such as Matlab, ImageJ or Excel, but aims to offer useful processing algorithms developed for the microscope images. All the underlying algorithms are implemented from the OpenCV library. In this section, we will describe the different functions available, and how to use them.

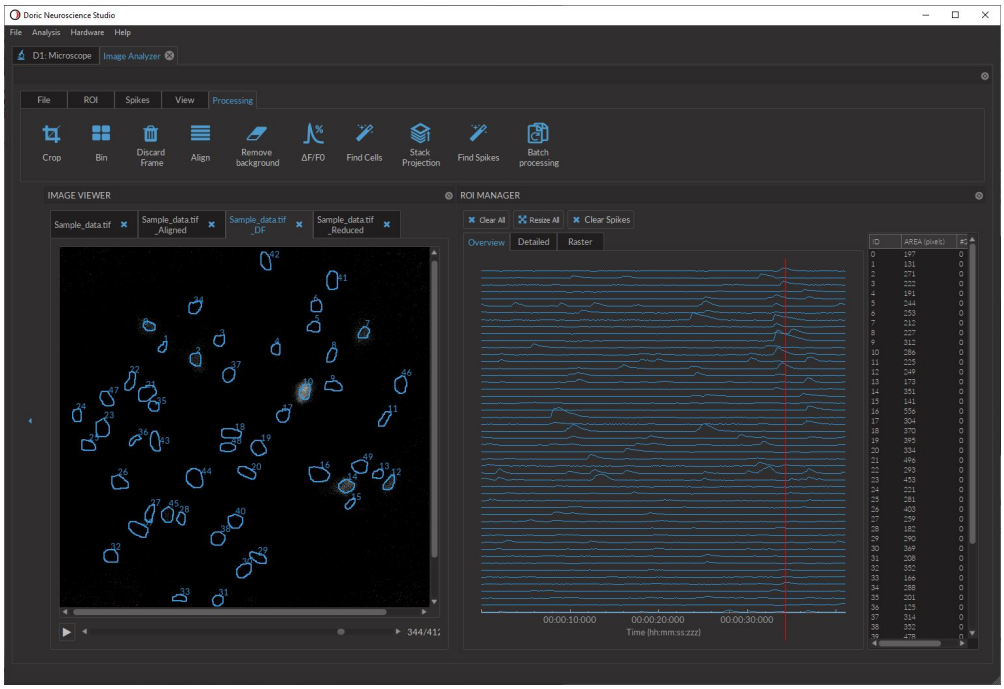


Figure 3.27: *Image Analysis Module Interface*

1. The **Image Viewer** displays the loaded images, allows navigation through the image stack and the drawing of regions of interest (ROIs) by clicking and dragging the mouse over the image. Multiple image sets can be opened, appearing as tabs in the upper left of the image box.
2. The **ROI Manager** displays the different ROI parameters and traces the mean signal intensity over time for each ROI.
3. The **Function Toolbar** contains all the buttons and functions accessible.

3.6.1 Function Toolbar

1. The **File tab** (Fig. 3.28) is used to save/load data.

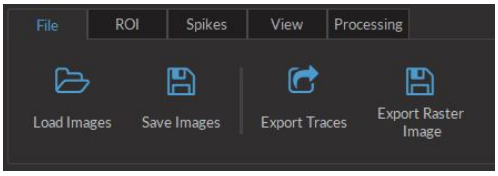


Figure 3.28: *File Tab*

- The **Load Images** function loads a square, 16 bit .tif file or .doric file.
- The **Save Images** function saves the current image tab to a 16 bit TIF multipage file or .doric file.

- The **Export Traces** function saves the average fluorescence intensity values for each ROI of the current tab to a .CSV or .doric file.
- The **Export Raster Image** function saves the raster plot of the ROIs of the current image tab to a .jpg file.

2. The **ROI tab** (Fig. 3.29) is used to save/load data relating to regions of interest drawn on an image.

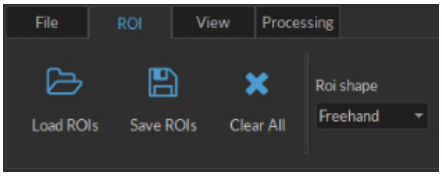


Figure 3.29: *ROI Tab*

- The **Load ROIs** function loads .CSV file containing informations about the saved ROIs.
- The **Save ROIs** function saves the current ROIs information to a .CSV file.
- The **Clear All** button clears all ROIs.
- The **ROI shape** function is a drop-down list that allows the selection of the **ROI** shape. These include **Freehand, Circle, Rectangle** and **Square**.

3. The **Spikes tab** (Fig. 3.30) is used to save/load data relating to regions of interest drawn on an image.

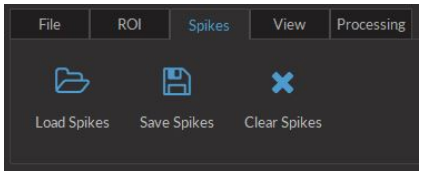


Figure 3.30: *Spikes Tab*

- The **Load Spikes** function loads .CSV file containing informations about the saved Spikes.
- The **Save Spikes** function saves the current Spikes information to a .CSV file.
- The **Clear All** button clears all Spikes.

4. The **View tab** (Fig. 3.31) is used to manipulate the appearance of an image without changing base data.

Figure 3.31: *View Tab*

- The **Fit In View** check box adjusts automatically the size of the current image to the **Image Viewer** window.
- The **Reset Zoom** and **Zoom factor** functions adjusts the display size of the current image.
- The **Contrast** function applies a different luminance response curve (gamma). See section 3.6.3 for details.
- The **Min** function applies a lower threshold with the cut-off value defined by the slider. See section 3.6.3 for details.
- The **Max** function applies an upper threshold with the cut-off value defined by the slider. See section 3.6.3 for details.
- The **Autocontrast** function directly applies the equalizeHist function of the OpenCV library.
- The **Reset** function returns the contrast and range values to their default.

- The **Image Info** button displays the image information window.
- The **Pseudocolor** function is a drop-down list for selecting alternate coloring schemes for the images presented.
- The **Frame Display Time** function adjust the frame rate in **Play** mode.

5. The **Processing tab** (Fig. 3.32) is used to process the image data.

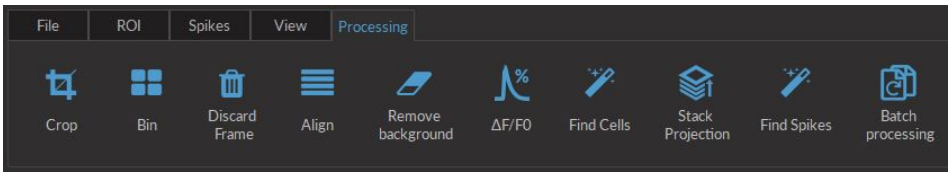


Figure 3.32: *Processing Tab*

- The **Crop** function allows to crop the current image in smaller dimensions to reduce the amount of data and facilitate the processing.
- The **Bin** function combines a cluster of pixels in a single pixel to reduce the amount of data and facilitate the processing. Note: in 2x2 binning, an array of 4 pixels becomes a single larger pixel.
- The **Discard Frame** function allows to remove user-defined frames in a data set. Note: The timestamps of the remaining frames stay the same when discarding frames.
- The **Align** function aligns the image stack to the user-defined key frame. See section 3.6.3 for computational details. Selecting this button will open the **Align Images** window (Fig. 3.33). By selecting the **Save Alignement Values** checkbox, the image alignement values will be preserved when saving the processed images. There are 4 different methods available.

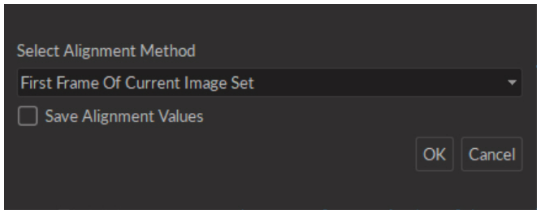
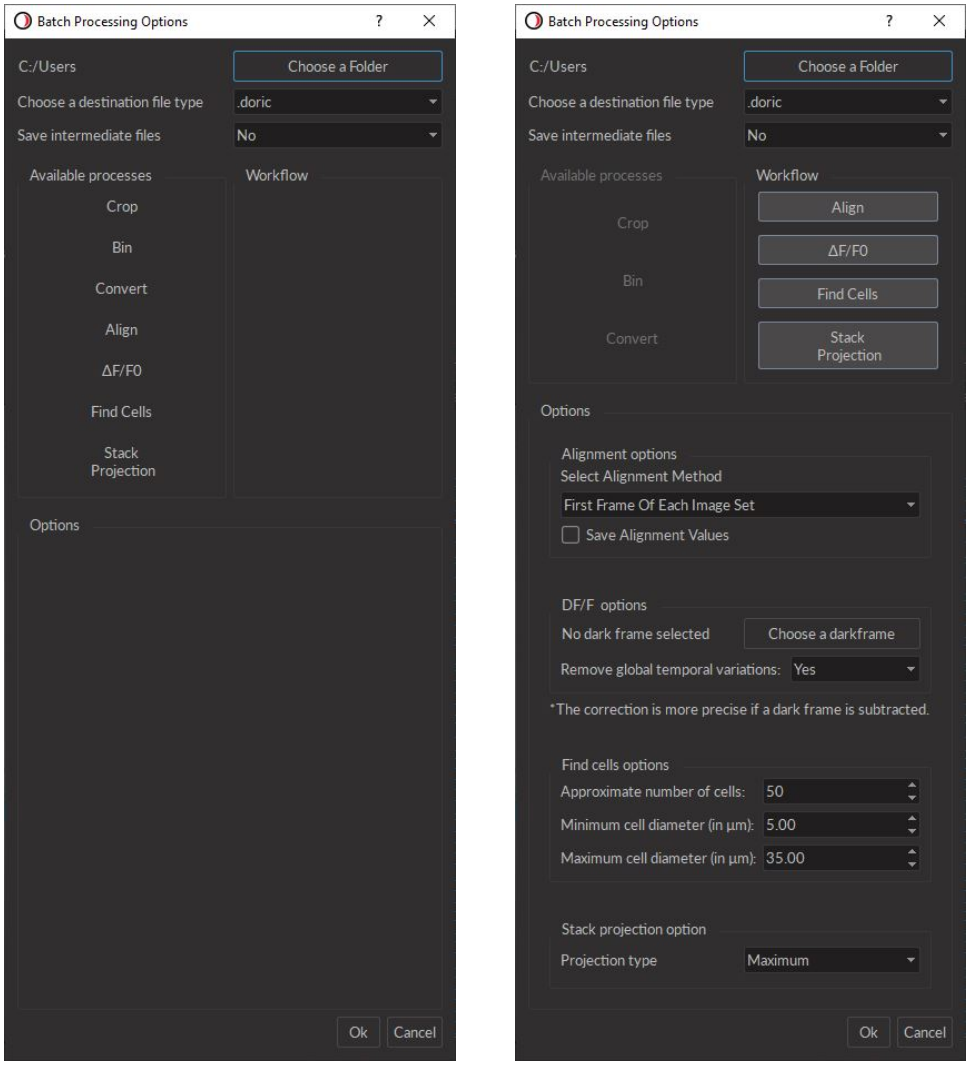


Figure 3.33: *Align Images Window*

  - The **First Frame Of Current Image Set** method uses the first image in the set to align the rest.
  - The **Select Frame From Current Image Set** method allows the selection of a single image in the set to use for alignment of all other frames.
  - The **Select Other Image Set And Frame** method aligns the current set using data from a different image set.
  - The **Select From Alignment Value File** method uses a previously-defined alignment for another image set. This method is most valuable when trying to align images from the *2-color fluorescence microscope*, to align one color channel using the data from the other.
- The **Remove Background** function removes the average value of a selected ROI from all images in the stack. Note: it is not recommended to use the **Remove Background** function before with the $\Delta\mathbf{F/F}_0$ function.
- The $\Delta\mathbf{F/F}_0$ function calculates the normalized fluorescence variation of the images and displays the results in a new tab. When selected, the See section 3.6.3 for details.
- The **Find Cells** function detects the cells and creates the ROI automatically. See section 3.6.3 for details.
- The **Stack Projection** function projects all movie frames to a single frame using the method selected in the Settings dialog. See section 3.6.3 for details.
- The **Find Spikes** function detects the spikes of the traces calculated from the ROI. The positions of the spikes are indicated by a red dot in the **ROI Manager**. See section 3.6.3 for details.

- The **Batch Processing** function opens the **Batch Processing Window** (Fig. 3.34). This allows the processing of large datasets in sequential order, without needing to activate each individual function. The processing defined in the batch processing window is applied to all the data saved in the destination file.



(a) *Batch processing window* (b) *Typical batch processing sequence*

Figure 3.34: *Batch Processing Window*

a) The **Available processes** box lists all processes available. Processes on the list will be greyed out if the work-flow order prevents them from being used. Each process has a number of parameters that are identical to those used outside of batch processing.
   - The **Crop** function allows to crop the current image in smaller dimensions to reduce the amount of data and facilitate the processing.
   - The **Bin** function combines a cluster of pixels in a single pixel to reduce the amount of data and facilitate the processing.
   - The **Convert** process is used to convert an image stack to **.doric** or **.tif** format.
   - The **Align** process aligns the image stack to the user-defined key frame. See section 3.6.3 for computational details.
   - The $\Delta\mathbf{F/F_0}$ process calculates the normalized fluorescence variation of the images and displays the results in a new tab. See section 3.6.3 for details.
   - The **Find Cells** process detects the cells and creates the ROI automatically. See section 3.6.3 for details.

- The **Stack Projection** process projects all image frames to a single frame using the method selected in the Settings dialog. See section 3.6.3 for details.

b) The **Workflow** box displays the order in which image processing actions will be taken. The parameters of the selected functions are adjusted in the **Options** box.

c) The **Choose a Folder** button allows the selection of a folder to save batch processing results.

d) The **File Type** list is used to defined the file extension used when the images are saved.

e) The **Save intermediate files** option will save intermediary files in the image processing process alongside the completed files.

3.6.2 ROI Manager

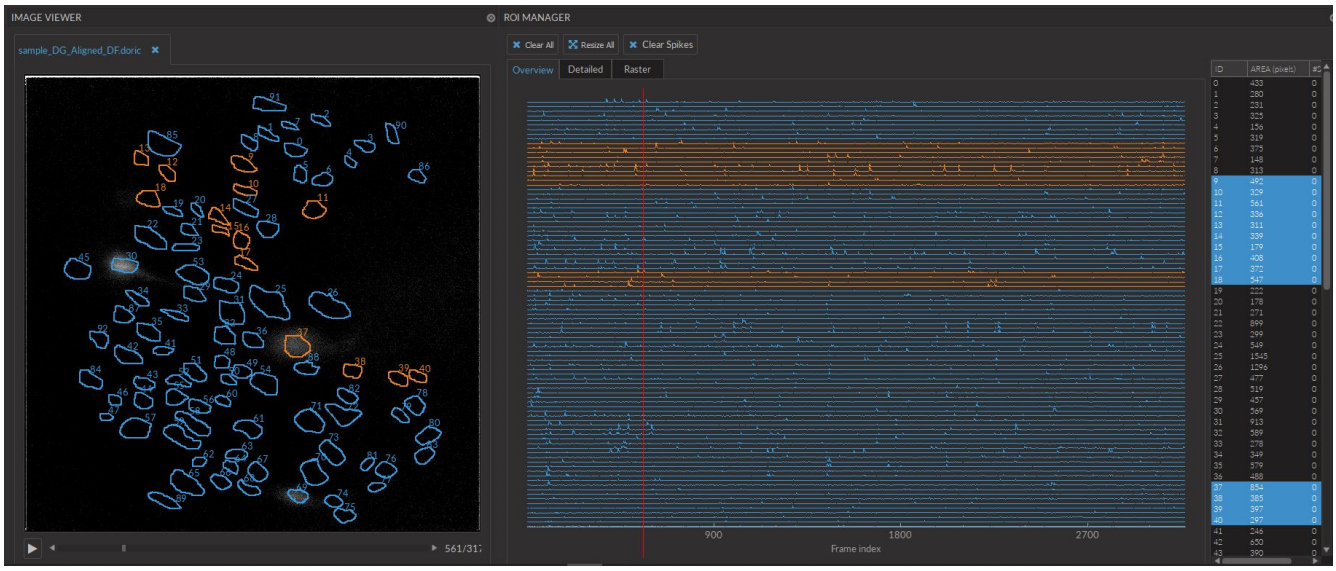


Figure 3.35: *ROI Manager*

The ROI manager extracts average intensity of a defined section of the image over an entire image stack. There is no limit to the number of ROI allowed per image stack.

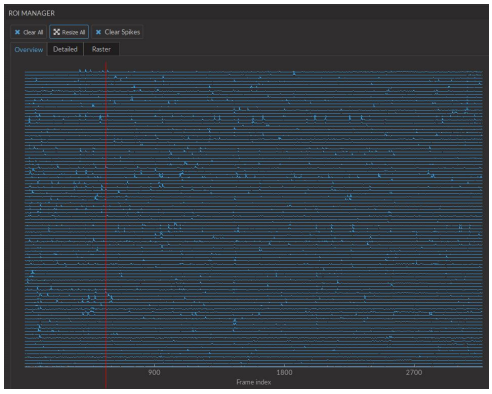
1. The **Image Viewer** contains the image stack and the ROI, numbered according to the order they where set. The ROI can be saved independently from the image stack on the ROI toolbar. The ROI are drawn directly on the *Image Viewer* in a *freehand* manner. All selected ROI can be moved together directly in the *Image Viewer*.

2. The **Intensity Plot** panel shows the plot of average intensity as a function of the frame index. The Y-axis represent the average count of all the pixels of the ROI. It is separated in *Overview* and *Detailed* tabs.

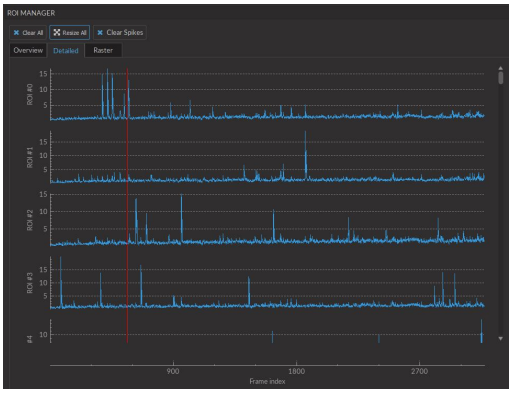
   - The **Overview** tab displays all the traces on the same graph, on the same scale (see Fig. 3.36a).
   - The **Detailed** tab displays each trace on a separate graph, allowing for precise intensity measurements (see Fig. 3.36b).
   - The **Raster** tab displays all the traces on the same color coded graph. In a raster plot each row (y-axis) corresponds to an ROI . The columns (x-axis) corresponds to the current time (see Fig. 3.36c).

3. The **ROI Data** list shows the parameters defining each ROI. Selected items will be displayed in orange on the Image Viewer and in the Overview graph.

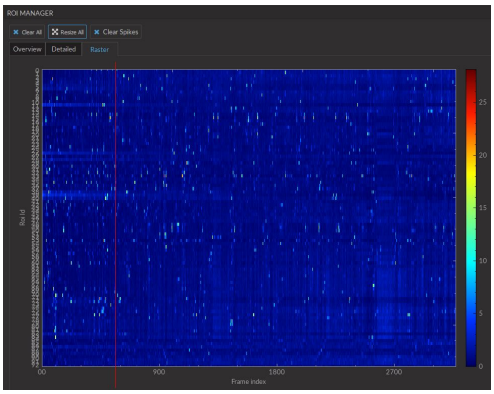
   - The **ID** shows the order of the ROI (starting at 0).
   - The **Area** shows the area (in pixels) contained in the ROI.



(a) *Overview Graph*



(b) *Detailed Graph*



(c) *Raster plot*

Figure 3.36: *ROI Manager Graph Tabs*

3.6.3 Algorithms

Contrast

The contrast adjustment applies the following operation to each pixel of the image: $V_{out} = AV_{in}^{\gamma}$, where V_{out} is the corrected pixel value, $A = 1$, V_{in} is the initial pixel value, and γ is the value as selected by the contrast slider.

Min and Max ranges

When the values of the display range are other than the default $min = 0$ and $max = 1020$, the following operation is applied to each pixel: $V_{out} = 1020 * (V_{in} - min)/(max - min)$, where V_{out} is the corrected pixel value, V_{in} is the initial pixel value, min and max are respectively the minimum and maximum slider values.

Image Alignment

The algorithm is inspired from Manuel Guizar-Sicairos, Samuel T. Thurman, and James R. Fienup, *Efficient subpixel image registration algorithms*, Opt. Lett. 33, 156-158 (2008). The basic idea is to obtain an initial estimate of the crosscorrelation peak by a Fourier transform and then refine the shift estimation by upsampling the Fourier transform only in a small neighborhood of that estimate by means of a matrix-multiply Fourier transform. With this procedure, all the image points are used to compute the upsampled crosscorrelation. In order to increase the precision of the algorithm, we use the laplacian of the images as inputs, instead of using the raw images. Briefly, the algorithm applies the following steps:

1. Calculate gaussian blur of the reference image with window of size 39 to smooth high frequency noise.
2. Calculate the laplacian of the blurred reference image.
3. Use the absolute values as the final reference image.

4. Reproduce steps 1 to 4 for the following image.
5. Calculate the 2D Fourier transform of the reference and the target image.
6. Multiply both images.
7. Calculate the inverse Fourier transform of the product image.
8. Get the position of the maximum correlation peak.
9. Create an upsample array around the maximum correlation peak to refine the shift calculations.
10. Calculate the Fourier transform of the larger array.
11. Do the matrix multiplication.
12. Locate the maximum correlation and map it back to the original space.

ΔF/F$_0$

The algorithm calculates a standard ΔF/F$_0$ with F_0 corresponding to the temporal average intensity, with an optional preprocessing step to remove the illumination variation artefacts. In order to properly calculate the $\Delta F/F_0$, the algorithm uses a dark frame to account for the sensor electronic offset. Calculating the $\Delta F/F_0$ without subtracting the offset will lead to artificially lower values. To record a dark frame, set the microscope driver to the desired exposure and gain, the LED power to zero and take a snapshot. Before calculating the F_0, the average temporal variations can be compensated to get a flat temporal average profile (Fig. 3.37). Keep in mind that removing the average temporal profile can also remove global activity patterns.

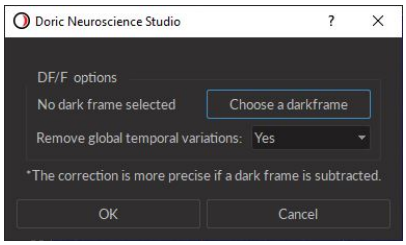


Figure 3.37: $\Delta F/F_0$ *Settings*

Briefly, the algorithm applies the following steps:

1. Calculate the average image intensity as a function of time (C).
2. If the global variation removal option is selected, apply the following correction to each image: $I_{out} = (I_{in} - I_{dark}) * (mean(C - I_{dark})/(C - I_{dark}))$ where I_{out} is the LED illumination corrected image, I_{in} the input image and C is the average temporal trace.
3. Calculate F_0 as the average projection of the movie.
4. Calculate the relative change $R(t)$ of fluorescence signal $R(t) = (F(t) - F_0)/F_0$.

Find Cells

The algorithm is inspired by Eran A. Mukamel, Axel Nimmerjahn and Mark J. Schnitzer, *Automated analysis of cellular signals from large-scale calcium imaging data*, Neuron 63(6), 747-760 (2009). The basic idea is to use a principal component analysis (PCA) as input of an independent component analysis (ICA) to separate the different temporal signals contained in the movie. This method is used as a starting point to determine the position of the different active cells. It is coupled with a segmentation routine optimized for reducing the false positives. The *Find Cells* algorithm uses user-defined boundaries shown in Fig. 3.38. The first parameter is an estimate of the number of cell present in the movie. By design, it must be lower than the number of frames minus five. The next parameters are the smallest and biggest object diameter in microns. These values are used to filtered the object found by the PCA/ICA.

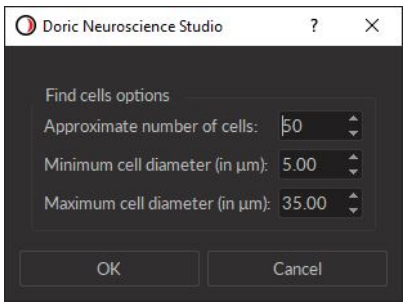


Figure 3.38: *Find Cells Settings*

Briefly, the algorithm applies the following steps:

1. Calculate and remove the spatiotemporal average from the movie, as the PCA/ICA algorithm requires zero-mean data.
2. Run OpenCV PCA algorithm on the centered data.
3. Normalize data by standard variation.
4. Calculate ICA with PCA as input data.
5. Apply segmentation to each ICA found.
6. Filter contours found at the previous step using user-defined boundaries.

Stack Projection

This function can be used to help for ROI drawing. It calculates a temporal projection using the user-defined method (see Fig. 3.39).

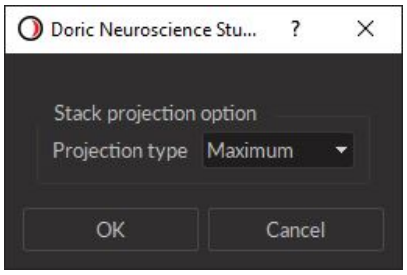


Figure 3.39: *Stack Projection Settings*

Maximum: the output is the maximum value found in all frames for each pixel.

Average: the output is the mean value of all frames for each pixel.

Sum: the output is the sum of all frames for each pixel.

Minimum: the output is the minimum value found in all frames for each pixel.

Find Spikes

This function detects the spikes of the traces calculated from the ROIs. The detection threshold is defined as a factor of the standard deviation of the trace.

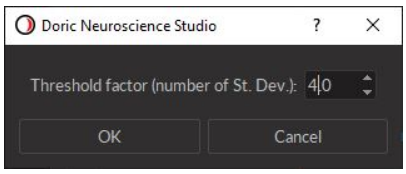


Figure 3.40: *Spike finder Settings*

4

Specifications

Table 4.1: *2-color Fluorescence Microscope Body General Specifications*

SPECIFICATIONS	VALUE	NOTES
Dimensions	18 x 17 x 9.5 mm^3	Without cannula and cables
Mass	3.7 g	Type-L
	3.0 g	Type-S
Excitation wavelength	465 nm and 561 mm	-
Collection wavelength	520/35 nm and 615/45 nm	-
Field of view (model L)	320 x 320 μm^2/600 x 600 $pixels^2$	-
Field of view (model S)	730 x 730 μm^2/600 x 600 $pixels^2$	-
Lens magnification	3 x	Type-L
	6.5 x	Type-S
Frame rate	45	-
Objective lense NA	0.5	-
Exposure	22 ms minimum	-
Gain (on-chip)	0-2	

Table 4.2: *Model L Imaging Cannula Range of Penetration Depth*

CANNULA TYPE	RANGE OF PENETRATION DEPTH (mm) [1]
LD	0 - 3.2
LV	3.0 - 6.0

Table 4.3: *Model L Imaging Cannula General Specifications*

SPECIFICATIONS	VALUE
Lens diameter	500 µm
Working distance	80 µm

[1] Including a working distance of 80 µm

Table 4.4: *Model S Imaging Cannula General Specifications*

SPECIFICATIONS	VALUE
Magnification	2 x
Working distance	2.4 mm

Table 4.5: *2-color Fluorescence Microscope Driver General Specifications*

SPECIFICATIONS	VALUE	NOTES
Power supply	110 - 240 VAC, 50 - 60 Hz	
DC Power supply	12 VDC	150 W
Dimensions	334 x 86 x 51 mm^3	Excluding connectors
Data link	Gigabit ethernet	
TTL input voltage	0 to +5 V	
Ce:YAG Analog input voltage	270 mA/V (typical)	See data sheet
Ce:YAG Monitor output voltage	3.7 V/A (typical)	See data sheet
Ce:YAG Maximum output current range	1200 mA	See data sheet
Ce:YAG Maximum forward voltage	32 V	Typical; see data sheet
Ce:YAG Minimum output current	40 mA	
Ce:YAG Rise/Fall time	<10 µs	
LED Analog input voltage	400 mA/V light source current 40 mA/V light source current	Standard 1 A LED Driver 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

Table 4.6: *2-color 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

[2]For all operation modes, except the internal complex mode

Table 4.7: *Ce:YAG + LED Optical Head General Specifications*

SPECIFICATIONS	VALUE	NOTES
Electrical connector	HDB15	Non-standard cable
Mass	1300 g	Approximate
Dimensions	150 x 100 x 80 mm^3	Including connectors
Output NA	0.63	
Output Optical Fiber Core Size	<600 µm	Power scales up to this core size

Table 4.8: *Doric Neuroscience Studio Hardware Requirements*

SPECIFICATIONS	VALUE	NOTES
Operating System	Windows 7, 8 , 10	64-bit
Memory (Minimum/Recommended)	8 GB/16 GB	
Processor Speed (Minimum/Recommended)	2 Ghz Quad-Core i5/ 3.46 Ghz Eight-core i7	
Hard Drive	500 MB	
Ethernet Card	Gigabit Card, Jumbo Frame compatible, dedicated card recommended	
Graphic Card	2 GB Memory, OpenGL v4.6 compatible	

5

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 optic and electronic components and should always be handled with care. While not in use, the microscope body with its protective 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.**
- **Objective 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 are made of glass, metal and plastic, and the contact with organic tissues or liquids like blood and saline solution is not recommended. If the microscope comes in contact with these substances, clean the optics (section 5.2) to avoid the appearance of stains.

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

5.2 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.

6

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 frames[1]?

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

[1] Dropped 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.

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.

7

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:

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