doric

Bundle-imaging Fluorescence Mini Cubes

User Manual

Version 1.1

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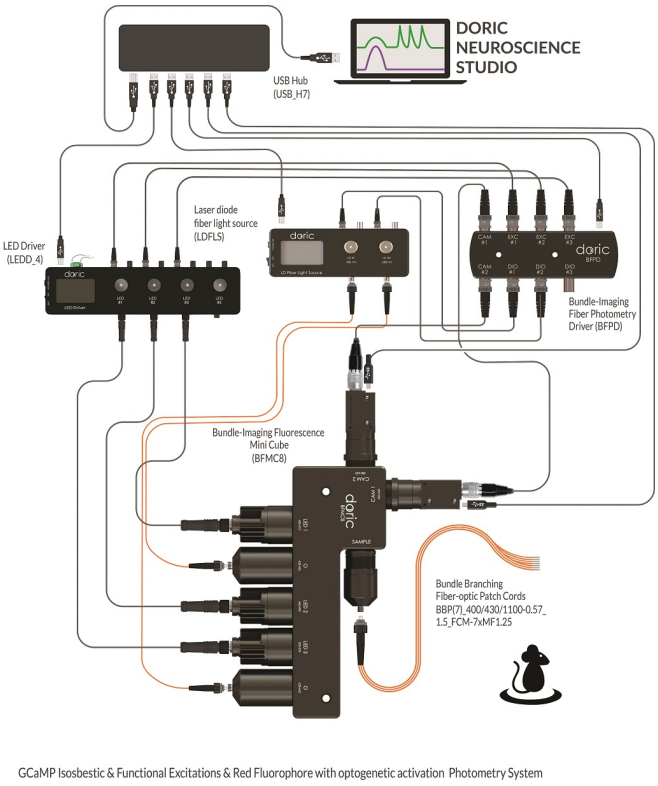
Introduction

The ***Bundle-imaging Fiber Photometry System (BFPS)*** is an elegant alternative for multiple site measurements. By bundling individual fiber together in a SMA connector, separate experiment sites are imaged onto a CMOS detector simultaneously which greatly simplified parallel fiber photometry measurements. The overall fluorescence signal from each site is recorded from pixel intensity variations within Doric Neuroscience Studio 1.1b.

The system is available for single and dual color measurements with isosbestic reference excitation as well as optogenetical synchronized experiments 1.1a.



(a) *GCaMP, RFP, Optogenetics 450 and 638 nm configuration*

(b) *Doric Neuroscience Studio BFPS interface*

Figure 1.1: *Bundle-Imaging Fiber Photometry System overview*

2

System Overview

2.1 Bundle-imaging Fluorescence Cube (BFMC) : Port type and description

The *Bundle-imaging Fluorescence Cube* have four types of optical port : Sample, Excitation, Camera and Optogenetic. According to the experiment, the number of ports and their design are modified to achieve the desired purpose (Fig 2.1).

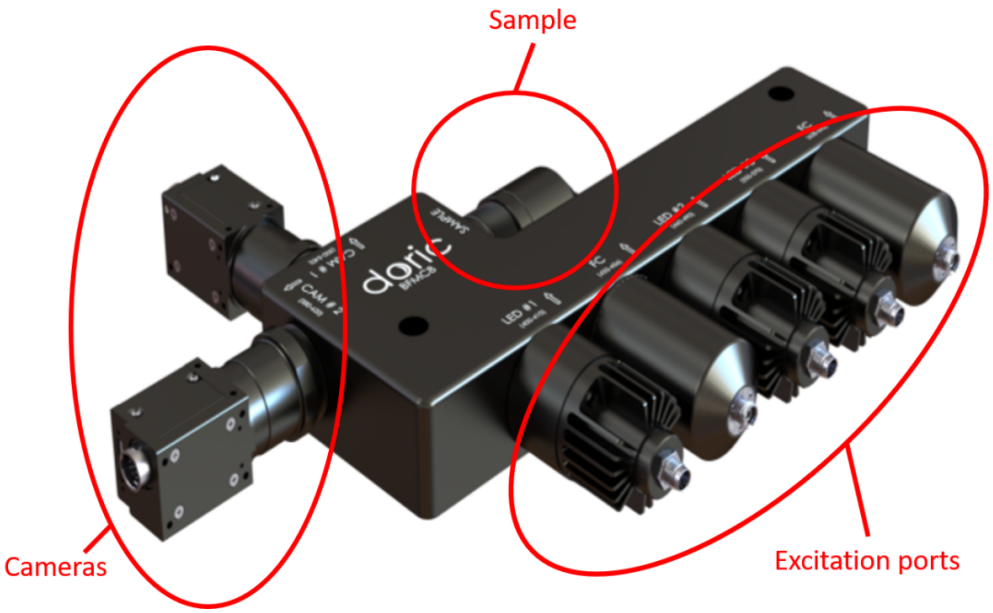


Figure 2.1: *BFMC 8 overview. Note that the cube are classified by their number of ports. In this case the cube has 8 ports : 5 excitation ports, 2 cameras and 1 port sample.*

2.1.1 Sample port

Each mini fluorescence cube has a single sample port. This is the only port without any spectral filtering, all wavelengths can pass freely through it. The sample port consists of a microscope lens and a fiber adapter to image and focus the fiber bundle onto the cameras. To accommodate larger fiber bundles (up to 2.5 mm), an SMA receptacle is used on the sample port.

A fiber bundle has two or more optical fibers bundled together in an SMA optical connector at one end. The other end consists of loose optical fibers with individual connectors. Low autofluorescence materials and black epoxy are used to reduce background fluorescence and prevent cross-talk between each fiber.

2.1.2 Excitation ports

To obtain a stable and uniform illumination, LEDs are favored. Excitation ports are designated as LED on the top engraving. If there is more than one excitation port, they are labelled as LED1, LED2 and potentially LED3. Each excitation port contains a filter chosen to correspond to the excitation peaks of the fluorescent protein the BFMC is designed to measure.

2.1.3 Camera ports

Camera ports are designated as CAM on the top engraving. If there is more than one camera port, they are labelled as CAM1 and CAM2. Each detector port contains a very wide filter to maximize the detection of the fluorescence.

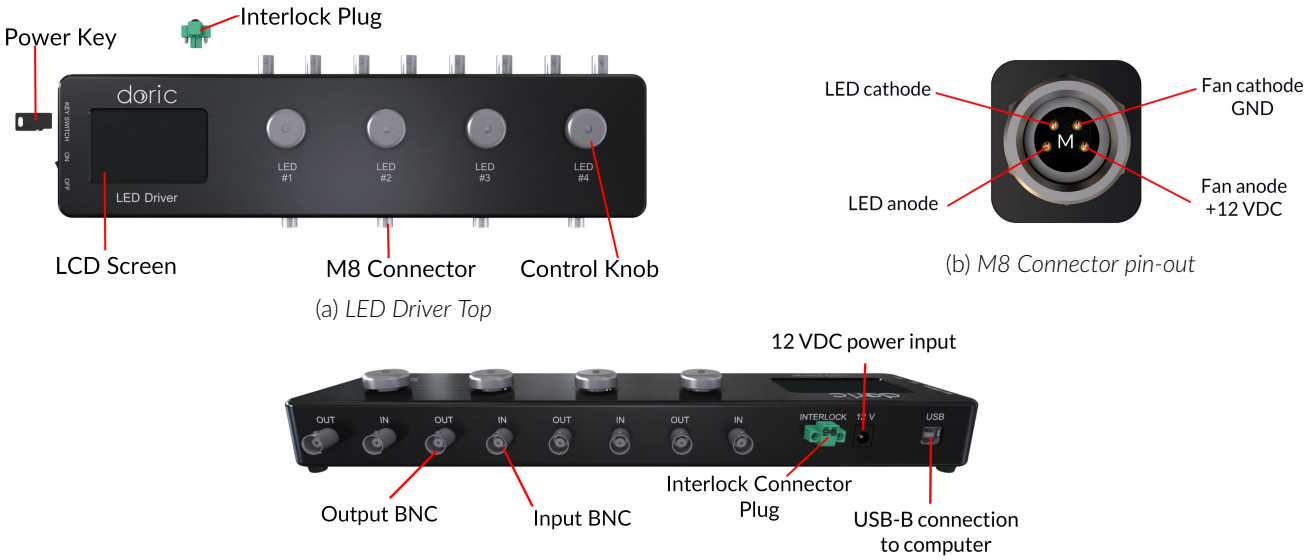
2.1.4 Opsin ports

If required, BFMC with Optogenetics excitation ports are available. Opsin ports are designated as O on the top engraving and contains a filter chosen to match the excitation spectrum of an opsin.

2.2 Bundle-imaging Fiber Photometry Subsystems

2.2.1 LED driver

LEDs are connected to the LED driver (Fig.2.2), which deliver the excitation current, with a M8 cable(Fig.2.2b). The LED number designated on the BFCM top engraving should be the same as the channel number of the LED driver to which it is connected. For more information on the LED driver, see the related User Manual.



(a) *LED Driver Top*

(b) *M8 Connector pin-out*

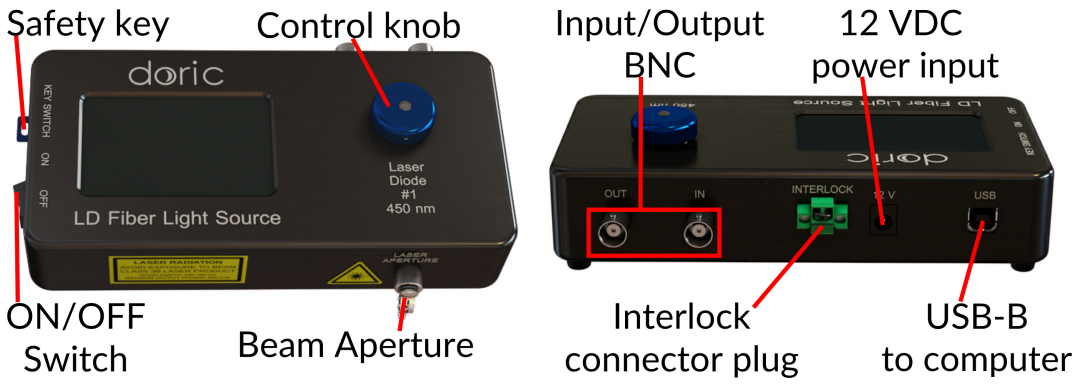
(c) *LED Driver Rear*

Figure 2.2: *LED Driver*

2.2.2 Laser Diode Fiber Light source

The Doric Laser Diode Fiber Light Source is a compact multiple-source laser system, available with 1, 2 or 4 channels (Fig.2.3). Laser diode outputs are FC/APC optical fiber receptacle. Optogenetic excitation is injected into the cube via a mono fiber-optic patch cord.

For more information on the Laser Diode Fiber Light source, see the related driver User Manual.



(a) *Fiber Light Source Top* (b) *Fiber Light Source Rear*

Figure 2.3: *Laser Diode Fiber Light Source Views*

2.2.3 Bundle imaging Fiber Photometry Driver (BFPD)

The Bundle-imaging Fiber Photometry Driver coordinates the BFMC system with Doric Neuroscience Studio (Fig.2.4). The BFPD synchronizes the LED and Laser drivers as well as the CMOS cameras to allow interleaved acquisitions.

LEDs must be connected to the EXC entries, Cameras must be connected to the CAM entries and the Optogenetic ports to the DIO entries of the BFPD. Available digital inputs or outputs (DIO) can also be used to synchronize other equipments.

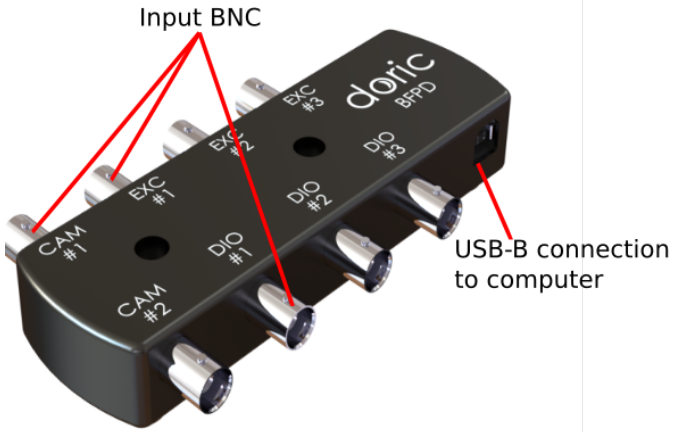


Figure 2.4: *BFP Driver*

3

Getting Started : General Setup Guidelines

3.1 Connecting the Bundle-Imaging Fiber Photometry System

All cables, power supply splitters as well as the USB hub are included with the Bundle-imaging Fiber Photometry System. Figure 3.1 illustrates connections between all subsystems.

If the Bundle-Imaging Fiber Photometry system has been ordered with a rack, must connections are already done. Skip to step 9.

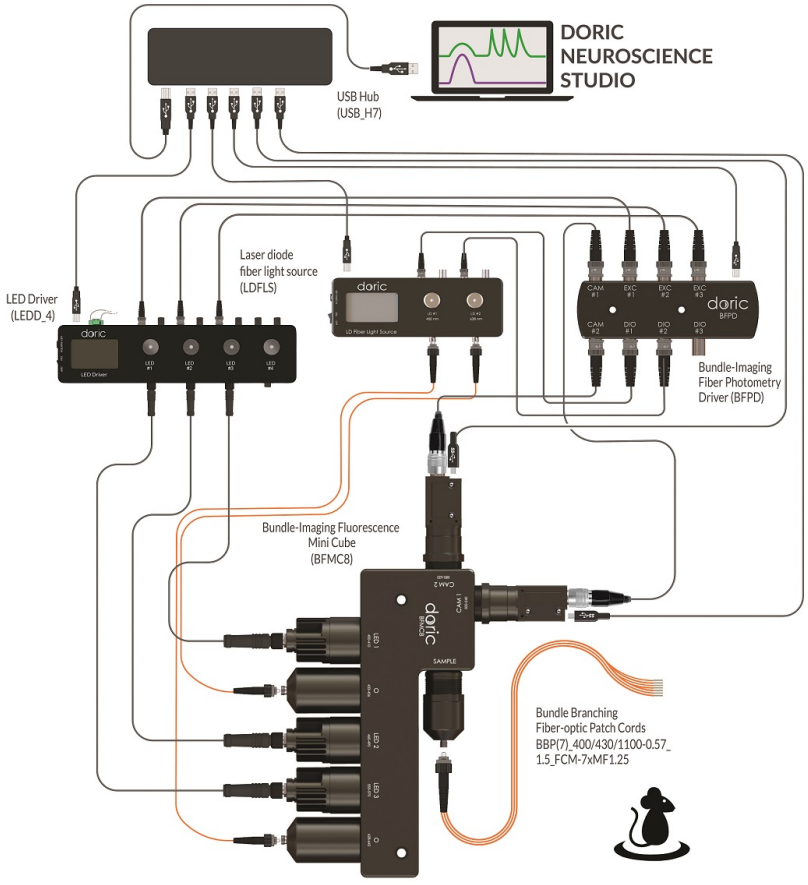


Figure 3.1: *Bundle-imaging Fiber Photometry System : Connections between subsystems.*

1. **Connect** a USB 3.0 cable between the cameras and the USB hub ports 1 and potentially 2, according to the number of detector ports.
2. **Connect** a USB 2.0 cable between the LED driver and the USB hub port 3
3. **Connect** a USB 2.0 cable between the BFPD and the USB hub port 4.
4. If the BFMC cube has Opsin ports, **connect** a USB 2.0 cable between the laser driver and the USB hub port 5.
5. **Connect** the integrated LEDs to the corresponding channel number of the LED driver with M8 cables.
6. If the BFMC cube has Opsin ports, **connect** the appropriate optical fiber between the laser source output and the optogenetic port. **FC/APC connector is identified by a green strain relief and should be connected to the laser diode light source.**
7. **Connect** the LED driver and Laser driver digital inputs as well as the camera to the BFPD with BNC cable. To ease experiment configuration in Doric Neuroscience Studio, we recommend connecting CAM1 with CAM1, LED1 with EXC1, Laser1 with DIO1 and so on.
8. **Connect** a USB 3.0 cable from the USB Hub to the PC.
9. **Connect** the LED driver, the Laser driver and the USB Hub to the 12 V AC/DC and 60W power supply with the power supply splitters.
10. **Open** Doric Neuroscience Studio. To set up an experiment refer to chapter 4.

3.2 Optical fiber patch cord

- **Clean** the optical fiber connector before insertion. Use isopropanol and a lint-free wipe.
- With an FC connector (Opsin ports), the **connector key must be oriented to enter within the receptacle slot** to ensure proper connection (Fig. 3.2).

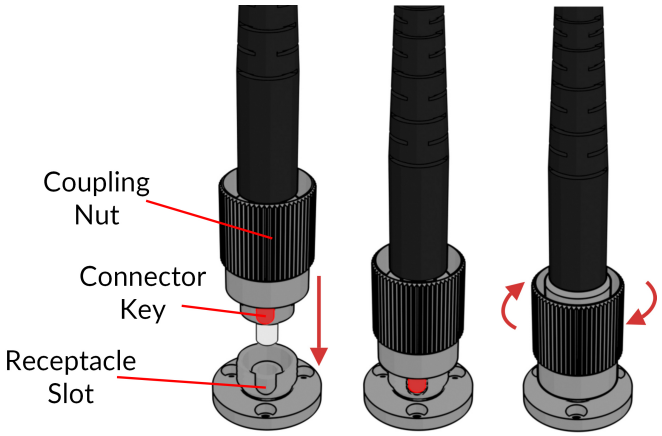


Figure 3.2: *FC connector, Fiber Installation*

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

3.2.1 Focus Adjustement

The BFMC fiber adapter allow to adjust the focus of the fiber bundle image on the camera (Fig.3.3). Cube are adjusted at factory but manual adjustment may be required over time.

1. **Connect** the optical fiber bundle to the fiber adapter and start an acquisition.
2. **Loosen** the counter-nut and **rotate** the fiber adapter until you get a clear image of the fiber bundle. It may be necessary to rotate the fiber adapter several times to adjust focus. **To avoid twisting the cable, disconnect and reconnect optical fiber cable during this alignment process.**

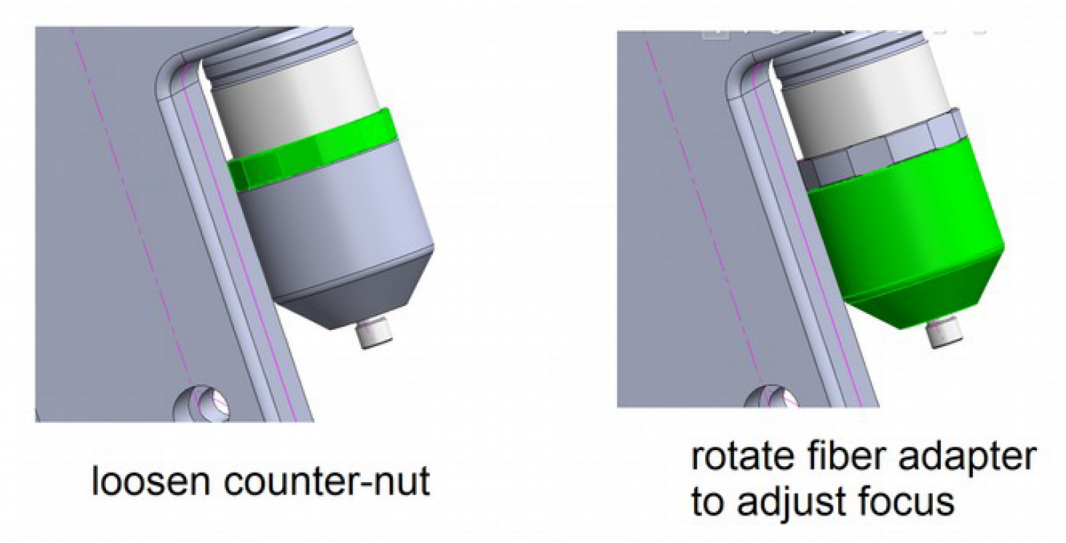


Figure 3.3: *Fiber adapter components to adjust focus*

4

Using Doric Neuroscience Studio

Doric Neuroscience Studio (DNS) provides an interface to control the Bundle-Imaging Fiber Photometry System. Here is an overview of DNS user interface. The following sections will introduce a brief description of the drivers functionalities as well as the experiment view window.

For more information about the Doric Neuroscience Studio, consults the software User Manual.

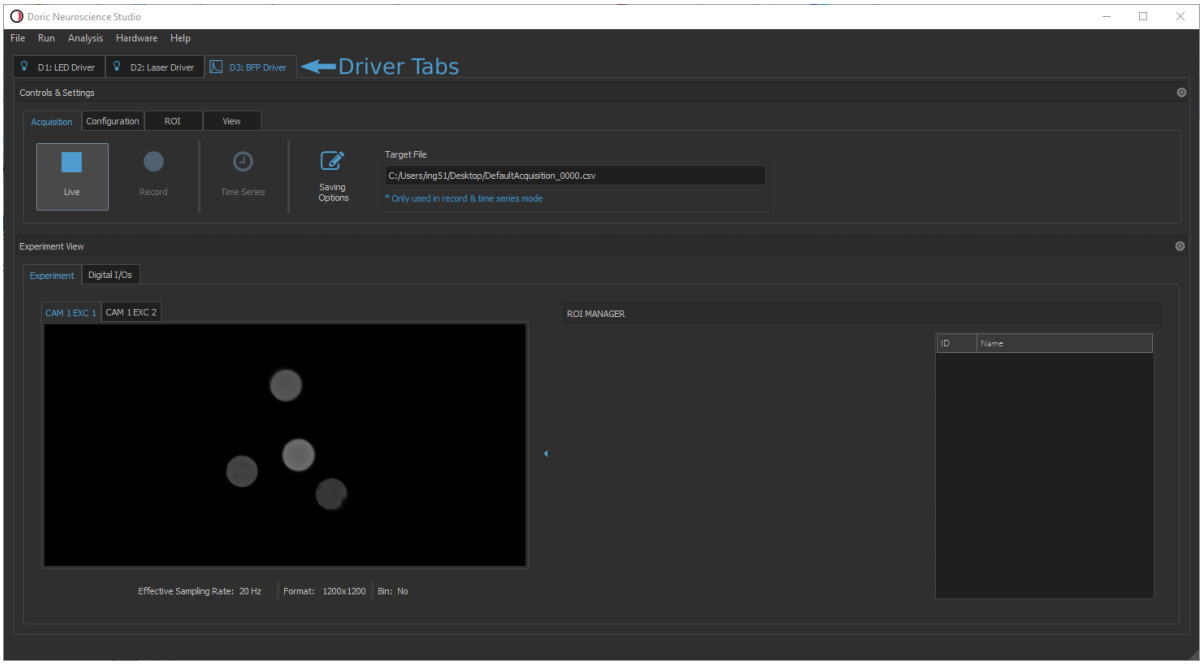


Figure 4.1: *Doric Neuroscience Studio Overview*

4.1 LED driver configuration

4.1.1 To manually control the LED driver,

1. Turn on the LED driver.
2. Push the knob of the LED to be configured. From the off mode :

1 push	Continuous Mode (CW)	Light will be emitted continuously
2 pushes	External TLL (TLL)	Uses for interleaved acquisition. Light will be emitted only if an external trigger is programmed.
3 pushes	External Analog (MOD)	Do not use. The camera acquisition rate is too low to use this mode.

Table 4.1: *LED driver mode from the off mode*

In the case of the Bundle-Imaging Fiber Photometry System always configure the LEDs in TTL mode.

3. Rotate the knob to adjust the current to the desired value

4.1.2 To control the LED driver with the Doric Neuroscience Studio

Select **Add Channel** in the Configuration tab under the LED driver module.
A new window will open where the mode and current of the selected channel can be configured (Figure 4.2).

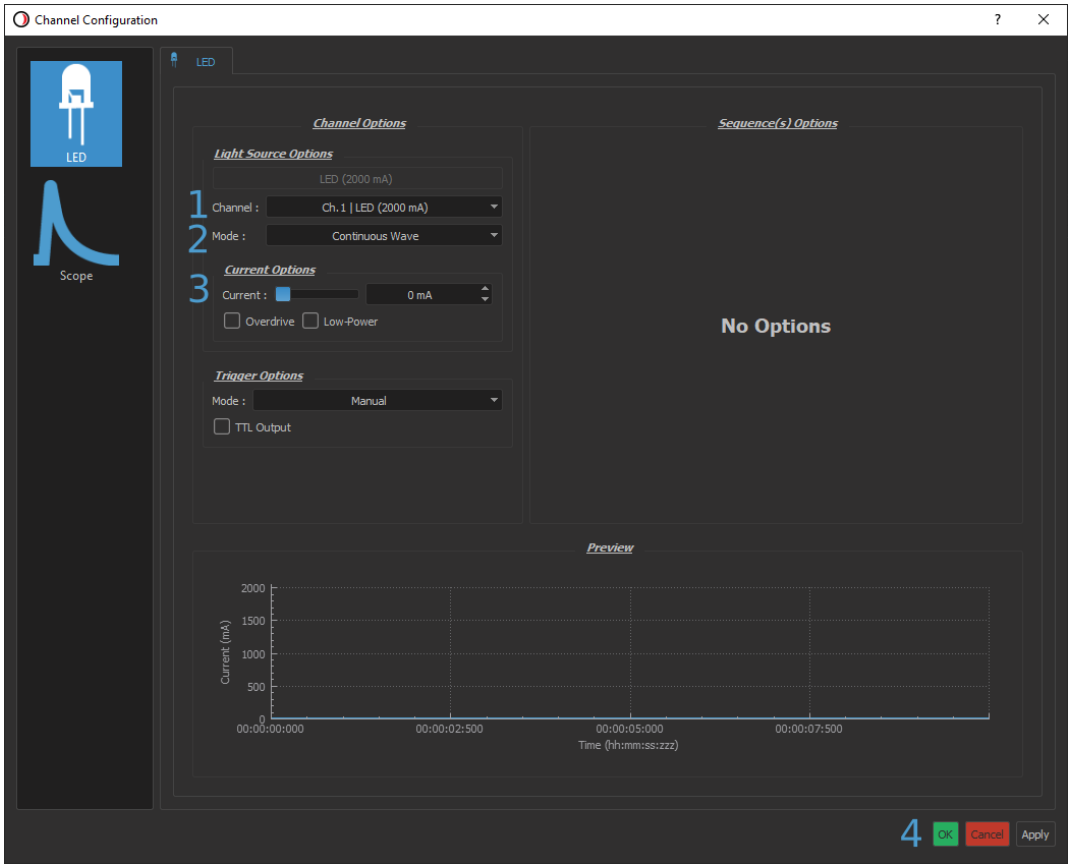


Figure 4.2: *LED driver, Channel Configuration*

1. **Channel** : Select the LED to be configured.
2. **Mode** : Select the External TTL mode.
3. **Current** : Set the current sends to the light source with the **current slider**.
    - The **Overdrive** checkbox, when selected, allows the system to exceed the normal safe current limit of the light source. **This should only be used with pulsed signals, as it can otherwise damage the light source.**
    - The **Low-Power** checkbox allows reduced power signalling for the same voltage. This allows low-power signals to be more stable in time. The maximal current is reduced to one tenth of light source normal maximal current. For example, a driver with a normal maximum current of 2000 mA for a 5 V signal (400 mA/V) will have a maximum current of 200 mA for a 5 V signal (40 mA/V).
4. Click **OK** at the bottom right corner.

The configured LED channel will appear in the Acquisition View (Fig 4.3).
At this point, no light is transmitted through the sample port.

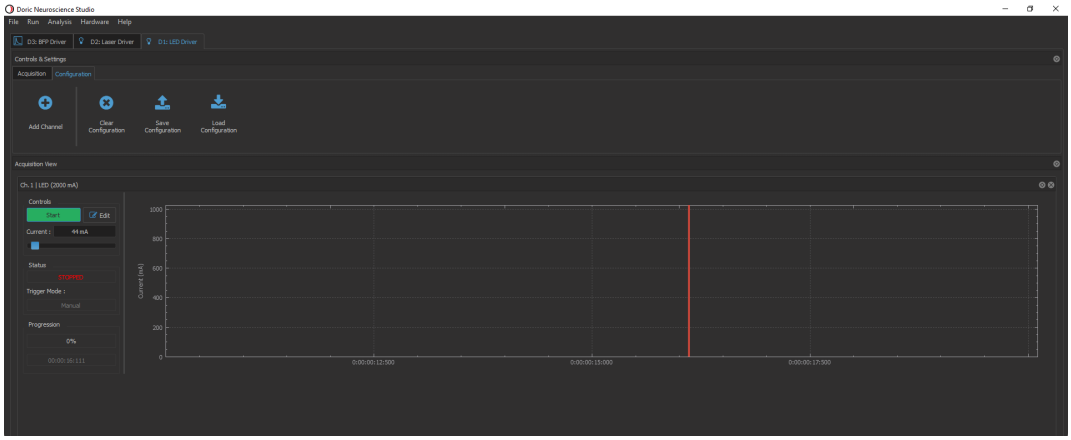


Figure 4.3: *LED driver, Acquisition View*

4.1.3 To correlate the LED current with the excitation power,

1. Select the LED to set in continuous mode "CW".
2. Measure the optical power output from the patch cord connected to the port sample with a power meter.

4.1.4 Low current Mode

If low current (< 100 mA) is needed, set the LED driver in low power mode, this allows low power signals to be more stable. To do that, see section 4.1.2, 3. **Current**.

4.2 Laser Diode Fiber Light configuration

To manually control the Laser driver, refer to section 4.1.1

4.2.1 To control the Laser driver with the Doric Neuroscience Studio

Select **Add Channel** in the Configuration tab under the Laser driver module. A new window will open where the mode and current of the selected channel can be configured (Figure 4.4).

1. **Channel** : Select the laser module to be configured.
2. **Mode** : Select the external TTL mode.
3. **Current** : Set the current sends to the laser module with the **current slider**.
4. Click **OK** at the bottom right corner.

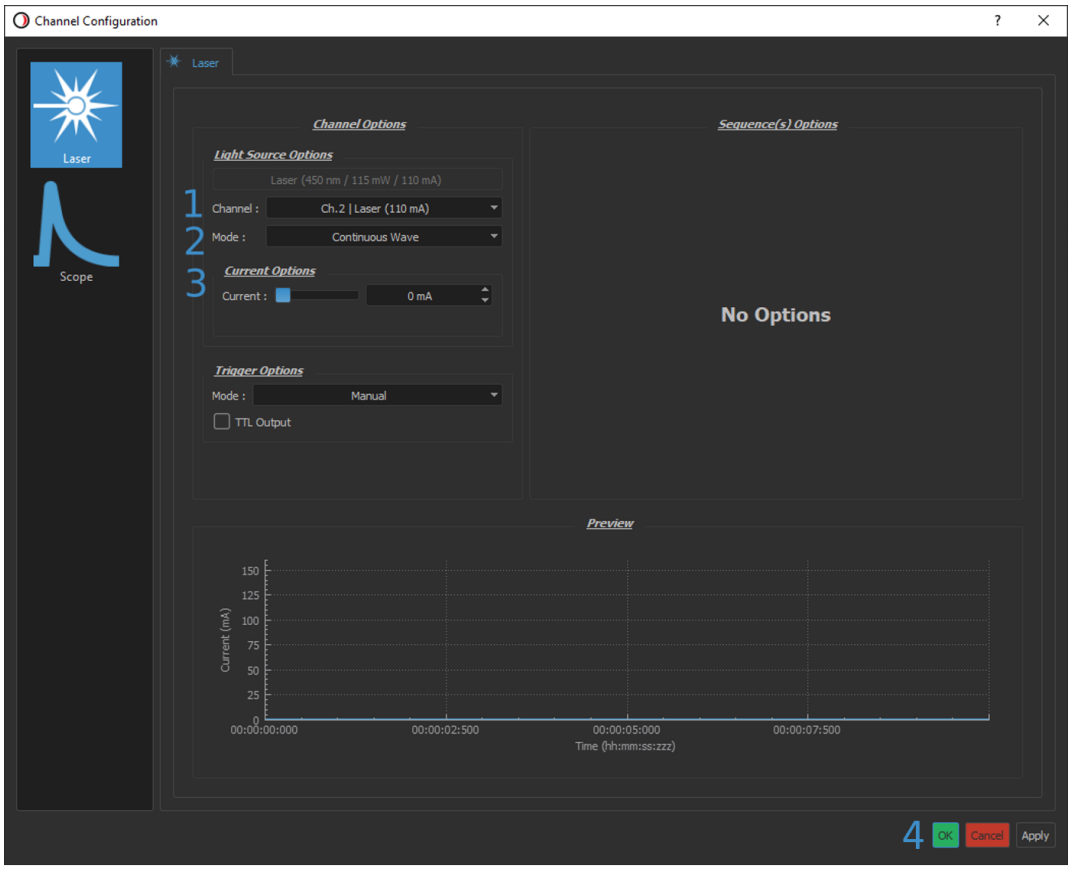


Figure 4.4: *Laser driver, Channel configuration*

The configured Laser channel will appear in the Acquisition View.
At this point, no light is transmitted through the port sample.

4.2.2 To correlate the Laser module current with the excitation power,

1. Set the Laser module in continuous mode ”CW”.
2. Measure the optical power output from the patch cord connected to the port sample with a power meter.

4.3 Bundle-Imaging Fiber Photometry (BFPD) configuration

4.3.1 Set Photometry Measurements

To start a new experiment with the Doric Neuroscience Studio, select Add Channel in the Configuration Tab under the BFPD module. A new window will open where the following parameters can be modified (Fig.4.5).

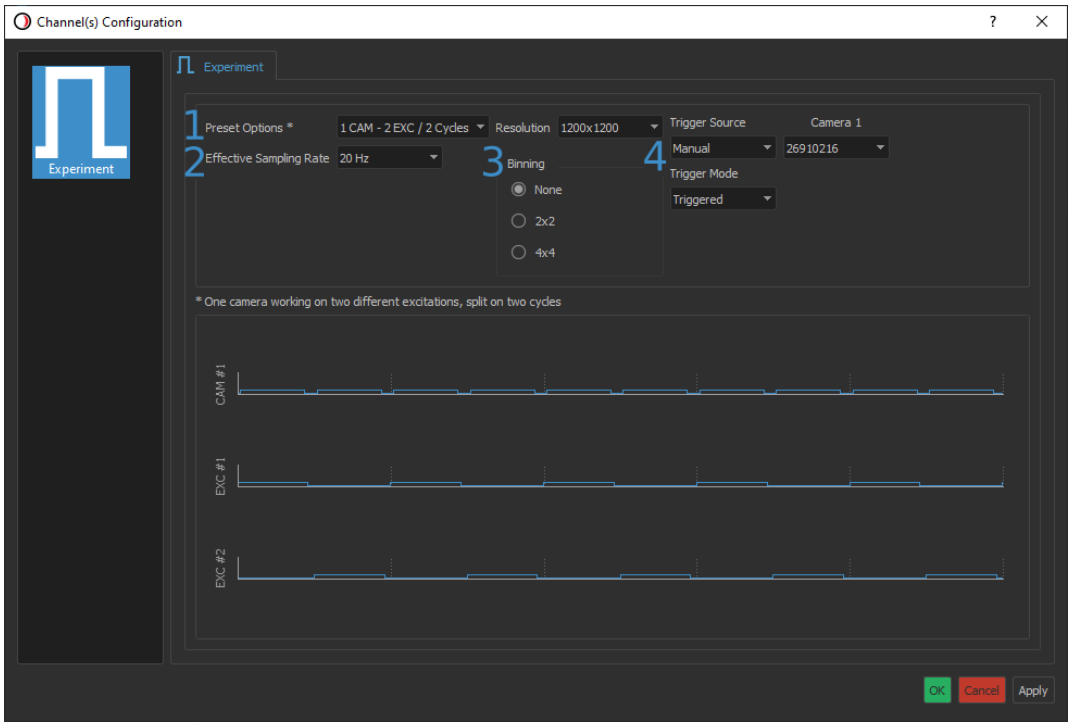


Figure 4.5: *BFP driver : Set photometry measurements*

1. **Preset Options :** Four interleaved configurations are preset (see table : 4.2).

Table 4.2: *Preset option explanations*

Name	Number of Camera	Number of LED	Cycles[1]	Note[2]
1 Cam - 2 Exc/2 Cycles	1	2	2	One camera working on two different excitations, split on two cycles.
2 Cam - 3 Exc/2 Cycles	2	3	2	Two cameras with three excitations, split on two cycles. Where excitaion two and three are on the same cycle.
2 Cam - 3 Exc/3 Cycles	2	3	3	Two cameras with three excitations, split on three cycles.
1 Cam - 1 Exc	1	1	1	One camera, one excitation.

[1] Serie of events that occur during one measurement
[2] For a preset configuration, an overview of each camera recording and LED excitation cycle is available in DNS.

2. **Effective Sampling Rate :** Frequency of each LED excitation pulse per second.
3. **Resolution and Binning : Resolution** is the width and height of the image and the total number of pixels in the image. **Binning** allows to reduce the number of pixels by combining a cluster of pixels into a single pixel.
4. **Trigger Source :** If the trigger source is set to **manual**, the experiment recording will start when the live or record button in the acquisition tab is clicked and stop when the stop button is pressed.

   To trigger the recording with an **external signal**, set the trigger source to the same DI/O number as the port that will receive the TTL signal on the BFPD. Two Trigger modes are available.

   4.1. **Trigger Mode : Triggered**
   The device is ready to record once the live or record button in the acquisition tab is clicked. The recording start as soon as the selected DIO receive a high TTL signal (5V). The recording has to be stopped manually with the stop button in the acquisition tab.

   4.2. **Trigger Mode : Gated**
   The device is ready to record once the live or record button in the acquisition tab is clicked. The recording start when the selected DI/0 receives a high TTL signal (5V). The recording continue as long as the DI/O is set to High, then the data stream will be interrupted when the DI/O signal is set to low (0V). Time will continue to be recorded. Once the DI/O is high again, the recording will restart. The recording will go on and off until the stop button in the acquisition tab is clicked manually.

Once a photometry experiment has been set up, run a live acquisition (section 4.5) to validate that the interleaved LEDs excitation is emitted at the port sample output. If no light is going through the port sample, ensure that the LED driver is well configured (section 4.1).

4.4 Set Optogenetic Measurements

To configure optogenetic excitation, select Digital I/O from the Configuration Tab under the BFPD module. A new window will open where the following parameters have to be set (Fig.4.6).

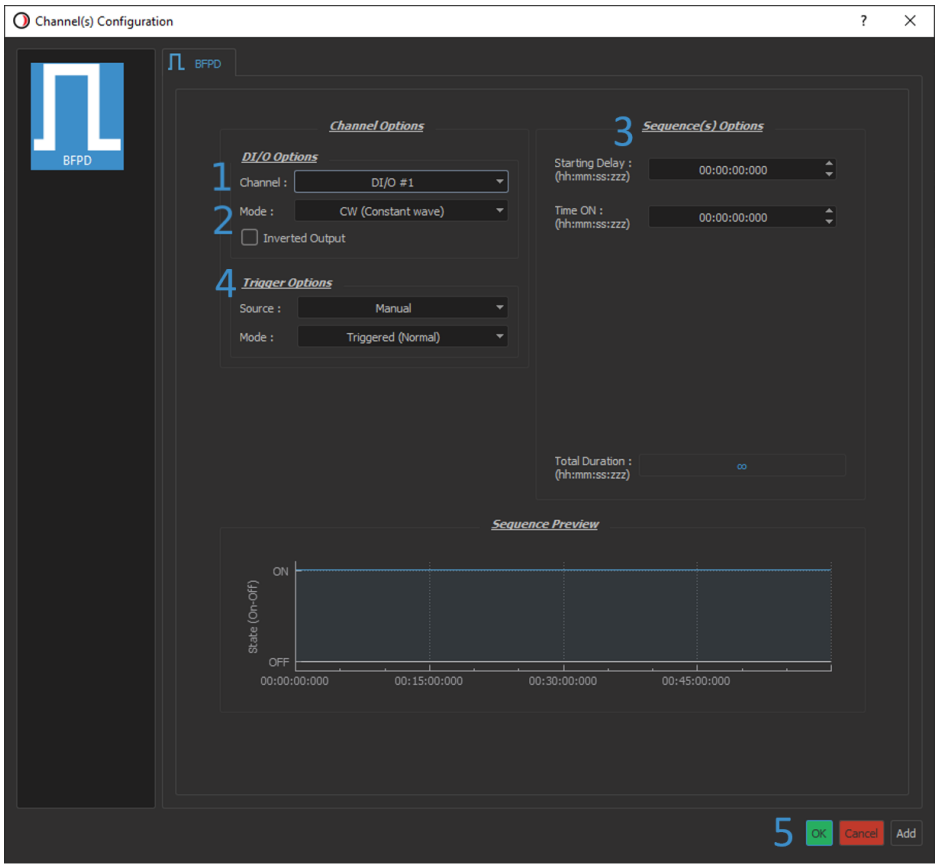


Figure 4.6: *BFP driver : Set optogenetic measurements*

1. **Channel** : Select the **BFPD DIO channel** connected to the laser driver input to be configured.
2. **Mode** : **CW and Square** modes are available in order to have continued or sequential optogenetic excitations.
3. The **Sequence(s) Options** section allows the excitation sequence customization.
4. The **Trigger Options** section allows the trigger customization manual by default.
5. Click **OK** at the bottom right corner.

Start a live acquisition (section 4.5) to validate that the opsin excitation is emitted at the port sample output. If no light is transmitted through the port sample, check the Laser driver configuration (section 4.2).

4.5 Recording and Data Saving

To start an acquisition, go to the Acquisition Tab under the BFPD module.

Four options are available (Fig.4.7).

1. **Live :** Live images are acquired by the software, but these are not saved on the computer. This mode is mainly used to set up an experiment.
2. **Record :** This mode allows single experiment recording.
3. **Time Series :** Time Series mode enables to perform long term recording with long delay. For example, 1 minute of recording every hour for 12 hours Note that the data will be saved in a single file.
4. **Saving Options** : The Saving Options button opens the **Saving Menu** window (fig:4.8).
   4.1. The **Save file Settings** box is used to define how and where the file is saved. The name is taken from the **Base Name** box, while the saving location can be chosen by clicking the **...** button. The **File Index** box is used to define the current indexation number used for multiple files saving during the same measurement session. All this information is summarized in the Target File box in the main tab. There are two option for the format, the **.doric** and the **.csv** .
   4.2. By default, **video files** are not saved. To save these data, check the **Save Video** box in **Save Options** (video are save in TIFF format).

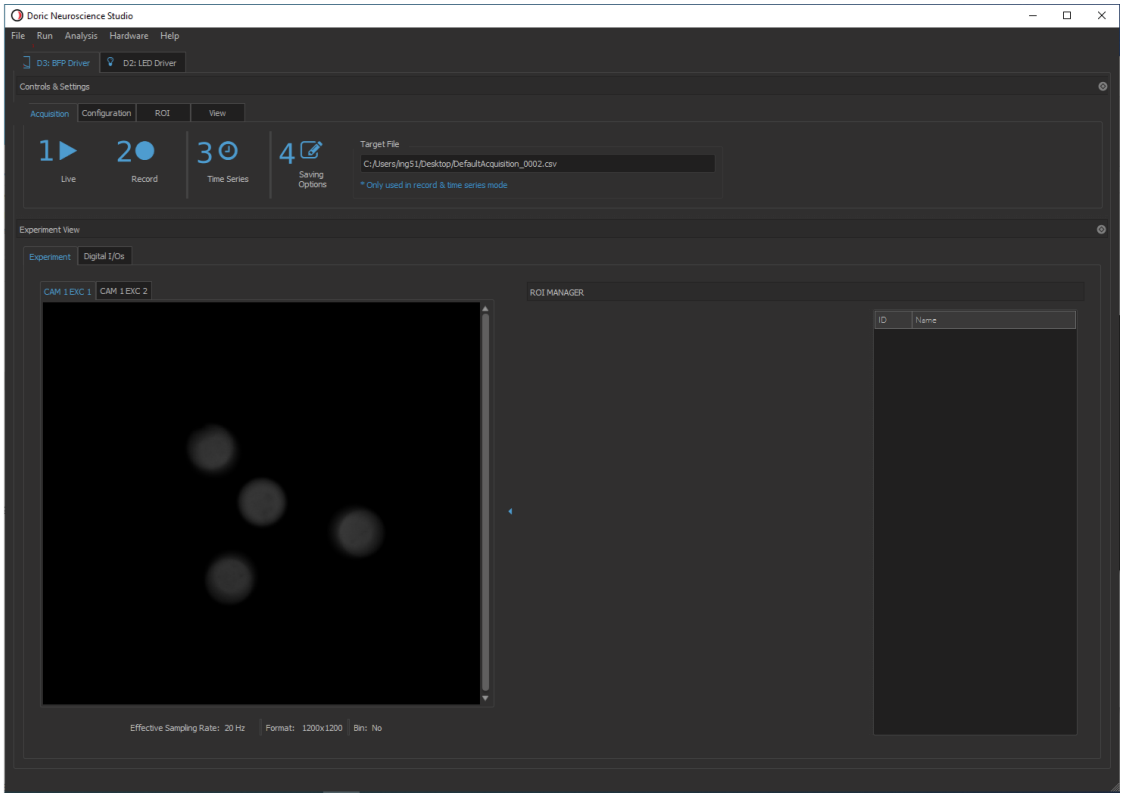


Figure 4.7: *Acquisition Setting Option*

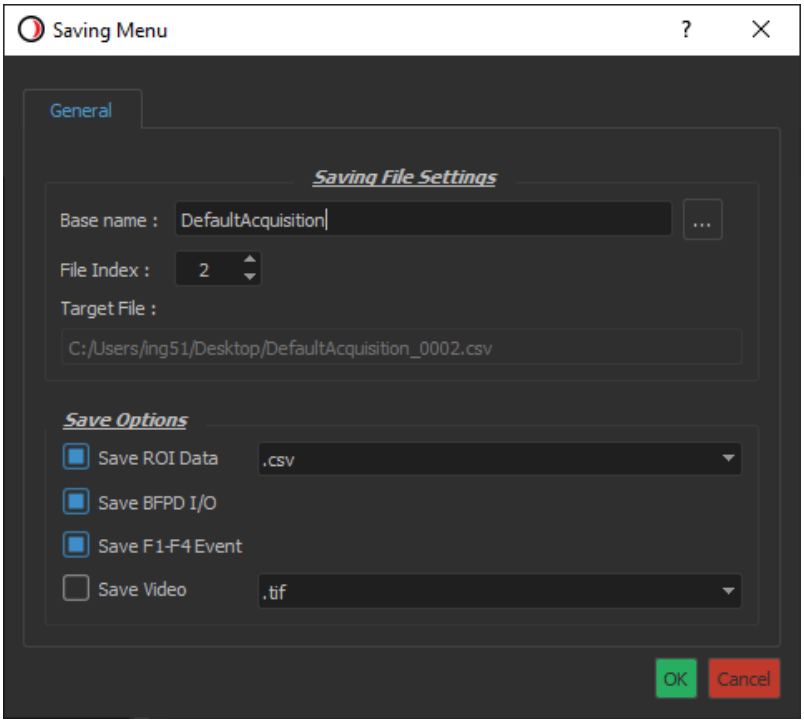


Figure 4.8: *Saving Menu tab*

4.6 Camera Settings

Under the BFPD configuration tab the options to set the cameras are listed (Fig. 4.9).

1. **Exposure** : The **Exposure** (in ms) slider adjusts the exposure time of the pixels.
2. **Gain** : The **Gain** (in dB) slider adjusts the gain of the pixels.
3. **Auto Center** : The **Auto Center** toggle center the image seen by the camera in the experiment window.
4. **X and Y offset** : If the **Auto Center** is unchecked, the **X and Y offset** sliders, allows the user to change the position of the image seen by the camera in the experiment view. The sliders are particularly useful when **two cameras** are used to superpose both images and ROIs.

Prior to experiment start, camera **exposure** time should be maximized and analog **gain** set to 0 db. Afterwards, if the signal is too strong or the camera is saturated, it will be preferable to reduce the excitation power before the exposure to minimize fluorophore bleaching. If the detected signal is too weak, the gain should be increased. However, increase the gain will also amplify electronic noise and reduce signal noise ratio.

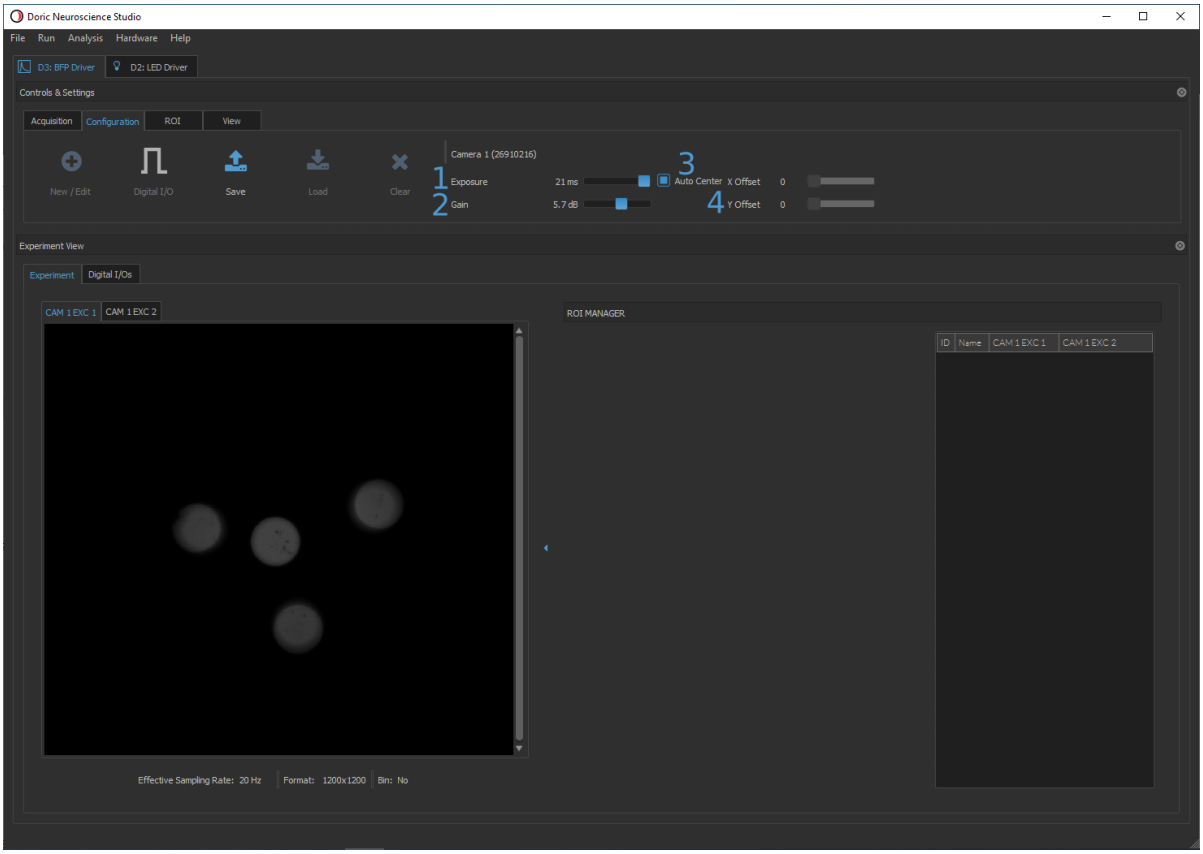


Figure 4.9: *Camera Settings*

4.7 Region of Interest (ROI)

Once a clear image of the connecting patch cord is obtained, it is possible to draw as many ROI region as needed. Pixels inside a ROI are averaged to get the region intensity which corresponds to the detected fluorescence signal from each site.

To set ROI (Fig.4.10),

1. Start a live acquisition (Section 5.4) and adjust the focus if needed (Section 4.2.1).
2. Left click on the camera view to draw a ROI. Next to the camera view, a graph of the ROI average count vs time will appear with a table of the average ROI count.
   We suggest drawing one ROI for each optical fibre, plus one outside to monitor the background.
3. Rename ROI by double clicking on its name in the table.
4. Go to the BFPD ROI Tab to save the configured ROI positions.

With a configuration using 2 cameras, when a ROI is added, it is added on both camera window at the same position. It is possible to keep them synchronized, by checking Sync ROI between Camera (3). However, it is preferred to uncheck Sync ROI between Camera in order to move and to resize ROI on each camera window independently to properly select the optical fibers.

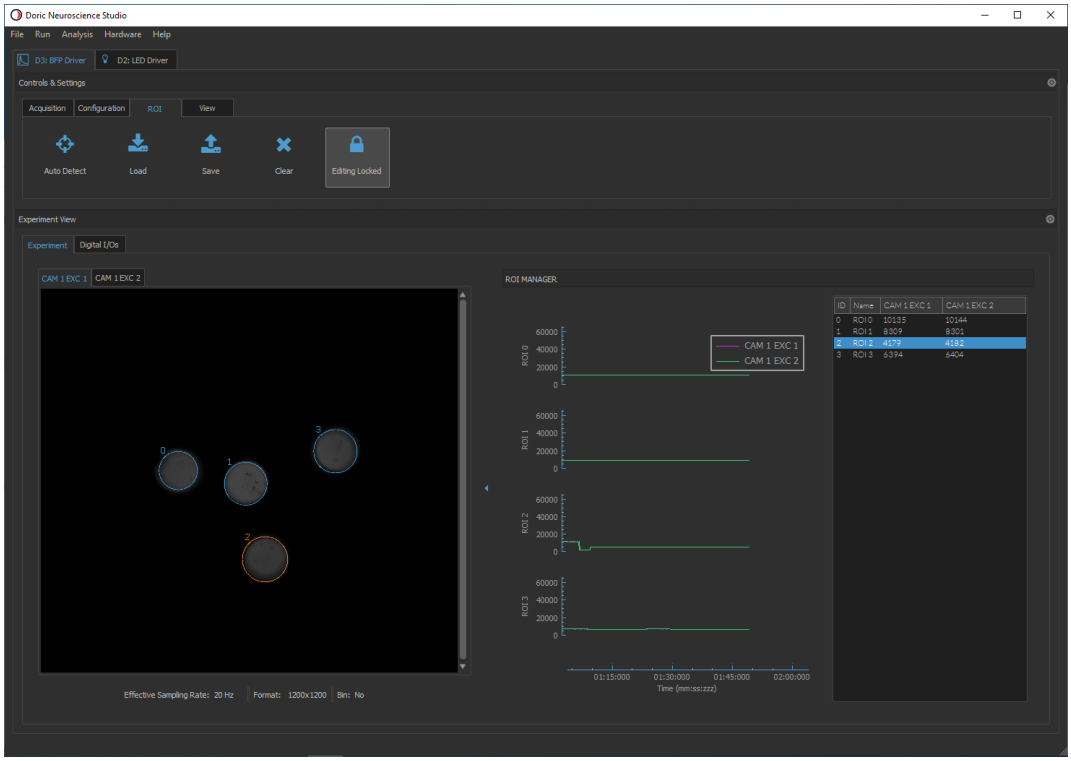


Figure 4.10: *ROI Settings*

4.8 View Options (View Tab)

To change the visualization settings of the camera view, select the tabs BFPD View tab.

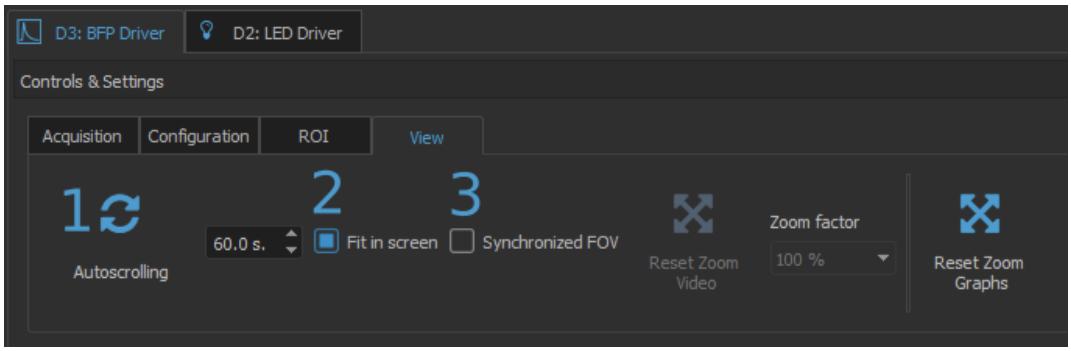


Figure 4.11: *View Options window*

1. **Autoscrolling** : The autoscrolling button makes the graph in the ROI manager scroll as new data appear. The duration (in secondes) kept on display is defined in the box beside the button.
2. **Fit in Screen** : When Fit in Screen is selected, the camera field of view is shown in the experiment view. When Fit in Screen is unchecked, it is possible to zoom on some part of the field of view with the **Zoom Factor** scrolling list (2.1).
3. **Synchronized Field of View** : If the BFMC has two cameras and the synchronized field of view box is checked, both camera experiment views are modified similar if the zoom factor is changed.

5

Specification

5.1 General specifications

Table 5.1: *General specifications for BFMC, connectorized LEDs and cameras*

Specification	Value	Unit
Bundle-imaging Fluorescence Mini Cube		
Wavelength range	350 to 1100	nm
Field of view	2.5	mm
Optical Fiber connector	SMA	-
Connectorized LEDs		
Input Current	Min : 0 Max : 1000	mA
Cable connector	M8-4pins Male	-
Output NA	0.55	-
Maximum Output Power	See Table 5.2	-
Cameras		
CMOS image sensor	Sony IMX249LLJ	-
Pixel Size	5.86 x 5.86	μm
Quantum Efficiency	82% at 520nm	
Computer interface	USB3.0	-
I/O Connector	Hirose	-
	HR10A-10P-125(73)	-
Power consumption (supplied by USB)	200	mA

5.2 Optical specifications

Table 5.2: *Typical Connectorized LED Output Power vs Optical Fiber Core Diameter*

LED		TYPICAL OUTPUT POWER @1000 mA (mW)[1]	
Central Wavelength (nm)	Bandwidth FWHM (nm)	Core 200 µm 0.37 NA	Core 400 µm 0.57 NA
405	10	~0.100	~0.470
415	10	~0.130	~0.500
474	23	~0.180	~0.700
563	9	~0.020	~0.090

Table 5.3: *Typical filter configuration of BFMC*

Fluorescence Mini Cubes	Excitation (nm)	Fluorescence (nm)	Opsin (nm)
BFMC4			
GCAMP Isosbestic and Functional	400-410[2] 460-490	500-550	
BFMC5/BFMC6[3]			
GCaMP Isos. + Func. and RFP	400-410[2] 460-490 555-570	500-550 580-680	
GCAMP Isos. + Func. and Opsin	400-410[2] 460-490	500-550	580-650
GFP + RFP and Opsin	460-490 555-570	500-550 580-680	628-642
BFMC7/BFMC8[3]			
Three-fluorophore Fluorescence and Opsin	400-410[2] 460-490 555-570	500-540 580-620	433-456 628-642

[1] All power values taken at a maximum current of 1000 mA, except for 405 and 415 nm LEDs (500 mA).
[2] GCAMP Isosbestic excitation can be modified to 410-420 nm.
[3] These configurations are offer with one or two cameras to detect the fluorescence bands.

Table 5.4: *Filter transmission and blocking band for standard filters (in nanometer)*

Port	**Blocking bands 1** ($T < 10^{-5}$)	**Transmission band** ($T > 0.9$)	**Blocking bands 2** ($T < 10^{-5}$)
400-410	350-393	398-411	415-850
420-445	200-415	418-444	449-1000
460-490	291-451	463-498	497-710
460-500	279-459	463-498	507-962
500-540	268-493	499-539	549-935
500-550	298-496	500-549	559-953
528-556	268-521	526-555	566-938
540-570	274-536	540-567	570-885
555-570	303-552	556-569	572-784
580-650	269-568	576-549	670-1000
580-680	320-575	582-678	688-975

5.3 Mechanical specifications

Please consult the customer drawing of each BFMC for more detailed dimension of the products. They are available for download on the corresponding product page on the website.

Table 5.5: *Overall size*

Model	**Depth (mm)**	**Width (mm)**
BFMC4, 1 Camera 2 Port	154.6	189.4
BFMC5, 1 Camera 3 Port	159.6	188.4
BFMC6, 2 Camera 3 Port	238.6	246.6
BFMC7, 1 Camera 5 Port	159.6	293.0
BFMC8, 2 Camera 5 Port	238.6	327.0

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Support

6.1 Maintenance

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

6.2 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.

6.3 Contact us

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

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