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Twist-on efocus Fluorescence Microscope System

User Manual

Version 1.4.0

Contents

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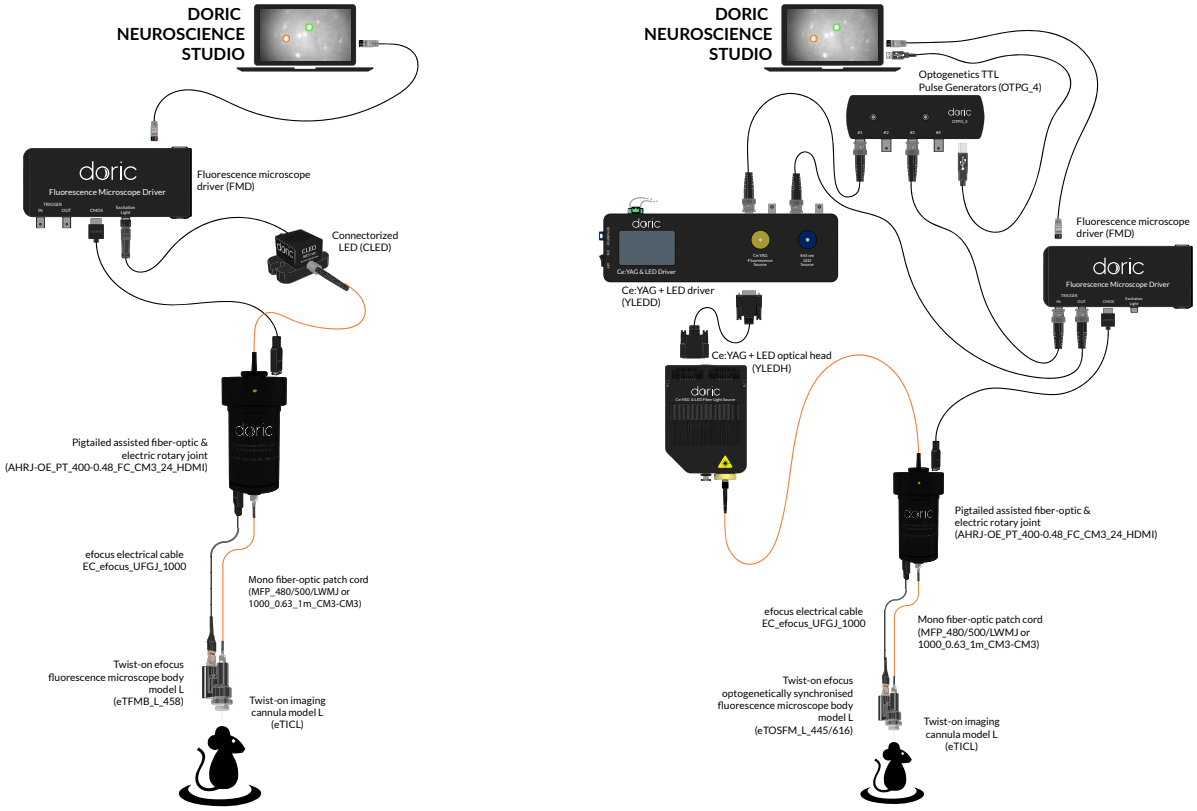
System Overview

The *Twist-on efocus Fluorescence Microscopy Systems (eTFMS)* are configured to image a single fluorophore within small animal subjects brains. An *Imaging Cannula* is used to send excitation light to fluorophores and receive the resulting fluorescence that is recorded in the microscope body with a CMOS sensor. *Twist-on efocus Fluorescence Microscope Body* also features an electronic focusing system to adjust the imaging depth via the software. The microscope is available for single-color (Fig. 1.1a) and optogenetically synchronized (Fig. 1.1b) calcium imaging.



(a) *GCaMP6 imaging configuration*

(b) *GCaMP6 imaging and NpHR3 activation configuration*

Figure 1.1: *Twist-on efocus Fluorescence Microscopy System Configurations*

1.1 Fluorescence Microscopy System: GCaMP6 filter set

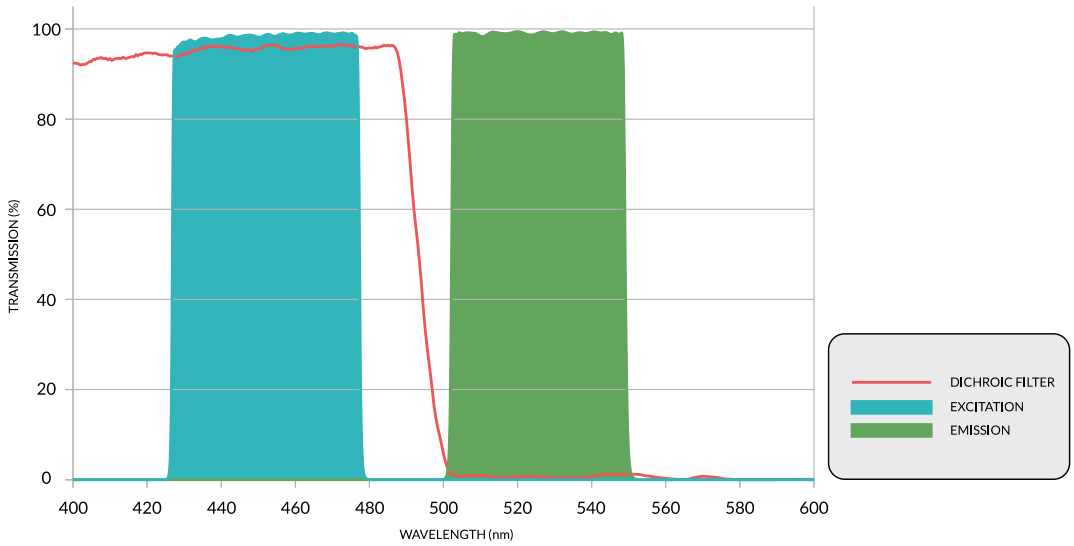


Figure 1.2: *GCaMP6 Excitation Filter Set Spectra*

The **Twist-on efocus Fluorescence Microscopy System (eTFMS)** (Fig. 1.1a) has been designed for the imaging of a single fluorophore using a single wavelength light source. This system is available in freely-moving and head-fixed configurations. The standard **GCaMP6** system (Fig. 1.2) uses a *Connectorized LED* as a light source.

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

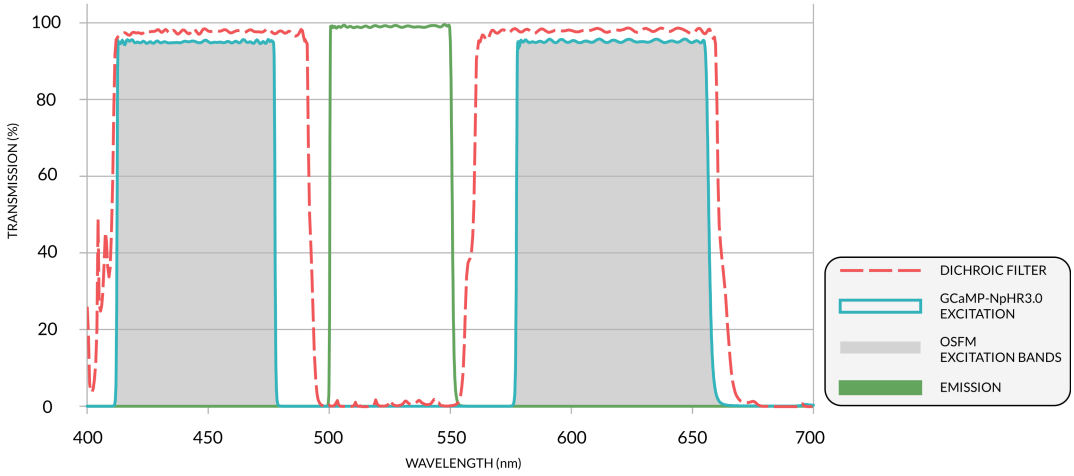


Figure 1.3: *GCaMP6+NpHR3.0 Excitation Filter Set Spectra*

The **Twist-on efocus Optogenetically Synchronized Fluorescence Microscopy System (eTOSFS)** (Fig. 1.1b) allows the imaging of 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, a *Ce:YAG + LED optical head* is needed for illumination (Fig. 1.3) .

2

Device Overview

2.1 Twist-on efocus Fluorescence Microscope Body

The *Twist-on efocus Fluorescence Microscope Body* 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 image from the deep structures of the brain to the microscope body. As there are no notable outward differences between the *Twist-on efocus Fluorescence Microscope Body (eTFMB)* and the *Twist-on efocus Optogenetically Synchronized Fluorescence Microscope Body (eTOSFM)*, these two products are not addressed separately.

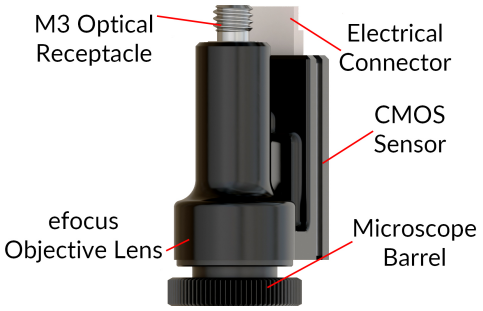


Figure 2.1: *Twist-on efocus microscope body*

- The **M3 Optical Receptacle** (Fig. 2.1) is used to receive light from a chosen light source using a fiber-optic patch cord.
- The **Electrical Connector** (Fig. 2.1) sends and receives electrical signal for the microscope using a 12-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** (Fig. 2.1) secures the microscope on a *Twist-on Imaging Cannula* (see 3.14, and *Imaging Cannula Implantation & Microscope Installation for eTFMB / eTOSFM application note* for more information on the microscope clamping procedure).
- The **CMOS Sensor** (Fig. 2.1) captures fluorescence images. Each *Microscope Body* has a serial number stored in memory that links each microscope to a specific set of mask correction filters associated with its **CMOS Sensor** that must be installed before use.
- The **efocus Objective Lens** (Fig. 2.1) transmits the image from the **Imaging Cannula** to the **CMOS Sensor**.

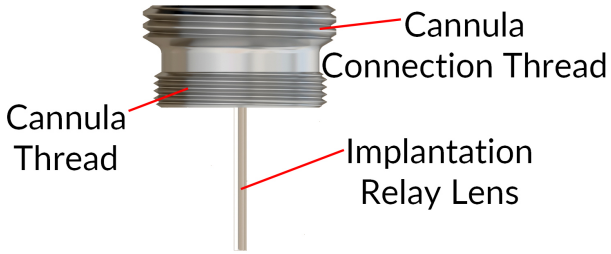


Figure 2.2: *Twist-on efocus Type-L Imaging Cannula*

2.2 Twist-on efocus Imaging Cannula

Imaging cannulas transmit images of structures located inside the brain to the surface of the skull. An imaging cannula 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** (Fig. 2.2) is used to secure the microscope body in place using the **Microscope Barrel**.
- The **Relay lens** (Fig. 2.2) 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** (Fig. 2.2) 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. 2.3).

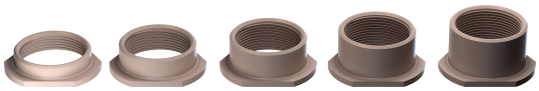


Figure 2.3: *Model L Imaging Cannula Protrusion Adjustment Rings. Height From Left to Right: 2.05 mm, 2.77 mm, 3.48 mm, 4.20 mm and 4.92 mm*

2.3 Twist-on efocus Dummy Microscope

Figure 2.4: *Dummy Microscope*

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

2.4 Pigtailed Assisted Fiber-optic & Electric Rotary Joint

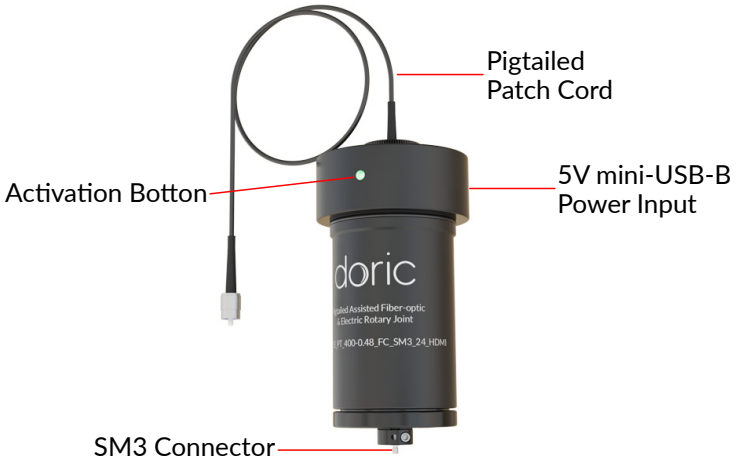


Figure 2.5: *24 contacts Pigtailed Assisted Fiber-optic & Electric Rotary Joint*

To use the microscope in experiments using freely-moving animals, the *24 contacts Pigtailed Assisted Fiber-optic & Electric Rotary Joint* (Fig. 2.5) 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 **CM3-CM3 Fiber-Optic Patch Cord connected to the SM3 connector**[2].
- The **HDMI Connectors** are used to provide electrical communication between the driver and the microscope.
- The **5 V mini-USB-B Connector** connects to the power supply for the assisted rotation.
- The **Activation Button** is used to activate/deactivate the assistance function. When the rotary assistance is activated, the button's light 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_CM3-CM3 or MFP_480/500/1000-0.63_1m_CM3-CM3

2.5 Fluorescence Microscope Driver

This driver allows for computer control over the excitation LED light source as well as microscope image capturing and broadcast at video rate to the computer via high speed Ethernet communication (Fig. 2.6). It can be triggered by or synchronized with external recording devices and it can also trigger other devices. This Fluorescence Microscope Driver is used with the *eTFMB*, and the *eTOSFM* microscope bodies. The standard model has an integrated LED light source driver.

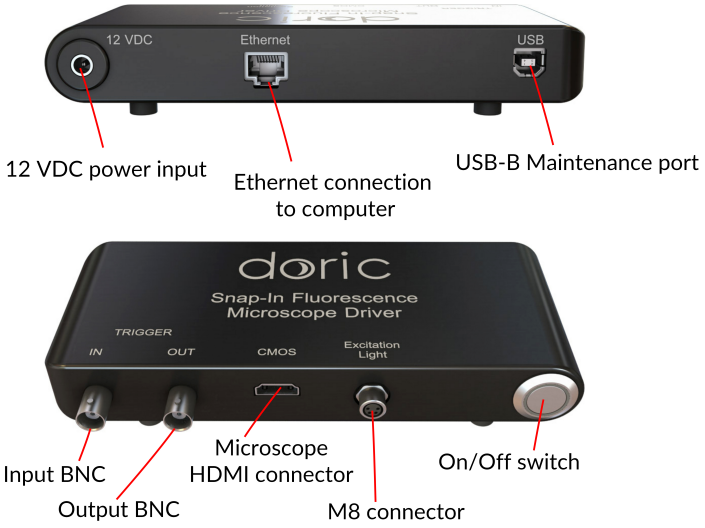
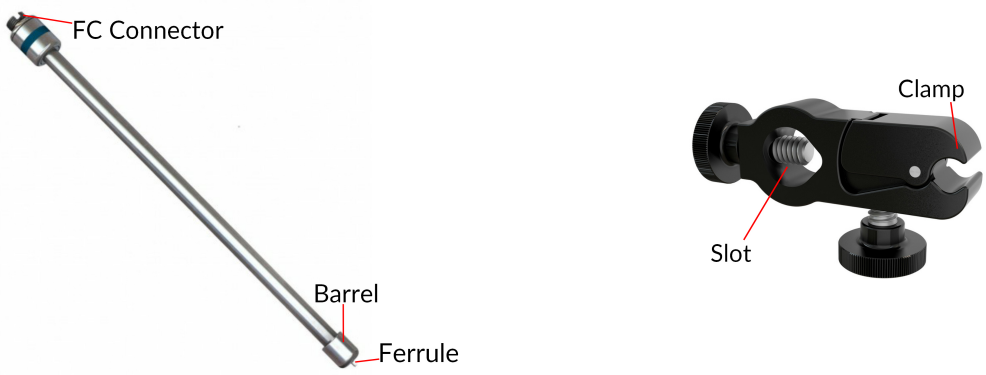


Figure 2.6: *Fluorescence Microscope Driver*

2.6 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 2.7: *Fluorescence Microscope Holder Elements*

- The *Clamp for Fluorescence Microscope Holder* (Fig. 2.7b) 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 using a FC-FC optical patch cord. This provides illumination to the microscope while is 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**.

2.7 efocus Electrical Cable

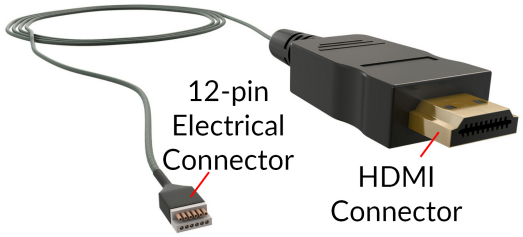


Figure 2.8: *efocus Electrical Cable*

The *efocus Electrical Cable* is used to connect the microscope and the driver. The **HDMI connector** is inserted into the driver, and the **12-pin Electrical Connector** is connected to the microscope electrical connector. When used with a *Pigtailed Assisted Fiber-optic & Electric Rotary Joint* (Fig. 2.5), the *efocus Electrical Cable* is connected to the HDMI connector located at the base of the rotary joint. To ensure a durable connection between the **12-pin Electrical Connector** and the microscope **Electrical Connector**, secure them together using the orange tape provided with the microscope.

3

Operations Guide

3.1 Connecting the Microscope Driver

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

3.2 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 6.5 for computer requirements.
2. **Select** the language to use during the installation.
3. In the license agreement window (Fig. 3.1), accept the agreement and click **Next** to continue the process.

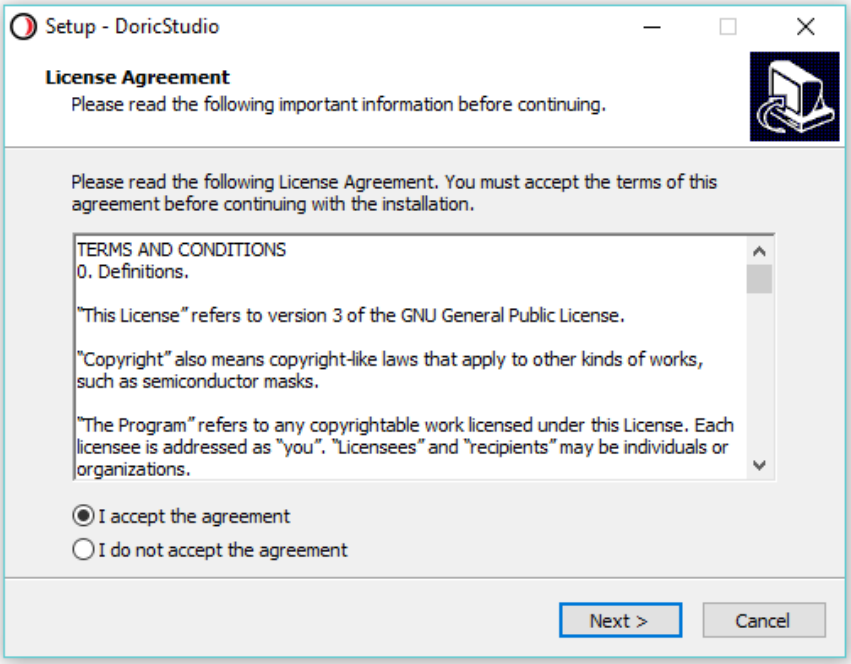


Figure 3.1: *Doric Neuroscience Studio License Agreement*

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

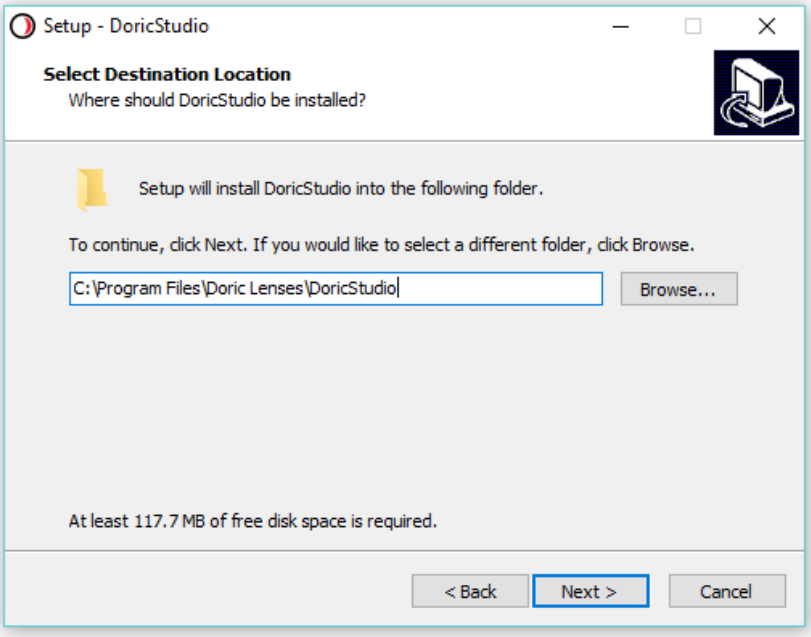


Figure 3.2: *Select Destination Location*

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

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

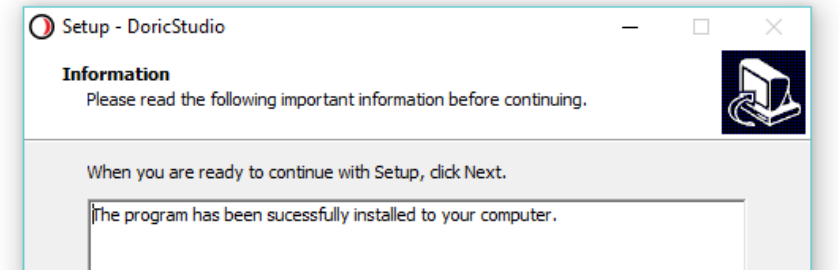


Figure 3.3: *Successful Installation of the Doric Neuroscience Studio*

9. Click **Next** and **Finish** to exit the setup.
10. Now the software is ready for use.

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, it will be on the Start Screen itself (Fig. 3.4).

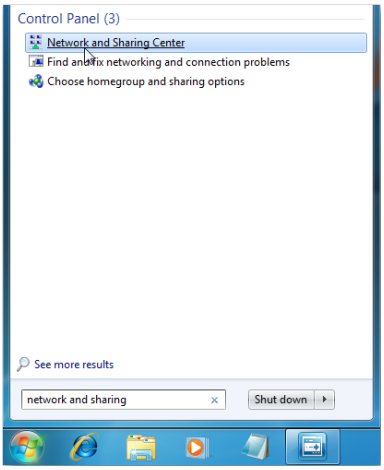


Figure 3.4: *Open Network and Sharing Center.*

On the side menu, select Change Adapter Settings (Fig. 3.5).

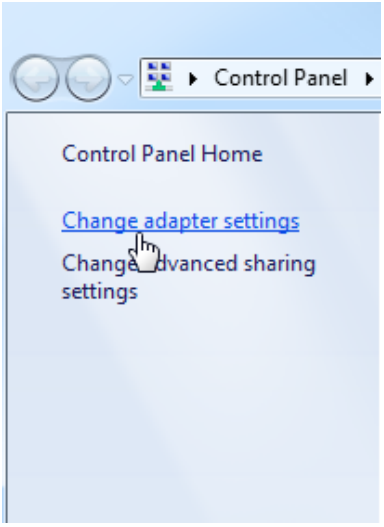


Figure 3.5: *Click on Change Adapter Settings.*

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

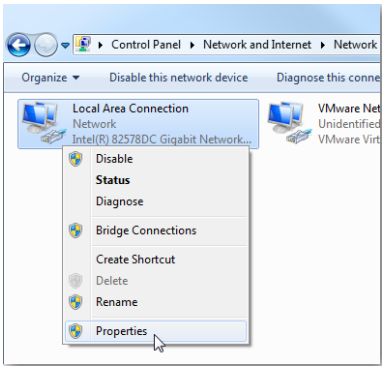


Figure 3.6: *Right-click on local adapter Properties.*

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

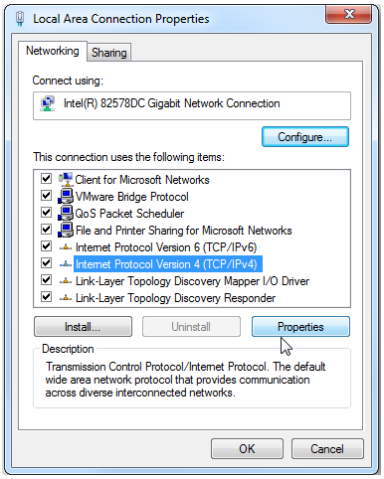


Figure 3.7: *Open IPv4 Properties.*

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).

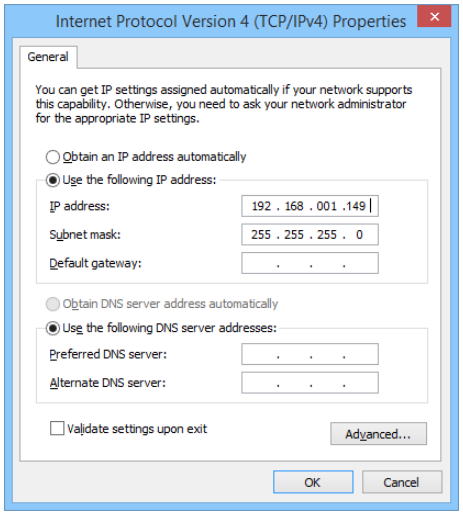


Figure 3.8: *Static IP settings.*

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).

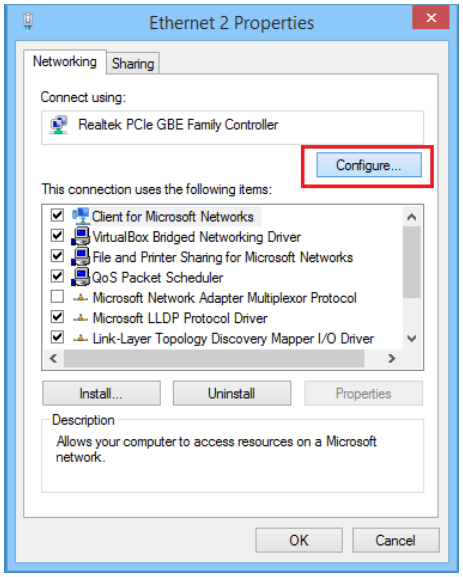


Figure 3.9: *Interface configuration.*

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).

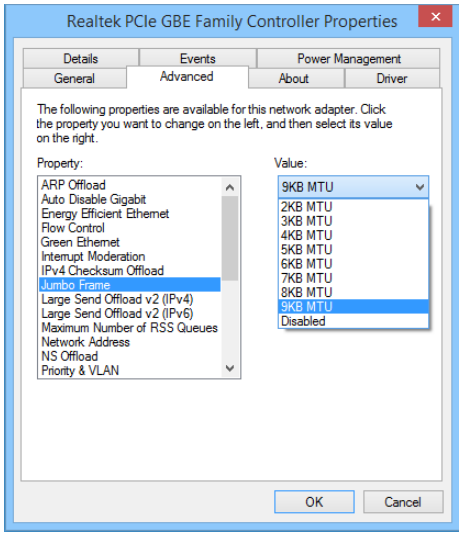


Figure 3.10: *Jumbo frame configuration.*

3.3.3 Configuring The Driver IP Address

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

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

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

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

3.4 Updating The Driver Firmware

To update the driver firmware version, contact us at sales@doriclenses.com. The updated firmware and an installation guide will be provided.

3.5 Updating Doric Neuroscience Studio

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

3.6 General Setup Guidelines

3.6.1 Optical fiber patch cord use

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

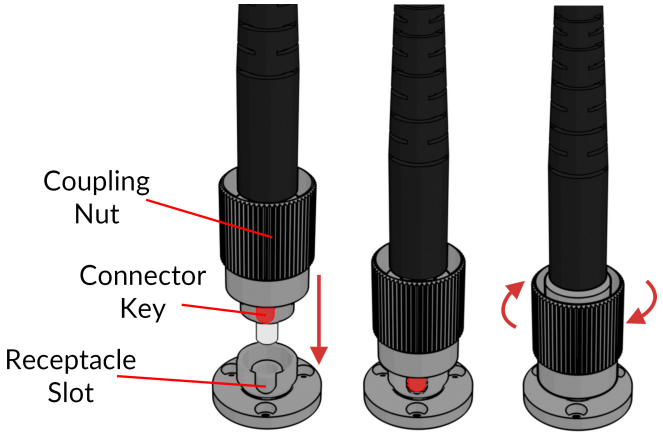


Figure 3.11: *FC connector, Fiber Installation*

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

3.6.2 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*.

3.7 Connecting the Twist-on efocus Microscopy System

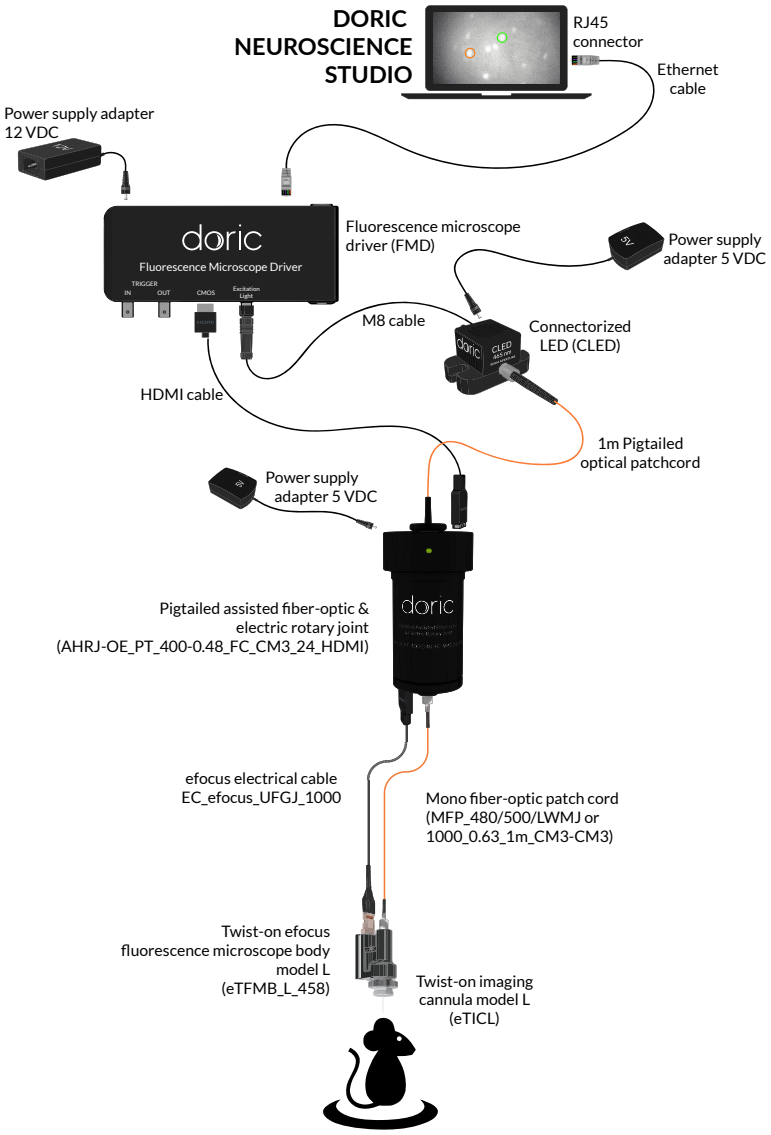


Figure 3.12: *Full GCaMP6 Freely-Moving Configuration, combination of (Fig. 3.13b) and (Fig. 3.15)*

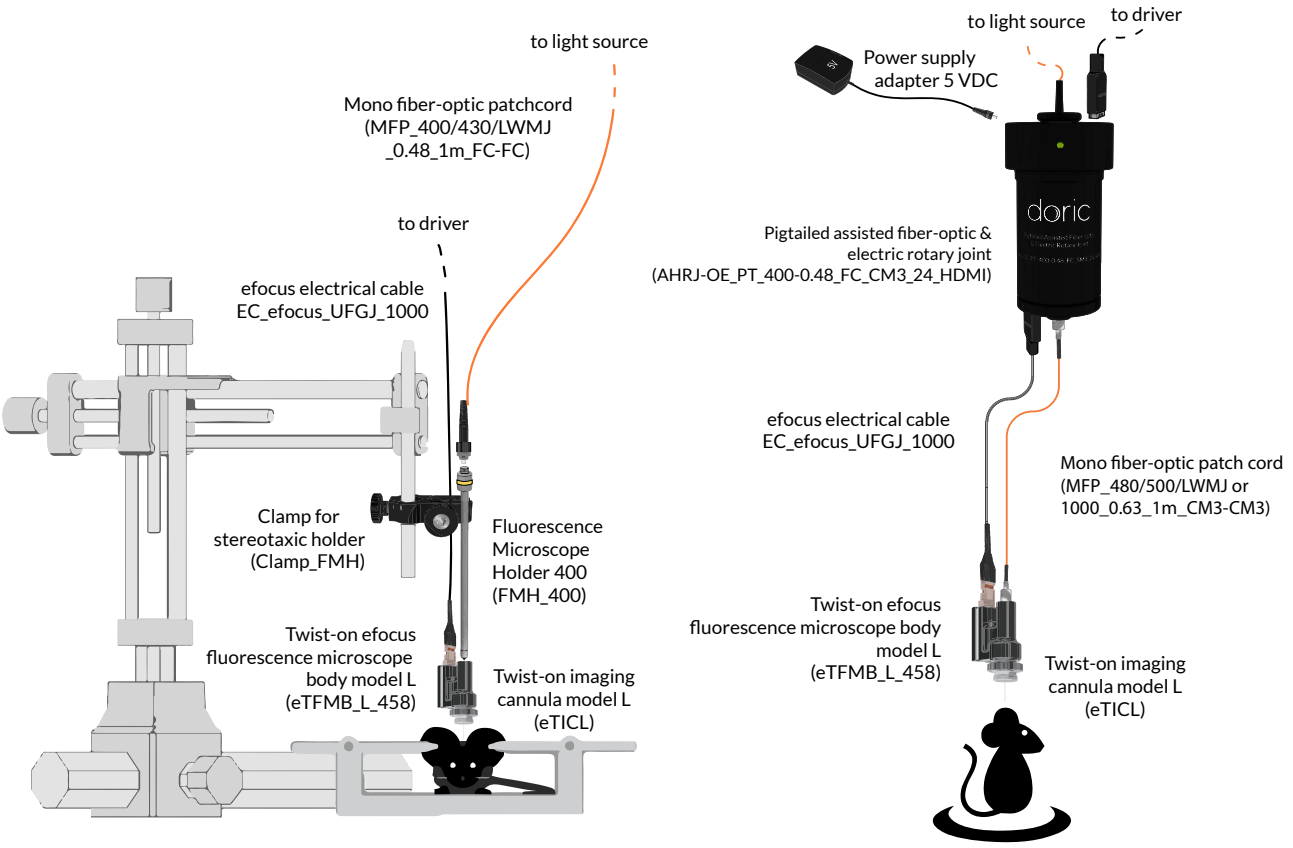
There are multiple different fluorescence microscope system configurations depending on the desired freedom of the animal and the fluorophores/opsin targeted. The animal must be held in one of the two following configurations.

- The **Head-fixed** configuration (Section 3.7.1, Fig. 3.13a) allows the observation of the animal while placed in a stereotaxic apparatus.
- The **Freely-Moving** configuration (Section 3.7.2, Fig. 3.13b) allows the observation of the animal while moving freely in a cage.

Depending on the *Microscope Body* type, two different lighting configurations are used:

- The **eTFMB GCaMP6** configuration (Section 3.7.3, Fig. 3.15) allows the imagery of **GCaMP6** fluorescence.
- The **eTOSFM GCaMP6 + NpHR3.0** configuration (Section 3.7.4, Fig. 3.16) allows the imagery of **GCaMP6** fluorescence with **NpHR3.0** activation.

Figure 3.13 shows the head-fixed and freely-moving system configurations. Each microscope body and lighting configuration uses the same devices in each of these configurations.



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

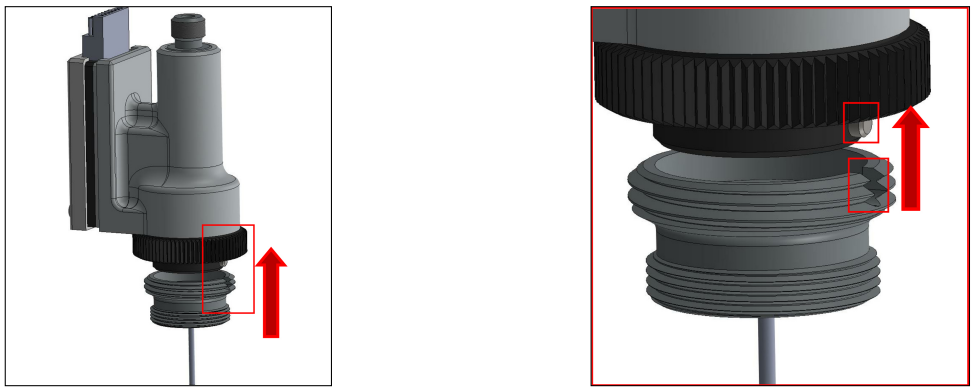
Figure 3.13: *Twist-on Microscopy Systems Connections*

3.7.1 Head-fixed Configuration Installation

1. Install the *Fluorescence Microscope Holder (FMH_400)* into the *Stereotaxic Clamp* (Fig. 3.13a). 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 **12 pins Electrical Connector** of the *efocus Electrical Cable* to the electrical connector of the microscope body, and its **HDMI** end to the CMOS port of the driver (*FMD*).
6. Unscrew the Input Protective Cap from *Twist-on imaging cannula* and connect the cannula to the microscope body (Fig. 3.14a) and ensure the microscope key is properly inserted in the cannula slot (Fig. 3.14b, 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 3.14: *Twist-on Microscopy Systems Connections*

3.7.2 Freely-moving configuration installation

1. Connect the microscope driver to the top end of the *Pigtailed Assisted Fiber-optic and Electric Rotary Joint* using the HDMI cable (Fig. 3.13b, 3.15).
2. Connect the pigtailed patch cord (top end of the rotary joint) to the light source **Beam Aperture**.
3. Connect the **12 pins Electrical Connector** of the *efocus Electrical Cable* to the electrical connector of the microscope body, and its **HDMI** end 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 (CM3-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. 3.14, 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_CM3-CM3 or MFP_480/500/1000-0.63_1m_CM3-CM3

3.7.3 Connecting the eTFMB

The *eTFMB_L_458* body is designed for **GCaMP6** fluorescence imaging. The indications of section 3.7.1 and 3.7.2 are to be followed for its installation.

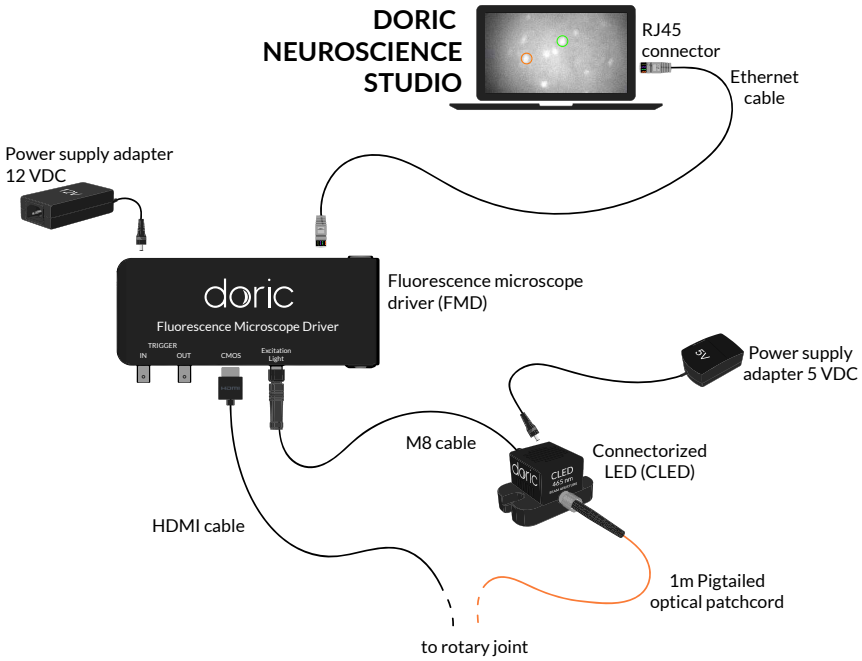


Figure 3.15: *GCaMP6 system configuration*

Microscope Driver and Light Source Installation

1. Connect the *12 VDC power supply* to the microscope driver **12 VDC power input**.
2. Connect the *5 VDC power supply* to the LED **5 VDC power input**.
3. Connect the driver to the LED using the **M8 connector**.
4. Connect the driver to the computer using the *CAT5E Ethernet Cable*.
5. Turn on the *Fluorescence Microscope Driver*.

3.7.4 Connecting the eTOSFM system

The indications in section 3.7.1 and 3.7.2 are to be followed for the installation of the **eTOSFM**. However, as the **eTOSFM** uses two light sources (*Ce:YAG + LED fiber Light Source* [3]), and two drivers to illuminate the sample, the installation is more elaborate.

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

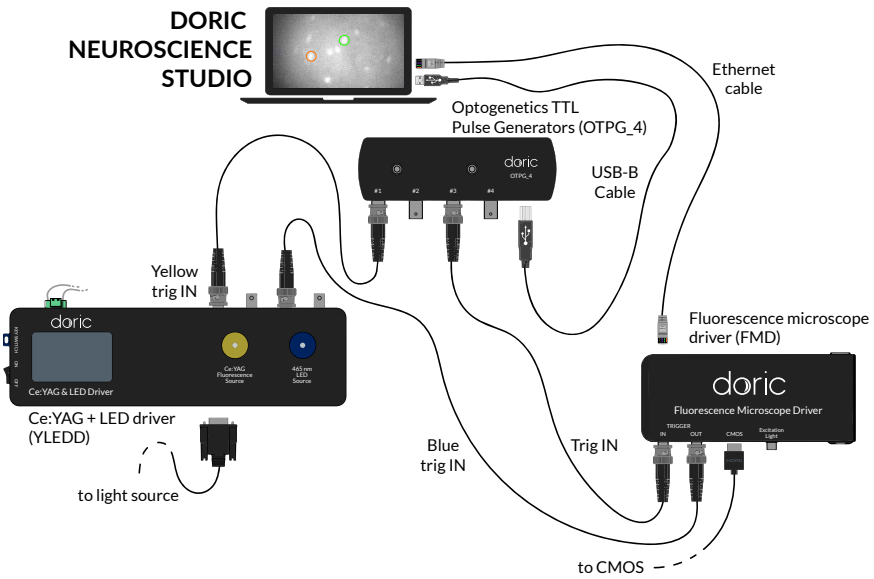


Figure 3.16: *GCaMP6 + NpHR3.0 configuration*

GCaMP6 + NpHR3.0 configuration

For the **GCaMP6 + NpHR3.0 configuration** (Fig. 3.16), the *Ce:YAG + LED optical head* is used as the light source. The LED light source is used for excitation, while the Ce:YAG light source is used for opsin activation.

1. Connect the *OTPG* to the computer using a **USB-A/USB-B cable**.
2. In this configuration, the LED light source needs to be triggered by the *Fluorescence microscope driver*. Connect the driver **BNC output** to the *Ce:YAG + LED driver* **LED BNC input**.
3. Connect the *Ce:YAG + LED driver* **Ce:YAG BNC input** to an *OTPG* channel.
4. Connect the *Fluorescence microscope driver* **Input BNC** to an *OTPG* channel.
5. When installing in a head-fixed or freely-moving configuration, the rest of the system is connected as described in section 3.7.1 and 3.7.2.

[3] For details and specifications concerning the Ce:YAG + LED Fiber Light Source, see its user manual.

4

Using Doric Neuroscience Studio

4.1 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. 4.1) and a complete description of all the functions.

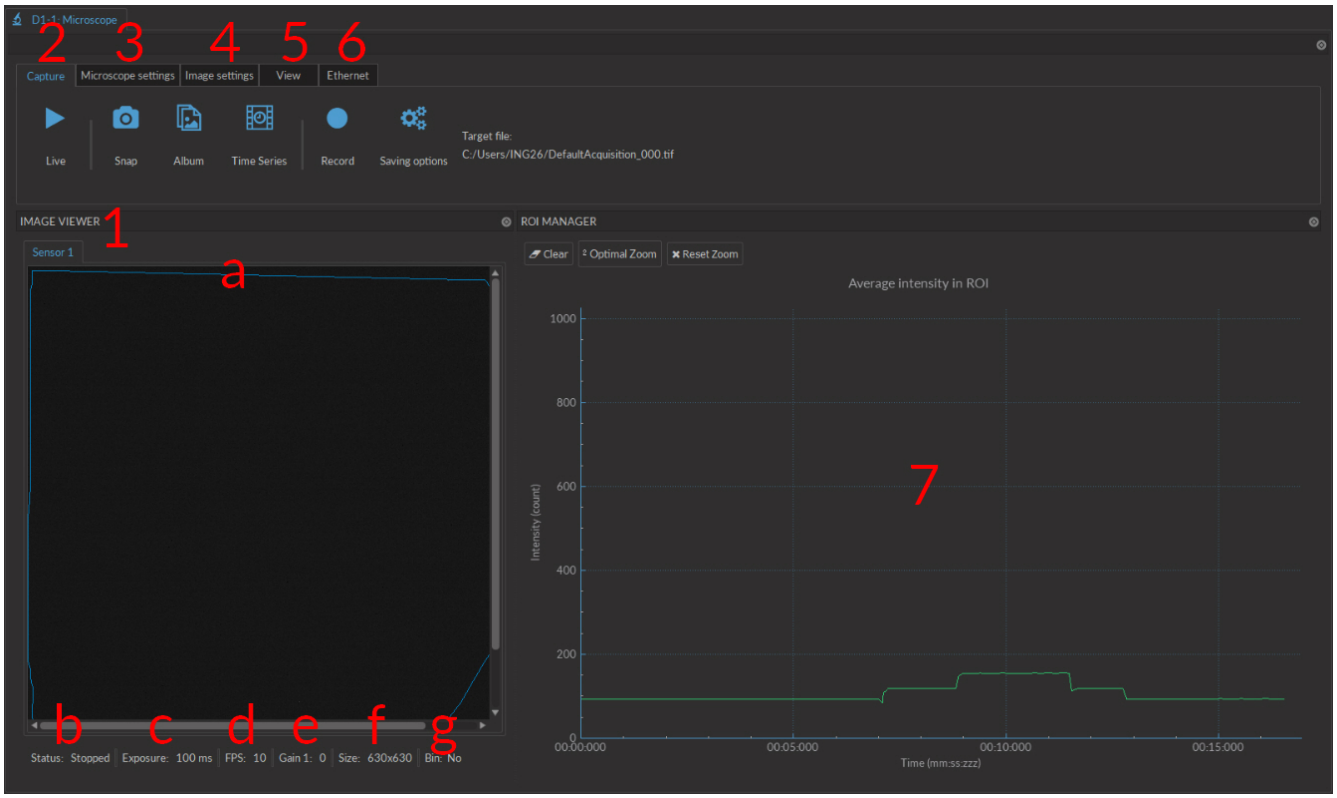


Figure 4.1: *Microscope Module Interface*

1. The **Image Box** (Fig. 4.1) 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. 4.1) 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. 4.1) will indicate the current microscope state (Live/Stopped).

c) The **Exposure** (in ms) (Fig. 4.1) indicates the exposure time of the microscope sensor.

d) The **FPS** (Frames Per Second) (Fig. 4.1) indicates the number of frames per second taken by the sensor.

e) The **Gain** (Fig. 4.1) indicates the electrical gain of the sensor.

f) The **Size** (Fig. 4.1) indicates the resolution of the sensor images (in Pixels x Pixels).

g) The **Bin** (Fig. 4.1) status indicates whether or not the sensor image is being binned (yes/no).

2. The **Capture** tab (Fig. 4.1) contains different image-capturing functions of the microscope.

Figure 4.2: *Capture Tab*

a) The **Live** button (Fig. 4.2), when pressed, displays images from the microscope. These images are not saved.

b) The **Snap** button (Fig. 4.2), 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. 4.2), 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. 4.2), 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 4.3: *Time Series Window*

e) The **Time Series** button (Fig. 4.2), when pressed, opens the time series interface (Fig. 4.3).

i. The **Number of time points** (Fig. 4.3) defines the number of moments when a set of images will be recorded.

ii. The **Images per time point** (Fig. 4.3) defines the number of images taken in each set.

iii. The **Time interval between points** (Fig. 4.3), 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. 4.3) 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. 4.3) displays the progress (in %) of the time series.

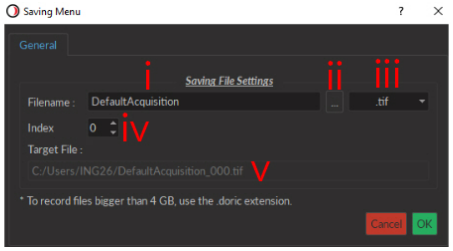


Figure 4.4: *Saving Options Window*

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

   i. The **Filename** box (Fig. 4.4) is used to define the recorded file name.

   ii. The **...** button (Fig. 4.4) opens a window used to choose the save file location.

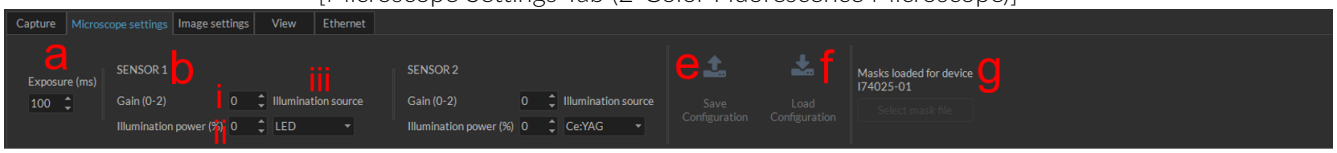
   iii. The **File type** drop-down menu (Fig. 4.4) is used to decide which file type is used to save images. For files larger than 4 GB, the .doric extension is recommended.

   iv. The **Index** box (Fig. 4.4) displays the current index that will be added to the filename.

   v. The **Target File** box (Fig. 4.4) shows the full location and name of the file being saved when an image sequence is recorded.

3. **Microscope settings** tab (Fig. 4.1) 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)]

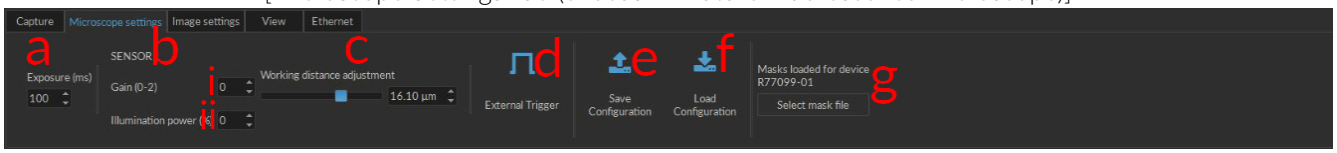


Figure 4.5: *Microscope Settings Tab*

a) The **Exposure** box (Fig. 4.5) sets the exposure time of the sensor. The time can be set between 22 and 1000 ms.

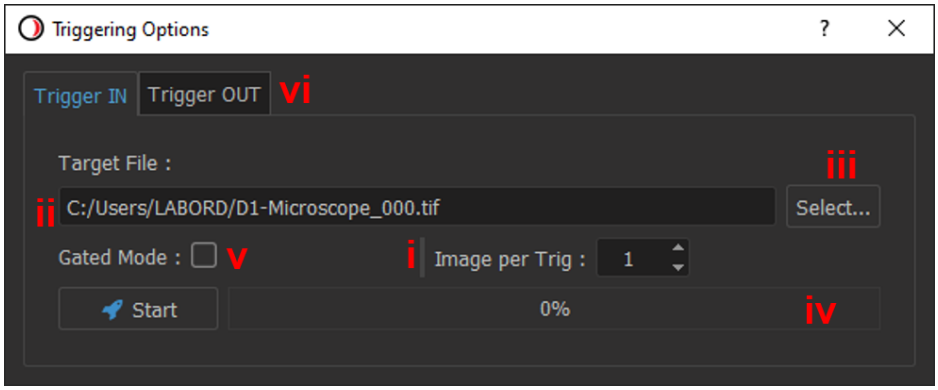
b) The **SENSOR** section (Fig. 4.5) defines characteristics for a single sensor and the associated excitation source. When a microscope used has multiple sensors, multiple **SENSOR** sections will be displayed, one for each sensor.

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

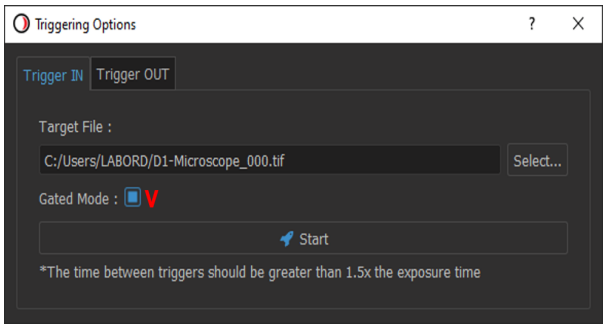
   ii. The **Illuminator power (%)** box defines the power emitted by the excitation light source. The light sources will be activated when the image acquisition is started. The maximum optical power (in mW) depends on the light source model.

   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.

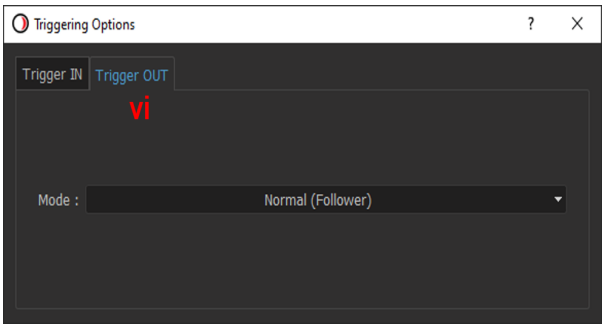
c) The **Working Distance Adjustment Slider** appears when an eFocus Miniature Fluorescence Microscope is connected to the driver. This slider will adjust the working distance from -45 to 45 um for snap-in fluorescence microscope bodies and from 0 to 350 um for twist-on fluorescence microscope bodies.



(a) *External Trigger Settings Window*



(b) *Gated Mode*



(c) *Trigger Out Mode*

Figure 4.6: *External Trigger Settings Windows*

d) The **External Trigger** button (Fig. 4.5) opens the **external trigger** window.
   i. The **Number of images per trig** box (Fig. 4.6a) defines the number of images acquired at each trigger pulse.
   ii. The **File name/location** (Fig. 4.6a) box displays the location where the images are saved as well as their file name.
   iii. The **Select...** (Fig. 4.6a) button allows the selection of the **File name/location**.
   iv. The **Progression bar** (Fig. 4.6a) displays the advancement of the triggered sequence (in %).
   v. The **Gated mode** checkbox (Fig. 4.6a) will change the external trigger to gated mode (Fig. 4.6b). 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. 4.5) will save all **Microscope settings** and **Image settings** in a **.doric** format file.

f) The **Load configuration** button (Fig. 4.5) 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 4.1.1.

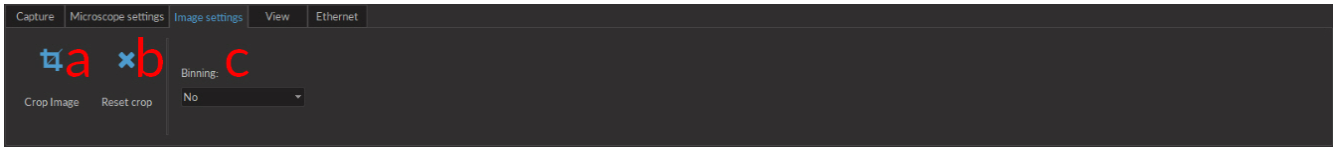


Figure 4.7: *Image Settings Tab*

4. The **Image settings** tab (Fig. 4.7) is used to define certain settings related to the displayed and recorded images.
    a) The **Crop Image** button (Fig. 4.7) 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. 4.7) 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. 4.7) allows the binning of pixels. This reduces the number of pixels for smaller save file sizes.
5. The **View** tab (Fig. 4.1) 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 4.8: *View Tab*

    a) The **Zoom In/Zoom Out** buttons (Fig. 4.8) will increase/decrease the zoom of the sensor image.
    b) The **Reset Zoom** button (Fig. 4.8) will reset the **Zoom factor** to 100%.
    c) The **Zoom Factor** drop-down list (Fig. 4.8) 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. 4.8) resize the image to fit the size of the Image Viewer box.
    e) The **Roi shape** drop-down list (Fig. 4.8) 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. 4.8) 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. 4.8) allows the adjustment of contrast from 0.1 to 5.
        ii. The **Min/Max** sliders (Fig. 4.8) 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. 4.8) 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. 4.8) allow the sensor image color palette to be changed.
    h) The **Show saturation** checkbox (Fig. 4.8) allows all saturation on the sensor image to be displayed in red. This function is only available if no pseudocolor is selected.

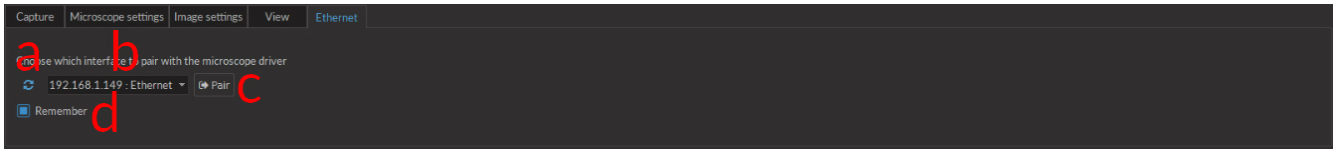


Figure 4.9: *Microscope Ethernet Tab*

6. The **Ethernet** tab (Fig. 4.1) is used to define the ethernet connection used to connect the computer to the microscope driver.
    a) The **Refresh** button (Fig. 4.9) will identify any accessible IP addresses and add them to the drop-down list.
    b) The **Ethernet** drop-down list (Fig. 4.9) 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. 4.9) connects the software to the driver.
    d) The **Remember** checkbox (Fig. 4.9) will keep the chosen IP address so that the chosen microscope driver will be connected automatically next time the software is opened.

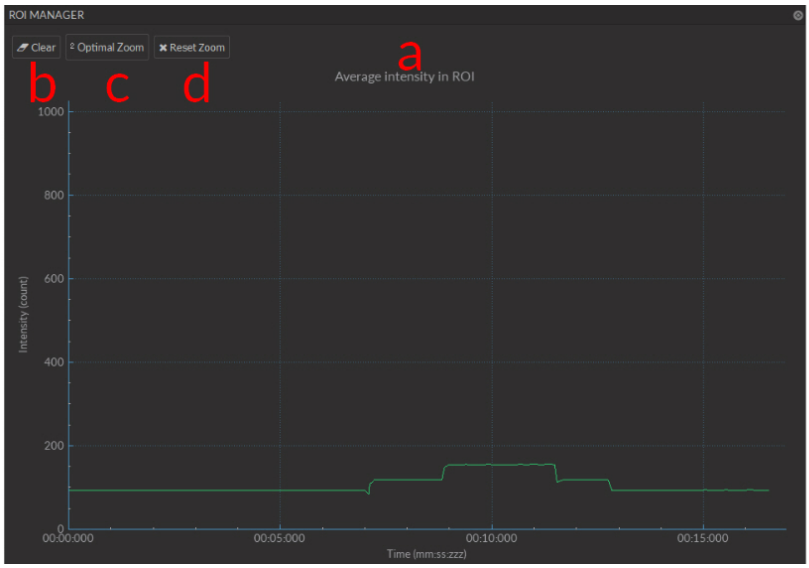


Figure 4.10: *ROI Manager*

7. The **ROI Manager** (Fig. 4.1) displays the live mean pixel intensity from a drawn ROI.
    a) The **Average Intensity in ROI** plot (Fig. 4.10) 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. 4.10) will clear any data displayed in the ROI manager and the ROI on the **Image Viewer**.
    c) The **Optimal Zoom** button (Fig. 4.10) sets the zoom factor on the plot to best display all data.
    d) The **Reset Zoom** button (Fig. 4.10) resets the zoom to its default setting.

4.1.1 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.

5

Using the Image Analysis Module

5.1 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.

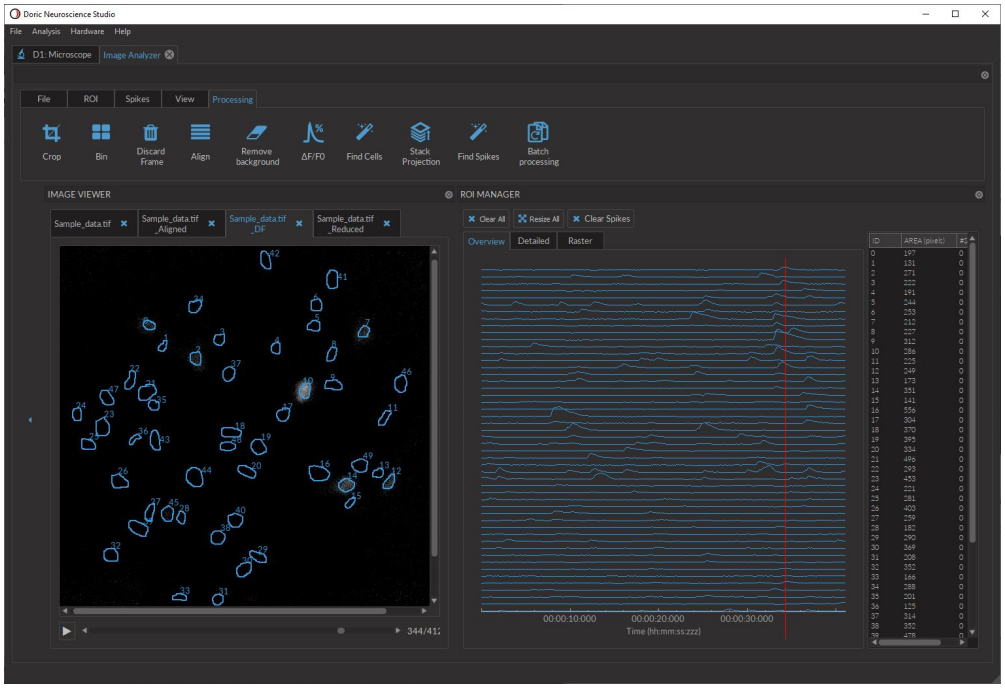


Figure 5.1: *Image Analysis Module Interface*

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.

5.1.1 Function Toolbar

1. The **File tab** (Fig. 5.2) is used to save/load data.

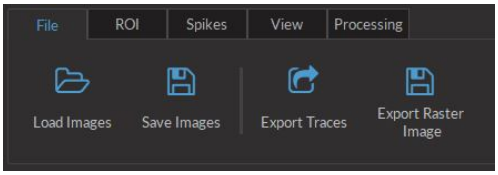


Figure 5.2: *File Tab*

   - 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. 5.3) is used to save/load data relating to regions of interest drawn on an image.

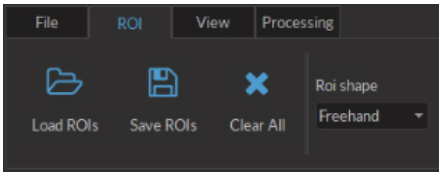


Figure 5.3: *ROI Tab*

   - 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. 5.4) is used to save/load data relating to regions of interest drawn on an image.

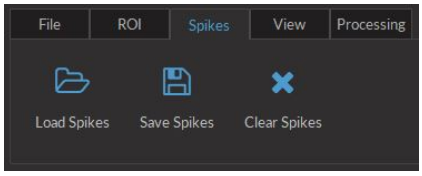


Figure 5.4: *Spikes Tab*

   - 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. 5.5) is used to manipulate the appearance of an image without changing base data.

Figure 5.5: *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 5.1.3 for details.
- The **Min** function applies a lower threshold with the cut-off value defined by the slider. See section 5.1.3 for details.
- The **Max** function applies an upper threshold with the cut-off value defined by the slider. See section 5.1.3 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. 5.6) is used to process the image data.

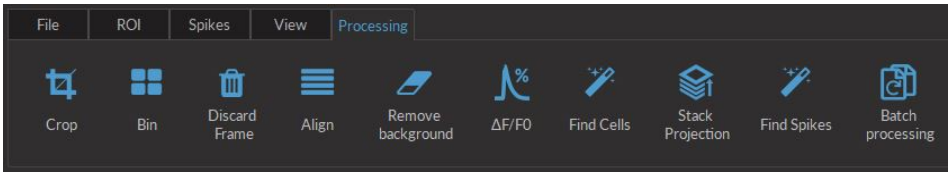


Figure 5.6: *Processing Tab*

- 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 5.1.3 for computational details. Selecting this button will open the **Align Images** window (Fig. 5.7). 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.

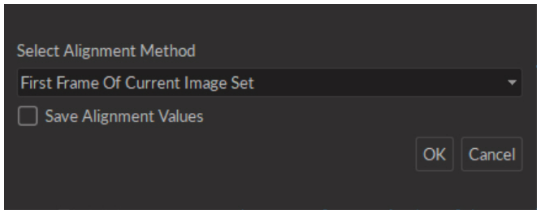
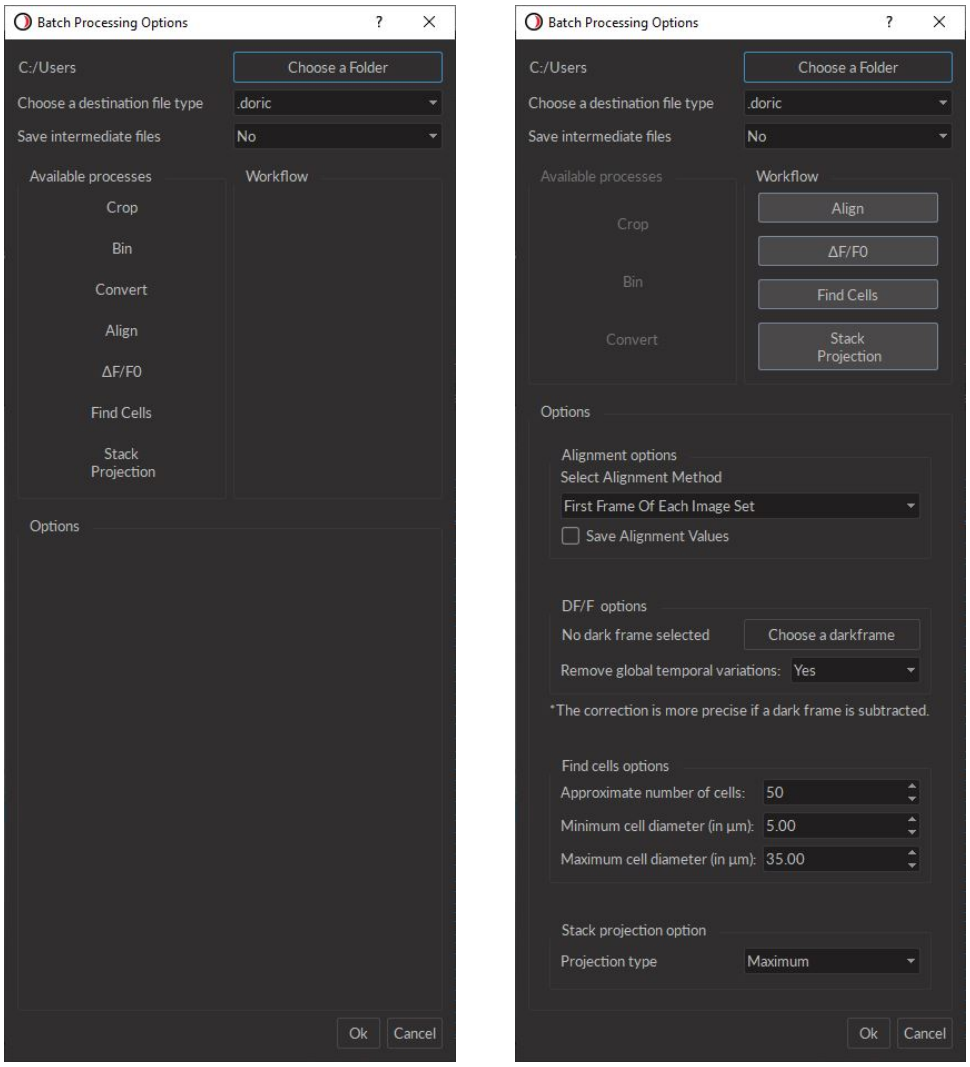


Figure 5.7: *Align Images Window*

  - 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 $\Delta F/F_0$ function.
- The $\Delta F/F_0$ function calculates the normalized fluorescence variation of the images and displays the results in a new tab. When selected, the See section 5.1.3 for details.
- The **Find Cells** function detects the cells and creates the ROI automatically. See section 5.1.3 for details.
- The **Stack Projection** function projects all movie frames to a single frame using the method selected in the Settings dialog. See section 5.1.3 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 5.1.3 for details.
- The **Batch Processing** function opens the **Batch Processing Window** (Fig. 5.8). 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 5.8: *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 5.1.3 for computational details.
- The $\Delta F/F_0$ process calculates the normalized fluorescence variation of the images and displays the results in a new tab. See section 5.1.3 for details.
- The **Find Cells** process detects the cells and creates the ROI automatically. See section 5.1.3 for details.
- The **Stack Projection** process projects all image frames to a single frame using the method selected in the Settings dialog. See section 5.1.3 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.

5.1.2 ROI Manager

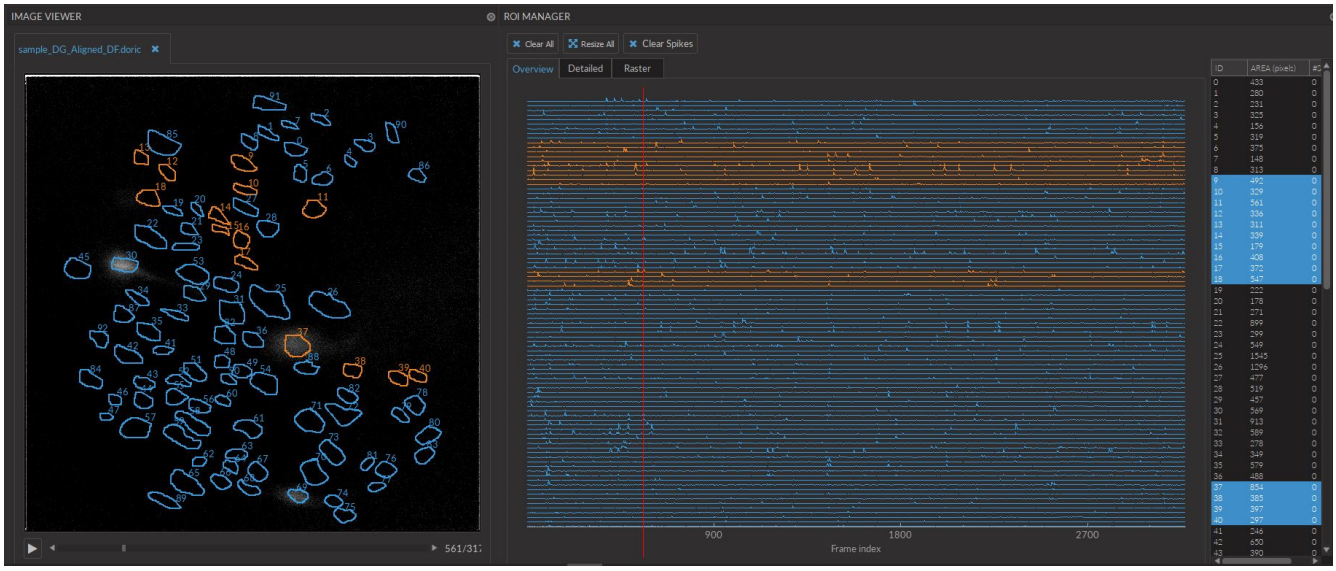


Figure 5.9: *ROI Manager*

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.

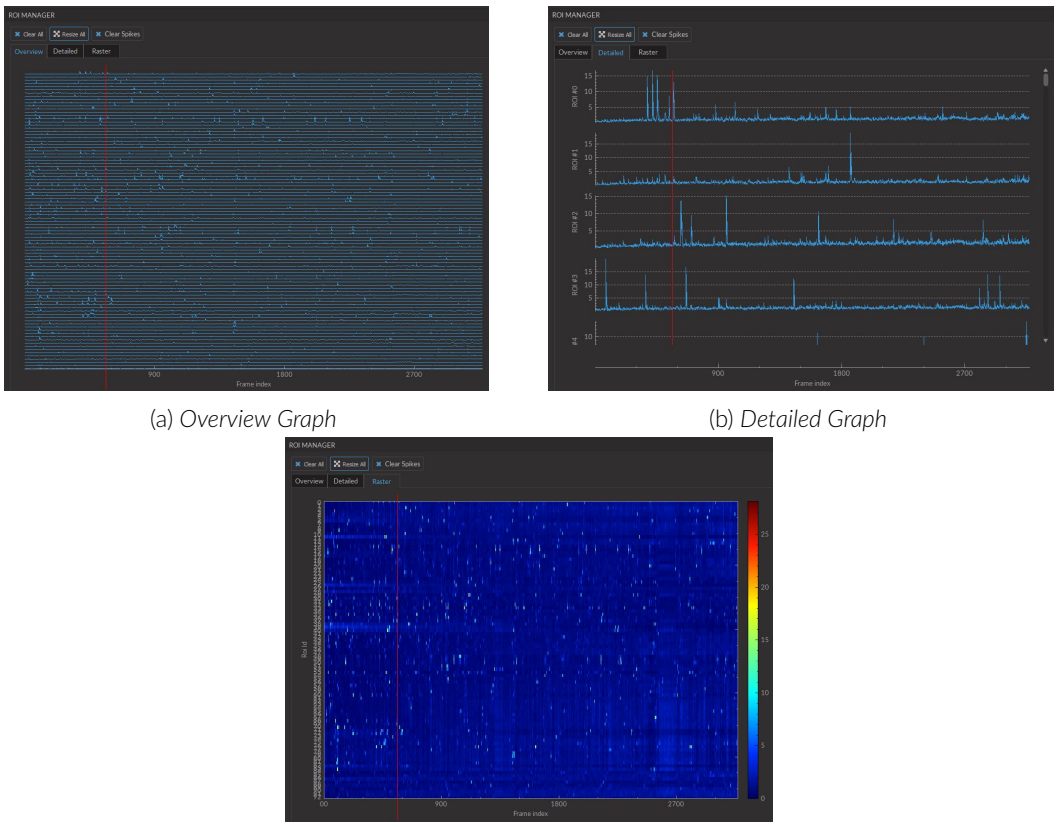
1. The **Image Viewer** contains the image stack and the ROI, numbered according to the order they where set. The ROI can be saved independently from the image stack on the ROI toolbar. The ROI are drawn directly on the *Image Viewer* in a *freehand* manner. All selected ROI can be moved together directly 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. 5.10a).
   - The **Detailed** tab displays each trace on a separate graph, allowing for precise intensity measurements (see Fig. 5.10b).

- 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. 5.10c).

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 5.10: *ROI Manager Graph Tabs*

5.1.3 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. 5.11). Keep in mind that removing the average temporal profile can also remove global activity patterns.

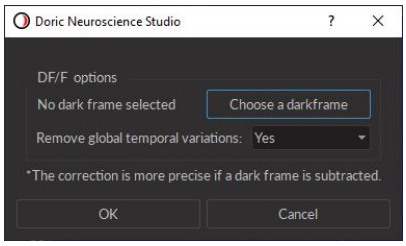


Figure 5.11: $\Delta F/F_0$ *Settings*

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

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. 5.12. 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 filtered the object found by the PCA/ICA.

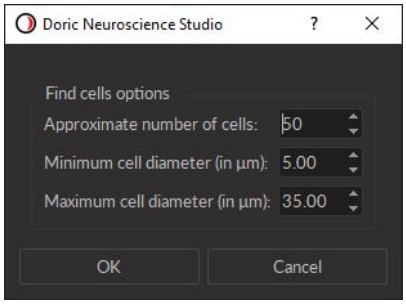


Figure 5.12: *Find Cells Settings*

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.

Stack Projection

This function can be used to help for ROI drawing. It calculates a temporal projection using the user-defined method (see Fig. 5.13).

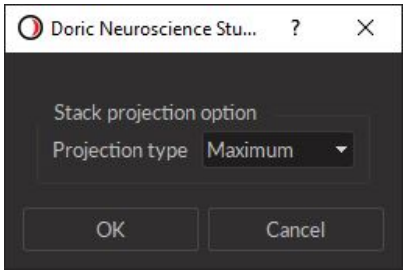


Figure 5.13: *Stack Projection Settings*

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.

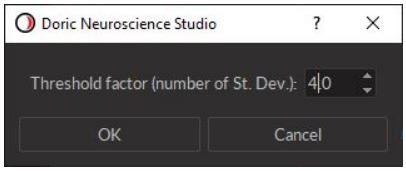


Figure 5.14: *Spike finder Settings*

6

Specifications

Table 6.1: *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	630 x 630 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 6.2: *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 6.3: *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 465 nm LED light source
[3] Including the thickness of the skull

Table 6.4: *Fluorescence Microscope Driver General Specifications*

SPECIFICATIONS	VALUE	NOTES
Power supply	110 - 240 VAC, 50 - 60 Hz	
DC Power supply	12 VDC	
Dimensions	186 x 90 x 77 mm^3	Including connectors
Data link	Gigabit ethernet	
TTL input voltage	0 to +5 V	
LED Analog input voltage	400 mA/V light source current	Standard 1 A LED Driver
	40 mA/V light source current	Low power mode enabled
LED BNC output voltage	2.5 V/A	
LED Maximum output current range	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
LED Connector	M8	See Figure 6.1

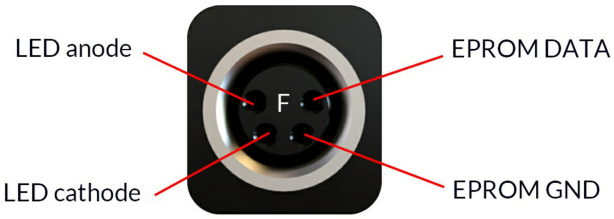


Figure 6.1: *M8 Female Pinout (Microscope Driver)*

Table 6.5: *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 7, 8 , 10	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

7

Annex 1: Cleaning and Handling

7.1 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 7.2) to avoid the formation of stains.

7.2 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.

8

Annex 2: Troubleshooting Guide

8.0.1 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.

8.0.2 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.

8.0.3 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.

9

Support

9.1 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.

9.2 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.

9.3 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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