doric

efocus Fluorescence Microscope System

Gen3

User Manual

Version 1.5.0

Contents

1

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*^{TM} *& 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 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 ⋆*LISER*^{TM} *& 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*^{TM} *& 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*^{TM} *& LED/LD Light Source* or from any optical fiber connected to its output FC connector. The ⋆*LISER*^{TM} *& 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*^{TM} *& 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

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

set a proper laser safety interlock circuit for your application and laboratory installation. The *⋆LISER™ & 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*.

(a) *Laser Classification Label Example*

(b) *Laser Warning Label*

LASER APERTURE

(c) *Laser Aperture Identification*

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).

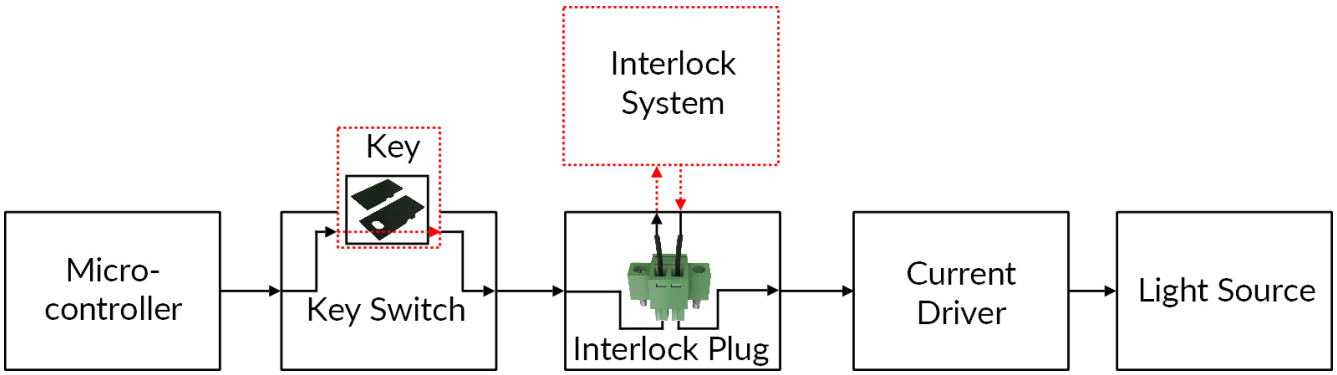
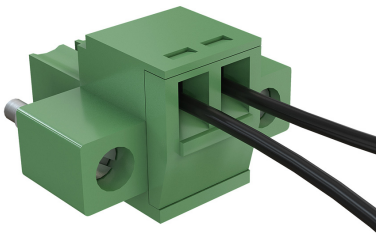


Figure 1.2: *Safety feature block diagram*

- 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.

(a) *Key Switch*



(b) *Interlock Plug*

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

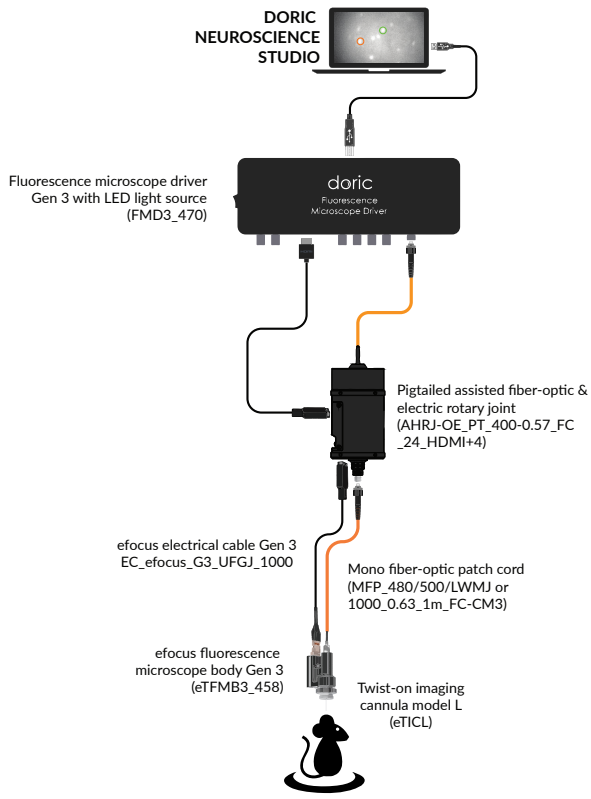
For light sources emitting invisible laser radiation, a dedicated LED indicator is ON when the driver is outputting an electrical current. When the driver is outputting current, the light source will emit light from the aperture.

2

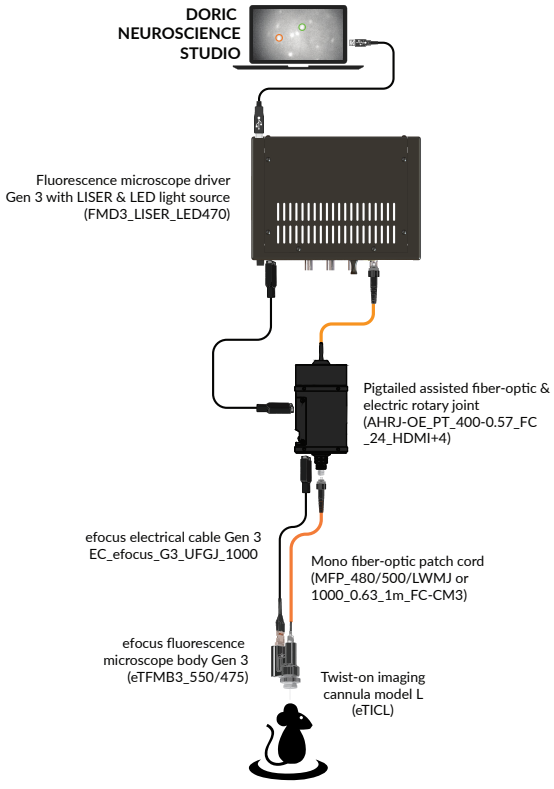
System Overview

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

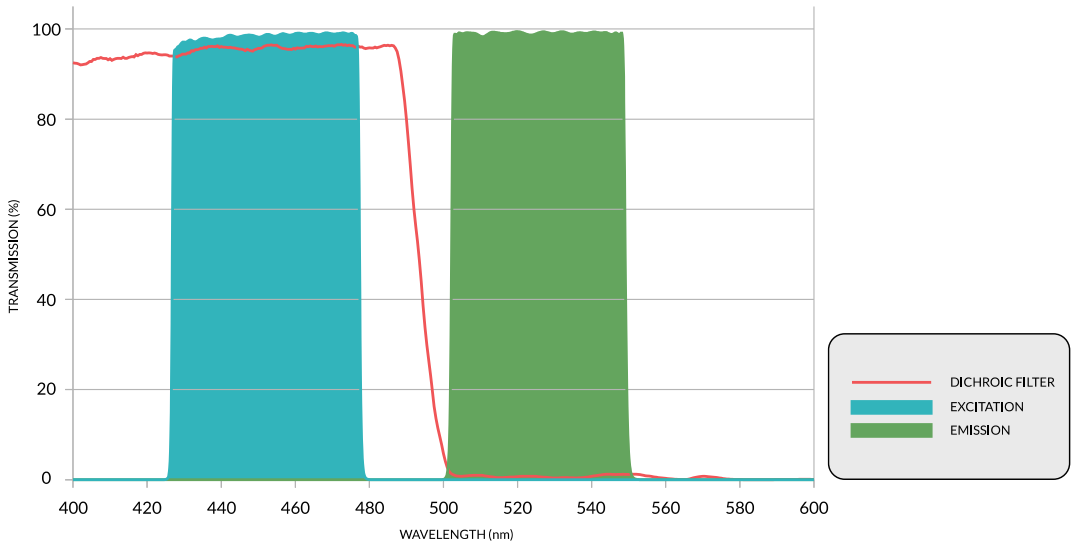


Figure 2.2: *GCaMP6 Excitation Filter Set Spectra*

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

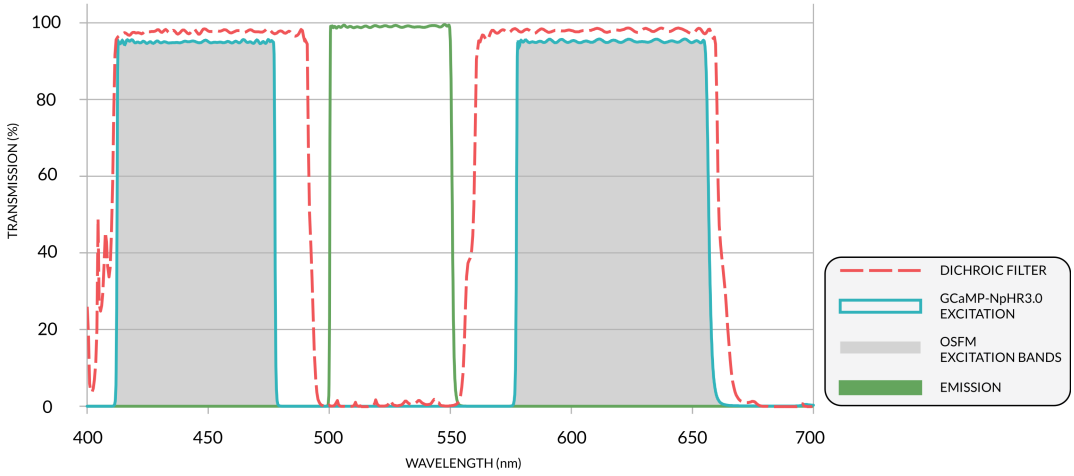


Figure 2.3: *GCaMP6+NpHR3.0 Excitation Filter Set Spectra*

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* ^{TM} with *LED* is integrated to the microscope driver (Fig. 2.3) .

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.

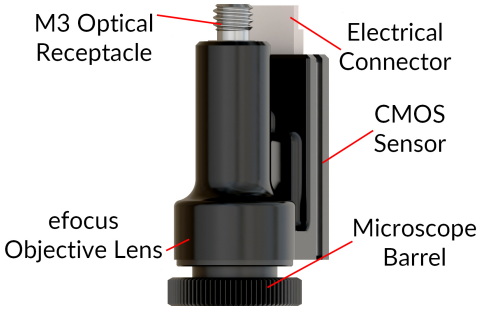


Figure 3.1: *efocus microscope body Gen 3*

- 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

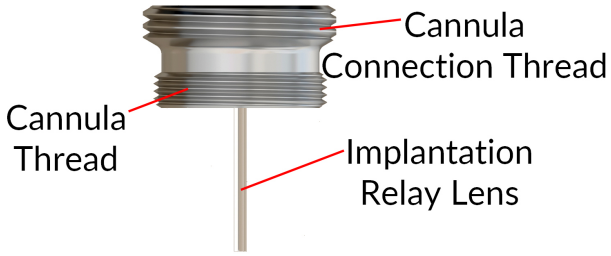


Figure 3.2: *Twist-on efocus Type-L Imaging Cannula*

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).

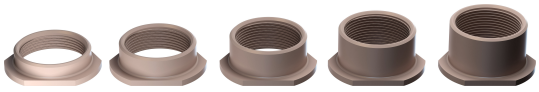


Figure 3.3: *Model L Imaging Cannula Protrusion Adjustment Rings. Height From Left to Right: 2.05 mm, 2.77 mm, 3.48 mm, 4.20 mm and 4.92 mm*

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

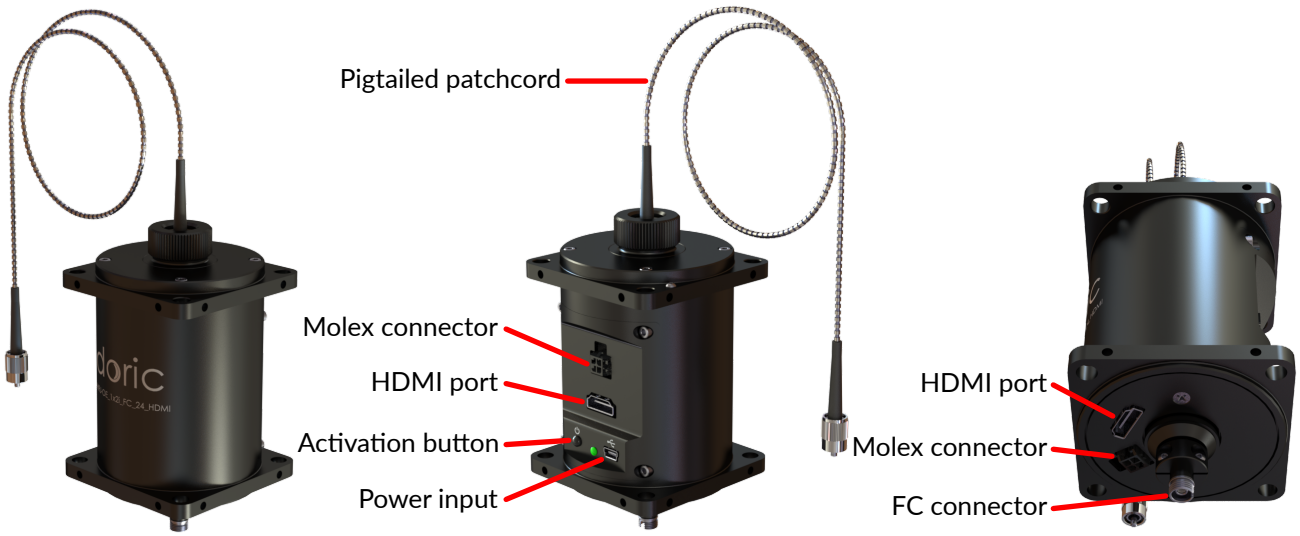


Figure 3.5: *24 contacts Pigtailed Assisted Fiber-optic & Electric Rotary Joint*

To use the microscope in experiments using freely-moving animals, the *24 contacts Pigtailed Assisted Fiber-optic & Electric Rotary Joint* (Fig. 3.5) is provided[1]. 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.

[1] To 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.

[2] MFP_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^{TM} (section 3.6) is designed to be used with the eTOSFM3 microscope.

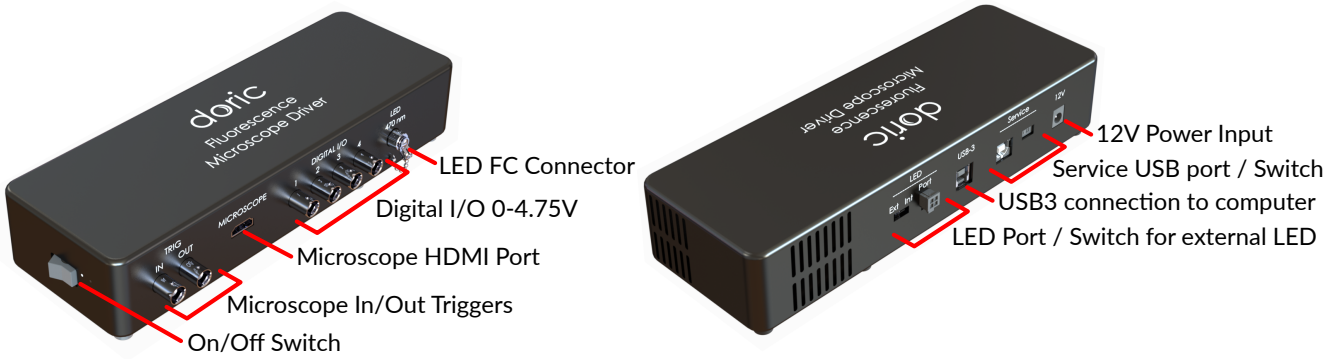


Figure 3.6: *Fluorescence Microscope Driver*

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 the 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^{TM}

This driver is used for computer control over the excitation of the ⋆LISER^{TM} 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^{TM} is designed to be used with the eTOSFM3 microscope.

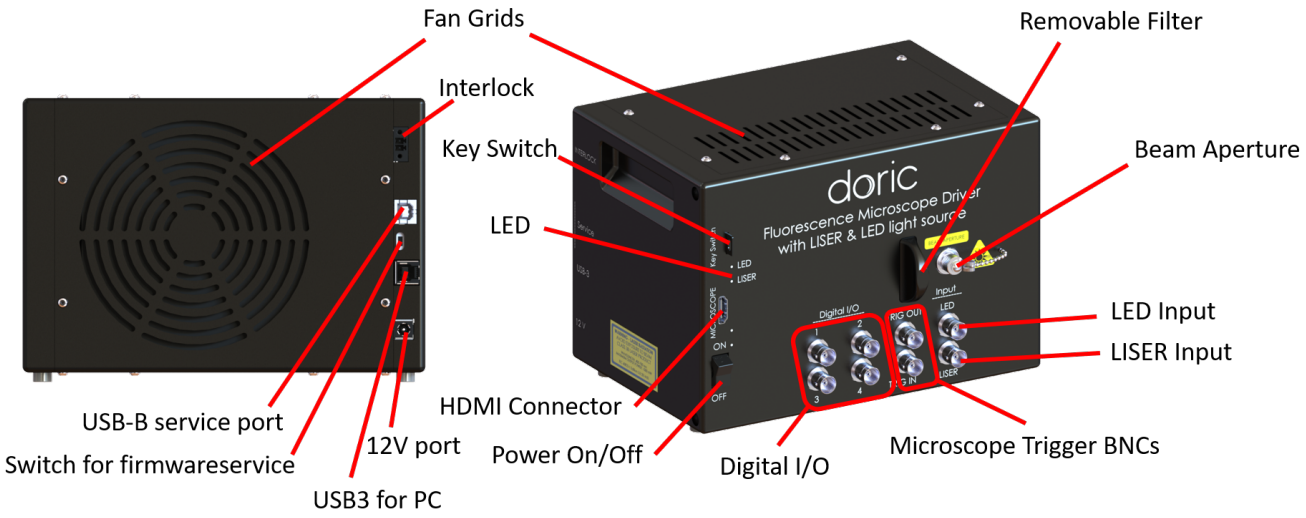


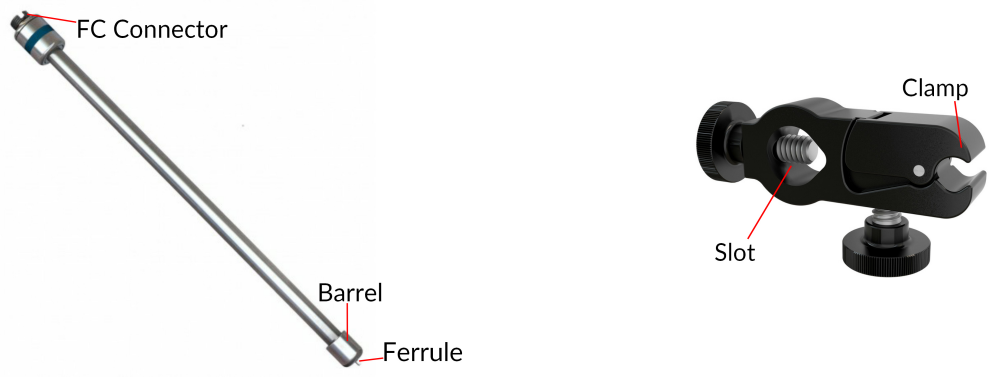
Figure 3.7: *Fluorescence Microscope Driver with ⋆LISER™*

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

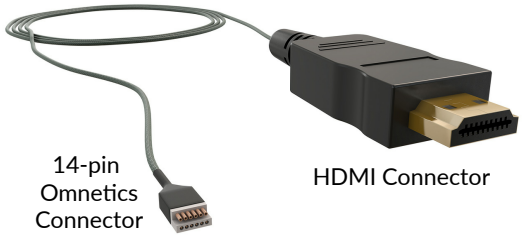


Figure 3.9: *efocus Electrical Cable Gen 3*

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.

4

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 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. See Table 7.5 for computer requirements.
2. **Select** the language to use during the installation.
3. If a previous version is already installed, the software proposes to remove all components from your computer before starting the installation of the new version (Fig. 4.1). This step is necessary to avoid any problems during the use of the new version. If it is the case, select **Yes**.

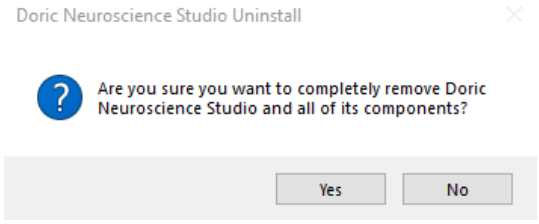


Figure 4.1: *Doric Neuroscience studio Uninstall previous version*

4. In the license agreement window (Fig. 4.2), accept the agreement and click **Next** to continue the process.

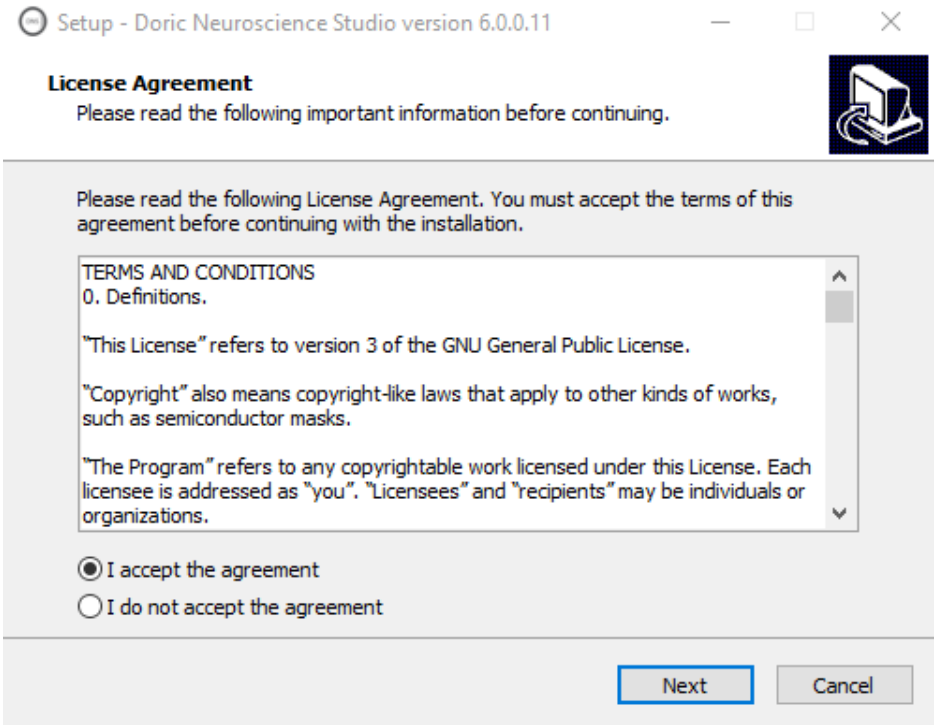


Figure 4.2: *Doric Neuroscience Studio License Agreement*

5. Click **Next** in the Information window.
6. **Choose** where to install the software (Fig. 4.3) and click **Next**.

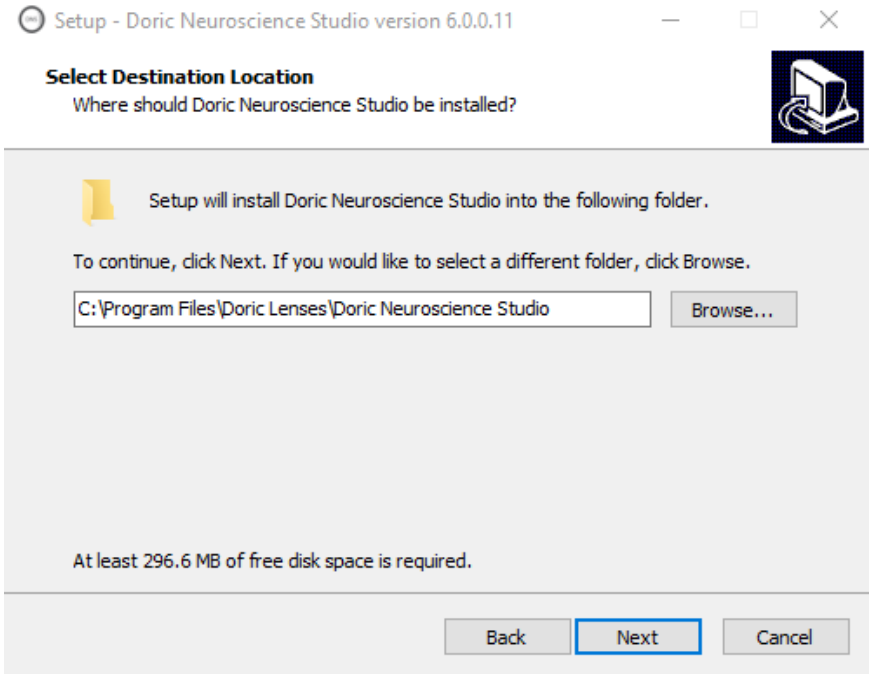


Figure 4.3: *Select Destination Location*

7. **Choose**, if desired, to create a shortcut in the Start Menu folder and click **Next**.
8. **Choose**, if desired, to create a desktop icon and click **Next**.

9. We recommend installing the **Doric Maintenance Tool**, which can be used to perform future firmware updates if necessary. The **External Driver** for the camera is needed if your setup is used with an external camera. When you have ticked the additional components you want to install in complement of **Doric Neuroscience Studio** (Fig 4.4), click **Next**.

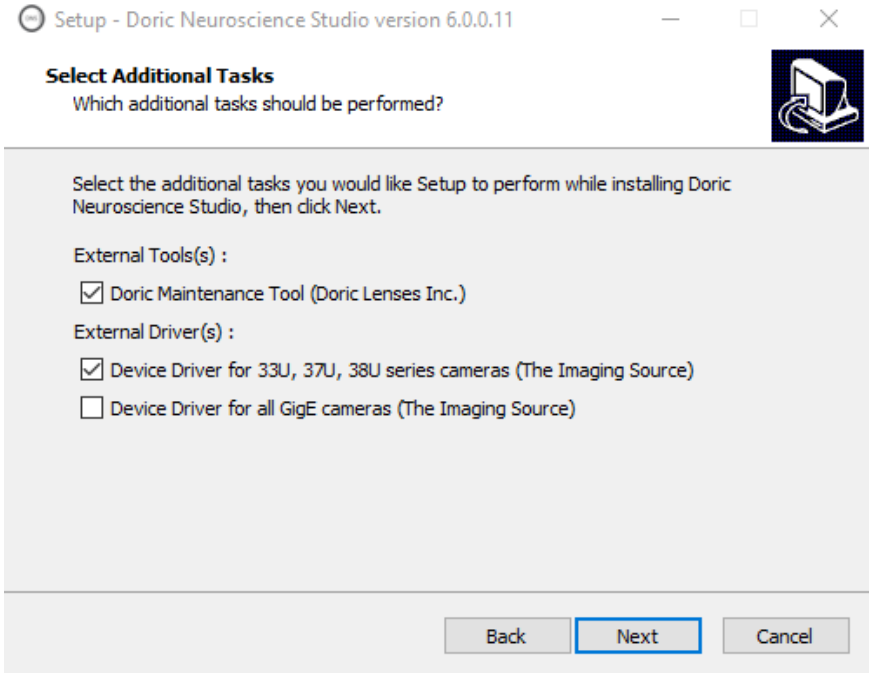


Figure 4.4: *Successful Installation of the Doric Neuroscience Studio*

10. When ready, click **Install** to begin the process. This should take a few moments. When the installation is done, the message in figure 4.5 will show up. If Doric Maintenance Tool has been selected to be installed, steps 2 to 10 also need to be done for Doric Maintenance Tool.

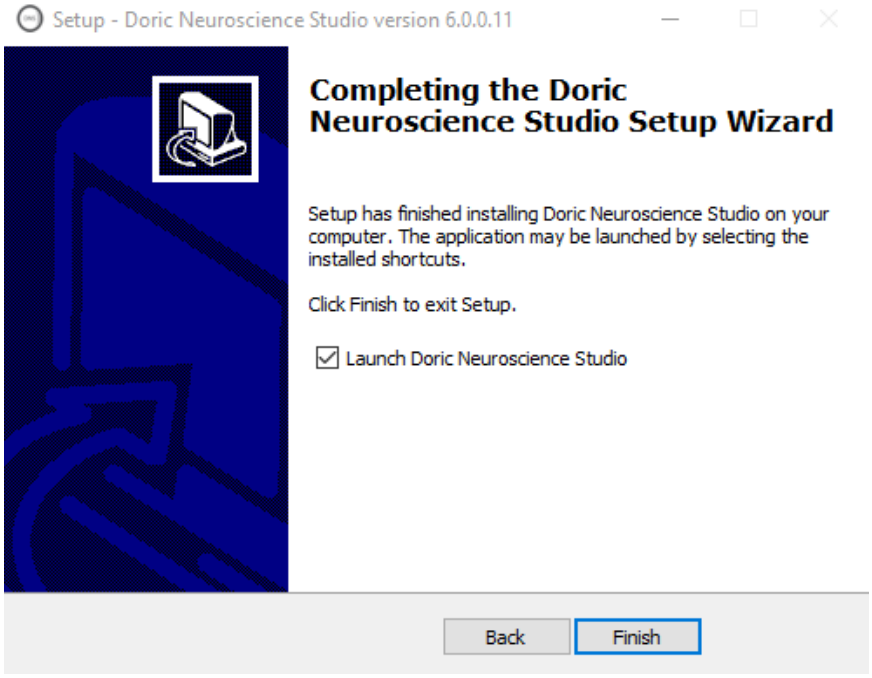


Figure 4.5: *Successful Installation of the Doric Neuroscience Studio*

11. Click **Next** and **Finish** to exit the setup.
12. Now the software is ready to use.

4.3 Updating The Driver Firmware

To update the driver firmware version, close Doric Neuroscience Studio.

1. Open Doric Maintenance Tool. (The software is installed at the same time as Doric Neuroscience Studio).
2. Turn On the Microscope Driver.

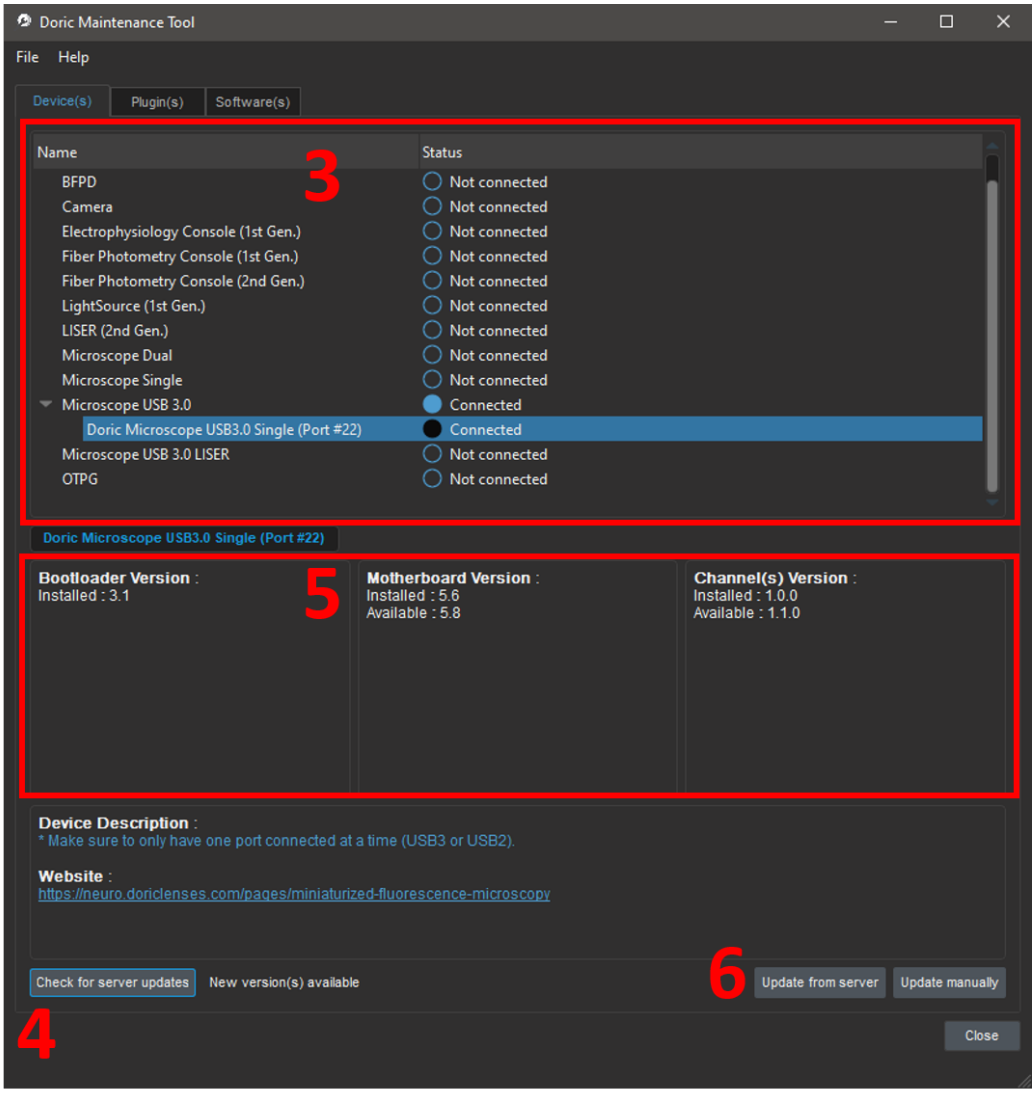


Figure 4.6: *Doric Maintenance Tool home page*

3. In the list under **Name**, select the device to update (its status need to be **Connected**) (Fig. 4.6).
4. Select **Check for server updates**. **Doric Maintenance Tool** will connect to the server and verify if an update is available (Fig. 4.6).
5. Under **Motherboard Version** and **Channel(s) Version**, the present version and the available versions are displayed (Fig. 4.6).

6. Select **Update from the server** to launch the update. (In some cases, an update can be necessary without using the server version. In this case, a representative of Doric Lenses will send you the update file and you can use **Update manually** instead of **Update from server**) (Fig. 4.6).

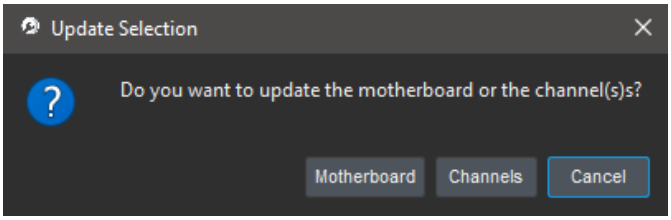


Figure 4.7: *Doric Maintenance Tool Update Selection*

7. A window ask you to choose between **Motherboard** or **Channels** to update (if you need to update both, select one of them and repeat the process after the end of the first update) (Fig. 4.7).
8. Wait the end of the installation and select **OK**.
9. Wait 10 seconds and turn OFF the Microscope Driver. Turn ON the Microscope Driver.

4.4 Updating Doric Neuroscience Studio

To update *Doric Neuroscience Studio* see the Doric Neuroscience Studio Manual.

4.5 General Setup Guidelines

4.5.1 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. 4.8).

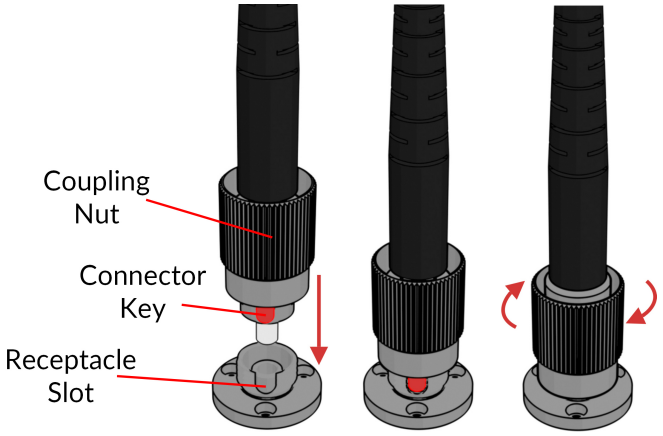


Figure 4.8: *FC connector, Fiber Installation*

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

4.5.2 Microscope Clamping Procedure

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

4.6 Connecting the efocus Microscopy System Gen 3

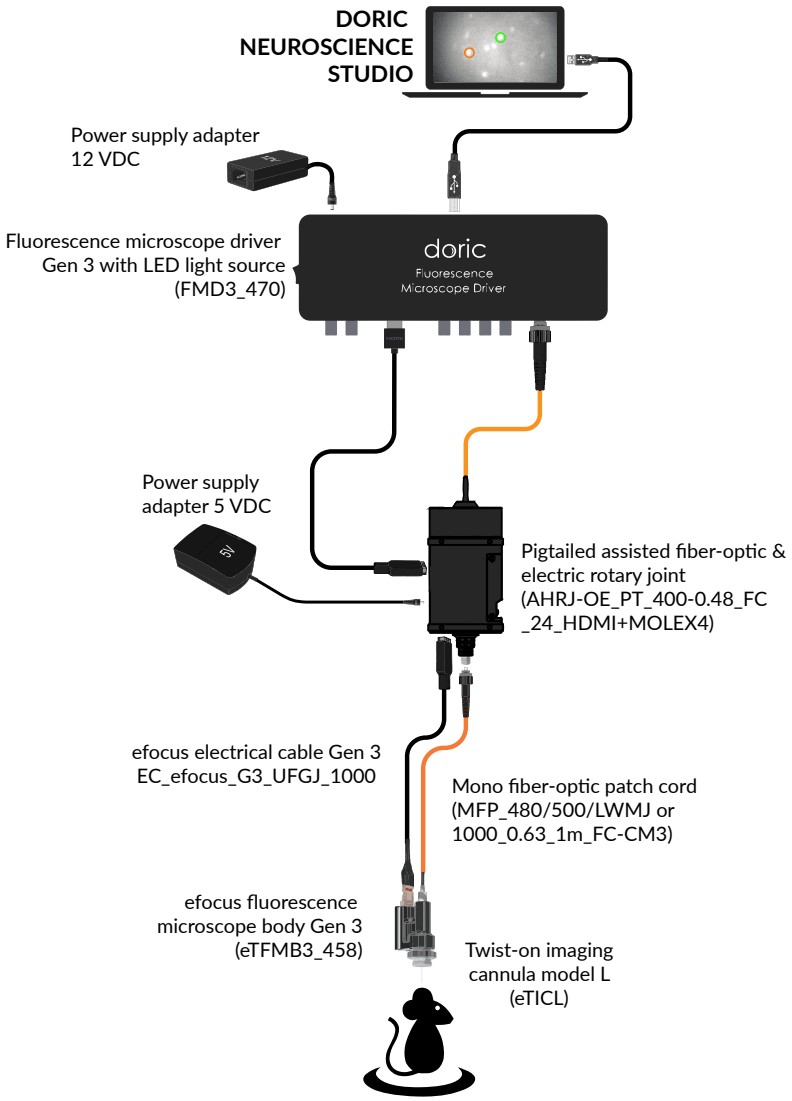


Figure 4.9: *Full GCaMP6 Freely-Moving Configuration, combination of Fig. 4.10a and Fig. 4.11b*

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.

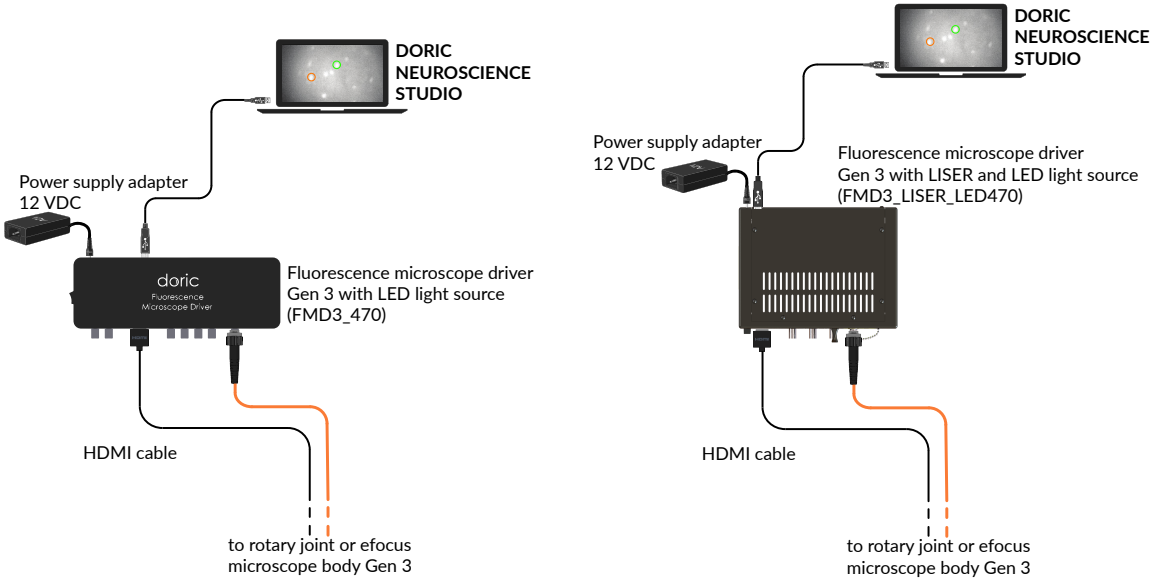
- The *eTFMB3_L_458* body Gen 3 is designed for **GCaMP** fluorescence imaging and uses the Fluorescence Microscope Driver with LED light source.
- The *eTFMB3_L_550* body Gen 3 is designed for **RCaMP** fluorescence imaging and uses the Fluorescence Microscope Driver with LISER and LED light source.
- 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 4.6.2, Fig. 4.11a) allows the observation of the animal while placed in a stereotaxic apparatus.
- The **Freely-Moving** configuration (Section 4.6.3, Fig. 4.11b) allows the observation of the animal while moving freely in a cage.

Figure 4.11 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 4.6.2 and 4.6.3 are to be followed for its installation.

4.6.1 Microscope Driver and Light Source Installation



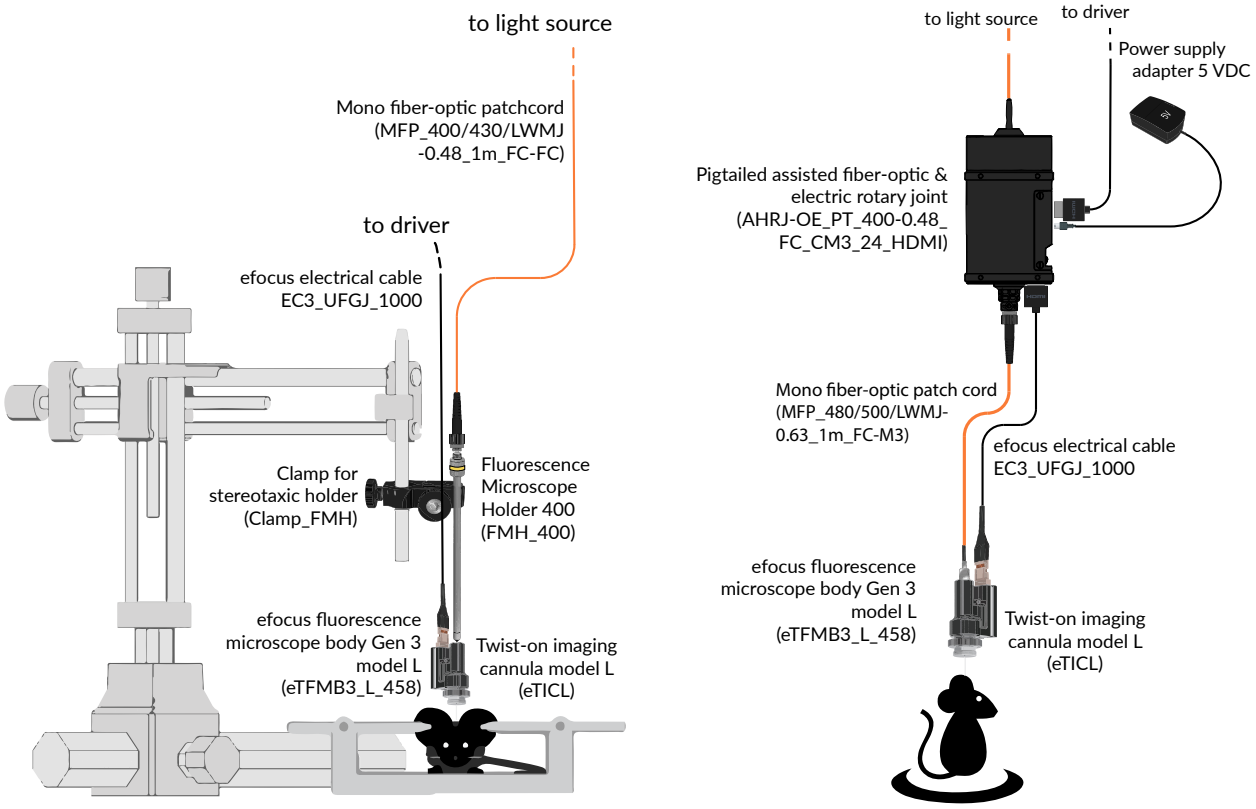
(a) *Microscope Driver with LED connections* (b) *Microscope Driver with LISER and LED connections*

Figure 4.10: *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.

4.6.2 Head-fixed Configuration Installation



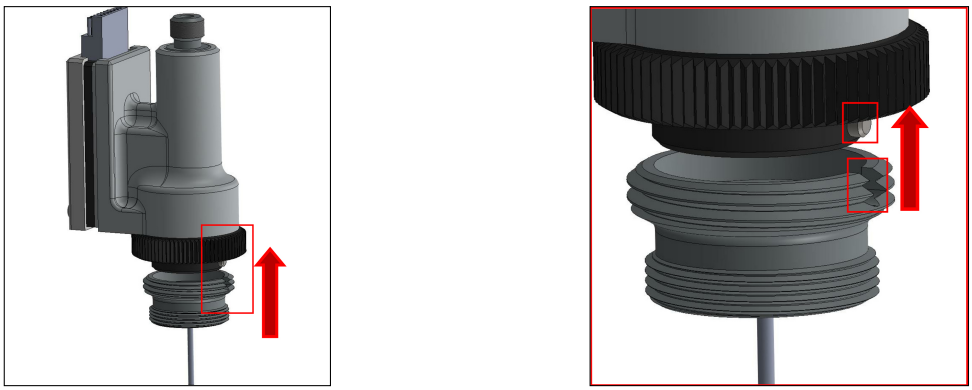
(a) *Head-fixed configuration* (b) *Freely-moving configuration*

Figure 4.11: *efocus Microscopy Systems Connections*

1. Install the *Fluorescence Microscope Holder (FMH_400)* into the *Stereotaxic Clamp* (Fig. 4.11a). 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 characteristics[1] 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. 4.12a). Ensure the microscope key is properly inserted in the cannula slot before screwing the microscope barrel on the cannula (Fig. 4.12b, 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.

[1] MFP_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 4.12: *Twist-on Microscopy Systems Connections*

4.6.3 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. 4.11b).
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 cord[2] 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. 4.12, 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.

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

5

Using Doric Neuroscience Studio

The Microscope module of the Doric Neuroscience Studio provides an interface to control our Fluorescence Microscope Driver Gen 3. The module enables image acquisition and its export in 12 bit .doric (hdf5-based) files. Doric files can be read by the Doric Neuroscience Studio **Image Analysis Module** or using an HDF5 library. Pixel gray values are contained between 0 and 4095 counts.

A complete description of the interface (Fig. 5.1) and functions of the user interface is done below. The description will consider 2 parts for the interface: the **Controls & Settings** (1 in Fig. 5.1) developed in section 5.2 and the **Acquisition View** (2 in Fig. 5.1) developed in section 5.3.

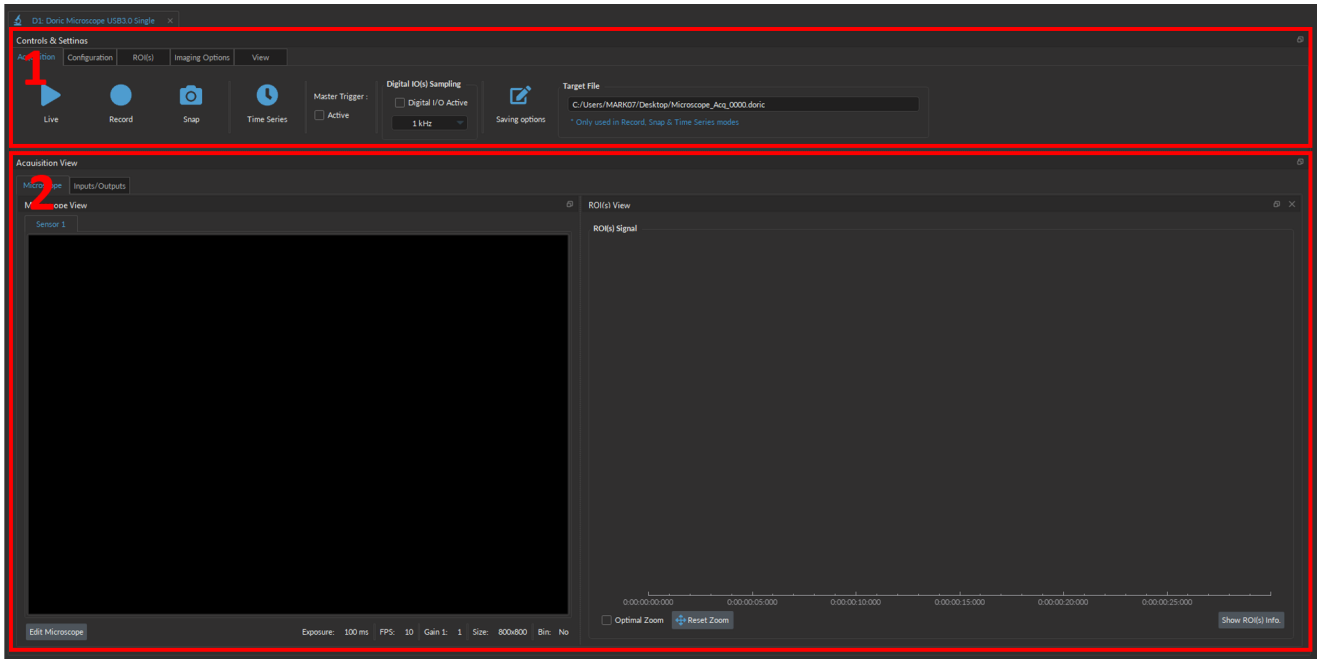


Figure 5.1: *Microscope Module Interface*

5.1 Channel Configuration

5.1.1 Microscope Channel Configuration Window Overview

The Microscope Configuration window can be accessed by clicking on the Edit Microscope button at the bottom left of the Acquisition View interface. It is used to edit the different microscope acquisition parameters.

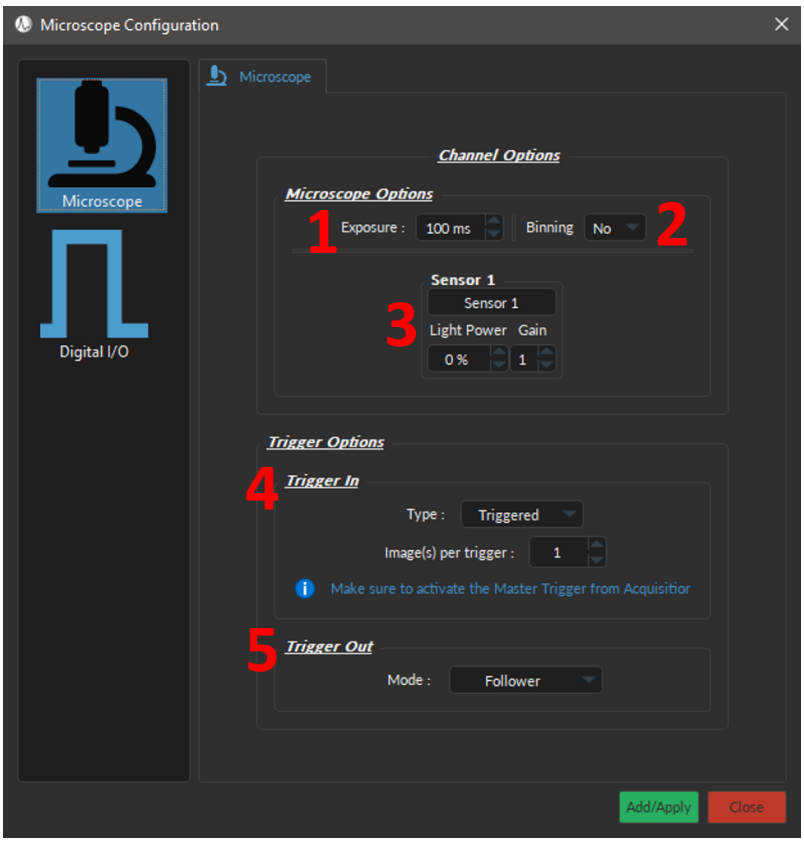


Figure 5.2: *Microscope Channels Configuration Main Interface*

Microscope Options

1. The **Exposure** sets the exposure time of the sensor. The time can be set between 20 and 1000 ms.
2. The **Binning** drop-down list allows the binning of pixels. This reduces the number of pixels for smaller save file sizes.
3. The **Sensor** section defines characteristics for a single sensor and the associated excitation source.
    - The **Sensor 1** editable name field.
    - The **Light 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.
    - The **Gain** box defines the sensor gain.

Trigger Options

4. Trigger In
    - The **Type** defines the type of trigger that is used to start a sequence. The **Triggered** type can starts a sequence at a rising edge while the **Gated** type can starts the sequence at a rising edge and stops it at a falling edge.

- The **Image(s) per trigger** box defines the number of images acquired at each trigger pulse. This option is only available if **Type** is defined as **Triggered**.

5. Trigger Out

   - The **Mode** drop down menu is used to select the type of TTL signal generated at the output of the Microscope Trigger Out BNC. In **Follower** 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.

5.1.2 Digital I/O Channel Configuration Window Overview

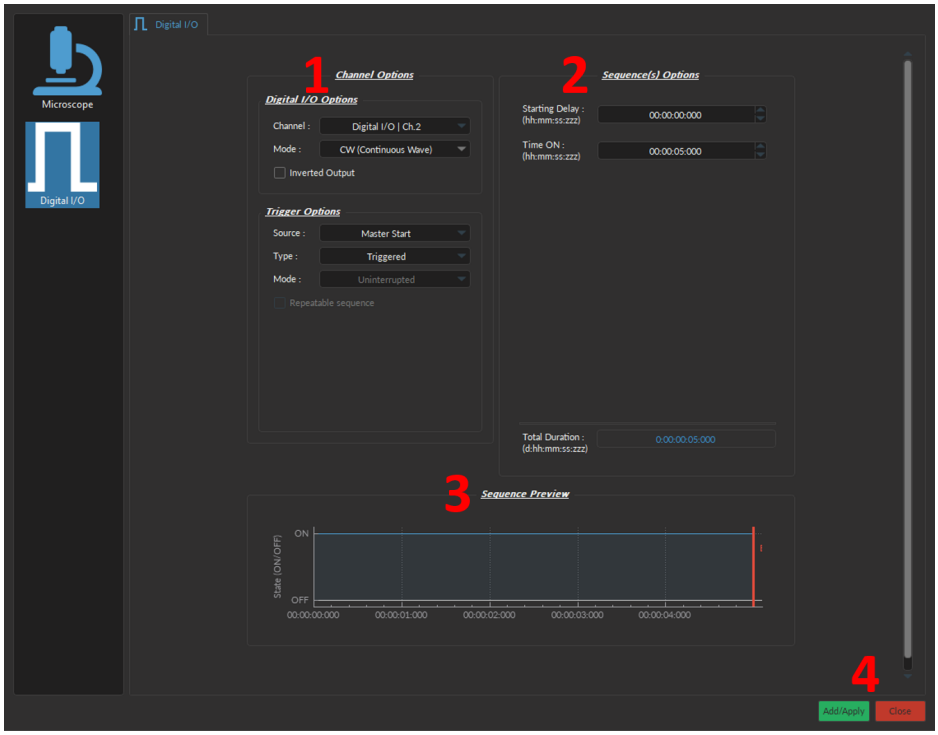


Figure 5.3: *Digital I/O Channels Configuration Main Interface*

The **Channels configuration** window is used to configure each channel. The window can be accessed by using either the **Add channel** or **Edit** buttons. This window is separated into multiple sections shown in Figure 5.3 that are defined below.

1. The **Channel Options** section allows you to define the Channels Type and the Triggering Options. The different fields of this section are explained in more detail in section 5.1.3.

2. The **Sequence Options** defines the parameters of each pulse sequence for the channel. These parameters are different for each Channel Mode. Should a parameter chosen be impossible to apply to a sequence (For example, a **Time ON** greater than 1/**Frequency**), the color of the option boxes will turn **RED**. The different fields for the different Channel Mode are explained in more detail in section 5.1.4

3. The **Sequence Preview** section shows a visualization of the output sequence that will be generated by the current configuration.

4. The **Add** button will save the current channel configuration and enables a new channel to be configured. The **Close** button will close the window without saving the current channel configuration.

5.1.3 Channel Options Section

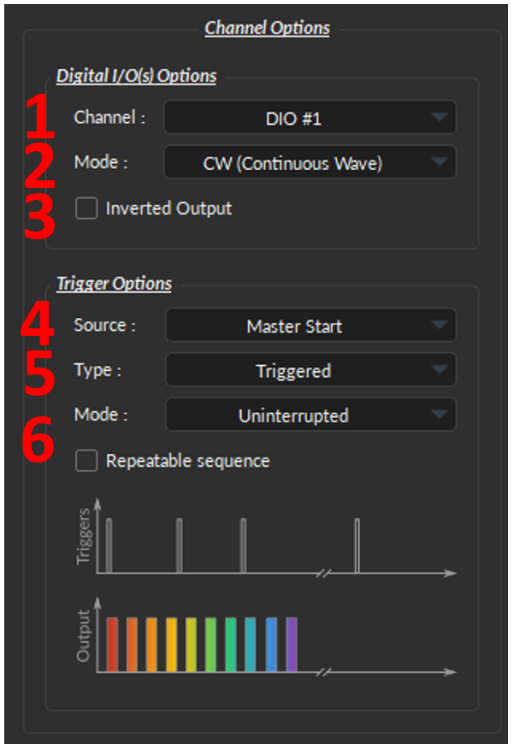


Figure 5.4: *Channel Options of the Channel Configuration Window*

The Channel Option section is separated in 2 sub-section, the **Digital I/O Options** section that defines the channel and its mode and the **Trigger Options** section that control the trigger method of the selected channel. The Trigger Options are not available when the channel is in Input mode.

Digital I/O Options

1. The **Channel** field identifies which of the available channels is currently being modified. The channel can be changed by selecting a new one from the drop-down list.
2. The **Mode** field identifies the type of signal sent. Three modes are available, **CW (Continuous Wave)**, **Square**, and **Input** mode. Each mode enables different options of the Sequence Option section that are explained in more detail in section 5.1.4.
3. The **Inverted Output** checkbox reverse the signal output. When selected, the ON TTL signal will send 0 V, while the OFF TTL signal will send 5 V.

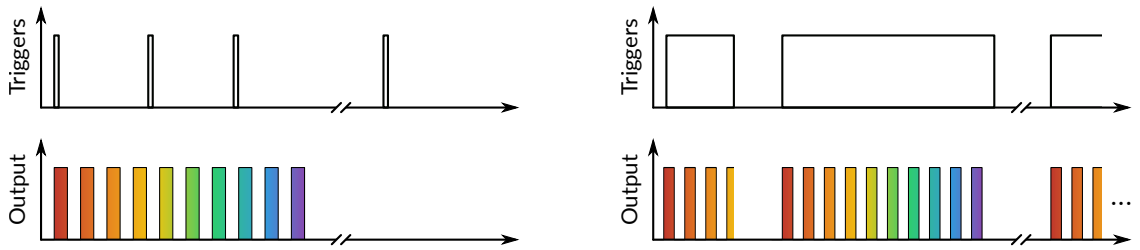
Trigger Options

4. The **Source** trigger option allows to chose between a **Master Start** (activated by the user) or another **Input** trigger, which is coming from a channel input.
5. The **Type** defines the type of trigger that is used to start/stop a sequence. The **Triggered** type can starts and stops a sequence at a rising edge while the **Gated** type can starts the sequence at a rising edge and stops it at a falling edge. A more refined interaction of the trigger with the defined sequence can be set up using the **Mode** field when it was available. Not all Trigger Type are available for each combination of Trigger Mode and Repeatability. The different combinations are shown in figure 5.5 and 5.6.

   For the eTFMB3 and the eTOSFM3, only two Type/Mode are available, the *Triggered Non-Repeatable Uninterrupted Sequence* and the *Gated Repeatable Restart Sequence*.

- Triggered Non-Repeatable Uninterrupted Sequence: This mode activates the channel sequence when an input greater than 3.3 V is detected by the BNC input. Following input pulses will be ignored (Fig. 5.5a).
- Gated Repeatable Restart Sequence: This mode activates the channel sequence when a rising edge higher than 3.3 V is detected on the BNC input. The falling edge will stop the sequence and the sequence will restart from the beginning when the next rising edge is received. When the sequence is completed, it will restart with the next input pulse (Fig. 5.5b).

6. The **Sequence Visualisation** shows a graphical representation of the behavior of the selected Trigger Option Type, Mode and Repeatability.



(a) *Triggered Non-Repeatable Uninterrupted Sequence* (b) *Gated Repeatable Restart Sequence*

Figure 5.5: *Sequence available for the eTFMB3 and eTOSFM3*

	Triggered		Gated	
	Non-repeatable sequence	**Repeatable sequence**	**Non-repeatable sequence**	**Repeatable sequence**
Uninterrupted	✓			
Pause				
Continue				
Restart				✓

Figure 5.6: *Trigger options possibilities for eTFMB3 and eTOSFM3 Digital I/O*

5.1.4 Sequence Options Section

CW (Continuous Wave) Mode

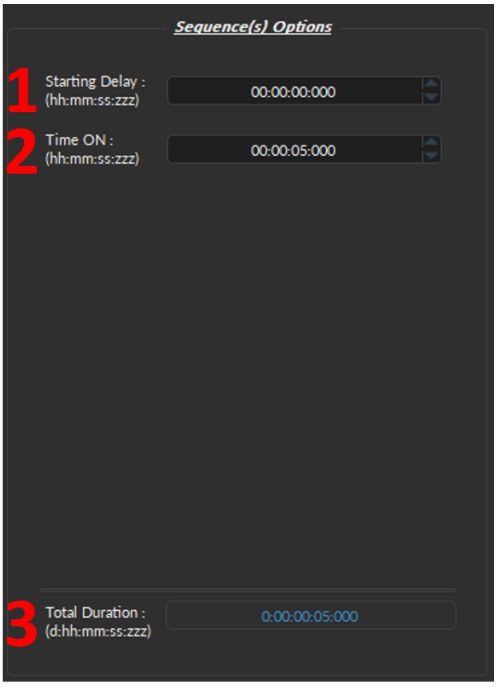


Figure 5.7: *Sequence Options of the CW Channel Mode*

The **CW (Continuous Wave)** channel mode allows the creation of a continuous TTL signal. The following elements appear in the **Sequence Options** section (Fig. 5.7).

1. The **Starting Delay** defines the time between the activation of the pulse sequence and the beginning of the signal.
2. The **Time ON** defines the length of time the continuous signal is active. Should the time chosen be 0, the signal will continue until the pulse is stopped manually.
3. The **Total Duration** shows the total expected duration of the pulse sequence. Should the duration be infinite, the box will display ∞. If there is an error in parameter selection, this box will display **N/A**.

Square Mode

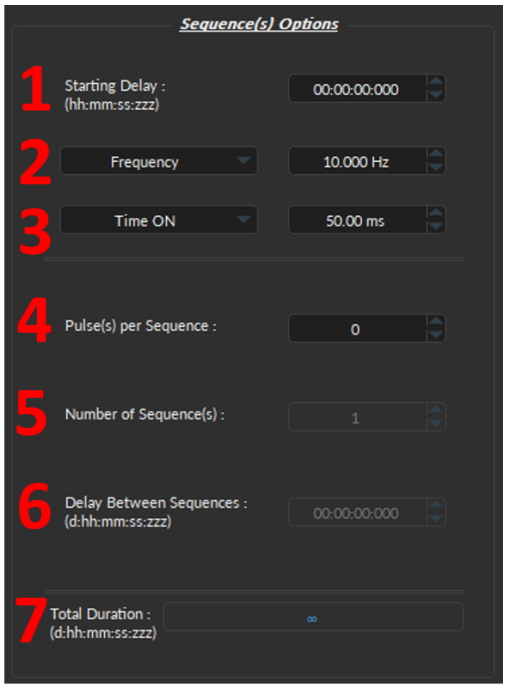


Figure 5.8: *Sequence Options of the Square Channel Mode*

The **Square** channel mode allows the creation of a square TTL pulse sequence. The Sequence Options of this mode are shown in Figure 5.8 and are explained below.

1. The **Starting Delay** defines the time between the activation of the pulse sequence and the beginning of the signal.
2. The **Frequency** sets the frequency (in Hz), which is the number of pulses per second. The frequency can also be changed to the **Period**. For example, a signal at 10 Hz (frequency) will output one pulse every 100 ms (period), whereas a signal at 0.5 Hz (frequency) will output one pulse every 2 seconds (period).
3. The **Time ON** defines the length of a single pulse. This time can also be converted to a **Duty Cycle**, which represents the % of the period the pulse duration corresponds to.
4. The **Pulse(s) per sequence** set the number of pulses per sequence. If it is set to 0, the number of pulses will be infinite.
5. The **Number of sequence(s)** sets the number of times that the sequence will be repeated.
6. The **Delay between sequences** sets the delay between each sequence.
7. The **Total Duration** shows the total expected duration of the pulse sequence. Should the duration be infinite, the box will display ∞. If there is an error in parameter selection, this box will display **N/A**.

Input Mode

The **Input** mode (Fig 5.9) records a signal as long as there is a high TTL signal on the chosen console channel. The channel can then be used as a trigger source for all the other channels of the Digital I/O. No **Trigger Options**, **Sequence Options** or **Sequence Previews** are available for this mode.

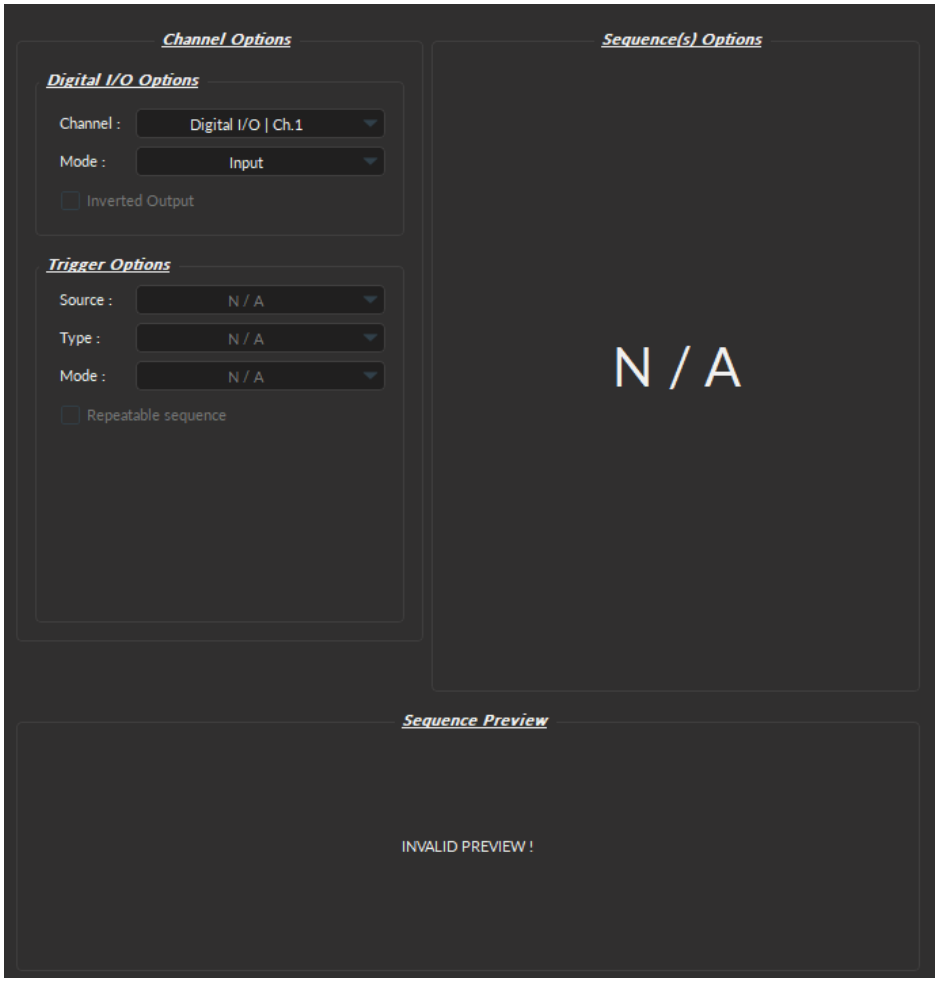


Figure 5.9: *Channels Configuration of the Input Mode Interface*

5.2 Controls & Settings

The controls & Setting are divided in 5 parts (Fig. 5.10): **Acquisition** (1), **Configuration** (2), **ROI(s)** (3), **Imaging Options** (4), and **View** (5), each ones of them will be explore in next subsections.

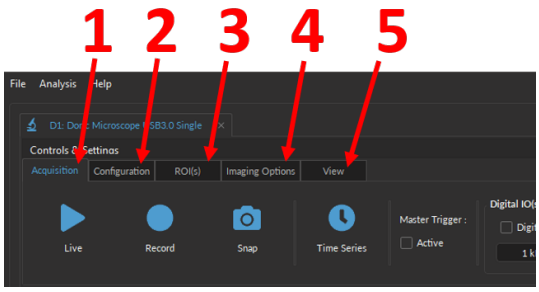


Figure 5.10: *Microscope Module Interface - Controls & Settings*

5.2.1 Acquisition

The **Acquisition** tab (Fig. 5.11) contains different image-capturing functions of the microscope.

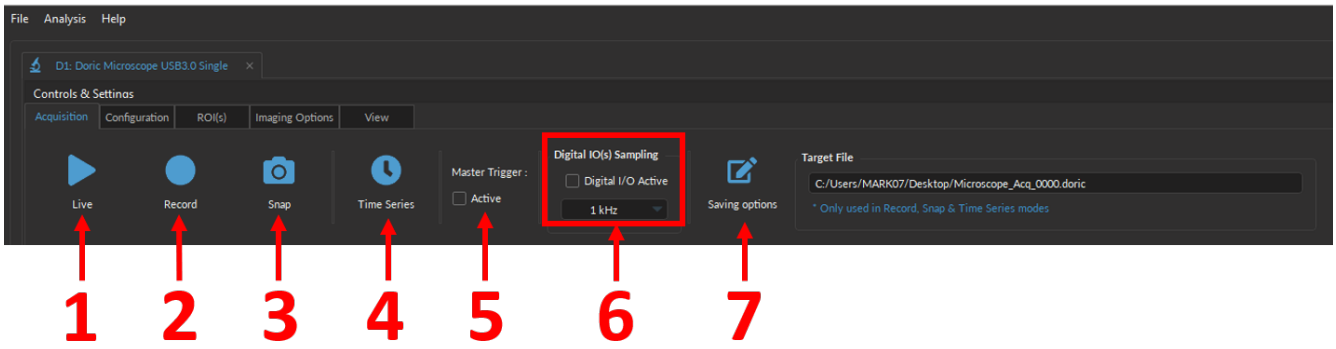


Figure 5.11: *Acquisition Tab*

1. The **Live** button (Fig. 5.11), when pressed, displays images from the microscope. These images are not saved.
2. The **Record** button (Fig. 5.11), 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.
3. The **Snap** button (Fig. 5.11), 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.

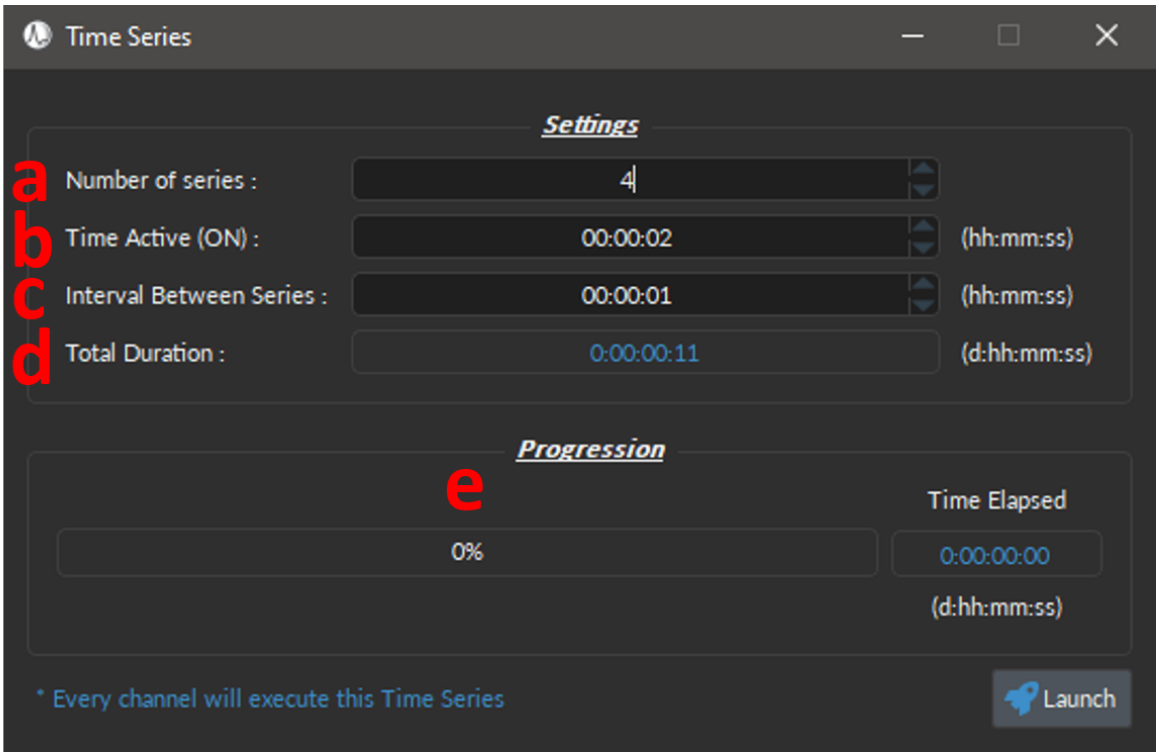


Figure 5.12: *Time Series Window*

4. The **Time Series** button (Fig. 5.11), when pressed, opens the time series interface (Fig. 5.12).
    a) The **Number of series** (Fig. 5.12) defines the number of images series will be recorded.
    b) The **Time active (ON)** (Fig. 5.12) defines the time of acquisition on each series.
    c) The **Interval Between Series** (Fig. 5.12), defines the duration between each series. This duration always has a minimum value of 1 second.
    d) The **Total Duration** displays the total duration of the full series.

e) The **Progression bar** (Fig. 5.12) displays the progress (in %) of the time series.

Note: If a precision under the second is necessary, the use of complex sequence can be used.

5. The **Master Trigger** checkbox activates or enables the Trigger option for the microscope. The trigger signal needs to be sent to the TRIG IN port of the microscope driver. Others parameters of triggering options will be explain in section 5.3.

6. The **Digital IO(s) Sampling** section is composed of two part: the **Digital I/O Active** checkbox to activate the Digital I/O and the **Digital I/O sampling rate** drop down menu to select the sampling rate (1kHz, 5kHz or 10 kHz).

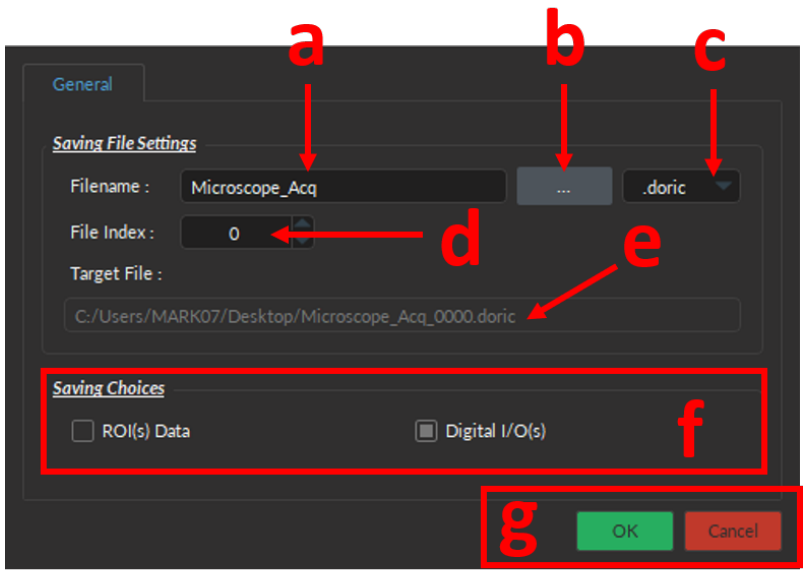


Figure 5.13: *Saving Options Window*

7. The **Saving options** button opens the **Saving options window** (Fig 5.13).

   a) The **Filename** box (Fig. 5.13) is used to define the recorded file name.
   b) The **...** button (Fig. 5.13) opens a window used to choose the save file location.
   c) The **File type** drop-down menu (Fig. 5.13) is used to decide which file type is used to save images. For files larger than 4 GB, the .doric extension is recommended.
   d) The **Index** box (Fig. 5.13) displays the current index that will be added to the filename.
   e) The **Target File** box (Fig. 5.13) shows the full location and name of the file being saved when an image sequence is recorded.
   f) The **Saving Choices** box allow to add **ROI(s)** Data and the **Digital I/O(s)** in the file to the data saved.
   g) At the end, select **OK** to save the selection or **Cancel** to discard them.

5.2.2 Configuration

The **Configuration** tab (Fig. 5.14) is used to set the channels and their configuration.

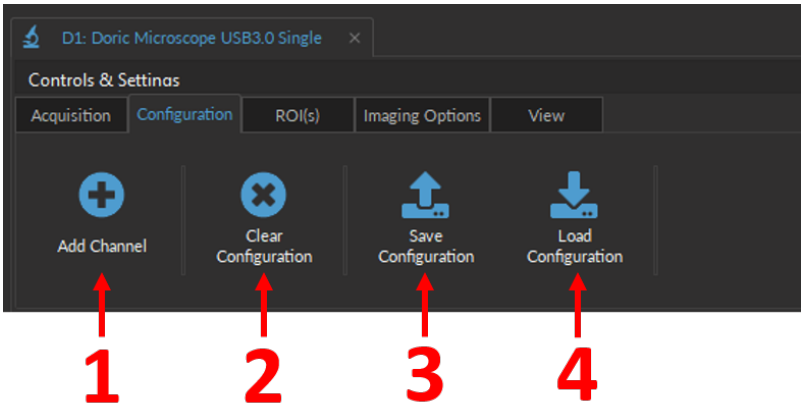


Figure 5.14: *Configuration Tab*

1. The **Add Channel** button (Fig. 5.14) is used to add the Digital I/O.
2. The **Clear Configuration** button (Fig. 5.14) is used to delete all the Digital I/O configured and reinitialize the microscope parameters.
3. The **Save Configuration** button will save all **Digital I/O** and **Image Options** in a **.doric** format file.
4. The **Load configuration** button (Fig. 5.14) will load a selected configuration file.

5.2.3 ROI(s)

The **ROI(s)** tab is only used to clear, save or load ROI(s) selection or to enable the tracing of ROI(s). The drawing of ROI(s) will be present in the section 5.3.

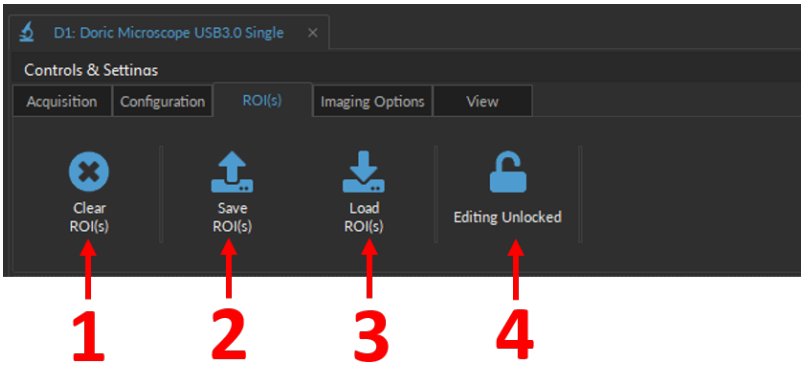


Figure 5.15: *Microscope ROI(s) Tab*

1. The **Clear ROI(s)** button (Fig. 5.15) is used to delete all the ROI(s) drawn.
2. The **Save ROI(s)** button (Fig. 5.15) will save all **ROI** configuration in a **.doric** format file.
3. The **Load configuration** button (Fig. 5.15) will load the ROI(s) contained in a **.doric** file.
4. The **Editing Unlocked/Locked** button (Fig. 5.15) is used to block ROI edition.

5.2.4 Imaging Options

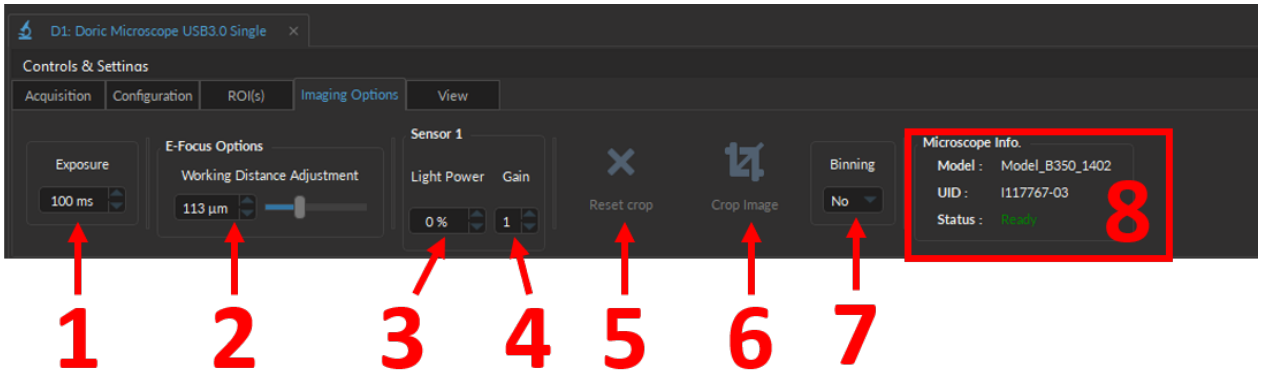


Figure 5.16: *Microscope Imaging Options Tab*

1. The **Exposure** box (Fig. 5.16) sets the exposure time of the sensor. The time can be set between 22 and 1000 ms.
2. 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 efocus fluorescence microscope bodies.
3. The **Light 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.
4. The **Gain** box defines the sensor gain.
5. The **Reset crop** button (Fig. 5.16) resets the cropped image to its original state. The change will only appear when a new **Capture** sequence is activated.
6. The **Crop Image** button (Fig. 5.16) allows a square to be drawn onto the image. When a new **Capture** sequence is activated, only the cropped region will be captured.
7. The **Binning** drop-down list (Fig. 5.16) allows the binning of pixels. This reduces the number of pixels for smaller save file sizes.
8. The **Mircoscope Info.** display information about the **Model** and **UID** of the connected microscope. It also display it **Status**: **Ready** if the microscope is ready to use and **Disconnected** if the microscope is disconnected.

5.2.5 View

The **View** tab (Fig. 5.1) 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.

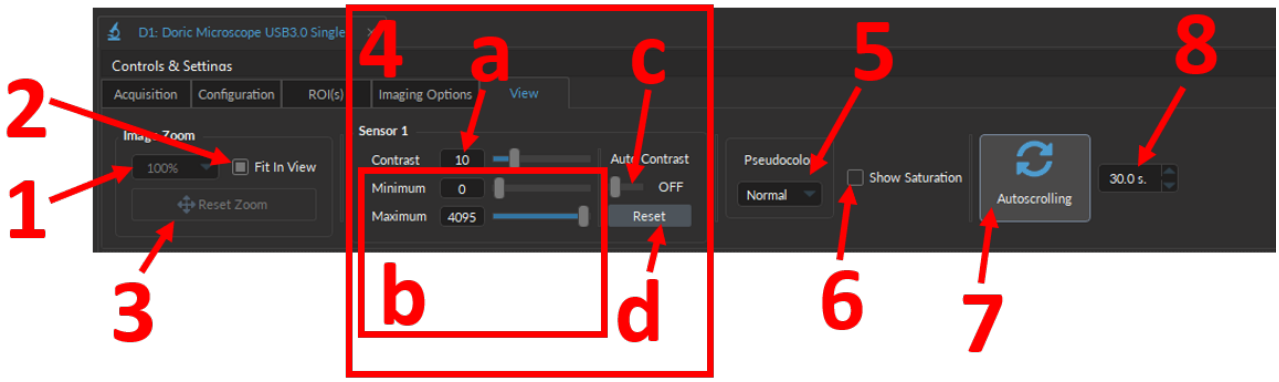


Figure 5.17: *View Tab*

1. The **Zoom Factor** drop-down list (Fig. 5.17) 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.
2. The **Fit In View** button (Fig. 5.17) resize the image to fit the size of the Image Viewer box.
3. The **Reset Zoom** button (Fig. 5.17) will reset the **Zoom factor** to 100%.
4. The **Sensor** section (Fig. 5.17) is used to adjust the contrast on a given sensor. When a microscope used has multiple sensors, multiple **Sensor** sections will be displayed, one for each sensor.
    a) The **Contrast** slider (Fig. 5.17) allows the adjustment of contrast from 0.1 to 5.
    b) The **Min/Max** sliders (Fig. 5.17) 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 4095, all pixels with a higher count will appear saturated.
    c) The **Auto contrast** slider button (Fig. 5.17) will activate an automatic contrast adjustment algorithm.
    d) The **Reset** button resets contrast functions to their default settings.
5. The **Pseudocolor** drop-down list (Fig. 5.17) allow the sensor image color palette to be changed.
6. The **Show saturation** checkbox (Fig. 5.17) allows all saturation on the sensor image to be displayed in red. This function is only available if no pseudocolor is selected.
7. The **Autoscolling** button activates the autoscrolling of the **ROI(s) View** (See section 5.3) in **Live** or **Reccord** mode.
8. The **Autoscrolling** box is used to adjust the temporal size of the **ROI(s) View** in the **Acquisition View** (See section 5.3 for more detail about the **ROI(s) View**).

5.3 Acquisition View

5.3.1 Microscope View

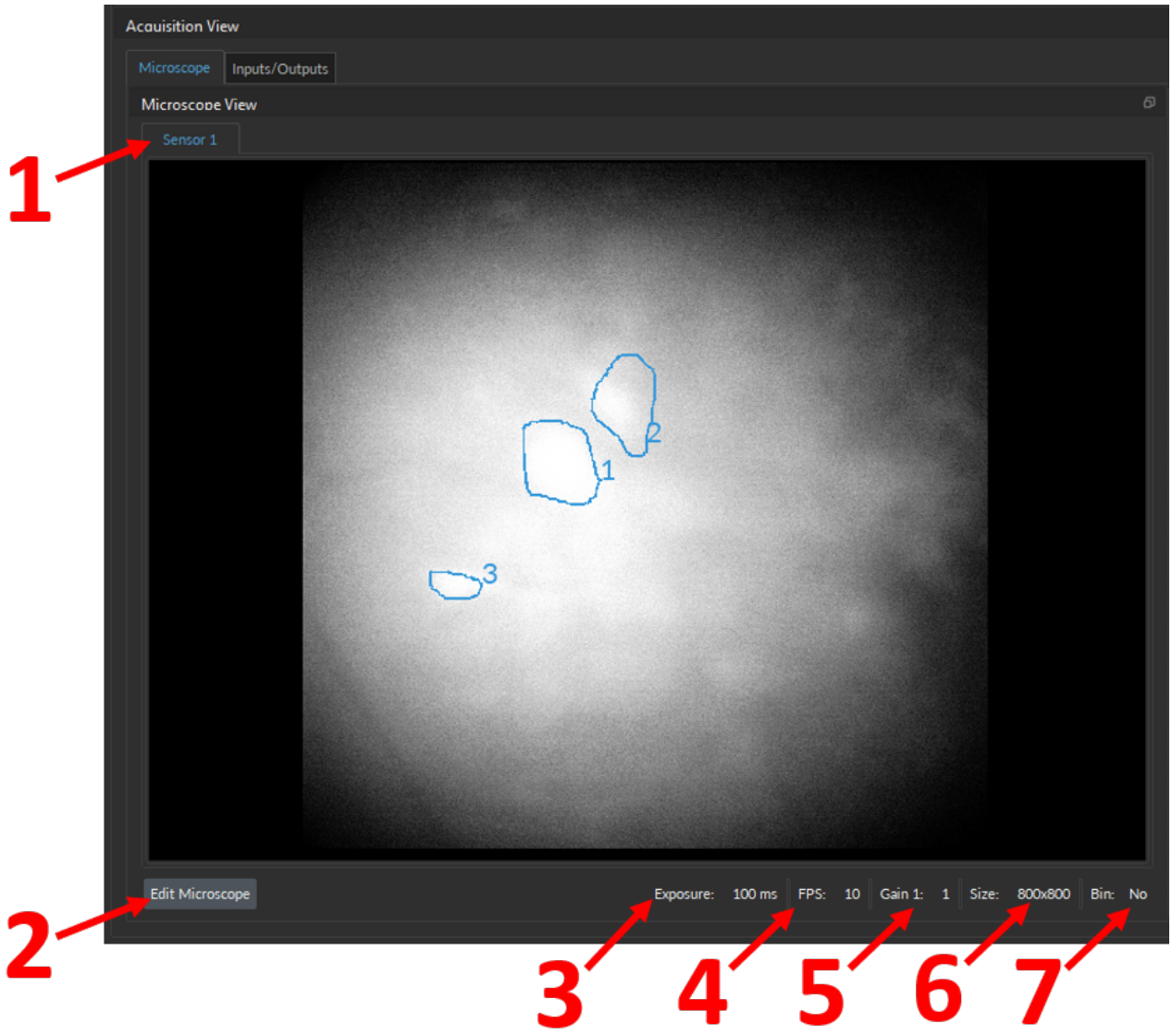


Figure 5.18: *Aquisition View section*

The **Microscope View** (Fig. 5.18) displays images from the microscope and allows region of interest (ROI) drawing by clicking and dragging the mouse over the image.

1. The **Sensor Tabs** (Fig. 5.18) displays the sensors available to view. For multi-sensor microscopes, changing tabs allow you to see the image available for each.
2. The **Edit Microscope** (Fig. 5.18) button opens the configuration window of the microscope (see section 5.1.1).
3. The **Exposure** (in ms) (Fig. 5.18) indicates the exposure time of the microscope sensor.
4. The **FPS** (Frames Per Second) (Fig. 5.18) indicates the number of frames per second taken by the sensor.
5. The **Gain** (Fig. 5.18) indicates the electrical gain of the sensor.

6. The **Size** (Fig. 5.18) indicates the resolution of the sensor images (in Pixels x Pixels).
7. The **Bin** (Fig. 5.18) status indicates whether or not the sensor image is being binned (yes/no).

5.3.2 Inputs/Outputs View

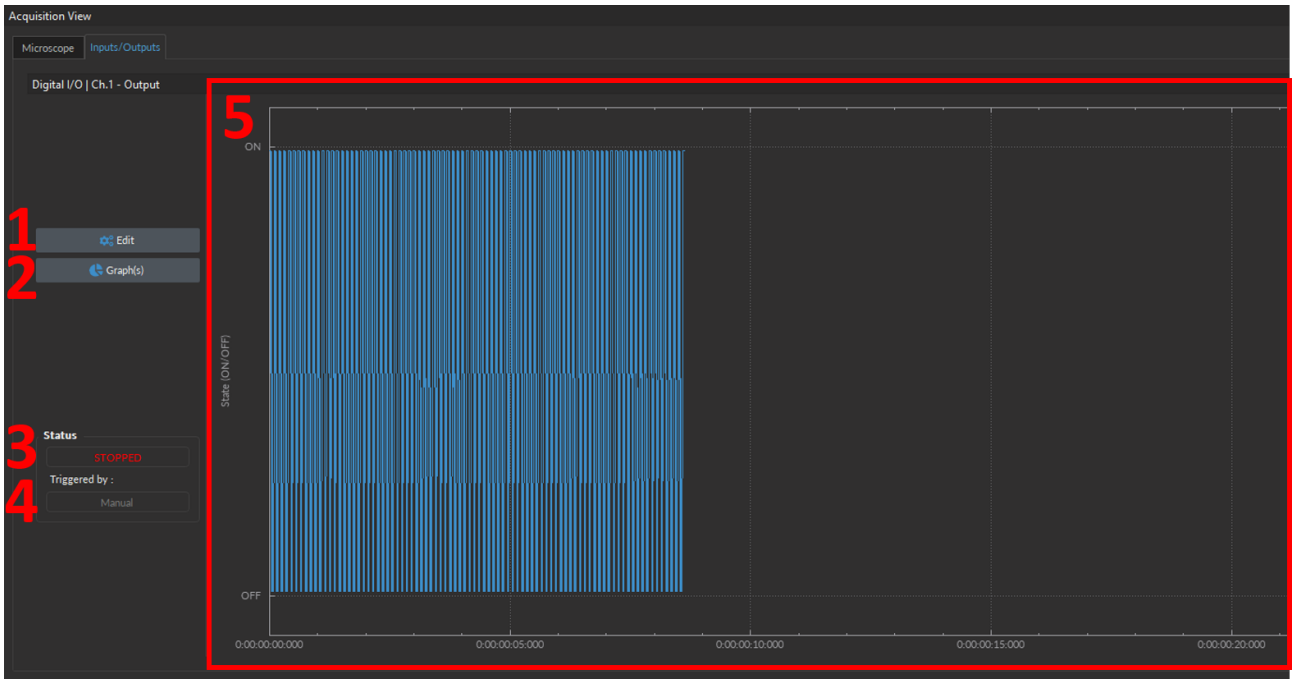


Figure 5.19: *Inputs/Outputs tab*

The **Inputs/Outputs View** (Fig. 5.19) displays trace from the Digital Input/Output.

1. The **Edit** button opens the configuration of the Digital Input/Output channel (Fig. 5.19).
2. The **Graph(s)** button opens a window to change graph display options (Fig. 5.19).
3. The **Status** displays the information if the channel is Running (in acquisition), Waiting (the trigger signal) or Stopped (Fig. 5.19).
4. The **Triggered by:** displays the option chosen to trigger the channel (Fig. 5.19).
5. The **Digital I/O plot** displays the signal sent or received on the programmed channel (Fig. 5.19).

5.4 ROI(s) View

5.4.1 ROI(s) Signal

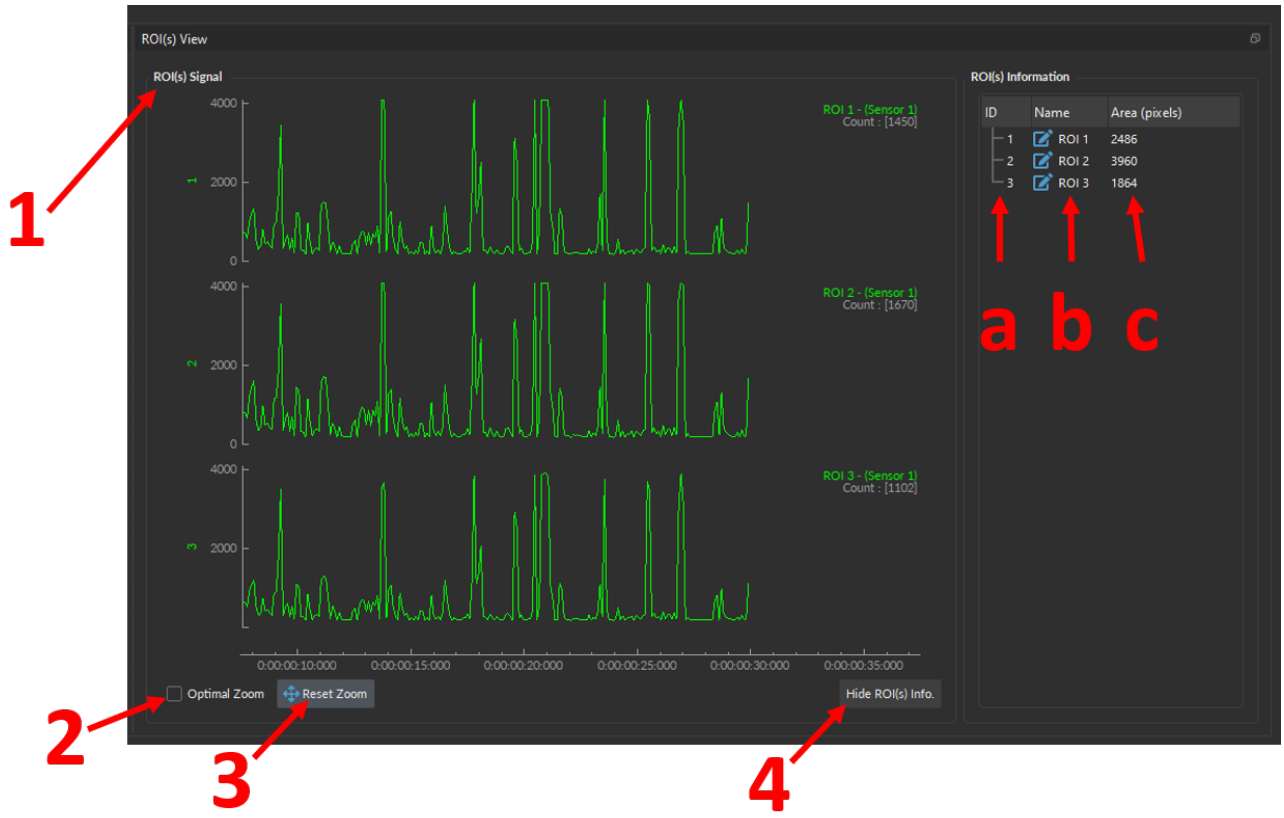


Figure 5.20: *ROI Manager*

The **ROI(s) View** (Fig. 5.20) displays the live mean pixel intensity from a drawn ROI(s).

1. The **ROI(s) Signal** plot (Fig. 5.20) displays the average intensity over time inside drawn ROI(s). *CTRL + mouse wheel* will adjust the x-axis zoom, while *SHIFT + mouse wheel* will adjust the y-axis zoom.
2. The **Optimal Zoom** checkbox (Fig. 5.20) sets the zoom factor on the plot to best display all data.
3. The **Reset Zoom** button (Fig. 5.20) resets the zoom to its default setting.

5.4.2 ROI(s) Information

4. The **Show/Hide ROI(s) Info.** (Fig. 5.20) open the panel with ROI(s) information:
    a) The **ID** column displays the number attributed to the ROI. It corresponds to the same number displayed next to each ROI drawn in the microscope view.
    b) The **Name** column displays the name attributed to the ROI. By default, the name is ROI and the ID number. Name can be changed by quickly clicking twice on the name .
    c) The **Area (pixels)** column displays the size of the ROI in pixels.

6

Using the Image Analysis Module

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 .doric formats, implements image processing functions and an export tool saves the fluorescence data in .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. To open Image Analyzer, select Analysis in the tab and choose Image Analyzer(Fig: 6.1).

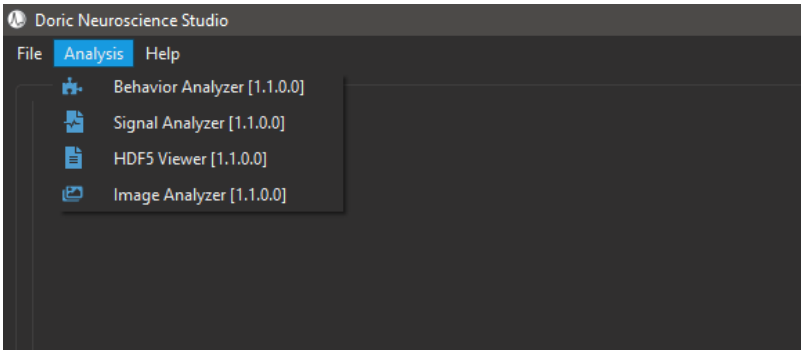


Figure 6.1: *Image Analyzer*

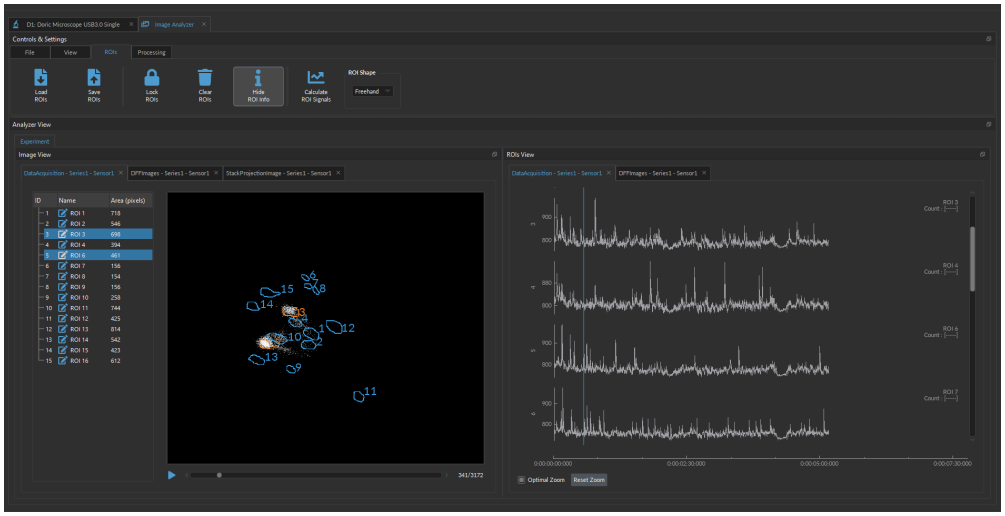


Figure 6.2: *Image Analysis Module Interface*

The Image Analyzer is composed of 2 main parts:

1. The **Controls & Settings** which regroups four tabs developed further in the document: **File** (section 6.1.1), **View** (section 6.1.2), **ROIs** (section 6.1.3), and **Processing** (section 6.1.4).
2. The **Analyzer View** 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, and displays the average intensity in each ROIs.

6.1 Controls & Settings

6.1.1 File

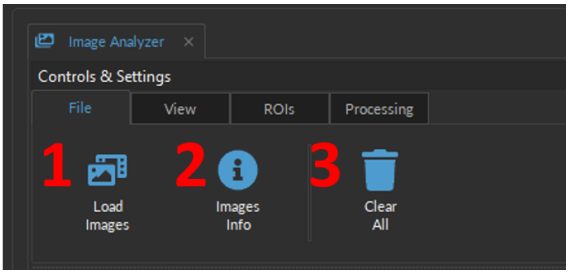


Figure 6.3: *File Tab*

The **File tab** (Fig. 6.3) is used to load data, obtain information about data and clear the **Analyser View**.

1. The **Load Images** button loads the images that must be a .doric containing images in a square, 16 bits format.
2. The **Images Info** displays a window with information about the images (Width x Height, Bits Count, Timestamp, Sensor ID, LED power, Exposure, and the Gain).
3. The **Clear All** button clears the **Analyser View** and close the analysis in progress.

6.1.2 View

The **View tab** (Fig. 6.4) is used to manipulate the appearance of an image without changing the base data.

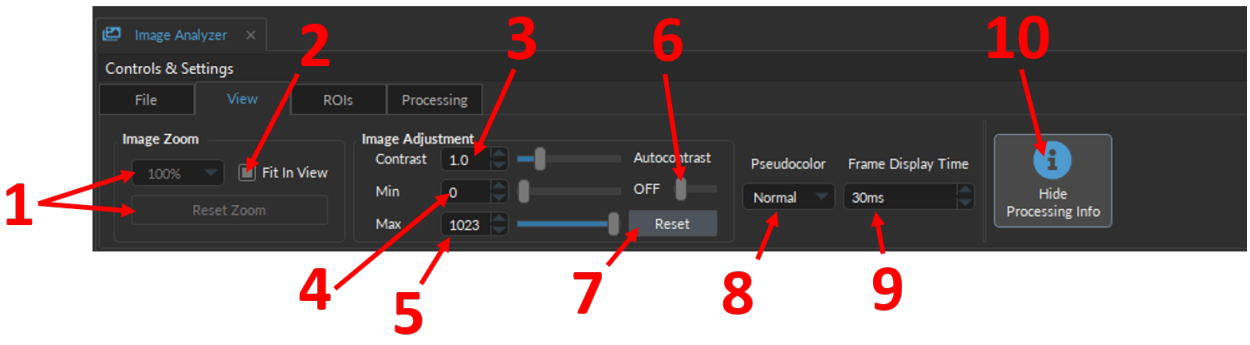


Figure 6.4: *View Tab*

1. The **Reset Zoom** and **Zoom factor** functions adjusts the displayed size of the current image. **Fit in View** needs to be unselected to use these functions.
2. The **Fit In View** check box adjusts automatically the size of the current image to the **Image Viewer** window.

3. The **Contrast** function applies a different luminance response curve (gamma). See section 6.3.1 for details.
4. The **Min** function applies a lower threshold with the cut-off value defined by the slider. See section 6.3.2 for details.
5. The **Max** function applies an upper threshold with the cut-off value defined by the slider. See section 6.3.2 for details.
6. The **Autocontrast** function directly applies the equalizeHist function of the OpenCV library.
7. The **Reset** function returns the contrast and range values to their default.
8. The **Pseudocolor** function is a drop-down list for selecting alternate coloring schemes for the images presented.
9. The **Frame Display Time** function adjust the frame rate in **Play** mode.
10. The **Show/Hide Processing Info** can be selected to display, near the images in the **Analyzer View**, the list of the process operated on the images in the order in which they are applied.

6.1.3 ROI

The **ROI** tab (Fig. 6.5) is used to save/load data relating to regions of interest drawn on an image.

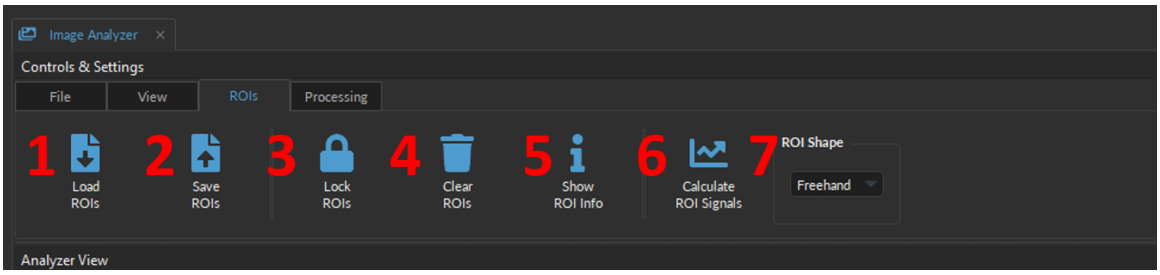


Figure 6.5: *ROI Tab*

1. The **Load ROIs** function loads .doric file containing informations about the saved ROIs.
2. The **Save ROIs** function saves the current ROIs information to a .doric file.
3. the **Lock/Unlock ROIs** can be selected to lock and unlock changes for ROIs. When it is active, you can not move or draw a ROI.
4. The **Clear ROIs** button clears all ROIs.
5. The **Show ROI Info** function display near to the images in the **Analyzer View** the **ID**, **Name**, and size (in pixels) of each ROIs.
6. The **Calculate ROI Signals** start the computing of ROI Signals depending of the ROI(s) drawn in the **Image Viewer**.
7. The **ROI shape** function is a drop-down list that allows the selection of the **ROI** shape. These include **Freehand, Circle, Rectangle** and **Square**.

6.1.4 Processing

The **Processing** tab (Fig. 6.6) is used to process the image data.

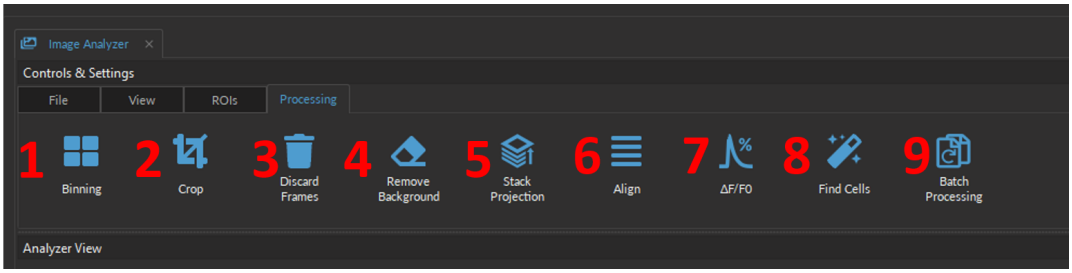


Figure 6.6: *Processing Tab*

1. The **Binning** 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.

2. The **Crop** function allows to crop the current image in smaller dimensions to reduce the amount of data and facilitate the processing.

3. 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.

4. 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 the $\Delta F/F_0$ function.

5. The **Stack Projection** function projects all movie frames to a single frame using the method selected in the Settings dialog. See section 6.3.6 for details.

6. The **Align** function aligns the image stack to the user-defined key frame. See section 6.3.3 for computational details. Selecting this button will open the **Align Images** window (Fig. 6.7). 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.

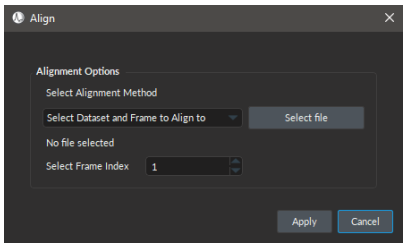


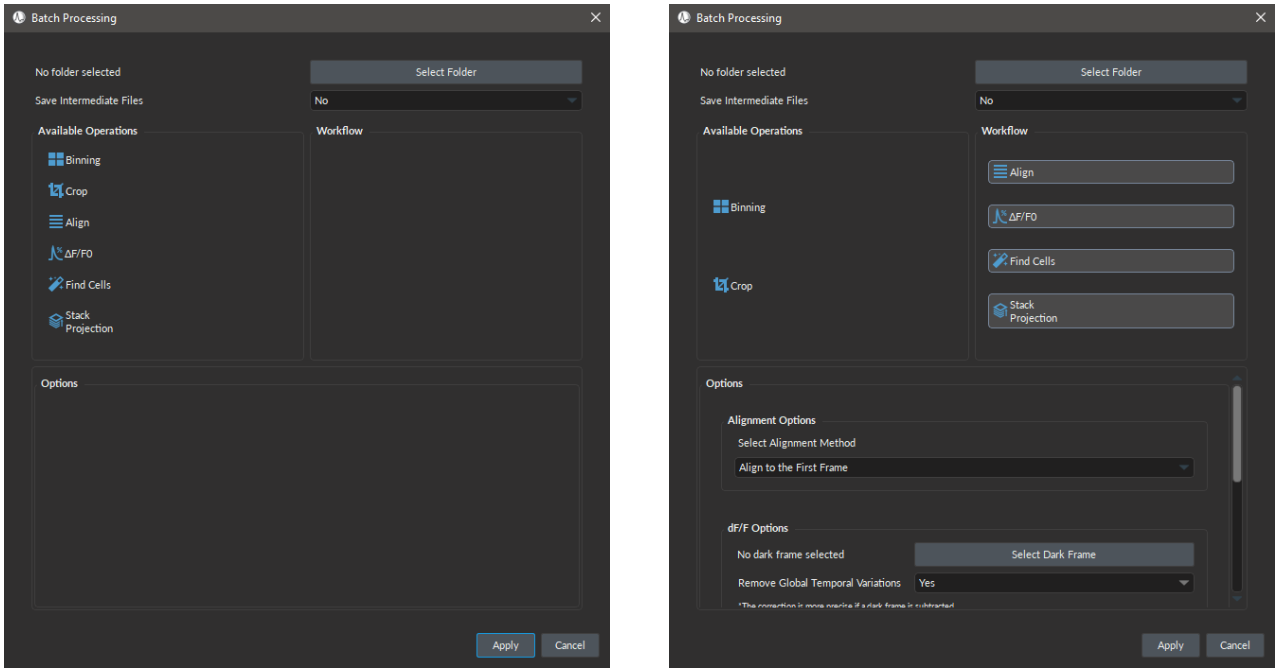
Figure 6.7: *Align Images Window*

   - The **Align to the First Frame** method uses the first image in the set to align the rest.
   - The **Select Frame to Align to** method allows the selection of a single image in the set to use for alignment of all other frames. Select the frame in the **Select Frame Index** display under **Select Alignment Method**.
   - The **Select Dataset And Frame to Align to** method aligns the current set using data from a different image set.
   - The **Select Alignment Shifts from Other 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.

7. The $\Delta F/F_0$ function calculates the normalized fluorescence variation of the images and displays the results in a new tab. See section 6.3.4 for details.

8. The **Find Cells** function detects the cells and creates the ROI automatically. See section 6.3.5 for details.

9. The **Batch Processing** function opens the **Batch Processing Window** (Fig. 6.8). 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 6.8: *Batch Processing Window*

- The **Available Operations** 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 **Binning** function combines a cluster of pixels in a single pixel to reduce the amount of data and facilitate the processing.
  - The **Crop** function allows to crop the current image in smaller dimensions to reduce the amount of data and facilitate the processing.
  - The **Align** process aligns the image stack to the user-defined key frame. See section 6.3.3 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 6.3.4 for details.
  - The **Find Cells** process detects the cells and creates the ROI automatically. See section 6.3.5 for details.
  - The **Stack Projection** process projects all image frames to a single frame using the method selected in the Settings dialog. See section 6.3.6 for details.
- 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.
- The **Select a Folder** button allows the selection of a folder to save batch processing results.
- The **Save intermediate Files** option will save intermediary files in the image processing process alongside the completed files.

6.2 Analyzer View

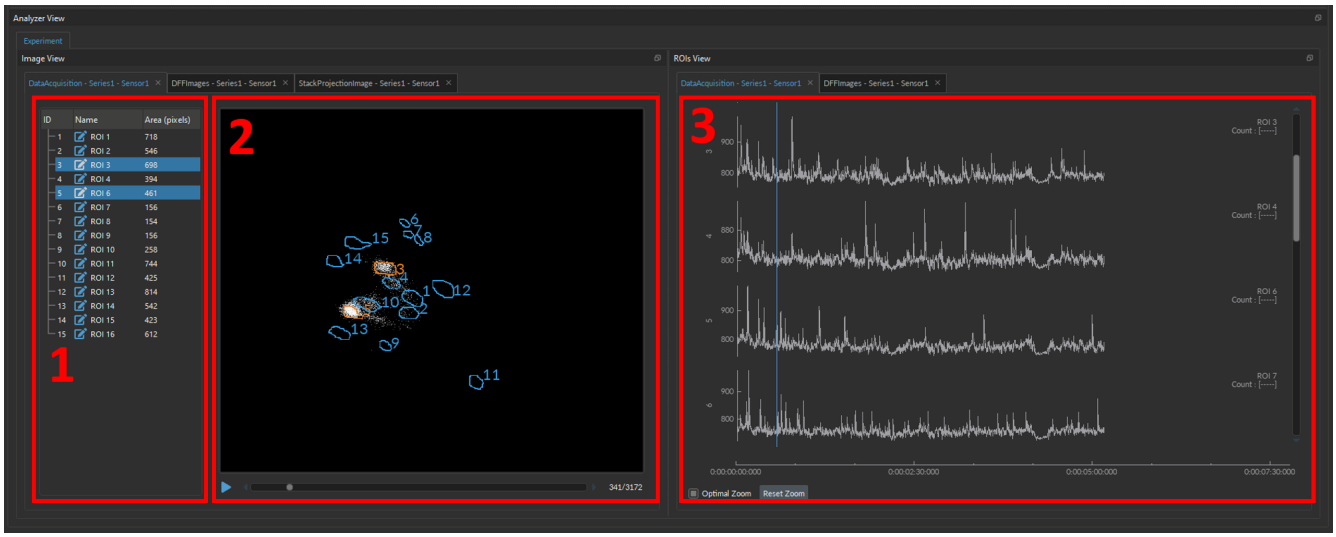


Figure 6.9: *ROI View*

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 **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.
   a) The **ID** shows the order of the ROI (starting at 1).
   b) The **Name** of the ROI, by default ROI ROI_ID. It can be change by clicking twice on the name.
   c) The **Area** shows the area (in pixels) contained in the ROI.
2. 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*.
3. The **ROIs View** panel shows the plot of average intensity as a function of the frame index. The Y-axis represents the average count of all the pixels of the ROI, or the variation to the baseline for ROI on normalised images. Each trace on a separate graph represent an ROI, allowing for precise intensity measurements (see Fig. 6.10)

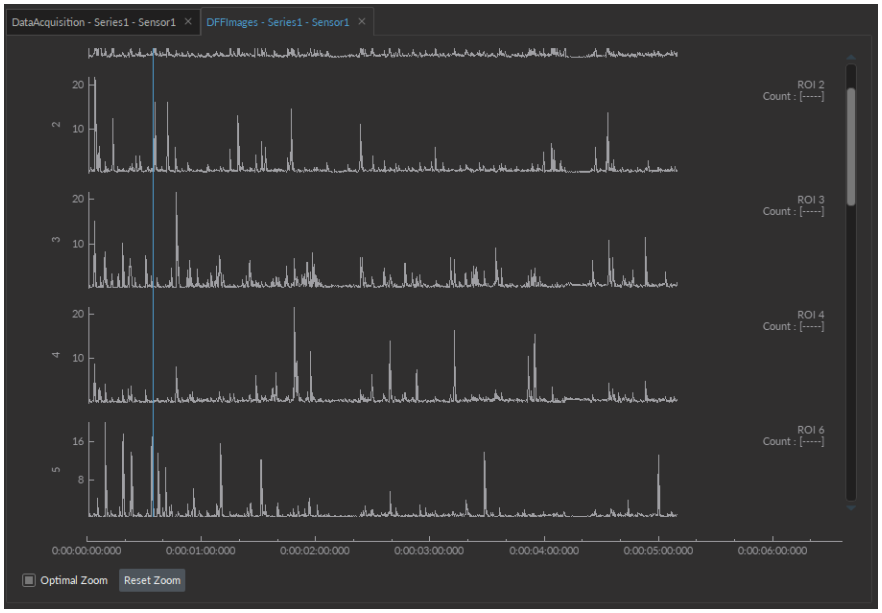


Figure 6.10: *ROI View Graph*

6.3 Algorithms

6.3.1 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.

6.3.2 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.

6.3.3 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.

6.3.4 $\Delta F/F_0$

The algorithm calculates a standard $\Delta F/F_0$ 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. 6.11). Keep in mind that removing the average temporal profile can also remove global activity patterns.

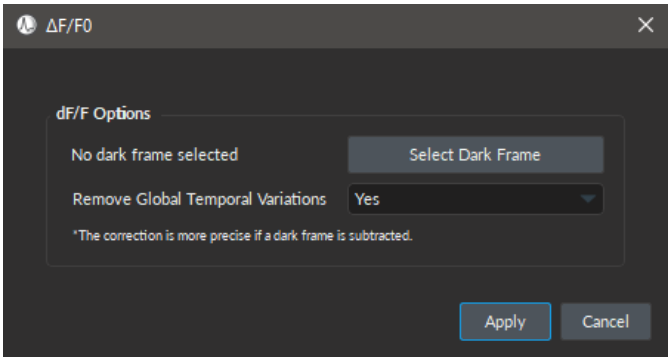


Figure 6.11: $\Delta F/F_0$ *Settings*

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 $R(t) = (F(t) - F_0)/F_0$.

6.3.5 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. 6.12. 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 filter the object found by the PCA/ICA.

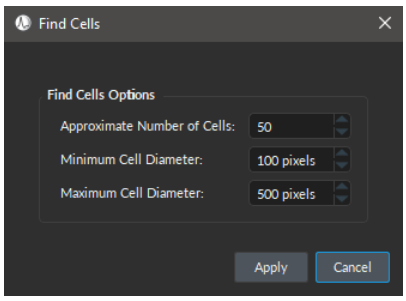


Figure 6.12: *Find Cells Settings*

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.

6.3.6 Stack Projection

This function can be used to help for ROI drawing. It calculates a temporal projection using the user-defined method (see Fig. 6.13).

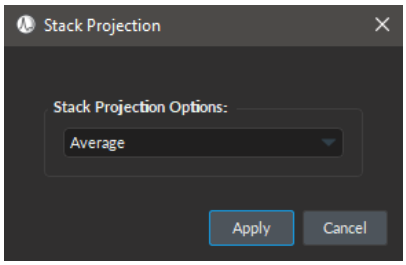


Figure 6.13: *Stack Projection Settings*

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

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

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

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

7

Specifications

Table 7.1: *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 µm	
GRIN lens diameter	500 µm	1000 µm
FOV at object plane	500 µm diameter (circular)	650 µm x 650 µm

Table 7.2: *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 7.3: *Twist-on Imaging Cannula Model L Specifications*

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

[1] Center wavelength/bandwidth
[2] With a 470 nm LED light source
[3] Including the thickness of the skull

Table 7.4: *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 µs	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 µs	

Table 7.5: *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 µsec[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	

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

8

Annex 1: Cleaning and Handling

8.1 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 8.2) to avoid the formation of stains.

8.2 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.

9

Annex 2: Troubleshooting Guide

9.1 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 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
    - *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.

[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.

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

9.2 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.

9.3 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.

10

Support

10.1 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.

10.2 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.

10.3 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.

10.4 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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